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Role of antibody-secreting cells and tertiary lymphoid structures in breast tumors immunosurveillance

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**Role of antibody-secreting cells and tertiary
lymphoid structures in breast tumors
immunosurveillance**

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"I learned that courage was not the absence of fear, but the triumph over it. The brave man is not he who does not feel afraid, but he who conquers that fear."

Nelson Mandela

Résumé scientifique (Français)

Les lymphocytes B (LB) sont des acteurs émergents de l'immuno-surveillance tumorale mais leur diversité et mécanismes d'action sont méconnus. Par des analyses en cytométrie de flux, par imagerie tissulaire par immunofluorescence multiplex, biochimiques et transcriptomiques, nous montrons que les tumeurs du sein (BT) sont infiltrées par des cellules productrices (ASC) d'IgG, mais également d'IgA de sous classes IgA1 et IgA2, d'isoformes mono- et dimériques et ciblant des antigènes souvent différents de ceux des IgG et impliqués dans des voies biologiques distinctes. Nous montrons par ailleurs une évolution drastique des ASC entre des tumeurs *in situ* précoces dans lesquelles les IgA-ASC prédominent très largement et les tumeurs invasives enrichies en IgG ASC et appauvries en IgA2. Par ailleurs, l'analyse d'une banque de données publiques et d'une cohorte rétrospective de cancers du sein triple négatifs (TNBC) montrent qu'un fort infiltrat en IgA ASC corrèle avec une survie accrue indépendamment des IgG ASC et est associé à un enrichissement en gènes de mastocytes et neutrophiles. L'autre versant de ma thèse a consisté à déterminer le lien entre l'organisation des LB en structures lymphoïdes tertiaires (TLS), l'antigénicité des tumeurs et la qualité de la réponse immunitaire grâce à des analyses multi-IF couplées au séquençage de l'ARN et de l'exome tumoral. Nous montrons dans une cohorte de TNBC qu'une forte densité en TLS est associée à un meilleur pronostic des patientes mais ne corrèle pas avec la charge mutationnelle des tumeurs ni le nombre de néoépitopes T prédits à partir des mutations. Cependant, nous démontrons que les TLS se développent préférentiellement dans les tumeurs surexprimant des gènes de cancer-testis antigènes (CTA) et/ou des fragments de rétrovirus endogènes humains (HERV). Les patients avec une forte densité en TLS sur-expriment des gènes associés à la cytotoxicité, à la voie Th1, au recrutement de lymphocytes T et B et à la région variable des immunoglobulines suggérant une meilleure réponse antitumorale. Nous avons également défini une signature génique de TLS adaptée au cancer du sein, qui, une fois validée, nous permettra de confirmer nos résultats à l'aide de bases de données publiques comme le TCGA. Ces résultats démontrent d'une part l'hétérogénéité des ASC, leur évolution au cours de la progression tumorale et un rôle bénéfique des IgA ASC sur la survie des patients et d'autre part que les TLS se développent principalement dans les tumeurs surexprimant certains types d'Ag tumoraux et sont associés à la mise en place d'une réponse anti-tumorale. Ces résultats devraient permettre à terme d'identifier de nouveaux mécanismes d'immunosurveillance médiée par les ASC et les TLS et manipulables à des fins thérapeutiques.

Scientific résumé (English)

B cells emerge as critical actors in the tumor microenvironment, but their diversity and clinical impact is still poorly investigated and remain controversial. Multiparametric flow cytometry and multiplexed *in situ* immunofluorescence imaging, antibody specificity profiling and transcriptome analysis were performed on fresh and FFPE breast tumor (BT) samples showed that BT were infiltrated by cells producing (ASC) IgG, but also IgA of IgA1 and IgA2 subclasses, of monomeric and dimeric isoforms and targeting different antigens involved in distinct pathways. Besides, we observed a drastic evolution of ASC between *in situ* carcinoma (early stage) where IgA-ASC predominate in all cases, and invasive tumors that were enriched in IgG ASC. Moreover, the analysis of publicly available database and a retrospective cohort of triple negative breast cancer (TNBC) revealed that a high density of IgA ASC correlated with increased survival in invasive breast tumors irrespectively of IgG ASC and were associated with an enrichment of mast cell and neutrophil genes. Besides, we aimed to study the link between the organization of B cells in tertiary lymphoid structures (TLS), tumor antigenicity and the quality of the immune response using multi-IF staining on tissue coupled to RNA-sequencing and whole-exome sequencing of the tumor. We showed that TLS are strongly associated with a favorable prognosis, but did not correlate with tumor mutational burden and predicted neoantigens count. However, we demonstrated that TLS develop preferentially in tumors that overexpress cancer-testis antigens (CTA) and/or human endogenous retroviral elements (HERV) genes. Patients exhibiting a high TLS density, upregulated genes of Th1-oriented immune response, cytotoxicity and variable regions of immunoglobulins suggesting that an efficient anti-tumor immune response is ongoing. Eventually, we defined a new TLS gene signature more adapted to breast tumor, which once validated, will allow to confirm our results using public databases such as the TCGA. These data demonstrate that IgA ASC have a positive impact on patient survival and that TLS develop preferentially in tumors overexpressing some types of tumor antigens and are associated to induction of anti-tumor immune response. This may lead to identify new immune surveillance mechanisms mediated by ASC and TLS that can be manipulated for therapeutic purposes.

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ABBREVIATIONS

AID	Activation Induced cytidine Deaminase	iBALT	inducible Bronchus-Associated Lymphoid Tissue
APC	Antigen Presenting Cell	IBC	Invasive Breast cancer
ASC	Antibody-secreting cell	IC	Immune-Complex
BCR	B Cell Receptor	ICPL	Immune Checkpoint Ligand
BLNK	B-cell linker protein	ICP	Immune Checkpoint
Breg	Regulatory B cell	IDC	Invasive Ductal Carcinoma
CAF	Cancer-Associated Fibroblasts	IF	Immunofluorescence
cDC	Conventional DC	Ig	Immunoglobulin
CRC	Colorectal carcinoma	IHC	Immunohistochemistry
CSR	Class Switching Recombination	IM	Invasive Margin
CTL	Cytotoxic T Lymphocyte	iNOS	inducible Nitric Oxide Synthase
DC	Dendritic cell	LN	Lymph Node
DCIS	Ductal carcinoma <i>in situ</i>	LTi	Lymphoid Tissue inducer
DC-Lamp	Dendritic Cell/Lysosomal	LTo	Lymphoid-Tissue organizing
EAE	Experimental Autoimmune Encephalomyelitis	LT α	Lymphotoxin alpha
ER	Estrogen Receptor	LT α 1 β 2	Lymphotoxin α 1 β 2
FACS	Flow cytometry	LT β R	Lymphotoxin- β receptor
Fas-L	Fas ligand	LV	Lymphatic Vessel
FDC	Follicular Dendritic Cell	mAb	Monoclonal Antibody
FFPE	Formalin-fixed paraffin-embedded	MDSC	Myeloid-derived suppressor cells
FRC	Fibroblastic Reticular Cell	MHC	Major Histocompatibility Complex
GC	Germinal Center	MMR	Mismatch Repair
GrB	Granzyme B	MSI	Microsatellite instability
HCC	HepatoCellular Carinoma	NAC	Neoadjuvant Chemotherapy
HE	Hematoxylin and Eosin	NK	Natural Killer
HER2	Human epidermal growth factor	NKT	Natural killer T
HERV	Human Endogeneous Retroviral Elements	NSCLC	Non-Small-Cell Lung Cancer
HEV	High Endothelial Venule	PCR	Pathologic complete response
HIF	Hypoxia-Inducible Factor	PDAC	Pancreatic Ductal Adenocarcinoma
HLA	Human Leucocyte Antigen	pDC	Plasmacytoid DC
HPV	Human Papillomavirus	PNAd	Peripheral-Node Addressin
		PR	Progesteron Receptor

SHM	Somatic Hypermutation	TMA	Tissue MicroArray
SLO	Secondary Lymphoid Organ	TMB	Tumor Mutational Burden
STAT3	Signal transducer and activator of transcription 3	TME	Tumor microenvironment
TAA	Tumor-associated antigens	TNBC	Triple-negative breast cancer
TAM	Tumor-Associated Macrophages	Treg	Regulatory T cells
T-bet	T-box transcription factor	TSA	Tumor-specific antigens
TC	Tumor Center	VEGFR2	Vascular Endothelial Growth Factor Receptor
TCGA	The Cancer Genome Atlas	VSCM	Vascular Smooth Muscle Cells
Tcm	Central memory T cells	VTP	Vascular Targeting Peptide
TCR	T-cell receptor		
Tfh	T follicular helper cell		
Tfr	T follicular regulatory		
Th	T helper		
TIL-B	Tumor-Infiltrating B cell		
TIL	Tumor Infiltrating Lymphocyte		
TLS	Tertiary lymphoid structure		

PREAMBLE

Several populations of immune cells infiltrate malignant tissues and they can either inhibit or promote tumor growth. These cells fall into two categories: innate and adaptive immune cells. T and B cells belong to the adaptive immune response, which is antigen-specific, needs a lag time between antigen exposure and immune response development and have the capacity for memory. In the context of cancer, the role of T lymphocytes has been well characterized. Several T cell subsets have been identified in the tumor microenvironment (TME) including those killing directly or indirectly malignant cells such as cytotoxic T cells (CTL) and Th1, and those helping their escape from the immune response like regulatory T cells (Treg). These basic discoveries led to the development of new strategies to fight against cancer including immune checkpoint blockade. On the other hand, B cell role is just emerging and still remains unclear, even if recent publications strongly support their critical role in the TME. Several B cell subsets have been described in tumors ranging from naïve B cells to terminally differentiated antibody-secreting cells (ASC) and memory B cells. Besides that, B cells can form dense aggregates adjacent to a T zone in so-called tertiary lymphoid structures (TLS).

My thesis project aimed to better characterize ASC heterogeneity in breast tumors, to determine their impact on cancer progression and patient survival and to give some insight into the underlying mechanisms that explain their differential impact. My second objective was to study the link between TLS and tumor immunogenicity and the quality of the immune response developed intratumorally.

In the first part of my introduction, I describe the immune microenvironment of breast tumors, mostly in Humans, with an emphasis on the current knowledge of the pro- VS anti-tumor roles of B cells. Then, I will discuss different aspects of TLS including their composition, heterogeneity, impact on the antitumor immune response and patient's prognosis, and finally the key actors involved in their development. The last chapter of this introduction is presented as a review article that will discuss recent data on the diversity of ASC and produced antibodies in the TME of breast and ovarian cancers and how the different ASC subsets and antibody isotypes and isoforms can contribute to the pro- vs anti- tumor effects.

The second part is dedicated to the results that were presented as a manuscript prepared for journal submission that focuses on disentangling ASC phenotypic and functional heterogeneity and their clinical impact on breast cancers. This was followed by complementary data, which evaluate the link between TLS and tumor antigens including neoantigens, endogeneous retroviral elements (HERV) and cancer testis antigens (CTA). Eventually, a discussion of the results as well as a conclusion and perspectives ended up this section.

Introduction

CHAPTER 1: BREAST CANCER-INFILTRATING B CELLS: KEY ACTORS IN THE TUMOR MICROENVIRONMENT

1. Breast cancer subtypes, prognosis and treatment

1.1. The epidemiology of breast cancers

In France, more than 58000 individuals were diagnosed with breast cancer in 2020, accounting for approximately 28% of all new cancer cases among women. Nearly 14000 patients die of breast cancer making it the first-leading cause of cancer death among French women (International Agency for Research on Cancer, IARC). Less than 1% of breast cancers occur in men according to the world health organization (WHO).

Various factors increase the incidence of breast cancer such as aging, genetic factors (dominant mutations in BRCA1, BRCA2 and PALB-2), lifestyle (obesity, alcohol, tobacco, hormones) and the environment (history of radiation exposure) (Hortobagyi et al. 2005) (WHO). Although the prevalence of breast cancer is increasing, improvement of patient survival is also observed thanks to early detection programs combined with innovative therapeutics including targeted therapies. Survival from breast cancer for at least 5 years after diagnosis ranges from more than 90% in high-income countries to 40% in South Africa for example (WHO).

1.2. Invasive breast cancer is a heterogeneous disease

Depending on distinctive histological and molecular features, numerous entities of breast cancer were characterized. In fact, there are some evidences showing that breast cancer is a collection of different diseases affecting the same anatomical organ (Perou et al. 2000; « Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications | PNAS » s. d.; Z. Hu et al. 2006). Many factors can determine patient clinical outcome including the tumor size, invasion of axillary lymph nodes as well as histological and molecular tumor subtypes. Each of these parameters, alone or in combination, lead to differences in response patterns to various treatment strategies (Weigelt, Geyer, et Reis-Filho 2010).

1.2.1. Histological classification of breast cancers

Breast cancers are classified according to histological types (Ellis et al.1992) that refers to their architectural pattern, cytological features and immunohistochemical profile. Up to 80% of infiltrating breast cancers are ductal carcinomas (IDC). Infiltrating lobular carcinomas (ILC) account for 5-15% of breast cancers (**Figure1**) (Ellis et al. 1992). Other histological subtypes have been described, albeit less frequently including mucinous, tubular, medullary and papillary carcinoma. This histological classification may indicate the site of origin within the mammary ductal system (Weigelt, Geyer, et Reis-Filho 2010).

1.2.2. Biological VS intrinsic molecular subtypes of breast tumors

In light of targeted therapies advances in breast cancer, it has become necessary to accurately stratify patients. This leads to the generation of new classification systems that incorporate the expression of markers such as estrogen and progesterone receptors (ER/PR), human epidermal growth factor receptor 2 (HER2) and the Ki67 proliferation marker. Immunohistochemical techniques (T. O. Nielsen et al. 2004) are widely applied to classify patients in different biological subtypes (**Figure 1**) which are:

- **Luminal subtype:** characterized by the expression of ER and/or PR by malignant cells, and is the most frequent subtype (50% of breast cancers). The luminal A subtype is characterized by low expression of Ki67 and is associated to low-rate recurrence and good response to hormonotherapy. High proliferation of cancer cells (Ki67 \geq 20%) refers to luminal B subtype (20% of breast cancers) that correlates with less favorable outcome (Prat et al. 2013).
- **HER2-enriched subtype:** HER2, also designated ERBB2 gene amplification is the primary mechanism of HER2 overexpression. It accounts for 15% of total breast cancers, have a high level of Ki67 and are associated with poor prognosis despite of the development of targeted therapies directed against HER2 with monoclonal antibodies or EGFR/HER2 kinase inhibitors (Goldhirsch et al. 2013).
- **Triple negative breast cancer (TNBC):** Less than 20% of breast tumors lack the expression of ER, PR and HER2 with an elevated mitotic index. Most TNBC are aggressive tumors with few therapeutic options (Fragomeni, Sciallis, et Jeruss 2018; Kondov et al. 2018).

More than 20 years ago, Perou et al. used gene expression profiling studies to distinguish **intrinsic molecular** breast tumor classes. Using 496 genes and an unbiased hierarchical clustering, they classified tumors into an ER-positive group, corresponding to the luminal A and B subtypes, and an ER-negative group that was further divided into basal-like, ErbB2+ and normal-like breast tumors. More recently, a new subtype of "Claudin-low" has been characterized (**Figure1**)(Prat et al. 2010). It is worth mentioning that based on The Cancer Genome Atlas (TCGA) database, Garrido-Castro et al. showed that 86% of TNBC (characterized by IHC) correspond to basal-like tumors based on their transcriptomic profile (Garrido-Castro, Lin, et Polyak 2019).

Interestingly, the different molecular subtypes display highly significant differences in prediction of overall survival, with the basal-like subtype having the worst outcome. Despite the clear benefit of this approach for predicting patient's outcome, relapse risk and for orienting therapy, the prohibitive cost of gene expression profiling technique restricts its use in clinical practice. To counter this obstacle, investigators narrowed down a 50-genes signature, termed PAM50, that can quite reliably

differentiate the molecular subtypes using targeted microarrays or quantitative real time PCR (Parker et al. 2009; Yersal et al. 2019).

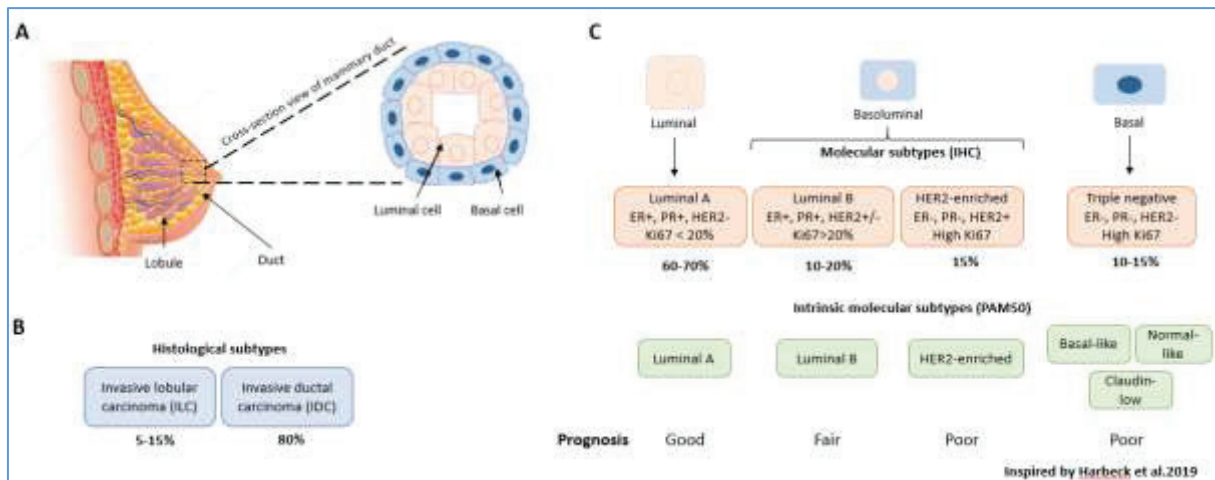


Figure 1: Histologic and molecular invasive breast cancer classification: (A) Graphical representation of the mammary gland and its duct. **(B)** Histological classification of breast cancer subtypes. **(C)** Molecular classification by IHC and PAM50 determination, showing their features, frequency and prognosis.

1.3. From *in situ* to invasive breast cancer

Ductal and lobular tumors are also categorized into *in situ* and invasive carcinoma. Ductal carcinoma *in situ* (DCIS) is considerably more common (80% of breast carcinoma *in situ*) than its lobular counterpart (20%). DCIS is defined as a premalignant proliferation of neoplastic mammary epithelial cells inside mammary ducts and surrounded by an intact basement membrane (Lopez-Garcia et al. 2010). Generally, patients diagnosed with DCIS have a favorable long-term breast-cancer-specific survival of 98% 10 years after surgery (Elshof et al. 2018).

A plethora of succeeding work studies to characterize DCIS and invasive breast carcinoma (IBC) at the molecular level, revealed their genetic similarity and potential common origin, suggesting that DCIS precedes development of invasive ductal carcinoma (IDC). Moreover, in many instances *in situ* and invasive lesions co-exist in IBCs at the time of diagnosis (Fisher et al. 1975). In most cases, these two components exhibit similar molecular subtype and comparable nuclear morphology and DNA ploidy, which strengthen this hypothesis (Tamimi et al. 2008; Giardina et al. 2003).

Erbas and collaborators, by reviewing breast biopsies, found that 14% to 53% of DCIS cases evolved to IDC over a minimum period of 10 years (Erbas et al. 2006). To decipher the mechanisms underlying the progression of DCIS to IDC (Figure 2), two main theories emerged: the first considers invasiveness to be driven by the selection of subclones with specific genetic aberrations (Doobar et al. 2019) while the second supports that the microenvironment, including immune elements and tumor stromal components, actively drives tumor progression (Kim et al. 2020; Lyons et al. 2011). There is

still open questions that need answers; probably the most important one being to identify biomarkers able to distinguish *in situ* breast carcinomas that will progress to invasive cancers from those that will not evolve.

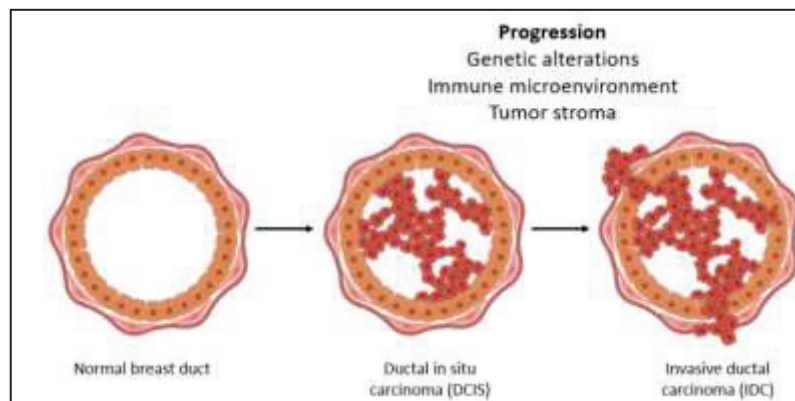


Figure 2: Ductal in situ carcinoma (DCIS) to invasive ductal carcinoma (IDC) progression hypothesis.

1.4. Invasive breast cancer: prognostic factors and treatment

1.4.1. Prognostic factors

Given the fact that breast cancer is a heterogeneous disease, identifying prognostic and predictive biomarkers is mandatory to optimize therapy decision and prevent cancer relapse in patients. Traditionally, the most widely used prognostic factors for the recurrence of breast cancer includes tumor size, histologic grade and the number of regional lymph nodes with metastases (Cianfrocca et Goldstein 2004; Donegan 1997). ER, PR, HER2, and Ki67 status determination in the context of breast cancer are also recommended for clinical use to evaluate patients' prognosis and to make treatment decision.

All these factors cited above are always useful but not sufficient and molecular tests have emerged for personalized and optimum patient management. In fact, molecular biomarkers had significantly improved ability to predict risk of relapse as compared to a model utilizing only clinical variables (tumor size, node status and histologic grade). Indeed, combining both clinical variables and molecular subtypes resulted in significantly better predictive value (Parker et al. 2009; Yersal et al. 2019). Moreover, they do not only provide prognostic information, but also predict response to therapy (Duffy et al. 2017; Györfy et al. 2010).

1.4.2. Treatment of breast malignancies

The therapeutic arsenal for non-metastatic breast cancer consists of both local – including surgical resection and postoperative radiation - and systemic therapies. The latter may be preoperative (neoadjuvant), postoperative (adjuvant) or both and is guided by the histological and molecular tumor classification. Hormonotherapy (+/- chemotherapy) is used for all patients with ER+ and/or PR+ tumors, anti-HER2 monoclonal antibody (trastuzumab) or a dual EGFR/HER2 kinase inhibitor plus chemotherapy for all HER2-positive tumors, while chemotherapy alone is used for triple negative breast cancer (TNBC). TNBC displays aggressive behavior, including earlier recurrence and metastasis with limited therapeutic options (Garrido-Castro, Lin, et Polyak 2019). Recently, immunotherapies blocking immune checkpoint inhibitors (ICPs) and their ligands (ICPL) such as PD1, CTLA4 and PDL1 have revolutionized patients care by inducing durable objective response across multiple tumor types, by re-invigorating the cytotoxicity and proliferative capacity of tumor infiltrating lymphocytes (TILs). Taking into account that TILs and PDL1 expression correlate with better response to immunotherapies and the fact that TNBC are highly infiltrated by TILs (Denkert et al. 2018) and express high level of PDL1, TNBC patients constitute good candidates for ICP/ICPL antagonists strategy. In spite of all these promising elements, clinical trials showed a low efficacy of these therapies when used alone. However, in early-stage TNBC, immunotherapy in combination with chemotherapy regimens demonstrated preliminary success. The I-SPY 2 trial on patients with stage II-III disease revealed that anti-PD1 combined to neo-adjuvant chemotherapy increased the estimated pathologic complete response (pCR) rates from 22% to 60%. Unfortunately, this was not confirmed by other clinical trials (Keenan et Tolaney 2020). Therefore, there is an urgent need for novel and more efficient immunotherapeutic agents to improve treatment of TNBC patients. It is also essential to develop biomarkers that predict response and resistance to therapy.

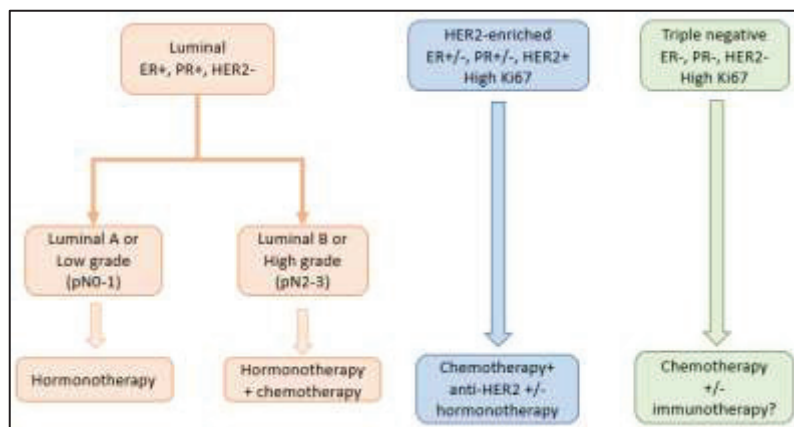


Figure 3: Therapy decision algorithm depending on molecular subtypes. ER: estrogen receptor, PR: Progesterone receptor, pN: nearby node metastasis.

2. The immune microenvironment of breast tumor

Tumor cells grow in a complex ecosystem composed of stromal cells, vessels, soluble factors, extracellular matrix and immune cells. The latter cells can indeed infiltrate the tumor microenvironment (TME) and evolve during cancer development according to the 3E theory: Elimination, Equilibrium and Escape. The **elimination** phase is defined by an efficient anti-tumor immune response against immunogenic abnormal cells preventing the emergence of tumors. During the **equilibrium** step that probably lasts for months or years in an asymptomatic form, there are cumulating advantageous tumor mutations under immune pressure in a so-called “immunoediting process”. During immunoediting, the balance shifts toward the “**escape** phase” as tumor cells continuously alter their immunogenicity and establish an immunosuppressive milieu within the TME. Tumor cells can diminish the expression of neoantigens and antigen presentation molecules, release suppressive cytokines and upregulate co-inhibitory immune ICPL on their surface to favor tumorigenesis (Dunn et al. 2002).

Leukocytes are rare but detectable in the healthy mammary gland tissue (Tower, Ruppert, et Britt 2019). During breast cancer development, both innate and adaptive immune cells are recruited. In DCIS, immune cells are usually abundant and mainly present in the stroma surrounding the ducts (Gil Del Alcazar et al. 2017). The composition and functions of leukocytes varies according to the stage of tumor development, molecular subtype, histologic stage and according to the treatment received by patients (Desmedt et al. 2018; Denkert et al. 2018; Massa et al. 2020). The following section provides an overview of what is known about the major BC-infiltrating immune cells, their impact on the tumor, and how they can influence patients’ prognosis.

Myeloid lineage immune cells represent a prominent cell population in the TME headed by tumor-associated macrophages (TAM). TAM are highly plastic in response to various stimuli and can play different roles in the TME ranging from priming antitumor response to pro-angiogenic and pro-tumor properties (Mantovani 2010). Indeed, M1-type macrophages, characterized by their secretion of pro-inflammatory cytokines and nitric oxide, elicit effective killing of malignant cells, whereas M2-type macrophages, which constitute the majority of TAM, support survival, invasiveness of cancer cells and attract regulatory T cells (Tregs) intratumorally (G. Solinas et al. 2009). A high TAM density is associated with a bad outcome in breast cancer (Tsutsui et al. 2005; Yue Zhang et al. 2013).

Myeloid-derived suppressor cells (MDSC) are a mixture of progenitor and immature myeloid-lineage cells, related to granulocytes and monocytes, which accumulate in the blood and tumor tissue of cancer patients. In breast cancer, high number of circulating MDSC is associated with poor prognosis (Safarzadeh et al. 2019). They are recruited by tumors to impair immune cell signaling by the secretion

of immunosuppressive soluble factors including IL10, TGF β and arginase. MDSC also promote angiogenesis, inflammation and metastasis (Baxevanis, Fortis, et Perez 2021).

Dendritic cells (DC) are critical in the TME since they enable to process tumor antigens, prime CTL and polarize CD4+ T cells to effective anti-tumor subsets. Different DC subtypes have been identified in human tumors at immature and mature states. In breast cancer, conventional DC (cDC) known as myeloid DC were described. This family of cells can be further subdivided into two distinct subpopulations: cDC1 and cDC2. Murine model showed that tumor-associated cDC1 engulf tumor material and migrate to secondary lymph nodes where they efficiently activate T cells by antigen presentation (Roberts et al. 2016; Broz et al. 2014). Recently, Hubert and colleagues demonstrated in human breast cancer that cDC1 subset play an important role in antitumor immune response through type III interferon production. This cytokine promotes a type 1 T helper (Th1) microenvironment through increased production of IFN γ , IL12p70, and cytotoxic lymphocyte-recruiting chemokines (Hubert et al. 2020). Moreover, DC-Lamp, a marker of activated DC strongly correlates with favorable prognosis (Martinet et Girard 2013). Nevertheless, cumulative evidence supports that the TME influences DC toward an immature and tolerogenic state expressing IL10 and TGF β , which trigger Tregs expansion (Aspord et al. 2007). Another DC subset, plasmacytoid DC (pDC), correlate with bad prognosis in breast tumors (Treilleux et al. 2004). Interestingly, Faget et al. reported an ICOS-mediated cross talk between CD4+ T cells and pDC leading to Treg amplification and patient survival impairment (Faget et al. 2013).

Lymphocytes are also present in breast tumors at variable proportions. In TNBC and HER2+ breast cancers, but not the luminal subtype, a high density of tumor-infiltrating lymphocytes (TILs) is associated with a favorable prognosis and a better response to chemotherapy (Dushyanthen et al. 2015; Pruneri, Vingiani, et Denkert 2018). TILs can consist of CD8+ cytotoxic T lymphocytes (CTLs), CD4+ T cell subsets, NK, NKT, T $\gamma\delta$ and B cells, which exert a wide range of functions and can thus differentially affect tumor growth. CTL are the most powerful effector in anti-tumor immune response. They recognize cancer-specific peptide MHC I complexes via their T-cell receptor (TCR) in cancer patients and effectively clear tumor cells. Two main mechanisms have been described to eliminate target cells, perforine/granzyme and death receptors (Fas/FasL and TRAIL/TRAIL-R) that induce apoptosis of malignant cells. The presence of CTL in cancer have a robust favorable prognostic value and recently it was considered as a predictive biomarker for response to anti-ICP/ICPL (Pruneri, Vingiani, et Denkert 2018; Bai et al. 2020). Th1 cells are a source of cytokines such as IFN γ which promotes antigen processing and presentation and activates DC, and stimulate M1 macrophages and NK cells to eliminate tumor cells (Emens 2012). In contrast, Th2 cells secrete IL4, IL10 and IL13, which can inhibit Th1 polarization and promote breast cancer development (Kohrt et al. 2005;

Kaewkangsadan et al. 2016). Tregs have broadly been described as tumor-promoting cells utilizing diverse mechanisms including cell contact and soluble factor secretion, thus associated with bad prognosis (Tanaka et Sakaguchi 2017; Ali et al. 2016; Cinier et al. 2021).

Although natural killer (NK) cells are generally considered as anti-tumor effectors due to their capacity to kill target cells, their role in breast cancer depends on their state of activation. High infiltration of HER2+ tumors with activated NK and a gene signature score of NK and DC in tumors of TNBC patients have been associated with better survival (Bense et al. 2017; Böttcher et al. 2018). Conversely, some authors demonstrated that resting NK in HER2+ and TNBC tumors correlate with bad prognosis in patients because of their altered functions and pro-angiogenic properties (A. Bruno et al. 2014; Bense et al. 2017).

Overlooked for a long time, B cells are increasingly emerging as critical actors of the TME and the next section will be dedicated to discuss their diversity and how they promote or suppress antitumor immunity.

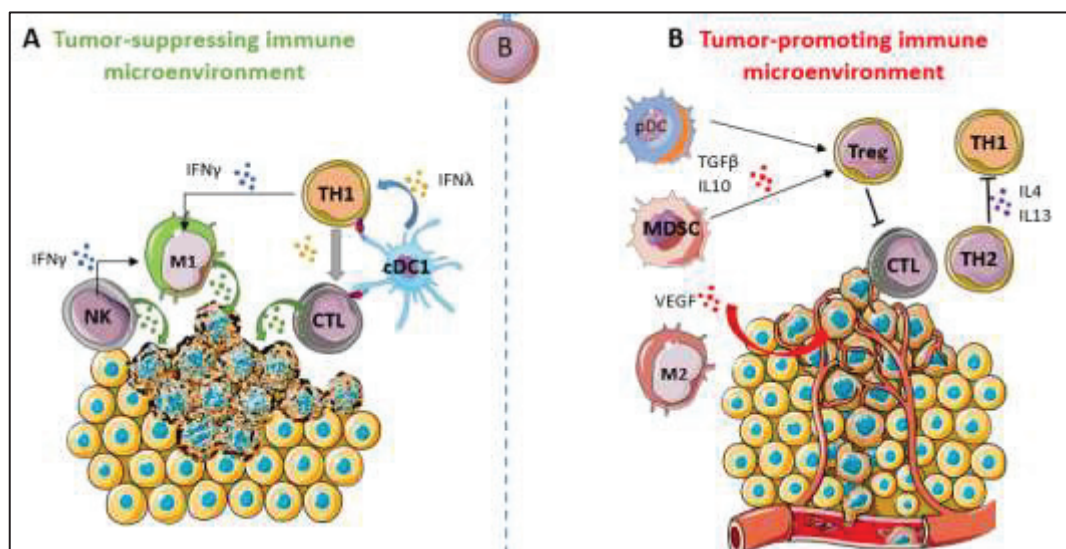


Figure 4: Breast tumor immune microenvironment. A shows antitumor immune cells including TH1, CTL, M1-macrophages, NK and cDC1 that can eliminate malignant cells efficiently. B Other cells harbor immunosuppressive properties Treg, MDSC, pDC and M2 macrophages and contribute to tumor growth. The role of B cells is still controversial.

Box1: Spatial architecture of the tumor immune microenvironment

A solid body of evidence has accumulated to demonstrate that the composition of tumor immune infiltrate provides many information on their mechanisms of action and their impact on patients' survival. However, patients with similar infiltrate compositions may have different prognosis, indicating further exploration is needed.

Thanks to innovative technologies and advances in bioinformatics analysis, a plethora of recent studies have revealed the primary importance of the spatial architecture of the immune TME, namely the location of immune cells, their interaction with their neighborhood and the spatial pattern of well-organized structures such as tumor-associated tertiary lymphoid structures (TLS) (See chapter 2) (Fu et al. 2021). The team of J. Galon pioneered this field through the development of an immunoscore for colorectal cancer patients, which is a simplified tool to measure the abundance of T cell populations located in the tumor center (TC) or invasive margin (IM). Remarkably, this immune-based classification has a superior prognostic value compared to usual clinical predictors (Galon et al. 2012). In breast cancer, a study demonstrated that a combined evaluation of CD8+ T cells and CD163+ macrophages densities in CT and IM allows a better stratification than the stage of the disease, tumor size and lymph node invasion (Fortis et al. 2017). Keren et al. used a multiplexed imaging platform able to simultaneously quantify *in situ* expression of 36 proteins covering identity, function and immune regulation of immune cells in TNBC patients. This work revealed that tumors with infiltrated immune cells have a better prognosis compared to those where the two compartments (immune and tumor) are segregated referred to as excluded tumors (Keren et al. 2018).

Quantifying immune cells without taking into account their distribution in the tissue and their interactions with their environment is not sufficient. Considering the spatial architecture of the TME will allow its better understanding in order to develop more accurate prognosis tools and discover new therapeutic targets including those that promote beneficial immune cells or prevent deleterious ones from infiltrating tumors.

3. B cells in breast tumors: good or bad guys?

B cells and the antibodies they produce are essential effectors of the adaptive immune system. Although their importance in infectious disease and vaccination is well recognized, their role in the TME, both in terms of influence on tumor growth, contribution to tumor immunity or immune escape and response to therapies, have been overlooked for a long time in favor of T cells. However, these cells are currently under the spotlight proved by the increasing number of studies published in the field in the recent years. Their impact on cancer patients' prognosis and their mechanisms of action is still a matter of debate and under active investigation.

In this part, we will discuss new findings on breast-cancer infiltrating B cells functions, their contribution in TME, and their impact on patients' survival.

3.1. Breast cancer-infiltrating B cells: wide range of subsets and diverse spatial organizations

Early studies, including those in breast cancer, reported that B cells can infiltrate tumors (TIL-B). Chin and Marsigliante provided the first evidence of the presence of BC-infiltrating B cells using flow cytometry (FACS) and immunohistochemistry (IHC). Although more than 70% of patients exhibited B cell infiltration, only 24% of tumors contain a large amount of these cells, which can reach 60% of the immune infiltrate (Chin et al. 1992; Marsigliante et al. 1999; Buisseret, Garaud, et al. 2017). They are detected at significantly higher densities in IBC compared to normal tissue. Using single-cell RNA-sequencing, Hu and colleagues identified multiple clusters/cell states in the B cell compartment including naïve, memory, GC-B cells and plasma cells indicating the presence of virtually all stages of B cell differentiation (Box2). Interestingly, CD25+B cells secreting IL10, so-called regulatory B cells (Bregs) were also identified in breast TME (Ishigami et al. 2019). Therefore, the breast tumor immune infiltrate contains phenotypically and functionally heterogeneous populations of B cells.

A second level of heterogeneity of the B cell compartment, besides its composition, relates to its spatial structuration in the TME. IHC analysis revealed that TIL-B in breast cancer can form dense aggregates in the periphery of the tumor (Grekou et al. 1996). Later on, Coronella and colleagues better characterized TIL-B clusters as containing, a T zone (CD3+) and B zone (CD20+) containing interdigitating follicular dendritic cells (CD21+) which function is to present native Ag to B cells (Coronella et al. 2002). These structures designated as tertiary lymphoid structure (TLS) are present in 25% to 60% of breast tumors and comprise also T follicular helper cells (Tfh) (Gu-Trantien et al. 2013; Coronella-Wood et Hersh 2003; Buisseret, Garaud, et al. 2017), high endothelial venules and mature DC (See Chapter 2).

In conclusion, the diversity of B cell subsets and their possible functional structuration with T cells in breast tumor TME may affect patient prognosis differently.

Box2: B cell ontogeny

The development of B cells requires two steps, which are explained briefly below:

- **Antigen-independent phase:** B cells are generated from a common lymphoid progenitor residing in the bone marrow. Pre- and pro-B cells undergo random rearrangements of V, D and J segments to create a functional μ heavy chain gene. Then, subsequent V and J recombination of light chain leads to formation of the B cell receptor (BCR) of the IgM isotype, which is expressed on immature B cells. Immature B cells then leave the bone marrow and migrate through the blood to the spleen to become a transitional B cells (Ti B cell). Finally, these cells migrate to secondary lymphoid organs where they undergo alternative splicing of a long primary mRNA transcript containing the rearranged VDJ exons and the $C\mu$ and $C\delta$ exons to co-express IgD and become a naïve B cell.

- **Antigen-dependent phase,** which corresponds to the activation and differentiation of naïve B cells into different types of effector cells in secondary lymphoid organs (SLO), following recognition of a cognate antigen and activation. B cells are able to directly bind soluble antigen or bind antigen presented on the surface of follicular dendritic cells (FDC), macrophages or DC. B cells take up the antigen through their BCR, process it and present it on major histocompatibility complex class-II (MHC II) molecules to CD4+ T cells. This cognate interaction results in CD40L surface expression by T cells as well as cytokine secretion (IL4 and IL21) leading to the proliferation of B cells, which then will either initiate the germinal center (GC) response (follicular response) or into short-lived extrafollicular plasma cells or memory B cells. Extrafollicular plasmablasts produce the first wave of antibodies, mainly IgM, before undergoing apoptosis within a few days. B cells that initiate GC response will migrate to the center of the follicle, begin to rapidly divide and undergo clonal expansion. Germinal center is divided into two distinct compartments known as the dark zone (DZ) and the light zone (LZ). DZ is the site where GC B cell named centroblasts, proliferate and undergo immunoglobulin class switching recombination (CSR) to express secondary Ig isotypes (IgG₁₋₄, IgA₁₋₂ and IgE in humans) and somatic hypermutation (SHM) allowing affinity maturation of their BCR. In the LZ, GC B cells referred to as centrocytes capture antigen presented on follicular dendritic cells (FDC), which they process and subsequently present to Tfh in order to select high-affinity centrocytes. The latter will re-enter the DZ for further rounds of proliferation and SHM before migrating back to the LZ for selection of variants with the highest affinity. This process is regulated by T follicular regulatory (Tfr) cells, which are also present in the LZ. Together, this will lead to the emergence of both memory B cells and long-lived antibody-secreting plasma cells that produce antibodies (Bemark 2015; Attaf et al. 2021; Stebegg et al. 2018) (Figure 5).

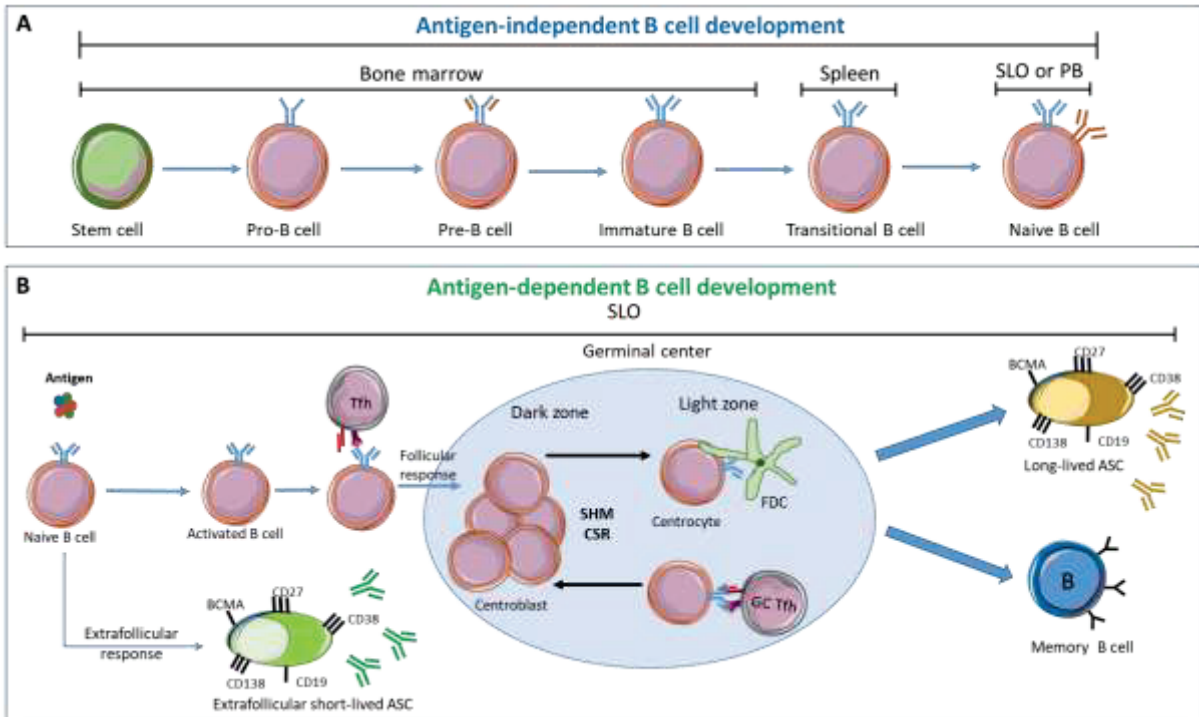


Figure 5: B cell differentiation. **A.** Antigen-independent B cell differentiation occurs in the bone marrow and the spleen. **B.** Ag-dependent B cell differentiation occurs in secondary lymphoid organs. After activation by antigens, B cells develop in a T-cell dependent way in the germinal center (follicular) and in a T-independent pathway (extrafollicular). SLO: secondary lymphoid organ. PB: peripheral blood. GC: Germinal center. ASC: antibody-secreting cell. SHM: somatic hypermutation. CSR: class switch recombination. Tfh: T follicular helper. FDC: follicular dendritic cell.

3.2. B cells can exert anti-tumor activities

3.2.1. B cell infiltration correlates with a good prognosis in breast cancer

High BC-infiltrating B cell density assessed by IHC is most often associated with a significantly better outcome in terms of patient survival, and multivariate analysis showed that CD20+ B cells are associated with better overall and disease-free survival, independently of CD8+ T cell infiltration (Mahmoud et al. 2012; H. Kuroda et al. 2021; Garaud et al. 2019). Transcriptomic studies also demonstrated that a B cell/plasma cell metagene score is strongly associated with better prognosis, confirmed by multivariate analysis (Schmidt et al. 2012; Gentles et al. 2015; Bianchini et al. 2010; Q. Hu et al. 2021). Further evidence supporting an anti-tumor effect of B cells comes from mouse models. DiLillo and colleagues reported an increased tumor growth and invasiveness in mice in which B cells have been depleted using antibodies (Abs). In fact, mice lacking B cells displayed an altered tumor-antigen specific CD8+ T cell proliferation and impairment of IFN γ - or TNF α -secreting CD4+ and CD8+ T cells induction (DiLillo, Yanaba, et Tedder 2010). Similarly, a mouse model with a knockout of the B-cell linker protein (BLNK) gene, required for B cell development, showed a decrease of tumor-infiltrating T cell number and increased tumor growth (Kobayashi et al. 2014). Furthermore, injection of pre-activated tumor specific B cells into mammary gland bearing tumors resulted in tumor regression associated with induction of tumor specific T cell immunity with IFN γ and GM-CSF production (Q. Li et al. 2011).

Moreover, B cells predict complete pathologic response in TNBC patients treated with neoadjuvant chemotherapy (H. J. Lee, Lee, et al. 2015). Recently, high expression of B cell and plasma cell gene signatures were associated with better response to anti-PD1 immunotherapy in patients affected by soft-tissue sarcoma and melanoma (Petitprez et al. 2020; Helmink et al. 2020). Remarkably, Hollern and collaborators showed, in mouse models of TNBC, that immunotherapy targeting ICP induced Tfh-mediated activation of B cells and antibody production, facilitating anti-tumor immune response (Hollern et al. 2019).

In summary, most human and murine studies revealed that BC-infiltrating B cells are associated with favorable patients' prognosis and can predict better response to neoadjuvant chemotherapy and to inhibitory immune checkpoint blockade immunotherapies, likely through different mechanisms.

3.2.2. Mechanisms underlying the anti-tumor functions of B cells

Multiple B-cell functions may contribute to limit tumor growth and promote anti-tumor immunity and extend well beyond the production of antibodies by plasma cells, a topic that will be developed in chapter 3.

3.2.2.1. B cells as antigen-presenting cells

Unlike DCs, B cells, through their BCR, can recognize antigens with high specificity allowing detection of low levels of antigens. This could be highly advantageous in poorly to moderately mutated tumors like BC where the antigenic load is low (Sautès-Fridman et al. 2020). Moreover, atypical memory B cell subset can express surface markers characteristic of antigen presenting cells (APC) such CD80 and CD86 in tumor context (J.-Y. Shi et al. 2013). In addition, it is well established that multivalent BCR ligation results in more efficient antigen presentation compared to conventional DC (Batista et Neuberger 1998). Once the antigen is recognized, it is internalized, processed and presented on MHC class II molecules to CD4+ T cells. T cells express CD40 ligand (CD40L) to stimulate in turn, CD40+ B cells. Activated B cells express CD80/CD86 that further stimulate CD4+ T cells triggering GC response (Elgueta et al. 2009). Tumor-infiltrating B cells (TIL-B) can efficiently present antigen to CD4+ T cells *in vitro*. Moreover, activated TIL-B were associated with an effector T-cell response (IFN γ + CD4+ T cells) (T. C. Bruno et al. 2017, 201). Interestingly, B cells are capable of antigen cross -presentation to CD8+ T cells on MHC class I, an essential phenomenon for anti-tumor CTL induction (DiLillo, Yanaba, et Tedder 2010). Thus, we can hypothesize that TIL-B can present tumor antigens locally to both CD4+ T cells and CD8+ T cells to induce a specific anti-tumor immune response.

3.2.2.2. B cells as a source of cytokines promoting anti-tumor immunity

Numerous studies on allograft rejection and in infectious or autoimmune diseases provide evidence on the great ability of B cells to secrete a large array of cytokines that might be beneficial in the context of cancer. B cells can contribute positively to Th1-type immunity through the production of CCL3, TNF α , GM-CSF, and IFN γ . IFN γ -secreting B cells, observed in a model of murine melanoma, promoted pro-inflammatory Th differentiation and tumor rejection (Ding et al. 2017). Moreover, B cells, once activated, can produce IL6 that facilitate differentiation into Tfh. Furthermore, in *cxc13*-transgenic murine models, LT α 1 β 2-expressing B cells at ectopic sites were sufficient to induce TLS (See chapter2) (P. Shen et Fillatreau 2015; Y. Shi 2021; Ansel et al. 2000). Nevertheless, it is likely that the type of secreted cytokines depends on the nature, dose and sequence of stimuli that B cells receive.

3.2.2.3. Cytotoxic B cells

In specific conditions, B cells can acquire cytotoxic properties. Hagn et al. induced differentiation of B cells into granzyme B (GrB)-secreting cytotoxic cells through T cell-derived IL21 and in the absence of CD40L costimulation, indicating an early cellular immune response mediated by B cells, before a fully activation of antigen-specific T cells. Surprisingly, these cells do not express perforin, yet they were able to induce apoptosis of tumor cell lines by effective GrB release (Hagn et al. 2012). Multi-immunofluorescent staining on IBC formalin-fixed paraffin-embedded (FFPE) sections confirmed the presence of GrB-secreting B cells in the TME in the vicinity of IL21-expressing T cells (Lindner et al. 2013). Additionally, CD19+ B cells from peripheral blood stimulated with CpG-containing oligodeoxynucleotides (CpG-ODN), upregulated the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/Apo2L) expression on their surface. These B cells triggered apoptosis of melanoma cells confirming their cytotoxic potential (Kemp, Moore, et Griffith 2004). Tao et al. highlighted another mechanism allowing B cells to kill directly tumor cells. They showed that IL10 gene silencing in tumor-draining lymph node B cells significantly increased their cytotoxic activity and their Fas ligand (Fas-L) expression. This result showed that these B cells can effectively kill tumor cells in an antigen-specific and Fas-L-dependent manner *in vitro* (Tao et al. 2015).

Collectively, these findings reveal the broad spectrum of anti-tumor properties exerted by B cells that may explain their association with a favorable patient outcome.

3.3. Tumor-promoting B cells

3.3.1. B cells can be associated with poor patient prognosis and promote tumor progression

Few studies in breast cancer found an association between B cells and adverse outcome. Miligy et al. showed in a cohort of pure DCIS and DCIS mixed with invasive cancer that an elevated tumor density of B cells was associated with invasion, larger tumor size, TNBC and HER2+ tumors, lymph-node metastases and shorter recurrence-free survival (RFS) (Miligy et al. 2017). No significant correlation was reported between higher CD20+ cell density assessed by IHC in tumor tissue of patients with IDC and patients' prognosis (Mohammed et al. 2012). In other solid cancers, different groups observed bad prognosis in patients highly infiltrated by B cells (Wouters et Nelson 2018; Sjöberg et al. 2018).

Many murine models lacking B-lymphocytes supported a pro-tumorigenic role of these cells in cancer. B cell-deficient mice immunized with melanoma-associated antigens were more protected against tumor development with potentiation of the magnitude and longevity of the specific CD8+ T cell immune response (Perricone et al. 2004). In another murine breast tumor model, Tadmor et al. observed that in the absence of B cells, tumor growth is inhibited accompanied with a decrease in

Tregs number and function (Tadmor et al. 2011), suggesting that B cells may favor Treg expansion and/or function. The presence of B cells was also associated with low tumor immunogenicity and inhibition of CD4+ T cell help for CTL mediated tumor immunity (Qin et al. 1998). Eventually, tumor-infiltrating B cells can mediate an acquired therapy resistance in melanoma patients treated with inhibitors to oncogenic BRAF^{V600E} (Somasundaram et al. 2017).

3.3.2. Mechanisms underlying the pro-tumor function of B cells

B cell subsets with tumor-promoting activities can be mediated by different mechanisms including pro-tumor produced antibodies (a topic underlined in chapter 3) and immunosuppressive B cells.

3.3.2.1. Immunosuppressive B cells:

B cells with immunosuppressive properties, also called Bregs, are composed of a heterogeneous group of cells capable of immunosuppressive activity in an inflammatory environment. In human cancer, a number of studies identified immature and mature B cells with a regulatory phenotype. Immunosuppressive plasma cells have also been described in tumors (see chapter 3). In invasive breast cancer patients, Guan et al. observed an elevated of CD19⁺CD24⁺CD38⁺IL10⁺Bregs, corresponding to immature/transitional B cells, in the circulation with a fraction of cells expressing PD-L1. The latter subpopulation correlated positively with Tregs and negatively with PD1^{hi} effector T cells and improved outcome (Guan et al. 2016). A recent study revealed the presence of BC-infiltrating Bregs identified by the expression of CD25 and IL10. This subset correlated strongly with Tregs and with shorter metastasis-free survival (Ishigami et al. 2019). IL10-producing Bregs can induce DC to produce IL4 and downregulate IL12, affecting the Th1/Th2 balance (Moulin et al. 2000). They can also suppress CD4+ T cell differentiation into Th1 and Th17 (Iwata et al. 2011). Moreover, this subset contributes to tumor progression by inducing macrophage polarization to M2 phenotype, that ultimately inhibit effector T and NK cells (Rosser et al. 2014). Lindner et al. described another Breg subset, which surprisingly express GrB, along with CD25, IL10, and indoleamine-2,3-dioxygenase (IDO). GrB+ Bregs are induced by IL21-secreting Tregs and integrate both BCR- and TLR-dependent signals. They harbor a CD19⁺CD38⁺CD1d⁺IgM⁺CD147⁺ phenotype (immature/transitional B cell phenotype). In contrast to cytotoxic activity of GrB from CTL, GrB secreted by Bregs is transferred to T cells and trigger the degradation of ζ chain of T-cell receptor (TCR) leading to T cell activity loss (Lindner et al. 2013). Moreover, aggressive gastric cancer had an increased proportion of Bregs expressing IL35 in the bloodstream (K. Wang, Liu, et Li 2018; Pylayeva-Gupta et al. 2016). IL35 is a suppressive cytokine of the IL12 family composed of EBI3 and IL12p35 chains and promote tumor growth, progression and metastasis by enhancing the secretion of IL6 and G-CSF and inhibiting IFN γ . Furthermore, IL35 is involved in myeloid cells recruitment and suppress CTL and NK activation (P. Shen et Fillatreau 2015;

K. Liu et al. 2021). It is worth mentioning that a recent single-cell RNA-sequencing on TNBC tumors-infiltrating B cells was unable to detect Breg cluster (Q. Hu et al. 2021).

Murine models described a wide spectrum of Breg subsets in tumors. Olkhanud and collaborators reported in a mouse model of breast cancer, the involvement of TGFβ-secreting Bregs in the polarization of Tregs from resting CD4+ T cells. This results in pulmonary metastasis that can be prevented in the absence of Bregs (Olkhanud et al. 2011). Later, another study detected PD-L1 expression on TGFβ-secreting Bregs in mammary tumors. This population was able to suppress effector T cell proliferation, Th1 cytokine secretion and NK proliferation in response to IL15. Strikingly, inhibition of TGFβ or PDL1 was sufficient to reject tumors *in vivo*, suggesting that current immunotherapies do not affect only T cells, but also B cell function (Yu Zhang et al. 2016).

3.3.2.2. Other pro-tumorigenic functions of B cells

B cells can secrete pro-inflammatory cytokines that may play important roles in cancer progression. Ou et al. observed that tumor infiltrating B cells increased invasiveness of primary human bladder cancer cells through their production of IL8 chemokine. IL8 upregulate androgen/androgen receptor signaling in tumor cells, which is involved in cancer cell metastasis (Ou et al. 2015). In the TME, B cells can be a source of IL1β, which potentiates HIF-2α expression with downstream Notch1 signaling activation in renal cancer cells leading to tumor-metastasis (S. Li et al. 2020). Somasundaram et al. reported that B cell-derived IGF-1, ligand of IGF1-R expressed on melanoma cancer cells induces resistance to BRAF and MEK inhibitors (Somasundaram et al. 2017).

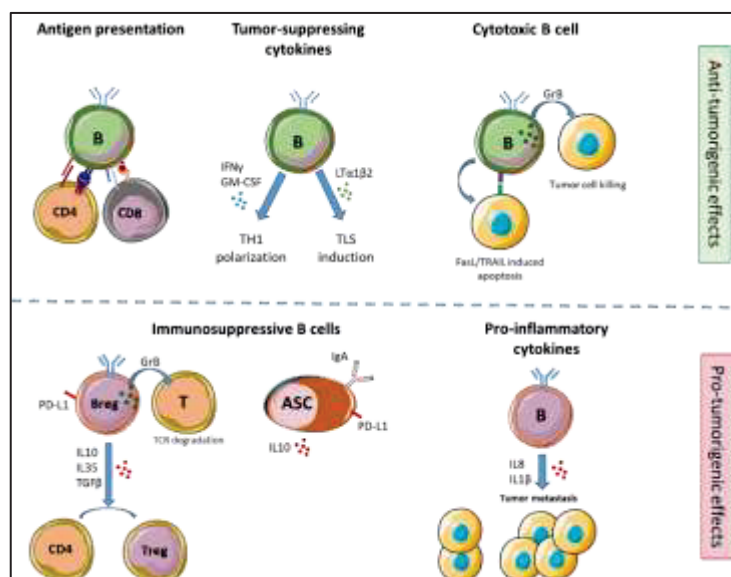


Figure 6: Anti- and pro-tumor B cell effects. B cells can suppress tumor growth through antigen presentation, secretion of tumor-suppressing cytokines and by direct cytotoxic effect. They can also exert pro-tumorigenic effects through their regulatory functions and the secretion of pro-inflammatory cytokines that enhance metastasis. TLS: tertiary lymphoid structure.

Therefore, the tumor microenvironment contains a heterogeneous population of B cells that could behave differently contributing to both pro- or anti-tumor immune response.

CHAPTER II: TUMOR-ASSOCIATED TERTIARY LYMPHOID STRUCTURES

As already mentioned in the first chapter, tumors are often infiltrated by immune cells that could organize themselves anatomically and functionally as in secondary lymphoid structures (SLO) in a so-called tertiary lymphoid structure (TLS). TLS are inducible and transient structures that were initially described in inflamed tissues associated with autoimmune diseases, infections and graft rejection. In the context of cancer, Coronella et al. were the first to report the presence of B cell aggregates containing follicular dendritic cells (FDC) adjacent to a T zone in breast tumors (Coronella et al. 2002). Later on, TLS were detected in non-small-cell lung cancer (NSCLC) patients, further characterized and were demonstrated to be associated with a more favorable prognosis (Dieu-Nosjean et al. 2008).

In this chapter, we will discuss the cellular composition of TLS, the different markers and techniques used to identify them, their impact on patients' prognosis and on the quality of the antitumor immune response. Eventually, we will discuss the mechanisms leading to their generation and maintenance.

1. Tumor-associated TLS: composition, location and level of structuration

The cellular composition of TLS varies depending on cancer types, stages, and primary or metastatic lesions, their location and level of structuration (Goc et al. 2013; Engelhard et al. 2018). Indeed, they have a variable degree of organization ranging from simple B and T cells clusters with rudimentary segregation to more complex, mature structures that contain high endothelial venules (HEVs), B cell follicles with FDCs, germinal centers and occasionally lymphatic vessels. In this section, we will describe all the components of fully developed TLS.

1.1. Cellular components of TLS

TLS contain key elements for immune response development to antigens. T and B cells are segregated into 2 adjacent regions: B follicles and T-cell rich area.

1.1.1. B cell follicle

Typical TLS-associated B follicle exhibits features of SLO-B cell follicle that encompasses a ring of naïve B cells around a GC that mainly contains B cells, T cells including Tfh, networks of FDC, and macrophages with scattered memory B cell and plasma cells.

- **B cell subsets:** SLO-associated GC is further compartmentalized into a dark zone where B cells, named centroblasts, proliferate actively and hypermutate their Ig genes, and a light zone where B lymphocytes, termed centrocytes, undergo isotype switching and can test their mutated BCR to select those with the highest affinity for a specific antigen. This minimizes the

risk of expanding poorly reactive B cells that can also recognize auto-antigens with low affinity. This step is safeguarded by Tfh and FDC. In the cancer context, the presence of highly proliferative B cells expressing Ki67 and BCL6 corresponding to CXCR5⁺ GC B cells, surrounded by IgD⁺ naïve B cells have been identified (Garaud et al. 2019; Werner et al. 2021; J Gunderson et al. 2021). Multi-parametric flow cytometry analysis of cell suspensions obtained from breast tumors allowed the detection of GC B cells identified as CD38⁺IgD⁻B cells (Garaud et al. 2019). In addition, different studies highlighted the functionality of TLS-associated GC B cells by detecting activation induced cytidine deaminase (AID) expression, an enzyme involved in SHM and CSR (Cipponi et al. 2012).

- **Tfh and Tfr:** Tfh are a specialized subset of CD4⁺ T cells providing help to B cells. They constitute key actors in GC through their secretion of various cytokines, including IL21, and expression CD40L, which will interact with CD40 on antigen-specific B cell to drive their proliferation, isotype switching, affinity maturation and differentiation to ASC and memory B cells. Moreover, GC B cells outnumber Tfh, indicating that Tfh help is limited, which allows efficient selection of B cells with the highest affinity to the antigen. This prevents cross-reactivity with self-antigens. Classical Tfh are characterized by the expression of Bcl-6, PD-1, ICOS and CXCR5, the latter chemokine receptor enabling their movement into the B cell zone in response to CXCL13 gradients (Hutloff 2018). Functional CXCR5⁺ Tfh, co-expressing PD1 and ICOS, were found in BC-infiltrating TLS and their presence was associated with TLS harboring GC (Noël et al. 2021). Moreover, this group demonstrated by flow cytometry, transcriptomics, and *in situ* IHC staining the presence, in breast tumors, of a non-typical PD1^{Hi}ICOS^{int}-Tfh population lacking CXCR5 expression and producing high amount of CXCL13, referred to as TfhX13. They were found in the tumor bed, albeit they are not GC-restricted. *In vitro* assays revealed that these cells were not capable of providing help to B cells, however, they may promote TLS/GC formation through CXCL13 secretion (Gu-Trantien et al. 2013; 2017; Noël et al. 2021). T follicular regulatory (Tfr) characterized by a CD25⁺CXCR5⁺FoxP3⁺GITR⁺ phenotype are also present in TLS and can suppress Tfh cells and humoral immunity with a higher efficacy than Tregs (Noël et al. 2021; Eschweiler et al. 2021).
- **FDC:** FDC are stromal cells owing the ability to present native antigens, retained in the form of immune-complexes (ICs) on their surface, to B cells resulting in the proliferation of GC B cells and the prevention of their apoptosis (Park et Choi 2005). FDC are present in both primary and secondary follicles, co-express CD21 and CD35 molecules and were detected by IHC in various cancers, including breast cancers (Coronella et al. 2002; Nzula, Going, et Stott 2003; Garaud et al. 2019). However, they upregulate their CD23 (low affinity Fc receptor for IgE) and CD32 (low affinity Fc receptor for IgG) in secondary B follicles. CD23⁺ FDC are more dense in the light

zone of the GC (C. D. C. Allen et Cyster 2008), and were detected in TLS with secondary follicles in melanoma (Werner et al. 2021). Additionally, a recent study in ovarian cancer showed that FDC in mature TLS are the main source of the B/Tfh/Tfr cell attracting chemokine CXCL13 (Ukita s. d.).

1.1.2. T cell rich area

The T cell zone of TLS comprises a majority of T cells, some B cells and to a lesser extend mature dendritic cells (DC). There is also an extensive network of fibroblastic reticular cell (FRC) and specialized blood vessels called high endothelial venules (HEVs).

- **T cells:** naïve CD8⁺CD45RA⁺T cells and central memory CD4⁺CD45RO⁺T cells (Tcm) are present in this zone. Both populations express CCR7 and CD62L, key molecules for their attraction, extravasation through HEV and maintenance in T cell rich area. Mature DC are also attracted to this zone favoring antigen presentation to T cells (Becht et al. 2016). T cell rich area is usually identified in tumors in the vicinity of B follicles constituting T cell rich region (Coronella et al. 2002; Gu-Trantien et al. 2013). It was initially thought that all tumor infiltrating T cells are effector ones that differentiate in tumor-draining LN then home to inflamed tissue. Nevertheless, the discovery of the presence TLS and HEV in tumors gave a new dimension to this notion. Indeed, naïve T cells and Tcm were observed in lung tumors, and appear to be restricted to TLS areas and colocalized with HEV. (de Chaisemartin et al. 2011). Eventually, a few Tregs were detected in melanoma-associated TLS (Messina et al. 2012).
- **Mature dendritic cells (DC):** immature DC, recruited from blood in inflamed tissue, are stimulated by antigens and inflammatory cytokines to undergo maturation and migration process (Banchereau et Steinman 1998). Thus, they upregulate CCR7, the receptor for the CCL19 and CCL21 chemokines that drive their migration to the T cell zone of SLO where antigen presentation to CD4⁺ and CD8⁺ T cells occurs. Mature DC express also the dendritic cell/lysosomal associated membrane protein (DC-Lamp) that concentrates in perinuclear lysosomes (de Saint-Vis et al. 1998). DC-Lamp⁺ mature DC were detected in multiple tumors and correlate with T and B cell infiltration, HEV density and presence of TLS (Martinet et Girard 2013; Goc et al. 2014; Truxova et al. 2018). Nevertheless, this association is not absolute as exemplified in renal cell carcinoma where most mature DC are found in TLS-negative stromal areas (Giraldo et al. 2015). Martinet et al. showed, in breast tumors, that mature DC are the main source of lymphotoxin β that is instrumental for HEV differentiation and maturation (Martinet et Girard 2013), suggesting that they could contribute to TLS induction and/or maintenance.

- **High endothelial venules (HEV):** lymphocytes transmigrate from the blood into canonical lymphoid organs through HEV. These specialized blood vessels are located mainly in the T cell zone and secrete CCL21, a key cytokine for chemoattraction of CCR7⁺ lymphocytes. HEV express peripheral-node addressins (PNAd), ligand of L-selectin expressed on the surface of naïve T and B lymphocytes and central memory T cells initiating their extravasation (Miyasaka et Tanaka 2004). A solid body of evidence showed that blood vessels with HEV characteristics develop also in various solid tumors. Martinet et al. did the first observation of the presence of HEV in solid tumors including breast tumors. They used an HEV-specific monoclonal antibody that recognizes an epitope on PNAd (MECA-79) to visualize HEV by IHC, and they were able to observe CD3⁺ T cells frequently extravasating or attached to the luminal surface of endothelial cells. They also showed that these vessels were a strong predictor of CD8⁺ T, Th1 and B cell infiltration (Martinet et al. 2011). Furthermore, DC-Lamp⁺ DC are frequently found around tumor HEV (Martinet et al. 2013). Although Song et al. demonstrated a robust correlation between HEV and TLS in a TNBC cohort, HEV appear to be more frequently present in tumors than TLS (Song et al. 2017; Martinet et al. 2011). Surprisingly, HEV of inflamed lymph nodes upregulate P-selectin, E-selectin, CXCL9 and MadCAM-1 resulting in the recruitment of activated/effector T cells, pDC, monocytes and neutrophils indicating that HEV function can be modulated by its environment (G. Vella, Guelfi, et Bergers 2021). It is likely that in tumor context, the immune cell composition influx through HEV can evolve.
- **Fibroblastic reticular cell (FRC):** FRC are stromal cells that originate from mesenchymal precursors in the microenvironment of lymph node (LN) during ontogeny. Engagement of the lymphotoxin β receptor on these precursors leads to their differentiation into lymphoid-tissue organizing cells (LTo), and then gives rise to mature FRC. Present in LN-T cell zones, they surround HEV to maintain their integrity, and their secreted chemokines and cytokines are crucial for naïve T cell recruitment and survival. FRC secrete CCL19 and CCL21 that attract CCR7⁺ T cell across HEV and retain them in the T cell zone. Moreover, they create a conduit system to increase potential interaction between T cell and DC facilitating antigen presentation. FRC have also immunoregulatory functions by: 1) presenting self-antigens inducing the anergy of CD8⁺ T and CD4⁺ T cells participating to peripheral tolerance, 2) limiting newly activated T cell expansion through their expression of inducible nitric oxide synthase (iNOS) (F. D. Brown et Turley 2015). Podoplanin-positive immunofibroblasts that closely resemble FRC phenotypically and functionally have been identified in TLS in the context of autoimmune diseases (Nayar et al. 2019). Rodriguez et al. showed recently in a murine model of melanoma that tumor-associated TLS development is orchestrated by podoplanin⁺ cancer-associated fibroblasts (CAF) with lymphoid tissue organizer cells (LTo) characteristics through

the expression of tumor necrosis factor- α (TNF α) (Rodriguez et al. 2021). Unfortunately, few data are available on FRC in human tumors, and further studies are needed to understand their role in cancer, especially that they could harbor suppressive activities.

In conclusion, tumor-associated TLS share many features with SLO in terms of composition and structuration. Nevertheless, TLS are not supplied by afferent lymph vessels and are not encapsulated resulting in a probable direct exposition of these structures to tumor antigens and secreted cytokines in the TME.

1.2. Tumor TLS are heterogeneous

Considerable heterogeneity in the level of structuration of tumor-infiltrating immune cells have been observed in cancer, ranging from tight clusters of B and/or T cells to fully developed TLS with secondary follicles. A second level of heterogeneity is due to their possible differential location in the tumor mass.

1.2.1. Various levels of TLS structuration are encountered in tumors

Four subtypes of lymphoid aggregates were initially described in human cancer based on their size, cellular composition, and degree of organization ranging from small clusters of approximately 20 to 50 cells (comprising CD4⁺ and CD8⁺ T cells, B cells and occasional DC) to fully developed TLS that exhibit prominent B-cell follicle with GC-like structures. In ovarian cancer, GC-containing TLS were found in almost 25% of patients and were strongly associated with other types of aggregates (Kroeger, Milne, et Nelson 2016). In oral squamous cell carcinoma, Li et al. observed the presence of CD3⁺ T cells, CD20⁺ B cells, PNA⁺ HEV and DC-Lamp⁺ cells in both immature and mature TLS, however, T and B cells were scattered diffusely in immature TLS without follicular structure, but seemed to be more abundant in DC and HEV than mature TLS (H. Li et al. 2021). This categorization is becoming increasingly important since it has a probable impact on the anti-tumor immune response intratumorally. Nevertheless, the methodology and markers used to assess the degree of TLS maturation are not yet standardized. In breast cancer, most TLS (>95%) do not contain a germinal center based on their morphology as can be judged on an HPS-colored tissue section (Song et al. 2017). Noël et al. defined active TLS as those with Ki67⁺ and PD1⁺ B cell follicle(s) and the presence of PD1⁺GZMB⁺TILs in T cell zone. Significantly higher expression of Tfh genes, Th1-oriented response, cytotoxic functions, B cell differentiation, and antigen presentation were observed in active TLS indicating that these structures are functional (Noël et al. 2021). Moreover, the proportion of GC-associated TLS varies depending on the type of cancer. In metastatic melanoma, less than 25% of TLS harbored a secondary B-cell follicle, as determined by the presence of CD21⁺CD23⁺FDC (characteristic of GC light zone), whereas more than 50% of TLS exhibit at least one GC in lung cancer and colorectal carcinoma (CRC) (Lynch et al. 2021; Posch et al. 2018; Siliņa

et al. 2018). Eventually, a recent study identified SEMA4A (Semaphorin 4A) as an upregulated marker in GC TIL-B compared to other TIL-B subsets adding another surrogate GC marker (Ruffin et al. 2021).

In summary, distinct states of maturation of TLS exist in solid tumors suggesting a possible developmental continuum of these structures intratumorally. Importantly, these levels of organization influence probably the quality of the immune response locally, and thus the impact on patient prognosis.

1.2.2. Location of TLS in tumor tissue:

The majority of studies have described TLS in the peritumoral areas and in the invasive margin, forming a kind of wall around the cancer tissue. In breast cancer, Sofopoulos et al. considered adjacent TLS as those situated in the invasive front and distant TLS as those that have normal breast tissue interpositioned between them and the tumor-infiltrating border. This study revealed that more than 90% of patients have at least one adjacent TLS, whereas only 25% of patients harbored distant TLS (Sofopoulos et al. 2019). A few studies reported intratumoral TLS (Hiraoka, Ino, et Yamazaki-Itoh 2016). In hepatocellular carcinoma (HCC), TLS in the non-neoplastic liver parenchyma were showed to serve as a niche for malignant hepatocyte progenitors (Finkin et al. 2015). Recent studies identified peritumoral TLS in most HCC cases, whereas intratumoral TLS were observed in only 30% of HCC. Unlike the first findings, high peritumoral TLS density showed significantly higher intratumoral immune infiltration leading to an efficient anti-tumor immune response (H. Li et al. 2021). Moreover, in melanoma and oral squamous cell carcinoma tissues, TLS seems to be more organized in peritumoral locations than within tumor nests (Engelhard et al. 2018; S. Li et al. 2020). Much remained to be done to fully understand underlying mechanisms leading to peritumoral and/or intratumoral TLS formation, their immunological properties and probable distinct prognostic values.

Therefore, with TLS being an important part of the immune contexture of tumors, it has become essential to analyze not only their density, but also their composition, location and degree of maturation.

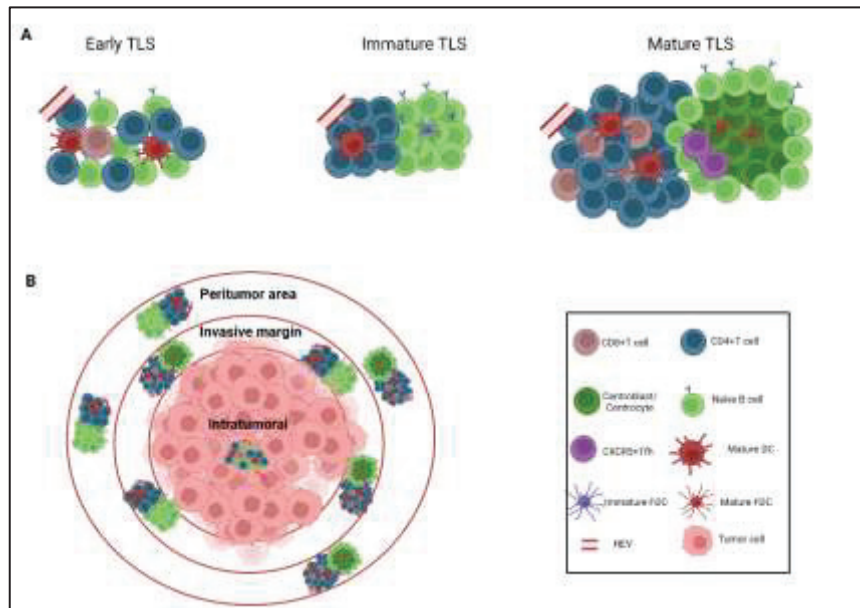


Figure 7: Tumor-infiltrating TLS are heterogeneous. A TLS formation ranges from early TLS with intermixing of T, B and DC cells to fully developed TLS harboring a GC. B TLS can be present at different locations: intratumoral, in the invasive margin or at peritumor area.

2. Methods for TLS identification and characterization in tumors

There is still no consensus regarding the best method and markers for TLS assessment in tumor tissues. Three main strategies have been used for TLS detection and quantification:

- Histopathological evaluation of TILs using an hematoxylin and eosin (HE) colored tissue section.
- Immunohistochemistry/Immunofluorescence (IHC/IF) on paraffin-embedded (FFPE) tumor sections.
- Gene expression on tumor samples (FFPE or frozen specimen).

Because TLS can have different localizations and are usually present in the invasive margin and peritumoral tissue, their detection cannot be performed on core biopsies nor tissue-microarray (TMA), and should be preferentially done on surgical specimens. For example, in breast cancer, 50% of the tumors that were found to contain TLS upon analysis of the whole tumor section were TLS-negative when using the core biopsy (Buisseret, Desmedt, et al. 2017). In addition, TLS distribution in the tumor can be highly heterogeneous suggesting that multiple sections at distance from each other should reduce false-negative risk. Eventually, due to TLS preferential location in the periphery of the tumor, it is recommended to evaluate them inside and outside the tumor border. It is also crucial to determine the TLS state of maturation using distinctive markers.

2.1. Histopathological/IHC characterization of TLS

Numerous studies relied only on an expert histopathological evaluation of TLS using an hematoxylin and eosin (HE) colored tissue section, which has the advantages to be an easy, rapid and low cost technique (Salgado et al. 2015; H. J. Lee et al. 2016; Figenschau et al. 2015). However, the comparative analysis of HE coloration and IHC (CD3/CD20 dual staining) on FFPE breast tumor sections revealed that TLS detection is underestimated on hematoxylin eosin compared to immunohistochemically stained tissues (Buisseret, Desmedt, et al. 2017). Moreover, this technique does not allow the distinction of the various immune populations constituting the TLS, and it can be very difficult to determine the level of TLS structuration even for an experienced pathologist.

Nowadays, most studies use single or multiparametric IHC/IF approaches to detect the different components of TLS, including B cell follicles, T cell rich zones, HEV, mature DC, and FDC, by immune-labeling of CD20+, CD3+, DC-Lamp+, MECA-79+ and CD21+ cells, respectively. However, we should be cautious with some markers that can be applied to limited types of cancer. DC-Lamp marker is a good example since, in lung carcinoma, it is observed only in TLS, whereas in renal cell carcinoma, most mature DC are found outside of TLS (Goc et al. 2014; Giraldo et al. 2015). Therefore, it is preferable to combine several markers to identify and quantify these structures. In addition, there is a need to assess the level of TLS organization and recent studies on different cancer types used the CD21 and CD23 markers to distinguish FDC present in primary (CD21+CD23-) vs secondary GC-containing (CD21+CD23+) follicles of TLS (Lynch et al. 2021; Posch et al. 2018; Siliņa et al. 2018).

The quantification of TLS also lacks standardization. In the literature, distinct methods to assess the amount of tumor-infiltrating TLS were utilized: (1) the density of DC-Lamp+ cells (Goc et al. 2014; Truxova et al. 2018); (2) HEV density (Martinet et al. 2011); (3) B follicle or TLS area/total area (Germain et al. 2014; Ruffin et al. 2021); (4) Number of lymphoid aggregates/total area (Tang et al. 2020). As mentioned above, DC-Lamp+ cells and HEV are also present in non-TLS regions, suggesting that they may not be the best surrogate indicator of TLS presence in all cancer types. In our experience, determination of B-cell follicle or TLS density are probably the most robust and specific approach for TLS quantification, at least in breast cancer.

Another key issue is the choice of the threshold allowing TLS stratification. The absence *VS* the presence of at least one lymphoid aggregate in a commonly used method (Sofopoulos et al. 2019). However, this is of limited value for tumors with a high frequency of TLS among patients, such as breast cancer. For these cases, the median value of the selected TLS marker is privileged (**Figure 8**).

IHC/IF approaches for TLS assessment are currently the most used, and likely most accurate, technique. However, computational methods to infer TLS presence/density from bulk transcriptomic data are increasingly being used.

2.2. TLS gene signature expression

Coppola et al. were the first group to define a gene signature that predicts the presence of TLS in CRC. This signature is composed of 12 genes coding for chemokines highly related to TLS neogenesis like CXCL13, CCL19 and CCL21 (detailed in TLS neogenesis part). It is noteworthy that the authors compared tumors with a high organized immune infiltrate to tumors with a minimal or absent lymphocytic infiltration, suggesting that this gene signature might not be specific of a structured immune infiltrate, but rather associated to highly infiltrated tumors (Coppola et al. 2011). This signature has been evaluated in other solid cancers including breast cancer (Prabhakaran et al. 2017). The same kind of strategy was used recently to develop new TLS gene signature in melanoma patients where they compared gene expression between highly *VS* negligibly infiltrated tumors by CD20+ and CD8+ T cells. They identified a 9-genes TLS signature that correlates with a gene signature constructed from TLS-related hallmark genes (Cabrita et al. 2020). Other studies used key TLS-associated cells or molecules as a good reflection of TLS/GC presence such as Tfh, FDC or CXCL13 (Gu-Trantien et al. 2013; Cipponi et al. 2012; Ukita s. d.). Recently, using spatial transcriptomics, Meylan et al. defined a 29-gene TLS imprint signature by comparing TLS area gene expression to non-TLS region on the same tumor section (Meylan et al. 2022) (Table 1).

Despite the multiple TLS gene signatures available, we do believe that there is still a need for the development of a robust and standardized TLS assessment assay based on bulk transcriptome analysis of tumor specimens. In my opinion, establishing a more accurate TLS gene signature would require to compare TLS-rich tumors to tumors without TLS matched for TIL content, in order to reveal genes that are specific of lymphocytes structuration and not to lymphocyte infiltration.

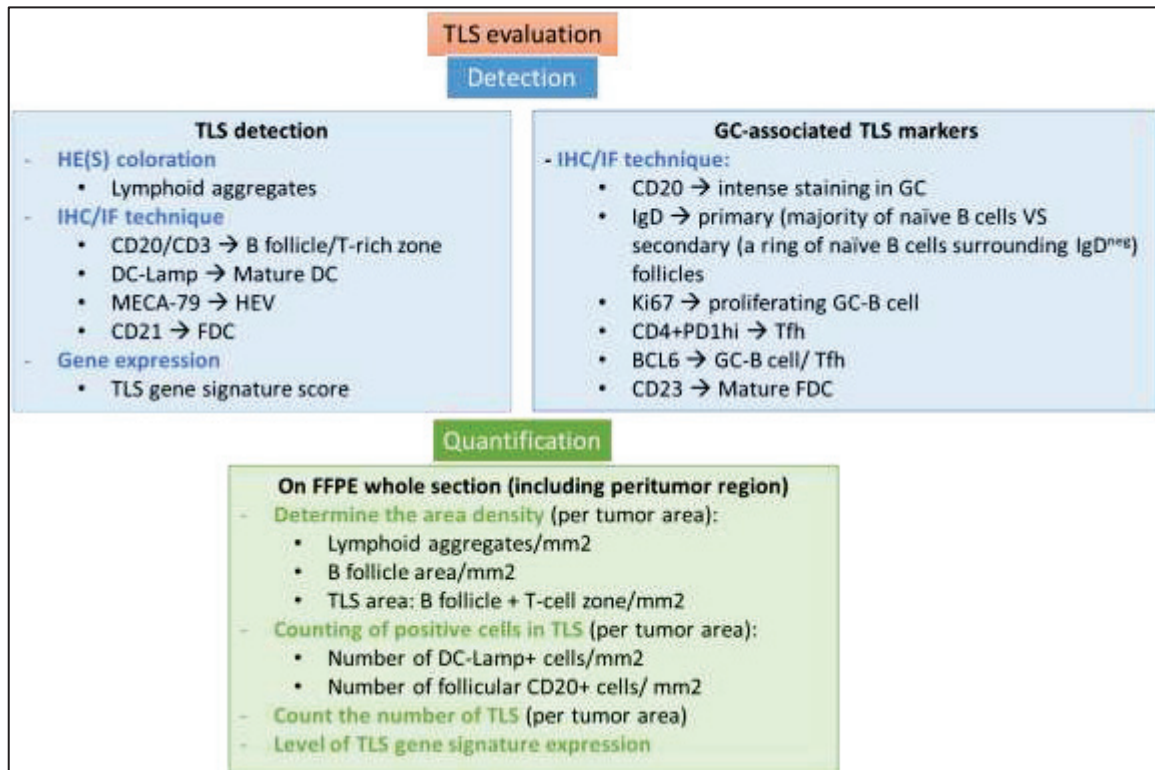


Figure 8: TLS evaluation in human tumor sections. TLS are generally detected using HE coloration, IHC/IF technique and recently transcriptomic analysis (blue left). TLS-containing GC can be identified with different markers using IHC/IF technique (blue right). Different strategies are currently used to determine TLS density (Green).

3. TLS are associated with better cancer patient prognosis and improved responses to therapies

The presence of TLS in tumors was reported, however their frequency tends to be dependent upon the type of cancer. Histological evaluation of TLS revealed that more than 90% of cases of colorectal, pancreatic and breast cancer exhibit at least one TLS, whereas these structures are found in less than 40% of skin and oral mucosal cancers (Hiraoka, Ino, et Yamazaki-Itoh 2016). Furthermore, assessment of RNAseq data from various cancer types using the 12-chemokine TLS gene signature showed an extremely heterogeneous distribution of signature scores according to cancer types, with TLS-High tumors such as breast and lung tumors, and TLS-Low tumors like brain tumors (Sautès-Fridman et al. 2019).

The presence of TLS is often associated with favorable prognosis in solid malignancies studied so far. Moreover, TLS predicts response to cancer therapies including immunotherapies.

3.1. TLS association with clinicopathological parameters and their impact on patients' prognosis

Despite the different strategies of TLS detection and quantification, tumor-associated TLS correlate with favorable prognosis in untreated primary and metastatic breast cancer (Table2). Most studies used immunohistochemical staining to identify TLS, albeit using different markers including B-cell follicle, HEV and FDC. Prabhakaran and colleagues assessed TLS presence using the 12-chemokine TLS gene signature (Prabhakaran et al. 2017), whereas Lee et al. used four genes associated with TLS (M. Lee et al. 2019). Only one study reported an unfavorable outcome for patients harboring at least one TLS; however, the authors observed the presence of at least one TLS in most patients (>80%) and their stratification were based on the presence of absence of TLS leading to unbalanced groups (98 TLS+ vs 14 TLS-) (Sofopoulos et al. 2019). A threshold for TLS quantification to compare these 2 groups would be more fitting as done by Martinet et al. who compared the highest tercile of HEV density to the two lowest terciles (Martinet et al. 2011). Moreover, Sofopoulos et al. focused on TLS located in peritumoral and/or invasive margin region neglecting those present intratumorally (Sofopoulos et al. 2019). HCC is a good example highlighting the impact of TLS location on prognosis. Finkin et al. showed that TLS detected in non-neoplastic liver tissue was linked to an increase risk of recurrence. They also demonstrated using a mouse model that peritumoral TLS development is driven by chronic inflammation in the pre-malignant phase and can serve as expansion/survival niches for malignant hepatocyte progenitors (Finkin et al. 2015). By contrast, TLS located in the tumor core of HCC were recently described as predictive of a decreased risk of early relapse (Calderaro et al. 2019) indicating that the location of TLS is of utmost importance to determine the impact on patients.

A second important feature to consider for tumor-associated TLS is their degree of maturation. Numerous studies in multiple cancer types showed that mature TLS exhibiting active secondary follicles provide a better protection than aggregates with intermixing of T cells within B cell clusters (Calderaro et al. 2019; Meylan et al. 2022; Posch et al. 2018; Ruffin et al. 2021; Noël et al. 2021). In breast cancer, high density of active TLS, characterized by the presence of germinal center, were associated with a better outcome (Noël et al. 2021).

Scarce data are available on the role of TLS in metastatic sites. TLS density, composition and state of maturation in metastases seem to depend on the primary tumor and the organ in which the metastasis is located. In breast cancer, Lee et al. analyzed a series of 355 metastases localized in lung, liver, brain and ovary. They showed that TLS were present only in lung and liver metastases, and matched TLS-associated primary tumors characteristics regarding their density. Surprisingly, patients lacking TLS in primary tumors but with TLS in metastatic sites showed significantly better overall survival (M. Lee et al. 2019).

Eventually, the link between TLS and clinicopathological parameters has also been investigated and revealed an association with more aggressive tumors, which is discordant with their positive impact on prognosis. TLS were more frequently present in tumors with a more aggressive grade, high proliferation index and absence of hormonal receptors expression, parameters that are usually associated with a worse patient survival. Moreover, higher PD1 and PDL1 positivity prevalence on immune cells was associated with tumor aggressiveness and TLS density (Buisseret, Garaud, et al. 2017). Despite this, TLS retain their positive prognostic value in patients (Table2). This indicates the need to better characterize TLS state of maturation and location that could explain these contradictory data.

3.2. TLS as a predictive biomarker for treatment response

Gu-Trantien and colleagues were the first group to address the question of TLS predictive impact of response to chemotherapy in breast cancer patients. In a cohort of 996 patients, they showed that high expression of an eight-gene Tfh cell signature, as well as of the single *CXCL13* gene, in pre-treatment biopsies predicted pathological complete response (pCR) to neoadjuvant chemotherapy (NAC). In addition, in TNBC patients treated with NAC, pCR was associated with higher densities of HEV and elevated expression of the *CXCL13* gene (Table2) (Gu-Trantien et al. 2013). Treatment of HER2-enriched breast tumors with anti-HER2 mAb correlated with a better disease-free survival in tumors with high TLS density (H. J. Lee, Kim, et al. 2015). Altogether, these data indicate that pre-existing TLS may favor response to chemotherapies and targeted therapies.

The development of immunotherapies targeting inhibitory immune checkpoints and their ligands (anti-ICP/ICPL) has revolutionized cancer patients' treatment. Anti-ICP/ICPL showed remarkable anti-cancer activity, however, there is only a fraction of patients that experience long-term benefits from this therapy. Therefore, it is essential to identify robust predictive biomarkers to select patients who are more likely to benefit from these therapies. Recent studies revealed a strong association between high TLS density and positive outcome of anti-ICP/ICPL treatment in sarcoma, melanoma and renal cell carcinoma patients (Petitprez et al. 2020; Helmink et al. 2020; Cabrita et al. 2020; Vanhersecke et al. 2021; Asrir et al. 2022). Moreover, increase of CD20 and TLS densities were observed after treatment in responding but not nonresponding patients (Helmink et al. 2020), indicating that immunotherapy favor TLS development. In line with that, the induction of TLS in a murine model was associated with a better response to immunotherapy (Johansson-Percival et al. 2017) (Box3). Furthermore, Vanhersecke et al. evaluated TLS degree of maturation on a retrospective cohort of 328 patients with various cancer types including breast cancer and treated with anti-PD1 or anti-PDL1 immunotherapies. They reported that the presence of mature TLS was associated with improved overall survival and objective response rates irrespective of PDL1 expression, a routine predictive biomarker (Vanhersecke et al. 2021). Therefore, it is essential to further refine TLS characterization by identifying their location and state of maturation to best appreciate their impact on patient prognosis and response to therapies.

Overall, these data indicate that the development of novel therapeutic strategies to induce TLS in patients may improve their prognosis and response to therapies.

Table 2: Prognostic and predictive impact of BC-associated TLS

Breast tumor type	TLS detection approach	TLS stratification	Nb of patients	Correlation with clinico-pathological parameters	Prognostic/predictive impact of TLS	Reference
Prognostic impact of TLS						
Untreated primary IBC	IHC B cell follicle/T cell zone	Presence VS absence	290	Association with higher histological grade and absence of expression of HR	NA	(Figenschau et al. 2015)
Primary IBC	IHC B cell follicle/ T cell zone/ FDC	Presence VS absence	248	Association with higher histological grade, higher TIL and HER2+ tumors	Favorable in HER+ tumors	(X. Liu et al. 2017)
Primary IBC	IHC HEV	Highest tertile VS two lowest tertiles	146	NA	Favorable	(Martinet et al. 2011)
Primary IBC	IHC B cell follicle/ T cell zone/ FDC	Presence VS absence	125	Association with TILs, higher histological grade and HR-	NA	(Buisseret, Garaud, et al. 2017)
Primary TNBC	HE: lymphocytic aggregates IHC: HEV	No to little TLS VS moderate to abundant	769	Association with TILs	Patients with moderate to abundant TLS have better DFS	(H. J. Lee et al. 2016)
Primary IBC	HE: lymphocytic aggregates IHC: B cell follicle/T cell zone/ FDC	Presence VS absence	112	NA	Peritumoral TLS vs no TLS associated with worse DFS and OS	(Sofopoulos et al. 2019)
Primary IBC	Transcriptomics 12-chemokine signature	Median of signature score	366	Association with undifferentiated tumor grade, ER- and HER+	Favorable	(Prabhakaran et al. 2017)
Metastatic IBC (lung, liver, brain and ovary)	HE: lymphocytic aggregates Transcriptomics: CXCL12, CXCL13, CCL2 and LTB	Presence VS absence	335	NA	TLS absent from primary tumors and present in metastases are associated with better OS	(M. Lee et al. 2019)
Predictive value of TLS on response to therapies						
HER2+ IBC under Trastuzumab	HE: lymphocytic aggregates	No or little TLS compared to moderate or abundant	447	Association with TILs and higher tumor grade	High amount of TILs correlates with high TLS density and is associated with better DFS	(H. J. Lee, Kim, et al. 2015)
Primary IBC under NAC	Transcriptomics	Tertiles	996	NA	Strongly associated with higher rate of pCR	(Gu-Trantien et al. 2013)
TNBC patients under NAC	IHC: B-cell follicle/ T-cell zone/ HEV Transcriptomics: CXCL13 mRNA	None to moderate VS abundant	108	NA	HEV density, CXCL13 mRNA and CD20+ cell density correlated with pCR	(Song et al. 2017)

4. Impact of TLS on the quality of the immune response to tumors

Numerous recent studies allow us a better comprehension of the immune response generated locally in these structures.

4.1. TLS are sites of anti-tumor immune response development

In multiple cancer types, the prognostic value of tumor-infiltrating T cells is usually stronger when B cells and/or antibody-secreting cells are also present in the TME, supporting a probable coordination of cellular and humoral immunity for efficient tumor control (Kroeger, Milne, et Nelson 2016; Wouters et Nelson 2018). TLS are sites where T and B cells functionally interact and cooperate to induce effectors capable of eliminating tumor cells. TLS containing tumors are indeed usually strongly infiltrated by CD8 and CD4 T cells, B cells, plasma cells and M1-type macrophages, immune cells with known anti-tumor functions (Goc et al. 2014; Ukita s. d.). In this section, I will discuss the different ways by which TLS may boost local and systemic immune responses to malignant cells.

4.1.1. TLS-associated vasculature allow immune cells recruitment in tumors

As previously mentioned, **HEV** formation is a key step in TLS maturation since they provide a gateway for the entry of circulating lymphocytes with naïve and central memory phenotype to the TME. Their density is an independent predictive indicator of better survival in solid cancers including breast tumors, and are usually associated with CD4+ Th1, cytotoxic CD8+ T cells, memory T and B cells, mature DC but not Tregs. Transcriptomic analysis revealed that HEV presence coincides with overexpression of T cell recruiting chemokines and receptors such as CXCL9, CXCL10, CXCL13, CCL19, CCL21 and PNAAd (Martinet et al. 2011; 2013, 201). Furthermore, single cell RNAseq data from melanoma tumors revealed that B-cell rich samples contained more CD4+ and CD8+ T cells with naïve and memory-like features (TCF7+, IL7R+ and CD62L) as compared to B cell poor samples, supporting an influx of CD62L+ naïve and memory T cells to TLS (Cabrita et al. 2020).

In addition to HEV, TLS development is also accompanied by **lymphatic vessels** (LV) neogenesis, which has been well described in autoimmune diseases and chronic kidney rejection. LV express CCL21, LYVE-1, podoplanin, VEGFR-2, and VEGFR-3. They provide communication of the lymphoid organs with the rest of the body. Afferent LV are the initial entry into lymph nodes (LN) from the tissues for antigen and APCs, whereas efferent vessels allow egress of activated cells from LN to the next LN and eventually into the blood stream. LV-associated TLS frequently contain cells and express CCL21 indicating that they may constitute a means for the entrance of APC from draining-lymph node, complementing HEV function (Ruddle 2016).

4.1.2. TLS as sites of tumor antigen presentation

TLS could contribute to mount an anti-tumor immune response through their ability to present tumor antigens locally. Breast tumors harboring active TLS show upregulation of genes involved in antigen presentation, including LAMP3, CD80 and various HLA molecules (Noël et al. 2021). T cell rich zone of TLS contains mature DC-Lamp⁺ DC that are in close contact with CD4⁺ T and CD8⁺ T cells suggesting local presentation of antigens captured from surrounding tumor cells, leading to i) the priming, proliferation and differentiation of naïve T cells into effector cells, and/or ii) to the reactivation and amplification of effector cells primed elsewhere (for example in draining lymph nodes) (Martinet et al. 2011; Germain et al. 2014; Kroeger, Milne, et Nelson 2016; Goc et al. 2014; Di Caro et al. 2015). Whether DC cross-present tumor antigens to CD8⁺ T cells or whether Th help is a prerequisite for an efficient generation of CTL occurring inside TLSs remains to be determined. However, intratumoral IgG directed against tumor antigen can increase costimulatory molecule CD86 on DCs and potentiate the capacity of DC to perform cross-presentation (discussed in chapter 3). Mature DC have also been detected in the germinal centers of ovarian cancer metastasis, indicating that they may also be involved in native antigen delivery to B cells (Montfort et al. 2017). Numerous studies showed that tumor-infiltrating B cells (TIL-B) with an activated memory phenotype express molecules involved in antigen presentation, including MHC class I and II, CD40, CD80 and CD86, suggesting their ability to present antigens to T cells. Bruno et al. indeed demonstrated, using an *ex vivo* culture system, that activated TIL-B can efficiently present antigen (captured *in vivo*) to CD4⁺ T cells leading to their proliferation and differentiation into IFN γ -producing effector cells (J. S. Nielsen et al. 2012; J.-Y. Shi et al. 2013; T. C. Bruno et al. 2017; Rossetti et al. 2018). Furthermore, B cells are able to perform cross-presentation of the cancer testis antigen NY-ESO1 to CD8⁺T cells (Gnjatic et al.2003). Accordingly, high density of B cells found in lung tumor-associated TLS correlated with greater CD4⁺ T cell and CD8⁺ T cell clonality in the tumor and draining LN (Zhu et al. 2015). In conclusion, TLS represent active sites for the priming/activation, expansion and differentiation of tumor-specific T cells.

4.1.3. TLS generate memory B cells and antibody-secreting cells

An increasing body of circumstantial evidence suggests that tumor-associated TLS are drivers of *in situ* activation, proliferation and differentiation of naïve/memory B cells to memory B cells and ASC, as might be expected for a structured ectopic lymphoid tissue: (1) All B-cell differentiation stages from naïve B cells through activated B cells and GC B cells to memory B cells and terminally differentiated ASCs were identified by flow cytometry and/or transcriptome analysis of epithelial tumors. In addition, a significant associations between TLS and ASC densities were observed (Wieland et al. 2021; Garaud et al. 2019; Kroeger, Milne, et Nelson 2016; Meylan et al. 2022). (2) Numerous studies showed that some TLS contain B cells that express Ki67 and BCL6 corresponding to bona fide GC B cells. Moreover, expression of AID was observed in GC B cells indicating that these cells undergo somatic hypermutation

and Ig class-switch recombination. Interestingly, isotype switching occurred not only to IgG, but also to IgA, even in non-mucosal tumors (Coronella et al. 2002; Germain et al. 2014; J. S. Nielsen et al. 2012; Montfort et al. 2017; Cipponi et al. 2012). (3) Single-cell RNA coupled with BCR sequencing of TNBC-infiltrating B cells revealed that they were mostly CD27+ memory B cells and had higher clonality, CSR and SHM than those in the blood (Q. Hu et al. 2021). Furthermore, memory B cells located within TLS in omental metastases of ovarian cancer had a high clonality and SHM rate (Montfort et al. 2017). Using spatial BCR profiling, a recent study showed in renal carcinoma tumors that the highest numbers of unique light chain clonotypes were found in TLS areas, whereas highly mutated sequences of variable regions are at distance from TLS, indicating that SHM occurs in TLS and mutated ASC disseminate throughout the tumors (Meylan et al. 2022). (4) Conventional CXCR5+ Tfh cells expressing PD1 and ICOS were detected predominantly within a fully developed TLS. Functional assays demonstrated that they were capable through CD40-CD40L interaction with B cells and cytokine production to trigger IgG and IgA production. In breast tumors, the group of Willard-gallo identified another Tfh subset, referred to as “TfhX13”, lacking CXCR5 expression, but expressing both PD1 and ICOS and producing CXCL13. These cells, mainly located outside of TLS, are associated with the presence of TLS, GC B cells and ASC and could contribute, through CXCL13 production, to the recruitment and activation of B cells and TLS formation (Gu-Trantien et al. 2013; 2017; Noël et al. 2021). (5) The presence of tumor-antigen specific ASC was recently demonstrated in HPV+ head and neck tumors, with the presence of a dominant population of HPV-specific ASC associated with an active *in situ* production of IgG targeting HPV (Wieland et al. 2021). Besides, Meylan and collaborators observed higher proportions of IgG labeled apoptotic tumor cells in TLS⁺ as compared to TLS⁻ tumors, illustrating that TLS induce/sustain the production of antibodies reacting with tumor cells and leading to their elimination (Meylan et al. 2022).

4.1.4. TLS is a privileged site for the generation of Th1-orientated immune response and cytotoxic CTL

Following antigen presentation, TLS can initiate/potentiate immune response through the local generation of tumor-specific T cell effectors. *In vivo* studies demonstrated that in the absence of secondary lymphoid organs, TLS in mouse melanoma lesions were able to generate CD8+ T cells specific for melanoma-associated antigen (Schrama et al. 2008), indicating that TLS can function independently of SLO. That TLS are sites of induction of T cell responses is supported by studies of T cell clonality. Indeed, TLS are associated with increased T cell receptor (TCR) clonality of CD4+ T cells in lung tumors (Zhu et al. 2015). In ovarian cancer, analysis of macrodissected TLS versus non-TLS tumor regions (matched for lymphocyte infiltration) revealed preferential T cell oligoclonal amplification in TLS (Ukita s. d.). Importantly, the clones with the highest amplification in non-TLS region were consistent with the clones found in TLS, suggesting that antigen-driven activation and

expansion of T cells initially takes place in TLS, and then effector cells disseminate to the tumor bed and circulation to eradicate malignant cells. Furthermore, multiple studies showed that TLS-enriched tumors, are characterized by increased proportions of CD38⁺CD69⁺ activated T cells, cytotoxic CD8⁺ T cells and NK along with a low infiltration of immunosuppressive cells (Goc et al. 2014; Truxova et al. 2018; Hiraoka, Ino, et Yamazaki-Itoh 2016). Cabrita et al. compared T cells isolated from TLS⁺ melanoma tumors vs TLS⁻ tumors and observed distinct transcriptional profile with an increased expression of TIM3 and PD1 in T cells infiltrating tumors lacking TLS (Cabrita et al. 2020). In addition, a spatial profiling analysis revealed increased activation markers on CD8⁺ T cells positioned inside TLS compared to those localized outside TLS, suggesting that these structures promote T cell responses inside TLS (Helmink et al. 2020). Eventually, compared to their TLS-negative counterpart, TLS-enriched breast tumors upregulated expression of *IFNG*, *TBX21*, *TCF7* genes, indicating a higher Th1-orientated immune response. Remarkably, in contrast to tumors containing immature TLS, those with mature TLS highly express genes associated with cytotoxic CD8⁺ T cells, such as CD8A, GZMB, PRF1, IL12 pathway indicating that TLS with active GC may promote an anti-tumor CD8 immune response (Noël et al. 2021). Moreover, in a murine lung adenocarcinoma model, the interaction between tumor-specific GC B cells and Tfh as well as IL21 produced by Tfh are essential for effector CD8⁺ T cell function increasing granzyme B expression (Cui et al. 2021, 20).

Altogether, these data demonstrate that TLS is a site of induction of an efficient cellular immune response directed against tumor antigens.

Box3: learning from studies on the effects of cancer therapies on TLS to better understand the roles of TLS in cancer

Cancer treatments may affect TLS composition and functions, which can influence patients' prognosis. Boivin et al. were the first to provide a descriptive view of TLS modulation occurring in tumors after treatment. In breast tumors, hypo-fractionated **radiotherapy** induced an early depletion of TLS followed by their restoration. This phase was accompanied by a significantly increase in memory CD8+ T cells (Boivin et al. 2018). Later, TLS density and their state of maturation in lung squamous cell carcinoma patients were analyzed in chemotherapy plus **corticosteroids**-treated VS chemotherapy-treated patients. Corticosteroid delivery during chemotherapy abrogated the positive prognostic value of TLS. Indeed, the authors reported similar TLS density but impaired GC formation in patients treated with corticosteroids, suggesting that mature TLS are crucial for the generation of efficient anti-tumor immune response (Siliņa et al. 2018). A recent study conducted on breast cancer patients showed that **chemotherapy** drastically remodels the tumor infiltrating B cell compartment with the emergence of ICOSL⁺ B cells and diminution of IL10 producing cells, the former population being associated with improved therapeutic efficacy and long-term survival. Strikingly, ICOSL⁺ B cells accumulated in TLS and had significantly closer contacts with T cells compared to ICOSL⁻ B cells. Moreover, in a mouse models of breast cancer it was shown that elevation of ICOSL⁺ B cells post-chemotherapy in responsive animals was associated with increased frequencies of granzyme B or perforin-expressing CD8+ T cells and Th1 cells, as well as decreased Tregs in tumors (Y. Lu et al. 2020). This could be the consequence of an immunogenic cell death induction leading to the release of tumor antigens that are then captured by DC initiating an anti-tumor immune response in TLS. The combination of **antiangiogenic** monoclonal antibody (mAb) and an **immunotherapy** targeting PDL1 in mouse models of breast cancer induced an increase of HEV that promoted lymphocyte infiltration and subsequent TLS formation and enhanced cytotoxic T cell activity (E. Allen et al. 2017). Moreover, in a murine melanoma model, it was shown that tumor-associated TLS are increased following **anti-ICP/ICPL immunotherapy**. Interestingly, this augmentation in TLS number and size were correlated with overall response to treatment (Rodriguez et al. 2021). Eventually, Helmink et al. observed that melanoma patients treated with **anti-ICP/ICPL** exhibit more abundant TLS in post-treatment and were more likely to respond to immunotherapy (Helmink et al. 2020). These findings support the fact that TLS elicit optimal activation and maintenance of anti-tumor immune responses. Future therapeutic strategies in solid malignancies should therefore preserve pre-existing TLS, and even harness their formation and increase their state of maturity to favor efficient anti-tumor immunity. This topic will be detailed in the last part of this chapter.

4.2. TLS can promote tumor progression

Scarce studies showed that lymphoid aggregates are associated with bad prognosis in tumors. Nevertheless, thanks mainly to animal models, varying mechanisms were discovered including inflammation, immunosuppression and immune exhaustion.

4.2.1. Tumor-associated TLS may serve as an inflammatory niche nursing malignant cells

TLS can harbor pro-tumorigenic effects supporting abnormal cells until they eventually acquire malignant properties. Finkin et al. demonstrated in human and mouse model of HCC that TLS constitute a unique immune microniche that provides early tumor progenitors with crucial survival and growth factors such as lymphotoxin β (LT β), CCL17 and CCL20. Remarkably, LT β blockade in early periods of tumorigenesis dramatically reduced HCC burden in mouse model. These findings were confirmed on a cohort of patients with preneoplastic/early hepatic lesions, showing elevated mRNA levels of various pro-inflammatory cytokines including IL6 and LT β in TLS-high tumors. It is worth noting that most detected TLS were found in the peritumoral area (Finkin et al. 2015; Meylan et al. 2020). In contrast to previous works, a study observed a favorable prognosis for HCC patients that display intratumoral mature TLS suggesting the importance of TLS localization and state of maturation in dictating TLS effect (Calderaro et al. 2019). Yamaguchi et al. divided TIL aggregates in CRC tumors, in 5 categories according to their immune composition. Unlike TLS displaying an active GC, TLS formation enriched in Th populations was associated with the worst prognosis. These structures were enriched in GATA3+ Th2 cells, which through their secretion of pro-inflammatory and pro-angiogenic cytokines, including IL4, IL5 and IL13, enhance tumor growth. Importantly, Th2 may also suppress T cell differentiation toward Th17 cells, known to favor TLS neogenesis and maintenance (discussed later in the next section), indicating that TLS maturation could be impaired in patients displaying a high density of Th2 within their TLS (Yamaguchi et al. 2020). It therefore appears that in some cancer types, peritumoral immature TLS may sustain a pro-inflammatory microenvironment that nourish tumor cells leading to their growth and proliferation. In contrast, intratumoral and mature TLS may display anti-tumor properties. This could be confirmed by comparing gene expression profiles between the different TLS subtypes and locations.

4.2.2. TLS may imprint an immunosuppressive microenvironment

Evidence that TLS can be associated with suppression of the immune response were first provided in models of renal allograft. Brown et al. reported that TLS may enhance allograft tolerance instead of an active local immune response leading to chronic rejection (K. Brown, Sacks, et Wong 2011). Joshi and colleagues demonstrated in mouse models that Tregs in TLS-associated lung tumors prevented CD4+ T and CD8+ T cells proliferation and infiltration in the tumor parenchyma. Nevertheless, the depletion of Tregs restored TLS anti-tumor activity and facilitated influx of new

effector T cells in the tumor site leading to tumor regression (Joshi et al. 2015). In addition, in CRC primary tumors, TLS-associated HEV, found mainly in the invasive margin, were surprisingly associated with a more advanced disease and did not correlate with intratumoral lymphocyte infiltration (Bento et al. 2015). The location and state of maturation of TLS seem to significantly influence their functions. Indeed, in contrast to intratumoral Tregs, those found in close contact with DC-Lamp⁺ DC, CD3⁺ and CD8⁺ T cells within peritumoral lymphoid aggregates in breast tumors were predictive of relapse and death (Gobert et al. 2009). Furthermore, recent studies in HCC primary tumors showed an increased expression of genes encoding several immunoregulatory molecules such as *TGFB1*, *IL10RA* in TLS-high versus TLS-Low tumors. It is worth mentioning that most (23/24) lymphoid aggregates considered as TLS in these patients lack the presence of primary or secondary B follicle (Meylan et al. 2020).

4.2.3. TLS can be associated with CD8⁺ T cell exhaustion

Successful elimination of malignant cells by TILs can be abolished by their upregulation of inhibitory immune checkpoint (ICP) proteins such as PD1, CTLA4 and TIM3, which engagement reduce their capacity to secrete cytokines. These so called “exhausted T cells” are defined as hypofunctional effector T cells that differentiate from normal effector T cells in response to chronic antigen stimulation like in tumors. Meylan et al. reported in HCC patients an overexpression of immune exhaustion markers including Tim3 and PD-L2 in TLS-high tumors (Meylan et al. 2020). Solinas et al. detected cells expressing ICP or their ligands in some breast tumors-associated TLS including LAG3⁺, TIM3⁺, PD1⁺, CTLA4, PDL1⁺ and PDL2⁺. Unexpectedly, these ICP/ICPL were associated with good prognosis (C. Solinas et al. 2017). It is worth mentioning that ICP are also markers of T cell activation and their presence may be linked with active anti-tumor immune response. Nevertheless, Thommen and colleagues identified a subset of CD8⁺ T cells infiltrating lung tumors that highly express PD1, TIM3 and LAG3 and fail to produce cytokines consistent with an exhausted state. Surprisingly, this subset was characterized by a significantly higher clonality, CXCL13 upregulation and was mainly positioned within TLS suggesting a possible involvement of these cells in the formation of TLS by recruiting CXCR5⁺ cells (Thommen et al. 2018). In accordance with this study, transcriptomic analysis revealed in breast tumors the presence of tumor-reactive PD1^{high}CD8⁺ T cells expressing CTLA4, TIM3 and CXCL13, however, they also expressed a high level of FASL, granzyme B, IFN γ consistently associated with cytotoxic activity (Noël et al. 2021). Obviously, it seems that tumor-reactive PD1^{Hi}-CD8⁺T cells lost classical effector functions, probably following a chronic antigen stimulation, but they have developed novel functions through their secreted cytokines.

4.2.4. Other mechanisms

A few studies also showed that TLS can play a role in cancer metastasis. The TLS vasculature is necessary for the neogenesis, maturation and maintenance of these structures, however, we cannot exclude that it can provide alternative routes for tumor cell dissemination. In a cohort of oral and pharyngeal primary cancer patients, an elevated HEV density correlated with an increased frequency of LN metastasis (H. Shen et al. 2014). Besides, higher LV density correlated with metastasis and poor prognosis in lung adenocarcinoma patients (Han et al.2018). In mouse models of breast cancer, lymphatic endothelial cells upregulated PDL1 in response to IFN γ , which consequently inhibited T cell mediated anti-tumor immunity suggesting that tumor lymphatic vasculature may ensure an immunological tolerance allowing safe transportation for malignant cells that can utilize this route to disseminate (Dieterich et al. 2017; Mustapha et al. 2021).

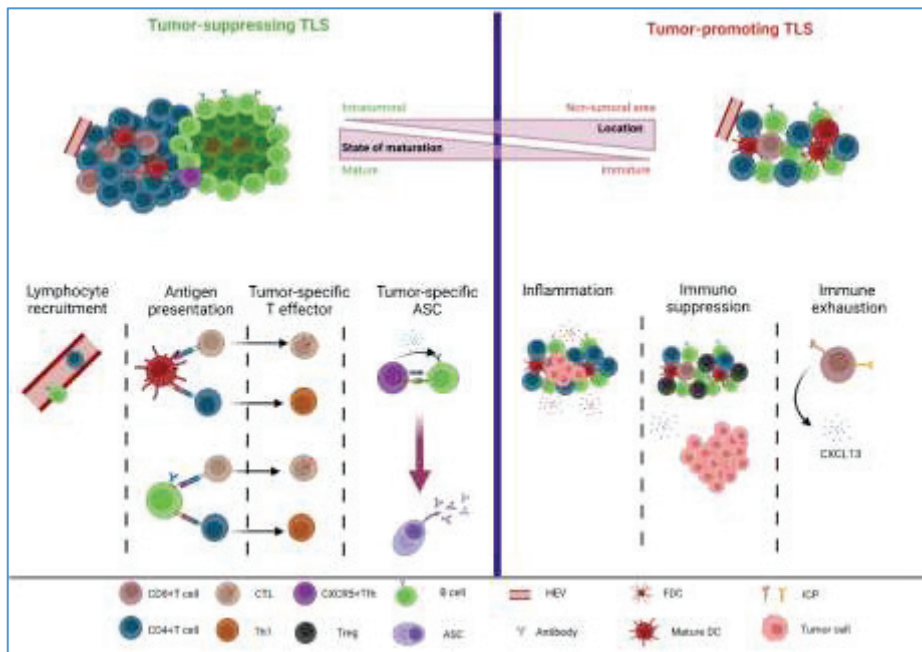


Figure 9: Tumor-associated TLS can anti or pro-tumor effects. TLS can exert anti-tumor role by recruiting immune cells, favoring tumor antigen presentation to T and B cells which induce their expansion and differentiation in CTL and ASC. Mechanisms of pro-tumorigenic effects of TLS include inflammation, immunosuppression and immune exhaustion.

To conclude, strong evidence indicate that tumor-associated TLS are in most cases a site of anti-tumor immune response development. They recruit lymphocytes that they prime and/or activate against tumor antigens to differentiate into effector cells including CD4+ T cells, CTL, memory B cells and ASC capable of eliminating malignant cells. Nevertheless, using multiple mechanisms including inflammation and immunosuppression, TLS can acquire detrimental functions. This dual role may be dictated not only by cancer types and stages, but also by the cellular composition, state of maturation, and location of TLS, as well as the TLS cytokine environment which only start to be studied (Hoch et al., s. d.).

5. TLS neogenesis and their therapeutic induction

The mechanisms responsible for TLS development in cancer are not yet fully understood, but lessons can be learnt from preclinical mouse models of autoimmunity, allograft rejection and infection. Furthermore, the molecular mechanisms responsible for the formation and maintenance of secondary lymphoid organs (SLO) and TLS share many similarities, although the initial cascade of events likely differs (**Box 4**).

5.1. TLS inducers

5.1.1. Cells

Mouse model experiments revealed that several immune cell types can promote TLS formation in tumors by mimicking functions of lymphoid tissue inducer cells (LTi) and organizer cells (LTo) that are both essential for neogenesis of SLO (**Box4**).

5.1.1.1. LTi-like cells

Studies on mouse model showed that ectopic lymphoid tissue can develop following inflammation or infection in the absence of classical CD45⁺CD4⁺CD3⁻ LTi, suggesting involvement of surrogate cells that can do the same functions (Rangel-Moreno et al. 2011). Activated B and T cells, macrophages, and DC indeed substitute for LTi cells in the initiation of TLS formation.

- **Activated B and T cells.** Th17 share many developmental and effector markers with LTi, including expression of the master transcription factor ROR γ T, the capacity to produce IL17 and IL22 and expression of CCR6, the chemokine receptor that favors their recruitment in inflammatory sites. In a model of experimental autoimmune encephalomyelitis (EAE), the induction of TLS was demonstrated to depend on Th17 expressing IL17 and podoplanin (Rangel-Moreno et al. 2011; Pikor et al. 2015). Podoplanin has a critical role for the development of the lymphatic system and is expressed on lymphatic endothelial cells and FRC of lymphoid organs. Moreover, during chronic kidney rejection, Th17 polarization of CD4⁺ T cells within the graft was associated with increased TLS development through their secretion of IL17 and IL21 (Deteix et al. 2010). Besides, under inflammatory stimuli, **B cells** can express membrane-bound LT α 1 β 2 (Figure 10) in an IL4R α -dependent manner, able to engage LT β R on CCL19⁺ stromal cells to promote GC-containing TLS formation that supported antibody production (Dubey et al. 2016). Recently, it was shown that intratumoral **CD8⁺ T cells** and **B cells** act coordinately as LTi cells to trigger tumor-associated TLS. While T cells promote initial aggregation and cancer-associated fibroblasts (LTo-like cells) organization into the reticular network, LT α 1 β 2⁺ B cells drive their expansion through LT β R signaling (Rodriguez et al. 2021).
- **Macrophages.** M1-polarized macrophages can work as LTi independently of LT β R signaling by producing TNF α and LT α , two key pro-inflammatory cytokines inducing TLS (Guedj et al. 2014;

Bénézech et al. 2015; Furtado et al. 2014). They also conferred to vascular smooth muscle cells (VSMC) an LTo phenotype, in a model of atherosclerosis (Guedj et al. 2014).

- **Dendritic cells** have been demonstrated to play critical role in the *de novo* formation of lymphatic vessels and local lymphoid tissue in infectious and auto-immune mouse models (Muniz et al. 2011). They are a source of LT β and homeostatic chemokines like CXCL12, CXCL13, CCL19 and CCL21 known to contribute to TLS organization (GeurtsvanKessel et al. 2009). In breast cancer, mature DC-Lamp⁺ DC are the main producers of LT β in breast tumors and their density correlates with tumor HEV density (Martinet et al. 2013).

Other immune cells have been reported to play the role LTI in inflamed tissues such as $\gamma\delta$ T cells, activated NKT and neutrophils. Through their secretion of IL22, $\gamma\delta$ T cells were described to induce TLS formation in the salivary glands in a mouse model of autoimmune disease (Barone et al. 2015). **NKT** cells through the activation of their TCR by synthetic glycolipids, were sufficient for inducible formation of fat-associated lymphoid clusters (FALC) through their secretion of IL4 (Bénézech et al. 2015). Eventually, **neutrophils** play a role in inducible bronchus-associated lymphoid tissue (iBALT) development since their depletion decreased IL21 and CCL19 gene expression and attenuated iBALT formation (Foo et al. 2015).

5.1.1.2. LTo-like cells

Surrogate LTI interact with local stromal cells that have characteristics of LTo such as fibroblasts.

- **Fibroblasts.** Podoplanin⁺ stromal cells at sites of autoimmune inflammation can differentiate to form a network of immunofibroblasts in an IL13-dependent manner to initiate TLS genesis. Expansion and stabilization of the fibroblast network depend on IL22 (Nayar et al. 2019). Recently, it was demonstrated in a murine melanoma model that tumor-associated TLS development was orchestrated by cancer-associated fibroblasts (CAF) with characteristics of LTo cells by a process involving TNFR, but not LT β R, signaling. These cells expressed substantially higher levels of CXCL13 that promoted B cell accumulation and increased proliferation of CAF and TLS expansion (Rodriguez et al. 2021).

5.1.2. Molecules

- Molecules involved in SLO genesis.** Several molecules involved in SLO development have been described to also drive TLS formation. Transgenic expression of CCL21 in mouse thyroid induced the recruitment of T cells able to interact with DC, leading to HEV formation and production of chemokines attracting more lymphocytes and DC to form TLS (L. Fan et al. 2000; Marinkovic et al. 2006). LIGHT (TNFS14), a member of the TNF superfamily of cytokines, can bind to LT β R and provide a crucial signal for TLS induction (Figure 10). Johansson-Percival and colleagues delivered LIGHT directly into the TME and observed the influx of T cells and TLS neogenesis (Johansson-Percival et al. 2017). A model of murine EAE confirmed that LT α /LT $\alpha\beta$ /LIGHT-LT β R signaling pathway is essential for TLS induction and its blockade prevented TLS formation (Columba-Cabezas et al. 2006).

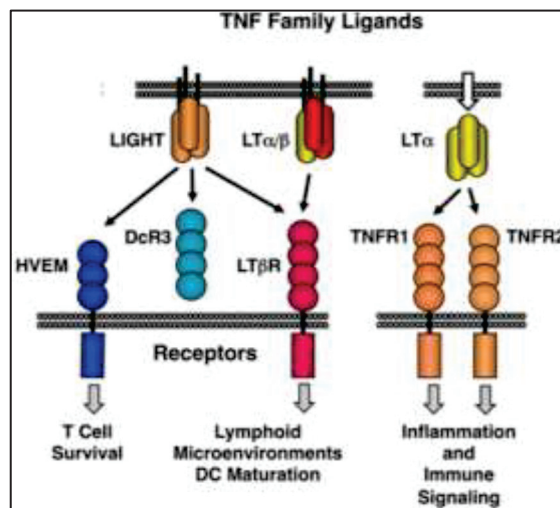


Figure 10: Map of the interaction between LT β R, HVEM, and TNF receptors with the LT/LIGHT family and the Ig-superfamily members CD160 and BTLA. LIGHT is shown in its membrane bound form, but it is also readily secreted like homotrimeric LT α (Cartoon from (T. T. Lu et Browning 2014))

- Inflammatory cytokines.** Multiple studies reported TLS induction upon inflammatory stimuli in mice lacking LT α cells (e.g. ROR γ T $^{-/-}$ mice). In a mouse model of gastric tumors driven by a mutated form of the common IL6 family receptor “gp130”, Hill and colleagues observed hyperactive STAT3 signaling leading to TLS formation supporting the key role of inflammation in TLS induction (Hill et al. 2018). TNF α , another member of TNF superfamily, have redundant physiological functions with other lymphotoxins and could act as an alternative trigger for TLS formation. Increased TNF α in inflamed tissue is indeed sufficient for TLS development and its blockade induced a decrease in TLS incidence (Guedj et al. 2014; Furtado et al. 2014). In a mouse model of pulmonary viral infection, IL1 α signaling triggered CXCL13 production and subsequent iBALT formation with presence of germinal centers (Neyt et al. 2016; E. Kuroda et al. 2016). Moreover, Denton et al. demonstrated in the same model the role of type I IFN in CXCL13 production by lung fibroblasts, which initiates the formation of functional TLS

suggesting that redundant mechanisms are in place to ensure iBALT genesis (Denton et al. 2019). To support that, Gunderson et al. observed an upregulation of type I IFN response genes in pancreatic tumor associated-TLS (J Gunderson et al. 2021). **IL17A** signaling also promoted the expression of CXCL13 and CCL19 and induced TLS formation in neonatal mice exposed to endotoxin inhalation. Interestingly, this study showed that IL17A is required to initiate but not to maintain these structures (Rangel-Moreno et al. 2011). Furthermore, IL-17 deficient mice fail to recruit B and T cells (Fleige et al. 2014). **IL22**, another cytokine that can be produced by Th17 cells, induced CXCL13 and CXCL12 expression leading to TLS formation in a mouse model of Sjogren's syndrome, an autoimmune disease. In this model, IL22 derived mainly from $\gamma\delta$ cells, however, we cannot exclude that both T cell subsets are source of IL22 production in an inflammatory site (Barone et al. 2015). IL17 and IL22 have also a role in the formation of FRC network (Pikor et al. 2015). It is likely that these molecules play an important role in TLS development in cancer, which is a highly inflamed tissue.

5.1.3. Antigenic stimulation

Chronic bacterial and viral infections can trigger inflammation that results in the development of TLS leading to the generation of efficient anti-pathogen immune responses. For example, Mycobacterium tuberculosis, influenza and hepatitis C viruses have been linked with TLS formation in mice and humans (Jones et Jones 2016). Recently, it was demonstrated in a mouse model of intestinal infection that antigen presentation is essential for TLS maintenance but not induction (Koscsó et al. 2020). In the context of cancer, we can hypothesize that tumor antigens can also play a role in TLS development. High tumor mutational burden (TMB) has been reported to promote the accumulation of neoantigens on tumor cells and to enhance anti-tumor immune response (Feng et al. 2021). Various classes of tumor antigens exist in the TME such as tumor-specific antigens (TSA) including neoantigens and tumor-associated antigens (TAA) (**Box 5**). The relationship between TLS and tumor antigens is rarely investigated. Recently, in a model of virus-induced cancer, Ruffin et al. showed an increase of GC B cells and TLS in HPV⁺ head and neck squamous cell carcinoma compared to HPV⁻ tumors, suggesting that HPV may induce TLS formation (Ruffin et al. 2021). Using the genomic and transcriptome data of solid tumors on TCGA, Lin et al. quantified TLS based on the 12-chemokine gene signature and evaluated its correlation with TMB, neoantigens and driver mutations. They reported that high TLS score positively correlated with TP53 mutation, higher TMB and increased predicted neoantigens counts in breast cancer, albeit the correlation was low (Lin et al. 2020). Besides, mutation in gene encoding BRCA that is involved in the detection and reparation of DNA alterations do not affect TLS density, location and composition in TNBC tumors (C. Solinas et al. 2019). Microsatellite instability (MSI) is the consequence of alteration of the DNA mismatch repair (MMR) system and are found in

several types of cancers such as CRC. Mature TLS are increased in MSI positive CRC tumors. Moreover, BRAF driver mutations were associated with higher TLS density and increased TLS maturity compared to non-mutated BRAF (Posch et al. 2018). Eventually, in pancreatic tumor patients, only mature TLS+ patients had a significantly increased number of predicted high and low-affinity neoantigens supporting that tumor antigens play a role in TLS maturation (J Gunderson et al. 2021). These data suggest a possible link between TLS and expression of tumor antigens, albeit, it is not clearly demonstrated. This may be due to the considerable diversity of antigens in the TME that can differ between cancer types and even between patients affected by the same cancer. It is also likely that TLS are not triggered/maintained by the same category of antigens in all patients.

It thus appear that TLS induction and/or maintenance does not depend on a single set of triggers, but rather results from a complex signaling networks involving antigens, molecules/cytokines and immune and stromal cells. These signals probably vary with the tumor type and tissue location and possibly from patient to patient.

5.2. TLS regulators:

TLS development can also be negatively regulated. **Treg** can interfere with lymphoid neogenesis by dampening neutrophilic inflammation, which is critical for iBALT neogenesis and humoral immune function (Foo et al. 2015). Indeed, in mice bearing breast tumors, Treg depletion resulted in an increased HEV density within the tumor facilitating T cell entrance (Hindley et al. 2012). In another model of genetically engineered mouse lung adenocarcinoma, Treg decreased costimulatory ligand expression by DC and T cells proliferation rates, usually increased in tumor-associated TLS (Joshi et al. 2015). **IL27**, a regulator of adaptive immunity may also counteract TLS formation through its capacity to inhibit expansion of Th17 cell that are potent TLS inducers. In a model of inflammatory arthritis, mice deficient for the IL27 receptor display increased synovial TLS formation (Jones et al. 2015). High expression of the transcription factor forkhead box protein 1 (**FoxP1**) impaired TLS formation in breast tumors and was inversely correlated with CXCL13 expression (De Silva et al. 2019). In addition, hypoxia within the TME restrains TLS formation. Lee et al. indeed showed in a mouse model of pancreatic cancer that the deletion of hypoxia-inducible factor-1 α (**HIF-1 α**) induced CXCL13 secretion and was accompanied by a prominent influx of B cells (K. E. Lee et al. 2016). Eventually, some **driver mutations** negatively correlated with TLS density such as CTNNB1 and IDH1 (Lin et al. 2020).

Collectively, these data demonstrate that TLS development in tumors is regulated by a balance between inducing and inhibiting molecules.

Box4: Roles of LTi and LTo in SLO neogenesis

Secondary lymphoid organs (SLO) development occurs during embryogenesis at predetermined sites independently of antigen recognition or inflammatory signals. It results from complex interactions between hematopoietic, mesenchymal and endothelial cells (Randall, Carragher, et Rangel-Moreno 2008; van de Pavert et Mebius 2010). SLO neogenesis can be divided in two consecutive steps:

- **Induction phase.** This step depends on CD45⁺CD4⁺CD3⁻ **lymphoid tissue inducer** (LTi) cells originating from the fetal liver. LTi express ROR γ T and membrane-bound lymphotoxin, an heterodimer of lymphotoxin α 1 and lymphotoxin β 2 (LT α 1 β 2) that can bind to lymphotoxin β receptor (LT β R) expressed on stromal cells, referred to as **lymphoid tissue organizer** (LTo) cells. Signaling through LT β R favors the expression of several adhesion molecules by LTo such as VCAM1, ICAM1, and MadCAM1, which together with the homeostatic chemokines CCL19, CCL21 and CXCL13, promotes the retention of LTi and the recruitment of immune cells leading to the next phase. This interaction also promotes HEV formation.
- **Organization phase.** During this phase, there is a large influx of cells from the bloodstream that coincides with the appearance of HEV. It is governed by chemokines, which not only recruit T cells, B cells and DC but also contribute to their spatial organization within nascent lymphoid tissue. CXCL13 attracts circulating CXCR5⁺ B cells and Tfh-like cells to what will become the B cell area, while CCL19 and CCL21 attract naïve and central memory T cells and DC to form the T cell rich zone. LTo can also differentiate into follicular dendritic cells (FDC) and fibroblastic reticular cells (FRC) that provide a conduit system on which T and B cells migrate and interact with each other.

5.3. Key steps in TLS formation

Based on the model previously established by Buckley et al. and later by Gago de Garça et al. (Buckley et al. 2015; Gago da Graça, van Baarsen, et Mebius 2021), TLS neogenesis can be divided in three main steps:

- **Resident stromal cell activation.** Following antigen exposure, persistent inflammation ensured by different hematopoietic cells induces local stromal cells, including fibroblasts, to acquire lymphoid tissue-like stromal cell characteristics, such as the expression of podoplanin, CCL19, CXCL13 and adhesion molecules ICAM1 and VCAM1 and lead to their expansion. Primed stromal cells create then a chemokine niche that attracts and spatially organizes hematopoietic cells.
- **Recruitment of immune cells.** Interactions between primed stromal cells and $LT\alpha 1\beta 2+$ activated immune cells, exerting LTi-like functions, results in their switch to functional LTos secreting the homeostatic chemokines CXCL13, CCL19, CCL21 and CXCL12 that are essential for immune cell recruitment and structuration within the TLS. In addition, local and recruited immune cells can acquire chemoattractive properties such as activated $CD8+$ T cells and $CD4+$ T cells that can secrete important amounts of CXCL13 creating a redundant environment that promote and sustain TLS formation, as shown in breast tumors (Gu-Trantien et al. 2017; 2013; Noël et al. 2021). Eventually, antigen presentation by myeloid and B cells contribute to TLS maturation with the formation of GC.
- **TLS maintenance and maturation.** Although $LT\beta R$ signaling can be dispensable for TLS induction, recent work demonstrated its importance in TLS maintenance and maturation. Indeed, in the absence of this signal, TLS became disorganized (Pikor et al. 2015). In line with that, $LT\alpha 1\beta 2+$ B cells were critical for skewing immature TLS into mature TLS in a murine model (McDonald, McDonough, et Newberry 2005). $LT\beta R$ signaling is also required for HEV formation, which is important for further TLS maturation since it constitutes the gateway for the entry of more lymphocytes from the bloodstream (Martinet et al. 2013). A final key cell for TLS maturation is the formation of FDC within the B cell follicles allowing the GC reaction and antigen delivery to B cells. The progenitors of TLS FDC remain so far unknown.

We can therefore postulate that the disparity in tumor-associated TLS frequency among patients and their heterogeneity regarding their maturation stage may depend on the nature of antigens, the type of inflammatory molecules and activated stromal and immune cells.

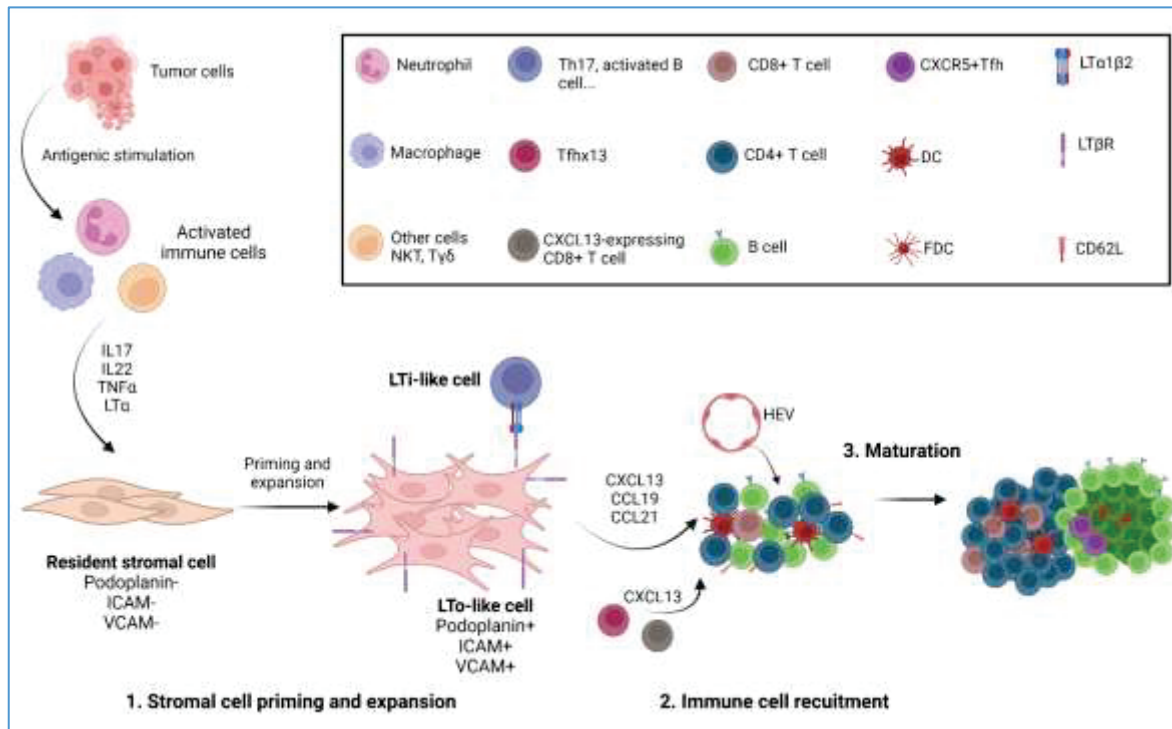


Figure 11: Model describing key steps of the tumor-associated TLS neogenesis. 1. Resident stromal cells are activated by inflammatory stimuli, then expanded and display LTo cell characteristics. 2. Following LT-LT β R signaling, LTo-like cells produce homeostatic cytokines allowing immune cell recruitment. HEV are formed in parallel. 3. Immune cells influx and antigen presentation leads to TLS maturation.

Box5: Classification of tumor antigens (Feola et al. 2020; Smith et al. 2019)

Based on the expression of parental gene, tumor antigens can generally be categorized into:

- **Tumor-associated antigens (TAA)** are self-proteins over-expressed in cancer cells. Upon malignant transformation, we can observe the overexpression of normal proteins such as p53 (antigen overexpressed), expression of proteins with tissue-specific gene patterns such as prostate-specific antigen (differentiation antigen), or the expression of proteins derived from gene expression restricted to the testes such as MAGE family (cancer-testis antigens).
- **Tumor-specific antigens (TSA) refer to** a class of antigens which expression is restricted to tumor cells and absent in healthy cells. They result from malignant mutations or the expression of viral elements.
 - o **Neoantigens** can emerge as a direct consequence of tumor DNA mutations (non-synonymous single point mutations, frameshifts, insertion/deletion) in case the corresponding mutated peptide present a high affinity for MHC-class_I or -II molecules and can initiate specific CD4 and/or CD8 T cell responses. This class antigens is mostly patient-specific. Tumor with a high tumor mutational burden are most likely to generate neoantigens.
 - o **Oncoviral antigens** are proteins derived from viruses endowed with an oncogenic transformation potential. As oncogenic viruses can drive the same kind of tumors, such Ab can be shared between patients.
 - o **Human endogenous retroviral elements (HERV)** are fragments of genomic DNA derived from exogenous retroviruses that have been incorporated into the genome through evolution. Their transcription arise through epigenetic dysregulation of the cancer genome leading to HERV-containing genomic regions expression. They represent 8% of the human genome.

5.4. Therapeutic manipulation for tumor-associated TLS induction

Because TLS usually associate with better patient survival and improved anti-tumor immune responses, manipulation of TLS neogenesis is considered as a promising therapeutic strategy for cancer patients.

Different approaches has been described in preclinical mouse models developed to induce TLS formation. Since TLS develop in inflammatory sites, and lymphotoxin involved in SLO formation are members of the **TNF superfamily**, these molecules are interesting for TLS induction. Suematsu introduced murine LT α in a stromal cell line that they transplanted under the capsule of left kidney in mice triggering TLS formation (Suematsu et Watanabe 2004). More recently, in a mouse model of pancreatic cancer, poorly infiltrated by immune cells, the use of a chimeric protein composed of LIGHT and a vascular targeting peptide (VTP) resulted in its delivery directly into the tumor and led to intratumoral T cell infiltration, TLS formation and an enhanced responsiveness to anti-PD1/CTLA4 therapies (Johansson-Percival et al. 2017). Delivery of DC engineered to express T box transcription factor (T-bet) into mouse sarcoma tumors enhanced lymphocyte infiltration, Th1 cell polarization and the development of TLS that led to tumor burden reduction. **T-bet expressing DC** were shown to produce TNF α , IL12p40 and IL36 γ and IL36R-deficient mice lost the ability to develop TLS and to control tumor growth, indicating a crucial role of T-bet/IL36 γ in therapeutic TLS induction (Weinstein et al. 2017). It was indeed found in human CRC that IL36 γ was highly expressed by M1-macrophages and endothelial cells and correlated with TLS formation (Weinstein et al. 2019). Other strategies aimed to induce **HEV formation** in order to favor lymphocyte entry into tumors. Therapies combining anti-vascular endothelial growth factor receptor (VEGFR2) Ab, anti-PDL1 Ab and a LT β R agonist were found to enhance HEV in tumors resulting in the conversion of non-immune infiltrated tumors (cold tumors) into immune-rich neoplasia (E. Allen et al. 2017).

In humans, **therapeutic vaccination** using HPV16 E6/E7 antigens in patients with high-grade cervical intraepithelial neoplasia induced increased CD8+ T cell infiltrates and TLS formation. Transcriptomic analysis revealed an upregulation of genes associated with immune activation (CXCR3) and effector functions (Tbet and IFN β). Remarkably, T cell clonal expansion was observed in the tissue of vaccinated patients, suggesting that the generated TLS may be a major component in the induction of antitumor immunity (Maldonado et al. 2014). Furthermore, in PDAC tumors, which are considered to be poorly immunogenic, vaccination with irradiated allogeneic GM-CSF-secreting pancreatic tumor cells (GVAX) induced intratumoral TLS in 85% of patients, in association with improved antigen-specific T cell response and patient survival. These findings support the potential role of TLS in transforming cold tumors (non infiltrated) into infiltrated malignancies (Lutz et al. 2014).

It is well established that immunotherapies targeting ICP/ICPL reinvigorate pre-existing antitumor “exhausted” T cells into effector cell that kill malignant cells. Therefore, combining a strategy to favor TLS neogenesis with anti-ICP/ICPL therapies is highly promising to increase the response rate especially in patients with cold tumors.

Chapter III: Heterogeneity and functions of antibody producing cells in breast and ovarian cancers

The last chapter of my introduction summarizes the current state of knowledge regarding the impact of antibody-secreting cells and their produced antibodies in breast and ovarian cancers.

This section was written together with Dr Olivia Le Saux (Oncologist and PhD student in the team working on ASC in ovarian cancer) in the form of a manuscript to be submitted shortly as a review to the “Cancers” journal. It will cover both breast and ovarian cancers, which have been described as being infiltrated by B cells at various stages of differentiation including ASC. Moreover, several mechanisms underlying the pro- or anti-tumor effects of ASC and their produced antibodies have been discovered in these cancer types.

Heterogeneity and functions of antibody secreting cells in breast and ovarian cancers

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Breast cancer, ovarian cancer, antibody-secreting cells, antibodies, heterogeneity

Abstract

Neglected for a long time in cancer, B cells and antibody secreting cells (ASC) have recently emerged as critical actors of the tumor microenvironment with an important role in shaping the antitumor immune response. ASC indeed exert a major influence on tumor growth, patient survival and response to therapies. The mechanisms underlying their pro- vs anti-tumor roles are starting to be elucidated, revealing contribution of their secreted antibodies as well as of their emerging non-canonical functions. Here, concentrating mostly on ovarian and breast cancers, we summarize the current knowledge on the heterogeneity of tumor-infiltrating ASC, we discuss their possible local or systemic origin in relation to their immunoglobulin repertoire and we review the different mechanisms by which antibody (Ab) subclasses and isoforms differentially impact tumor cells and anti-tumor immunity. We also discuss the emerging role of cytokines and other immune modulators produced by ASC in cancer. Finally, we propose strategies to manipulate the tumor ASC compartment to improve cancer therapies.

Introduction

B cells and antibody secreting cells (ASC) are critical effectors of the adaptive immune system. Although their importance in infectious disease and vaccination is well recognized, their role in cancer and response/resistance to immunotherapies have been overlooked for a long time in favor of T cells. More than 70% of tumors exhibit B cell infiltration, accounting for up to 60% of the immune infiltrate (Garaud et al.2019, Marsigliante et al.1999, Milne et al.2009, Chin et al.1992, Bruisset et al.2017). In a fraction of tumors, B cells can functionally organize with other immune cells in so-called tertiary lymphoid structures (TLS), resembling secondary lymphoid organs, with segregated B cell and T cell zones, mature dendritic cells (DC) and specialized blood vessels. Such structures can initiate and/or amplify powerful *in situ* cellular and antibody responses and are associated with better patient prognosis and response to immune therapies (Fridman et al.2019). In most tumor types, tumor-infiltrating (Ti) B cells mainly consist of naïve and antigen-experienced memory B cells, and to a lesser extent of ASC (Garaud et al.2019, Hu et al.2020, Kroeger et al.2016). Ti-ASC constitute a multifunctional B cell subset that can impact tumor cells in multiple ways. Indeed, each ASC can not only produce a huge quantity of monoclonal Immunoglobulins of a unique isotype that recognize a specific (tumor) antigen (Ag), but can also exert non-canonical functions through the release of pro- or anti-inflammatory cytokines and/or the expression immune checkpoint ligands able to modulate anti-tumor immunity. In this review, we will highlight, mainly in breast and ovarian cancers (BC and OC), the phenotypic and functional diversity of Ti ASC and how both their Ab-dependent and -independent functions shapes antitumor immunity and impact patients prognosis.

Humoral response development and prognosis impact in breast and ovarian cancers

ASC infiltrate breast and ovarian tumors

Antibody-secreting cells, identified using morphological features or by expression of CD38 or CD138 through immunohistochemistry or immunofluorescence, can be detected in approximately 30% of BC and high grade serous OC (range from 12% to 70% according to studies) and their density within the tumor infiltrate can strikingly vary from patient to patient (1–5). Such variations can be explained by several factors, including i) the type of tissue analyzed (e.g. ASCs were found to be increased in ovaries and metastases compared to fallopian tubes (6)), ii) the histology and molecular classification of the tumor (e.g. ASC infiltration is typically higher in medullary (3,5), HER2+ and triple-negative BC compared to ER+ BC (7)), iii) tumor-specific characteristics, such as expression of tumor-associated Ags as described in OC where ASC infiltration was positively associated with the expression of the cancer–testis antigens NY-ESO-1, MAGEA1 and CTAG2 (1), and iv) lack of a truly specific ASC marker (CD138, often used as a biomarker to identify ASC, can be expressed by other cells and is poorly detectable on immature ASC, e.g. plasmablasts (PBs), which infiltrate up to 80% of tumors (4,8)). However, the degree of ASC infiltration does not seem to vary with tumor mutation load, *BRCA1/2* status and P53 mutations (9). Several general features are associated with Ti-ASC. They preferentially localize in the tumor stroma (10,11) in the vicinity of CD8⁺ and CD4⁺ T cells, B cells and TLS (3,9,12). In most cancer types, Ti ASC produce mostly IgG antibodies (Abs), although this may vary depending on the type of tissue (non-mucosal vs mucosal origin of the cancer tissue) (5,9,13–21). Recently, Biswas et al., reported intriguing results showing that ASC infiltrating OC were mainly producing IgA (22), contrasting with other studies indicating a dominance of IgG-expressing cells (9,13). The reasons for these discrepancies remain currently unknown but could be linked to the use of tissue from different anatomical locations, to different treatments received by patients and/or to the possible presence of adjacent normal mucosal tissue.

Antibodies in breast and ovarian cancer patients

Antibodies directed against a broad array of tumor- and self-Ag are frequently detected in the serum and the tumor microenvironment (TME) of cancer patients. These Ags include aberrantly- and over-expressed proteins, onco-viral and intracellular proteins, endogenous retro-elements, but occasionally also neoantigens derived from tumor mutations. Early approaches to study the repertoire of antibodies in cancer patients include serological expression cloning (SEREX) (23) and phage display (24) methods. For example, NY-ESO-1, a well-known cancer testis antigen aberrantly expressed in various solid tumors, including BC and OC, was discovered by SEREX based on its capacity to induce

potent humoral response in cancer patients (25). The magnitude of such antibody responses strikingly varies according to the cancer type and from patient to patient. Profiling of serum antibody specificities using microarrays assembling more than 8,000 proteins has indeed revealed more than 200 Ags were targeted by IgG in OC, compared to less than 30 in pancreatic cancers, revealing their difference of immunogenicity (26). Levels of circulating antibodies to tumor and self-Ags can have some prognostic and diagnostic value. For instance, antibodies against mucin 1 (MUC1) can be detected in the serum of patients at early stages of BC and OC and serve as a marker of good prognosis (27,28), whereas anti-p53 antibodies have been associated with an unfavorable outcome in BC (29,30). Few studies have so far investigated other antibody isotypes than IgG. Interestingly, anti-calreticulin IgG and IgA were reported in various solid cancers, and IgA were more often associated to breast tumor metastasis than were IgG (31), suggesting a differential role of the 2 Ab isotypes. In BC, Ab levels to different sets of Ag have been proposed for early detection of the disease, including the *in situ* stage (32). Such diagnostic value of circulating Ab is best exemplified in paraneoplastic neurological diseases, which are rare autoimmune diseases that develop in a fraction of cancer patients. Elevated levels of Ab to so-called onconeural-Ag, e.g. Ags expressed physiologically by neuronal cells and aberrantly by tumor cells, are not only used for the diagnosis of the neurological disease but can also predict the type and tissue location of tumors, which can be indolent making it difficult to diagnose in a significant proportion of patients (33–35).

Circulating tumor-specific antibodies likely originate from plasma cells (PCs) residing in classical niches, such as the bone marrow and spleen, but also from PCs present in the TME. Profiling of supernatants of B cells isolated from lung tumors and activated *in vitro* and of supernatants obtained during dissociation of breast tumors with focused sets of Ag, revealed IgG and IgA against one or several tumor Ag in each patient, indicating local production of tumor-specific Ab in the TME (36,37). Interestingly, while most antibody reactivities were detected in both the serum and tumor, some were restricted to a single compartment, indicating that systemic and local Ab responses in cancer patients are partly disconnected. It is indeed likely that the repertoire of Ab in the TME is more restricted and focused on tumor Ag, especially in tumor with TLS, which promote Ag-driven expansion B cells and their differentiation into PCs (38,39).

Origin of tumor-infiltrating ASC

There is increasing evidence that Ti-ASC consist not only of cells differentiated outside the TME and attracted from blood to the tumor bed but also of cells that have locally differentiated from recruited naive and/or memory B cells. It is now well established that PB generated in secondary lymphoid organs (SLO) can acquire different chemokine receptors, like CXCR4, CCR9 and CCR10

allowing their migration to the bone marrow to constitute a contingent of long-lived PC and to different effector sites including mucosal tissues. Studies in vaccination models revealed that PB can also express CXCR3 allowing their migration to inflammatory sites in response to gradients of the inflammatory chemokines CXCL9, CXCL10 or CXCL11 (40). In OC, Kroeger et al. indeed showed that Ti-IgG⁺ ASC universally expressed CXCR3 suggesting that this chemokine receptor may contribute to their recruitment (1).

With the discovery of TLS in certain tumors, it is now admitted that inflammation-driven recruitment from blood does not account for all ASCs present in the TME. TLS can develop in chronic inflammatory sites and are organized similarly to SLO, with B cell follicles adjacent to T cell rich areas that contain mature DC and high endothelial venules. They are associated with better patient prognosis in many solid cancers including BC and OC (41–44). Numerous evidence support that TLS are sites where B cells differentiate into ASC in response to local presentation of tumor Ags. Indeed, in many epithelial tumors, virtually all B-cell differentiation stages, e.g. naive B cells, activated B cells, germinal center (GC) B cells, memory B cells, PB and terminally differentiated ASC (PC), have been identified by flow cytometry and bulk or single transcriptome profiling (8,22,45,46), arguing for an ongoing local humoral immune response in certain tumors. In line with this, TLS can harbor B cell follicles with GC containing B cells expressing Ki67 and AID, revealing ongoing Ag-driven expansion, somatic hypermutation (SHM) and class-switch recombination (36,46–48). In addition, a strong association between the presence of TLS and elevated ASC numbers have been reported in various solid cancers (1).

Examination of the immunoglobulin repertoire of B cells and ASC from TLS-positive tumors revealed a more oligoclonal response compared to tumors with an unstructured immune infiltrate (1,39,49,50). Using spatial BCR profiling, a recent study in renal carcinoma revealed clonal selection and expansion in TLS areas and detected the presence of fully mature ASC clonotypes at distance from TLS, indicating that SHM occurs in TLS and that mutated ASC disseminate throughout the tumors (47). Our own study in ovarian tumors from patients with paraneoplastic cerebellar degeneration, a rare autoimmune neurological disease associated to cancer, revealed tumor Ag deposits in B cell follicles of TLS (51). TLS may thus allow emergence of clonally expanded tumor-Ag specific ASC. The latter have been recently detected in several cancers, including human papillomavirus (HPV)+ head and neck (48) and ovarian (50) cancers. Mazor et al. showed that ASC that infiltrate ovarian tumors were mutated, clonally expanded and produced antibodies reacting against metalloproteinase (MMP) 14 - an autoAg that is overexpressed in ovarian tumor cells – and able to bind the surface of tumor cells (50). Besides, Meylan and collaborators observed higher proportions of IgG labeled apoptotic tumor cells in TLS⁺

tumors as compared to TLS⁻ tumors (47), indicating that TLS sustain the production of antibodies reacting with tumor cells and possibly leading to their elimination.

Isotype switching to both IgG and IgA can occur in tumors (13,52,53). It requires two signals: CD40L provided by T follicular helper (Tfh) cells and specific cytokines (produced by Tfh or surrounding cells), which dictate the nature of the switched isotype (54,55). Three Tfh subsets can be distinguished according to their chemokine receptor and cytokine profiles, namely CXCR3⁺CCR6⁻ IFN γ -producing Tfh1, CXCR3⁻CCR6⁻ IL-4/IL-13-producing Tfh2 and CXCR3⁻CCR6⁺ IL-17/IL-22-producing Tfh17 cells (56). In breast tumors, Tfh are detected predominantly within fully developed TLS, mostly consist of Tfh1 cells and can trigger IgG and IgA production by B cells *in vitro* by a process involving CD40-CD40L interaction (57). Therefore, it is more likely that the type of intratumorally produced cytokines largely dictates the class/subclass of antibodies expressed by newly differentiated ASC. Karagiannis and colleagues showed in melanoma tissues that the presence of IgG4 ASC coincides with high levels of IL4 and IL10, two cytokines promoting Th2 polarization and isotype switching toward IgG4 (58–60). Another study in a mouse model of prostate cancer demonstrated that TGF- β R signaling in B cells is mandatory for the induction of IgA PCs with immunosuppressive properties (61). We can therefore reasonably suppose that the Ab isotype expressed by locally generated ASCs largely depends on the functional profile of Tfh cells and reflects the cytokine TME.

Altogether, our current knowledge indicates that Ti-ASC may contain cells recruited from the circulation – possibly including some tumor-Ag specific cells generated in SLO - and cells that have differentiated locally in TLS from naïve and/or memory B cells and that are thus likely enriched in tumor Ag-specific cells (Figure 1). A recent study further supports this hypothesis by showing that tumors from patients with HPV-positive head and neck cancers were infiltrated by HPV-specific ASC with minimal bystander recruitment of influenza-specific ASC (48).

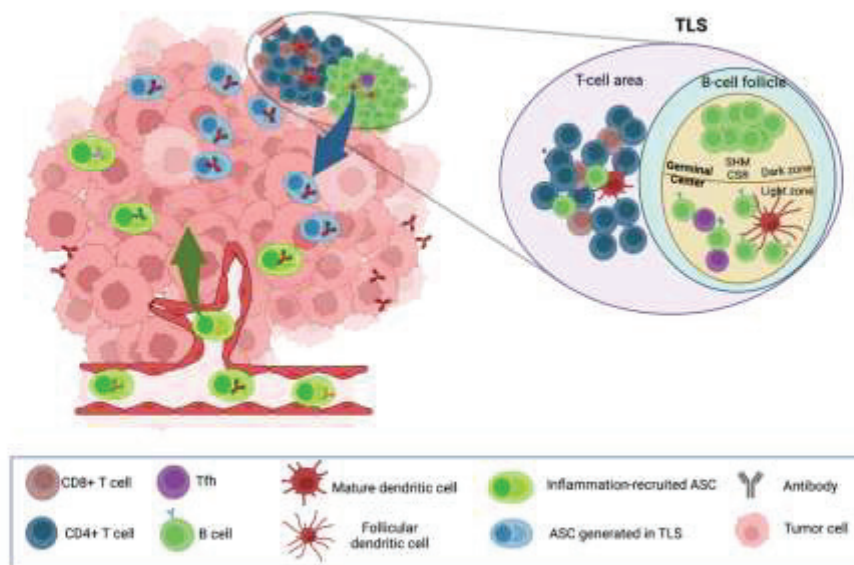


Figure 1: Possible origins of tumor infiltrating ASC. Circulating polyclonal ASC can be recruited to the tumor site in response to inflammation, whereas oligoclonal ASC can be locally generated from naïve and/or memory B cells in TLS in response to stimulation by Ags, including tumor Ags.

ASC and Antibodies influence cancer patient survival and response to immunotherapies

Numerous studies have evaluated the association between tumor infiltration by ASC at the time of diagnosis and patient survival after surgery (Table 1). Overall, ASC are usually associated with a better prognosis that manifests by increased disease-free and/or overall patient survival. This positive correlation is reinforced in case of tumor co-infiltration by CD8⁺ T cells (1) and when tumor cells are coated with endogenous immunoglobulins, a situation that is usually accompanied by increased intra-epithelial T cells (22). Nonetheless, a minority of studies reported an association with a poor prognosis (62–65). These discordant results may be linked to the phenotypic and functional heterogeneity of the tumor ASC infiltrate as well as to methodological issues linked to the detection of ASC, which range from a simple morphological identification, IHC/IF analysis with different markers like CD138 and IRF4, to computational analysis of ASC gene signature score from tumor bulk RNA-seq data using diverse algorithms. The use of CD138 as a marker of PC should indeed take into account that other cells, including epithelial cells, can express this cluster of differentiation and could bias prognosis studies (66). Combining this marker with a morphological identification of PCs or with another ASC marker like the Ig Kappa light chain (IGKC) should therefore be privileged (62,67,68). Another possibility would be that the prognosis impact of ASC may vary with their state of maturation,

as CD138 is upregulated during ASC maturation into PC but is poorly expressed at the PB stage (69). The respective impact of PB versus PC in cancer remains so far largely unexplored.

Limited information is so far available regarding the impact of ASC according to the Ab isotype they produce. Most studies reported in the literature have analyzed the relation between the relative proportion in the TME of Ig subtypes and patient survival, assuming that Ig detected in the TME, at the RNA or protein level, are produced by T_H-B cells. Overall, in most cancers, including melanoma (52,70), lung cancer (71), bladder cancer (72) and prostate cancer (61), a high expression of IgG1 is associated with longer survival whereas IgG4 and IgA expressions correlate with bad outcome. However, IgA were recently reported in OC as associated with positive impact by impairing tumor growth (22). In addition, in BC, it was shown that NY-ESO-1, more frequently elicited IgG response, which was associated with poorer prognosis, albeit IgG subclasses were not identified (37). Moreover, it is difficult to interpret these data as NY-ESO-1, per se, is associated with shorter survival and this result may only highlight the cancer aggressiveness (73). These results highlight the need to deeply characterize the diversity of ASC in the TME to better appreciate their impact on anti-tumor immunity and patient outcome.

Beyond their usual association with increased patient survival following surgery and chemotherapy, ASC were recently showed to be also associated with better response to checkpoint blockade. By comparing the transcriptome of tumors from patients treated with nivolumab (anti-PD-1) +/- ipilimumab (anti-CTLA4), Helmink et al. documented significantly higher expression of ASC-related genes MZB1, JCHAIN and Immunoglobulin Lambda Like polypeptide 5 (IGLL5) in patients that responded to treatment compared to non-responsive patients (74). Patil et al. showed that PCs, identified with a transcriptional signature including MZB1, DERL3, TNFRSF17, JSRP1, SLAMF7 and immunoglobulin genes, predicted a better overall survival to atezolizumab (anti-PD-L1) in non-squamous cell lung cancer independently of intra-tumoral CD8⁺ T cells and PD-L1 expression (75). In addition, Meylan et al. showed in clear cell renal cancers that patients with a high frequency of IgG-labeled tumor cells had a high response rate to nivolumab +/- ipilimumab and prolonged PFS (38). The predictive impact of ASC on immune checkpoint inhibitors may be explained in part by the activation of T_{fh}, which strongly express PD1 leading to B cell activation and anti-tumor immune response, as suggested in murine models (76,77).

Table 1: ASC impact on patient prognosis					
<i>Studies reporting a positive impact are highlighted in blue, while those documenting a negative impact are highlighted in salmon.</i>					
Author/Year	Histological tumor type	Number of patients	Identification of ASC	Prognosis	Reference
Kroeger et al. 2016	HGSOC	30	CD20 ⁺ CD38 ⁺ CD138 ⁺ cytosolicCD79a ⁺ IHC CD19 ⁺ IgD ⁺ CD38 ⁺ Flow Cytometry <i>TNFRSF17/IGJ</i> PC gene signature	Good	(9)
Lundgren et al. 2016	OC	209	CD138 IHC <i>IGKC</i> gene expression	Poor Neutral	(6)
Yang et al. 2021	HGSOC	351	Gene signature (CIBERSORT)	Poor	(65)
Biswas et al. 2021	HGSOC	534	CD19 ⁺ CD138 ⁺ (multiplex IHC) Internalized IgA in tumor cells	Good (total area and epithelial tumor islets) Good	(22)
Schmidt et al. 2012	BC OC	1810 426	<i>IGKC</i> expression	Good Neutral	(68)
Iglesia et al. 2014	BC OC	728 266	IgG cluster	Good (non-luminal BC) Good (mesenchymal and immunoreactive molecular subtypes)	(78)
Gentles et al. 2015	Pan-cancer	796 BC 1127 OC	Plasma cell gene signature (Cibersort)	Good (BC) Neutral (OC)	(79)
Ridolfi et al. 1977	Infiltrating ductal carcinoma	192	Morphological identification on hematoxylin and eosin stained slides	Neutral (Medullary carcinoma) Good (others)	(80)
Yeong et al. 2018	Triple-negative BC	269	intra-tumoral CD38 ⁺ IHC stromal CD38 ⁺ IHC	Good Neutral	(12)
Mohammed et al. 2012	Invasive ductal breast cancer	468	CD138 ⁺ IHC and morphological identification (H&E)	Poor	(63)
Miligy et al. 2017	Invasive BC	44	CD138 ⁺ IHC	Neutral	(10)
Kuroda et al. 2021	TNBC	114	Stromal CD38 ⁺ IHC Intra-tumoral CD38 ⁺ IHC, stromal and intra-tumoral CD138 ⁺ IHC	Good Neutral	(81)
Fan et al. 2011	BC	550	IGG gene cluster expression	Good	(82)
Harris et al. 2021	TNBC	69	Plasma cell signature (Cibersort)	Neutral	(83)
Wei et al. 2016	BC	92	Morphological identification (typical “cart-wheel” nucleus)	Poor	(64)

Antibodies functions in cancer

The canonical function of PCs is to produce antibodies. Depending on their isotype, isoform and/or Ag target, they can differentially impact tumor cells and anti-tumor immunity and may thus account for the anti- or pro-tumor properties of tumor infiltrating ASC.

Antibody isotypes and isoforms determine their functions

Antibody Fab regions are involved in recognition and possible modulation or neutralization of Ags on infectious particles or tumor cells, whereas antibody Fc regions can trigger a wide scope of immune effector functions, such as complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) or phagocytosis (ADCP) that can lead to tumor cell killing. It is well documented that the latter functions contribute to the efficacy of several cancer therapeutic monoclonal antibodies (mAbs) such as Rituximab (anti-CD20) and trastuzumab (anti-HER2). Antibody isotypes and isoforms vary in their ability to activate or inhibit immune system components including the formation of the complement complex or the engagement of Fc receptors (FcRs) on the surface of effector cells (84). This section will focus on antibody functions through their Fc region (Figure 2).

Anti-tumor effects

Antibody-mediated killing of tumor cells

Mouse models and use of therapeutic monoclonal Abs (anti-HER2/neu, -EGFR) in humans have revealed that Ab specific to tumor Ag can kill tumor cells. Whether endogenously produced antibody contribute to tumor cell control in cancer patients remains however to be formally demonstrated. Nonetheless, the fact that Ab from patients can exert cytotoxicity against tumor cells was established in 1979. Wood et al. indeed showed that the serum of patients with brain tumors, but not that of healthy volunteers, exerted significant cytotoxicity against allogeneic astrocytoma cells (85). Later, other groups demonstrated that tumor-specific antibodies isolated from patients were effectively able to kill neoplastic cells *in vitro* and *in vivo* (86,87). Recently, Meylan and collaborators found an association between IgG-stained malignant cells and apoptotic tumor cells in favor of an anti-tumor effector activity (47). Such capacity to kill tumor cells strikingly varies depending on the Ab class and subclass.

IgM. Secreted IgM harbor a pentameric form displaying 10 Ag-binding sites and thus constitute the isotype with the highest valency (88). It is the first isotype produced after an initial immunological challenge. "Natural IgM" - which are polyreactive, mostly recognize self-Ags with low affinity (89) and are present in the circulation even without Ag challenge (90) - seem to play a crucial role in the immunosurveillance of precancerous and cancerous cells (91). Atif et al. indeed showed that mice

lacking a diverse natural IgM repertoire display exacerbated tumor growth and described a mechanism by which interstitial macrophages and monocytes cleared neoantigen-expressing cells coated with natural IgM antibodies (92). Additionally, natural or adaptive IgM able to opsonize tumor Ags can directly kill malignant cells via the activation of the complement classical pathway (93).

IgG. There are four subclasses of IgG (IgG1, IgG2, IgG3, and IgG4) that differ by their Fc region and capacity to exert a cytotoxic function. That response to mAb therapy is usually higher in patients with Fc receptor polymorphisms that increase affinity for IgG (FcγRIIa-131H/R and FcγRIIIa-158V/F) supports the fact that the anti-tumor function of antibodies requires Fcγ receptor expressing cells (94), for instance NK cells, monocytes/macrophages and/or neutrophils. IgG1 and IgG3 can induce ADCC by engaging FcγRIIIa on CD16+ **NK** cells (95) to trigger cell cytotoxicity through perforin and granzyme release (96). Different studies proved that NK are able to induce ADCC in tumors. In a model of HER2+/neu breast tumor xenograft, it was indeed shown that an anti-HER2/neu engineered antibody lacking the ability to bind FcγRIIIa, was inefficient to prevent tumor growth, suggesting a potential role of NK in mAb efficacy (97). Moreover, Hubert and collaborators observed formation of ADCC synapses in immunocompetent mice bearing syngeneic breast tumor treated with an antibody directed against Tn, a specific breast cancer Ag (98). In humans, anti-HER2 IgG1 does not only suppress HER2 signaling in breast cancer cells but also mediates ADCC through NK cells, increasing the efficacy of HER2 therapy (99). Besides, **monocytes** and **macrophages** can mediate ADCP of IgG1 opsonized tumor cells. By engineering different IgG subclasses directed against a melanoma Ag, Karagiannis et al. showed that IgG1, but not IgG4, induced phagocytosis of tumor cells by monocytes *in vitro* and reduced tumor growth *in vivo* in a mouse model of subcutaneous human melanoma xenograft supplemented with human immune effector cells (58). Additionally, by intravital microscopy in mice, Gül et al. were able to visualize phagocytosis of opsonized circulating tumor cells by macrophages resulting in the prevention of liver metastases (100). That macrophages may induce tumor cell killing through ADCC in humans is suggested by the recent observation of Meylan and colleagues in renal cancer of the close proximity of macrophages and apoptotic tumor cells harboring IgG deposits (47). **Neutrophils** are also efficient to perform cytotoxicity in an FcγRIIIa (CD32a)-dependent manner (101). Intriguingly, neutrophils use a mechanism different from that of NK cells or monocytes to kill tumor cells. In a model of HER2/neu+ breast cancer cells opsonized by anti-HER2 IgG1 Ab, Matlung et al. indeed demonstrated that this cytotoxicity was not dependent on lytic granule release and involved an active mechanic destruction of the target cell plasma membrane, leading to a form of immune cell-mediated necrotic type of cell death referred to as trogoptosis (102). This method of killing is restricted to neutrophils, requires neutrophil/target cell conjugate formation dependent on CD11b/CD18 interaction, and is weakened by CD47-SIRPα interactions. Another important component that can mediate tumor cell

killing, albeit frequently neglected in the TME, is the complement system. Complement cascades can be initiated by 3 different pathways: classical, alternative and lectin pathways. Complement activation through the different pathways results in the generation of the terminal complement complex C5b-9, also called membrane attack complex (MAC) that elicits target cell lysis by creating transmembrane pores (103). Deposition of MAC on tumor tissue has been described in different cancers including breast and ovarian malignancies (104,105) suggesting that CDC is occurring in the TME. Cumulative evidence suggests that the classical pathway, which is mainly activated by IgM or IgG-containing immune complexes (ICs), is implicated in complement cascade initiation in the TME (106,107). Yet, CDC has been so far mainly demonstrated for therapeutic anti-cancer mAbs. Rituximab, an anti-CD20 mAb, was capable to induce activation of the classical complement pathway on malignant B cells *in vitro* and *in vivo* (108,109). Nevertheless, tumor cells can increase the expression of inhibitory molecules of complement activation on their surface to escape CDC, as demonstrated in patients whose breast cancer cells overexpress CD55 and CD59, two molecules that negatively regulate the complement system. These patients displayed a higher rate of relapse after anti-HER2 treatment compared to patients with lower expression (110). Inhibition of the expression of these molecules on breast cancer cell line induces a significantly enhanced anti-HER2-induced CDC-dependent lysis (111).

Among IgG subclasses, IgG1 and IgG3 demonstrate the highest affinity for most FcγRs and are potent activator of ADCC, ADCP and CDC. Such cytotoxicity can induce the release of danger-associated molecular patterns (DAMPs) and tumor Ags from necrotic tumor cells and may initiate durable adaptive antitumor immunity, including a cytotoxic T cell response. Besides, Gordan and colleagues revealed that a complex set of factors, including the organ environment, the level of Ag expression and the antibody concentration, determines the activation of different FcγRs and/or the classical complement pathway resulting in the cytotoxic IgG activity (112).

IgA. A role of IgA in tumor immunosurveillance is suggested by the fact that individuals with IgA deficiency are at (moderate) increased risk of cancer, especially in the gastrointestinal tract (HR=1.64) (113). The functions of IgA are highly diverse and depend on their subclass, isoform and the nature of accessory cells expressing IgA receptors in the TME. Two IgA subclasses co-exist, IgA1 and IgA2 that differ by the size of their hinge region, their shape and their glycosylation profile (114). In addition, IgA can exhibit a monomeric form (mIgA) dominant in serum, a dimeric (dIgA) form containing the J-chain and characteristics of mucosal tissues, and a secretory form (SIgA) consisting of dIgA assembled to the secretory component extracted from the polymeric Ig receptor "PIgR" during epithelial IgA transcytosis (115). Depending on their isoform and subclass, human IgA can bind with different affinities to several specific receptors, including FcαRI/CD89 (116) and the polymeric Ig receptor (PIgR), as well as to accessory receptors like DECTIN-1, DC-SIGN and CD71. FcαRI is expressed

by monocytes, macrophages, kupffer cells, eosinophils, and neutrophils (117,118). Both mIgA₁₋₂ and dIgA₁₋₂ bind to FcαRI with moderate affinity, while IgAICs bind avidly. By engaging FcαRI on neutrophils, IgA can induce effective ADCC of various tumor targets, including HER2+/neu breast cancer cells (119–121). Importantly, while neutrophils express high affinity receptors for both IgG and IgA, IgA appears more efficient than IgG to kill tumor cells, as shown with therapeutic antibodies *in vitro* and *in vivo* (120–122). This may be due to a stronger ITAM signaling in neutrophils induced by IgA compared to IgG. Indeed, whereas FcγRIIIa, the main FcγR expressed by neutrophils, can signal through only one ITAM, FcαRI can engage four ITAMs on the FcαRI-associated Fcγ-chains. As demonstrated for IgG, IgA induced neutrophil-dependent tumor cell killing likely involves a mechanism of trogocytosis that can be potentiated by the blockade of CD47-SIRPα interaction (123).

IgE. IgE plays a pathogenic role in allergy by triggering the rapid degranulation of FcεR-expressing cells including basophils and mast cells, and a protective effector in parasitic infections, but their role in cancer remains poorly understood. Interestingly, omalizumab, a therapeutic mAb blocking IgE binding to its high affinity receptor FcεRI and administered to severe cases of asthma, has been associated with higher risk of solid cancers development, suggesting that endogenous IgE may be part of the immunological host defense against tumors (124). Interestingly, Crawford and colleagues reported that topical exposure to an environmental DNA-damaging xenobiotic can induce an IgE response, depending on γδ intraepithelial T cells. This IgE response can protect against epithelial carcinogenesis by a mechanism involving engagement of FcεRI, possibly on basophils (125). Moreover, Josephs et al., by engineering mAbs specific for folate receptors (FRα) - which are widely expressed by human ovarian tumor cells - demonstrated the superior antitumor efficacy of IgE compared to IgG in a syngeneic rat model of cancer (126). IgE, but not IgG, promotes MCP-1 production by monocytes resulting in increased recruitment of macrophages and their polarization towards M1-like type cells expressing CD80 and TNFα. Besides, genes associated with the FcεR complex, including *FCER1* (α subunit of the IgE receptor) and *MS4A2* (β subunit of the IgE receptor), were associated to a favorable prognosis of patients with lung cancer(127). These data support a tumor-protective role of IgE but further studies are needed to determine whether an IgE response to tumor Ag develops in cancer patients and can be manipulated.

Antibody can promote anti-tumor immunity by favoring antigen presentation to T cells

Ag presentation is a critical step for inducing anti-tumor immunity and usually requires specialized Ag presenting cells (APC), notably for CD8⁺ T cells in the case of soluble or membrane bound Ags that require cross-presentation. Ab, able to form immune complexes (IC), change the way tumor

Ag are captured and presented to T cells and have been shown to be instrumental for inducing protective T cell mediated immunity.

When complexed to **IgG**, Ags can be internalized by APC, processed and presented on major histocompatibility (MHC) class-II molecules to CD4⁺ T cells (128). Importantly, IgG can also deliver exogenous Ags into the MHC class-I processing pathway of DC for presentation to CD8⁺ T cells in a process known as Ag “cross-presentation”. Ag/IgG ICs have been shown to allow cross-priming of tumor-specific CD8⁺ T cells able to protect against the development of colorectal cancer and lung metastases, by a process involving the surface FcγR and cytoplasmic FcRn (neonatal Fc receptor for IgG) of DC. FcRn ligation by IgG-IC induces IL12 production by DC allowing CD8⁺ T cell polarization into IFNγ producing cytotoxic cells (129,130). Such process might be facilitated by TLS thanks to the presence of a contingent of mature DC in the vicinity of T cells and of ASC producing anti-tumor Abs.

It is possible that such capacity to favor Ag presentation is not limited to IgG. Indeed, certain DCs can use FcεRI for directing soluble Ags complexed to **IgE** into the cross-presentation pathway to prime anti-tumor CD8⁺ T cells able to eliminate cancer cells (131). An *in vivo* study also recently revealed that natural **IgM** allows monocytes to present tumor neoantigens to CD4⁺ T cells leading to the emergence of activated T helper cells expressing CD40L with the capacity to license Ag-cross-presenting Batf3⁺ conventional type 1 DC for induction of a cytotoxic CD8⁺ T cell response (92). Although FcαRI is expressed on immature DC and can internalize IgA1 or IgA2 complexes, Ag presentation to T cells appears rather inefficient through this pathway (132). Yet, engagement of IgA receptors on inflammatory-type DC may indirectly enhance Ag-specific CD8⁺ T cells responses through its capacity to activate DC as recently shown for IgA2 (133).

Thus, antibodies may play an essential role for bridging innate and adaptive immunity to initiate or amplify efficient anti-tumor immune responses contributing *in fine* to tumor cell elimination.

IgA transcytosis in tumor cells may increase their sensitivity to CD8⁺ T lymphocytes cytotoxicity

Early studies have revealed the existence of IgG and/or IgA deposits in/on tumors cells in certain tumors, including breast and ovarian primary tumors (22,72). The team of Conejo-Garcia recently provided insights into the mechanism underlying IgA penetration in tumors cells and its consequences. Using tumor cell lines, they showed that polyclonal IgA can be internalized by ovarian and endometrial tumor cells after binding to the polymeric IgA receptor (PIgR) resulting in drastic transcriptional changes in malignant cells (22,134). In ovarian tumor cells, these changes included inhibition of the RAS pathway and upregulation of inflammatory genes, and were associated with an increased sensitivity of tumor cells to cytolytic killing by T cells *in vitro* and *in vivo* (22). While both irrelevant and tumor specific IgA can mediate these effects, they were more pronounced when tumor

cells expressed the cognate Ag. Whether a similar mechanism exists for IgG is currently unknown. In breast tumors, FcRn, an essential IgG transporter, can be expressed in malignant epithelial cells of mammary glands, but IgG internalization through this receptor have never been demonstrated (135).

Pro-tumor effects

Antibodies can suppress tumor killing

IgG4 is the less abundant subclass of IgG in the serum and has limited effector functions due to its negligible binding ability to C1q and Fcγ receptors compared to other IgG subclasses. There is evidence that this IgG subclass dampens anti-tumor immunity. Karagiannis et al. indeed reported that metastatic melanoma patients with a higher serum IgG4/total IgG ratio had significantly lower survival rates and that tumor specific IgG4 were not only unable to activate monocytes to kill tumor cells but also suppressed tumor specific IgG1-induced ADCP of tumor cells, indicating that IgG4 can suppress tumor-cell killing likely by competition with IgG1 for FcγRI binding (136). Non-tumor specific IgG4 also exerted a partial suppressive function. In addition, Alberse et al. reported that IgG4 can interact with IgG1 and IgE through their Fc domains, potentially influencing their antitumor effector functions (137). Besides the identity of the Ab subclass, several post-translational modifications can drastically affect the antitumor function of IgG. A substantial fraction of IgG in cancer patients are indeed sialylated in their Fc part, conferring to these Abs a potential suppressive function (138). Zhang et al. provided evidence that the cleavage of single peptide bond in the hinge region of endogenous IgG1 (sc-IgG1) by breast cancer-associated MMP constitutes an evasion mechanism to humoral immunity in cancer. ScIgG1 are significantly higher in tumors than in normal tissues, positively correlates with adverse clinical factors, such as frequent local metastasis to axillary lymph nodes, and display reduced Fc immune effector functions, including ADCC (139). Such IgG alterations have also been observed for an anti-HER2 mAb infused in patients with BC leading to a reduction of its therapeutic efficacy (140). How the TME affects the functions of the different Ab isotypes and what are the consequences of these modifications on tumor immune evasion needs to be analyzed.

IgA. Polymeric IgA have been involved in several inflammatory diseases, including IgA nephropathy, since their aggregation strongly increases their affinity to FcαR1 allowing potent cell activation and initiation of an inflammatory response. Conversely, serum mIgA in steady state are endowed with powerful anti-inflammatory properties toward the immune system. IgA poorly activate the complement pathway and have been showed to inhibit NK-dependent ADCC (141) . Moreover, in the absence of bound Ag, mIgA impair IgG-mediated phagocytosis, chemotaxis, bactericidal activity, oxidative burst activity, and cytokine release (142). Pasquier and colleagues further demonstrated that mIgA binding to FcαRI inhibits IgG-mediated phagocytosis in human monocytes, by a process involving

intracellular signaling through the associated Fc γ R adaptor that contains an inhibitory ITAM configuration (143). Eventually, once bound to Fc α RI, these Abs may induce death of activated neutrophils instead of tumor cell trogoptosis (144). Therefore, the dual role of IgA largely depends on their isoform, the presence or absence of target Ag and the nature of accessory cells that they can engage.

Antigen/antibody immune complexes tumor-promoting inflammation

Tumor-promoting inflammation is the seventh hallmark of cancer and contributes to various aspects of solid tumor development including growth and survival of cancer cells (145). Numerous studies highlighted that IgG-containing ICs can trigger inflammation in the TME, which in turn can promote carcinoma development. Indeed, high levels of circulating ICs are associated with increased tumor burden and poor prognosis in various cancers, including BC (146). Using a transgenic mouse model of epithelial carcinogenesis, Andreu and colleagues showed a reduction of both leukocyte recruitment and tumor growth in the absence of B cells or activating Fc γ R. Such experiments revealed that IgG-mediated stimulation of mast cells and macrophages can promote, in certain settings, inflammation and a subsequent increase of angiogenesis, survival and proliferation of tumor cells (147). Furthermore, Ags opsonized by IgG can activate the classical complement pathway leading to the release of the C3a and C5a anaphylatoxins, whose levels correlate with decreased effector T cell numbers and increased tumor burden in a mouse model of OC (148). C3a and/or C5a can indeed directly enhance tumor cell survival, proliferation and metastasis after binding to their receptors on tumor cells. Moreover, they can recruit myeloid-derived suppressor cells, tumor-promoting macrophages, stimulate the pro-tumorigenic properties of mast cells resulting in chronic inflammation, suppress CD8⁺ T cell cytotoxicity and promote formation of pro-tumoral neutrophil extracellular trap (106).

Modulation of tumor-associated antigens by antibodies

Antibodies may also affect tumor cells, independently of the immune system, by directly exerting an agonist or antagonist function on target Ags, like growth factors or surface receptors that are essential for tumor cell survival, expansion or dissemination, or by directly triggering tumor cell apoptosis upon binding to cell surface proteins. These properties are largely exploited for cancer therapy with the success of mAbs targeting HER2, EGFR and other growth factor receptors. There is some evidence that such Ab can naturally be generated in cancer patients. For example, natural IgM are able to induce tumor-specific apoptosis by cross-linking of complement inhibitory proteins, blocking growth factor receptors or by increasing the intracellular level of neutral lipids (91,149,150). In addition, a monoclonal antibody directed against the 78kDa glucose-regulated protein (GRP78) - an

Ag highly expressed by breast tumor cells (151) – and isolated from a gastric cancer patient (150) was shown to be able to directly kill cancer cells by cellular lipotoxicity involving intracellular lipids, cholesterylester and triglycerides accumulation and leading to cell apoptosis. Another example relies to the pathologic effects of Abs that can be observed in cancer patients with a paraneoplastic syndrome. In these rare autoimmune disease, high levels of auto-Ab directed against self-Ags aberrantly expressed in tumor cells are usually observed. In the case of ovarian tumors with Abs to N-methyl-D-aspartate receptor (NMDAR) (152,153), incubation of the Ab on neuronal cells has been shown to result in the rapid internalization of the extracellular GluN1 NMDAR subunit and decrease of glutamatergic synaptic function, possibly explaining neurological clinical manifestations observed in patients (154,155). A minor fraction of patients affected by lung or breast cancer, ovarian teratoma or thymoma can develop a paraneoplastic syndrome caused by the humoral and cellular immune response against GABA-B receptor, expressed by both neurons and tumor cells. Anti-GABA-B autoantibody found in patients' serum were shown to colocalize with GABA-B receptor and to disrupt its structure resulting in seizures and changes in memory, learning and behavior in animal models (156,157).

Overall, antibodies can have both beneficial and deleterious roles in the TME and their better characterization and the understanding of their mode of action will be essential to manipulate them for therapeutic purposes.

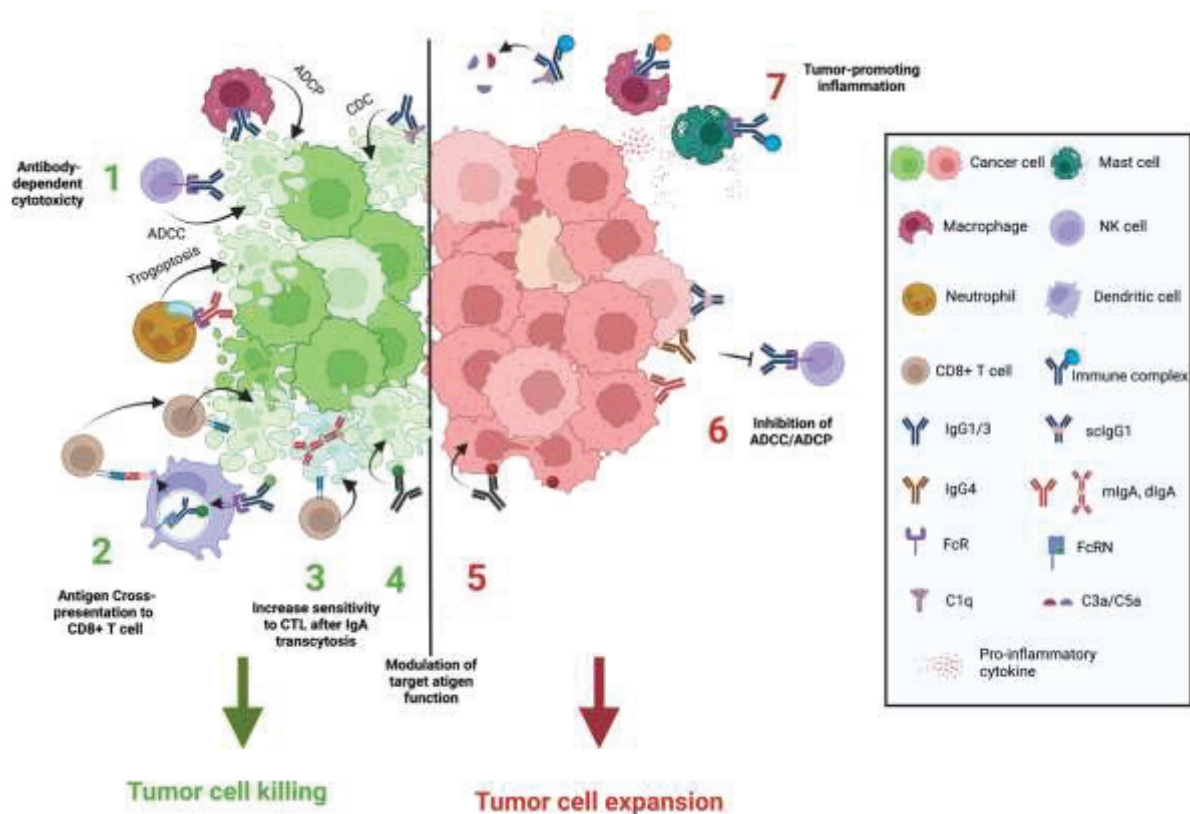


Figure 2: Mechanisms of action of antibodies in cancer. Antibodies can use several mechanisms to suppress (1-4) or promote (5-7) tumor growth. (1) Certain subclasses of antibodies can mediate tumor cell killing by ADCC mediated by NK cells, ADCP by macrophages, trogoptosis by neutrophils and CDC. (2) Capture of antibody/antigen complex by DC can prime anti-tumor immunity through antigen cross-presentation to CD8+ T cells. (3) Transcytosis of dimeric IgA in tumor cells increases their sensitivity to CD8+ T cell mediated cytotoxicity. (4) Direct modulation of target antigen function leading to tumor cell dysfunction/elimination. (5) Modulation of tumor antigens leading to survival/proliferation of tumor cells. (6) IgG4, sIgG1 and IgA can suppress the effector functions of cytotoxic antibody isotypes. IgG4 compete with IgG1 for FcγR binding. SclgG1 has a reduced ability to induce ADCC compared to IgG1. IgA inhibit NK-dependent ADCC mediated by IgG1/IgG3. (7) IgG-antigen immune complexes can induce tumor-promoting inflammation by stimulation of macrophages and mast cells and/or by the activation of the classical complement pathway and the release of pro-inflammatory anaphylatoxins.

Antibody-independent pro- vs anti-tumor functions of antibody-producing cells

Besides the production of antibodies, there is increasing evidence that ASC display several non-canonical functions that can impact tumor growth and antitumor immunity.

Cytokine secretion

In addition to antibody production, accumulating evidence demonstrate that certain human ASC subsets can produce a diverse array of cytokines or molecules endowed with either pro- or anti-inflammatory functions. Nevertheless, the cytokine expression profile of Ti-ASC remains so far poorly documented, possibly due to their usual low frequency and to the fact that detecting intracellular cytokines in B cells can be technically challenging. Inflammatory bowel disease associated-ASC can produce **granzyme B** (158,159), traditionally known for its perforin-dependent pro-apoptotic function underlying the capability of cytotoxic immune cells to kill tumorigenic cells (160,161). Moreover, in infectious disease, ASC can express the inducible Nitric Oxid Synthase (iNOS) (162), which was reported in BC to elicit cancer progression through regulation of cell adhesion and motility (163,164). ASC can also secrete immunosuppressive molecules such as **IL-35** (165) that inhibit anti-tumor cytokines including IFN-γ (166). Interestingly, PCs in human lung cancers can express EB13, a component of IL-27 and IL-35 heterodimers (167), and **IL-10** (61,168,169) a cytokine with both tumor-promoting properties through inhibition of tumor Ag presentation (170,171) and tumor-inhibiting activities through anti-angiogenic functions via down-regulation of VEGF, IL-1b, TNF-α, IL-6, and MMP-9 (172)). IL-10-expressing PB have been described in human renal cell carcinoma and appeared to be enriched in IgA producing cells (173). IL-10⁺ PB infiltration was associated with an up-regulation of the T cell exhaustion marker Tim-3 and higher tumor size and stage, suggesting that they may suppress T cell immunity. Using mouse models of prostate and liver cancers, Shalpour and colleagues showed that IL10-producing IgA⁺ secreting cells are induced in the TME, especially after chemotherapy, by a process requiring TGFβ-receptor signaling in B cells, and can directly suppress cytotoxic CD8⁺ T lymphocytes in an IL-10 dependent manner (61,168). Thus far, little is known about the specific markers that could distinguish suppressive IL-10⁺ ASC from their non-IL-10-producing (potentially antitumor)

counterparts. Regulatory IL-10⁺ ASCs producing IgM or IgG isotypes have also been described in autoimmune and infectious diseases (61,168,174,175)

Other functions of ASC in the TME

IgA⁺ ASCs in prostate and liver cancers also express high levels of PD-L1 on their surface (61,168). PD-L1 neutralization did not decrease tumor burden in mice models deficient for IgA or CD8 T cells, indicating that it acts via IgA⁺ cells and targets CD8⁺ T cells. PD-L1 expression on ASC was confirmed in human samples from BC (176). In infectious disease, the Fillatreau group recently described LAG3 as another immune checkpoint expressed by regulatory ASCs (177). These cells were a major source of IL-10 early after infection with *Salmonella* (177). They also express the PD-L1, PD-L2, and CD200 (Lino et al. 2018) inhibitory receptors that are upregulated upon activation and frequently associated with T cell. Regulatory ASCs, possibly present in tumors, may use these inhibitory receptors as additional mechanisms to suppress an immune response in addition to secretion of IL-10 or other suppressive cytokines.

The increased expression of immune checkpoint ligands on ASCs may suggest close interactions between ASCs and other immune cells in the TME. Recent evidences also suggest a role for exosome transfer in the function of Ti ASC. In OC, it has been shown that ASCs were enriched in the mesenchymal subtype of HGSOC and that the epithelial to mesenchymal phenotypic switch of tumor cells *in vitro* and *in vivo*, which controls tumor metastasis and patient prognosis, was mediated by the transfer of PC-derived exosomes containing miR-330-3p into non-mesenchymal ovarian cancer cells inducing their transition (65) (Figure 3).

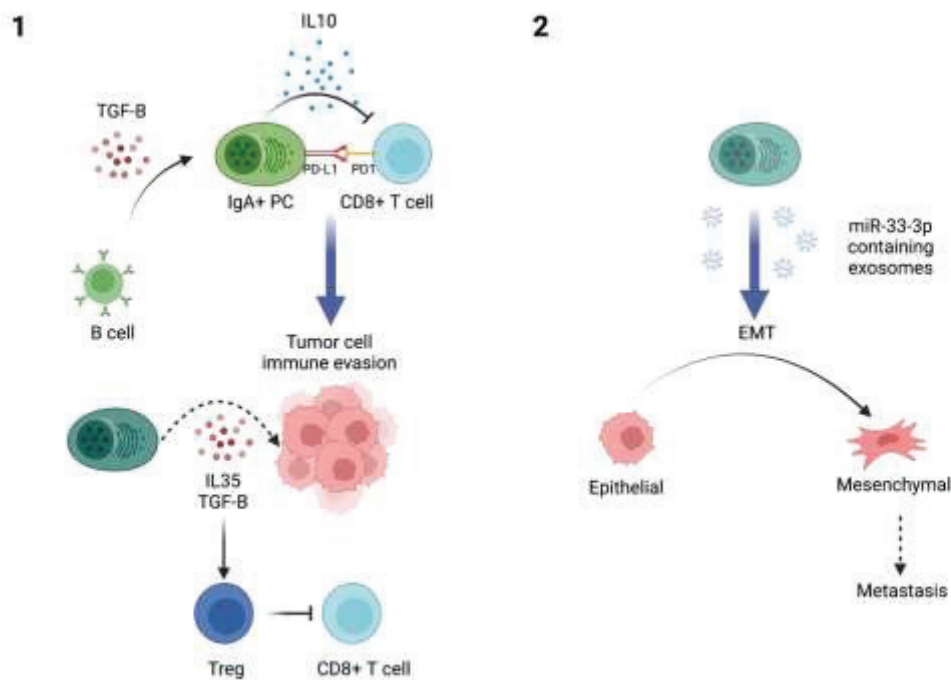


Figure 3. Non-canonical functions of antibody-secreting cells involved in tumor promotion. (1) IgA+ PC can secrete IL-10 and express the inhibitory immune check-point ligand “PD-L1” and suppress CD8+ T cell function. PC may secrete other suppressive cytokines, like IL-35 and TGFb, involved in tumor promotion by stimulating tumor cell proliferation and/or suppression of antitumor immunity (2) PC are able to deliver miR-330-3p containing exosomes to tumor cells initiating an epithelial to mesenchymal phenotypic switch and tumor dissemination.

Conclusion

In this review, we have emphasized that ASC can exhibit both beneficial and detrimental roles in the TME. These pro- and anti-tumor functions depend not only on the class and subclass of Ab they produce, the identity of their target Ag, the nature and functions of the accessory cells present in the TME, but also on their non-canonical activities such as cytokine production and immune checkpoint expression, the latter remaining to be better characterized. ASC thus emerge as critical actors of the TME, but many questions remain to address to envisage their therapeutic manipulation. To what extent is the ASC infiltrate fed by cells differentiated in secondary lymphoid organs vs locally in TLS, and, in the latter case, do all ASC originate from a germinal center reaction or can they also be generated in extrafollicular areas? Does the origin of ASC impact their functional properties and Ag specificity? Although the diversity of T_H ASC starts to be revealed, a more comprehensive analysis of the Ab classes (including IgE) and subclasses, isoforms and glycosylation pattern is required to better appreciate how ASC may impact tumor immunity through Ab production. A particular attention should be paid to the contribution of the different IgA isoforms to activation versus immune tolerance. In

addition, because Ab-related ASC functions largely depend on engagement of Ig Fc receptors on accessory cells, which identity will dictate Ab effector function, a comprehensive spatial and functional characterization of cells (neutrophils, NK cells, mast cells, macrophages) expressing the different Fc receptors should be performed to better elucidate/predict elucidate the function of ASC. Furthermore, the presence of Ti-ASC and TLS has recently been associated to better response to immune checkpoint inhibitors. Determining whether ASC and Ab play an active role in this response and identification of the underlying mechanism could pave the way for defining strategies to improve efficacy of immunotherapies. Increasing recruitment, neo-differentiation and/or function of antitumor ASC could indeed complete the anti-cancer therapeutic arsenal. Inducing TLS could be an option since these structures favor emergence of ASCs enriched in tumor Ag-specific cells and production of antibodies able to bind to tumor cells. This will require a better understanding of how cancer-associated TLS are initiated and maintained in an active form. Finally, although evidence of a detrimental effect of ASC are still scarce in human, one therapeutic avenue in certain cancers could consist in inducing their specific depletion. This could be achieved through proteasome inhibition with the risk of also depleting anti-tumor ASC. Another important challenge will thus consist in identifying reliable markers able to distinguish pro- from anti-tumoral ASC and the signals involved in their development in order to identify the best therapeutic strategies.

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References

1. Kroeger DR, Milne K, Nelson BH. Tumor-Infiltrating Plasma Cells Are Associated with Tertiary Lymphoid Structures, Cytolytic T-Cell Responses, and Superior Prognosis in Ovarian Cancer. *Clin Cancer Res.* 15 juin 2016;22(12):3005-15.
2. Milne K, Köbel M, Kalloger SE, Barnes RO, Gao D, Gilks CB, et al. Systematic Analysis of Immune Infiltrates in High-Grade Serous Ovarian Cancer Reveals CD20, FoxP3 and TIA-1 as Positive Prognostic Factors. *PLoS One* [Internet]. 29 juill 2009 [cité 10 avr 2020];4(7). Disponible sur: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2712762/>
3. Mohammed ZMA, Going JJ, Edwards J, Elsberger B, Doughty JC, McMillan DC. The relationship between components of tumour inflammatory cell infiltrate and clinicopathological factors and survival in patients with primary operable invasive ductal breast cancer. *Br J Cancer.* 21 août 2012;107(5):864-73.
4. Nielsen JS, Sahota RA, Milne K, Kost SE, Nesslinger NJ, Watson PH, et al. CD20+ tumor-infiltrating lymphocytes have an atypical CD27- memory phenotype and together with CD8+ T cells promote favorable prognosis in ovarian cancer. *Clin Cancer Res.* 15 juin 2012;18(12):3281-92.
5. Wang Y, Ylera F, Boston M, Kang SG, Kutok JL, Klein-Szanto AJP, et al. Focused antibody response in plasma cell-infiltrated non-medullary (NOS) breast cancers. *Breast Cancer Res Treat.* 17 juill 2007;104(2):129-44.
6. Lundgren S, Berntsson J, Nodin B, Micke P, Jirstrom K. Prognostic impact of tumour-associated B cells and plasma cells in epithelial ovarian cancer. *J Ovarian Res.* 6 avr 2016;9:21.
7. A single-cell RNA expression atlas of normal, preneoplastic and tumorigenic states in the human breast. *The EMBO Journal.* juin 2021;40(11):e107333.
8. Hu Q, Hong Y, Qi P, Lu G, Mai X, Xu S, et al. Atlas of breast cancer infiltrated B-lymphocytes revealed by paired single-cell RNA-sequencing and antigen receptor profiling. *Nat Commun.* 12 avr 2021;12(1):2186.
9. Kroeger DR, Milne K, Nelson BH. Tumor-Infiltrating Plasma Cells Are Associated with Tertiary Lymphoid Structures, Cytolytic T-Cell Responses, and Superior Prognosis in Ovarian Cancer. *Clin Cancer Res.* 15 2016;22(12):3005-15.
10. Miligy I, Mohan P, Gaber A, Aleskandarany MA, Nolan CC, Diez-Rodriguez M, et al. Prognostic significance of tumour infiltrating B lymphocytes in breast ductal carcinoma in situ. *Histopathology.* 2017;71(2):258-68.
11. deLeeuw RJ, Kroeger DR, Kost SE, Chang PP, Webb JR, Nelson BH. CD25 Identifies a Subset of CD4⁺ FoxP3⁻ TIL That Are Exhausted Yet Prognostically Favorable in Human Ovarian Cancer. *Cancer Immunol Res.* mars 2015;3(3):245-53.
12. Yeong J, Lim JCT, Lee B, Li H, Chia N, Ong CCH, et al. High Densities of Tumor-Associated Plasma Cells Predict Improved Prognosis in Triple Negative Breast Cancer. *Front Immunol.* 2018;9:1209.

13. Montfort A, Pearce O, Maniati E, Vincent BG, Bixby L, Böhm S, et al. A Strong B-cell Response Is Part of the Immune Landscape in Human High-Grade Serous Ovarian Metastases. *Clin Cancer Res.* 1 janv 2017;23(1):250-62.
14. Wieczorek M, Braicu EI, Oliveira-Ferrer L, Sehoul J, Blanchard V. Immunoglobulin G Subclass-Specific Glycosylation Changes in Primary Epithelial Ovarian Cancer. *Frontiers in Immunology* [Internet]. 2020 [cité 7 mars 2022];11. Disponible sur: <https://www.frontiersin.org/article/10.3389/fimmu.2020.00654>
15. Gerçel-Taylor C, Bazzett LB, Taylor DD. Presence of aberrant tumor-reactive immunoglobulins in the circulation of patients with ovarian cancer. *Gynecol Oncol.* avr 2001;81(1):71-6.
16. Ruhaak LR, Kim K, Stroble C, Taylor SL, Hong Q, Miyamoto S, et al. Protein-Specific Differential Glycosylation of Immunoglobulins in Serum of Ovarian Cancer Patients. *J Proteome Res.* 4 mars 2016;15(3):1002-10.
17. Qian Y, Wang Y, Zhang X, Zhou L, Zhang Z, Xu J, et al. Quantitative Analysis of Serum IgG Galactosylation Assists Differential Diagnosis of Ovarian Cancer. *J Proteome Res.* 6 sept 2013;12(9):4046-55.
18. Alley WR, Vasseur JA, Goetz JA, Svoboda M, Mann BF, Matei DE, et al. N-linked Glycan Structures and Their Expressions Change in the Blood Sera of Ovarian Cancer Patients. *J Proteome Res.* 6 avr 2012;11(4):2282-300.
19. Saldova R, Royle L, Radcliffe CM, Abd Hamid UM, Evans R, Arnold JN, et al. Ovarian Cancer is Associated with Changes in Glycosylation in Both Acute-Phase Proteins and IgG. *Glycobiology.* 28 août 2007;17(12):1344-56.
20. Gaffey MJ, Frierson HF, Mills SE, Boyd JC, Zarbo RJ, Simpson JF, et al. Medullary carcinoma of the breast. Identification of lymphocyte subpopulations and their significance. *Mod Pathol.* nov 1993;6(6):721-8.
21. Ito T, Saga S, Nagayoshi S, Imai M, Aoyama A, Yokoi T, et al. Class distribution of immunoglobulin-containing plasma cells in the stroma of medullary carcinoma of breast. *Breast Cancer Res Treat.* 1986;7(2):97-103.
22. Biswas S, Mandal G, Payne KK, Anadon CM, Gatenbee CD, Chaurio RA, et al. IgA transcytosis and antigen recognition govern ovarian cancer immunity. *Nature.* 2021;591(7850):464-70.
23. Sahin U, Türeci O, Schmitt H, Cochlovius B, Johannes T, Schmits R, et al. Human neoplasms elicit multiple specific immune responses in the autologous host. *Proc Natl Acad Sci U S A.* 5 déc 1995;92(25):11810-3.
24. Fosså A, Alsøe L, Cramer R, Funderud S, Gaudernack G, Smeland EB. Serological cloning of cancer/testis antigens expressed in prostate cancer using cDNA phage surface display. *Cancer Immunol Immunother.* mai 2004;53(5):431-8.
25. Chen YT, Scanlan MJ, Sahin U, Türeci Ö, Gure AO, Tsang S, et al. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *Proc Natl Acad Sci U S A.* 4 mars 1997;94(5):1914-8.
26. Gnjjatic S, Ritter E, Büchler MW, Giese NA, Brors B, Frei C, et al. Seromic profiling of ovarian and pancreatic cancer. *Proc Natl Acad Sci U S A.* 16 mars 2010;107(11):5088-93.

27. Blixt O, Bueti D, Burford B, Allen D, Julien S, Hollingsworth M, et al. Autoantibodies to aberrantly glycosylated MUC1 in early stage breast cancer are associated with a better prognosis. *Breast Cancer Res.* 8 mars 2011;13(2):R25.
28. Richards ER, Devine PL, Quin RJ, Fontenot JD, Ward BG, McGuckin MA. Antibodies reactive with the protein core of MUC1 mucin are present in ovarian cancer patients and healthy women. *Cancer Immunol Immunother.* juill 1998;46(5):245-52.
29. Kulić A, Sirotković-Skerlev M, Jelisavac-Cosić S, Herceg D, Kovac Z, Vrbanec D. Anti-p53 antibodies in serum: relationship to tumor biology and prognosis of breast cancer patients. *Med Oncol.* sept 2010;27(3):887-93.
30. Lenner P, Wiklund F, Emdin SO, Arnerlöv C, Eklund C, Hallmans G, et al. Serum antibodies against p53 in relation to cancer risk and prognosis in breast cancer: a population-based epidemiological study. *Br J Cancer.* févr 1999;79(5-6):927-32.
31. Erić-Nikolić A, Milovanović Z, Sánchez D, Pekáriková A, Džodić R, Matić IZ, et al. Overexpression of calreticulin in malignant and benign breast tumors: relationship with humoral immunity. *Oncology.* 2012;82(1):48-55.
32. Desmetz C, Bascoul-Mollevi C, Rochaix P, Lamy PJ, Kramar A, Rouanet P, et al. Identification of a New Panel of Serum Autoantibodies Associated with the Presence of In situ Carcinoma of the Breast in Younger Women. *Clinical Cancer Research.* 15 juill 2009;15(14):4733-41.
33. Honnorat J, Cartalat-Carel S, Ricard D, Camdessanche JP, Carpentier AF, Rogemond V, et al. Onco-neural antibodies and tumour type determine survival and neurological symptoms in paraneoplastic neurological syndromes with Hu or CV2/CRMP5 antibodies. *J Neurol Neurosurg Psychiatry.* avr 2009;80(4):412-6.
34. Eichler TW, Totland C, Haugen M, Qvale TH, Mazengia K, Storstein A, et al. CDR2L Antibodies: A New Player in Paraneoplastic Cerebellar Degeneration. *PLOS ONE.* 18 juin 2013;8(6):e66002.
35. Jarius S, Wildemann B. « Medusa head ataxia »: the expanding spectrum of Purkinje cell antibodies in autoimmune cerebellar ataxia. Part 2: Anti-PKC-gamma, anti-GluR-delta2, anti-Ca/ARHGAP26 and anti-VGCC. *J Neuroinflammation.* 17 sept 2015;12:167.
36. Germain C, Gnjatic S, Tamzalit F, Knockaert S, Remark R, Goc J, et al. Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. *Am J Respir Crit Care Med.* 1 avr 2014;189(7):832-44.
37. Garaud S, Zayakin P, Buisseret L, Rulle U, Silina K, de Wind A, et al. Antigen Specificity and Clinical Significance of IgG and IgA Autoantibodies Produced in situ by Tumor-Infiltrating B Cells in Breast Cancer. *Front Immunol.* 2018;9:2660.
38. Meylan M, Petitprez F, Becht E, Bougoüin A, Pupier G, Calvez A, et al. Tertiary lymphoid structures generate and propagate anti-tumor antibody-producing plasma cells in renal cell cancer. *Immunity.* 24 févr 2022;S1074-7613(22)00081-4.
39. Coronella JA, Spier C, Welch M, Trevor KT, Stopeck AT, Villar H, et al. Antigen-driven oligoclonal expansion of tumor-infiltrating B cells in infiltrating ductal carcinoma of the breast. *J Immunol.* 15 août 2002;169(4):1829-36.

40. Odendahl M, Mei H, Hoyer BF, Jacobi AM, Hansen A, Muehlinghaus G, et al. Generation of migratory antigen-specific plasma blasts and mobilization of resident plasma cells in a secondary immune response. *Blood*. 15 févr 2005;105(4):1614-21.
41. Martinet L, Garrido I, Filleron T, Le Guellec S, Bellard E, Fournie JJ, et al. Human solid tumors contain high endothelial venules: association with T- and B-lymphocyte infiltration and favorable prognosis in breast cancer. *Cancer Res*. 1 sept 2011;71(17):5678-87.
42. Truxova I, Kasikova L, Hensler M, Skapa P, Laco J, Pecen L, et al. Mature dendritic cells correlate with favorable immune infiltrate and improved prognosis in ovarian carcinoma patients. *J Immunother Cancer*. 4 déc 2018;6(1):139.
43. Ukita. Tertiary lymphoid structures induced by CXCL13-producing CD4+ T cells increase tumor infiltrating CD8+ T cells and B cells in ovarian cancer | bioRxiv [Internet]. [cité 7 mars 2022]. Disponible sur: <https://www.biorxiv.org/content/10.1101/2021.12.01.470493v1>
44. Sautès-Fridman C, Verneau J, Sun CM, Moreira M, Chen TWW, Meylan M, et al. Tertiary Lymphoid Structures and B cells: Clinical impact and therapeutic modulation in cancer. *Semin Immunol*. avr 2020;48:101406.
45. Griss J, Bauer W, Wagner C, Simon M, Chen M, Grabmeier-Pfistershammer K, et al. B cells sustain inflammation and predict response to immune checkpoint blockade in human melanoma. *Nat Commun*. déc 2019;10(1):4186.
46. Garaud S, Buisseret L, Solinas C, Gu-Trantien C, de Wind A, Van den Eynden G, et al. Tumor infiltrating B-cells signal functional humoral immune responses in breast cancer. *JCI Insight*. 13 août 2019;5:129641.
47. Meylan M, Petitprez F, Becht E, Bougoüin A, Pupier G, Calvez A, et al. Tertiary lymphoid structures generate and propagate anti-tumor antibody-producing plasma cells in renal cell cancer. *Immunity*. 24 févr 2022;S1074-7613(22)00081-4.
48. Wieland A, Patel MR, Cardenas MA, Eberhardt CS, Hudson WH, Obeng RC, et al. Defining HPV-specific B cell responses in patients with head and neck cancer. *Nature*. sept 2021;597(7875):274-8.
49. Nzula S, Going JJ, Stott DI. Antigen-driven clonal proliferation, somatic hypermutation, and selection of B lymphocytes infiltrating human ductal breast carcinomas. *Cancer Res*. 15 juin 2003;63(12):3275-80.
50. Mazor RD, Nathan N, Gilboa A, Stoler-Barak L, Moss L, Solomonov I, et al. Tumor-reactive antibodies evolve from non-binding and autoreactive precursors. *Cell*. 31 mars 2022;185(7):1208-1222.e21.
51. Small M, Treilleux I, Couillault C, Pissaloux D, Picard G, Paindavoine S, et al. Genetic alterations and tumor immune attack in Yo paraneoplastic cerebellar degeneration. *Acta Neuropathol*. avr 2018;135(4):569-79.
52. Bosisio FM, Wilmott JS, Volders N, Mercier M, Wouters J, Stas M, et al. Plasma cells in primary melanoma. Prognostic significance and possible role of IgA. *Mod Pathol*. avr 2016;29(4):347-58.

53. Cipponi A, Mercier M, Seremet T, Baurain JF, Théate I, van den Oord J, et al. Neogenesis of lymphoid structures and antibody responses occur in human melanoma metastases. *Cancer Res.* 15 août 2012;72(16):3997-4007.
54. Aversa G, Cocks BG, Punnonen J, Carballido JM, de Vries JE. Contact-mediated signals and cytokines involved in B-cell activation and isotype switching in pre-B and mature B cells. *Res Immunol.* avr 1994;145(3):222-6; discussion 244-249.
55. Coffman RL, Leberman DA, Rothman P. Mechanism and regulation of immunoglobulin isotype switching. *Adv Immunol.* 1993;54:229-70.
56. Morita R, Schmitt N, Bentebibel SE, Ranganathan R, Bourdery L, Zurawski G, et al. Human blood CXCR5(+)CD4(+) T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. *Immunity.* 28 janv 2011;34(1):108-21.
57. Noël G, Fontsa ML, Garaud S, De Silva P, de Wind A, Van den Eynden GG, et al. Functional Th1-oriented T follicular helper cells that infiltrate human breast cancer promote effective adaptive immunity. *J Clin Invest.* 1 oct 2021;131(19):e139905.
58. Karagiannis P, Gilbert AE, Josephs DH, Ali N, Dodev T, Saul L, et al. IgG4 subclass antibodies impair antitumor immunity in melanoma. *J Clin Invest.* 1 avr 2013;123(4):1457-74.
59. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today.* mars 1996;17(3):138-46.
60. Kurte M, López M, Aguirre A, Escobar A, Aguillón JC, Charo J, et al. A synthetic peptide homologous to functional domain of human IL-10 down-regulates expression of MHC class I and Transporter associated with Antigen Processing 1/2 in human melanoma cells. *J Immunol.* 1 août 2004;173(3):1731-7.
61. Shalpour S, Font-Burgada J, Di Caro G, Zhong Z, Sanchez-Lopez E, Dhar D, et al. Immunosuppressive plasma cells impede T-cell-dependent immunogenic chemotherapy. *Nature.* mai 2015;521(7550):94-8.
62. Lundgren S, Berntsson J, Nodin B, Micke P, Jirström K. Prognostic impact of tumour-associated B cells and plasma cells in epithelial ovarian cancer. *J Ovarian Res.* 6 avr 2016;9:21.
63. Mohammed ZMA, Going JJ, Edwards J, Elsberger B, Doughty JC, McMillan DC. The relationship between components of tumour inflammatory cell infiltrate and clinicopathological factors and survival in patients with primary operable invasive ductal breast cancer. *Br J Cancer.* 21 août 2012;107(5):864-73.
64. Wei H, Fu P, Yao M, Chen Y, Du L. Breast cancer stem cells phenotype and plasma cell-predominant breast cancer independently indicate poor survival. *Pathol Res Pract.* avr 2016;212(4):294-301.
65. Yang Z, Wang W, Zhao L, Wang X, Gimple RC, Xu L, et al. Plasma cells shape the mesenchymal identity of ovarian cancers through transfer of exosome-derived microRNAs. *Sci Adv.* févr 2021;7(9):eabb0737.
66. Saqi A, Yun SS, Yu GH, Alexis D, Taub RN, Powell CA, et al. Utility of CD138 (syndecan-1) in distinguishing carcinomas from mesotheliomas. *Diagn Cytopathol.* août 2005;33(2):65-70.

67. Lohr M, Edlund K, Botling J, Hammad S, Hellwig B, Othman A, et al. The prognostic relevance of tumour-infiltrating plasma cells and immunoglobulin kappa C indicates an important role of the humoral immune response in non-small cell lung cancer. *Cancer Letters*. juin 2013;333(2):222-8.
68. Schmidt M, Hellwig B, Hammad S, Othman A, Lohr M, Chen Z, et al. A Comprehensive Analysis of Human Gene Expression Profiles Identifies Stromal Immunoglobulin κ C as a Compatible Prognostic Marker in Human Solid Tumors. *Clin Cancer Res*. 1 mai 2012;18(9):2695-703.
69. McCarron MJ, Park PW, Fooksman DR. CD138 mediates selection of mature plasma cells by regulating their survival. *Blood*. 18 mai 2017;129(20):2749-59.
70. Bolotin DA, Poslavsky S, Davydov AN, Frenkel FE, Fanchi L, Zolotareva OI, et al. Antigen receptor repertoire profiling from RNA-seq data. *Nat Biotechnol*. 11 oct 2017;35(10):908-11.
71. Isaeva OI, Sharonov GV, Serebrovskaya EO, Turchaninova MA, Zaretsky AR, Shugay M, et al. Intratumoral immunoglobulin isotypes predict survival in lung adenocarcinoma subtypes. *Journal for ImmunoTherapy of Cancer*. 29 oct 2019;7(1):279.
72. Welinder C, Jirström K, Lehn S, Nodin B, Marko-Varga G, Blixt O, et al. Intra-tumour IgA1 is common in cancer and is correlated with poor prognosis in bladder cancer. *Heliyon*. août 2016;2(8):e00143.
73. Wang H, Chen D, Wang R, Quan W, Xia D, Mei J, et al. NY-ESO-1 expression in solid tumors predicts prognosis. *Medicine (Baltimore)*. 27 nov 2019;98(48):e17990.
74. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, et al. B cells and tertiary lymphoid structures promote immunotherapy response. *Nature*. 2020;577(7791):549-55.
75. Patil NS, Nabet BY, Müller S, Koeppen H, Zou W, Giltmane J, et al. Intratumoral plasma cells predict outcomes to PD-L1 blockade in non-small cell lung cancer. *Cancer Cell [Internet]*. 24 févr 2022 [cité 6 mars 2022]; Disponible sur: <https://www.sciencedirect.com/science/article/pii/S1535610822000356>
76. Hollern DP, Xu N, Thennavan A, Glodowski C, Garcia-Recio S, Mott KR, et al. B Cells and T Follicular Helper Cells Mediate Response to Checkpoint Inhibitors in High Mutation Burden Mouse Models of Breast Cancer. *Cell*. 14 nov 2019;179(5):1191-1206.e21.
77. Sánchez-Alonso S, Setti-Jerez G, Arroyo M, Hernández T, Martos MI, Sánchez-Torres JM, et al. A new role for circulating T follicular helper cells in humoral response to anti-PD-1 therapy. *J Immunother Cancer*. sept 2020;8(2):e001187.
78. Iglesia MD, Vincent BG, Parker JS, Hoadley K, Carey LA, Perou CM, et al. Prognostic B-Cell Signatures using mRNA-Seq in Patients with Subtype-Specific Breast and Ovarian Cancer. *Clin Cancer Res*. 15 juill 2014;20(14):3818-29.
79. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat Med*. août 2015;21(8):938-45.
80. Ridolfi RL, Rosen PP, Port A, Kinne D, Miké V. Medullary carcinoma of the breast. A clinicopathologic study with 10 year follow-up. *Cancer*. 1977;40(4):1365-85.

81. Kuroda H, Jamiyan T, Yamaguchi R, Kakumoto A, Abe A, Harada O, et al. Prognostic value of tumor-infiltrating B lymphocytes and plasma cells in triple-negative breast cancer. *Breast Cancer*. juill 2021;28(4):904-14.
82. Fan C, Prat A, Parker JS, Liu Y, Carey LA, Troester MA, et al. Building prognostic models for breast cancer patients using clinical variables and hundreds of gene expression signatures. *BMC Med Genomics*. 9 janv 2011;4:3.
83. Harris RJ, Cheung A, Ng JCF, Laddach R, Chenoweth AM, Crescioli S, et al. Tumor-Infiltrating B Lymphocyte Profiling Identifies IgG-Biased, Clonally Expanded Prognostic Phenotypes In Triple-Negative Breast Cancer. *Cancer Res*. 15 août 2021;81(16):4290-304.
84. Steplewski Z, Sun LK, Shearman CW, Ghrayeb J, Daddona P, Koprowski H. Biological activity of human-mouse IgG1, IgG2, IgG3, and IgG4 chimeric monoclonal antibodies with antitumor specificity. *Proc Natl Acad Sci U S A*. juill 1988;85(13):4852-6.
85. Wood WC, Kornblith PL, Quindlen EA, Pollock LA. Detection of humoral immune response to human brain tumors: specificity and reliability of microcytotoxicity assay. *Cancer*. janv 1979;43(1):86-90.
86. Gilbert AE, Karagiannis P, Dodev T, Koers A, Lacy K, Josephs DH, et al. Monitoring the systemic human memory B cell compartment of melanoma patients for anti-tumor IgG antibodies. *PLoS One*. 29 avr 2011;6(4):e19330.
87. DeFalco J, Harbell M, Manning-Bog A, Baia G, Scholz A, Millare B, et al. Non-progressing cancer patients have persistent B cell responses expressing shared antibody paratopes that target public tumor antigens. *Clin Immunol*. févr 2018;187:37-45.
88. Bendtzen K, Hansen MB, Ross C, Svenson M. High-avidity autoantibodies to cytokines. *Immunol Today*. mai 1998;19(5):209-11.
89. Lopes-Carvalho T, Kearney JF. Development and selection of marginal zone B cells. *Immunol Rev*. févr 2004;197:192-205.
90. Elkon K, Casali P. Nature and functions of autoantibodies. *Nat Clin Pract Rheumatol*. sept 2008;4(9):491-8.
91. Vollmers HP, Brandlein S. Death by stress: natural IgM-induced apoptosis. *Methods Find Exp Clin Pharmacol*. avr 2005;27(3):185-91.
92. Atif SM, Gibbings SL, Redente EF, Camp FA, Torres RM, Kedl RM, et al. Immune Surveillance by Natural IgM Is Required for Early Neoantigen Recognition and Initiation of Adaptive Immunity. *Am J Respir Cell Mol Biol*. nov 2018;59(5):580-91.
93. Díaz-Zaragoza M, Hernández-Ávila R, Viedma-Rodríguez R, Arenas-Aranda D, Ostoa-Saloma P. Natural and adaptive IgM antibodies in the recognition of tumor-associated antigens of breast cancer (Review). *Oncol Rep*. sept 2015;34(3):1106-14.
94. Musolino A, Naldi N, Bortesi B, Pezzuolo D, Capelletti M, Missale G, et al. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. *J Clin Oncol*. 10 avr 2008;26(11):1789-96.

95. Banks ND, Kinsey N, Clements J, Hildreth JEK. Sustained antibody-dependent cell-mediated cytotoxicity (ADCC) in SIV-infected macaques correlates with delayed progression to AIDS. *AIDS Res Hum Retroviruses*. 1 nov 2002;18(16):1197-205.
96. Hatjiharissi E, Xu L, Santos DD, Hunter ZR, Ciccarelli BT, Verselis S, et al. Increased natural killer cell expression of CD16, augmented binding and ADCC activity to rituximab among individuals expressing the Fc γ R11a-158 V/V and V/F polymorphism. *Blood*. 1 oct 2007;110(7):2561-4.
97. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. *Nat Med*. avr 2000;6(4):443-6.
98. Hubert P, Heitzmann A, Viel S, Nicolas A, Sastre-Garau X, Oppezio P, et al. Antibody-dependent cell cytotoxicity synapses form in mice during tumor-specific antibody immunotherapy. *Cancer Res*. 1 août 2011;71(15):5134-43.
99. Muntasell A, Cabo M, Servitja S, Tusquets I, Martínez-García M, Rovira A, et al. Interplay between Natural Killer Cells and Anti-HER2 Antibodies: Perspectives for Breast Cancer Immunotherapy. *Front Immunol*. 2017;8:1544.
100. Gül N, Babes L, Siegmund K, Korthouwer R, Bögels M, Braster R, et al. Macrophages eliminate circulating tumor cells after monoclonal antibody therapy. *J Clin Invest*. févr 2014;124(2):812-23.
101. Albanesi M, Mancardi DA, Jönsson F, Iannascoli B, Fiette L, Di Santo JP, et al. Neutrophils mediate antibody-induced antitumor effects in mice. *Blood*. 31 oct 2013;122(18):3160-8.
102. Matlung HL, Szilagyi K, Barclay NA, van den Berg TK. The CD47-SIRP α signaling axis as an innate immune checkpoint in cancer. *Immunol Rev*. mars 2017;276(1):145-64.
103. Merle NS, Noe R, Halbwachs-Mecarelli L, Fremeaux-Bacchi V, Roumenina LT. Complement System Part II: Role in Immunity. *Front Immunol*. 2015;6:257.
104. Niculescu F, Rus HG, Retegan M, Vlaicu R. Persistent complement activation on tumor cells in breast cancer. *Am J Pathol*. mai 1992;140(5):1039-43.
105. Cho MS, Vasquez HG, Rupaimoole R, Pradeep S, Wu S, Zand B, et al. Autocrine effects of tumor-derived complement. *Cell Rep*. 27 mars 2014;6(6):1085-95.
106. Roumenina LT, Daugan MV, Petitprez F, Sautès-Fridman C, Fridman WH. Context-dependent roles of complement in cancer. *Nat Rev Cancer*. déc 2019;19(12):698-715.
107. Kwak JW, Laskowski J, Li HY, McSharry MV, Sippel TR, Bullock BL, et al. Complement Activation via a C3a Receptor Pathway Alters CD4⁺ T Lymphocytes and Mediates Lung Cancer Progression. *Cancer Res*. 1 janv 2018;78(1):143-56.
108. Taylor RP, Lindorfer MA, Cook EM, Beurskens FJ, Schuurman J, Parren PWHI, et al. Hexamerization-enhanced CD20 antibody mediates complement-dependent cytotoxicity in serum genetically deficient in C9. *Clinical Immunology*. 1 août 2017;181:24-8.
109. Gaetano ND, Cittera E, Nota R, Vecchi A, Grieco V, Scanziani E, et al. Complement Activation Determines the Therapeutic Activity of Rituximab In Vivo. *The Journal of Immunology*. 1 août 2003;171(3):1581-7.

110. Liu M, Yang YJ, Zheng H, Zhong XR, Wang Y, Wang Z, et al. Membrane-bound complement regulatory proteins are prognostic factors of operable breast cancer treated with adjuvant trastuzumab: a retrospective study. *Oncol Rep.* déc 2014;32(6):2619-27.
111. Wang Y, Yang YJ, Wang Z, Liao J, Liu M, Zhong XR, et al. CD55 and CD59 expression protects HER2-overexpressing breast cancer cells from trastuzumab-induced complement-dependent cytotoxicity. *Oncol Lett.* sept 2017;14(3):2961-9.
112. Gordan S, Albert H, Danzer H, Lux A, Biburger M, Nimmerjahn F. The Immunological Organ Environment Dictates the Molecular and Cellular Pathways of Cytotoxic Antibody Activity. *Cell Rep.* 3 déc 2019;29(10):3033-3046.e4.
113. Ludvigsson JF, Neovius M, Ye W, Hammarström L. IgA deficiency and risk of cancer: a population-based matched cohort study. *J Clin Immunol.* févr 2015;35(2):182-8.
114. Woof JM, Russell MW. Structure and function relationships in IgA. *Mucosal Immunol.* nov 2011;4(6):590-7.
115. Brandtzaeg P, Kvale D, Sollid LM, Thrane PS. The Poly-Ig Receptor — Functional Aspects of Secretory Component Expression. In: Fossum S, Rolstad B, éditeurs. *Histophysiology of the Immune System: The Life History, Organization, and Interactions of Its Cell Populations* [Internet]. Boston, MA: Springer US; 1988 [cité 20 avr 2022]. p. 9-20. (Advances in Experimental Medicine and Biology). Disponible sur: https://doi.org/10.1007/978-1-4684-5535-9_2
116. Lu J, Marjon KD, Marnell LL, Wang R, Mold C, Du Clos TW, et al. Recognition and functional activation of the human IgA receptor (FcαRI) by C-reactive protein. *Proc Natl Acad Sci U S A.* 22 mars 2011;108(12):4974-9.
117. Monteiro RC, Van De Winkel JGJ. IgA Fc receptors. *Annu Rev Immunol.* 2003;21:177-204.
118. Brandsma AM, Jacobino SR, Meyer S, ten Broeke T, Leusen JHW. Fc receptor inside-out signaling and possible impact on antibody therapy. *Immunol Rev.* nov 2015;268(1):74-87.
119. Boross P, van Lent PL, Martin-Ramirez J, van der Kaa J, Mulder MHCM, Claassens JWC, et al. Destructive arthritis in the absence of both FcγRI and FcγRIII. *J Immunol.* 1 avr 2008;180(7):5083-91.
120. Borrok MJ, Luheshi NM, Beyaz N, Davies GC, Legg JW, Wu H, et al. Enhancement of antibody-dependent cell-mediated cytotoxicity by endowing IgG with FcαRI (CD89) binding. *MAbs.* 2015;7(4):743-51.
121. Lohse S, Loew S, Kretschmer A, Jansen JHM, Meyer S, Ten Broeke T, et al. Effector mechanisms of IgA antibodies against CD20 include recruitment of myeloid cells for antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity. *Br J Haematol.* mai 2018;181(3):413-7.
122. Boross P, Lohse S, Nederend M, Jansen JHM, van Tetering G, Dechant M, et al. IgA EGFR antibodies mediate tumour killing in vivo. *EMBO Mol Med.* août 2013;5(8):1213-26.
123. Treffers LW, Ten Broeke T, Rösner T, Jansen JHM, van Houdt M, Kahle S, et al. IgA-Mediated Killing of Tumor Cells by Neutrophils Is Enhanced by CD47-SIRPα Checkpoint Inhibition. *Cancer Immunol Res.* janv 2020;8(1):120-30.

124. Strunk RC, Bloomberg GR. Omalizumab for asthma. *N Engl J Med.* 22 juin 2006;354(25):2689-95.
125. Crawford G, Hayes MD, Seoane RC, Ward S, Dalessandri T, Lai C, et al. Epithelial damage and tissue $\gamma\delta$ T cells promote a unique tumor-protective IgE response. *Nat Immunol.* août 2018;19(8):859-70.
126. Josephs DH, Bax HJ, Dodev T, Georgouli M, Nakamura M, Pellizzari G, et al. Anti-Folate Receptor- α IgE but not IgG Recruits Macrophages to Attack Tumors via TNF α /MCP-1 Signaling. *Cancer Res.* 1 mars 2017;77(5):1127-41.
127. Ly D, Zhu CQ, Cabanero M, Tsao MS, Zhang L. Role for High-Affinity IgE Receptor in Prognosis of Lung Adenocarcinoma Patients. *Cancer Immunol Res.* sept 2017;5(9):821-9.
128. Amigorena S, Lankar D, Briken V, Gapin L, Viguier M, Bonnerot C. Type II and III receptors for immunoglobulin G (IgG) control the presentation of different T cell epitopes from single IgG-complexed antigens. *J Exp Med.* 16 févr 1998;187(4):505-15.
129. Baker K, Rath T, Flak MB, Arthur JC, Chen Z, Glickman JN, et al. Neonatal Fc Receptor Expression in Dendritic Cells Mediates Protective Immunity against Colorectal Cancer. *Immunity.* déc 2013;39(6):1095-107.
130. Rafiq K, Bergtold A, Clynes R. Immune complex-mediated antigen presentation induces tumor immunity. *J Clin Invest.* 1 juill 2002;110(1):71-9.
131. Platzer B, Elpek KG, Cremasco V, Baker K, Stout MM, Schultz C, et al. IgE/Fc ϵ RI-Mediated Antigen Cross-Presentation by Dendritic Cells Enhances Anti-Tumor Immune Responses. *Cell Rep.* 10 mars 2015;10(9):1487-95.
132. Otten MA, Groenveld I, van de Winkel JGJ, van Egmond M. Inefficient antigen presentation via the IgA Fc receptor (FcaRI) on dendritic cells. *Immunobiology.* 2006;211(6-8):503-10.
133. Gayet R, Michaud E, Nicoli F, Chanut B, Paul M, Rochereau N, et al. Impact of IgA isoforms on their ability to activate dendritic cells and to prime T cells. *Eur J Immunol.* sept 2020;50(9):1295-306.
134. Mandal G, Biswas S, Anadon CM, Yu X, Gatenbee CD, Prabhakaran S, et al. IgA-Dominated Humoral Immune Responses Govern Patients' Outcome in Endometrial Cancer. *Cancer Res.* 1 mars 2022;82(5):859-71.
135. Cianga P, Cianga C, Cozma L, Ward ES, Carasevici E. The MHC class I related Fc receptor, FcRn, is expressed in the epithelial cells of the human mammary gland. *Hum Immunol.* déc 2003;64(12):1152-9.
136. Karagiannis P, Gilbert AE, Josephs DH, Ali N, Dodev T, Saul L, et al. IgG4 subclass antibodies impair antitumor immunity in melanoma. *J Clin Invest.* avr 2013;123(4):1457-74.
137. Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. *Clin Exp Allergy.* avr 2009;39(4):469-77.
138. Oaks M, Taylor S, Shaffer J. Autoantibodies targeting tumor-associated antigens in metastatic cancer: Sialylated IgGs as candidate anti-inflammatory antibodies. *Oncoimmunology.* 1 juin 2013;2(6):e24841.

139. Zhang N, Deng H, Fan X, Gonzalez A, Zhang S, Brezski RJ, et al. Dysfunctional Antibodies in the Tumor Microenvironment Associate with Impaired Anticancer Immunity. *Clin Cancer Res.* 1 déc 2015;21(23):5380-90.
140. Fan X, Brezski RJ, Fa M, Deng H, Oberholtzer A, Gonzalez A, et al. A single proteolytic cleavage within the lower hinge of trastuzumab reduces immune effector function and in vivo efficacy. *Breast Cancer Res.* 8 août 2012;14(4):R116.
141. Tomaras GD, Ferrari G, Shen X, Alam SM, Liao HX, Pollara J, et al. Vaccine-induced plasma IgA specific for the C1 region of the HIV-1 envelope blocks binding and effector function of IgG. *Proc Natl Acad Sci U S A.* 28 mai 2013;110(22):9019-24.
142. Monteiro RC. Role of IgA and IgA fc receptors in inflammation. *J Clin Immunol.* janv 2010;30(1):1-9.
143. Pasquier B, Launay P, Kanamaru Y, Moura IC, Pfirsch S, Ruffié C, et al. Identification of Fc α RI as an inhibitory receptor that controls inflammation: dual role of Fc γ ITAM. *Immunity.* janv 2005;22(1):31-42.
144. Wehrli M, Cortinas-Elizondo F, Hlushchuk R, Daudel F, Villiger PM, Miescher S, et al. Human IgA Fc receptor Fc α RI (CD89) triggers different forms of neutrophil death depending on the inflammatory microenvironment. *J Immunol.* 1 déc 2014;193(11):5649-59.
145. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 4 mars 2011;144(5):646-74.
146. Dass TK, Aziz M, Rattan A, Tyagi SP. Clinical utility and monitoring of breast cancer by circulating immune complexes. *Indian J Pathol Microbiol.* oct 1992;35(4):298-307.
147. Andreu P, Johansson M, Affara NI, Pucci F, Tan T, Junankar S, et al. Fc γ activation regulates inflammation-associated squamous carcinogenesis. *Cancer Cell.* 17 févr 2010;17(2):121-34.
148. Gunn L, Ding C, Liu M, Ma Y, Qi C, Cai Y, et al. Opposing roles for complement component C5a in tumor progression and the tumor microenvironment. *J Immunol.* 15 sept 2012;189(6):2985-94.
149. Vollmers HP, Brändlein S. Natural human immunoglobulins in cancer immunotherapy. *Immunotherapy.* mars 2009;1(2):241-8.
150. Pohle T, Brändlein S, Ruoff N, Müller-Hermelink HK, Vollmers HP. Lipoptosis: tumor-specific cell death by antibody-induced intracellular lipid accumulation. *Cancer Res.* 1 juin 2004;64(11):3900-6.
151. Gazit G, Hung G, Chen X, Anderson WF, Lee AS. Use of the glucose starvation-inducible glucose-regulated protein 78 promoter in suicide gene therapy of murine fibrosarcoma. *Cancer Res.* 1 juill 1999;59(13):3100-6.
152. Dalmau J, Tüzün E, Wu H yan, Masjuan J, Rossi JE, Voloschin A, et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann Neurol.* janv 2007;61(1):25-36.

153. North WG, Liu F, Tian R, Abbasi H, Akerman B. NMDA receptors are expressed in human ovarian cancer tissues and human ovarian cancer cell lines. *Clin Pharmacol.* 23 oct 2015;7:111-7.
154. Hughes EG, Peng X, Gleichman AJ, Lai M, Zhou L, Tsou R, et al. Cellular and Synaptic Mechanisms of Anti-NMDA Receptor Encephalitis. *J Neurosci.* 28 avr 2010;30(17):5866-75.
155. Lynch DR, Rattelle A, Dong YN, Roslin K, Gleichman AJ, Panzer JA. Anti-NMDA Receptor Encephalitis: Clinical Features and Basic Mechanisms. *Adv Pharmacol.* 2018;82:235-60.
156. Lancaster E, Lai M, Peng X, Hughes E, Constantinescu R, Raizer J, et al. Antibodies to the GABA(B) receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. *Lancet Neurol.* janv 2010;9(1):67-76.
157. Zhu F, Shan W, Lv R, Li Z, Wang Q. Clinical Characteristics of Anti-GABA-B Receptor Encephalitis. *Front Neurol.* 21 mai 2020;11:403.
158. Xu W, Narayanan P, Kang N, Clayton S, Ohne Y, Shi P, et al. Human plasma cells express granzyme B. *European Journal of Immunology.* 2014;44(1):275-84.
159. Cupi ML, Sarra M, Marafini I, Monteleone I, Franzè E, Ortenzi A, et al. Plasma Cells in the Mucosa of Patients with Inflammatory Bowel Disease Produce Granzyme B and Possess Cytotoxic Activities. *Jl.* 15 juin 2014;192(12):6083-91.
160. Adrain C, Murphy BM, Martin SJ. Molecular ordering of the caspase activation cascade initiated by the cytotoxic T lymphocyte/natural killer (CTL/NK) protease granzyme B. *J Biol Chem.* 11 févr 2005;280(6):4663-73.
161. Sharif-Askari E, Alam A, Rhéaume E, Beresford PJ, Scotto C, Sharma K, et al. Direct cleavage of the human DNA fragmentation factor-45 by granzyme B induces caspase-activated DNase release and DNA fragmentation. *EMBO J.* 15 juin 2001;20(12):3101-13.
162. Neumann L, Mueller M, Moos V, Heller F, Meyer TF, Loddenkemper C, et al. Mucosal Inducible NO Synthase–Producing IgA+ Plasma Cells in Helicobacter pylori–Infected Patients. *The Journal of Immunology.* 1 sept 2016;197(5):1801-8.
163. Glynn SA, Boersma BJ, Dorsey TH, Yi M, Yfantis HG, Ridnour LA, et al. Increased NOS2 predicts poor survival in estrogen receptor–negative breast cancer patients. *J Clin Invest.* 1 nov 2010;120(11):3843-54.
164. Lahiri M, Martin JHJ. Nitric oxide decreases motility and increases adhesion in human breast cancer cells. *Oncol Rep.* févr 2009;21(2):275-81.
165. Jing L, Kim S, Sun L, Wang L, Mildner E, Divaris K, et al. IL-37- and IL-35/IL-37-Producing Plasma Cells in Chronic Periodontitis. *J Dent Res.* 1 juill 2019;98(7):813-21.
166. Liu X, Ren H, Guo H, Wang W, Zhao N. Interleukin-35 has a tumor-promoting role in hepatocellular carcinoma. *Clin Exp Immunol.* févr 2021;203(2):219-29.
167. Kurebayashi Y, Emoto K, Hayashi Y, Kamiyama I, Ohtsuka T, Asamura H, et al. Comprehensive Immune Profiling of Lung Adenocarcinomas Reveals Four Immunotypes with Plasma Cell Subtype a Negative Indicator. *Cancer Immunology Research.* 29 févr 2016;4(3):234-47.

168. Shalapour S, Lin XJ, Bastian IN, Brain J, Burt AD, Aksenov AA, et al. Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity. *Nature*. 16 nov 2017;551(7680):340-5.
169. Machado-Santos J, Saji E, Tröscher AR, Paunovic M, Liblau R, Gabriely G, et al. The compartmentalized inflammatory response in the multiple sclerosis brain is composed of tissue-resident CD8+ T lymphocytes and B cells. *Brain*. 1 juill 2018;141(7):2066-82.
170. Beissert S, Hosoi J, Grabbe S, Asahina A, Granstein RD. IL-10 inhibits tumor antigen presentation by epidermal antigen-presenting cells. *The Journal of Immunology*. 1 févr 1995;154(3):1280-6.
171. Yue FY, Dummer R, Geertsen R, Hofbauer G, Laine E, Manolio S, et al. Interleukin-10 is a growth factor for human melanoma cells and down-regulates HLA class-I, HLA class-II and ICAM-1 molecules. *International Journal of Cancer*. 1997;71(4):630-7.
172. Huang S, Ullrich SE, Bar-Eli M. Regulation of tumor growth and metastasis by interleukin-10: the melanoma experience. *J Interferon Cytokine Res*. juill 1999;19(7):697-703.
173. Cai C, Zhang J, Li M, Wu ZJ, Song KH, Zhan TW, et al. Interleukin 10-expressing B cells inhibit tumor-infiltrating T cell function and correlate with T cell Tim-3 expression in renal cell carcinoma. *Tumor Biol*. 1 juin 2016;37(6):8209-18.
174. Neves P, Lampropoulou V, Calderon-Gomez E, Roch T, Stervbo U, Shen P, et al. Signaling via the MyD88 adaptor protein in B cells suppresses protective immunity during *Salmonella typhimurium* infection. *Immunity*. 24 nov 2010;33(5):777-90.
175. Fillatreau S, Sweeney CH, McGeachy MJ, Gray D, Anderton SM. B cells regulate autoimmunity by provision of IL-10. *Nat Immunol*. oct 2002;3(10):944-50.
176. Sakaguchi A, Horimoto Y, Onagi H, Ikarashi D, Nakayama T, Nakatsura T, et al. Plasma cell infiltration and treatment effect in breast cancer patients treated with neoadjuvant chemotherapy. *Breast Cancer Research*. 29 oct 2021;23(1):99.
177. Lino AC, Dang VD, Lampropoulou V, Welle A, Joedicke J, Pohar J, et al. LAG-3 Inhibitory Receptor Expression Identifies Immunosuppressive Natural Regulatory Plasma Cells. *Immunity*. 17 juill 2018;49(1):120-133.e9.

THESIS HYPOTHESIS AND OBJECTIVES

B cells are increasingly recognized as essential actors of the tumor microenvironment with evidence that they can contribute to both anti- and pro-tumor immune responses. The tumor-infiltrating B cell contingent contains a mixture of subsets, including ASC that can produce antibodies of different isotypes and isoforms and also likely exert non-canonical functions through secretion of immune-modulatory cytokines and expression of ICP ligands. The heterogeneity of ASC in cancer, in particular in BC, and the impact of the different subpopulations on clinical outcome is however yet to be elucidated. In addition, antibody response have been described both at early (*in situ*) and late (invasive) stage of breast tumor progression although poorly characterized. Thus, we hypothesize that the ASC compartment evolves during tumor development, which may contribute to the immune escape of malignant cells and that the pro- vs anti-tumor role of ASC and produced antibodies depends on their phenotypic and functional diversity.

Besides, B cells can also functionally organize with T cells to form TLS, which are generally associated with better patient prognosis and increased responses to immunotherapies targeting inhibitory immune checkpoints. TLS develop only in a fraction of cancer patients and are sites of oligoclonal T and B cell responses. Therefore, we postulate that TLS are privileged sites for induction and/or amplification of anti-tumor immune response and may develop preferentially in the most immunogenic tumors. Yet, the nature of the antigens that drive such TLS remain poorly documented. Different classes of tumor antigens can indeed be evaluated including those resulting from mutations (neoantigens), cancer-testis antigens and HERV.

A better understanding of ASC diversity, their mechanism of action and TLS drivers could allow the development of new immunotherapeutic strategies.

In this context, my thesis project had 2 main objectives:

- i) deeply characterize the diversity of ASC infiltrating human breast tumors regarding their state of maturation and the isotypes and isoforms of produced antibodies at different stages of tumor development, identify the proteins targeted by IgG and IgA in the circulation and the tumor microenvironment and define the respective impact of IgG- vs IgA-ASC on tumor progression and clinical outcome
- ii) study the link between TLS and tumor antigenicity and the quality of the anti-tumor immune response in breast cancer.

To deeply characterize tumor ASC heterogeneity, we exploited fresh and/or fixed tumor samples from patients with an *in situ* or invasive breast carcinoma and used different complementary techniques, including multicolor flow cytometry, multiplex immunofluorescence tissue staining (multi-IF), whole peptide arrays for profiling antibody specificities and differential gene expression profiling. The respective impact of tumor infiltrating IgG vs IgA ASC on patient survival was determined using the breast cancer dataset of the TCGA as well as an in-house assembled TNBC cohort. The link between TLS, tumor antigenicity and the quality of immune response was performed with samples from the TNBC cohorts, by combining multi-IF staining to stratify patients according to their TLS density, whole exome sequencing (WES) and MHC-peptide affinity prediction algorithms to quantify the tumor mutational burden and candidate T cell neoepitopes, and RNA-seq to identify tumor-associated antigens and to assess anti-tumor immune response in TLS-enriched patients.

RESULTS

Complementary but distinct protective roles of tumor-infiltrating IgG and IgA producing cells in breast cancer

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Footnotes

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Keywords

Invasive breast cancer, ductal carcinoma in situ, antibody-secreting cells, IgG, IgA

Abbreviations

ADCC	Antibody-Dependent Cell-mediated Cytotoxicity	ICPL	Immune CheckPoint Ligand
Ag	Antigen	IF	Immunofluorescence
ASC	Antibody-secreting cells	IG	Immunoglobulin
BC	Breast Cancer	IHC	Immunohistochemistry
CTL	Cytotoxic CD8+ T cell	KM	Kaplan-Meier
DC	Dendritic Cell	NTT	Non Tumor Tissue
DCIS	Ductal Carcinoma in situ	STM	Soluble Tumor Medium
FCS	Fetal Calf Serum	TCGA	The Cancer Genome Atlas
FFPE	Formaldehyde Fixed-Paraffin Embedded	Tfh	T follicular helper cells
FMO	Fluorescence Minus One	Ti	Tumor-infiltrating
GC	Germinal Center	TLS	Tertiary lymphoid structure
HD	Healthy Donors	TMA	Tissue Micro Arrays
IBC	Invasive Breast tumors	TNBC	Triple negative breast cancer

Abstract

Antibody-secreting cells (ASC) emerge as critical actors of the tumor microenvironment of breast cancers, but their impact on patient outcome remains controversial and their diversity is still poorly understood. Here, using samples from retrospective and prospective cohorts of patients and by combining flow cytometry and multi-immunofluorescence tissue staining, we show that ASC account for about 8% of B cells infiltrating invasive breast tumors, consist of cells at different maturation stages, producing not only IgG but also IgA1 and IgA2 in monomeric and dimeric forms. We also demonstrate a drastic remodeling of the ASC compartment between *in situ* and invasive breast carcinomas, manifested by an overdominance of IgA ASC over IgG ASC in all early-stage tumors, versus only 15% of invasive tumors, and a drop of the proportion of IgA2 producing cells in advanced tumors. Ab specificity profiling using an overlapping peptide array covering the whole proteome revealed that several hundred of proteins are targeted by IgG and IgA in each patient, with an increased number of serum IgA reactivities in cancer patients compared to healthy individuals. Furthermore, the antibody response in tumor and serum are partly disconnected, notably for IgA, and the 2 isotypes appear to target largely non-overlapping sets of antigens, involved in distinct immune and molecular pathways. Finally, using a cohort of triple negative breast cancers and the TCGA database, we demonstrate that IgA-ASC and IgG ASC are both associated with better patient prognosis but typified distinct tumor immune microenvironment. Indeed, comparison of IgA-rich only tumors with their counterpart revealed increased expression of genes associated to mast cells and neutrophils in IgA-rich tumors, while IgG-rich only were enriched in genes of activated adaptive immune cells. Overall, our results extend our understanding of ASC heterogeneity in tumors, reveal a drastic change of the local antibody response during breast tumor progression and demonstrate that IgA and IgG ASC play complementary, yet distinct, positive roles on patient survival.

Introduction

Breast cancer (BC) is a highly complex disease not only due to the existence of various histological and molecular subtypes, but also because of complex immune cell networks operating inside the tumor microenvironment. BC patient prognosis strongly relies on the cellular composition, density, local structuration and function of tumor-infiltrating (Ti) immune cells (1). As in many other malignancies, a high density of cytotoxic CD8⁺ T cells and Th1 cells is associated with a favorable clinical outcome in terms of overall and disease-free patient survival (2). In contrast, the role played by B cells and the antibody response in solid cancers, and in BC in particular, remains still poorly understood. In BC, as in many other malignancies, tumor infiltration by B cells is usually predictive of a favorable clinical outcome (3–6), especially when they functionally organized with T cells in so-called tertiary lymphoid structures (TLS) (7–9). Such TLS were also recently associated with an increased response rate to immunotherapies targeting inhibitory immune checkpoints (10–12). Breast tumor-infiltrating (Ti) B cells has been shown to mainly consist of naïve and memory B cells, and to a much lesser extent of antibody-secreting cells (ASC) (13,14).

The role played by ASC in solid cancers, and in BC in particular, remains controversial and still poorly understood. Several studies reported that an increased ASC density/gene signature score was either neutral for prognosis or was associated with reduced patient survival (3,15,16), while other reports documented an exact opposite positive clinical impact (17–20). Besides differences in the methodologies of types of patient cohorts, these discrepant results may be linked to the heterogeneity of the Ti-ASC contingent with existence of phenotypically and functionally distinct subsets. It is indeed likely that the class and subclass of antibodies produced by ASC as well as the identity of the targeted antigens (Ag) critically condition their local functions. Both IgG and IgA recognizing tumor antigens were reported in breast tumors (21). Antibody (Ab) isotypes exert different functions depending on the affinity of their Fc region for receptors expressed on accessory cells and for components of the complement system. These strongly condition their capacity to i) kill tumor cells (22) ii) sensitize malignant cells to the cytotoxic action of CD8⁺ T cells (CTL) (23,24), iii) promote dendritic (DC) mediated Ag cross-presentation to CD8⁺ T cells (25), iv) activate or suppress Ag presenting cells (Pasquier et al.2005) and v) trigger local tumor-promoting inflammation (26). Besides these canonical functions, ASC are also an important source of cytokines and can express inhibitory immune checkpoint ligand (ICPL). In this respect, Shalpour et al. have recently identified a suppressive role of IgA ASC on CD8⁺ T cell-mediated anti-tumor immunity that relies on expression of PDL1 and production of IL10 (27).

There is thus increasing evidence that ASC are critical actors of the tumor immune microenvironment but the cellular and molecular basis of their pro- versus anti-tumoral roles remains incompletely understood. The present study was aimed to disentangle the heterogeneity of the ASC compartment in breast tumors and its evolution during cancer progression, the diversity of locally produced antibodies, and the respective impact of IgA vs IgG ASC on patient outcome .

Material and methods

Patient cohorts

For the prospective cohort of patients used for flow cytometry, IHC/multi-IF tissue immunofluorescence and Ig titration, fresh and fixed tissue specimen were collected from 92 primary breast cancer patients diagnosed and treated at the Centre Léon Bérard (CLB) between 2012 and 2018. Inclusion criteria included a size estimated at diagnosis greater than 2.5 cm and an SBR grade of I-III and included all histological subtypes. The characteristics of patients are provided in supplementary table S1. All samples were prepared from surgical pieces by a pathologist and provided by the CLB tissue bank within the frame of a protocol approved by the Institutional review board and ethics committee (L-06-36 and L-11-26) and following patients' written informed consent, in accordance with the Declaration of Helsinki. A series of 15 ductal in situ carcinomas was also used for immunofluorescence tumor tissue staining and their characteristics are described in supplementary table S2.

Two retrospective cohorts of patients were also used. The PACS08 trial (28) included, between October 2007 and September 2010, 592 patients with triple negative (TNBC, ER-PR-HER2-) breast cancer and 170 patients with luminal (ER+PR-HER2-) tumors and aimed to compare two different adjuvant chemotherapies but did not reveal major differences between the two arms of the study. All tumors were thus considered equally and the impact of IgG and IgA ASC on patient survival was determined by IHC staining of formalin-fixed paraffin-embedded (FFPE) Tissue MicroArrays. An additional cohort of patients was specifically assembled for the present study and exclusively included untreated TNBC patients, diagnosed at the CLB between 2005 and 2013, with a tumor size >10mm and with available FFPE surgical tumor tissue (Supplementary fig. 7).

Preparation of tumor cell suspensions and supernatants

Tumor pieces were first mechanically dissociated into small fragments, with 1 mL of RPMI 1640 supplemented with antibiotics per 500 mg of tumor, and tissue supernatant was collected, clarified by centrifugation and 0.22 µm filtered to obtain Soluble Tumor Medium (STM) containing the soluble factors of the tumor microenvironment. Tumor fragments were then incubated for 45 minutes at 37°C

with an enzymatic cocktail of collagenase IA (1 µg/mL, Sigma) or collagenase IV (6.9mg/mL, Gibco) and DNase I (250 µg/mL, Sigma) in medium with antibiotics under gentle magnetic shaking. Digested samples were filtered using a 70 µm cell-strainer, washed and resuspended in RPMI 1640 medium supplemented with 10% Fetal Calf Serum (FCS) until use for further analysis.

Flow Cytometry

Single cell suspensions from primary breast tumors were incubated with various combinations of fluorescently labeled Ab for 30 minutes at 4°C in PBS 10% FCS 0.5 mmol/L EDTA. Cells were then fixed in PBS 4% formalin for 20 minutes at 4°C until analysis. In addition to cell surface staining, an intracellular staining was performed for Foxp3, Ki67, IgG, IgA and IgM using a fixation/permeabilization kit (ThermoFisher, Saint Aubin, France) right before analysis on the flow cytometer. Antibodies and viability dyes are listed in supplementary table S3. Appropriate fluorescence minus one (FMO) controls were used in each experiment. Analysis of stained cells was performed using an LSR Fortessa cell analyzer (BD Biosciences) and data were processed using the FlowJo Software (Tree Star).

IF and IHC staining of FFPE sections

Four-colors IF staining. After deparaffinization and antigen retrieval, 4 µm-thick FFPE tissue sections were incubated with various combinations of up to 3 primary antibodies (Supplementary table S4) for 2 hours at RT, washed and then incubated with Alexa-Fluor (-488, -555 and -647, ThermoFischer Scientific, Saint Aubin, France) coupled secondary Abs specific for primary Ab species and/or subclasses. DAPI was then added to stain nuclei and slides were mounted with Prolong Gold Medium (ThermoFischer Scientific). Multi-IF staining was performed manually except for pan-CK/IgG/IgA and CD3/CD20/PNA_d, which were performed on the Discovery XT autostainer (Roche Ventana, Meylan, France). Stained slides were scanned with the Panoramic Scan II device (3D Histech, Sysmex, Villepinte, France).

Automated seven-colors multiplex IF staining. After deparaffinization, rehydration and antigen retrieval, FFPE tumor sections were sequentially stained with each primary antibody (Supplementary Table S4), followed by OPAL-HRP secondary antibody incubation and then revealed with tyramide signal amplification and OPAL fluorophores (Akoya Biosciences) and the same cycle was reproduced until staining with the last Ab of the panel. The sections were then counterstained with spectral DAPI (Akoya Biosciences) and mounted with a coverslip. Whole slides were imaged at a 20x magnification using the Vectra Polaris multispectral scanner (Akoya Biosciences) and digital images were visualized with the Phenochart viewer (Akoya Biosciences) and unmixed using the spectral library from the software.

Analysis of multi-IF digital images. The tumor area was first annotated on an HPS colored section of each tumor by a trained pathologist and annotations were transferred to multi-IF digital images for immune cell quantification. IgA and IgG ASC were quantified in the stroma versus the tumor area of 30 patients using the Halo software (Indica Labs) using the CK/IgG/IgA staining. More than 10 square regions of 1 mm², representing about one-tenth of the entire tissue section, were defined and tissue segmentation was performed according to CK staining (Supplementary Fig.2A). A machine learning algorithm was then set up to exclude artifacts and necrosis or fibrous areas, and to identify IgG and IgA ASC based on a strong intracytoplasmic staining. Cells positive for pan-CK or displaying a membrane IgG or IgA staining were not counted. The density of each cell phenotype was then calculated and expressed as the number of cells per mm² of stroma or tumor tissue. For the TNNC cohort (n=120), a manual semi-quantitative scoring of IgG and IgA-ASC was performed independently by 3 persons using a 4-point scale allowing to categorize samples as IgA/GHi or IgA/GLo. For panels involving IgA subclasses, Ig-J chain and PC transcription factors, a manual quantification was performed for IgA1, IgA2, IgA+IgJ+, IgA+IgJ-, Blimp1+ and/or IRF4+ cells using representative regions.

TLS were identified as structures harboring dense and segregated B and T cell areas. To quantify them, we first manually annotated B cell follicles, defined as dense CD20+ cell aggregates with few T cells, and quantify their surface using Halo. TLS-B follicle density was then calculated as the % of the total tumor surface occupied by all annotated B cell follicles (e.g. Surface TLS-B Foll / Total Surface x 100).

IHC. Single IHC staining of 4 µm thick plain or TMA (PACS08 cohort) tumor sections was performed with Ab (Sup. Table 1) to IgA, IgG, and CD20 (DAKO) using a Discovery XT autostainer (Roche Ventana). Sections were deparaffinized, heated in an antigen retrieval solution, incubated with the primary antibody for 30 to 60 minutes, and were then revealed with a biotinylated secondary Ab bound to streptavidin peroxidase conjugate (Ultratech HRP DAB kit, Coulter Immunotech) followed by DAB substrate. Sections were then counterstained with hematoxylin and mounted. For TMA, a trained pathologist manually counted the number of positive cells per spot and the mean value of the three independent spots per patient was calculated.

Quantification and analysis of immunoglobulins

ELISA and multiplex Ab quantification. Ab classes were quantified in STM from breast tumors with IgA, IgG, and IgM ELISA kits from Invitrogen (ThermoFischer Scientific) according to the manufacturer's instructions. The four subclasses of IgG were measured in the same fluids with the Bio-Plex Pro™ Human Isotyping Panel (Bio-Rad).

Western Blot. Soluble tumor milieu, previously titrated by ELISA for their IgA content, were loaded at an equivalent IgA quantity on Mini-PROTEAN TGX Precast 4%–15% Gels (Bio-Rad) and transferred after migration to a Trans-Blot Turbo Mini PVDF membrane (Bio-Rad). IgA were detected using a goat anti-human IgA (1/2 000, Southern Biotech). Serum IgA and colostrum IgA (Sigma-Aldrich, l'Isle d'Abeau, France), and recombinant dimeric IgA (a kind gift of S. Paul, IMAP Saint Etienne) were used as positive controls. Membranes were then incubated with a rabbit anti-goat antibody for IgA (1/5000, DAKO) followed by Luminata™ Crescendo Reagent (Millipore), and revealed on the chemidoc-MP.

Fluorescent ELISPOT. IgG, IgA, and IgM producing cells were quantified with a three-color fluorospot kit (Immunospot©, Cellular Technology Limited, Cleveland, USA). Serial 2-fold dilutions of cells (starting at 4.10⁵ cells per well) prepared from breast tumors were incubated in already coated ELISPOT plates overnight without any stimulus. The remaining steps were performed according to the manufacturer's instructions and spots were counted using the ImmunoSpot® S6 ULTRA-V.

Antibody profiling by peptide arrays

IgG and IgA antibodies present in STM (n=72) and matched patient serum (n=53) and in a series of sera from healthy donors (n=23) were profiled for their antigen specificity in an unbiased way using overlapping peptide arrays. The 1st step was performed only on STM and involved a whole-proteome peptide arrays that was designed based on 20,246 protein sequences obtained from the Universal Protein Resource (UniProt) downloaded on April 12, 2013. The sequences were tiled as overlapping 16-mer linear peptides at a 2 amino acid tiling interval and then randomly distributed on the array leading to >5.5 million peptides. Samples were diluted 1:100 in binding buffer (0.01M Tris-Cl, pH 7.4, 1% alkali-soluble casein (Novagen, EMD chemicals, San Diego, CA), 0.05% Tween-20) and bound to arrays overnight at 4 °C. After sample binding, the arrays were washed 3× in wash buffer (1× TBS, 0.05% Tween-20), 10 min per wash. IgG, IgM and IgA detection was performed by incubation for 3h at RT with Alexa Fluor® 647-conjugated goat anti-human IgG secondary antibody (Jackson ImmunoResearch), Cy3-conjugated goat anti-human IgM secondary antibody (Jackson ImmunoResearch), and goat anti-IgA secondary antibody. After washing and drying, fluorescent signal of the secondary antibody was detected by scanning at 635 nm at 2 μm resolution and 15% PMT gain,

using an MS200 microarray scanner (Roche NimbleGen). All analyses were performed in the R statistical programming environment (www.cran.r-project.org) using an R package “RNGdat”. Briefly, raw array signal intensities were spatially corrected via a 2-dimensional loess smoother (15) and background was corrected by deconvolution (16). A 1-sided Kolmogorov-Smirnov test was used to assess whether the signal within an 8-mer sliding window centered on a specific probe was above the sampled background. For each sample, any signal above the means of all peptide signals + 3 SD was considered significant and the peptide probe was highlighted as Ab reactive. An epitope was defined as ≥ 2 contiguous probes with significant reactivity. For the 2nd step, a focused peptide arrays was designed by aggregating all significant epitopes found during the whole proteome analysis and tiled up- and downstream 10 amino acids with 16-mer linear peptides at a 2 amino acid tiling interval. This array was used to profile all STM and serum samples.

Gene expression analysis in the TCGA

Patient data from the TCGA BRCA project was obtained from the GDC portal repository (<https://portal.gdc.cancer.gov/>). We downloaded the HTSeq-FPKM files and transformed the transcript-level data into gene level data by summarizing alternative transcripts. FPKM were then transformed to TPM. Samples from FFPE tumor tissue, normal tissue or metastatic lesions, as well as entities with warnings were removed. One sample for each patient was selected in accordance with GDC recommendations (<https://confluence.broadinstitute.org/display/GDAC/FAQ#FAQ>). After such filtration, a cohort of 938 patients was formed. IGHA and IGHG expressions were calculated as a sum of expression values for the IGHA1 and IGHA2 genes, and for IGHG1, IGHG2, IGHG3, and IGHG4 genes, respectively. Kaplan-Meier (KM) plotter tool was utilized to generate survival plots. The statistical comparison of the survival curves was done using the log-rank test. Multivariable analysis was performed with Cox proportional hazard regression. The R package MCP-counter was applied to produce the absolute abundance scores of major immune cells (B_lineage, NK, monocytic_lineage, mDC, CD8_T and neutrophil) and of a published TLS gene signature (Cabrita et al.2020). We also constructed signatures for Plasma cells (HSP90B1, IGJ, MZB1, PRDM1, PRDX4, SSR4, TNFRSF17, XBP1, IGF1) and mast cells (MS4A2, GATA2) using hallmark genes, that we validated on publicly available RNAseq and single-cell datasets (Zilionis et al.2019). Forest plots were obtained using R packages and represent for the univariate and multivariate analysis the hazard ratios and their 95% CI. Boxes represent hazard ratios and are inversely proportional to the width of the CI, horizontal lines are 95% CI.

Nanostring nCounter mRNA analysis

Based on digital images of IgG-IgA-Ck-DAPI multi-IF staining of the TNBC cohort (n=120), 6 IgAHi-IgGLo and 6 TNBC samples were selected and RNA was extracted from 2-10 5µm-thick FFPE slides using the Qiagen 100% RNeasy FFPE kit according to the manufacturer's protocol. We then used the PanCancer immune Profiling kit from Nanostring technologies. to simultaneously quantify 770 immune-related genes. Briefly, 100 ng of purified RNA was added to 3µL of reporter CodeeSet and 2µL Capture ProbeSet, as recommended (Nanonstring technologies) and samples were processed on an nCounter platform (BioGenet, Lyon Sud, Lyon, France). Data were normalized using housekeeping genes (full gene list and controls available on manufacturer's website). Differentially expressed genes between IgA-dominant tumors and IgG-dominant tumors were identified using the DESeq2 package in R.

Results

Invasive breast tumors are infiltrated by B cells at different stages of differentiation including IgG and IgA producing plasmablasts and plasma cells

Flow cytometry analysis of cells prepared from freshly resected invasive breast tumors (n=42) by mechanical dissociation and enzymatic digestion revealed that B cells were detected in all patients, albeit at highly variable frequencies among CD45+ cells, with a median value of 8% (Fig 1A, Supplementary data Fig.1A). On the basis of surface expression of CD20, CD38, IgD and IgM, three major B cell subpopulations were identified among CD19+ B cells: memory B cells (CD20+CD38-IgD-), naive B cells (CD20+CD38-IgD+IgM+) and antibody-secreting cells (ASC) (CD20-CD38hi) (Fig.1B), which accounted on average for about 6.2%, 2.7% and 0.9 % of the total immune infiltrate, respectively (Fig.1C). As expected, the vast majority of ASC expressed high level of CD27 (Fig.1B). A minor population of CD20posCD38int cells was also detected in some tumors and consisted of germinal center (GC)-like B cells (IgD-IgM-) and IgD+IgM+ cells, most likely corresponding to pre-GC B cells and/or transitional (Ti) B cells (Fig.1C, Supplementary data Fig.1B). As terminally differentiated ASC can downregulate CD19 expression, we also analyzed CD38Hi cells among total CD45+ cells. Both CD19+ and CD19- CD27+CD38Hi ASC were detected in most tumors and their density was significantly correlated ($r=0,68$, $p < 0.0001$), CD19+ ASC being usually the dominant population. Although CD138 is affected by the enzymes used for cell preparation (data not shown), this ASC marker was still detected on 30-40% of CD27+CD38Hi cells. Analysis of Ki67 expression revealed that roughly half of the ASC expressed Ki67 on average, indicating that they still proliferate and thus correspond to plasmablasts (PB) (Supplementary data Fig.1C).

Analysis of IgG, IgA and IgM expression revealed that in most patients the tumor ASC compartment consisted of a majority of IgG- and IgA-producing cells but few IgM-producing cells (Fig.1E, Supplementary data Fig. 1F), with an overdominance of IgG ASC except in 15% of patients, where IgA ASC outnumbered IgG ASC (Fig.1F-G, Supplementary data Fig. 1D) Intriguingly, isotype repartition was different in the ASC and the memory B cell compartments of the same patient (Fig.1F). A 3-colors Ig fluorescent ELISPOT confirmed tumor infiltration by ASC producing IgG and to a lesser extend IgA (Fig.1F). ELISA quantification of the different Ig isotypes in tumor supernatants obtained during mechanical tumor dissociation revealed higher concentrations of IgG followed by IgA and IgM (Supplementary data Fig. 1E), confirming local production of Ab.

Collectively, these findings demonstrate breast tumor infiltration by B cells at different stages of differentiation including a heterogeneous ASC population consisting of both PB and PC producing mainly IgG and IgA antibodies.

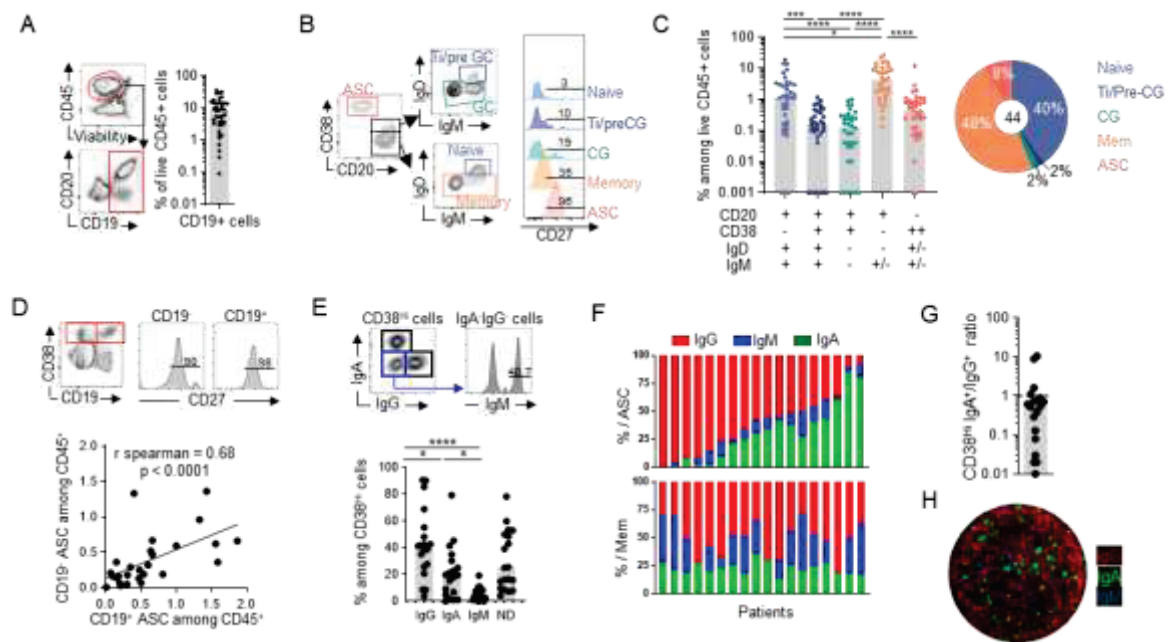


Figure 1: Invasive breast tumors are infiltrated by a heterogeneous population of antibody-secreting cells. **A** Illustration of FCM gating strategy of cell suspensions obtained with enzymatic digestion of fresh human invasive breast tumors to identify CD19+B cells among viable CD45+ cells (Left). Graph representing CD19+B cells frequency among CD45+ immune infiltrate (n=42). Due to logarithmic scale, null values were arbitrary replaced by 0.01 (Right). **B** Illustration of FCM gating strategy allowing to distinguish the different B cell maturation stages among viable CD45+CD19+ cell, e.g. Naïve B cells (CD38-IgD+IgM+), GC B cells (CD38+IgD-), memory B cells (CD38-IgD-), ASC (CD38HiCD20-) (Left). Illustration of histograms depicting CD27 expression by different B cells populations (Right). **C** Percentage among viable CD45+ cells of each B cell subset in all analyzed invasive breast tumors. Due to logarithmic scale, null values were arbitrary replaced by 0.001 (Left). Pie chart depicting the median of percentage of each B cell subset that infiltrate tumors (Right). **D** Illustration of FCM gating strategy to identify CD19+ and CD19- ASC (Left). Illustration of histograms depicting CD27 expression on CD19+ and CD19- ASC. **E** Illustration of FCM gating strategy to analyze expression of IgG and IgA on CD38Hi ASC (Top left). Illustration of histogram depicting IgM on IgG-IgA- CD38Hi ASC (Top right). Graph representing the percentage among CD38Hi cells of each subtype of ASC on tumors with functional IgG staining. **ND**: Non determined gated as IgG-IgA-IgM- (n=19) (Bottom). **F** Bar graphs representing the percentage of each isotype produced by ASC (top) and memory B cells (bottom) in the same tumors. **G** IgA/IgG CD38Hi cells ratios calculated using the percentages of each subset determined by FCM. Due to logarithmic scale, null values were arbitrary replaced by 0.01. **H** Representative image of a fluorescent ELISPOT assay performed with cells isolated from breast tumors and allowing to visualize IgG (Red), IgA (Green), and IgM (Blue)-secreting cells as spot-forming cells.

IgG and IgA ASC preferentially localize in the tumor stroma and are increased in TLS-containing tumors

To get insight into the density and location of ASC in tumors, we performed multi-IF staining of tumor tissue sections (Fig. 2A-C). IgG and IgA co-staining with IRF4 and Blimp1, the 2 master transcription factors of ASCs, showed that almost all IgHi cells expressed IRF4 and/or Blimp1 with little if any double negative cells, indicating that they correspond to tumor infiltrating ASC. Interestingly, 89.5% of IgG ASCs and 77.7% of IgA ASCs express IRF4 with no detectable levels of Blimp1, suggesting

they might be at an early stage of maturation (Fig.2A). Contrary to healthy breast tissues that are exclusively infiltrated by IgA and few IgM ASC (Fig.2B), invasive tumors exhibited a majority of IgG and IgA ASCs, as well as very few IgM-secreting cells (Fig.2D, Supplementary Fig.2B). Segmentation of the tumor vs stromal areas using the CK tumor marker and cell phenotyping (Supplementary Fig.2A) revealed that these ASCs were preferentially located in the tumor stroma, but some also infiltrate tumor islets and are found in close contact with tumor cells (Fig.2D). Calculation of the IgA to IgG cell density ratio revealed a dominance of IgG ASC in all but 3 tumors (20%), which on the opposite were IgA ASC high (Fig.2E). It is worth noting that a diffuse IgG and/or IgA positive staining was also detected in a fraction of CK+ cells (Supplementary Fig.2C), suggesting the existence of Ab deposits in tumor cells.

To determine whether ASC infiltration varies according to the tumor subtypes, we used samples from the UNICANCER-PACS08 multi-centric phase-III trial that included 308 triple negative (TN) and 95 luminal breast tumors. IHC staining of Tissue Micro Arrays (TMA) for CD20, IgG and IgA (Supplementary data Fig.2D) revealed heterogeneous infiltration by B cells, IgG-ASC and IgA-ASC and statically significant higher densities of ASC in TN tumors compared to their luminal counterpart (Supplementary data Fig.2E). We then focused on TN tumors to study the link between ASC infiltration and lymphocyte organization in TLS by multi-IF staining of whole tissue sections for IgG-IgA-CK and CD3-CD20-HEV. Analysis of a retrospective cohort of 120 patients revealed that >80% of patients had at least one TLS-B cell follicle and that a third of those harbored TLS with a characteristic morphology of germinal center (GC) (Supplementary data Fig. 2G). Importantly, a statistically significant association is observed between the density of TLS, as measured by the % of the tumor area occupied by B cell follicles, and that of IgG ($p=0.0062$) and IgA ($p < 0.0001$) ASC (Fig.2G, Fig.2H). Computational analysis of breast tumor samples from the TCGA databases revealed a significant positive correlation between IGHG of IGHA mRNA levels and the expression score of a commonly-used TLS gene signature (Fig. 2I), further supporting the link between presence of TLS and ASC infiltration.

To further explore the link between presence of TLS and ASC infiltration, we examined T follicular helper cells (Tfh) in our tumor samples by flow cytometry. Tfh, identified as CD4+CXCR5+ T cells, accounted on average for about 30% of CD4+ T cells (range 7.9%-52.5%), consisted mostly of CXCR3+CCR6- Tfh1-like cells (range 68.7%-97.8%) and contained a minor fraction of PD1HiICOS+ cells, a phenotype usually ascribed to GC-Tfh (Supplementary data Fig. 2I). Importantly, Tfh frequency positively correlated with the density of TLS determined by IF staining and both metrics also correlated with the density of ASC (Fig.2J). Overall, these data highlight a close association between the presence of TLS and the density of tumor-infiltrating ASC.

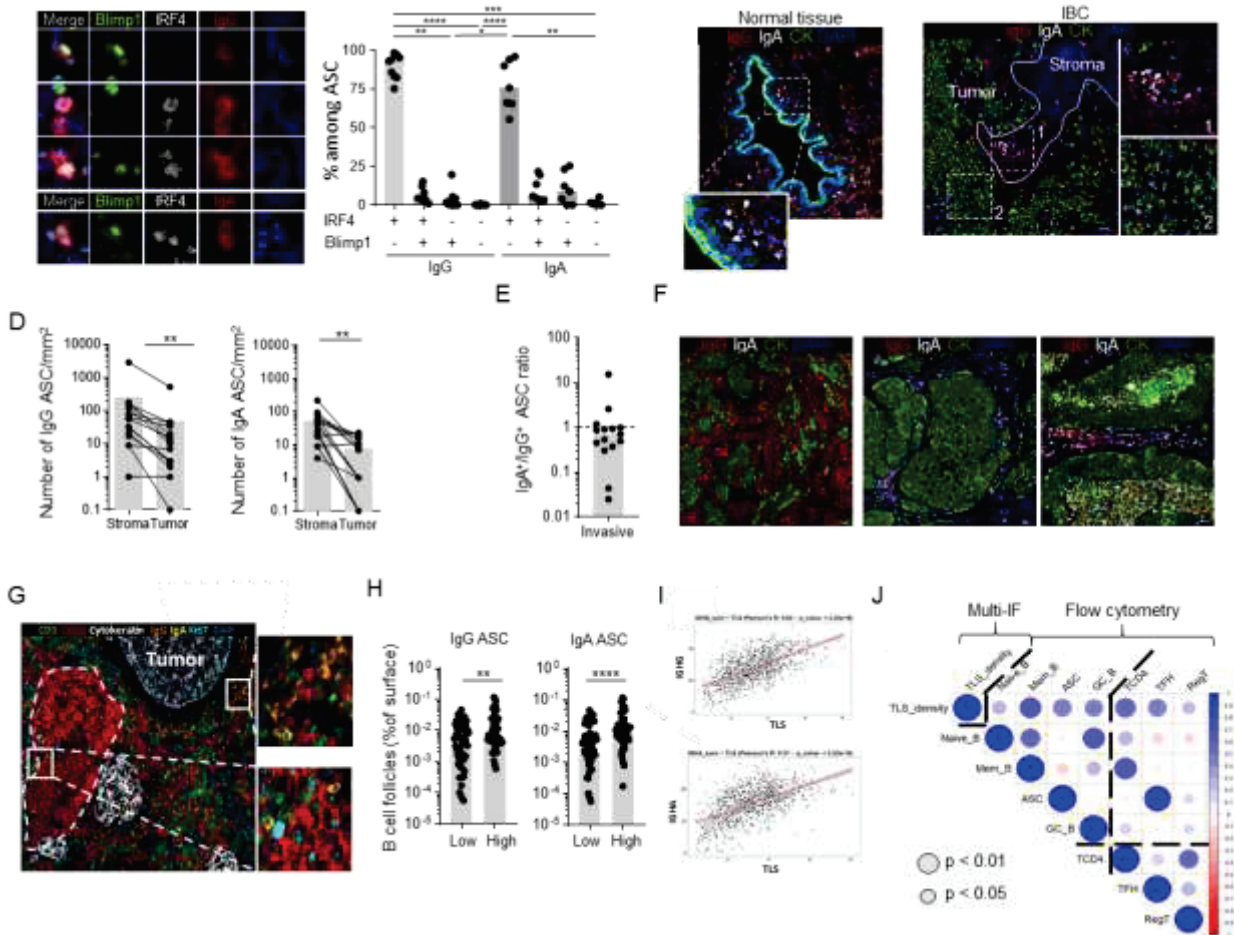


Figure 2: IgG- and IgA-ASC infiltrate mainly the stroma and correlate with TLS density. **A** Representative images of IRF4/Blimp1/IgG or IgA staining on FFPE tissue sections showing infiltration of breast tumors by cells with typical morphology of ASC expressing IRF4 and/or Blimp1 (Left). Percentage among IgG- and IgA-ASC of IRF4+Blimp1-, IRF4+Blimp1+, IRF4-Blimp1- and IRF4-Blimp1- expressing cells (n=7) (Right). **B** Illustration of a pan-CK/IgG/IgA IF staining of a tumor-distant normal mammary tissue area showing IgA ASC beneath the ductal epithelium. **C** Representative images of pan-CK/IgG/IgA IF staining on FFPE tissue section showing breast tumor infiltration by IgG- and IgA-ASC in the stroma (square1) and in close contact with tumor cells (square2). **D** Graphs depicting the density of IgG-ASC (left) and IgA-ASC (right) in tumor VS stroma areas in invasive breast tumors (n=15) using the Halo software. Due to logarithmic scale, null values were arbitrary replaced by 0.1. **E** Graph depicting IgA-ASC/IgG-ASC ratios determined by quantification of IgG-ASC and IgA-ASC cells on FFPE sections of invasive ductal carcinomas (n=15) using the Halo software (Left). Due to logarithmic scale, null values were arbitrary replaced by 0.01. **F** Representative images of pan-CK/IgG/IgA representing IgG-ASC dominant (left), IgA-ASC dominant (center) and mixed IgG and IgA-ASC tumors (right). **G** Representative multiplexed immunofluorescent staining of TLS, IgG- and IgA-ASC on FFPE sections of invasive breast tumors. Tumor-infiltrating lymphocytes were revealed by CD20 (Red), CD3 (Green), IgA ASC (Yellow) and IgG ASC (Orange). Tumor cells were revealed by panCK (White). **H** Graphs depicting the density of IgG- (left) and IgA-ASC (right) determined semi-quantitatively in B-cell follicles high VS low densities determined by manual annotation on Halo software. Due to logarithmic scale, null values were arbitrary replaced by 0.0001. **I** Correlation graph between TLS gene signature score and IGHG (left) and IGH A (right) on breast cancer cohort of TCGA database. **J** Correlation plot using bubble representation depicting correlations between TLS density determined by IF and T and B cell subsets determined by flow cytometry for the same patients (n=24).

ASCs from in situ carcinomas strikingly differ from those of invasive carcinomas by over-representation of IgA-producing cells and higher proportion of IgA2

To get insight into the evolution of the ASC compartment during tumor development, we compared non-tumor tissue (NTT), ductal carcinoma in situ (DCIS) and invasive breast tumors (IBC) for IgG- and IgA- ASC densities and IgA isoforms and subclasses expression using multi-IF tissue staining. IgG- and IgA- ASC were detected in most DCIS samples, exclusively localized within immune cell aggregates surrounding tumor ducts, and their density was higher in high grade compared to low grade DCIS (Fig.3A). The stage of maturation of ASC, as can be judged by IRF4/Blimp1 expression, appeared similar between DCIS and IBC, while a higher proportion of Blimp1+ ASC was seen in NTT, reflecting increased infiltration by mature plasma cells (Supplementary data Fig.3A & 3B). Interestingly, in all DCIS samples, IgA ASC by far outnumbered IgG ASC (IgA/IgG ratio range from 1.6 to 21.8 with a mean of 9.7) highly dominant profile (Fig. 3B), a profile that is only occasionally observed in IBC, suggesting a potential differential role of IgG and IgA ASCs during breast cancer progression.

Because IgA functions strikingly differ between IgA subclasses and isoforms, due to their capacity to engage specific and accessory receptors with differential affinities, we quantified IgA1 and IgA2 ASC as well as IgA ASC expressing the IgJ chain that is necessary for the assembly of dimeric IgA. Multi-IF staining showed that while IgA ASC in NTT and DCIS consisted of IgA1 and IgA2 producing cells in virtually equal proportions, IgA1 ASC dominated in invasive breast tumors (Fig. 3C). This finding in IBC was confirmed by flow cytometry analysis of fresh tumor cell suspension (Fig. 3D) and similar results were found in memory B cell compartment (Supplementary data Fig.3C). Consistently, analysis of the TCGA dataset showed a higher expression of IGHA1 than IGHA2 in breast tumors (Supplementary data Fig. 3D). We then assessed expression of the IgJ chain in IgA ASC by IF tissue staining as an indicator of their capacity to produce dIgA. Whereas >85% of IgA ASC expressed IgJ in NTT on average, this proportion dropped to roughly 30% in DCIS and IBC (Fig. 3E) revealing a drastic evolution of the IgA ASC compartment and suggesting production of both mIgA and dIgA in the cancerous tissue with a dominance of the former isoform. Western blot analysis of the molecular weight of IgA indeed revealed the presence of both mIgA (150kd) and dIgA (300kd), but no secretory IgA, in the tumor of most patients. dIgA accounted for 10 to 55% of total IgA, proportions that are usually higher than in matched patient serum samples (Fig. 3F).

Altogether, these data show that the ASC compartment significantly differs between DCIS and IBC with an inversion of the IgA to IgG ratio and a decrease of IgA2 producing cells among IgA ASC in advanced tumors.

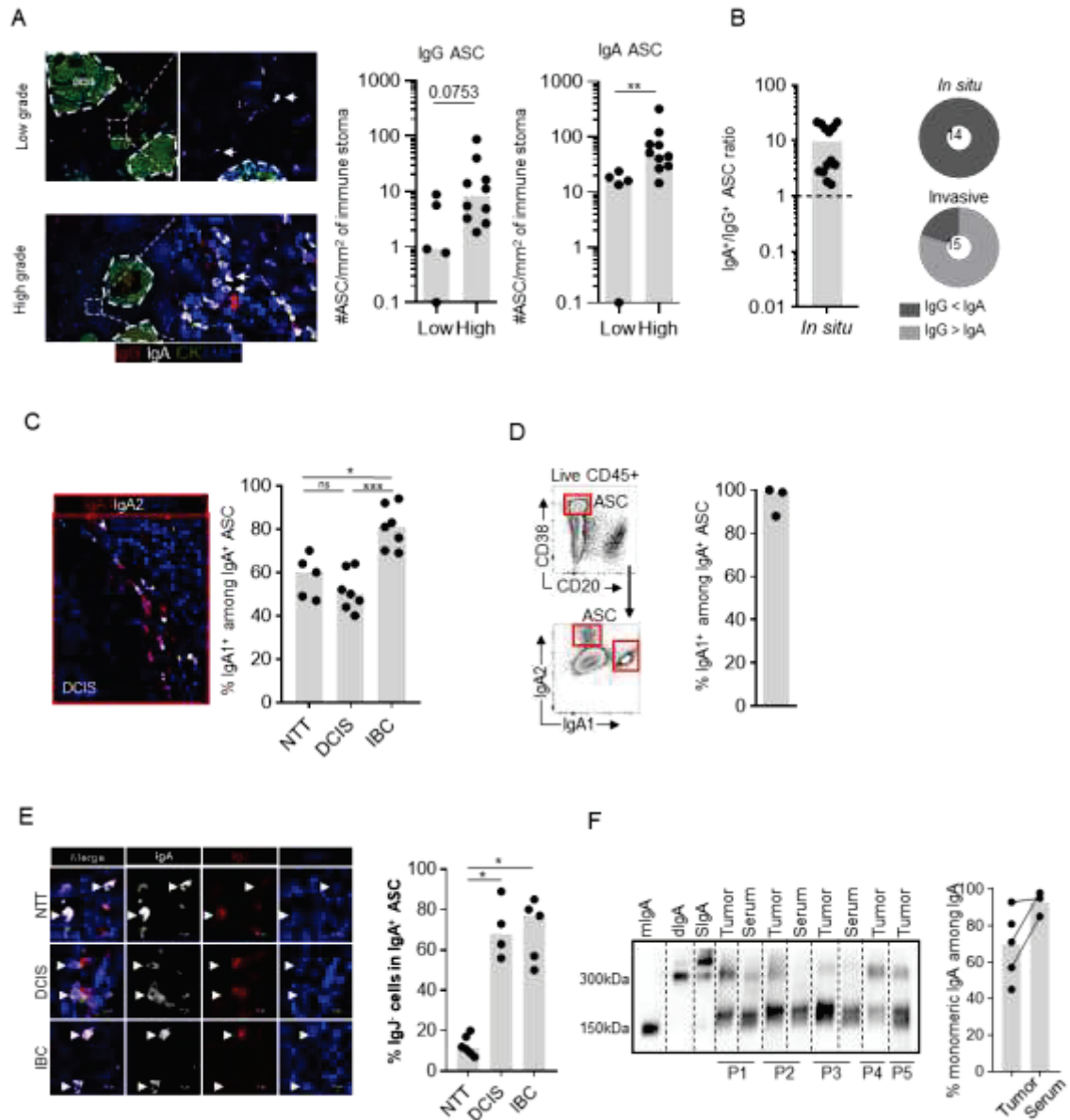


Figure 3: Ductal carcinoma in situ displayed a dominance of IgA-ASC dominance over IgG-ASC compared to invasive breast tumors. **A** Representative illustration of pan-CK/IgG/IgA staining of low-grade VS high-grade DCIS showing IgA (white arrows) and IgG (red arrows) ASC located exclusively in the peri-tumor stroma next to tumor cells (left). Graphs depicting the number of IgG- and IgA-ASC per mm² of tissue in low (n=5) VS high (n=10)-grade DCIS. Due to logarithmic scale, null values were arbitrary replaced by 0.1 (right). **B** Graph representing IgA-ASC/ IgG-ASC ratios in DCIS samples (n=14). Due to logarithmic scale, null values were arbitrary replaced by 0.1 (left). Pie charts showing IgA-ASC dominant tumors over IgG-ASC (dark gray) in DCIS and IBC (right). **C** Representative image of IgA1/IgA2 immunofluorescence staining (left). Graph representing the percentages of IgA1⁺ ASC among IgA-ASC in non-tumor tissue (n=4), DCIS (n=7) and IBC (n=7). **D** Illustration of the strategy to analyze by flow cytometry IgA2 and IgA2-ASC on viable CD45⁺CD38⁺ cells (left). Bar plot depicting the percentages of IgA1-ASC among IgA-ASC in IBC patients (n=3) (right). **E** Representatives images of IgA/IgJ staining in NTT, DCIS and IBC (left). Graph depicting the percentages of IgJ⁺ cells among IgA-ASC in NTT (n=5), DCIS (n=4) and IBC (n=5). **F** Western-blot analysis of patient's tumor supernatants (n=5) and matched sera (n=3) to distinguish monomeric (150kDa) from dimeric IgA (300kDa) based on their molecular weight (left). Graph representing the percentages of monomeric IgA among IgA in tumor supernatants and sera. NTT= Non-tumor tissue. DCIS = Ductal carcinoma in situ. IBC = Invasive breast carcinoma.

IgG and IgA target mostly different proteins involved in distinct pathways

To examine the repertoire of antibodies produced in breast cancer patients, we comprehensively profiled IgG and IgA reactivity using overlapping peptide arrays. A series of 71 STM was first profiled with an array covering the whole proteome and all detected IgG and IgA positive protein regions were then tiled and included in a focused array to subsequently analyze simultaneously both STM (n=71) and matched patient sera when they were available (n=52). A series of sera from healthy donors (HD) was also included as controls (Supplementary data Fig. 4A). Several hundreds of protein and epitope reactivities were detected for each patient in both the STM and serum (Fig. 4A, supplementary data Fig 4B) and for >90% of these proteins, only a single peptide region, referred to as an epitope thereafter was targeted (Supplementary data Fig.4C). Overall, we observed a higher number of proteins targeted by IgG compared to IgA in both cancer patients and HD, except for lobular cancers where a substantial fraction of patients presented an elevated ratio of IgA to IgG reactivities number, differentiating them from patients with a ductal carcinoma (Supplementary Fig.4D). While the number of IgG protein reactivities per patient was comparable in STM and serum and was in the same range as that observed in HD, the number of IgA targets in the serum of cancer patients was higher than that of STM and HD' serum (Fig. 4A) indicating a cancer driven systemic IgA response. Unexpectedly, only a minor fraction of IgG/IgA target proteins was recognized by both isotypes in both BC patients and HD (Fig.4B), indicating that IgG and IgA mostly target different protein antigens.

We then compared IgG and IgA reactivities in the serum VS STM of cancer patients. While roughly 70% of all detected IgG protein reactivities were present in both the serum and STM, this frequency dropped to less than 30% for IgA (Fig. 4C), indicating that the systemic and tumor IgA responses are partly disconnected. Compared to IgG and IgA specificities present in serum and STM, those found exclusively in one or the other compartment displayed an increased counts of reactivities, which are absent from healthy donors and detected in only one patient (private reactivities) (Fig.4D, Supplementary data Fig.4E).

In order to get insight into the type proteins targeted by IgG vs IgA in cancer patients, we focused on proteins with no IgG/IgA reactivity detected in HD and performed pathways analysis for proteins with specific IgG/IgA detected in at least 2 patients (Supplementary data Fig.4F). While some common pathways were found, such as ATPase and GTPase activities, proteins inducing IgG vs IgA Ab were involved in separate biological pathways. IgG-targeted proteins were mainly involved in cell metabolism pathways, while those recognized by IgA related to the mammary gland, carcinogenesis and immune cells like Tfh, memory B cell and basophils, further supporting that IgG and IgA target distinct sets of proteins (Fig.4E).

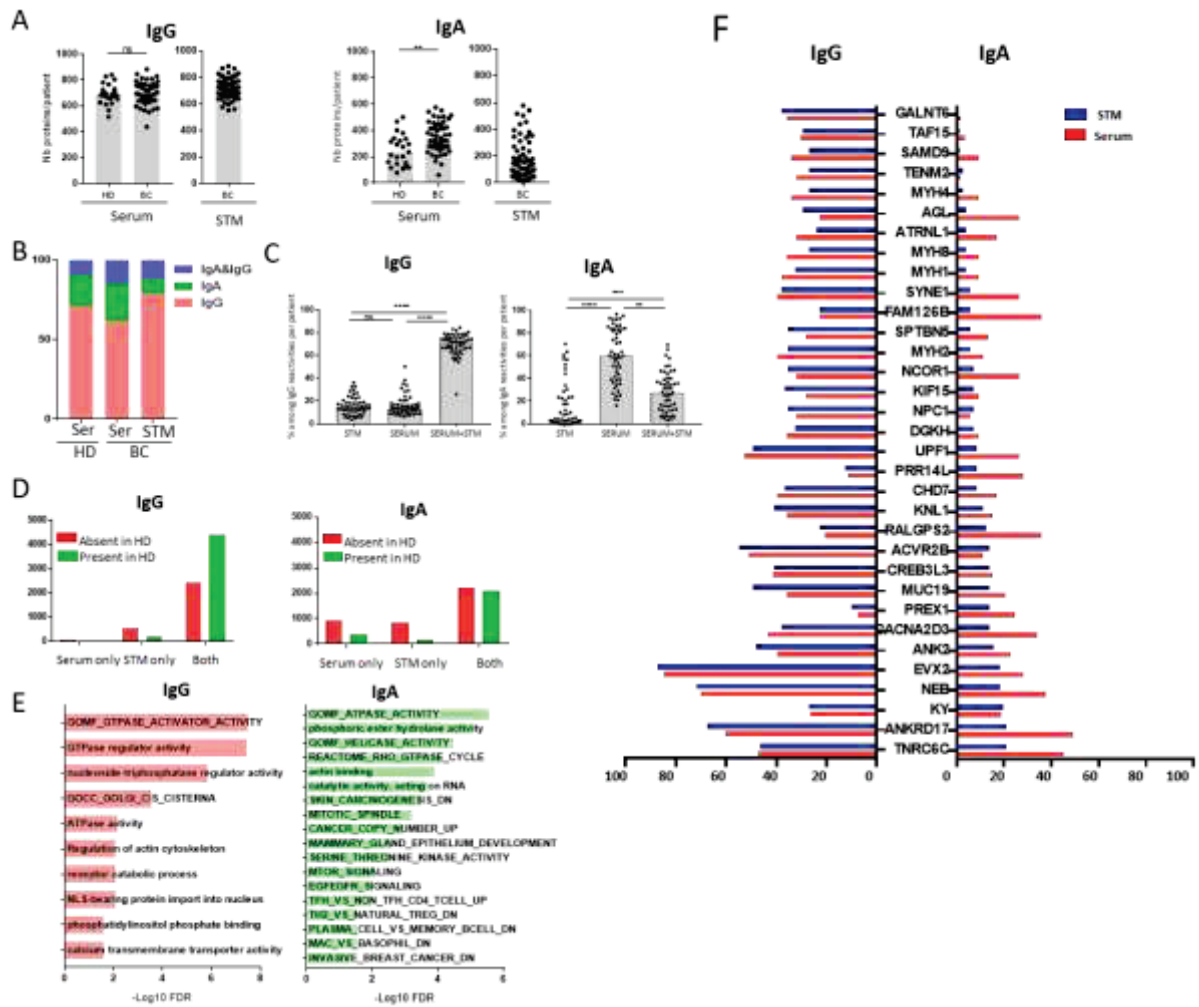


Figure 4: IgG and IgA antibodies target proteins involved in distinct pathways. **A** Graph depicting the number of targeted proteins per patient for both IgG (left) and IgA (right) in the sera of HD, BC and STM of patients. **B** Graph representing the mean proportion of IgG, IgA and shared IgG and IgA reactivities in HD and BC patients. **C** Bar plots showing the repartition of IgG (left) and IgA (right) reactivities proportions per patient present in STM, sera and in both fluids. **D** Cumulative number in all patients of targeted proteins present VS absent from HD for IgG (top) and IgA (bottom) in patients' sera, STM and both fluids. **E** Pathway enrichment analysis (hallmark sets and Gene Ontology) of IgG reactivities (left) VS IgA reactivities (right). **F** More frequent targeted proteins among patients for IgG and/or IgA in STM and/or serum.

Finally, we identified the proteins with IgG and/or IgA reactivities that were the more shared between cancer patients and which prevalence in patients was at least twice that observed in HD serum (Supplementary data. Fig. 4G). Strikingly, we observed similar frequencies between the serum and STM for IgG, whereas, IgA response in the TME was uncoupled from systemic response. Among these proteins, there is one cancer testis antigen named "KNL1" and other proteins already described in cancer, albeit not breast malignancies such as: MUC19, NPC1, PREX1. The whole list is in supplementary table 3.

IGHA and IGHG expression level differentially impact patient prognosis and associate with distinct immune environment

In order to evaluate the possible differential impact of IgG and IgA ASC on patient survival, we used the invasive breast carcinoma dataset from the TCGA. Patients with high expression of a plasma-cell gene signature had a statistically significant better progression free survival ($p=0.0016$) (Fig. 5A). Considering that the plasma cell gene signature nicely correlated with the expression of the IgG and IgA heavy chain genes (IGHG1-4, IGHA1-2) (Supplementary data Fig.5A), we analyzed their impact on survival. IGHA and, to a lesser extent, IGHG expression was associated with better relapse-free survival (Fig. 5B), but only IGHA expression retained a significant impact on overall survival (Supplementary data Fig. 5B). Multivariate analysis considering classical clinical data showed that IGHA expression emerged as an independent prognostic marker in addition to the tumor stage (Fig.5C). A tendency for IGHG, albeit not significant, was also observed (Supplementary data Fig.5C). Patients were then categorized according to the combined expression IGHG and IGHA genes, resulting in 4 groups. Patients that were high for both IGH isotypes had the best prognosis, followed by IGHA-Hi only patients, whereas those with a low expression of both IGHG and IGHA or a high expression of only IGHG had a reduced progression free and overall survival (Fig. 5D and Supplementary data Fig.5D). Intriguingly, whereas 3 out of the 4 groups had comparable proportions of the different tumor histological and molecular subtypes, the IGHA-Hi only group was strongly enriched in luminal A tumors (85%, $p<0.0001$, Fig. 5E) and contained an increased proportion of lobular tumors (<0.0001) (Supplementary Fig.5E), suggesting that the latter tumors favor emergence of an IgA-dominant ASC compartment.

To infer the immune microenvironment of the 4 categories of tumors and get insight into the possible mechanism associated with IgG versus IgA clinical impact, we used the MCP Counter algorithm (29) to estimate the abundance of different immune cell types from transcriptomic profiles. A supervised hierarchical clustering revealed the G-Hi_A-Hi group had increased expression scores for most of the analyzed cell types - including B cells, CD8 T cells, monocytes/macrophages and dendritic cells – and for TLS (Fig.5F), possibly explaining its good prognosis. A univariate logistic regression further revealed a strong association of mast cell and neutrophil gene signature scores with G-Lo_A-Hi tumors compared to G-Hi_A-Lo tumors, which conversely were associated with monocytic and B cell lineage gene expression (Fig. 5G). These associations remained statistically significant in a multivariate analysis (Supplementary Fig. 5F). Furthermore, a positive correlation was found between IGHA, but not IGHG, expression and neutrophil and mast cell gene signature scores (Supplementary Fig.5G & 5H). Moreover, the level of mast cell gene expression is higher in luminal A tumors than in other molecular subtypes, as already described by other studies (30) (Supplementary Fig. 5I).

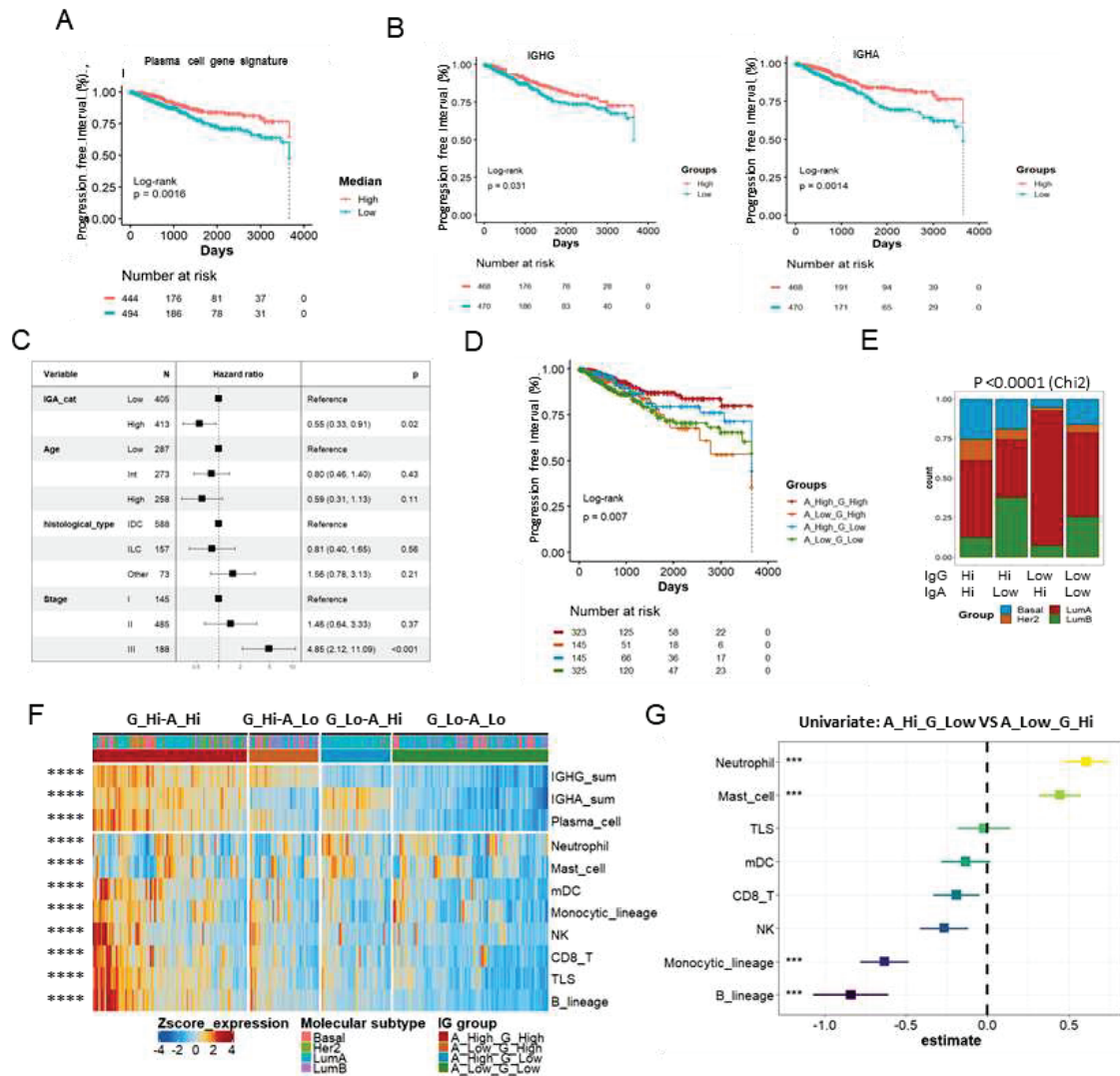


Figure 5: IGHA^{Hi}-IGHG^{Low} breast tumors are associated with favorable prognosis and are enriched in innate immune cell gene signatures. A-B Kaplan-Meier analysis of the prognosis impact in the breast carcinoma cohort of TCGA database of **A** plasma cell gene signature **B** IGHG representing the sum of IGHG1, IGHG2, IGHG3 and IGHG4 genes (left) and IGHA representing the sum of IGHA1 and IGHA2 genes (right). High and low levels of expression was determined using the median. Statistical significance was evaluated by the log-rank test. **C** Multivariate Cox analysis showing the effect of breast cancers prognostic factors on IGHA prognostic value. **D** Kaplan-Meier survival curves display PFI for IGHG and IGHA on TCGA database. Data were divided into four groups based on IGHG and IGHA level of expression. **E**. Graph depicting the repartition of breast cancer molecular subtypes in the four categories defined in D. **F** Supervised heatmap displaying levels of expression on immune cell gene signature scores in the four categories defined in D. **G** Graph depicting the association of each immune gene signature with the studied groups: IGHA^{Hi}-IGHG^{Low} and IGHA^{Low}-IGHG^{Hi}. Analysis was performed using univariate logistic regression.

In conclusion, IGHA expression in tumors correlated with increased patient survival independently of IGHG expression and each Ab isotype typified a distinct tumor immune microenvironment.

IgA ASC ameliorate TNBC prognosis and coincides with mast cell and neutrophil infiltration

To confirm and extend the results obtained with the TCGA, we measured the impact of IgG versus IgA ASC density (assessed on TMA) on patient prognosis using the PACS08 cohort. No association with survival was observed (data not shown). To avoid the possible sampling bias linked to TMA and because we showed that ASC infiltration is higher in TNBC than in luminal tumors, we performed the same analysis with a retrospective series of 120 untreated TNBC stained for IgG and IgA on whole-sections. Patients with high IgG-ASC tumor infiltration tended to have prolonged survival but this did not reach statistical significance (Fig.6A, Supplementary data Fig.6A). In contrast, a significantly increased progression free survival ($p = 0.0036$) was observed for IgA ASC-high patients (Fig.6B, Supplementary data fig. 6B), indicating that IgA ASC associate with a better prognosis in TNBC.

To compare the immune microenvironment of IgG ASC rich tumors from that of their IgA rich counterpart we profiled the RNA of a series of A-Hi and G-Hi only tumors using the 770 genes NanoString immune-oncology panel. Differential gene expression analysis revealed an upregulation of cancer-testis antigens and genes associated with adaptive immune cells and their activation in tumors dominated by IgG-ASC. In contrast, tumors categorized as IgG-Low and IgA-High displayed increased expression of genes related to innate immune cells and their chemoattraction, including mast cell (FCER1, MS4A2, CCL14) and neutrophil (MME, CEACAM6, CXCL14) genes (Fig.6C, Supplementary Fig.6C). 7-colors immunofluorescent staining of tumor tissue sections revealed the presence of mast cells (MS4A2) in regions highly infiltrated by IgA-ASC (Fig.6D). In these tumors, close contacts between neutrophils and IgA-labeled tumor cells can be observed, with evidence of CK in neutrophils (CD66b+/CK+) (Fig.6E), reminiscent of membrane tumor exchange occurring during Ab-dependent tumor cell trogocytosis by neutrophils (31).

The results revealed that high densities of IgA ASC correlated with increase TNBC patient survival and tumor infiltration by mast cells and neutrophils.

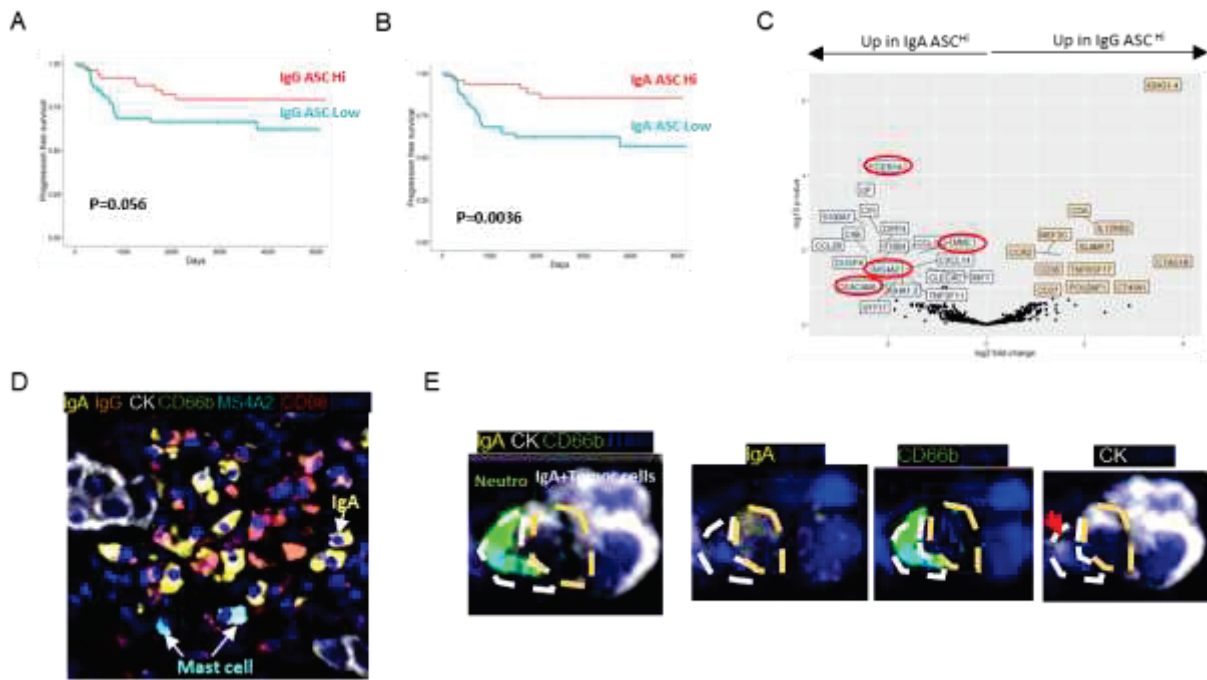


Figure 6: IgA-ASC dominant triple-negative breast tumors are associated with favorable prognosis and with innate immune response **A-B** Kaplan-Meier analysis of the prognosis impact in TNBC cohort (n=120) of **A** IgG-ASC density and **B** IgA-ASC density. ASC densities were determined semi-quantitatively using pan-CK/IgG/IgA staining on FFPE section. Statistical significance was evaluated by the log-rank test. **C** Volcano plot depiction of DEG between IgG-ASC dominant (n=4) VS IgA-ASC dominant tumors (n=6). Gene expression was determined using Nanostring technology and Pan-cancer immune profile panel (770 genes + 10 added genes) **D** Representative multiplexed immunofluorescent staining of IgG/IgA/CD66b/MS4A2/CD68/pan-CK to visualize IgG-ASC (orange), IgA-ASC (yellow), neutrophil (green), mast cell (cyan), macrophage (red) and tumor cell (white). White arrows show the presence of mast cells in IgA-ASC enriched regions. **E** Representative multiplexed immunofluorescent staining showing close contact between a neutrophil (white dashed line) and an IgA-labeled tumor cell (yellow dashed line). Red arrow shows the presence of cytokeratin fragments on neutrophil.

Discussion

A flurry of recent studies in human cancers have shown that ASC and humoral immune responses are key contributors in controlling tumor growth. IHC/multi-IF and bulk tumor transcriptome analysis have indeed revealed that elevated tumor infiltration by ASC densities correlates with longer survival in multiple cancer types (1), although opposite associations have also been reported (3,27). This might be explained by our current poor understanding of the phenotypic and functional heterogeneity of tumor-infiltrating ASC and its possible evolution during cancer progression. Here, using samples from retrospective and prospective cohorts of patients and by combining multiplex FCM, IF tissue staining and Ab specificity profiling, we show that ASC infiltrating invasive breast cancers consists of cells at different maturation stages, producing not only IgG but also monomeric or dimeric IgA1/IgA2 that target a set of antigens largely different from that of IgG. We also reveal for the first time a drastic change of the ASC compartment between *in situ* carcinomas and invasive carcinomas with an over dominance of IgA-ASC in all early tumors to a dominance of IgG-ASC in advanced tumors associated with a drop of the proportion of IgA2 producing cells. Finally, we demonstrated that tumor infiltration by IgG ASC and IgA ASC are both positively associated with better patient survival but typified different tumor immune microenvironments.

Our flow cytometry analysis of single cell suspensions prepared from invasive breast tumors showed that B cells accounted for about 10% of immune cells and consisted mostly of memory and naïve B cells, as well as ASC, in line with previous observations (13,14). Some breast tumors were also infiltrated by a small fraction of GC B cells and by CD38⁺IgM⁺IgD⁺ cells, which could not be further deconvoluted due their scarcity but likely correspond to transitional and/or pre-GC B cells. ASC were identified as CD19⁺CD20⁻CD38^{hi} cells, expressed surface CD27 and intracytoplasmic Ig as expected and accounted for about 8% of tumor infiltrating immune cells. Interesting, they contained 10 to 34 % of KI67⁺ cells indicating that they consisted on proliferating plasmablasts and plasma cells. We also describe for the first time breast tumor infiltration by a population of CD19⁻ ASC in most patients, albeit their frequency was 2-3 fold lower than that of conventional CD19⁺ ASC. Loss of the B cell lineage CD19 marker has been usually observed upon terminal differentiation/maturation of ASC (32). CD19⁻ ASC have been described as non-cycling long-lived PC in bone marrow of healthy individuals (33,34) but are also detectable in inflamed tissues (35). Whether CD19⁻ ASC originate from CD19⁺ ASC or from separate precursors remains unknown; however, some published data suggested that CD19⁺ ASC differentiate into CD19⁻ ASC during childhood, and once the pool of CD19⁻ cells is established, this differentiation is inhibited (35). Therefore, both subsets are observed in breast tumors suggesting that CD19⁺ ASC would stem from recent local antigen stimulation, whereas CD19⁻ ASC might not be antigen-specific and infiltrate tumors in response to inflammatory signals.

The function of ASC is in a large part dictated by their subclass of Ab owing to their different tumor cell killing abilities via activation of the complement pathway or triggering of the cytotoxic and/or phagocytic potential of accessory cells through Fc receptors. Our flow cytometry, multi-IF and ELISPOT data revealed that the ASC contingent in invasive breast tumors consisted mostly of IgG-, and to a lesser extent of IgA-, producing cells with few IgM and no IgD ASC. Interestingly, this isotype profile appeared disconnected from that of the memory B cell compartment, which showed an increase in IgM-expressing cells. It was demonstrated that the switch to IgG or IgA mediated selection into ASC fate rather than memory B cells (36,37), which may explain the higher proportion of IgM-expressing memory B cells compared to IgM ASC. The fact that a fraction of the ASC appeared IgG⁺IgA⁻IgM⁻ by flow cytometry may be due to the presence IgE producing cells and/or, more likely, to the technical issues in measuring intracytoplasmic Ig, especially IgG, in tumor single cell suspensions. Multi-IF tissue staining revealed that IgG and IgA ASC, identified by their high intracytoplasmic Ig content, typical morphology and expression of IRF4 and/or Blimp1, the 2 key transcription factors required for ASC generation and antibody production (38), mostly localized in the tumor stroma within the immune infiltrate. Interestingly, about 15-20% of these ASC were also present in close contact with tumor cells, suggesting the possible existence of niches favoring ASC attraction and survival. Finally, our Flow cytometry and multi-IF data both revealed a dominance of IgG-ASC except in 15-20% of patients, where IgA ASC outnumbered IgG ASC, reminiscent of recent published results in TNBC (16). This contrasts with ovarian and endometrial tumors, which are usually enriched in IgA PB and PC (23,24). Altogether, these data indicate the presence of an inter-patient heterogeneity regarding ASC isotypes, which may be the consequence of distinct tumor microenvironments leading potentially to different prognosis impacts. We also report that more than 80% of ASC express exclusively IRF4 consistent with an early stage of maturation, which is discordant with the fact that up to 34% of ASC express Ki67. Blimp1 expression were detected in a few cells that could result from a lack of multi-IF technique sensitivity particularly when considering that the difference in Blimp1 expression between PB and PC is about fivefold (39). Other groups showed the presence of both ASC subsets at varying proportions (13,23,40). This, together with fact that our flow cytometry data revealed the presence of B cells at all stages of differentiation suggest a local differentiation of ASC. This is supported by i) multi-IF detection of TLS harboring GC with the presence of ASC inside these structures, ii) TLS correlation with ASC, GC B cells and Tfh (CXCR5+), and iii) the presence of PD1^{Hi}ICOS⁺ Tfh, similar to GC Tfh found in SLO, consistent with a recent study in breast cancer (41). Collectively, these data showed that TLS are sites for ASC generation, which was demonstrated recently in renal carcinoma (42).

One of the most striking observation of the present study is the drastic change of the ASC compartment between *in situ* and invasive breast carcinomas. DCIS is a non-invasive and poorly

evolutionary form of breast cancer characterized by the confinement of proliferating malignant cells in the mammary ducts. Compared to their invasive counterparts, DCIS usually present a more active immune infiltrate (43,44) frequently organized in TLS, known to favor Th1 and cytotoxic CD8+ T cell immunity (41,45) suggesting more efficient tumor immune surveillance. Strikingly, all DCIS tumors displayed a high predominance of IgA ASC over IgG ASC while less than 20% of IBC tumors did so. It is most likely that this imbalance in ASC populations is the consequence of tumor development and is not due to the persistence of normal mammary tissue resident IgA ASC lining the duct epithelium (46). Indeed, IgA ASC almost exclusively localized in the immune stroma surrounding malignant lesions and their density was significantly increased in high-grade compared to low-grade tumors, suggesting a recruitment from the periphery in response to inflammation and/or a local generation following tumor antigen stimulation in TLS. Although IgA were believed for a long time to have mostly anti-inflammatory properties, their capacity to induce phagocytosis by monocyte/macrophage and to trigger more efficiently than IgG (47) tumor cell killing by trogoptosis via neutrophils (31), which are known to infiltrate DCIS (44), argue for a role of IgA in immune surveillance of early tumors. IgA consists of 2 subclasses, IgA1 and IgA2, which differ by their tissue distribution, hinge region and Fc glycosylations conferring them different capacities to bind Ag and distinct affinities for Fc receptors (48). We observed an increased proportion of IgA2 cells among ASC in DCIS and non tumoral tissue compared to IBC. Interestingly, IgA2 has a superior capacity to elicit ADCC by neutrophils upon CD89 engagement compared to IgA1 (49), suggesting that a more efficient IgA-mediated tumor cell killing may occur at the *in situ* versus invasive stage of breast cancer. Besides, IgA exist as several molecular isoforms, mostly monomers and dimers, which can exert pro- or anti-inflammatory functions through their capacity to engage distinct receptors or to trigger opposite signaling pathways after binding to CD89 (50). Using multi-IF tissue staining, we show that while most IgA ASC in NTT co-expressed the J chain, necessary for IgA dimer assembly, a substantial fraction of ASC in DCIS and IBC did not, suggesting a shift toward production of monomeric IgA during cancer development. This also provide further evidence that IgA ASC in DCIS differ from those present in normal ducts. In addition, western blot analysis of a series of invasive breast tumor supernatants demonstrated the presence of both IgA isoforms with a usual dominance of monomeric IgA (70%), whereas IgA in the serum of matched patients were mostly monomeric, as expected. Engagement of Fc α RI by monomeric IgA has been shown to suppress production of pro-inflammatory cytokines and to induce IL10 expression in human monocytes and monocyte-derived DC (51,52), suggesting that this IgA isoform may contribute to immune escape. Collectively, our data demonstrate a strong molecular diversity of IgA produced in tumors that evolves during tumor progression with an increased proportion of monomeric IgA1 in advanced breast tumors, which may explain decreased tumor immune control.

In parallel, we analyzed the antigens targeted by IgG and IgA in breast cancer patients using an overlapping peptide array. This was performed first with a whole-proteome array to identify all peptide reactivities in STM and then with a focused array covering all protein regions with positive hits from step 1 to compare STM, matched patient serum samples and serum from healthy donors. To our knowledge, this is the first study of such magnitude for comparing IgG and IgA reactivities, that goes far beyond the few reports documenting Ab reactivities against a limited set of proteins (21,53). Although the analysis was limited to small size linear epitopes, several hundreds of protein reactivities were detected in each patient, in both the STM and serum, with usually a single region with contiguous positive peptides - e.g. an “epitope” – for most proteins. Importantly, a higher number of proteins appear targeted by IgG compared to IgA. This may reflect the lower IgA titers in STM and sera compared to IgG (Supplementary figure 1E), differences in the detection threshold between the two isotypes, an increased recognition of linear epitopes by IgG, or a more restrained IgA repertoire. This latter possibility is consistent with the study of Garaud et al. showing that the quantity of IgA, but not IgG, Ab reactive to breast tumor-associated antigens is linked to the presence of TLS (21). Another striking observation was that only a minor fraction of all detected proteins was recognized both by IgG and IgA, indicating that the 2 Ab isotypes target poorly overlapping sets of Ag. Interestingly, while the mean number of proteins targeted by IgG in the serum was similar between cancer patients and HD, the number of IgA reactivities was significantly higher in cancer patients, indicating a cancer-driven systemic IgA response. This may be linked to the fact that breast tumors develop in a mucosal site, known to bias B cell responses towards IgA. Another major finding of this analysis is that while most IgG reactivities were found both in tumor and sera, a major proportion of IgA reactivities was only detected in serum, indicating that the systemic and tumor IgA responses are partly disconnected in cancer patients. Besides revealing a distinct distribution of IgG and IgA ASC, this could also be explained by a different capacity of the 2 Ab isotypes to diffuse from the serum to the tumor. Indeed, the neonatal Fc receptor FcRn is highly expressed in endothelial and epithelial cells of the mammary glands and has the ability to deliver serum IgG across cellular barriers to tissues (54). In contrast, there is not such a route by which circulating IgA can reach mucosal sites (55). A large majority of proteins targeted by IgG and/or IgA is predicted to have an intracellular location, consistent with numerous studies of circulating IgG in cancer patients (56). Such Ab may be generated following tumor cell death and release of their intracellular content and, in the case of IgG, could possibly promote anti-tumor immunity by favoring tumor Ag cross-presentation to CD8+ T cells (25). Interestingly, cancer cells can externalize certain intracellular proteins, referred to as “eProt”, making them accessible to autoantibodies (57) and conferring them new properties related to cell signaling and invasion (58). Importantly, we detected, exclusively in cancer patients, IgG and IgA against the known eProts nucleolin, estrogen receptor and GRP94 (59). To get insight of the type of Ag targeted by IgG vs IgA in

cancer patients, we first performed a pathway analysis with the proteins for which Ab were detected in patients but not in HD. Both sets of proteins had in common the ATPase and GTPase pathways that are crucial for cancer cell survival and growth (e.g replication, transcription). Interestingly, unlike IgG, IgA also targeted proteins related to mammary gland development, carcinogenesis and breast cancer invasiveness, as well as proteins associated to immune cell infiltration. This may result from abnormalities of the clearance of dead epithelial and immune cells in tumor bed that lead to the release of intracellular molecules triggering an immune response. Thus, even though the identity of the proteins targeted by IgG and/or IgA is significantly different from one patient to another, some of these proteins have in common to be involved in processes relevant to cancer. We further identified the top most frequent antigens recognized by IgG and/or IgA in cancer patients and which prevalence was at least 2-fold higher than in HD. 33 proteins were identified with these criteria, with usually the presence of specific IgG and IgA in both the tumor and serum, although the prevalence of IgA was usually lower than that of IgG especially in the tumor. Unexpectedly, none of these proteins has been so far associated to breast cancers except the cancer testis Ag “KLN1” (60). Consistent with the study of Garaud et al., no antibody reactivity was found against MUC1, but IgG and IgA against another MUC family member, MUC19 was detected in 30-50% of patients (21). Altogether, our findings revealed that IgG and IgA recognized different set of antigens involved in distinct pathways.

Tumor infiltration by ASC at the time of cancer diagnosis is usually associated with better patient overall and disease-free survival in most cancer types (19). In breast cancer however, the impact of ASC infiltration, measured by CD138 IHC staining or by the expression score of a PC gene signature or even the sole Ig kappa light chain, remains controversial (3,17,19). In addition, very few studies have so far addressed the possible differential contribution of ASC according to the class/subclass of Ab they produce. Elevated mRNA levels of the IgG1 heavy chain (IGHG1) among all IGH transcripts was associated with longer survival in melanoma, non-papillary bladder cancer and KRAS-mutated lung cancer, while on the opposite, a high IGHA/IGH ratio correlated with shorter survival in 2 out of the 3 cancers (61,62), suggesting opposite functions for IgG and IgA ASC. The deleterious role of IgA ASC is indeed corroborated by studies in mouse models of prostate cancer and hepatocellular carcinomas where they suppressed CD8+ T cells via PD-L1 and IL-10 (27,63). Yet, recent studies in ovarian and endometrial tumors revealed that IgA can also promote immunity by sensitizing malignant cells to T cell mediated killing after binding to the poly-Ig receptor and transcytosis (23,24). To address the respective roles of IgG and IgA ASC, we used the TCGA, which assembles RNA and clinical data from 939 sporadic breast cancers, as well as an in-house constituted cohort of 120 TNBC. We showed that patients with high mRNA levels of IGHA, IGHG or both isotypes, and patients with high densities of IgG or IgA ASC, assessed by multi-IF tissue staining, had increased survival compared to

their low counterpart, indicating that both types of ASC might be beneficial. However, analysis of immune gene signatures from the various subgroups in the TCGA revealed that IGHG^{Hi}-IGHA^{Hi} tumors were highly infiltrated by various immune cell subsets, including CD8+ T cells, and possibly contained TLS, 2 features known to be associated with increased patient survival (8), complicating data interpretation. Interestingly, while far less immune infiltrated, IGHG^{Lo}-IGHA^{Hi}, but not IGHG^{Hi}-IGHA^{Lo}, tumors were associated with a good patient survival, comparable to that of IGHG^{Hi}-IGHA^{Hi} patients, suggesting a beneficial role of IgA ASC independently of IgG ASC. Interestingly, the IGHG^{Lo}-IGHA^{Hi} group was substantially enriched in luminal A tumors and lobular tumors. Together with the fact that significantly more IgA reactivities than IgG reactivities were detected in lobular tumors compared to ductal carcinomas, these data indicate that the molecular and histological features of the tumor may influence the TME leading to the recruitment or generation of ASC producing distinct Ab isotypes. Altogether, these data indicate that IgA ASC, and to lesser extend, IgG ASC are associated with increased survival of patients with TNBC or non-TNBC tumors.

Importantly, IgG ASC and IgA ASC rich tumors appear to strikingly differ in their immune microenvironment. Indeed differential gene expression analysis between IgG^{Lo}-IgA^{Hi} and IgG^{Hi}-IgA^{Lo} tumors from both the TCGA and TNBC cohort revealed that IgG-ASC enriched tumors were associated with overexpression of Cancer Testis associated genes and genes related to activated adaptive immune cells suggesting the development of a specific immune response triggered by tumor antigens. In contrast, IgA-ASC dominant tumors exhibited increased expression of genes related to innate immune cells including mast cells and neutrophils. Such a link between mast cells, neutrophils and IgA ASC is supported by some evidence from the literature. Mast cells can indeed interact with B cells through CD40-CD40L together and trigger their differentiation into IgA-secreting plasma cells via production of IL6 and TGF β (64,65). Mast cells constitute an important source of cytokines and chemokines including CXCL8, a potent chemoattractant of neutrophils (66). Besides, consistent with the strong association between IGHG^{Lo}-IGHA^{Hi} and luminal and lobular breast tumor type, it was demonstrated that significantly higher numbers of mast cells were associated with luminal and lobular breast tumors. To support that, mast cell can stimulate estrogen receptor activity in breast malignant cells promoting the luminal phenotype (30,67). Preliminary multi-IF data further revealed mast cells near IgA-ASC rich areas. In addition, neutrophils were observed in close contact with IgA-stained tumor cells, with signs of membrane mixing between the 2 cell types, a finding reminiscent of neutrophil mediated trogoptosis of Ab-loaded tumor cells (31,68). Whether such a mechanism is operative in *in situ* and/or invasive tumors and accounts for the beneficial impact of IgA ASC on patient survival remains to be determined.

Overall, the present study demonstrated that breast tumors are infiltrated by a considerable heterogeneous pool of ASC that may have differential impact on prognosis. This diversity depends on the stage of cancer progression, molecular and histological types of tumor as well as antigenic stimulation and infiltrating immune cells. Finally, further studies should be performed to better understand what can favor IgA- VS IgG-dominant tumors and their consequences on the quality of the immune response.

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References:

1. Fridman WH, Petitprez F, Meylan M, Chen TWW, Sun CM, Roumenina LT, et al. B cells and cancer: To B or not to B? *J Exp Med.* 4 janv 2021;218(1):e20200851.
2. Ali HR, Provenzano E, Dawson SJ, Blows FM, Liu B, Shah M, et al. Association between CD8+ T-cell infiltration and breast cancer survival in 12,439 patients. *Ann Oncol.* août 2014;25(8):1536-43.
3. Mohammed ZMA, Going JJ, Edwards J, Elsberger B, Doughty JC, McMillan DC. The relationship between components of tumour inflammatory cell infiltrate and clinicopathological factors and survival in patients with primary operable invasive ductal breast cancer. *Br J Cancer.* 21 août 2012;107(5):864-73.
4. Mahmoud SMA, Lee AHS, Paish EC, Macmillan RD, Ellis IO, Green AR. The prognostic significance of B lymphocytes in invasive carcinoma of the breast. *Breast Cancer Res Treat.* avr 2012;132(2):545-53.
5. Arias-Pulido H, Cimino-Mathews A, Chaheer N, Qualls C, Joste N, Colpaert C, et al. The combined presence of CD20 + B cells and PD-L1 + tumor-infiltrating lymphocytes in inflammatory breast cancer is prognostic of improved patient outcome. *Breast Cancer Res Treat.* sept 2018;171(2):273-82.
6. Brown SD, Warren RL, Gibb EA, Martin SD, Spinelli JJ, Nelson BH, et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. *Genome Res.* mai 2014;24(5):743-50.
7. Figenschau SL, Fismen S, Fenton KA, Fenton C, Mortensen ES. Tertiary lymphoid structures are associated with higher tumor grade in primary operable breast cancer patients. *BMC Cancer.* 6 mars 2015;15(1):101.
8. Martinet L, Garrido I, Filleron T, Le Guellec S, Bellard E, Fournie JJ, et al. Human solid tumors contain high endothelial venules: association with T- and B-lymphocyte infiltration and favorable prognosis in breast cancer. *Cancer Res.* 1 sept 2011;71(17):5678-87.
9. Prabhakaran S, Rizk VT, Ma Z, Cheng CH, Berglund AE, Coppola D, et al. Evaluation of invasive breast cancer samples using a 12-chemokine gene expression score: correlation with clinical outcomes. *Breast Cancer Res.* 19 juin 2017;19(1):71.
10. Cabrita R, Lauss M, Sanna A, Donia M, Skaarup Larsen M, Mitra S, et al. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. *Nature.* janv 2020;577(7791):561-5.
11. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, et al. B cells and tertiary lymphoid structures promote immunotherapy response. *Nature.* janv 2020;577(7791):549-55.
12. Petitprez F, de Reyniès A, Keung EZ, Chen TWW, Sun CM, Calderaro J, et al. B cells are associated with survival and immunotherapy response in sarcoma. *Nature.* janv 2020;577(7791):556-60.

13. Garaud S, Buisseret L, Solinas C, Gu-Trantien C, de Wind A, Van den Eynden G, et al. Tumor infiltrating B-cells signal functional humoral immune responses in breast cancer. *JCI Insight*. 13 août 2019;5:129641.
14. Hu Q, Hong Y, Qi P, Lu G, Mai X, Xu S, et al. Atlas of breast cancer infiltrated B-lymphocytes revealed by paired single-cell RNA-sequencing and antigen receptor profiling. *Nat Commun*. 12 avr 2021;12:2186.
15. Miligy I, Mohan P, Gaber A, Aleskandarany MA, Nolan CC, Diez-Rodriguez M, et al. Prognostic significance of tumour infiltrating B lymphocytes in breast ductal carcinoma *in situ*. *Histopathology*. août 2017;71(2):258-68.
16. Harris RJ, Cheung A, Ng JCF, Laddach R, Chenoweth AM, Crescioli S, et al. Tumor-Infiltrating B Lymphocyte Profiling Identifies IgG-Biased, Clonally Expanded Prognostic Phenotypes in Triple-Negative Breast Cancer. *Cancer Res*. 15 août 2021;81(16):4290-304.
17. Schmidt M, Hellwig B, Hammad S, Othman A, Lohr M, Chen Z, et al. A Comprehensive Analysis of Human Gene Expression Profiles Identifies Stromal Immunoglobulin κ C as a Compatible Prognostic Marker in Human Solid Tumors. *Clin Cancer Res*. 1 mai 2012;18(9):2695-703.
18. Iglesia MD, Vincent BG, Parker JS, Hoadley KA, Carey LA, Perou CM, et al. Prognostic B-cell Signatures Using mRNA-Seq in Patients with Subtype-Specific Breast and Ovarian Cancer. *Clin Cancer Res*. 15 juill 2014;20(14):3818-29.
19. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat Med*. août 2015;21(8):938-45.
20. Yeong J, Lim JCT, Lee B, Li H, Chia N, Ong CCH, et al. High Densities of Tumor-Associated Plasma Cells Predict Improved Prognosis in Triple Negative Breast Cancer. *Front Immunol*. 30 mai 2018;9:1209.
21. Garaud S, Zayakin P, Buisseret L, Rulle U, Silina K, de Wind A, et al. Antigen Specificity and Clinical Significance of IgG and IgA Autoantibodies Produced *in situ* by Tumor-Infiltrating B Cells in Breast Cancer. *Front Immunol*. 2018;9:2660.
22. Sharonov GV, Serebrovskaya EO, Yuzhakova DV, Britanova OV, Chudakov DM. B cells, plasma cells and antibody repertoires in the tumour microenvironment. *Nat Rev Immunol*. mai 2020;20(5):294-307.
23. Biswas S, Mandal G, Payne KK, Anadon CM, Gatenbee CD, Chaurio RA, et al. IgA transcytosis and antigen recognition govern ovarian cancer immunity. *Nature*. 18 mars 2021;591(7850):464-70.
24. Mandal G, Biswas S, Anadon CM, Yu X, Gatenbee CD, Prabhakaran S, et al. IgA-Dominated Humoral Immune Responses Govern Patients' Outcome in Endometrial Cancer. *Cancer Res*. 1 mars 2022;82(5):859-71.
25. Baker K, Rath T, Flak MB, Arthur JC, Chen Z, Glickman JN, et al. Neonatal Fc Receptor Expression in Dendritic Cells Mediates Protective Immunity against Colorectal Cancer. *Immunity*. déc 2013;39(6):1095-107.
26. de Visser KE, Korets LV, Coussens LM. De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer Cell*. 1 mai 2005;7(5):411-23.

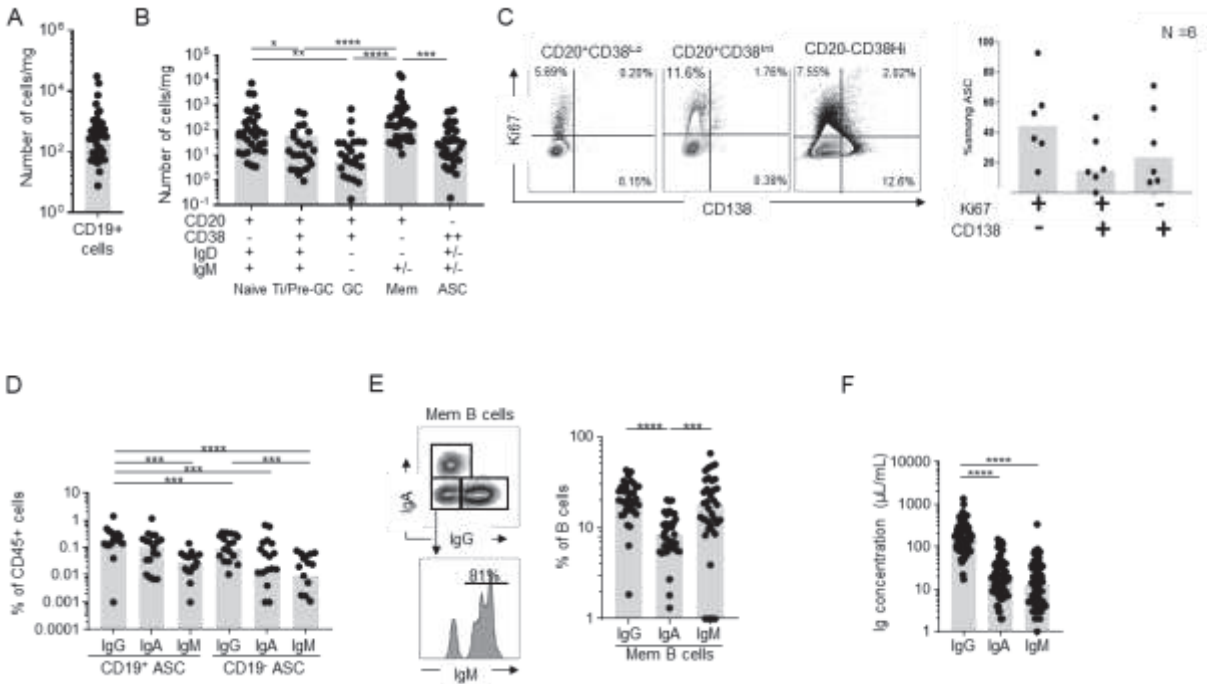
27. Shalpour S, Font-Burgada J, Di Caro G, Zhong Z, Sanchez-Lopez E, Dhar D, et al. Immunosuppressive plasma cells impede T-cell-dependent immunogenic chemotherapy. *Nature*. 7 mai 2015;521(7550):94-8.
28. Campone M, Lacroix-Triki M, Roca L, Spielmann M, Wildiers H, Cottu P, et al. UCBG 2-08: 5-year efficacy results from the UNICANCER-PACS08 randomised phase III trial of adjuvant treatment with FEC100 and then either docetaxel or ixabepilone in patients with early-stage, poor prognosis breast cancer. *Eur J Cancer*. nov 2018;103:184-94.
29. Becht E, Giraldo NA, Germain C, de Reyniès A, Laurent-Puig P, Zucman-Rossi J, et al. Chapter Four - Immune Contexture, Immunoscore, and Malignant Cell Molecular Subgroups for Prognostic and Theranostic Classifications of Cancers. In: Schreiber RD, éditeur. *Advances in Immunology* [Internet]. Academic Press; 2016 [cité 18 mars 2022]. p. 95-190. (Tumor Immunology; vol. 130). Disponible sur: <https://www.sciencedirect.com/science/article/pii/S006527761530002X>
30. Glajcar A, Szpor J, Pacek A, Tyrak KE, Chan F, Streb J, et al. The relationship between breast cancer molecular subtypes and mast cell populations in tumor microenvironment. *Virchows Arch*. mai 2017;470(5):505-15.
31. Matlung HL, Babes L, Zhao XW, van Houdt M, Treffers LW, van Rees DJ, et al. Neutrophils Kill Antibody-Opsonized Cancer Cells by Trogoptosis. *Cell Rep*. 26 juin 2018;23(13):3946-3959.e6.
32. Wang K, Wei G, Liu D. CD19: a biomarker for B cell development, lymphoma diagnosis and therapy. *Exp Hematol Oncol*. 29 nov 2012;1(1):36.
33. Halliley JL, Tipton CM, Liesveld J, Rosenberg AF, Darce J, Gregoret IV, et al. Long-Lived Plasma Cells Are Contained within the CD19(-)CD38(hi)CD138(+) Subset in Human Bone Marrow. *Immunity*. 21 juill 2015;43(1):132-45.
34. Groves CJ, Carrell J, Grady R, Rajan B, Morehouse CA, Halpin R, et al. CD19-positive antibody-secreting cells provide immune memory. *Blood Adv*. 27 nov 2018;2(22):3163-76.
35. Mei HE, Wirries I, Frölich D, Brisslert M, Giesecke C, Grün JR, et al. A unique population of IgG-expressing plasma cells lacking CD19 is enriched in human bone marrow. *Blood*. 12 mars 2015;125(11):1739-48.
36. Gitlin AD, von Boehmer L, Gazumyan A, Shulman Z, Oliveira TY, Nussenzweig MC. Independent Roles of Switching and Hypermutation in the Development and Persistence of B Lymphocyte Memory. *Immunity*. 19 avr 2016;44(4):769-81.
37. Palm AKE, Henry C. Remembrance of Things Past: Long-Term B Cell Memory After Infection and Vaccination. *Frontiers in Immunology* [Internet]. 2019 [cité 13 avr 2022];10. Disponible sur: <https://www.frontiersin.org/article/10.3389/fimmu.2019.01787>
38. Yu YH, Lin KI. Factors That Regulate the Generation of Antibody-Secreting Plasma Cells. *Adv Immunol*. 2016;131:61-99.
39. Low M, Infantino S, Grigoriadis G, Tarlinton D. Targeting plasma cells: are we any closer to a panacea for diseases of antibody-secreting cells? *Immunol Rev*. mars 2016;270(1):78-94.

40. Griss J, Bauer W, Wagner C, Simon M, Chen M, Grabmeier-Pfistershammer K, et al. B cells sustain inflammation and predict response to immune checkpoint blockade in human melanoma. *Nat Commun.* déc 2019;10(1):4186.
41. Noël G, Fontsa ML, Garaud S, De Silva P, de Wind A, Van den Eynden GG, et al. Functional Th1-oriented T follicular helper cells that infiltrate human breast cancer promote effective adaptive immunity. *J Clin Invest.* 1 oct 2021;131(19):e139905.
42. Meylan M, Petitprez F, Becht E, Bougoüin A, Pupier G, Calvez A, et al. Tertiary lymphoid structures generate and propagate anti-tumor antibody-producing plasma cells in renal cell cancer. *Immunity.* 24 févr 2022;S1074-7613(22)00081-4.
43. Ben-Hur H, Cohen O, Schneider D, Gurevich P, Halperin R, Bala U, et al. The role of lymphocytes and macrophages in human breast tumorigenesis: an immunohistochemical and morphometric study. *Anticancer Res.* avr 2002;22(2B):1231-8.
44. Gil Del Alcazar CR, Huh SJ, Ekram MB, Trinh A, Liu LL, Beca F, et al. Immune Escape in Breast Cancer During In Situ to Invasive Carcinoma Transition. *Cancer Discov.* oct 2017;7(10):1098-115.
45. Martinet L, Filleron T, Le Guellec S, Rochaix P, Garrido I, Girard JP. High endothelial venule blood vessels for tumor-infiltrating lymphocytes are associated with lymphotoxin β -producing dendritic cells in human breast cancer. *J Immunol.* 15 août 2013;191(4):2001-8.
46. Hsu SM, Raine L, Nayak RN. Medullary carcinoma of breast: an immunohistochemical study of its lymphoid stroma. *Cancer.* 15 sept 1981;48(6):1368-76.
47. Brandsma AM, Bondza S, Evers M, Koutstaal R, Nederend M, Jansen JHM, et al. Potent Fc Receptor Signaling by IgA Leads to Superior Killing of Cancer Cells by Neutrophils Compared to IgG. *Front Immunol.* 2019;10:704.
48. Woof JM, Russell MW. Structure and function relationships in IgA. *Mucosal Immunol.* nov 2011;4(6):590-7.
49. Dechant M, Beyer T, Schneider-Merck T, Weisner W, Peipp M, Winkel JGJ van de, et al. Effector Mechanisms of Recombinant IgA Antibodies against Epidermal Growth Factor Receptor. *The Journal of Immunology.* 1 sept 2007;179(5):2936-43.
50. Ben Mkaddem S, Rossato E, Heming N, Monteiro RC. Anti-inflammatory role of the IgA Fc receptor (CD89): From autoimmunity to therapeutic perspectives. *Autoimmunity Reviews.* 1 avr 2013;12(6):666-9.
51. Lecocq M, Detry B, Guisset A, Pilette C. Fc α RI-mediated inhibition of IL-12 production and priming by IFN- γ of human monocytes and dendritic cells. *J Immunol.* 1 mars 2013;190(5):2362-71.
52. Pilette C, Detry B, Guisset A, Gabriels J, Sibille Y. Induction of interleukin-10 expression through Fc α receptor in human monocytes and monocyte-derived dendritic cells: role of p38 MAPKinase. *Immunol Cell Biol.* juin 2010;88(4):486-93.
53. Germain C, Gnjjatic S, Tamzalit F, Knockaert S, Remark R, Goc J, et al. Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. *Am J Respir Crit Care Med.* 1 avr 2014;189(7):832-44.

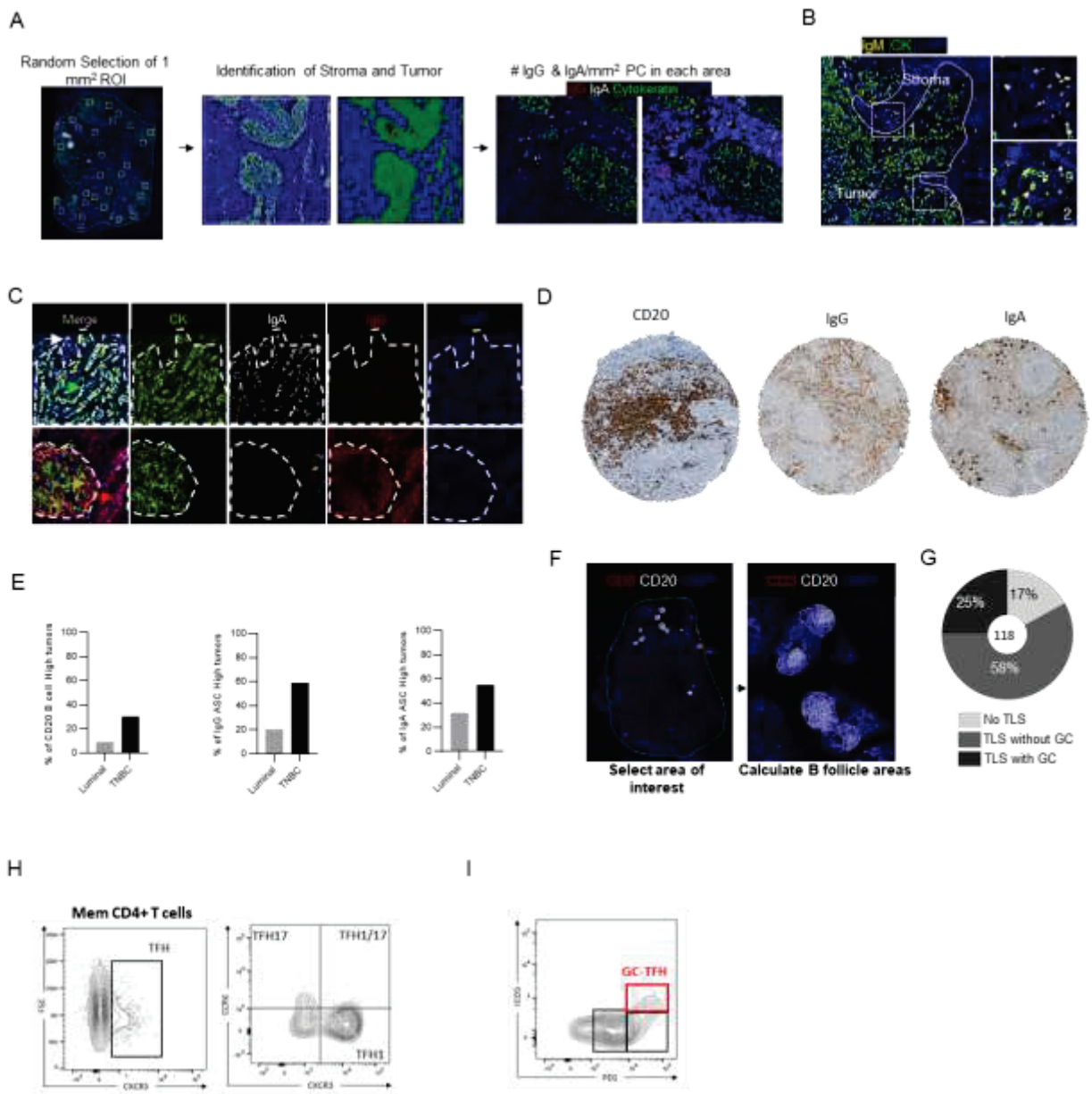
54. Spiekermann GM, Finn PW, Ward ES, Dumont J, Dickinson BL, Blumberg RS, et al. Receptor-mediated immunoglobulin G transport across mucosal barriers in adult life: functional expression of FcRn in the mammalian lung. *J Exp Med.* 5 août 2002;196(3):303-10.
55. Lamm ME. Current concepts in mucosal immunity. IV. How epithelial transport of IgA antibodies relates to host defense. *Am J Physiol.* avr 1998;274(4):G614-617.
56. Järås K, Anderson K. Autoantibodies in cancer: prognostic biomarkers and immune activation. *Expert Rev Proteomics.* oct 2011;8(5):577-89.
57. Hansen MH, Nielsen H, Ditzel HJ. The tumor-infiltrating B cell response in medullary breast cancer is oligoclonal and directed against the autoantigen actin exposed on the surface of apoptotic cancer cells. *Proceedings of the National Academy of Sciences.* 23 oct 2001;98(22):12659-64.
58. Albaret MA, Vermot-Desroches C, Paré A, Roca-Martinez JX, Malet L, Esseily J, et al. Externalized Keratin 8: A Target at the Interface of Microenvironment and Intracellular Signaling in Colorectal Cancer Cells. *Cancers.* 2018;10(11):452.
59. Weidle UH, Maisel D, Klostermann S, Schiller C, Weiss EH. Intracellular proteins displayed on the surface of tumor cells as targets for therapeutic intervention with antibody-related agents. *Cancer Genomics Proteomics.* avr 2011;8(2):49-63.
60. The Human Protein Atlas [Internet]. [cité 21 avr 2022]. Disponible sur: <https://www.proteinatlas.org/>
61. Isaeva OI, Sharonov GV, Serebrovskaya EO, Turchaninova MA, Zaretsky AR, Shugay M, et al. Intratumoral immunoglobulin isotypes predict survival in lung adenocarcinoma subtypes. *J immunotherapy cancer.* déc 2019;7(1):279.
62. Bolotin DA, Poslavsky S, Davydov AN, Frenkel FE, Fanchi L, Zolotareva OI, et al. Antigen receptor repertoire profiling from RNA-seq data. *Nat Biotechnol.* 11 oct 2017;35(10):908-11.
63. Shalpour S, Lin XJ, Bastian IN, Brain J, Burt AD, Aksenov AA, et al. Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity. *Nature.* 16 nov 2017;551(7680):340-5.
64. Merluzzi S, Frossi B, Gri G, Parusso S, Tripodo C, Pucillo C. Mast cells enhance proliferation of B lymphocytes and drive their differentiation toward IgA-secreting plasma cells. *Blood.* 8 avr 2010;115(14):2810-7.
65. Valeri V, Tonon S, Vibhushan S, Gulino A, Belmonte B, Adori M, et al. Mast cells crosstalk with B cells in the gut and sustain IgA response in the inflamed intestine. *European Journal of Immunology.* 2021;51(2):445-58.
66. Mukai K, Tsai M, Saito H, Galli SJ. Mast cells as sources of cytokines, chemokines and growth factors. *Immunol Rev.* mars 2018;282(1):121-50.
67. Majorini MT, Cancila V, Rigoni A, Botti L, Dugo M, Triulzi T, et al. Infiltrating Mast Cell-Mediated Stimulation of Estrogen Receptor Activity in Breast Cancer Cells Promotes the Luminal Phenotype. *Cancer Res.* 1 juin 2020;80(11):2311-24.

68. Treffers LW, Ten Broeke T, Rösner T, Jansen JHM, van Houdt M, Kahle S, et al. IgA-Mediated Killing of Tumor Cells by Neutrophils Is Enhanced by CD47-SIRP α Checkpoint Inhibition. *Cancer Immunol Res.* janv 2020;8(1):120-30.

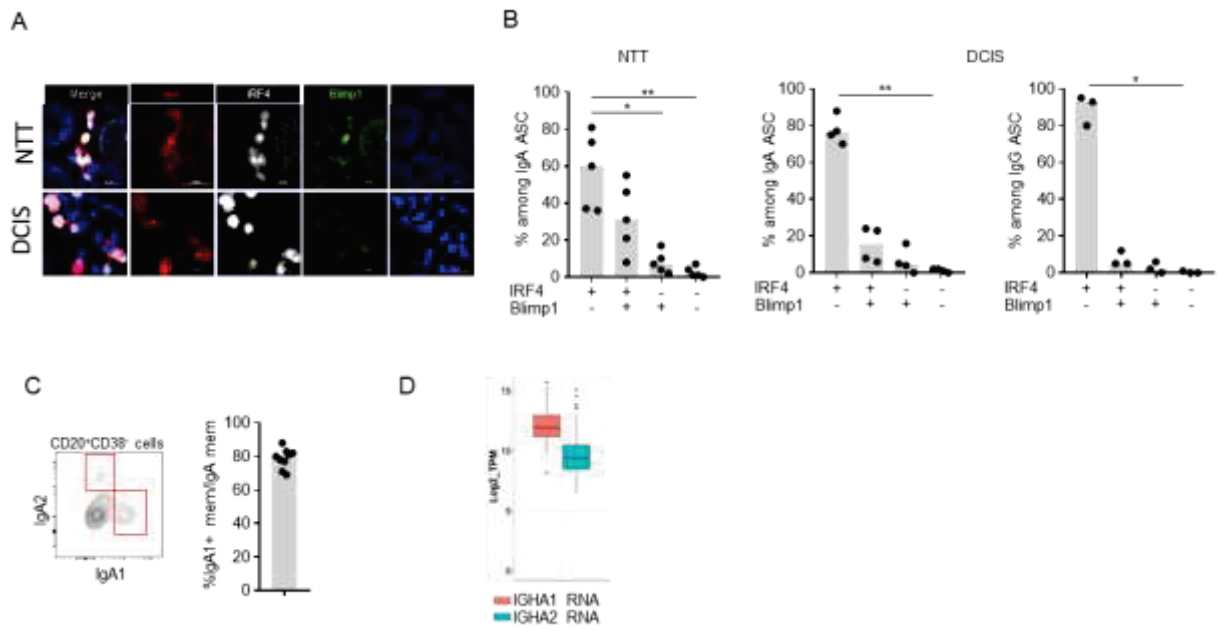
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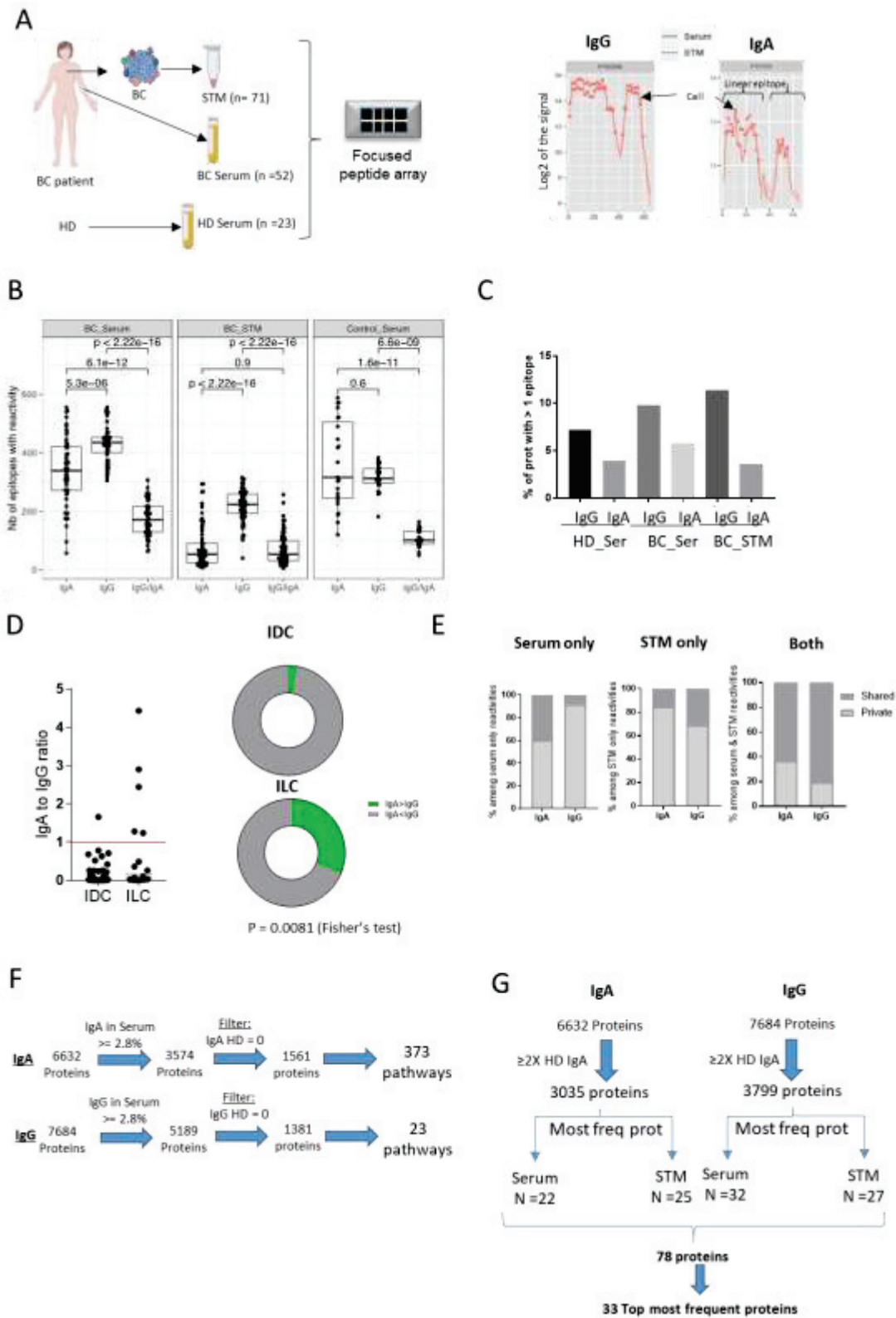
Supplementary figure 1: Invasive breast tumors are infiltrated by a heterogeneous population of antibody-secreting cells. A Graph representing CD19+ B cell number per mg of tissue (n=42). Due to logarithmic scale, null values were arbitrary replaced by 1 (Right). B Graph representing the number per mg of each CD19+B cell subsets C Illustration of the flow cytometry gating strategy to determine Ki67 and CD138 levels of expression on CD20⁺CD38⁻ (naïve and memory B cell), CD20⁺CD38^{int} (GC, pre-GC and Ti-B cell) and CD20⁻CD38^{hi} (ASC) (left). Graph representing the percentages of ASC depending on Ki67 and CD138 expression (n = 6) (right). D Graph depicting the percentage among CD45+ cells of each isotype on CD19⁺ and CD19⁻ ASC compartments. E FCM gating strategy to analyze IgG, IgA and IgM E Illustration of FCM gating strategy to analyze expression of IgG, IgA and IgM on memory B cells (left). Graph representing the percentage among B cells the percentage of each memory B cell subtype. Due to logarithmic scale, null values were arbitrary replaced by 1 (n=42) (right). F IgG, IgA and IgM quantification by ELISA in n = 72 soluble tumor medium (STM obtained during mechanical dissociation of fresh breast tumors).



Supplementary figure 2: Mainly IgG- and IgA-secreting cells infiltrate breast tumors and their density is associated with that of TLS. **A** Illustration of the strategy used to quantify IgG and IgA-ASC in IBC based on pan-CK/IgG/IgA staining using the HALO digital image analysis software. **B** Representative image of pan-CK/IgM IF staining showing low infiltration of IBC by IgM-ASC. **C** Representative images of pan-CK/IgG/IgA staining showing co-staining of CK with either IgA (top) or IgG (bottom). **D** Illustration of the staining of CD20⁺ B cells, IgG ASC and IgA ASC on TMA of the PACS08 Unicancer cohort using single color IHC. **E** Graphs representing the frequency of CD20⁺ B cells, IgG and IgA-secreting cells in TNBC VS luminal molecular subtypes. **F** Illustration of the strategy used to quantify B-cell follicle density in TN tumors based on CD20/CD3/DAPI IF staining using the HALO software. **G** Pie Chart showing the distribution of TNBC patients according to their TLS status. **H** Illustration of FCM gating strategy on cell suspensions prepared by enzymatic digestion of fresh invasive breast tumors to identify Tfh subsets among memory CD4⁺ T cells. **I** Illustration of FCM gating strategy to identify Tfh states of activation among CXCR5⁺ Tfh cells.



Supplementary figure 3: In situ breast tumors exhibited an elevated IgA- to IgG-ASC ratio. **A** Representative images of IRF4/Blimp1/ IgA staining on FFPE tissue sections NTT and DCIS. **B** Percentage of IgG- and/or IgA-ASC depending in Blimp1 or IRF4 expression in NTT (n=5) and DCIS (n=4). **C** Relative abundance of if IGHA1 and IGHA2 on the basis of TCGA transcriptional analysis (n=939). **D** Illustration of the strategy to analyze by flow cytometry IgA1 and IgA2- memory B cells on viable CD45⁺CD38^{Hi} cells (left). Bar plot depicting the percentages of IgA1-expressing B memory cells among IgA-memory B cells in IBC patients (n=8) (right).



Supplementary figure 4: IgG- and IgA antibody profiling using peptide array. **A** Study design to perform antibody profiling (left). Illustration of IgA and IgG positive signals in STM and serum for the same patient. P78358 uniprot code for NY-ESO1. **B** Box plot depicting the number of targeted epitopes for IgG, IgA and common to IgG and IgA in BC-sera (left), BC-STM (Middle) and HD-ser (right). **C** Graph representing the percentages of proteins with more than one peptide region recognized for IgG and IgA in the different fluids. **D** Scatter plot showing the ratio of IgA to IgG reactivities counts exclusively found in STM in ductal (IDC) VS lobular (ILC) invasive carcinoma (left). Pie chart depicting the percentage of IgA > IgG VS IgA < IgG reactivity counts in IDC and ILC (right). **E** Bar plots representing the percentage of private (present in one patient) VS shared (present in >1 patient) specificities for each isotype in each compartment. **F** Strategy adopted for pathways analysis. **G** Strategy adopted to determine a list for the top most frequent recognized proteins among patients.

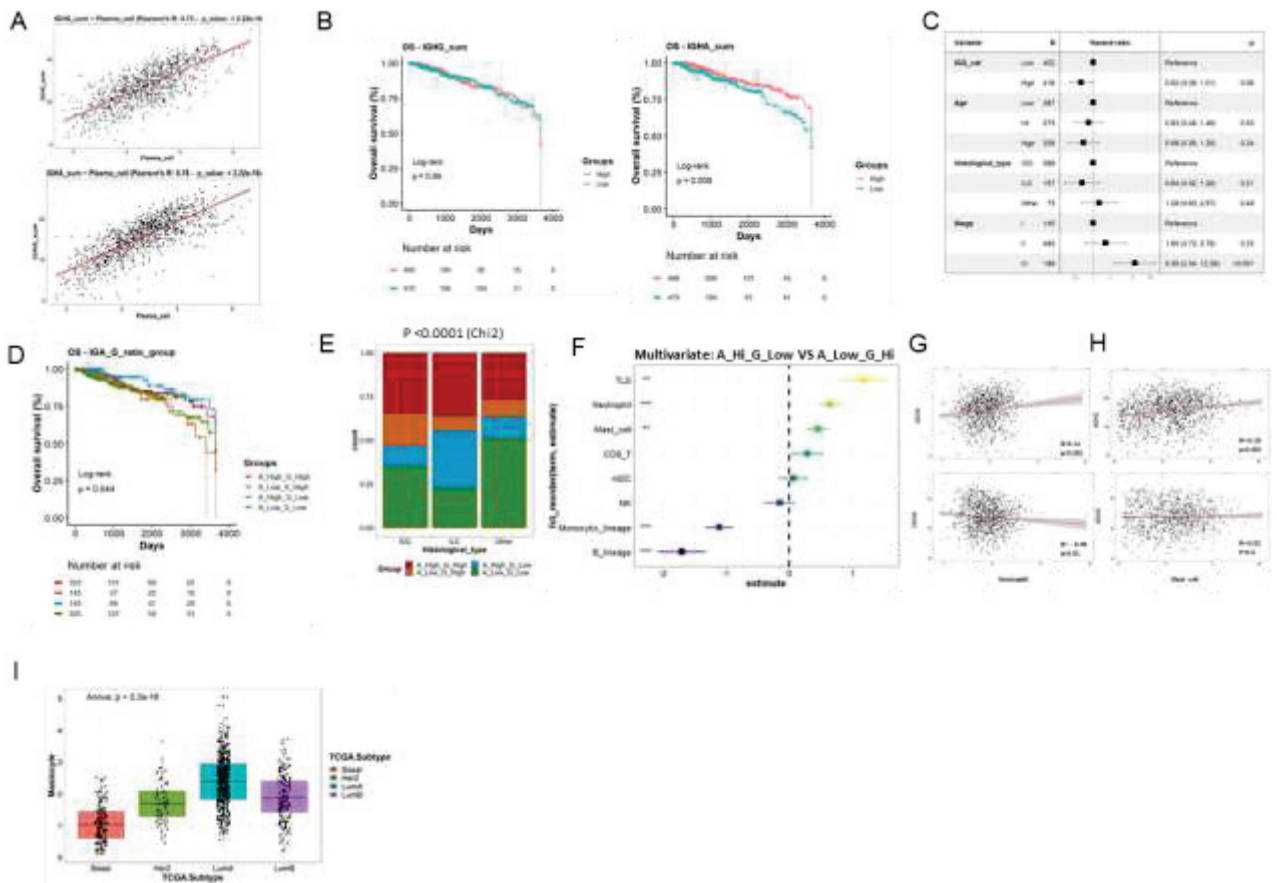
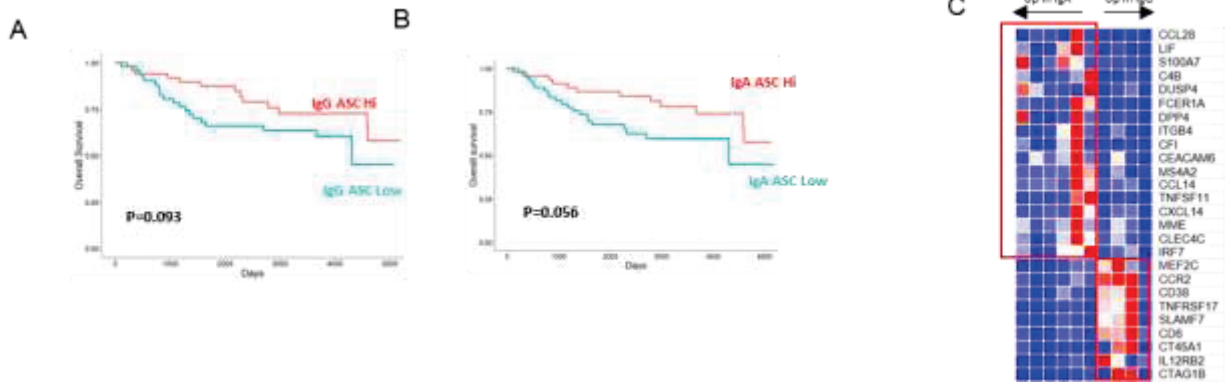
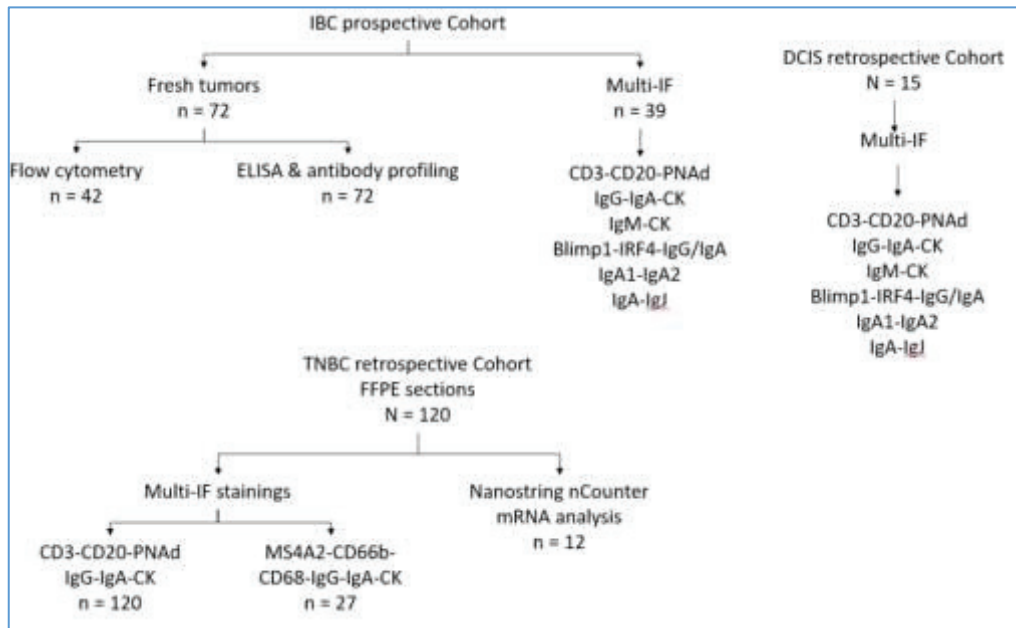


Figure 5: IGHA^{Hi}-IGHG^{Low} breast tumors are associated with favorable prognosis and are enriched in innate immune cell gene signatures. **A** Correlation plot between plasma cell gene signature and IGHG (top) and IGHA (bottom). **B** Kaplan-Meier analysis of the overall survival in the breast carcinoma cohort of TCGA database of IGHG representing the sum of IGHG1, IGHG2, IGHG3 and IGHG4 gene expression (left) and IGHA representing the sum of IGHA1 and IGHA2 gene expression (right). High and low levels of expression was determined using the median. Statistical significance was evaluated by the log-rank test. **C** Multivariate Cox analysis showing the effect of breast cancers prognostic factors on IGHG prognostic value. **D** Kaplan-Meier survival curves display OS for IGHG and IGHA on BC cohort of TCGA database. Data were divided into four groups based on IGHG and IGHA level of expression. **E**. Graph depicting the repartition of the four categories based on IGHG and IGHA depending on breast cancer histological types. **F** Graph depicting the association of each immune gene signature with the studied groups: IGHA^{Hi}-IGHG^{Low} and IGHA^{Low}-IGHG^{Hi}. Analysis was performed using multivariate logistic regression. **G** Correlation plots between neutrophil gene signature and IGHG (top) and IGHA (bottom) level of expression. **H** Correlation plots between mast cell gene signature score and IGHG (top) and IGHA (bottom) level of expression. **I** Box plot representing mast cell gene signature score in different breast tumor molecular subtypes.



Supplementary figure 6: IgA-ASC dominant TN breast tumors are associated with favorable prognosis and with innate immune response **A-B** Kaplan-Meier analysis of overall survival in TNBC cohort (n=120) of **A** IgG-ASC density and **B** IgA-ASC density. ASC densities were determined semi-quantitatively using pan-CK/IgG/IgA staining on FFPE section. Statistical significance was evaluated by the log-rank test. **D** Supervised heatmap representing significantly upregulated genes in IgA-dominant tumors (n=6) et in IgG-dominant tumors (n=4).



Supplementary figure 7: Retrospective and prospective cohorts used for flow cytometry, multi-IF staining, ELISA, antibody profiling and transcriptomic analysis for the present study

	Flow Cytometry n (%)	ELISA n (%)	Tissue multi-IF n (%)
Total	42 (100)	72 (100)	39 (100)
Age (median and range)	63 (35-90)	61.5 (32-91)	57 (39-88)
Histology			
Ductal	26 (62)	43 (60)	24 (62)
Lobular	11 (26)	23 (32)	9 (23)
Others	5 (12)	6 (8)	6 (15)
Histological grade (SBR)			
1	2 (5)	1 (1)	1 (3)
2	17 (40)	28 (39)	13 (33)
3	23 (55)	43 (60)	25 (64)
Size			
T1	2 (5)	1 (1)	2 (5)
T2	28 (66)	45 (63)	28 (72)
T3	10 (24)	23 (32)	8 (20)
ND	2 (5)	3 (4)	1 (3)
Node status			
negative	23 (55)	37 (51)	23 (59)
Positive	19 (45)	33 (46)	16 (41)
ND	0 (0)	2 (3)	0 (0)
Metastasis			
Yes	5 (12)	15 (21)	7 (18)
No	37 (88)	57 (79)	32 (82)
Lymphovascular Embolism			
Absent	26 (62)	43 (60)	24 (62)
Present	12 (28)	25 (35)	13 (33)
ND	4 (10)	4 (5)	2 (5)
HR			
ER-	8 (19)	21 (29)	13 (33)
ER+	34 (81)	51 (71)	26 (67)
PR-	11 (26)	26 (36)	18 (46)
PR+	31 (74)	46 (64)	21 (54)
HER2			
HER-	36 (86)	62 (86)	32 (82)
HER+	4 (9)	9 (13)	6 (15)
ND	2 (5)	1 (1)	1 (3)
Triple negative			
Yes	7 (17)	18 (25)	10 (26)
No	35 (83)	54 (75)	29 (74)
Neoadjuvant treatment			
Yes	0 (0)	11 (15)	4 (10)
No	42 (100)	61 (85)	35 (90)

Supplementary table S1: Characteristics of the prospective cohorts of breast cancer patients used for flow cytometry, ELISA and tissue multi-IF analysis

	n (%)
Total	15 (100)
Histological grade (SBR)	
1	3 (20)
2	2 (13)
3	10 (67)
Size	
<40mm	10 (67)
>40mm	5 (33)
Necrosis	
Yes	10 (67)
No	5 (33)

Supplementary table S2: Characteristics of the in situ ductal carcinoma samples used for immunofluorescence studies.

Antigen targeted	Conjugate	Dilution	Incubation temperature	Staining	Clone	Company
CCR6	PE-Cy7	1/20	37°	Surface	11A9	BD
CD138	PE	1/10	37°	Surface	44F9	Miltenyi
CD19	PE-CF594	1/50	4°	Surface	H1B19	BD
CD19	APC	1/10	4°	Surface	H1B19	BD
CD20	APC	1/10	4°	Surface	L27	BD
CD20	BB515	1/100	4°	Surface	2H7	BD
CD27	BV711	1/25	4°	Surface	O323	Biologend
CD27	BV421	1/25	4°	Surface	O323	Biologend
CD3	BV711	1/25	4°	Surface	UCHT1	BD
CD38	PerCP-Cy5;5	1/25	4°	Surface	HIT2	BD
CD4	APCef780	1/10	4°	Surface	RPA-T4	eBiosciences
CD45	AF700	1/25	4°	Surface	HI30	BD
CD45RA	BV650	1/25	4°	Surface	HI100	Biologend
CRTH2	PerCP-Cy5;5	1/20	37°	Surface	BM16	BD
CXCR3	VioBrightFITC	1/50	37°	Surface	REA232	Miltenyi
CXCR5	PE/Dazzle-594	1/10	37°	Surface	J252D4	Biologend
FoxP3	Ef660	1/20	4°	Intracellular	PCH01	Invitrogen
ICOS	Vioblue	1/10	4°	Surface	REA192	Miltenyi
IgA	FITC	1/200	4°	Surface & intracellular	Polyclonal	Southern Biotech
IgA1	FITC	1/25	4°	Surface & intracellular	B3506B4	Southern Biotech
IgA2	PE	1/25	4°	Surface & intracellular	A9604D2	Southern Biotech
IgD	BV510	1/10	4°	Surface	IA6-2	BD
IgG	PE	1/20	4°	Surface & intracellular	G18-145	BD
IgM	BV421	1/25	4°	Surface & intracellular	MHM-88	Biologend
Ki67	BV605	1/100	4°	Intracellular	Ki-67	Biologend
PD1	PE	1/20	4°	Surface	PD1.3.1.3	Miltenyi
Zombie Aqua	AF430	1/400	4°	Surface		Biologend
Zombie NIR	APCH7	1/2000	4°	Surface		Biologend
Zombie Yellow	BV570	1/400	4°	Surface		Biologend

Supplementary table S3: Antibodies used for flow cytometry staining

Antigen targeted	Species	Clone	Dilution	Company
Blimp1	Rat IgG2a	6D3	1/400	
CD20	Mouse IgG2a	L26	1/600	DAKO
CD3	Rabbit	Polyclonal	1/100	DAKO
CD66b	Mouse IgM	G10F5	1/200	BD
CD68	Mouse IgG3	PG-M1	1/80	DAKO
CK	Mouse IgG1	AE1/AE3	1/100	DAKO
IgA	Rabbit	Polyclonal	1/4000	DAKO
IgA1	Mouse IgG1	B3506B4	1/50	Abcam
IgA2	Rabbit	RM125	1/100	Abcam
IgG	Rabbit	Polyclonal	1/5000	DAKO
IgJ	Rabbit	Polyclonal	1/200	Atlas antibodies
IgM	Rabbit	Polyclonal	1/300	DAKO
IRF4	Mouse IgG	Mum1p	1/30	DAKO
Ki67	Mouse IgG2	MIB-1	1/100	DAKO
MS4A2	Rabbit	Polyclonal	1/200	Atlas antibodies
PNA _d	Rat			BD biosciences

Supplementary table S4: Antibodies used for multi-IF staining

Proteins	Extracell	CTA	IgA BC Ser	IgA BC STM	IgA HD Ser	IgG BC Ser	IgG BC STM	IgG HD Ser
ANKRD17	FALSE	FALSE	49%	21%	22%	60%	68%	74%
TNRC6C	FALSE	FALSE	45%	21%	30%	47%	46%	22%
NEB	FALSE	FALSE	38%	18%	9%	70%	72%	78%
FAM126B	FALSE	FALSE	36%	6%	9%	23%	23%	22%
RALGAP2	FALSE	FALSE	36%	13%	13%	21%	23%	13%
CACNA2D3	TRUE	FALSE	34%	14%	13%	43%	38%	17%
EVX2	FALSE	FALSE	28%	18%	13%	85%	87%	96%
PRR14L	FALSE	FALSE	28%	8%	13%	11%	13%	13%
AGL	FALSE	FALSE	26%	4%	4%	23%	30%	30%
NCOR1	FALSE	FALSE	26%	7%	13%	32%	35%	35%
SYNE1	FALSE	FALSE	26%	6%	9%	40%	38%	43%
UPF1	FALSE	FALSE	26%	8%	13%	53%	49%	43%
PREX1	FALSE	FALSE	25%	14%	9%	8%	10%	4%
SMC4	FALSE	FALSE	25%	3%	9%	6%	15%	22%
ACSM2B	FALSE	FALSE	23%	7%	4%	23%	20%	13%
ANK2	FALSE	FALSE	23%	15%	4%	40%	48%	57%
APC	FALSE	FALSE	23%	7%	9%	36%	38%	35%
ARHGAP29	FALSE	FALSE	23%	4%	9%	28%	23%	17%
CMPK1	FALSE	FALSE	23%	10%	4%	4%	13%	22%
DST	FALSE	FALSE	23%	13%	0%	43%	45%	48%
KIF3C	FALSE	FALSE	23%	11%	9%	36%	48%	52%
ZMIZ1	FALSE	FALSE	23%	8%	9%	13%	17%	9%
ZNF280C	FALSE	FALSE	23%	8%	9%	21%	24%	35%
CFAP47	FALSE	FALSE	21%	11%	9%	64%	69%	52%
DYNC2H1	FALSE	FALSE	21%	11%	9%	57%	48%	65%
MUC19	TRUE	FALSE	21%	14%	4%	36%	49%	57%
KY	FALSE	FALSE	19%	20%	9%	26%	27%	30%
ANK3	FALSE	FALSE	19%	11%	4%	15%	23%	26%
SIGLEC11	FALSE	FALSE	19%	11%	9%	64%	65%	65%
SYNE2	FALSE	FALSE	19%	11%	4%	47%	48%	35%
ATRNL1	TRUE	FALSE	17%	4%	13%	32%	24%	13%
CHD7	FALSE	FALSE	17%	8%	4%	40%	37%	17%
GLOD5	FALSE	FALSE	17%	4%	4%	19%	25%	9%
MYO16	FALSE	FALSE	17%	11%	4%	25%	30%	35%
CRER3L3	FALSE	FALSE	15%	14%	4%	42%	41%	43%
KNL1	FALSE	TRUE	15%	11%	13%	36%	41%	17%
RYR3	FALSE	FALSE	15%	11%	4%	40%	44%	26%
EFCAB13	FALSE	FALSE	13%	4%	0%	19%	25%	9%
KLC1	FALSE	FALSE	13%	4%	0%	28%	25%	9%
PLEKHA6	FALSE	FALSE	13%	6%	0%	28%	24%	13%
RTN3	FALSE	FALSE	13%	13%	4%	28%	30%	48%
SPTBN3	FALSE	FALSE	13%	6%	4%	28%	35%	13%
SRRM4	FALSE	FALSE	13%	11%	4%	28%	27%	17%
STK4	FALSE	FALSE	13%	3%	0%	30%	18%	9%
XKR4	FALSE	FALSE	13%	4%	9%	28%	23%	13%
ACVR2B	TRUE	FALSE	11%	14%	4%	51%	55%	57%
INCENP	FALSE	FALSE	11%	4%	0%	26%	27%	13%
MEGF8	TRUE	FALSE	11%	6%	13%	26%	25%	4%
MYH2	FALSE	FALSE	11%	6%	4%	40%	35%	17%
USP46	FALSE	FALSE	11%	11%	4%	15%	17%	35%
ZHX2	FALSE	FALSE	9%	13%	0%	23%	21%	0%
DGKH	FALSE	FALSE	9%	7%	9%	36%	32%	17%
KIF15	FALSE	FALSE	9%	7%	13%	28%	37%	13%
MYH1	FALSE	FALSE	9%	4%	4%	38%	32%	13%
MYH4	FALSE	FALSE	9%	3%	4%	34%	27%	9%
MYH8	FALSE	FALSE	9%	4%	0%	36%	27%	13%
MYO21	FALSE	FALSE	9%	1%	4%	28%	24%	9%
NAP1L1	FALSE	FALSE	9%	6%	0%	28%	18%	9%
NOG	TRUE	FALSE	9%	3%	4%	28%	21%	9%
NOTCH1	TRUE	FALSE	9%	4%	0%	30%	24%	13%
SAMD9	FALSE	FALSE	9%	1%	4%	34%	27%	13%
THOC2	FALSE	FALSE	9%	3%	4%	28%	15%	9%
XIRP2	FALSE	FALSE	9%	6%	13%	25%	25%	4%
ZNF483	FALSE	FALSE	9%	1%	0%	30%	18%	13%
NEDD9	FALSE	FALSE	8%	11%	0%	15%	20%	30%
BEST1	TRUE	FALSE	8%	6%	0%	28%	30%	9%
VWAB	FALSE	FALSE	8%	4%	0%	26%	25%	9%
NPC1	FALSE	FALSE	6%	7%	4%	32%	35%	13%
GOLGA5	FALSE	FALSE	6%	3%	0%	28%	14%	9%
KIF1A	FALSE	FALSE	6%	3%	0%	21%	25%	9%
SAFB	FALSE	FALSE	4%	4%	0%	28%	20%	13%
STKLD1	FALSE	FALSE	4%	4%	0%	28%	23%	13%
TAF15	FALSE	FALSE	4%	1%	9%	30%	30%	9%
ZNF397	FALSE	FALSE	4%	3%	0%	23%	25%	0%
STYXL2	FALSE	FALSE	2%	3%	0%	21%	25%	9%
TENM2	TRUE	FALSE	2%	3%	0%	32%	27%	9%
GALNT6	FALSE	FALSE	2%	0%	4%	36%	38%	17%
SNRK	FALSE	FALSE	2%	0%	0%	28%	21%	13%

Supplementary table S5: Most frequent targeted proteins among patients

2. Complementary results:

2.1. ASC at different stages of maturation infiltrate tumors

As already showed in our previous data (Figure 2 of ASC in breast tumors paper), we developed multiplex IF tissue staining involving Ab to IRF4 and Blimp1 to study ASC maturation state. Because this technique is likely not sensitive enough to detect low level of Blimp1 expression, we constructed a flow cytometry panel that includes Ab to IRF4, Blimp1 as well as the proliferation marker Ki67, with the aim to distinguish plasmablasts ($Ki67^+IRF4^+Blimp1^{Lo/-}$) from plasma cells ($Ki67^-IRF4^+Blimp1^+$) (Odendahl et al.2005). In tonsils, multi-IF staining revealed that most ASC were IRF4⁺ (white arrow) and Blimp1⁻ with a few detectable Blimp1⁺ ASC (green arrow) (Figure 13A). In contrast, flow cytometry analysis of tonsil cell suspensions showed that most ASCs expressed both transcription factors and that a minor fraction of them expressed Ki67 (Figure 13B). In both ovarian and colorectal tumors, 3 major populations of CD19⁺CD38^{hi} cells can be identified, e.g. IRF4⁺Blimp1⁻ cells likely corresponding to nascent ASCs, IRF4⁺Blimp1⁺ cells and IRF4⁻Blimp1⁺ cells (Figure 13C-D). There was also a significant proportion of cells expressing Ki67 indicating that some ASC are proliferating (Figure 13D). In addition, a fraction of CD38^{int} GC-like B cells, likely containing GC B cells, express highly Ki67 and were IRF4⁺Blimp1⁺ indicating their commitment to the ASC differentiation pathway. Overall, these results indicate that the ASC compartment present in ovarian and colorectal tumors is heterogeneous and composed of distinct stages of maturation. Such staining could not so far be performed on breast tumors due to absence of eligible samples in the last couple of months.

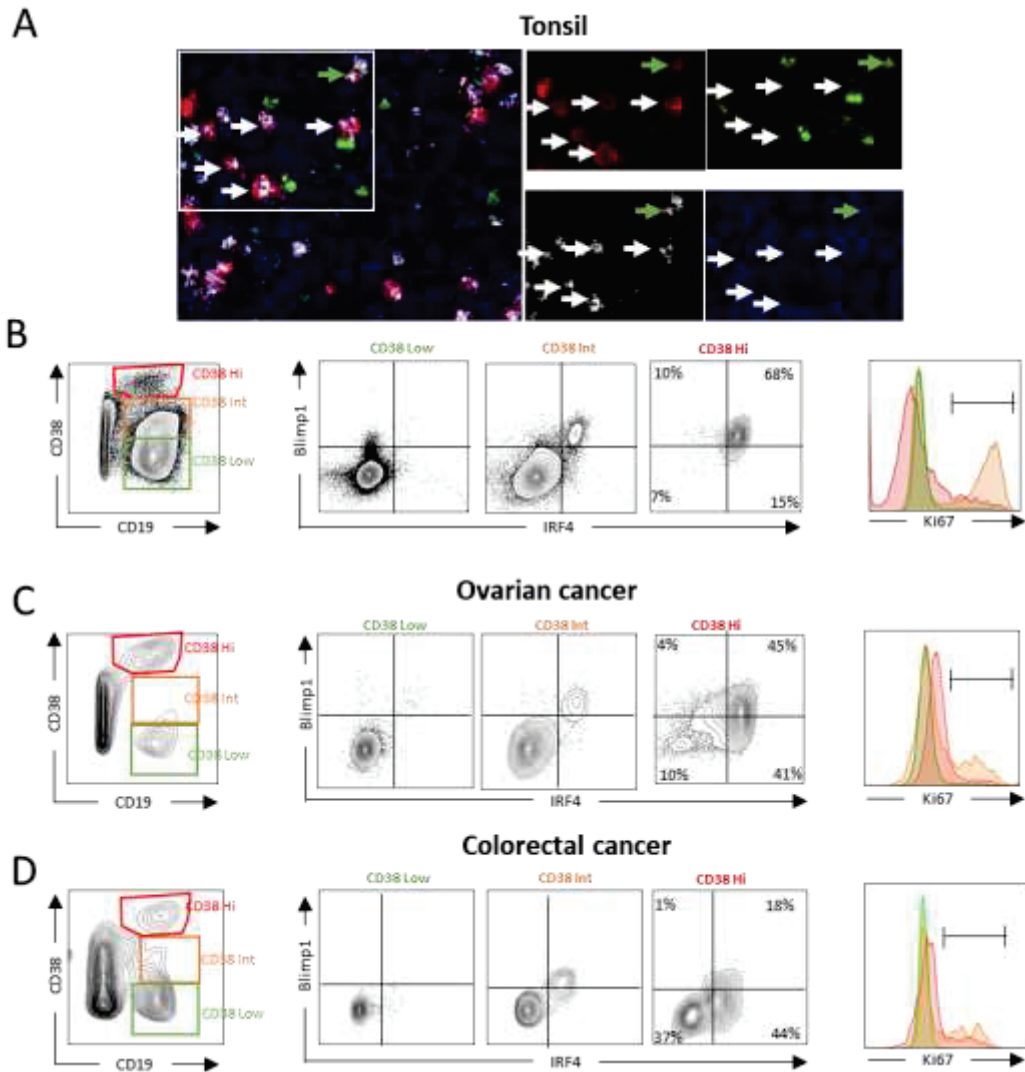


Figure13: ASC are present in tumors at different stages of differentiation. **A** Representative image of a tonsil FFPE section stained by multi-IF with Ab to IgG (Red), Blimp1 (Green), IRF4 (white) and counterstained with DAPI. IRF4+ ASC and Blimp1+ASC were highlighted with white and green arrows, respectively. **B-D** Cell suspensions obtained by enzymatic digestion of a fresh human tonsil (**B**), ovarian tumor (**C**) and colorectal tumor (n=1) were characterized by FCM with Ab to CD19, CD38 (surface staining) and Ab to Blimp1, IRF4 and Ki67 (intra-cytoplasmic/nuclear staining). Illustration of the gating strategy (left) and dotplots showing IRF4 vs Blimp1 expression on three populations of viable CD45+CD19+ cells: CD38low (containing naïve and memory B cells), CD38int (containing GC B cells) and CD38Hi (ASC). Representative overlaid histograms depicting Ki67 expression by the different populations (Right).

2.2. Breast tumors-infiltrating Tfh are composed of different subsets

We assessed by flow cytometry the composition of CD4⁺ T cell subpopulations on cell suspensions obtained after dissociation and enzymatic digestion of fresh breast tumors (n = 10). Among CD45⁺ live cells, memory CD4⁺ T cells were identified as CD45⁺ CD3⁺ CD4⁺ CD45RA⁻ cells and further separated into Foxp3⁺ regulatory T cells and Foxp3⁻ effector T cells (Figure 14A). Based on chemokine receptors expression, four Th subsets were identified e.g. Th1 (CXCR3⁺CCR6⁻CXCR5⁻), Th17 (CXCR3⁻CCR6⁺CXCR5⁻), Th1/17 (CXCR3⁺CCR6⁺CXCR5⁻) and Tfh (CXCR5⁺) cells. T follicular regulatory (Tfr) could also be distinguished based on FoxP3 and CXCR5 co-expression. Th1 predominated in most tumors with a mean of 78% and ranging from 58% to 99.2%. Th17, Th1/17, Tfh and Treg subsets were observed at much less proportions (Figure 14B). Next, we evaluated the percentage of each Tfh subset and Tfr within CD4⁺CD45RA⁻ T cells. Most tumors exhibited a high proportion of Tfh1 (mean = 22.6%, min = 5.2%, max = 40.7%), followed by Tfr (mean = 7%, min = 0.07%, max = 15.8%), Tfh1/17 (mean = 3.12%, min = 0.3%, max = 9%) and finally Tfh17 (mean = 0.35%, min = 0%, max = 0.8%) (Figure 14C). We observed a strong correlation between CXCR5⁺ Tfh population and Tfr subset (r=0.87, p = 0.001) (Figure 14D). Interestingly, the distribution of T cell subsets based on their profile of chemokine receptors expression in CXCR5⁻ Th cells mirrored that of CXCR5⁺ Tfh (Figure 14E). Tfh can display different states of activation that determine their function and several subsets have been identified in breast tumors according to PD1 and ICOS expression (Gu-Trantien et al.2017, Noël et al.2021). In our dataset, 3 populations of CXCR5⁺ Tfh can be distinguished according to PD1 expression: PD1^{Low} cells, PD1^{Int} cells and PD1^{Hi} cells (Figure 14F), the latter containing a substantial proportion of cells expressing ICOS (Figure 14G) and thus likely corresponding to GC-Tfh. Together, these data demonstrate that CXCR5⁺ Tfh infiltrating breast tumors are phenotypically heterogeneous suggesting that they display distinct functions.

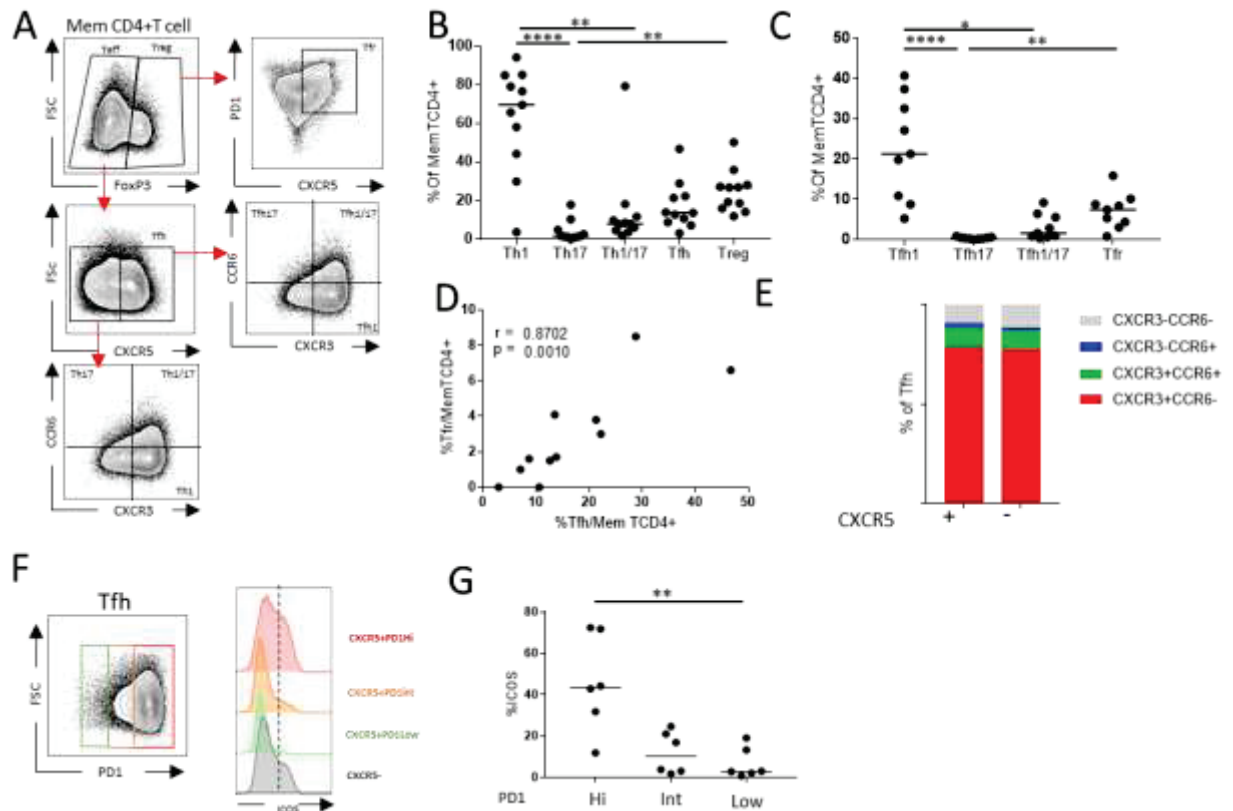


Figure14: Heterogeneous population of T follicular helper cells (Tfh) infiltrate breast tumors. **A** Illustration of FCM gating strategy of cell suspensions obtained with enzymatic digestion of fresh human invasive breast tumors to identify Tfh subsets among memory CD4⁺T cells. **B** Scatter plot depicting frequency of T helper cell subsets among memory CD4⁺ T cells (n=10). Only statistically significant associations are shown. **C** Scatter plot depicting the frequency of Tfh subsets among memory CD4⁺ T cells (n=10). Only statistically significant associations are shown. **D** Stacked bars showing the percentage of Th subsets regarding their CXCR3 and/or CCR6 expression in CXCR3⁺ and CXCR3⁻ Th populations. **E** Correlation of Tfh with T follicular regulatory cells (Tfr). **F** Illustration of FCM gating strategy to identify Tfh states of activation among CXCR5⁺ Tfh cells. **G** Scatter plot showing the frequency of ICOS expression depending on PD1 level in CXCR5⁺ Th population (n=6). Only statistically significant associations are shown.

3. TLS association with tumor antigens and the quality of the immune response

TLS are present in tumors at variable proportions and have been associated with a more favorable patients' prognosis and increased responses to therapies (Sautès-Fridman et al. 2020). Recently, several studies reported that TLS convey different prognostic significance according to their spatial distribution and maturation state. TLS, particularly those harboring GC, correlate with Th1, Tfh, T-cell activation and cytotoxic gene signature in malignancies indicating that TLS shape the anti-tumor immune response (Noël et al. 2021). Furthermore, they constitute privileged sites of interactions between T and B cells favoring oligoclonal responses (Coronella et al. 2002; Cipponi et al. 2012; Meylan et al. 2022). However, the nature of the Ags that drive or amplify such immune responses in TLS are not yet defined. It may be hypothesized that TLS preferentially develop in the most immunogenic tumors, e.g. those harboring high loads of mutation-derived neoantigens and/or overexpressing other types of tumor antigens like cancer testis Ags, differentiation Ags or retroviral endogenous elements.

To study the link between TLS and expression of tumor antigens and the quality of the immune response, we first established a retrospective cohort of non-treated TNBC patients (n=120) with available FFPE tumor samples. We performed a multi-IF/IHC staining on FFPE sections of all patients to determine their TLS status. In parallel, we generated whole exome sequencing (WES) and RNA sequencing (RNAseq) data from the patients with available frozen tumor material (n=45) to determine the tumor mutational burden (TMB), predict candidate neoantigens and measure expression of tumor associated Ags including HERVs and rely these different metrics to the presence of TLS. RNAseq data were also used to identify differentially expressed genes and pathways between TLS-Hi and TLS-Lo patients in order to gain insight into the favorable outcome of these structures. Eventually, we aimed to define a robust TLS gene signature in order to evaluate the link of TLS with expression of tumor antigens using public databases.

3.1. Link between TLS, mutational burden, neoantigens, CTA and HERV *TLS stratification and impact on patient survival.*

We stained FFPE tumor sections from our TNBC cohort (n=120) (see manuscript in preparation for detail on its clinical characteristics) with a multi-IF panel containing Ab to CD3, CD20, IgG, IgA and CK and used digital images to visualize and manually annotate TLS, based on the existence of segregated dense T cell aggregates and B cell aggregates/follicles (Figure 15A). IgG and IgA ASC were usually detected in the vicinity of these TLS. Some patients contained B cell follicles with a GC as judged i) by their higher CD20 intensity compared to surrounding B cells (Figure 15A) and ii) the presence of proliferating (Ki67+) cells and CD4⁺PD1^{Hi} cells (e.g. Tfh) in the same area of a serial section stained for CD4, CD8, PD1, Ki67 and CK (Figure 15B). Most patients (83%) exhibited at least one TLS and one fourth harbored TLS-containing GC (Figure 15C). Kaplan Meyer curves showed that patients with GC positive or negative TLS had prolonged OS (although not reaching statistical significance) and significantly increased PFS, with a tendency for a better impact of TLS with GC (Figure 15D). We next quantified the density of TLS by calculating the percentage of tumor surface occupied by B cell follicles, based on the total tumor surface and B cell follicle surface measured using the Halo software (Figure 15E). Categorization of patients as TLS^{Hi} and TLS^{Low} using the median of the afore-mentioned metric (Figure 15F) revealed a strong positive association of TLS density with overall survival (OS) (p=0.0034) and disease-free survival (DFS) (p=0.0043) (Figure 15G).

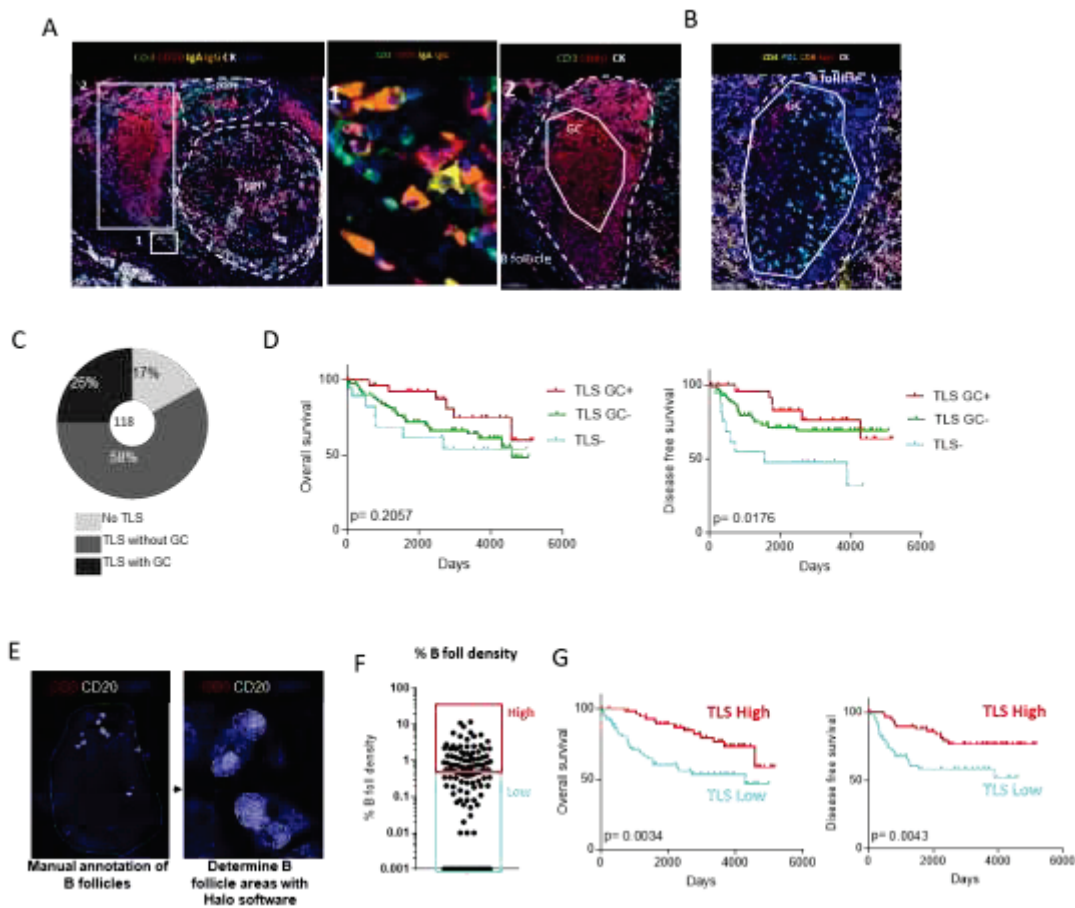


Figure 15: Tertiary lymphoid structures density correlates with better prognosis in TNBC patients. **A-B** Representative multiplexed immunofluorescent staining of tumor-associated TLS on FFPE sections of TNBC patients. **A** Tumor-infiltrating lymphocytes were revealed by CD20 (Red), CD3 (Green), IgA ASC (Yellow) and IgG ASC (Orange). Tumor cells were revealed by panCK (White). **B** Germinal centers were identified by the presence of T follicular helper CD4+PD1Hi (Cyan) cells, and proliferating GC-B cells Ki67+ (Red) cells. **C** Pie chart showing the distribution of TNBC patients according to their TLS status. **D** Overall survival (OS) and disease-free survival (DFS) analysis based on TLS status (n=118). Log-rank test was used for OS analysis. **E** Illustration of the strategy used to quantify B-cell follicle density in TN tumors based on CD20/CD3/DAPI IF staining using the HALO software. **F** Scatter plot representing B cell follicle densities for each patient used as a metric for TLS density. The median was selected to determine TLS High VS TLS Low patients. **G** Overall survival (OS) analysis derived from stratification of TNBC patients based on the median of TLS densities (n=118). Log-rank test was used for OS analysis.

We also quantified (in a qualitative manner) the density of the other cell types in the “TLS” multi-IF panel in order to analyze the association between TLS and T and B cells, as well as DC-LAMP+ mature DC that are commonly used in other tumor types as surrogates of TLS presence (Figure 16A). As expected, tumor with high densities of CD3+ T cells, CD20+ B cells or DC-LAMP+ DC displayed higher densities of TLS (Figure 16B). Moreover, B cell infiltration positively correlated with a better OS ($p=0.015$) and DFS, although the latter association was not statistically significant ($p=0.16$) (Figure 16C). Finally, we also observed a tendency for a positive impact of CD3+ T cells and DC-Lamp+ DC (Figure 16D, figure 16E). Altogether, these data demonstrate that TLS are preferentially present in tumors highly infiltrated by B cells, T cells and mature DC and that the density of B cell follicles better predicts patient survival than that of T cell zone and mature DC.

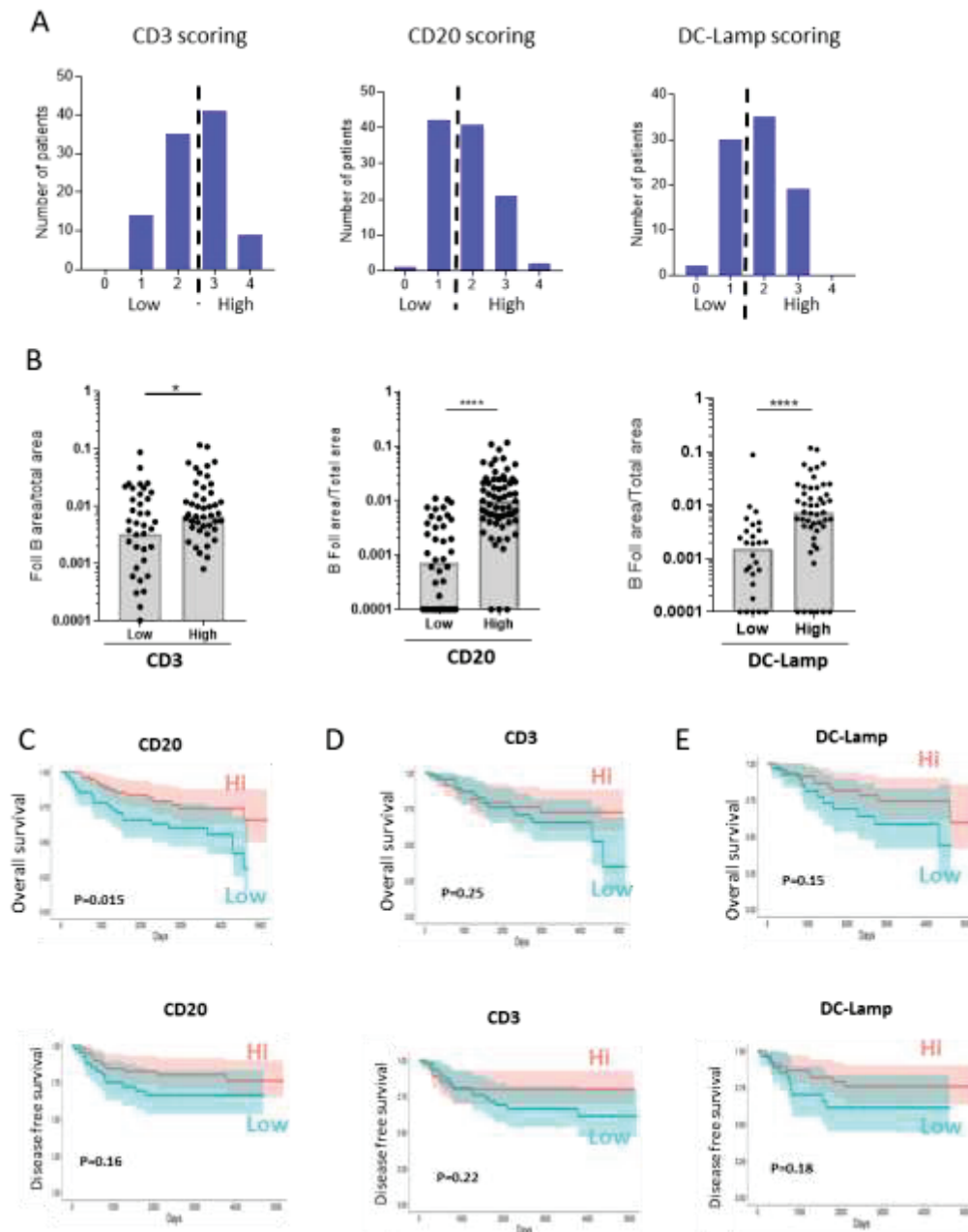


Figure 16: Tertiary lymphoid structures density are associated with immune infiltrate. A Semi-quantitative analysis of CD3+, CD20+ and DC-Lamp+ cells. Dashed lines represent the selected threshold to stratify patients in high and low. **B** Bar plot comparing the frequency B follicle density in high VS low CD3+, CD20+ and DC-Lamp+ cells. **C** OS and DFS analysis derived from stratification of TNBC patients based on high VS low CD20 densities (n =118). **D** OS and DFS analysis derived from stratification of TNBC patients based on high VS low CD20 densities (n = 99). **E** OS and DFS analysis derived from stratification of TNBC patients based on high VS low CD20 densities (n = 86). Log-rank test was used for OS and DFS analysis

Link between TLS, TMB and number of putative T cell neoepitopes

In order to evaluate a potential link between TLS and antigenicity, we profiled the TMB and neoantigen load in TNBC patients with available frozen tumor samples and matched PBMC (n = 45) by combining WES, RNAseq and computational analysis. The WES data were obtained using genomic DNA extracted from frozen tissue samples and patient-matched peripheral blood mononuclear cells and were analyzed to identify somatic mutations using Mutatec2. HLA alleles were predicted for each patient from RNA-seq data using Optitype. Finally, NetMHCpan4.0 was employed to predict affinity of mutated peptides to MHC-class I molecules and identify candidate CD8+ T cell neoepitopes (Figure 17A). We did not perform the same analysis for class II neoepitopes since most current publicly available algorithms cannot reliably predict peptide affinity to MHC class-II alleles. TLS densities determined by multi-IF on FFPE and frozen sections, TMB and class I neoepitopes numbers are shown for each patient in figures 17B and 17C. TLS densities determined from FFPE vs frozen tissue sections did not correlate, likely due to the small size of frozen sections compared to their FFPE counterparts and to the general bad quality of the staining for frozen samples, which complicated reliable identification and annotation of B cell follicles (data not shown). Therefore, we decided to use TLS densities determined on FFPE sections for further analysis.

As expected, the TMB and number of predicted class I neoepitopes was highly variable between patients but both metrics were positively correlated ($r = 0.79$, $p < 0.0001$) (Figure 17D). To investigate whether tumor antigenicity depended on tumor intrinsic molecular subtypes, we used the PAM50 tool and observed that 55% of tumors displayed a basal-like subtype (Figure 17E). TMB and class I neoepitopes number were increased in basal-like tumors compared to other subtypes indicating a higher antigenicity (Figure 17F). No significant correlation was observed between TLS density and either the TMB or number of predicted neoepitopes, although some patients with a high % of B cell follicles had an elevated TMB and a high number of putative CD8 epitopes (Figure 17G). However, because i) tumor cellularity, estimated by a bio-pathologist on hematoxylin & eosin (HE) stained slide, positively correlated with TMB ($r = 0.56$, $p = 0.0003$) (Figure 17H) and number of predicted neoepitopes (data not shown), and ii) about half of the TLS-Hi tumors have a low tumor cell purity (Figure 17I), we cannot exclude a technical bias, which could be bypassed by performing WES on laser micro-dissected tumor islets. Because TLS density (FFPE) and TMB/neoepitopes (Frozen) were not defined using the same block, we also calculated a TLS enrichment score using a 7-hallmark TLS gene signature (*CXCL13*, *CXCR5*, *CCL19*, *CCL21*, *CCR7*, *SELL*, *LAMP3*) (Cabrita et al. 2020) from frozen RNA-seq data. As showed in figure 17J, no correlation was found between the TLS gene signature score and either the TMB or number or putative neoepitopes.

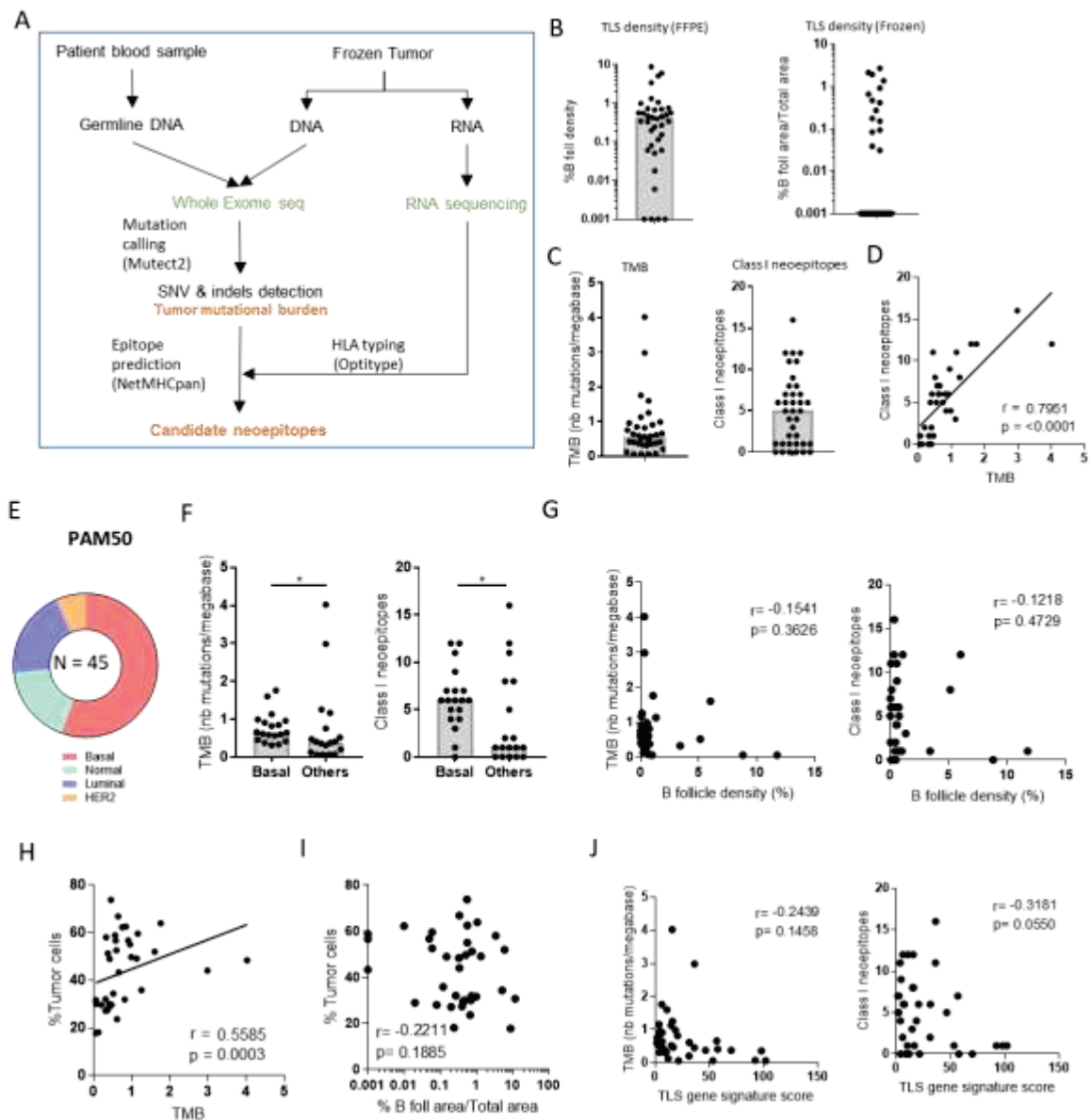


Figure 17: Tertiary lymphoid structures density is not linked with tumor mutational burden (TMB) and class I neoepitopes. **A** Integrative genomics and bioinformatics approach for the identification of neoantigens in TNBC patients (n = 45). **B** Bar plots representing TLS density determined by IF on FFPE and frozen sections, **C** Bar plots depicting TMB and class I neoepitopes number among TNBC patients. **D** Correlation plot between TMB and class I neoepitopes (n=37). **E** Pie chart depicting the distribution of molecular subtypes according to PAM50 technique (n=45). **F** Bar plots comparing TMB and number of class I neoepitopes between basal and other TNBC molecular subtypes (n=37). **G** Correlation plot between TMB and tumor cell fraction (n=37). **H** Correlation plot between TLS density and tumor cell fraction (n=37). **I** Correlation plot between TLS density (determined by IF on FFPE section) and TMB (left) and class I neoepitopes (right). **J** Correlation plot between TLS gene signature score (determined on frozen section) and TMB (left) and class I neoepitopes (right)

Link between TLS and other classes of tumor Ags

Next, we sought to assess the link between TLS and tumor-associated antigens, namely endogenous retroviral elements (HERV) and cancer-testis antigens (CTA). For this purpose, we stratified patients into TLS^{Hi} (n = 10) vs TLS^{Low} (n=17) by removing patients with intermediate TLS densities (Figure 18A) and performed a differential gene expression analysis between the two patients categories focusing on HERV gene families and CTA genes. Volcano plots revealed significant upregulation of 31 HERV genes and 10 CTA genes in TLS-Hi tumors, while only 5 HERV genes and 1 CTA gene were downregulated (Figure 18 B-C). Furthermore, supervised patient clustering showed that TLS-Hi patients usually overexpressed several HERV or CTA, including 3 patients that overexpressed most HERV genes (yellow rectangles) and 2 patients that upregulated 5-8 out of the 10 CTA genes (red rectangle) (Figure 18D). Quite interestingly, virtually all TLS-Hi patients displayed increased expression of HERV and/or CTA genes, indicating a link between these classes of tumor Ags and TLS development or maintenance.

Collectively, these data indicate that depending on patients, TLS may be associated to neoantigens, CTA or HERV but probably not all classes of tumor antigens in the same patient.

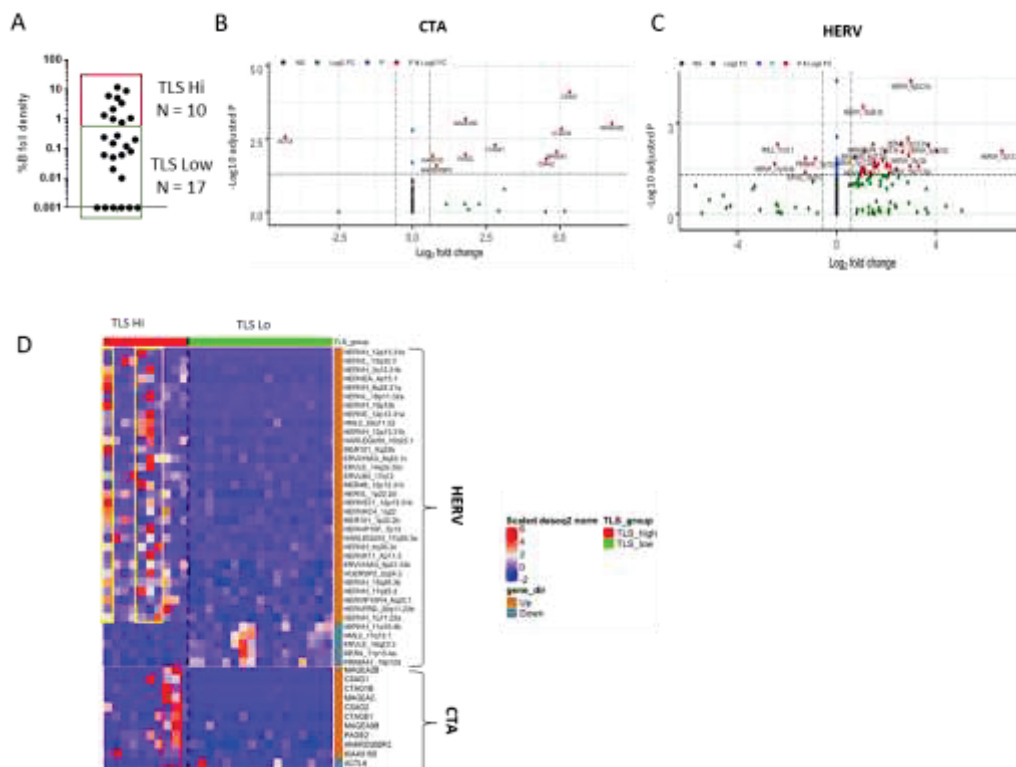


Figure 18: Link between TLS and other tumor antigens classes. **A** Scatter plot representing high VS low TLS density groups determined by IF on FFPE sections (n=27). **B** Volcano plot depiction of differential expression of CTA genes stratified on high VS low TLS density. **C** Volcano plot depiction of differential expression of HERV genes stratified on high VS low TLS density. **D** Heatmap of specifically up and downregulated HERV and CTA genes in TLS high patients.

3.2. Lymphocyte organization in TLS correlate with signs of an efficient anti-tumor response

We then used RNAseq data to infer the quality of the immune response associated with the presence of an organized immune infiltrate in TNBC patients. To do so, we first selected tumor samples with a similarly high T and B cell infiltrate and stratified them according to the density of B cell follicles determined by multi-IF analysis of FFPE tumor sections (Figure 19A, figure 19B). To avoid false TLS-Low tumors (due to sampling issues) we also took into account the multi-IF data on frozen sections that were prepared from a different tumor block. We ended up with 13 TLS^{Hi} and 8 TLS^{Lo} tumors matched for their immune infiltrate (Figure 19C). Remarkably, despite the small size of the cohort, organization of the immune infiltrate in TLS was strongly associated to a better OS ($p = 0.0052$) and DFS ($p = 0.0032$) (Figure 19D), revealing the mere importance of TLS over the density of immune cells.

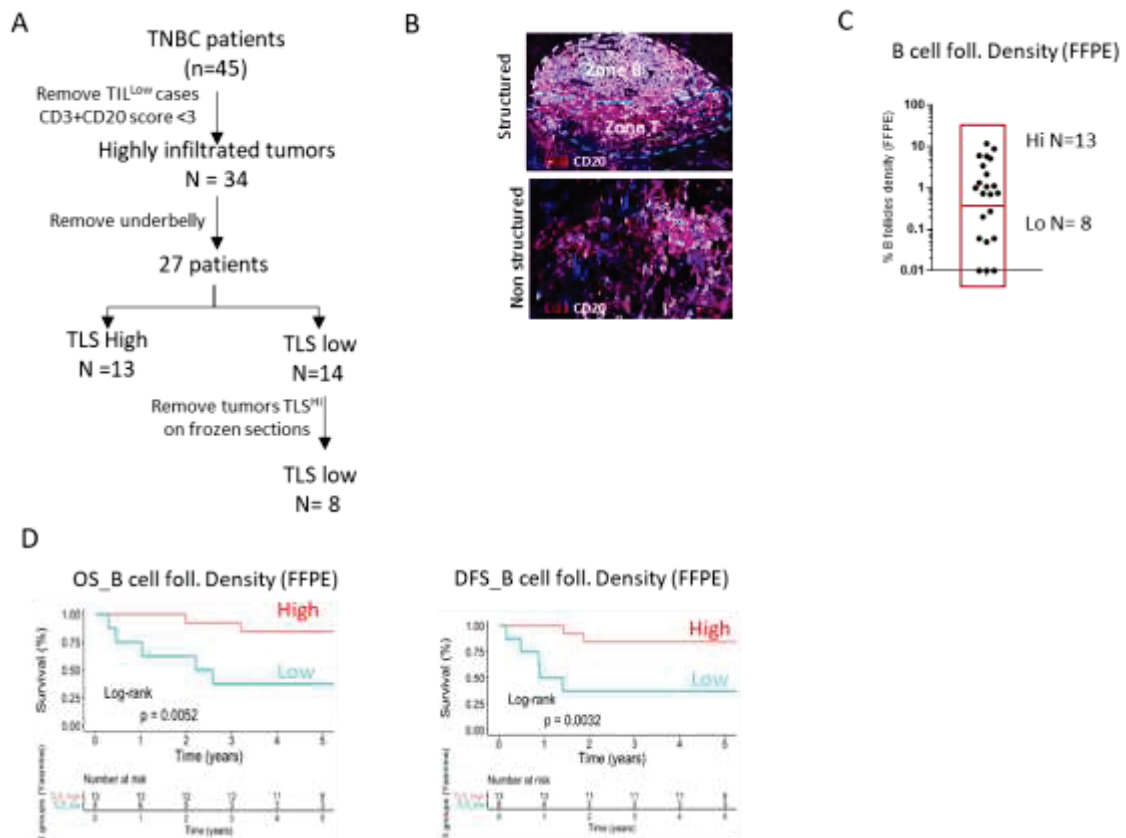


Figure 19: Structured immune infiltrate is strongly associated with a favorable outcome **A** Used strategy for patients' selection and stratification. TLS low group is similarly infiltrated to B follicle density high group. Patients with high B follicle density on frozen section and low density on FFPE section were eliminated to prevent false-negative cases. **B** Representative immunofluorescence images showing organized immune infiltrate in TLS (top) versus non-organized immune infiltrate (bottom). **C** Scatter plot representing high versus low B follicle density groups determined for RNAseq analysis (n=21). **D** OS and DFS analysis of TNBC patients based on high versus low B follicle densities (n=21). Log-rank test was used for DFS analysis.

Differential gene expression analysis revealed 128 upregulated and 12 downregulated genes in TLS-Hi tumors compared to TLS-Low tumors (Figure 20A). Significantly higher expression of lymphoid chemokines (*CXCL13*, *CCL19*, *LTA*, orange arrows), T/B cell homing receptors (*CCR7*, *SELL*, green arrows) and B cell related genes (*MS4A1*, *PAX5*, *IL21R* blue arrows) was observed in TLS^{Hi} patients. Genes involved in Th1-oriented immune response and cytotoxicity such as *IFNG*, *CXCR3*, *CXCL9* and *GZMB* (red arrows) also characterized breast tumors with a high TLS density. Similar trends were found for genes encoding immune checkpoint molecules, including *ICOS*, *PDCD1*, *CTLA4*, *TIGIT* and *LAG3* (grey arrows) (Figure 20B). Interestingly, many transcripts of heavy and light chains immunoglobulin (Ig) genes were upregulated in TLS^{Hi} patients (Table 20C), suggesting an oligoclonal B cell expansion. Gene set enrichment analysis revealed a variety of pathways in patients with high TLS density including those related to T-cell activation, differentiation and regulation, to Th1 cytotoxicity, cell killing and humoral immune response as top hits. Down-regulated genes were involved mainly in hypoxia pathways as well as oncogene/tumor suppressor pathways; P53 and CTNNB1 (Figure 20D). Overall, these findings demonstrate that the structuration of the immune infiltrate is associated with an upregulation of genes involved in lymphocyte migration, Th-1 oriented immune response, cytotoxicity and humoral immune response.

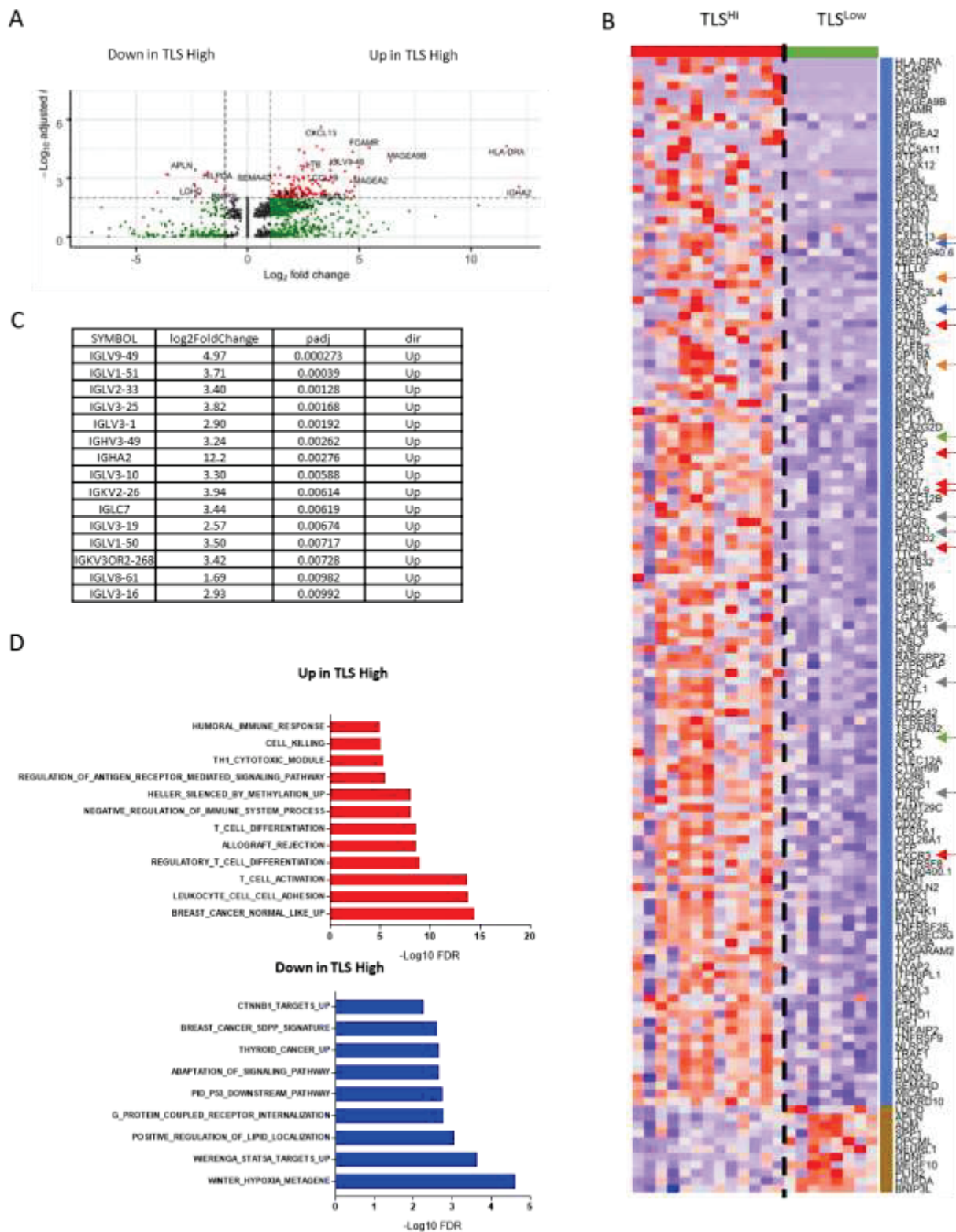


Figure 20: TLS-enriched tumors are associated with immunoglobulin genes, Th1-oriented immune response and cytotoxicity. A Volcano plot depicting differential expression genes between TLS^{Hi} and TLS^{Low} patients. Threshold $p \leq 0.01$, fold-change ≥ 2 . We found 128 genes upregulated and 12 genes downregulated. **B** Supervised heatmap of up and down regulated genes in TLS^{Hi} patients. Dashed line separate TLS^{Hi} VS TLS^{Low} tumors. **C** List of immunoglobulin genes found after DEG analysis. 15 upregulated and 0 downregulated in TLS^{Hi} tumors. **D** Pathway enrichment analysis (hallmark sets and Gene Ontology) of DEGs between TLS^{Hi} and TLS^{Low} patients. We found 396 upregulated pathways & 66 downregulated pathways.

3.3. Towards defining a robust TLS gene signature in TNBC

In order to further evaluate the relationship between TLS density and expression of tumor antigens using publicly available breast cancer databases, and possibly datasets from other tumor types, we aimed to develop a home-made TLS gene signature based on our matched RNA-seq/multi-IF TNBC dataset, that we expect to be more reliable than the signatures published so far. Based on the upregulated genes identified in TLS^{Hi} tumors (Figure 20B), we constituted combinations of 20 genes that we correlated with the density of B cell follicles (repeated 1000 times) to select the most correlated set of genes and then the most frequent genes in the different combinations (Figure 21A). The 9-genes signature that emerged included *TNFRSF9*, *ATF6B* and *SSTR3* that are known to play an important role in T cell activation, cytotoxicity and adhesion, respectively. The remaining genes included among others the CTA *MAGEA2* and *CSAG1* (Figure 21B). We found that our developed TLS gene signature correlated better with B cell follicle densities ($r = 0.73$, $p = 0.00023$) than the compendium of TLS-hallmark genes constructed by Cabrita et al. ($r = 0.43$, $p = 0.051$) (Figure 21C). Given the fact that our gene signature included two CTA, making it probably TNBC specific, we tried to exclude all CTA genes to define a more broadly applicable gene signature using the same approach (Figure 21A). We ended up with a 3-genes signature involving *ATF6B*, *ADD2* and *ZBED2* that correlated with TLS density quite well ($r=0.68$, $p = 0.00094$) (Figure 21D), albeit less than the 9-genes signatures. Finally, we evaluated the prognostic impact of these 2 TLS-gene signatures on TNBC patients of the TCGA cohort ($n=195$) (Figure 21F & 21G). High 9-gene TLS signature score tended to be associated with a better PFS and OS, although it did not reach significance, while the 3-gene TLS signature score correlated with a favorable outcome. Validation of the 2 gene signatures has to be performed with an independent TNBC data set and will be extended to other types of cancers, including melanoma and ovarian cancers.

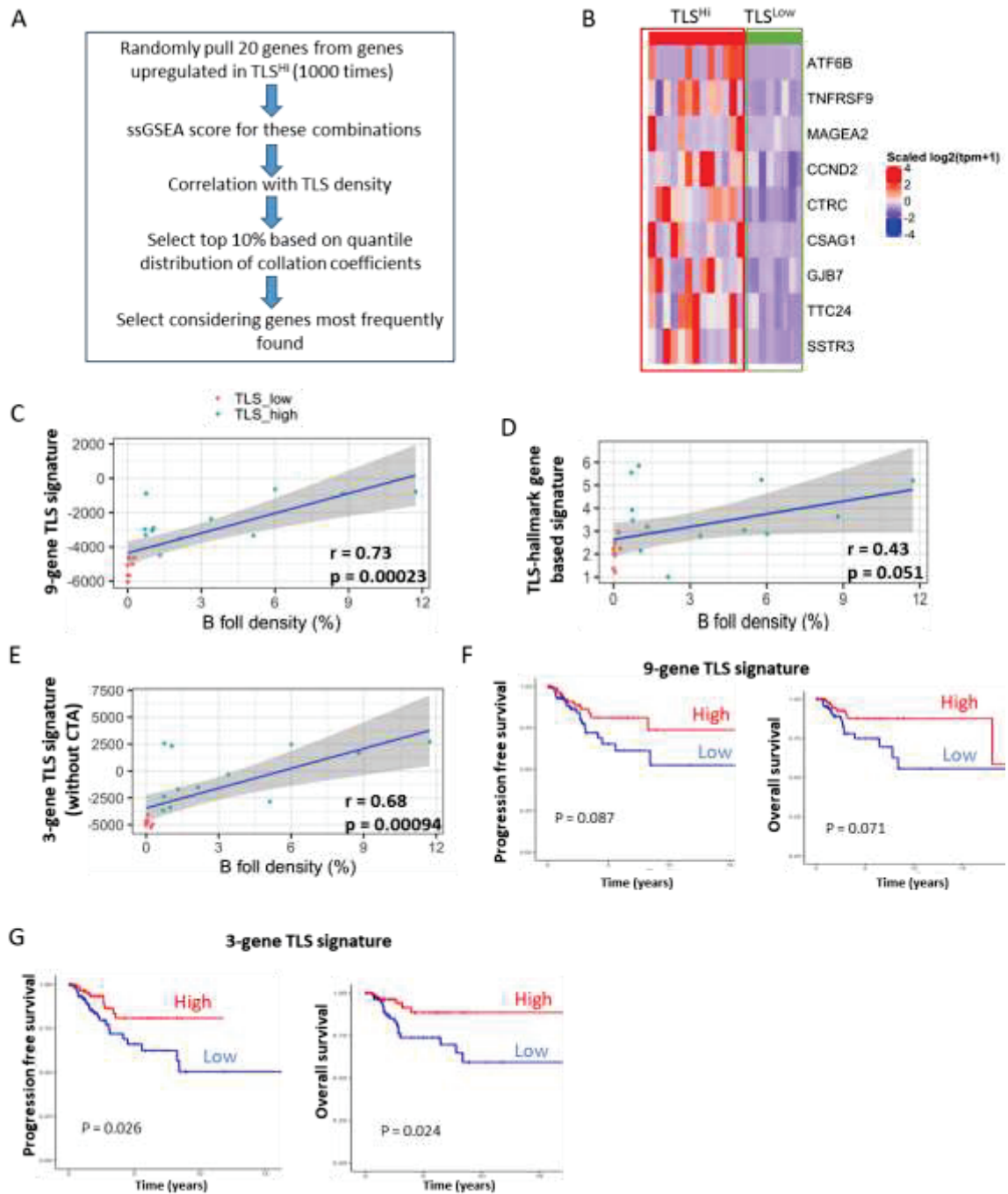


Figure 21: Tumor-associated TLS gene signature development. **A** Strategy utilized to identify TLS gene signature. **B** Heatmap of our built 9-gene TLS signature in tumors TLS^{Hi} VS TLS^{Lo}. **C** Correlation plot between B follicle densities of patients and our developed 9-gene TLS signature. **D** Correlation plot between B follicle densities and TLS-hallmark gene based signature. **E** Correlation plot between B follicle densities of patients and our developed 3-gene TLS signature (without CTA). **F-G** Kaplan-Meier analysis of the prognostic impact of **F** 9-gene TLS signature score and **G** 3-gene TLS gene signature score on TNBC cohort of TCGA (n = 195). The median was used as a cut-off of high and low score. Statistical significance was evaluated by the log-rank test.

DISCUSSION

1. IgG-dominant VS IgA ASC-dominant tumors are characterized by distinct immune microenvironments

We observed an interpatient heterogeneity regarding IgG and IgA ASC densities in breast tumors with a dominance of IgG ASC in most IBC cases, whereas a minor fraction of tumors were mainly infiltrated by IgA ASC. This could be linked to: 1) the nature of chemokine gradients in the TME that may drive a preferential recruitment of one or the other ASC population from blood and/or 2) the local factors that can condition isotype switching towards IgG vs IgA within TLS.

Both IgG and IgA ASC express CXCR4 allowing their migration to the bone marrow. Nevertheless, they also display distinct chemokine receptors. Whereas IgG ASC express CXCR3 (Lacotte et al. 2013; Chihara et al. 2013; Marques et al. 2011), IgA ASC upregulate CCR10 and/or CCR9 (Kunkel et Butcher 2003). Furthermore, Kroeger et al. showed that IgG PC infiltrating human ovarian tumors universally expressed CXCR3, suggesting that these cells might migrate from the periphery to the inflamed tissue in response to CXCL9, CXCL10, CXCL11, which are chemokines that are usually detected in cancer tissues (Kroeger, Milne, et Nelson 2016). CXCR3 ligands are also involved in the recruitment of activated Th1 cells, CTL and NK cells, and may thus be instrumental for assembling an anti-tumor immune microenvironment (Tokunaga et al. 2018). Differential gene expression analysis using the Nanostring technology between IgG and IgA ASC dominant tumors showed an upregulation of CXCR3 gene expression in IgG-rich tumors, albeit this did not reach statistical significance (log₂ fold-change = 0.9, Adj p= 0.3). Besides, this analysis revealed an upregulation of CCL28 expression in IgA ASC rich tumors. CCL28, which is CCR10 ligand, has been described as a potent and selective chemoattractant of IgA ASC in diverse mucosal tissues, including mammary glands, indicating that this chemokine may be involved in IgA ASC recruitment (Lazarus et al. 2003; Wilson et Butcher 2004). According to Gong et al., CCL28 expression was associated to better patient survival only in luminal like BC cases (Gong et al. 2019), strengthening the link that we found between IgA-enriched tumors, the luminal tumor subtype and favorable outcome of patients (Figure 5).

Besides local chemokines that might determine which ASC of specific isotype is recruited in tumors, we cannot exclude that local factors can orientate Ig class switch recombination of activated B cells in TLS. Immunoglobulin CSR is initiated by AID in activated B cells (Muramatsu et al. 2000). Interestingly, numerous studies observed AID expression in GC B cells of tumor-associated TLS indicating that an ongoing Ig CSR occurs (Coronella et al. 2002; Germain et al. 2014; J. S. Nielsen et al. 2012; Montfort et al. 2017; Cipponi et al. 2012). Besides, CSR requires the CD40L signal provided by Tfh and specific cytokines (Aversa et al. 1994; Coffman, Lebman, et Rothman 1993). Indeed, others and ourselves identified a heterogeneous population of Tfh infiltrating breast tumors including those highly expressing both PD1 and ICOS corresponding to GC Tfh (Noël et al. 2021) (Figure 14). Eventually,

cytokines produced locally may influence AID expression and Ig switch: IL10 and/or IL21 have been found to be switch factors for the *in vitro* production of IgG1 and IgG3, whereas TGF- β triggers a switch to IgA (Pène et al. 2004; Zan et al. 1998). The DEG based on RNA profiling using Nanostring technology was not enough informative to know what could explain the dominance of one or the other ASC population. This is likely due to the small number of patients (6 IgA-dominant tumors VS 4 IgG-dominant tumors) and an insufficient sensitivity of the technique. It would be interesting to rely IgA vs IgG expression to the type of cytokines and chemokines using the TCGA database. We will also evaluate cytokine and chemokine titers in tumor supernatants of a cohort of breast cancer patients with available flow cytometry data regarding ASC isotypes.

Importantly, we demonstrated that IgG and IgA ASC-enriched tumors greatly differ in their immune microenvironments. Our analysis of the breast cancer cohort of the TCGA RNAseq databasis showed that IGHG-Lo_IGHA-Hi tumors were enriched in neutrophils, mast cells and the luminal molecular subtype while the IGHG-Hi_IGHA-Lo tumor category was associated with higher expression of monocytic lineage related genes. Xu et al. demonstrated that human tonsillar macrophages can drive the differentiation of B cells into plasma cells through the secretion of CXCL10 that amplifies the production of IL6 by B cells leading to PC differentiation, which indicate that tumor-associated monocytes can locally drive B cell differentiation (Xu et al. 2012). Strikingly, Martin and colleagues identified in a fraction of Crohn disease patients a cellular module called GIMATS referring to the presence of IgG PC, Inflammatory Mononuclear phagocytes, Activated T cells and Stromal cells. Activated DC were also detected in these patients within lymphoid aggregates, indicating their potential role in activation, expansion and differentiation of T and B cells. Furthermore, they demonstrated that GIMATS module organization was initiated by activated stromal cells that strongly produce CCL2 and CCL7 leading to circulating CCR2+ monocytes attraction and their differentiation into inflammatory macrophages (Martin et al. 2019). Interestingly, our RNA profiling using Nanostring technology revealed an upregulation of CCR2 in IgG ASC rich tumors, in addition to genes reflecting the presence of activated T cells, plasma cells (CD27, CD38, TNFRSF17) and germinal center formation (POU2AF1). It is thus likely that a subset of breast tumors enriched in IgG ASC also exhibit the GIMATS cellular module, driven by similar mechanisms and leading to inflammation. Although this microenvironment is pathogenic in Crohn patients through the promotion of inflammation and autoimmune response, it could be beneficial in cancer patients via the local generation of tumor antigen specific immune effectors.

2. TLS link with tumor immunogenicity and their impact on the development of anti-tumor immune response

2.1. TLS are present in a high proportion of TN breast tumors and their density positively correlates with increased patient survival

Previous studies reported the presence of TLS in 38% to 90% of patients with breast cancer (Figenschau et al. 2015; X. Liu et al. 2017; Buisseret, Garaud, et al. 2017; Sofopoulos et al. 2019), with an increased incidence in TNBC and HER2-enriched tumors compared to other tumor molecular subtypes (Buisseret, Garaud, et al. 2017; Sofopoulos et al. 2019). There is currently no standardized approach for TLS detection and quantification. While Figenschau et al. used hematoxylin-colored slides to detect TLS with evidence of GC and then confirmed existence of segregated T and B cell zones by IHC (Figenschau et al. 2015), Martinet et al. quantified HEV as a surrogate metric of TLS density (Martinet et al. 2011). In lung tumors, density of mature DC-Lamp⁺ dendritic cells has been demonstrated to nicely correlate with TLS (Dieu-Nosjean et al. 2008). Not surprisingly, CD3/CD20 dual IHC staining appears more accurate than HE to identify TLS in tumors (Buisseret, Desmedt, et al. 2017). We thus privileged this strategy to detect TLS in our TNBC cohort using a 7-colors multi-IF staining involving CD3 and CD20 Abs and calculated the % of tumor surface occupied by dense B cell aggregates (e.g. follicles) as a metric of TLS density. Quantification of B cell follicles instead of the entire TLS B and T cell zone was preferred due to the difficulty to delineate with precisions the frontiers of the entire TLS. Presence of at least one TLS was detected in roughly 80% of TN breast tumors and about one third of TLS⁺ tumors harbored active TLS with a GC. TLS were mostly confined to peritumoral but were also occasionally detected in the tumor core, in accordance with the literature (X. Liu et al. 2017; Sofopoulos et al. 2019). Importantly, high B cell follicle densities significantly correlated with a more favorable prognosis in terms of overall and disease-free survival, with a tendency for a better impact of GC-containing TLS. To our knowledge, only one study found a negative impact of TLS in breast tumors (Sofopoulos et al. 2019). In addition to the fact that the authors excluded intratumoral TLS, patients were only categorized according to the sole presence or absence of TLS leading to unbalanced groups (98 TLS⁺ vs 14 TLS⁻). In line with previous observations, we found that TLS density is strongly associated with that of T cells and B cells (Martinet et al. 2011; Buisseret, Garaud, et al. 2017). Importantly, whereas B cell follicle density also correlated with tumor infiltration by mature DC-LAMP⁺ DC, only the former metric was associated with patient survival (data not shown). Together, these data indicate that B cell follicle density can be calculated as a proxy of TLS density by digital images analysis and has predict the prognosis of TNBC cancer patients with better accuracy than T cells and mature dendritic cells.

2.2. TLS formation/maintenance may be triggered by diverse classes of tumor antigens

Based on genomic data obtained from 45 frozen tumors of TNBC patients and the corresponding TLS density quantified on FFPE section, we evaluated the link between TLS and tumor immunogenicity. We confirmed that the TMB and neoepitopes load were increased in basal-like tumors compared to other subtypes (Haricharan et al. 2014). Unexpectedly, we did however not observe any significant correlation between TLS density, TMB and MHC-class-I neoantigen counts. This is consistent with recent observations made in melanoma patients (Cabrita et al. 2020). Karn et al. reported a strong inverse correlation between immune T and B cell infiltration and TMB in the TNBC patients from the TCGA database, but TLS were not explored in this study (Karn et al. 2017). In contrast, Lin et al. found a positive correlation between the 12-chemokine TLS gene signature score (Coppola et al. 2011) and neoantigen load in breast cancer, albeit the correlation coefficient was very low ($r=0.12$, $p < 0.001$). It should be however mentioned that relying TLS to TMB/neoepitopes is technically challenging as higher mutation loads are usually detected in samples with the highest tumor cell purities, while tumors highly infiltrated by immune cells organized in TLS have by definition a lower tumor cell purity than their TLS-negative counterparts. Such possible bias could be bypassed by performing WES on laser micro-dissected tumor islets. Furthermore, we have to acknowledge that TLS quantification and WES were performed on different tumor blocks, which may introduce a bias given the considerable tumor heterogeneity and non-homogeneous distribution of TLS inside tumors. Yet, using RNAseq and WES data performed on the same tumor blocks, we found no correlation between a published TLS gene signature and neither the TMB nor number of predicated T cell neoepitopes. Although, we could establish a bona fide correlation between TLS and tumor mutations, we observed, however that two out of the 5 tumors with very high B follicle densities had an elevated number of putative T cell neoepitopes. This indicates that in some, but not all patients, neoantigen load may play a role in the formation and/or maintenance of TLS. This observation led us to investigate other tumor-associated antigens, including HERV and CTA.

Different CTA have been shown to be expressed in cancers and are considered as interesting targets to trigger anti-tumor immune responses. Analysis of DEG between TLS-Hi vs TLS-Lo TNBC tumors revealed significant overexpression of 10 CTA family members in TLS^{Hi} patients. 6 out of the 10 patients with TLS^{Hi} tumors indeed overexpressed at least 1 CTA and 4 of them at least 2. Most of overexpressed CTA have been already described in breast tumors, such as CTAG1 that is expressed in 62% of patients (Taylor et al. 2007) and melanoma-associated antigen-A (MAGE-A) family members which are overexpressed in about 50% of TNBC (H. Wang et al. 2016). The only CTA that was found downregulated in TLS^{Hi} tumors, ACTL8, was reported as markedly upregulated in TNBC patients compared to other subtypes (S. Fan et al. 2021). The overexpression of this CTA was associated with

increased infection rate in colorectal cancer patients, which suggest that ACTL8 may suppress immune response (Han et al. 2019). T cells and antibodies directed against CTA are detectable in cancer patients, indicating that the aberrant expression of these antigens in tumor cells can result in the induction of adaptive immune responses (Mischo et al. 2006; Groeper et al. 2007; Gnjatic et al. 2003). Garaud et al. further reported that CTAG1B, which we found upregulated in TLS^{Hi} TN breast tumors, was one of the most frequently tumor antigen targeted by antibodies in both the serum and tumor of breast cancer patients (Garaud et al. 2018). Altogether, our data showing that about half of the TLS-Hi tumors overexpressed one or several CTA combined with the fact that CTA can elicit Ab response inside tumors, argues that this class of tumor Ag is associated with TLS formation and/or maintenance, at least in some patients.

As HERV genes are silenced by epigenetic mechanisms in the majority of healthy tissues but can be overexpressed in tumors, they are considered as a new class of tumor antigens (Szpakowski et al. 2009). HERV-specific IgG, Th1 cells and cytotoxic CD8+ T cells have indeed been detected in breast cancer patients (Wang-Johanning et al. 2008). These antigens are therefore of major interest for the development of cancer vaccines. In line with that, our team recently identified a series of MHC-class-I epitopes from HERV with shared expression between patients and showed that these peptides can induce cytotoxic CD8+ T cells able to lyse patient-derived tumor organoids (Bonaventura et al., s. d.). Although the link between HERV and the induction of an efficient adaptive immune response is emerging, whether HERV expression may contribute to TLS formation and/or maintenance and thus favor the generation of anti-tumor effectors remains unexplored. We demonstrated for the first time that a majority of TLS^{Hi} tumors overexpressed one to several (up to 16) viral retroelements, including a majority of HERV, compared to their TLS^{Lo} counterpart, indicating a close positive association between expression of these genes and the density of TLS. Interestingly, a different set of viral elements (up to 5) appeared upregulated in a minor fraction of TLS^{Lo} tumors, suggesting that not all endogenous retroelements are associated with TLS. Remarkably, virtually all tumors that were TLS high overexpressed one to several HERV and/or CTA, suggesting a primary role of these 2 classes of Ags in TLS development and/or maintenance. Yet, patients overexpressing several HERV are different from those overexpressing 2 or more CTA, indicating that the type of tumor antigens associated to TLS may differs from patient to patient. Together, these data indicate that TLS may mostly develop in tumors with high Ag loads, with a primary role of germ line encoded tumor Ag and endogenous viral retroelements in most patient and an apparent limited contribution of mutation-derived neoantigens.

2.3. TLS is a privileged site for the development of an efficient anti-tumor immune response

In this study, we demonstrated that the structuration of the immune infiltrate was associated with better outcome despite the small number of patients. The spatial organization of TLS is essential to enhance interactions between different immune cells in order to effectively develop anti-tumor response, which explains the positive prognosis impact of structured over non-structured immune infiltrates.

Previous findings showed that an elevated TLS density in breast tumors correlated with an upregulation of genes involved in Th1 and cytotoxicity pathways (Martinet et al. 2011; Noël et al. 2021). Goc et al. found that a high density of mature DC, a specific marker of TLS in lung tumors, correlated with a strong infiltration of effector-memory CD8⁺ T cells (Goc et al. 2014). In addition, TLS have been associated with increased TCR clonality of T cells (Zhu et al. 2015). The analysis of microdissected TLS versus non-TLS tumor regions revealed an oligoclonal amplification in TLS (Ukita s. d.). Similarly, we reported a higher expression of genes involved in T-cell homing, activation and differentiation, Th1-oriented immune response, cytotoxicity. Furthermore, genes reflecting an ongoing humoral immune response were increased in TLS-enriched tumors. This includes CXCL13, CCR7 and SELL (coding for CD62L) important chemokine and receptors for B cell recruitment in tumors. In addition, genes coding for heavy and light chains of Ig were found exclusively upregulated in TLS^{Hi} tumors suggesting an oligoclonal B cell expansion only in tumors with high TLS density. Higher clonality of memory B cells and ASC was detected in TLS regions indicating that SHM occurs within these structures (Coronella et al. 2002; Montfort et al. 2017; Meylan et al. 2022).

We also observed upregulated genes of Tfh cells (*CXCL13*, *IL21R*, *PCDC1*, *ICOS*), which are specialized CD4⁺ T cells that play an important role in B-cell differentiation within GC (Noël et al. 2021). Using multi-IF technique, we detected the presence of Tfh in GC as CD4⁺ T cells highly expressing PD1 (Figure 15B). We also identified by flow cytometry a heterogeneous population of CXCR5⁺ Tfh infiltrating breast tumors (Figure 14). Three subsets of Tfh Tfh1, Tfh2 and Tfh17 defined according to the expression of the CCR6 and CXCR3 chemokine receptors; Tfh1 cells are CXCR3⁺CCR6⁻ cells, Tfh2 cells are CXCR3⁻CCR6⁻ cells, Tfh17 are CXCR3⁻CCR6⁺ cells whereas Tfh1/17 are CXCR3⁺CCR6⁺ cells (Morita et al. 2011). Tfh1 was the dominant Tfh subset in our tumors. Consistently, analysis of cytokines secreted in breast tumor supernatants revealed that tumor-infiltrating functional Tfh produced higher amounts of IFN γ indicating that IFN γ -secreting Tfh may correspond to Tfh1 (Noël et al. 2021). We also detected Tfr at a significant proportion. Li et al. found that Tfr infiltrating ovarian tumors expressed high levels of IL10 and TGF β that suppress the activation of CD8⁺ T cells (L. Li, Ma, et Xu 2019). In breast tumors, significantly higher Tfh/Tfr ratio was observed in TLS-containing GC

compared to those without. Tfr were able to inhibit Tfh activities in TGF β -dependent mechanism highlighting the role played by Tfr in controlling Tfh-dependent B cell differentiation in TLS (Noël et al. 2021). Tfh subset can be further divided based on the level of expression of PD1 and ICOS. Tfh that express higher PD1 and ICOS have been shown to correlate with a functional Tfh defined as GC Tfh (L. A. Vella et al. 2019). We detected this subpopulation in breast tumors as CXCR5⁺PD1^{Hi}ICOS^{Int}. Strikingly, this subset was located within TLS and was the only one capable to provide help to B cells for IgG and IgA production *ex vivo* illustrating the presence of functional Tfh in tumor-associated TLS (Noël et al. 2021). Breast tumors are mostly infiltrated of Tfh1 with a significant fraction of functional Tfh indicating an ongoing effective humoral response in germinal centers.

Genes downregulated in TLS^{Hi} tumors were involved in hypoxia pathway. Interestingly, a hypoxic TME was reported to restrain TLS formation. Pancreas-specific hypoxia-inducible factor 1a (HIF1a) detection increased CXCL13 secretion and B cell infiltration (K. E. Lee et al. 2016). We also found an overexpression of the targets of CTNNB1 (encoding for β 1-catenin) pathway, whose mutation was negatively correlated with high TLS scoring (Lin et al. 2020).

Overall, our findings strengthen previous observations that showed that tumor-associated TLS are sites for the generation of tumor antigen-specific humoral and cellular immune response. We also highlight some pathways involved in blocking TLS formation, which paves the way for the discovery of new therapeutic targets. Nevertheless, further analysis are ongoing on this dataset to get a better understanding of new underlying immune and non-immune mechanisms related to TLS.

2.4. Towards defining a robust TLS gene signature

We also aimed to exploit our matched multi-IF and RNAseq data of TNBC tumors to build a robust TLS gene signature in breast cancer to validate our observations on the relationship between TLS and tumor antigen expression in public cancer databases. Unlike the strategies used for already published TLS gene signatures (Coppola et al. 2011; Cabrita et al. 2020), we restricted our analysis only to tumors highly infiltrated by T and B cells in order to define a gene signature accounting for organization of the immune infiltrate in TLS and not just increased immune cell infiltration. Starting from the genes overexpressed in TLS^{Hi} tumors, we randomly pooled down 20 genes and calculated a ssGSEA score to perform a correlation with the density of B cell follicles and repeated the process 1,000 times. The most frequent genes in the top 10% gene sets presenting a significant correlation with TLS were then selected. This yields a 9-genes signature that contained immune (TNFRSF9, CTSC) and non-immune (ATF6B, CCND2, GJB7, TTC24, SSTR3) genes, as well as two CTA (MAGEA2 and CSAG1), known to be highly expressed in breast cancer (Taylor et al. 2007; H. Wang et al. 2016). Interestingly, some, but not all, of these genes have already been linked to TLS. TNFRSF9 encodes CD137, a TNFR-superfamily member expressed by activated T cells and with costimulatory functions. CD137 engagement by its

ligand triggers T cell proliferation and survival, and enhances their effector functions. A recent study reported that CD137⁺CD8⁺ T cells found within TLS were associated with a better response to anti-PD1 therapy suggesting that CD137 expression is tightly linked to efficient anti-tumor immunity within TLS (Zizzari et al. 2022). ATF6 β is a transcription factor located within the endoplasmic reticulum (ER) and that can be cleaved in response to ER stress. In an ATF6 β -deficient murine model of parasitic infection, an impairment of IFN γ production by CD8⁺ T cell-mediated was observed, suggesting a role for ATF6 β in the activation and functions of CD8⁺ T cells (Yamamoto et Takeda 2012). Eventually, CCND2, encoding cyclin D2, was recently described as highly expressed in lung tumors enriched in B cells, CD8⁺ T cells and Tfh, e.g. likely to TLS-enriched tumors. Moreover, this gene is associated with good patient prognosis and its upregulation enhances T cell resistance to tumor-cell mediated apoptosis (Tian et al. 2021). This 9-genes TLS signature nicely correlated with the density of B follicles ($r=0,73$) and with the previously published TLS gene signature of Cabrita et al., although the 2 signatures have no gene in common. After validation with an independent cohort of breast cancer patients and cross-comparison with the different published signatures, this signature will be used to interrogate public databases to further explore the link between TLS, expression of different types of tumor Ags, patient survival and responses to therapies. It should also constitute a valuable tool to stratify patients according to their immune contexture. In addition, we will also determine whether this signature can be extended to ovarian and melanoma tumors, for which we have already generated matched multi-IF and RNAseq datasets. In this respect, it may be necessary to remove from the signature the two CTA that are quite specific of breast tumors.

CONCLUSION AND PERSPECTIVES

The present study brings new insight into the heterogeneity and critical role of B cells and ASC in the TME of breast cancer. We demonstrated that tumor-infiltrating ASC consist of both plasmablasts and plasma cells originating at least in part from TLS, localize in the stroma and the tumor bed, and can produce IgG but also monomeric and dimeric IgA that recognize largely non-overlapping sets of antigens. We also showed for the first time that the ASC compartment drastically evolves during cancer progression in terms and critically influence clinical patient outcome with a protective role of IgA ASC.

Nevertheless, there are several points in this study that need to be deepened. It would be indeed interesting to assess the role of IgM ASC, albeit they are present in much less proportion in breast tumors compared to IgG and IgA ASC. In addition, the examination of IgG subclasses is of utmost importance because they differ in their capacity to exert cytotoxic functions and may thus have differential clinical impacts. In models other than cancer, it is admitted that the state of maturation of ASC affects their functions, including antibody production, cytokine secretion and ICPL expression. Thus, we can examine ICP/ICPL expression profile on different tumor-associated ASC subsets using flow cytometry. Moreover, we may consider sorting tumor-associated ASC to perform single-cell RNA sequencing allowing the characterization of ASC maturation diversity and potentially study the link of different ASC subsets with ICP/ICPL, cytokine and chemokine expression. Also, our antibody profiling approach generated a huge amount of data that we are still analyzing. One of the limitations is that we detected linear epitopes indicating that we need to determine if these observations could be applied to conformational epitopes.

Strikingly, we showed a drastic change of the composition of the ASC compartment between pre-invasive and invasive tumors moving from IgA ASC-dominant to IgG ASC-dominant tumors suggesting a protective role of IgA ASC. To understand what could distinguish these two stages of progression in terms of immune microenvironment, we performed recently an RNA profiling using Nanostring technology to compare DCIS and IBC tumors and the analysis is still ongoing.

Unlike previous studies, we observed that IgA ASC, irrespective of IgG ASC, are associated with good prognosis in IBC patients strengthening their anti-tumor role. Nevertheless, we still need to answer critical questions that will help to decipher underlying mechanisms: how ASC of specific isotype are recruited and/or induced? Do they directly influence patients' survival, or is it related to the other immune cells present in the microenvironment? Do they communicate with other cells (e.g. immune, stromal, immune cells) by close contact or by secreting molecules (e.g. antibody, cytokine, exosome...), which may affect their functions? What would be the consequences of these interactions? The development of new technologies that combine spatial transcriptomics and proteomics and in vitro experiments could help addressing these questions.

On the other hand, we confirmed that TLS induce and/or amplify anti-tumor adaptive immune response since their presence is associated with CTA, Th1, cytotoxicity, antigen presentation and ICP. Furthermore, we highlight new pathways that may prevent TLS formation, although it needs to be confirmed by *in vivo* experiments. This may explain the variable proportion of TLS among cancer patients and can lead in the long term to improve therapeutic strategies promoting TLS induction. We also demonstrated a link between the density of TLS and tumor cell antigenicity; however, we showed that the type of tumor antigen that correlates with TLS varies from one patient to another. We did not observe a correlation between TLS and neoantigens, although this can be due to technical issue (sampling bias). Nevertheless, we showed an upregulation of CTA and HERV antigens in TLS-enriched tumors. Recent publications support the fact that HERV expression in tumor cells induce an efficient anti-tumor cellular and humoral immune response. Our results suggest that HERV may induce or sustain TLS, which constitutes the site where the antitumor immune response may be locally amplified. HERV are currently considered as relevant targets for innovative cancer vaccines, especially for tumors with low to intermediate TMB. It would be interesting to examine tumor tissues post-HERV vaccination to observe a potential formation of TLS.

The small number of patients in our cohort makes it impossible to conclude about a potential link between TLS and tumor immunogenicity. Thus, we are currently working on the construction of a robust home-made TLS gene signature that we still need to validate on an independent cohort of breast cancer with available RNA seq and multi-IF data. This will allow to explore public databases (TCGA, METABRIC, other databases) for associations of TLS with HERV and other tumor antigens classes.

REFERENCES

- Ali, H. Raza, Leon Chlon, Paul D. P. Pharoah, Florian Markowitz, et Carlos Caldas. 2016. « Patterns of Immune Infiltration in Breast Cancer and Their Clinical Implications: A Gene-Expression-Based Retrospective Study ». *PLoS Medicine* 13 (12): e1002194. <https://doi.org/10.1371/journal.pmed.1002194>.
- Allen, Christopher D C, et Jason G Cyster. 2008. « Follicular dendritic cell networks of primary follicles and germinal centers: phenotype and function ». *Seminars in Immunology* 20 (1): 14-25. <https://doi.org/10.1016/j.smim.2007.12.001>.
- Allen, Elizabeth, Arnaud Jabouille, Lee B. Rivera, Inge Lodewijckx, Rindert Missiaen, Veronica Steri, Kevin Feyen, et al. 2017. « Combined Antiangiogenic and Anti-PD-L1 Therapy Stimulates Tumor Immunity through HEV Formation ». *Science Translational Medicine* 9 (385): eaak9679. <https://doi.org/10.1126/scitranslmed.aak9679>.
- Ansel, K. Mark, Vu N. Ngo, Paul L. Hyman, Sanjiv A. Luther, Reinhold Förster, Jonathon D. Sedgwick, Jeffrey L. Browning, Martin Lipp, et Jason G. Cyster. 2000. « A Chemokine-Driven Positive Feedback Loop Organizes Lymphoid Follicles ». *Nature* 406 (6793): 309-14. <https://doi.org/10.1038/35018581>.
- Aspord, Caroline, Alexander Pedroza-Gonzalez, Mike Gallegos, Sasha Tindle, Elizabeth C. Burton, Dan Su, Florentina Marches, Jacques Banchereau, et A. Karolina Palucka. 2007. « Breast Cancer Instructs Dendritic Cells to Prime Interleukin 13-Secreting CD4+ T Cells That Facilitate Tumor Development ». *The Journal of Experimental Medicine* 204 (5): 1037-47. <https://doi.org/10.1084/jem.20061120>.
- Asrir, Assia, Claire Tardiveau, Juliette Coudert, Robin Laffont, Lucas Blanchard, Elisabeth Bellard, Krystle Veerman, et al. 2022. « Tumor-Associated High Endothelial Venules Mediate Lymphocyte Entry into Tumors and Predict Response to PD-1 plus CTLA-4 Combination Immunotherapy ». *Cancer Cell* 40 (3): 318-334.e9. <https://doi.org/10.1016/j.ccell.2022.01.002>.
- Attaf, Noudjoud, Sabrina Baaklini, Laurine Binet, et Pierre Milpied. 2021. « Heterogeneity of Germinal Center B Cells: New Insights from Single-Cell Studies ». *European Journal of Immunology* 51 (11): 2555-67. <https://doi.org/10.1002/eji.202149235>.
- Aversa, G., B. G. Cocks, J. Punnonen, J. M. Carballido, et J. E. de Vries. 1994. « Contact-Mediated Signals and Cytokines Involved in B-Cell Activation and Isotype Switching in Pre-B and Mature B Cells ». *Research in Immunology* 145 (3): 222-26; discussion 244-249. [https://doi.org/10.1016/s0923-2494\(94\)80188-6](https://doi.org/10.1016/s0923-2494(94)80188-6).
- Bai, Rilan, Zheng Lv, Dongsheng Xu, et Jiuwei Cui. 2020. « Predictive biomarkers for cancer immunotherapy with immune checkpoint inhibitors ». *Biomarker Research* 8 (1): 34. <https://doi.org/10.1186/s40364-020-00209-0>.
- Banchereau, J., et R. M. Steinman. 1998. « Dendritic Cells and the Control of Immunity ». *Nature* 392 (6673): 245-52. <https://doi.org/10.1038/32588>.
- Barone, Francesca, Saba Nayar, Joana Campos, Thomas Cloake, David R. Withers, Kai-Michael Toellner, Yang Zhang, et al. 2015. « IL-22 regulates lymphoid chemokine production and assembly of tertiary lymphoid organs ». *Proceedings of the National Academy of Sciences* 112 (35): 11024-29. <https://doi.org/10.1073/pnas.1503315112>.
- Batista, F. D., et M. S. Neuberger. 1998. « Affinity Dependence of the B Cell Response to Antigen: A Threshold, a Ceiling, and the Importance of off-Rate ». *Immunity* 8 (6): 751-59. [https://doi.org/10.1016/s1074-7613\(00\)80580-4](https://doi.org/10.1016/s1074-7613(00)80580-4).
- Baxevasis, Constantin N., Sotirios P. Fortis, et Sonia A. Perez. 2021. « The Balance between Breast Cancer and the Immune System: Challenges for Prognosis and Clinical Benefit from Immunotherapies ». *Seminars in Cancer Biology* 72 (juillet): 76-89. <https://doi.org/10.1016/j.semcancer.2019.12.018>.
- Becht, Etienne, Nicolas A. Giraldo, Claire Germain, Aurélien de Reyniès, Pierre Laurent-Puig, Jessica Zucman-Rossi, Marie-Caroline Dieu-Nosjean, Catherine Sautès-Fridman, et Wolf H. Fridman. 2016. « Chapter Four - Immune Contexture, Immunoscore, and Malignant Cell Molecular Subgroups for Prognostic and Theranostic Classifications of Cancers ». In *Advances in*

- Immunology*, édité par Robert D. Schreiber, 130:95-190. Tumor Immunology. Academic Press. <https://doi.org/10.1016/bs.ai.2015.12.002>.
- Bemark, Mats. 2015. « Translating transitions – how to decipher peripheral human B cell development ». *Journal of Biomedical Research* 29 (4): 264-84. <https://doi.org/10.7555/JBR.29.20150035>.
- Bénézech, Cécile, Nguyet-Thin Luu, Jennifer A. Walker, Andrei A. Kruglov, Yunhua Loo, Kyoko Nakamura, Yang Zhang, et al. 2015. « Inflammation-Induced Formation of Fat-Associated Lymphoid Clusters ». *Nature Immunology* 16 (8): 819-28. <https://doi.org/10.1038/ni.3215>.
- Bense, Rico D., Christos Sotiriou, Martine J. Piccart-Gebhart, John B. A. G. Haanen, Marcel A. T. M. van Vugt, Elisabeth G. E. de Vries, Carolien P. Schröder, et Rudolf S. N. Fehrmann. 2017. « Relevance of Tumor-Infiltrating Immune Cell Composition and Functionality for Disease Outcome in Breast Cancer ». *Journal of the National Cancer Institute* 109 (1). <https://doi.org/10.1093/jnci/djw192>.
- Bento, Diana Costa, Emma Jones, Syed Junaid, Justyna Tull, Geraint T. Williams, Andrew Godkin, Ann Ager, et Awen Gallimore. 2015. « High Endothelial Venules Are Rare in Colorectal Cancers but Accumulate in Extra-Tumoral Areas with Disease Progression ». *Oncimmunology* 4 (3): e974374. <https://doi.org/10.4161/2162402X.2014.974374>.
- Bianchini, Giampaolo, Yuan Qi, Ricardo H. Alvarez, Takayuki Iwamoto, Charles Coutant, Nuhad K. Ibrahim, Vicente Valero, et al. 2010. « Molecular Anatomy of Breast Cancer Stroma and Its Prognostic Value in Estrogen Receptor–Positive and –Negative Cancers ». *Journal of Clinical Oncology* 28 (28): 4316-23. <https://doi.org/10.1200/JCO.2009.27.2419>.
- Boivin, Gaël, Pradeep Kalambaden, Julien Faget, Sylvie Rusakiewicz, Pierre Montay-Gruel, Etienne Meylan, Jean Bourhis, Guy Lesec, et Marie-Catherine Vozenin. 2018. « Cellular Composition and Contribution of Tertiary Lymphoid Structures to Tumor Immune Infiltration and Modulation by Radiation Therapy ». *Frontiers in Oncology* 8 (juillet): 256. <https://doi.org/10.3389/fonc.2018.00256>.
- Bonaventura, Paola, Vincent Alcazer, Virginie Mutez, Laurie Tonon, Juliette Martin, Nicolas Chuvin, Emilie Michel, et al. s. d. « Identification of shared tumor epitopes from endogenous retroviruses inducing high-avidity cytotoxic T cells for cancer immunotherapy ». *Science Advances* 8 (4): eabj3671. <https://doi.org/10.1126/sciadv.abj3671>.
- Böttcher, Jan P., Eduardo Bonavita, Probir Chakravarty, Hanna Blees, Mar Cabeza-Cabrerizo, Stefano Sarmicelli, Neil C. Rogers, Erik Sahai, Santiago Zelenay, et Caetano Reis e Sousa. 2018. « NK Cells Stimulate Recruitment of CDC1 into the Tumor Microenvironment Promoting Cancer Immune Control ». *Cell* 172 (5): 1022-1037.e14. <https://doi.org/10.1016/j.cell.2018.01.004>.
- Brown, Flavian D., et Shannon J. Turley. 2015. « Fibroblastic Reticular Cells: Organization and Regulation of the T Lymphocyte Life Cycle ». *Journal of immunology (Baltimore, Md. : 1950)* 194 (4): 1389-94. <https://doi.org/10.4049/jimmunol.1402520>.
- Brown, Kathryn, Steven H. Sacks, et Wilson Wong. 2011. « Tertiary Lymphoid Organs in Renal Allografts Can Be Associated with Donor-Specific Tolerance Rather than Rejection ». *European Journal of Immunology* 41 (1): 89-96. <https://doi.org/10.1002/eji.201040759>.
- Broz, Miranda L., Mikhail Binnewies, Bijan Boldajipour, Amanda E. Nelson, Joshua L. Pollack, David J. Erle, Andrea Barczak, et al. 2014. « Dissecting the Tumor Myeloid Compartment Reveals Rare Activating Antigen-Presenting Cells Critical for T Cell Immunity ». *Cancer Cell* 26 (5): 638-52. <https://doi.org/10.1016/j.ccell.2014.09.007>.
- Bruno, Antonino, Guido Ferlazzo, Adriana Albini, et Douglas M. Noonan. 2014. « A Think Tank of TINK/TANKs: Tumor-Infiltrating/Tumor-Associated Natural Killer Cells in Tumor Progression and Angiogenesis ». *JNCI Journal of the National Cancer Institute* 106 (8): dju200. <https://doi.org/10.1093/jnci/dju200>.
- Bruno, Tullia C., Peggy J. Ebner, Brandon L. Moore, Olivia G. Squalls, Katherine A. Waugh, Evgeniy B. Eruslanov, Sunil Singhal, et al. 2017. « Antigen-Presenting Intratumoral B Cells Affect CD4+ TIL Phenotypes in Non-Small Cell Lung Cancer Patients ». *Cancer Immunology Research* 5 (10): 898-907. <https://doi.org/10.1158/2326-6066.CIR-17-0075>.

- Buckley, Christopher D., Francesca Barone, Saba Nayar, Cecile Bénézech, et Jorge Caamaño. 2015. « Stromal Cells in Chronic Inflammation and Tertiary Lymphoid Organ Formation ». *Annual Review of Immunology* 33: 715-45. <https://doi.org/10.1146/annurev-immunol-032713-120252>.
- Buisseret, Laurence, Christine Desmedt, Soizic Garaud, Marco Fornili, Xiaoxiao Wang, Gert Van den Eyden, Alexandre de Wind, et al. 2017. « Reliability of Tumor-Infiltrating Lymphocyte and Tertiary Lymphoid Structure Assessment in Human Breast Cancer ». *Modern Pathology* 30 (9): 1204-12. <https://doi.org/10.1038/modpathol.2017.43>.
- Buisseret, Laurence, Soizic Garaud, Alexandre de Wind, Gert Van den Eyden, Anais Boisson, Cinzia Solinas, Chunyan Gu-Trantien, et al. 2017. « Tumor-Infiltrating Lymphocyte Composition, Organization and PD-1/ PD-L1 Expression Are Linked in Breast Cancer ». *Oncoimmunology* 6 (1): e1257452. <https://doi.org/10.1080/2162402X.2016.1257452>.
- Cabrita, Rita, Martin Lauss, Adriana Sanna, Marco Donia, Mathilde Skaarup Larsen, Shamik Mitra, Iva Johansson, et al. 2020. « Tertiary Lymphoid Structures Improve Immunotherapy and Survival in Melanoma ». *Nature* 577 (7791): 561-65. <https://doi.org/10.1038/s41586-019-1914-8>.
- Calderaro, Julien, Florent Petitprez, Etienne Becht, Alexis Laurent, Théo Z. Hirsch, Benoit Rousseau, Alain Luciani, et al. 2019. « Intra-Tumoral Tertiary Lymphoid Structures Are Associated with a Low Risk of Early Recurrence of Hepatocellular Carcinoma ». *Journal of Hepatology* 70 (1): 58-65. <https://doi.org/10.1016/j.jhep.2018.09.003>.
- Chaisemartin, Luc de, Jérémy Goc, Diane Damotte, Pierre Validire, Pierre Magdeleinat, Marco Alifano, Isabelle Cremer, Wolf-Herman Fridman, Catherine Sautès-Fridman, et Marie-Caroline Dieu-Nosjean. 2011. « Characterization of Chemokines and Adhesion Molecules Associated with T cell Presence in Tertiary Lymphoid Structures in Human Lung Cancer ». *Cancer Research* 71 (20): 6391-99. <https://doi.org/10.1158/0008-5472.CAN-11-0952>.
- Chihara, Norio, Toshimasa Aranami, Shinji Oki, Takako Matsuoka, Masakazu Nakamura, Hitaru Kishida, Kazumasa Yokoyama, et al. 2013. « Plasmablasts as Migratory IgG-Producing Cells in the Pathogenesis of Neuromyelitis Optica ». *PLoS ONE* 8 (12): e83036. <https://doi.org/10.1371/journal.pone.0083036>.
- Chin, Y., J. Janseens, J. Vandepitte, J. Vandenbrande, L. Opdebeek, et J. Raus. 1992. « Phenotypic Analysis of Tumor-Infiltrating Lymphocytes from Human Breast Cancer ». *Anticancer Research* 12 (5): 1463-66.
- Cianfrocca, Mary, et Lori J. Goldstein. 2004. « Prognostic and Predictive Factors in Early-Stage Breast Cancer ». *The Oncologist* 9 (6): 606-16. <https://doi.org/10.1634/theoncologist.9-6-606>.
- Cinier, Justine, Margaux Hubert, Laurie Besson, Anthony Di Roio, Céline Rodriguez, Vincent Lombardi, Christophe Caux, et Christine Ménétrier-Caux. 2021. « Recruitment and Expansion of Tregs Cells in the Tumor Environment-How to Target Them? ». *Cancers* 13 (8): 1850. <https://doi.org/10.3390/cancers13081850>.
- Cipponi, Arcadi, Marjorie Mercier, Teofila Seremet, Jean-François Baurain, Ivan Théate, Joost van den Oord, Marguerite Stas, Thierry Boon, Pierre G. Coulie, et Nicolas van Baren. 2012. « Neogenesis of Lymphoid Structures and Antibody Responses Occur in Human Melanoma Metastases ». *Cancer Research* 72 (16): 3997-4007. <https://doi.org/10.1158/0008-5472.CAN-12-1377>.
- Coffman, R. L., D. A. Leberman, et P. Rothman. 1993. « Mechanism and Regulation of Immunoglobulin Isotype Switching ». *Advances in Immunology* 54: 229-70. [https://doi.org/10.1016/s0065-2776\(08\)60536-2](https://doi.org/10.1016/s0065-2776(08)60536-2).
- Columba-Cabezas, Sandra, Marilena Griguoli, Barbara Rosicarelli, Roberta Magliozzi, Francesco Ria, Barbara Serafini, et Francesca Aloisi. 2006. « Suppression of Established Experimental Autoimmune Encephalomyelitis and Formation of Meningeal Lymphoid Follicles by Lymphotoxin Beta Receptor-Ig Fusion Protein ». *Journal of Neuroimmunology* 179 (1-2): 76-86. <https://doi.org/10.1016/j.jneuroim.2006.06.015>.
- Coppola, Domenico, Michael Nebozhyn, Farah Khalil, Hongyue Dai, Timothy Yeatman, Andrey Loboda, et James J. Mulé. 2011. « Unique Ectopic Lymph Node-like Structures Present in

- Human Primary Colorectal Carcinoma Are Identified by Immune Gene Array Profiling ». *The American Journal of Pathology* 179 (1): 37-45. <https://doi.org/10.1016/j.ajpath.2011.03.007>.
- Coronella, Julia A., Catherine Spier, Matthew Welch, Katrina T. Trevor, Alison T. Stopeck, Hugo Villar, et Evan M. Hersh. 2002. « Antigen-Driven Oligoclonal Expansion of Tumor-Infiltrating B Cells in Infiltrating Ductal Carcinoma of the Breast ». *Journal of Immunology (Baltimore, Md.: 1950)* 169 (4): 1829-36. <https://doi.org/10.4049/jimmunol.169.4.1829>.
- Coronella-Wood, Julia A., et Evan M. Hersh. 2003. « Naturally Occurring B-Cell Responses to Breast Cancer ». *Cancer Immunology, Immunotherapy: CII* 52 (12): 715-38. <https://doi.org/10.1007/s00262-003-0409-4>.
- Cui, Can, Jiawei Wang, Eric Fagerberg, Ping-Min Chen, Kelli A. Connolly, Martina Damo, Julie F. Cheung, et al. 2021. « Neoantigen-Driven B Cell and CD4 T Follicular Helper Cell Collaboration Promotes Anti-Tumor CD8 T Cell Responses ». *Cell* 184 (25): 6101-6118.e13. <https://doi.org/10.1016/j.cell.2021.11.007>.
- De Silva, Pushpamali, Soizic Garaud, Cinzia Solinas, Alexandre de Wind, Gert Van den Eyden, Vinu Jose, Chunyan Gu-Trantien, et al. 2019. « FOXP1 Negatively Regulates Tumor Infiltrating Lymphocyte Migration in Human Breast Cancer ». *EBioMedicine* 39 (janvier): 226-38. <https://doi.org/10.1016/j.ebiom.2018.11.066>.
- Denkert, Carsten, Gunter von Minckwitz, Silvia Darb-Esfahani, Bianca Lederer, Barbara I. Heppner, Karsten E. Weber, Jan Budczies, et al. 2018. « Tumour-Infiltrating Lymphocytes and Prognosis in Different Subtypes of Breast Cancer: A Pooled Analysis of 3771 Patients Treated with Neoadjuvant Therapy ». *The Lancet. Oncology* 19 (1): 40-50. [https://doi.org/10.1016/S1470-2045\(17\)30904-X](https://doi.org/10.1016/S1470-2045(17)30904-X).
- Denton, Alice E., Silvia Innocentin, Edward J. Carr, Barry M. Bradford, Fanny Lafouresse, Neil A. Mabbott, Urs Mörbe, et al. 2019. « Type I interferon induces CXCL13 to support ectopic germinal center formation ». *The Journal of Experimental Medicine* 216 (3): 621-37. <https://doi.org/10.1084/jem.20181216>.
- Desmedt, Christine, Roberto Salgado, Marco Fornili, Giancarlo Pruneri, Gert Van den Eynden, Gabriele Zoppoli, Françoise Rothé, et al. 2018. « Immune Infiltration in Invasive Lobular Breast Cancer ». *Journal of the National Cancer Institute* 110 (7): 768-76. <https://doi.org/10.1093/jnci/djx268>.
- Deteix, Clémence, Valérie Attuil-Audenis, Aurélie Duthey, Natacha Patey, Brigitte McGregor, Valérie Dubois, Giuseppina Caligiuri, Stéphanie Graff-Dubois, Emmanuel Morelon, et Olivier Thauinat. 2010. « Intragraft Th17 Infiltrate Promotes Lymphoid Neogenesis and Hastens Clinical Chronic Rejection ». *Journal of Immunology (Baltimore, Md.: 1950)* 184 (9): 5344-51. <https://doi.org/10.4049/jimmunol.0902999>.
- Di Caro, Giuseppe, Giovanni Francesco Castino, Francesca Bergomas, Nina Cortese, Maurizio Chiriva-Internati, Fabio Grizzi, Alberto Mantovani, et Federica Marchesi. 2015. « Tertiary Lymphoid Tissue in the Tumor Microenvironment: From Its Occurrence to Immunotherapeutic Implications ». *International Reviews of Immunology* 34 (2): 123-33. <https://doi.org/10.3109/08830185.2015.1018416>.
- Dieterich, Lothar C., Kristian Ikenberg, Timur Cetintas, Kübra Kapaklikaya, Cornelia Huttmacher, et Michael Detmar. 2017. « Tumor-Associated Lymphatic Vessels Upregulate PDL1 to Inhibit T-Cell Activation ». *Frontiers in Immunology* 8: 66. <https://doi.org/10.3389/fimmu.2017.00066>.
- Dieu-Nosjean, Marie-Caroline, Martine Antoine, Claire Danel, Didier Heudes, Marie Wislez, Virginie Poulot, Nathalie Rabbe, et al. 2008. « Long-Term Survival for Patients with Non-Small-Cell Lung Cancer with Intratumoral Lymphoid Structures ». *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 26 (27): 4410-17. <https://doi.org/10.1200/JCO.2007.15.0284>.
- DiLillo, David J., Koichi Yanaba, et Thomas F. Tedder. 2010. « B Cells Are Required for Optimal CD4+ and CD8+ T Cell Tumor Immunity: Therapeutic B Cell Depletion Enhances B16 Melanoma Growth in Mice ». *Journal of immunology (Baltimore, Md. : 1950)* 184 (7): 4006-16. <https://doi.org/10.4049/jimmunol.0903009>.

- Ding, Qing, Kanishka Mohib, Vijay K. Kuchroo, et David M. Rothstein. 2017. « TIM-4 Identifies IFN- γ -Expressing Proinflammatory B Effector 1 Cells That Promote Tumor and Allograft Rejection ». *Journal of Immunology (Baltimore, Md.: 1950)* 199 (7): 2585-95. <https://doi.org/10.4049/jimmunol.1602107>.
- Donegan, W. L. 1997. « Tumor-Related Prognostic Factors for Breast Cancer ». *CA: A Cancer Journal for Clinicians* 47 (1): 28-51. <https://doi.org/10.3322/canjclin.47.1.28>.
- Dubey, Lalit Kumar, Luc Lebon, Ilaria Mosconi, Chen-Ying Yang, Elke Scandella, Burkhard Ludewig, Sanjiv A. Luther, et Nicola L. Harris. 2016. « Lymphotoxin-Dependent B Cell-FRC Crosstalk Promotes De Novo Follicle Formation and Antibody Production Following Intestinal Helminth Infection ». *Cell Reports* 15 (7): 1527-41. <https://doi.org/10.1016/j.celrep.2016.04.023>.
- Duffy, M. J., N. Harbeck, M. Nap, R. Molina, A. Nicolini, E. Senkus, et F. Cardoso. 2017. « Clinical Use of Biomarkers in Breast Cancer: Updated Guidelines from the European Group on Tumor Markers (EGTM) ». *European Journal of Cancer (Oxford, England: 1990)* 75 (avril): 284-98. <https://doi.org/10.1016/j.ejca.2017.01.017>.
- Dunn, Gavin P., Allen T. Bruce, Hiroaki Ikeda, Lloyd J. Old, et Robert D. Schreiber. 2002. « Cancer Immunoediting: From Immunosurveillance to Tumor Escape ». *Nature Immunology* 3 (11): 991-98. <https://doi.org/10.1038/ni1102-991>.
- Dushyanthen, Sathana, Paul A. Beavis, Peter Savas, Zhi Ling Teo, Chenhao Zhou, Mariam Mansour, Phillip K. Darcy, et Sherene Loi. 2015. « Relevance of tumor-infiltrating lymphocytes in breast cancer ». *BMC Medicine* 13 (août): 202. <https://doi.org/10.1186/s12916-015-0431-3>.
- Elgueta, Raul, Micah J. Benson, Victor C. de Vries, Anna Wasiuk, Yanxia Guo, et Randolph J. Noelle. 2009. « Molecular Mechanism and Function of CD40/CD40L Engagement in the Immune System ». *Immunological Reviews* 229 (1): 152-72. <https://doi.org/10.1111/j.1600-065X.2009.00782.x>.
- Ellis, I. O., M. Galea, N. Broughton, A. Locker, R. W. Blamey, et C. W. Elston. 1992. « Pathological Prognostic Factors in Breast Cancer. II. Histological Type. Relationship with Survival in a Large Study with Long-Term Follow-Up ». *Histopathology* 20 (6): 479-89. <https://doi.org/10.1111/j.1365-2559.1992.tb01032.x>.
- Elshof, Lotte E., Marjanka K. Schmidt, Emiel J. Th Rutgers, Flora E. van Leeuwen, Jelle Wesseling, et Michael Schaapveld. 2018. « Cause-Specific Mortality in a Population-Based Cohort of 9799 Women Treated for Ductal Carcinoma In Situ ». *Annals of Surgery* 267 (5): 952-58. <https://doi.org/10.1097/SLA.0000000000002239>.
- Emens, Leisha A. 2012. « Breast Cancer Immunobiology Driving Immunotherapy: Vaccines and Immune Checkpoint Blockade ». *Expert Review of Anticancer Therapy* 12 (12): 1597-1611. <https://doi.org/10.1586/era.12.147>.
- Engelhard, Victor H., Anthony B. Rodriguez, Ileana S. Mauldin, Amber N. Woods, J. David Peske, et Craig L. Slingluff. 2018. « Immune Cell Infiltration and Tertiary Lymphoid Structures as Determinants of Antitumor Immunity ». *The Journal of Immunology* 200 (2): 432-42. <https://doi.org/10.4049/jimmunol.1701269>.
- Erbas, Bircan, Elena Provenzano, Jane Armes, et Dorota Gertig. 2006. « The Natural History of Ductal Carcinoma in Situ of the Breast: A Review ». *Breast Cancer Research and Treatment* 97 (2): 135-44. <https://doi.org/10.1007/s10549-005-9101-z>.
- Eschweiler, Simon, James Clarke, Ciro Ramírez-Suástegui, Bharat Panwar, Ariel Madrigal, Serena J. Chee, Ioannis Karydis, et al. 2021. « Intratumoral Follicular Regulatory T Cells Curtail Anti-PD-1 Treatment Efficacy ». *Nature Immunology* 22 (8): 1052-63. <https://doi.org/10.1038/s41590-021-00958-6>.
- Faget, Julien, Vanja Sisirak, Jean-Yves Blay, Christophe Caux, Nathalie Bendriss-Vermare, et Christine Ménétrier-Caux. 2013. « ICOS Is Associated with Poor Prognosis in Breast Cancer as It Promotes the Amplification of Immunosuppressive CD4+ T Cells by Plasmacytoid Dendritic Cells ». *Oncoimmunology* 2 (3): e23185. <https://doi.org/10.4161/onci.23185>.

- Fan, L., C. R. Reilly, Y. Luo, M. E. Dorf, et D. Lo. 2000. « Cutting Edge: Ectopic Expression of the Chemokine TCA4/SLC Is Sufficient to Trigger Lymphoid Neogenesis ». *Journal of Immunology (Baltimore, Md.: 1950)* 164 (8): 3955-59. <https://doi.org/10.4049/jimmunol.164.8.3955>.
- Fan, Shaoxia, Shen Yan, Yang Yang, Jian Shang, et Min Hao. 2021. « Actin-Like Protein 8 Promotes the Progression of Triple-Negative Breast Cancer via Activating PI3K/AKT/mTOR Pathway ». *OncoTargets and therapy* 14 (avril): 2463-73. <https://doi.org/10.2147/OTT.S291403>.
- Feng, Hong, Fujun Yang, Lihong Qiao, Kai Zhou, Junfei Wang, Jiao Zhang, Tian Tian, Ying Du, et Hong Shangguan. 2021. « Prognostic Significance of Gene Signature of Tertiary Lymphoid Structures in Patients With Lung Adenocarcinoma ». *Frontiers in Oncology* 11: 693234. <https://doi.org/10.3389/fonc.2021.693234>.
- Feola, Sara, Jacopo Chiaro, Beatriz Martins, et Vincenzo Cerullo. 2020. « Uncovering the Tumor Antigen Landscape: What to Know about the Discovery Process ». *Cancers* 12 (6): 1660. <https://doi.org/10.3390/cancers12061660>.
- Figenschau, Stine L., Silje Fismen, Kristin A. Fenton, Christopher Fenton, et Elin S. Mortensen. 2015. « Tertiary lymphoid structures are associated with higher tumor grade in primary operable breast cancer patients ». *BMC Cancer* 15 (1): 101. <https://doi.org/10.1186/s12885-015-1116-1>.
- Finkin, Shlomi, Detian Yuan, Ilan Stein, Koji Taniguchi, Achim Weber, Kristian Unger, Jeffrey L. Browning, et al. 2015. « Ectopic Lymphoid Structures Function as Microniches for Tumor Progenitor Cells in Hepatocellular Carcinoma ». *Nature Immunology* 16 (12): 1235-44. <https://doi.org/10.1038/ni.3290>.
- Fisher, E. R., R. M. Gregorio, B. Fisher, C. Redmond, F. Vellios, et S. C. Sommers. 1975. « The Pathology of Invasive Breast Cancer. A Syllabus Derived from Findings of the National Surgical Adjuvant Breast Project (Protocol No. 4) ». *Cancer* 36 (1): 1-85. [https://doi.org/10.1002/1097-0142\(197507\)36:1<1::aid-cnrcr2820360102>3.0.co;2-4](https://doi.org/10.1002/1097-0142(197507)36:1<1::aid-cnrcr2820360102>3.0.co;2-4).
- Fleige, Henrike, Sarina Ravens, Georgios Leandros Moschovakis, Jasmin Bölter, Stefanie Willenzon, Gerd Sutter, Susanne Häussler, Ulrich Kalinke, Immo Prinz, et Reinhold Förster. 2014. « IL-17-Induced CXCL12 Recruits B Cells and Induces Follicle Formation in BALT in the Absence of Differentiated FDCs ». *The Journal of Experimental Medicine* 211 (4): 643-51. <https://doi.org/10.1084/jem.20131737>.
- Foo, Shen Yun, Vivian Zhang, Amit Lalwani, Jason P. Lynch, Aowen Zhuang, Chuan En Lam, Paul S. Foster, et al. 2015. « Regulatory T Cells Prevent Inducible BALT Formation by Dampening Neutrophilic Inflammation ». *The Journal of Immunology* 194 (9): 4567-76. <https://doi.org/10.4049/jimmunol.1400909>.
- Fortis, Sotirios P., Michael Sofopoulos, Nectaria N. Sotiriadou, Christoforos Haritos, Christoforos K. Vaxevanis, Eleftheria A. Anastasopoulou, Nicole Janssen, et al. 2017. « Differential Intratumoral Distributions of CD8 and CD163 Immune Cells as Prognostic Biomarkers in Breast Cancer ». *Journal for Immunotherapy of Cancer* 5: 39. <https://doi.org/10.1186/s40425-017-0240-7>.
- Fragomeni, Simona Maria, Andrew Sciallis, et Jacqueline S. Jeruss. 2018. « Molecular subtypes and local-regional control of breast cancer ». *Surgical oncology clinics of North America* 27 (1): 95-120. <https://doi.org/10.1016/j.soc.2017.08.005>.
- Fu, Tong, Lei-Jie Dai, Song-Yang Wu, Yi Xiao, Ding Ma, Yi-Zhou Jiang, et Zhi-Ming Shao. 2021. « Spatial Architecture of the Immune Microenvironment Orchestrates Tumor Immunity and Therapeutic Response ». *Journal of Hematology & Oncology* 14 (1): 98. <https://doi.org/10.1186/s13045-021-01103-4>.
- Furtado, Glauca C., Michelle E. Pacer, Gerold Bongers, Cecile Benezech, Zhengxiang He, Lili Chen, M Cecilia Berin, George Kollias, Jorge H. Caamaño, et Sergio A. Lira. 2014. « TNF α -dependent development of lymphoid tissue in the absence of ROR γ t+ Lymphoid Tissue Inducer cells ». *Mucosal immunology* 7 (3): 602-14. <https://doi.org/10.1038/mi.2013.79>.

- Gago da Graça, Catarina, Lisa G. M. van Baarsen, et Reina E. Mebius. 2021. « Tertiary Lymphoid Structures: Diversity in Their Development, Composition, and Role ». *Journal of Immunology (Baltimore, Md.: 1950)* 206 (2): 273-81. <https://doi.org/10.4049/jimmunol.2000873>.
- Galon, Jérôme, Franck Pagès, Francesco M. Marincola, Magdalena Thurin, Giorgio Trinchieri, Bernard A. Fox, Thomas F. Gajewski, et Paolo A. Ascierto. 2012. « The Immune Score as a New Possible Approach for the Classification of Cancer ». *Journal of Translational Medicine* 10 (janvier): 1. <https://doi.org/10.1186/1479-5876-10-1>.
- Garaud, Soizic, Laurence Buisseret, Cinzia Solinas, Chunyan Gu-Trantien, Alexandre de Wind, Gert Van den Eynden, Celine Naveaux, et al. 2019. « Tumor Infiltrating B-Cells Signal Functional Humoral Immune Responses in Breast Cancer ». *JCI Insight* 5 (août): 129641. <https://doi.org/10.1172/jci.insight.129641>.
- Garaud, Soizic, Pawel Zayakin, Laurence Buisseret, Undine Rulle, Karina Silina, Alexandre de Wind, Gert Van den Eyden, Denis Larsimont, Karen Willard-Gallo, et Aija Linē. 2018. « Antigen Specificity and Clinical Significance of IgG and IgA Autoantibodies Produced in Situ by Tumor-Infiltrating B Cells in Breast Cancer ». *Frontiers in Immunology* 9: 2660. <https://doi.org/10.3389/fimmu.2018.02660>.
- Garrido-Castro, Ana C., Nancy U. Lin, et Kornelia Polyak. 2019. « Insights into Molecular Classifications of Triple-Negative Breast Cancer: Improving Patient Selection for Treatment ». *Cancer Discovery* 9 (2): 176-98. <https://doi.org/10.1158/2159-8290.CD-18-1177>.
- « Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications | PNAS ». s. d. Consulté le 18 avril 2022. <https://www.pnas.org/doi/10.1073/pnas.191367098>.
- Gentles, Andrew J, Aaron M Newman, Chih Long Liu, Scott V Bratman, Weiguo Feng, Dongkyoon Kim, Viswam S Nair, et al. 2015. « The Prognostic Landscape of Genes and Infiltrating Immune Cells across Human Cancers ». *Nature Medicine* 21 (8): 938-45. <https://doi.org/10.1038/nm.3909>.
- Germain, Claire, Sacha Gnjjatic, Fella Tamzalit, Samantha Knockaert, Romain Remark, Jérémy Goc, Alice Lepelley, et al. 2014. « Presence of B Cells in Tertiary Lymphoid Structures Is Associated with a Protective Immunity in Patients with Lung Cancer ». *American Journal of Respiratory and Critical Care Medicine* 189 (7): 832-44. <https://doi.org/10.1164/rccm.201309-1611OC>.
- GeurtsvanKessel, Corine H., Monique A.M. Willart, Ingrid M. Bergen, Leonie S. van Rijt, Femke Muskens, Dirk Elewaut, Albert D.M.E. Osterhaus, Rudi Hendriks, Guus F. Rimmelzwaan, et Bart N. Lambrecht. 2009. « Dendritic cells are crucial for maintenance of tertiary lymphoid structures in the lung of influenza virus–infected mice ». *Journal of Experimental Medicine* 206 (11): 2339-49. <https://doi.org/10.1084/jem.20090410>.
- Giardina, C., G. Serio, G. Lepore, T. Lettini, A. M. Dalena, A. Pennella, G. D'Eredità, T. Valente, et R. Ricco. 2003. « Pure Ductal Carcinoma in Situ and in Situ Component of Ductal Invasive Carcinoma of the Breast. A Preliminary Morphometric Study ». *Journal of Experimental & Clinical Cancer Research: CR* 22 (2): 279-88.
- Gil Del Alcazar, Carlos R., Sung Jin Huh, Muhammad B. Ekram, Anne Trinh, Lin L. Liu, Francisco Beca, Xiaoyuan Zi, et al. 2017. « Immune Escape in Breast Cancer During In Situ to Invasive Carcinoma Transition ». *Cancer Discovery* 7 (10): 1098-1115. <https://doi.org/10.1158/2159-8290.CD-17-0222>.
- Giraldo, Nicolas A., Etienne Becht, Franck Pagès, Georgios Skliris, Virginie Verkarre, Yann Vano, Arnaud Mejean, et al. 2015. « Orchestration and Prognostic Significance of Immune Checkpoints in the Microenvironment of Primary and Metastatic Renal Cell Cancer ». *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* 21 (13): 3031-40. <https://doi.org/10.1158/1078-0432.CCR-14-2926>.
- Gnjatic, Sacha, Djordje Atanackovic, Mitsutoshi Matsuo, Elke Jäger, Sang Yull Lee, Danila Valmori, Yao-Tseng Chen, Gerd Ritter, Alexander Knuth, et Lloyd J. Old. 2003. « Cross-Presentation of HLA Class I Epitopes from Exogenous NY-ESO-1 Polypeptides by Nonprofessional APCs ». *The Journal of Immunology* 170 (3): 1191-96. <https://doi.org/10.4049/jimmunol.170.3.1191>.

- Gobert, Michael, Isabelle Treilleux, Nathalie Bendriss-Vermare, Thomas Bachelot, Sophie Goddard-Leon, Vanessa Arfi, Cathy Biota, et al. 2009. « Regulatory T Cells Recruited through CCL22/CCR4 Are Selectively Activated in Lymphoid Infiltrates Surrounding Primary Breast Tumors and Lead to an Adverse Clinical Outcome ». *Cancer Research* 69 (5): 2000-2009. <https://doi.org/10.1158/0008-5472.CAN-08-2360>.
- Goc, Jérémy, Wolf-Herman Fridman, Catherine Sautès-Fridman, et Marie-Caroline Dieu-Nosjean. 2013. « Characteristics of Tertiary Lymphoid Structures in Primary Cancers ». *Oncoimmunology* 2 (12): e26836. <https://doi.org/10.4161/onci.26836>.
- Goc, Jérémy, Claire Germain, Thi Kim Duy Vo-Bourgais, Audrey Lupo, Christophe Klein, Samantha Knockaert, Luc de Chaisemartin, et al. 2014. « Dendritic Cells in Tumor-Associated Tertiary Lymphoid Structures Signal a Th1 Cytotoxic Immune Contexture and License the Positive Prognostic Value of Infiltrating CD8+ T Cells ». *Cancer Research* 74 (3): 705-15. <https://doi.org/10.1158/0008-5472.CAN-13-1342>.
- Goldhirsch, A., E. P. Winer, A. S. Coates, R. D. Gelber, M. Piccart-Gebhart, B. Thürlimann, H.-J. Senn, et Panel members. 2013. « Personalizing the Treatment of Women with Early Breast Cancer: Highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013 ». *Annals of Oncology: Official Journal of the European Society for Medical Oncology* 24 (9): 2206-23. <https://doi.org/10.1093/annonc/mdt303>.
- Gong, Di-He, Yi-Yu Chen, Ding Ma, Hai-Yan Chen, Ke-Feng Ding, et Ke-Da Yu. 2019. « Complicated prognostic values of CCL28 in breast cancer by subtype ». *Journal of Thoracic Disease* 11 (3): 777-87. <https://doi.org/10.21037/jtd.2019.02.26>.
- Grekou, A. N., T. Toliou, P. Stravoravdi, F. Patakiouta, T. Tsoukalas, M. Pinakidis, et G. Keramidas. 1996. « Correlation of Apoptosis with the Distribution and Composition of Lymphocytic Infiltrate in Human Breast Carcinomas ». *Anticancer Research* 16 (6C): 3991-95.
- Groeneveld, Clarice S., Jacqueline Fontugne, Luc Cabel, Isabelle Bernard-Pierrot, François Radvanyi, Yves Allory, et Aurélien de Reyniès. 2021. « Tertiary Lymphoid Structures Marker CXCL13 Is Associated with Better Survival for Patients with Advanced-Stage Bladder Cancer Treated with Immunotherapy ». *European Journal of Cancer (Oxford, England: 1990)* 148 (mai): 181-89. <https://doi.org/10.1016/j.ejca.2021.01.036>.
- Groeper, Célia, Franco Gambazzi, Paul Zajac, Lukas Bubendorf, Michel Adamina, Rachel Rosenthal, Hans-Reinhard Zerkowski, Michael Heberer, et Giulio C. Spagnoli. 2007. « Cancer/Testis Antigen Expression and Specific Cytotoxic T Lymphocyte Responses in Non Small Cell Lung Cancer ». *International Journal of Cancer* 120 (2): 337-43. <https://doi.org/10.1002/ijc.22309>.
- Guan, Honggeng, Yuqiu Wan, Jing Lan, Qin Wang, Zhangyu Wang, Yecheng Li, Jiqing Zheng, et al. 2016. « PD-L1 is a critical mediator of regulatory B cells and T cells in invasive breast cancer ». *Scientific Reports* 6 (octobre): 35651. <https://doi.org/10.1038/srep35651>.
- Guedj, Kevin, Jamila Khallou-Laschet, Marc Clement, Marion Morvan, Anh-Thu Gaston, Giulia Fornasa, Jianping Dai, et al. 2014. « M1 Macrophages Act as LTβR-Independent Lymphoid Tissue Inducer Cells during Atherosclerosis-Related Lymphoid Neogenesis ». *Cardiovascular Research* 101 (3): 434-43. <https://doi.org/10.1093/cvr/cvt263>.
- Gu-Trantien, Chunyan, Sherene Loi, Soizic Garaud, Carole Equeter, Myriam Libin, Alexandre de Wind, Marie Ravoet, et al. 2013. « CD4⁺ Follicular Helper T Cell Infiltration Predicts Breast Cancer Survival ». *The Journal of Clinical Investigation* 123 (7): 2873-92. <https://doi.org/10.1172/JCI67428>.
- Gu-Trantien, Chunyan, Edoardo Migliori, Laurence Buisseret, Alexandre de Wind, Sylvain Brohée, Soizic Garaud, Grégory Noël, et al. 2017. « CXCL13-Producing TFH Cells Link Immune Suppression and Adaptive Memory in Human Breast Cancer ». *JCI Insight* 2 (11): 91487. <https://doi.org/10.1172/jci.insight.91487>.
- Györfy, Balazs, Andras Lanczky, Aron C. Eklund, Carsten Denkert, Jan Budczies, Qiyuan Li, et Zoltan Szallasi. 2010. « An Online Survival Analysis Tool to Rapidly Assess the Effect of 22,277 Genes on Breast Cancer Prognosis Using Microarray Data of 1,809 Patients ». *Breast Cancer Research and Treatment* 123 (3): 725-31. <https://doi.org/10.1007/s10549-009-0674-9>.

- Hagn, Magdalena, Kai Sontheimer, Karen Dahlke, Sabine Brueggemann, Christof Kaltenmeier, Tamara Beyer, Stefanie Hofmann, et al. 2012. « Human B Cells Differentiate into Granzyme B-Secreting Cytotoxic B Lymphocytes upon Incomplete T-Cell Help ». *Immunology and Cell Biology* 90 (4): 457-67. <https://doi.org/10.1038/icb.2011.64>.
- Han, Qiang, Ming-Li Sun, Wen-Si Liu, Hai-Shan Zhao, Long-Yang Jiang, Zhao-Jin Yu, et Min-Jie Wei. 2019. « Upregulated expression of ACTL8 contributes to invasion and metastasis and indicates poor prognosis in colorectal cancer ». *Oncotargets and therapy* 12 (mars): 1749-63. <https://doi.org/10.2147/OTT.S185858>.
- Haricharan, Svasti, Matthew N. Bainbridge, Paul Scheet, et Powel H. Brown. 2014. « Somatic mutation load of estrogen receptor-positive breast tumors predicts overall survival: an analysis of genome sequence data ». *Breast Cancer Research and Treatment* 146 (1): 211-20. <https://doi.org/10.1007/s10549-014-2991-x>.
- Helmink, Beth A., Sangeetha M. Reddy, Jianjun Gao, Shaojun Zhang, Rafet Basar, Rohit Thakur, Keren Yizhak, et al. 2020. « B Cells and Tertiary Lymphoid Structures Promote Immunotherapy Response ». *Nature* 577 (7791): 549-55. <https://doi.org/10.1038/s41586-019-1922-8>.
- Hill, David G., Liang Yu, Hugh Gao, Jesse J. Balic, Alison West, Hiroko Oshima, Louise McLeod, et al. 2018. « Hyperactive gp130/STAT3-driven gastric tumourigenesis promotes submucosal tertiary lymphoid structure development ». *International Journal of Cancer* 143 (1): 167-78. <https://doi.org/10.1002/ijc.31298>.
- Hindley, James P., Emma Jones, Kathryn Smart, Hayley Bridgeman, Sarah N. Lauder, Beatrice Ondondo, Scott Cutting, et al. 2012. « T-Cell Trafficking Facilitated by High Endothelial Venules Is Required for Tumor Control after Regulatory T-Cell Depletion ». *Cancer Research* 72 (21): 5473-82. <https://doi.org/10.1158/0008-5472.CAN-12-1912>.
- Hiraoka, Nobuyoshi, Yoshinori Ino, et Rie Yamazaki-Itoh. 2016. « Tertiary Lymphoid Organs in Cancer Tissues ». *Frontiers in Immunology* 7 (juin): 244. <https://doi.org/10.3389/fimmu.2016.00244>.
- Hoch, Tobias, Daniel Schulz, Nils Eling, Julia Martínez Gómez, Mitchell P. Levesque, et Bernd Bodenmiller. s. d. « Multiplexed imaging mass cytometry of the chemokine milieu in melanoma characterizes features of the response to immunotherapy ». *Science Immunology* 7 (70): eabk1692. <https://doi.org/10.1126/sciimmunol.abk1692>.
- Hollern, Daniel P., Nuo Xu, Aatish Thennavan, Cherise Glodowski, Susana Garcia-Recio, Kevin R. Mott, Xiaping He, et al. 2019. « B Cells and T Follicular Helper Cells Mediate Response to Checkpoint Inhibitors in High Mutation Burden Mouse Models of Breast Cancer ». *Cell* 179 (5): 1191-1206.e21. <https://doi.org/10.1016/j.cell.2019.10.028>.
- Hortobagyi, Gabriel N., Jaime de la Garza Salazar, Kathleen Pritchard, Dino Amadori, Renate Haidinger, Clifford A. Hudis, Hussein Khaled, et al. 2005. « The Global Breast Cancer Burden: Variations in Epidemiology and Survival ». *Clinical Breast Cancer* 6 (5): 391-401. <https://doi.org/10.3816/cbc.2005.n.043>.
- Hu, Qingtao, Yu Hong, Pan Qi, Guangqing Lu, Xueying Mai, Sheng Xu, Xiaoying He, et al. 2021. « Atlas of breast cancer infiltrated B-lymphocytes revealed by paired single-cell RNA-sequencing and antigen receptor profiling ». *Nature Communications* 12 (avril): 2186. <https://doi.org/10.1038/s41467-021-22300-2>.
- Hu, Zhiyuan, Cheng Fan, Daniel S. Oh, J. S. Marron, Xiaping He, Bahjat F. Qaqish, Chad Livasy, et al. 2006. « The Molecular Portraits of Breast Tumors Are Conserved across Microarray Platforms ». *BMC Genomics* 7 (avril): 96. <https://doi.org/10.1186/1471-2164-7-96>.
- Hubert, Margaux, Elisa Gobbin, Coline Couillault, Thien-Phong Vu Manh, Anne-Claire Doffin, Justine Berthet, Céline Rodriguez, et al. 2020. « IFN-III Is Selectively Produced by CDC1 and Predicts Good Clinical Outcome in Breast Cancer ». *Science Immunology* 5 (46): eaav3942. <https://doi.org/10.1126/sciimmunol.aav3942>.
- Hutloff, Andreas. 2018. « T Follicular Helper-Like Cells in Inflamed Non-Lymphoid Tissues ». *Frontiers in Immunology* 9. <https://www.frontiersin.org/article/10.3389/fimmu.2018.01707>.
- Ishigami, Emi, Masahiro Sakakibara, Junta Sakakibara, Takahito Masuda, Hiroshi Fujimoto, Shouko Hayama, Takeshi Nagashima, et al. 2019. « Coexistence of Regulatory B Cells and Regulatory

- T Cells in Tumor-Infiltrating Lymphocyte Aggregates Is a Prognostic Factor in Patients with Breast Cancer ». *Breast Cancer (Tokyo, Japan)* 26 (2): 180-89.
<https://doi.org/10.1007/s12282-018-0910-4>.
- Iwata, Yohei, Takashi Matsushita, Mayuka Horikawa, David J. Dilillo, Koichi Yanaba, Guglielmo M. Venturi, Paul M. Szabolcs, et al. 2011. « Characterization of a Rare IL-10-Competent B-Cell Subset in Humans That Parallels Mouse Regulatory B10 Cells ». *Blood* 117 (2): 530-41.
<https://doi.org/10.1182/blood-2010-07-294249>.
- J Gunderson, Andrew, Venkatesh Rajamanickam, Cynthia Bui, Brady Bernard, Joanna Pucilowska, Carmen Ballesteros-Merino, Mark Schmidt, et al. 2021. « Germinal Center Reactions in Tertiary Lymphoid Structures Associate with Neoantigen Burden, Humoral Immunity and Long-Term Survivorship in Pancreatic Cancer ». *Oncoimmunology* 10 (1): 1900635.
<https://doi.org/10.1080/2162402X.2021.1900635>.
- Johansson-Percival, Anna, Bo He, Zhi-Jie Li, Alva Kjellén, Karen Russell, Ji Li, Irma Larma, et Ruth Ganss. 2017. « De Novo Induction of Intratumoral Lymphoid Structures and Vessel Normalization Enhances Immunotherapy in Resistant Tumors ». *Nature Immunology* 18 (11): 1207-17. <https://doi.org/10.1038/ni.3836>.
- Jones, Gareth W., Michele Bombardieri, Claire J. Greenhill, Louise McLeod, Alessandra Nerviani, Vidalba Rocher-Ros, Anna Cardus, et al. 2015. « Interleukin-27 Inhibits Ectopic Lymphoid-like Structure Development in Early Inflammatory Arthritis ». *The Journal of Experimental Medicine* 212 (11): 1793-1802. <https://doi.org/10.1084/jem.20132307>.
- Jones, Gareth W., et Simon A. Jones. 2016. « Ectopic lymphoid follicles: inducible centres for generating antigen-specific immune responses within tissues ». *Immunology* 147 (2): 141-51.
<https://doi.org/10.1111/imm.12554>.
- Joshi, Nikhil S., Elliot H. Akama-Garren, Yisi Lu, Da-Yae Lee, Gregory P. Chang, Amy Li, Michel DuPage, et al. 2015. « Regulatory T Cells in Tumor-Associated Tertiary Lymphoid Structures Suppress Anti-Tumor T Cell Responses ». *Immunity* 43 (3): 579-90.
<https://doi.org/10.1016/j.immuni.2015.08.006>.
- Kaewkangsadan, Viriya, Chandan Verma, Jennifer M. Eremin, Gerard Cowley, Mohammed Ilyas, et Oleg Eremin. 2016. « Crucial Contributions by T Lymphocytes (Effector, Regulatory, and Checkpoint Inhibitor) and Cytokines (TH1, TH2, and TH17) to a Pathological Complete Response Induced by Neoadjuvant Chemotherapy in Women with Breast Cancer ». *Journal of Immunology Research* 2016: 4757405. <https://doi.org/10.1155/2016/4757405>.
- Karn, Thomas, Tingting Jiang, Christos Hatzis, Nicole Sängler, Ahmed El-Balat, Achim Rody, Uwe Holtrich, Sven Becker, Giampaolo Bianchini, et Lajos Pusztai. 2017. « Association Between Genomic Metrics and Immune Infiltration in Triple-Negative Breast Cancer ». *JAMA Oncology* 3 (12): 1707-11. <https://doi.org/10.1001/jamaoncol.2017.2140>.
- Keenan, Tanya E., et Sara M. Tolaney. 2020. « Role of Immunotherapy in Triple-Negative Breast Cancer ». *Journal of the National Comprehensive Cancer Network: JNCCN* 18 (4): 479-89.
<https://doi.org/10.6004/jnccn.2020.7554>.
- Kemp, Troy J., Jill M. Moore, et Thomas S. Griffith. 2004. « Human B Cells Express Functional TRAIL/Apo-2 Ligand after CpG-Containing Oligodeoxynucleotide Stimulation ». *The Journal of Immunology* 173 (2): 892-99. <https://doi.org/10.4049/jimmunol.173.2.892>.
- Keren, Leeat, Marc Bosse, Diana Marquez, Roshan Angoshtari, Samir Jain, Sushama Varma, Soo-Ryum Yang, et al. 2018. « A Structured Tumor-Immune Microenvironment in Triple Negative Breast Cancer Revealed by Multiplexed Ion Beam Imaging ». *Cell* 174 (6): 1373-1387.e19.
<https://doi.org/10.1016/j.cell.2018.08.039>.
- Kim, Milim, Yul Ri Chung, Hyun Jeong Kim, Ji Won Woo, Soomin Ahn, et So Yeon Park. 2020. « Immune microenvironment in ductal carcinoma in situ: a comparison with invasive carcinoma of the breast ». *Breast Cancer Research* 22 (1): 32.
<https://doi.org/10.1186/s13058-020-01267-w>.
- Kobayashi, Tadahiro, Yasuhito Hamaguchi, Minoru Hasegawa, Manabu Fujimoto, Kazuhiko Takehara, et Takashi Matsushita. 2014. « B Cells Promote Tumor Immunity against B16F10

- Melanoma ». *The American Journal of Pathology* 184 (11): 3120-29.
<https://doi.org/10.1016/j.ajpath.2014.07.003>.
- Kohrt, Holbrook E., Navid Nouri, Kent Nowels, Denise Johnson, Susan Holmes, et Peter P. Lee. 2005. « Profile of Immune Cells in Axillary Lymph Nodes Predicts Disease-Free Survival in Breast Cancer ». *PLoS Medicine* 2 (9): e284. <https://doi.org/10.1371/journal.pmed.0020284>.
- Kondov, Borislav, Zvonko Milenkovicj, Goran Kondov, Gordana Petrushevska, Neli Basheska, Magdalena Bogdanovska-Todorovska, Natasha Tolevska, et Ljube Ivkovski. 2018. « Presentation of the Molecular Subtypes of Breast Cancer Detected By Immunohistochemistry in Surgically Treated Patients ». *Open Access Macedonian Journal of Medical Sciences* 6 (6): 961-67. <https://doi.org/10.3889/oamjms.2018.231>.
- Koscsó, Balázs, Sravya Kurapati, Richard R. Rodrigues, Jelena Nedjic, Kavitha Gowda, Changsik Shin, Chetna Soni, et al. 2020. « Gut-resident CX3CR1hi macrophages induce tertiary lymphoid structures and IgA response in situ ». *Science immunology* 5 (46): eaax0062.
<https://doi.org/10.1126/sciimmunol.aax0062>.
- Kroeger, David R., Katy Milne, et Brad H. Nelson. 2016. « Tumor-Infiltrating Plasma Cells Are Associated with Tertiary Lymphoid Structures, Cytolytic T-Cell Responses, and Superior Prognosis in Ovarian Cancer ». *Clinical Cancer Research* 22 (12): 3005-15.
<https://doi.org/10.1158/1078-0432.CCR-15-2762>.
- Kunkel, Eric J., et Eugene C. Butcher. 2003. « Plasma-Cell Homing ». *Nature Reviews Immunology* 3 (10): 822-29. <https://doi.org/10.1038/nri1203>.
- Kuroda, Etsushi, Koji Ozasa, Burcu Temizoz, Keiichi Ohata, Christine X. Koo, Tomohiro Kanuma, Takato Kusakabe, et al. 2016. « Inhaled Fine Particles Induce Alveolar Macrophage Death and Interleukin-1 α Release to Promote Inducible Bronchus-Associated Lymphoid Tissue Formation ». *Immunity* 45 (6): 1299-1310. <https://doi.org/10.1016/j.immuni.2016.11.010>.
- Kuroda, Hajime, Tsengelmaa Jamiyan, Rin Yamaguchi, Akinari Kakumoto, Akihito Abe, Oi Harada, Bayarmaa Enkhbat, et Atsuko Masunaga. 2021. « Prognostic Value of Tumor-Infiltrating B Lymphocytes and Plasma Cells in Triple-Negative Breast Cancer ». *Breast Cancer* 28 (4): 904-14. <https://doi.org/10.1007/s12282-021-01227-y>.
- Lacotte, Stéphanie, Marion Decossas, Carole Le Coz, Susana Brun, Sylviane Muller, et Hélène Dumortier. 2013. « Early Differentiated CD138^{high}MHCII⁺IgG⁺ Plasma Cells Express CXCR3 and Localize into Inflamed Kidneys of Lupus Mice ». *PLoS ONE* 8 (3): e58140.
<https://doi.org/10.1371/journal.pone.0058140>.
- Lazarus, Nicole H., Eric J. Kunkel, Brent Johnston, Eric Wilson, Kenneth R. Youngman, et Eugene C. Butcher. 2003. « A Common Mucosal Chemokine (Mucosae-Associated Epithelial Chemokine/CCL28) Selectively Attracts IgA Plasmablasts ». *The Journal of Immunology* 170 (7): 3799-3805. <https://doi.org/10.4049/jimmunol.170.7.3799>.
- Lee, Hee Jin, Joo Young Kim, In Ah Park, In Hye Song, Jong Han Yu, Jin-Hee Ahn, et Gyungyub Gong. 2015. « Prognostic Significance of Tumor-Infiltrating Lymphocytes and the Tertiary Lymphoid Structures in HER2-Positive Breast Cancer Treated With Adjuvant Trastuzumab ». *American Journal of Clinical Pathology* 144 (2): 278-88. <https://doi.org/10.1309/AJCPIXUYDVZORZ3G>.
- Lee, Hee Jin, Jeong-Ju Lee, In Hye Song, In Ah Park, Jun Kang, Jong Han Yu, Jin-Hee Ahn, et Gyungyub Gong. 2015. « Prognostic and Predictive Value of NanoString-Based Immune-Related Gene Signatures in a Neoadjuvant Setting of Triple-Negative Breast Cancer: Relationship to Tumor-Infiltrating Lymphocytes ». *Breast Cancer Research and Treatment* 151 (3): 619-27.
<https://doi.org/10.1007/s10549-015-3438-8>.
- Lee, Hee Jin, In Ah Park, In Hye Song, Su-Jin Shin, Joo Young Kim, Jong Han Yu, et Gyungyub Gong. 2016. « Tertiary Lymphoid Structures: Prognostic Significance and Relationship with Tumour-Infiltrating Lymphocytes in Triple-Negative Breast Cancer ». *Journal of Clinical Pathology* 69 (5): 422-30. <https://doi.org/10.1136/jclinpath-2015-203089>.
- Lee, Kyoung Eun, Michelle Spata, Lauren J. Bayne, Elizabeth L. Buza, Amy C. Durham, David Allman, Robert H. Vonderheide, et M. Celeste Simon. 2016. « Hif1a Deletion Reveals Pro-Neoplastic

- Function of B Cells in Pancreatic Neoplasia ». *Cancer Discovery* 6 (3): 256-69. <https://doi.org/10.1158/2159-8290.CD-15-0822>.
- Lee, Miseon, Sun-Hee Heo, In Hye Song, Hajar Rajayi, Hye Seon Park, In Ah Park, Young-Ae Kim, Heejae Lee, Gyungyub Gong, et Hee Jin Lee. 2019. « Presence of Tertiary Lymphoid Structures Determines the Level of Tumor-Infiltrating Lymphocytes in Primary Breast Cancer and Metastasis ». *Modern Pathology: An Official Journal of the United States and Canadian Academy of Pathology, Inc* 32 (1): 70-80. <https://doi.org/10.1038/s41379-018-0113-8>.
- Li, Hui, Hailing Liu, Hongyuan Fu, Jiaxin Li, Lin Xu, Genshu Wang, et Hong Wu. 2021. « Peritumoral Tertiary Lymphoid Structures Correlate With Protective Immunity and Improved Prognosis in Patients With Hepatocellular Carcinoma ». *Frontiers in Immunology* 12. <https://www.frontiersin.org/article/10.3389/fimmu.2021.648812>.
- Li, Li, Yan Ma, et Yuan Xu. 2019. « Follicular Regulatory T Cells Infiltrated the Ovarian Carcinoma and Resulted in CD8 T Cell Dysfunction Dependent on IL-10 Pathway ». *International Immunopharmacology* 68 (mars): 81-87. <https://doi.org/10.1016/j.intimp.2018.12.051>.
- Li, Qiao, Xiangming Lao, Qin Pan, Ning Ning, Ji Yet, Yingxin Xu, Shengping Li, et Alfred E. Chang. 2011. « Adoptive Transfer of Tumor Reactive B Cells Confers Host T-Cell Immunity and Tumor Regression ». *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* 17 (15): 4987-95. <https://doi.org/10.1158/1078-0432.CCR-11-0207>.
- Li, Saiyang, Chi Huang, Guanghui Hu, Junjie Ma, Yonghui Chen, Jin Zhang, Yiran Huang, et al. 2020. « Tumor-Educated B Cells Promote Renal Cancer Metastasis via Inducing the IL-1 β /HIF-2 α /Notch1 Signals ». *Cell Death & Disease* 11 (3): 163. <https://doi.org/10.1038/s41419-020-2355-x>.
- Lin, Ziyang, Lixia Huang, ShaoLi Li, Jincui Gu, Xiaoxian Cui, et Yanbin Zhou. 2020. « Pan-Cancer Analysis of Genomic Properties and Clinical Outcome Associated with Tumor Tertiary Lymphoid Structure ». *Scientific Reports* 10 (1): 21530. <https://doi.org/10.1038/s41598-020-78560-3>.
- Lindner, Stefanie, Karen Dahlke, Kai Sontheimer, Magdalena Hagn, Christof Kaltenmeier, Thomas F.E. Barth, Tamara Beyer, et al. 2013. « Interleukin 21–Induced Granzyme B–Expressing B Cells Infiltrate Tumors and Regulate T Cells ». *Cancer Research* 73 (8): 2468-79. <https://doi.org/10.1158/0008-5472.CAN-12-3450>.
- Liu, Kewei, Ai Huang, Jun Nie, Jun Tan, Shijie Xing, Yue Qu, et Ke Jiang. 2021. « IL-35 Regulates the Function of Immune Cells in Tumor Microenvironment ». *Frontiers in Immunology* 12. <https://www.frontiersin.org/article/10.3389/fimmu.2021.683332>.
- Liu, Xia, Julia Y.S. Tsang, Thazin Hlaing, Jintao Hu, Yun-Bi Ni, Siu Ki Chan, Sai Yin Cheung, et Gary M. Tse. 2017. « Distinct Tertiary Lymphoid Structure Associations and Their Prognostic Relevance in HER2 Positive and Negative Breast Cancers ». *The Oncologist* 22 (11): 1316-24. <https://doi.org/10.1634/theoncologist.2017-0029>.
- Lu, Theresa T., et Jeffrey L. Browning. 2014. « Role of the Lymphotoxin/LIGHT System in the Development and Maintenance of Reticular Networks and Vasculature in Lymphoid Tissues ». *Frontiers in Immunology* 5: 47. <https://doi.org/10.3389/fimmu.2014.00047>.
- Lu, Yiwen, Qiyi Zhao, Jian-You Liao, Erwei Song, Qidong Xia, Jiayao Pan, Yihong Li, et al. 2020. « Complement Signals Determine Opposite Effects of B Cells in Chemotherapy-Induced Immunity ». *Cell* 180 (6): 1081-1097.e24. <https://doi.org/10.1016/j.cell.2020.02.015>.
- Lutz, Eric R., Annie A. Wu, Elaine Bigelow, Rajni Sharma, Guanglan Mo, Kevin Soares, Sara Solt, et al. 2014. « Immunotherapy Converts Non-immunogenic Pancreatic Tumors into Immunogenic Foci of Immune Regulation ». *Cancer immunology research* 2 (7): 616-31. <https://doi.org/10.1158/2326-6066.CIR-14-0027>.
- Lynch, Kevin T., Samuel J. Young, Max O. Meneveau, Nolan A. Wages, Victor H. Engelhard, Craig L. Slingluff, et Ileana S. Mauldin. 2021. « Heterogeneity in Tertiary Lymphoid Structure B-Cells Correlates with Patient Survival in Metastatic Melanoma ». *Journal for Immunotherapy of Cancer* 9 (6): e002273. <https://doi.org/10.1136/jitc-2020-002273>.
- Lyons, Traci R., Jenean O'Brien, Virginia F. Borges, Matthew W. Conklin, Patricia J. Keely, Kevin W. Eliceiri, Andriy Marusyk, Aik-Choon Tan, et Pepper Schedin. 2011. « Postpartum Mammary

- Gland Involution Drives Progression of Ductal Carcinoma in Situ through Collagen and COX-2 ». *Nature Medicine* 17 (9): 1109-15. <https://doi.org/10.1038/nm.2416>.
- Mahmoud, S. M. A., A. H. S. Lee, E. C. Paish, R. D. Macmillan, I. O. Ellis, et A. R. Green. 2012. « The Prognostic Significance of B Lymphocytes in Invasive Carcinoma of the Breast ». *Breast Cancer Research and Treatment* 132 (2): 545-53. <https://doi.org/10.1007/s10549-011-1620-1>.
- Maldonado, Leonel, Jessica E. Teague, Matthew P. Morrow, Iveta Jotova, T. C. Wu, Chenguang Wang, Cindy Desmarais, et al. 2014. « Intramuscular Therapeutic Vaccination Targeting HPV16 Induces T Cell Responses That Localize in Mucosal Lesions ». *Science translational medicine* 6 (221): 221ra13. <https://doi.org/10.1126/scitranslmed.3007323>.
- Mantovani, Alberto. 2010. « La Mala Educación of Tumor-Associated Macrophages: Diverse Pathways and New Players ». *Cancer Cell* 17 (2): 111-12. <https://doi.org/10.1016/j.ccr.2010.01.019>.
- Marinkovic, Tatjana, Alexandre Garin, Yoshifumi Yokota, Yang-Xin Fu, Nancy H. Ruddle, Glaucia C. Furtado, et Sergio A. Lira. 2006. « Interaction of Mature CD3⁺CD4⁺ T Cells with Dendritic Cells Triggers the Development of Tertiary Lymphoid Structures in the Thyroid ». *The Journal of Clinical Investigation* 116 (10): 2622-32. <https://doi.org/10.1172/JCI28993>.
- Marques, Cristina P., Parul Kapil, David R. Hinton, Claudia Hindinger, Stephen L. Nutt, Richard M. Ransohoff, Timothy W. Phares, Stephen A. Stohlman, et Cornelia C. Bergmann. 2011. « CXCR3-Dependent Plasma Blast Migration to the Central Nervous System during Viral Encephalomyelitis ». *Journal of Virology* 85 (13): 6136-47. <https://doi.org/10.1128/JVI.00202-11>.
- Marsigliante, S, L Biscozzo, A Marra, G Nicolardi, G Leo, G B Lobreglio, et C Storelli. 1999. « Computerised Counting of Tumour Infiltrating Lymphocytes in 90 Breast Cancer Specimens ». *Cancer Letters*, 9.
- Martin, Jerome C., Christie Chang, Gilles Boschetti, Ryan Ungaro, Mamta Giri, John A. Grout, Kyle Gettler, et al. 2019. « Single-Cell Analysis of Crohn's Disease Lesions Identifies a Pathogenic Cellular Module Associated with Resistance to Anti-TNF Therapy ». *Cell* 178 (6): 1493-1508.e20. <https://doi.org/10.1016/j.cell.2019.08.008>.
- Martinet, Ludovic, Thomas Filleron, Sophie Le Guellec, Philippe Rochoaix, Ignacio Garrido, et Jean-Philippe Girard. 2013. « High Endothelial Venule Blood Vessels for Tumor-Infiltrating Lymphocytes Are Associated with Lymphotoxin β -Producing Dendritic Cells in Human Breast Cancer ». *Journal of Immunology (Baltimore, Md.: 1950)* 191 (4): 2001-8. <https://doi.org/10.4049/jimmunol.1300872>.
- Martinet, Ludovic, Ignacio Garrido, Thomas Filleron, Sophie Le Guellec, Elisabeth Bellard, Jean-Jacques Fournie, Philippe Rochoaix, et Jean-Philippe Girard. 2011. « Human Solid Tumors Contain High Endothelial Venules: Association with T- and B-Lymphocyte Infiltration and Favorable Prognosis in Breast Cancer ». *Cancer Research* 71 (17): 5678-87. <https://doi.org/10.1158/0008-5472.CAN-11-0431>.
- Martinet, Ludovic, et Jean-Philippe Girard. 2013. « Regulation of tumor-associated high-endothelial venules by dendritic cells ». *OncImmunology* 2 (11): e26470. <https://doi.org/10.4161/onci.26470>.
- Massa, Chiara, Thomas Karn, Carsten Denkert, Andreas Schneeweiss, Claus Hanusch, Jens-Uwe Blohmer, Dirk-Michael Zahm, et al. 2020. « Differential Effect on Different Immune Subsets of Neoadjuvant Chemotherapy in Patients with TNBC ». *Journal for Immunotherapy of Cancer* 8 (2): e001261. <https://doi.org/10.1136/jitc-2020-001261>.
- McDonald, Keely G., Jacquelyn S. McDonough, et Rodney D. Newberry. 2005. « Adaptive Immune Responses Are Dispensable for Isolated Lymphoid Follicle Formation: Antigen-Naive, Lymphotoxin-Sufficient B Lymphocytes Drive the Formation of Mature Isolated Lymphoid Follicles ». *The Journal of Immunology* 174 (9): 5720-28. <https://doi.org/10.4049/jimmunol.174.9.5720>.
- Messina, Jane L., David A. Fenstermacher, Steven Eschrich, Xiaotao Qu, Anders E. Berglund, Mark C. Lloyd, Michael J. Schell, Vernon K. Sondak, Jeffrey S. Weber, et James J. Mulé. 2012. « 12-

- Chemokine Gene Signature Identifies Lymph Node-like Structures in Melanoma: Potential for Patient Selection for Immunotherapy? » *Scientific Reports* 2 (octobre): 765.
<https://doi.org/10.1038/srep00765>.
- Meylan, Maxime, Florent Petitprez, Etienne Becht, Antoine Bougoüin, Guilhem Pupier, Anne Calvez, Ilenia Giglioli, et al. 2022. « Tertiary Lymphoid Structures Generate and Propagate Anti-Tumor Antibody-Producing Plasma Cells in Renal Cell Cancer ». *Immunity*, février, S1074-7613(22)00081-4. <https://doi.org/10.1016/j.immuni.2022.02.001>.
- Meylan, Maxime, Florent Petitprez, Laetitia Lacroix, Luca Di Tommaso, Massimo Roncalli, Antoine Bougoüin, Alexis Laurent, et al. 2020. « Early Hepatic Lesions Display Immature Tertiary Lymphoid Structures and Show Elevated Expression of Immune Inhibitory and Immunosuppressive Molecules ». *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* 26 (16): 4381-89. <https://doi.org/10.1158/1078-0432.CCR-19-2929>.
- Miligy, Islam, Priya Mohan, Ahmed Gaber, Mohammed A Aleskandarany, Christopher C Nolan, Maria Diez-Rodriguez, Abhik Mukherjee, et al. 2017. « Prognostic Significance of Tumour Infiltrating B Lymphocytes in Breast Ductal Carcinoma *in Situ* ». *Histopathology* 71 (2): 258-68.
<https://doi.org/10.1111/his.13217>.
- Mischo, Axel, Boris Kubuschok, Kubilay Ertan, Klaus-Dieter Preuss, Bernd Romeike, Evi Regitz, Claudia Schormann, et al. 2006. « Prospective Study on the Expression of Cancer Testis Genes and Antibody Responses in 100 Consecutive Patients with Primary Breast Cancer ». *International Journal of Cancer* 118 (3): 696-703. <https://doi.org/10.1002/ijc.21352>.
- Miyasaka, Masayuki, et Toshiyuki Tanaka. 2004. « Lymphocyte Trafficking across High Endothelial Venules: Dogmas and Enigmas ». *Nature Reviews Immunology* 4 (5): 360-70.
<https://doi.org/10.1038/nri1354>.
- Mohammed, Z. M. A., J. J. Going, J. Edwards, B. Elsberger, J. C. Doughty, et D. C. McMillan. 2012. « The Relationship between Components of Tumour Inflammatory Cell Infiltrate and Clinicopathological Factors and Survival in Patients with Primary Operable Invasive Ductal Breast Cancer ». *British Journal of Cancer* 107 (5): 864-73.
<https://doi.org/10.1038/bjc.2012.347>.
- Montfort, Anne, Oliver Pearce, Eleni Maniati, Benjamin G. Vincent, Lisa Bixby, Steffen Böhm, Thomas Dowe, et al. 2017. « A Strong B-cell Response Is Part of the Immune Landscape in Human High-Grade Serous Ovarian Metastases ». *Clinical cancer research : an official journal of the American Association for Cancer Research* 23 (1): 250-62. <https://doi.org/10.1158/1078-0432.CCR-16-0081>.
- Morita, Rimpei, Nathalie Schmitt, Salah-Eddine Bentebibel, Rajaram Ranganathan, Laure Bourdery, Gerard Zurawski, Emile Foucat, et al. 2011. « Human Blood CXCR5(+)CD4(+) T Cells Are Counterparts of T Follicular Cells and Contain Specific Subsets That Differentially Support Antibody Secretion ». *Immunity* 34 (1): 108-21.
<https://doi.org/10.1016/j.immuni.2010.12.012>.
- Moulin, Véronique, Fabienne Andris, Kris Thielemans, Charlie Maliszewski, Jacques Urbain, et Muriel Moser. 2000. « B Lymphocytes Regulate Dendritic Cell (Dc) Function in Vivo ». *The Journal of Experimental Medicine* 192 (4): 475-82.
- Muniz, Luciana R., Michelle E. Pacer, Sergio A. Lira, et Glaucia C. Furtado. 2011. « A Critical Role for Dendritic Cells in the Formation of Lymphatic Vessels within Tertiary Lymphoid Structures ». *The Journal of Immunology* 187 (2): 828-34. <https://doi.org/10.4049/jimmunol.1004233>.
- Muramatsu, Masamichi, Kazuo Kinoshita, Sidonia Fagarasan, Shuichi Yamada, Yoichi Shinkai, et Tasuku Honjo. 2000. « Class Switch Recombination and Hypermutation Require Activation-Induced Cytidine Deaminase (AID), a Potential RNA Editing Enzyme ». *Cell* 102 (5): 553-63.
[https://doi.org/10.1016/S0092-8674\(00\)00078-7](https://doi.org/10.1016/S0092-8674(00)00078-7).
- Mustapha, Rami, Kenrick Ng, James Monypenny, et Tony Ng. 2021. « Insights Into Unveiling a Potential Role of Tertiary Lymphoid Structures in Metastasis ». *Frontiers in Molecular Biosciences* 8 (septembre): 661516. <https://doi.org/10.3389/fmolb.2021.661516>.

- Nayar, Saba, Joana Campos, Charlotte G. Smith, Valentina Iannizzotto, David H. Gardner, Frédéric Mourcin, David Roulois, et al. 2019. « Immunofibroblasts are pivotal drivers of tertiary lymphoid structure formation and local pathology ». *Proceedings of the National Academy of Sciences of the United States of America* 116 (27): 13490-97. <https://doi.org/10.1073/pnas.1905301116>.
- Neyt, Katrijn, Corine H. GeurtsvanKessel, Kim Deswarte, Hamida Hammad, et Bart N. Lambrecht. 2016. « Early IL-1 Signaling Promotes iBALT Induction after Influenza Virus Infection ». *Frontiers in Immunology* 7 (août): 312. <https://doi.org/10.3389/fimmu.2016.00312>.
- Nielsen, Julie S., Rob A. Sahota, Katy Milne, Sara E. Kost, Nancy J. Nessler, Peter H. Watson, et Brad H. Nelson. 2012. « CD20+ Tumor-Infiltrating Lymphocytes Have an Atypical CD27-Memory Phenotype and Together with CD8+ T Cells Promote Favorable Prognosis in Ovarian Cancer ». *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* 18 (12): 3281-92. <https://doi.org/10.1158/1078-0432.CCR-12-0234>.
- Nielsen, Torsten O., Forrest D. Hsu, Kristin Jensen, Maggie Cheang, Gamze Karaca, Zhiyuan Hu, Tina Hernandez-Boussard, et al. 2004. « Immunohistochemical and Clinical Characterization of the Basal-like Subtype of Invasive Breast Carcinoma ». *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* 10 (16): 5367-74. <https://doi.org/10.1158/1078-0432.CCR-04-0220>.
- Noël, Grégory, Mireille Langouo Fontsa, Soizic Garaud, Pushpamali De Silva, Alexandre de Wind, Gert G. Van den Eynden, Roberto Salgado, et al. 2021. « Functional Th1-Oriented T Follicular Helper Cells That Infiltrate Human Breast Cancer Promote Effective Adaptive Immunity ». *The Journal of Clinical Investigation* 131 (19): e139905. <https://doi.org/10.1172/JCI139905>.
- Nzula, Sazini, James J. Goings, et David I. Stott. 2003. « Antigen-Driven Clonal Proliferation, Somatic Hypermutation, and Selection of B Lymphocytes Infiltrating Human Ductal Breast Carcinomas ». *Cancer Research* 63 (12): 3275-80.
- Olkhanud, Purevdorj B., Bazarragchaа Damdinsuren, Monica Bodogai, Ronald E. Gress, Ranjan Sen, Katarzyna Wejksza, Enkhzol Malchinkhuu, Robert P. Wersto, et Arya Biragyn. 2011. « Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting CD4+ T cells to T regulatory cells ». *Cancer research* 71 (10): 3505-15. <https://doi.org/10.1158/0008-5472.CAN-10-4316>.
- Ou, Zhenyu, Yongjie Wang, Longfei Liu, Lei Li, Shuyuan Yeh, Lin Qi, et Chawnsang Chang. 2015. « Tumor Microenvironment B Cells Increase Bladder Cancer Metastasis via Modulation of the IL-8/Androgen Receptor (AR)/MMPs Signals ». *Oncotarget* 6 (28): 26065-78. <https://doi.org/10.18632/oncotarget.4569>.
- Park, Chan-Sik, et Yong Sung Choi. 2005. « How Do Follicular Dendritic Cells Interact Intimately with B Cells in the Germinal Centre? » *Immunology* 114 (1): 2-10. <https://doi.org/10.1111/j.1365-2567.2004.02075.x>.
- Parker, Joel S., Michael Mullins, Maggie C.U. Cheang, Samuel Leung, David Voduc, Tammi Vickery, Sherri Davies, et al. 2009. « Supervised Risk Predictor of Breast Cancer Based on Intrinsic Subtypes ». *Journal of Clinical Oncology* 27 (8): 1160-67. <https://doi.org/10.1200/JCO.2008.18.1370>.
- Pavert, Serge A. van de, et Reina E. Mebius. 2010. « New Insights into the Development of Lymphoid Tissues ». *Nature Reviews. Immunology* 10 (9): 664-74. <https://doi.org/10.1038/nri2832>.
- Pène, Jérôme, Jean-François Gauchat, Sandrine Lécart, Elodie Drouet, Paul Guglielmi, Vera Boulay, Adriana Delwail, Don Foster, Jean-Claude Lecron, et Hans Yssel. 2004. « Cutting Edge: IL-21 Is a Switch Factor for the Production of IgG1 and IgG3 by Human B Cells ». *The Journal of Immunology* 172 (9): 5154-57. <https://doi.org/10.4049/jimmunol.172.9.5154>.
- Perou, C. M., T. Sørlie, M. B. Eisen, M. van de Rijn, S. S. Jeffrey, C. A. Rees, J. R. Pollack, et al. 2000. « Molecular Portraits of Human Breast Tumours ». *Nature* 406 (6797): 747-52. <https://doi.org/10.1038/35021093>.
- Perricone, Michael A., Karen A. Smith, Kirsten A. Claussen, Malinda S. Plog, Donna M. Hempel, Bruce L. Roberts, Judith A. St George, et Johanne M. Kaplan. 2004. « Enhanced Efficacy of

- Melanoma Vaccines in the Absence of B Lymphocytes ». *Journal of Immunotherapy (Hagerstown, Md.: 1997)* 27 (4): 273-81. <https://doi.org/10.1097/00002371-200407000-00003>.
- Petitprez, Florent, Aurélien de Reyniès, Emily Z. Keung, Tom Wei-Wu Chen, Cheng-Ming Sun, Julien Calderaro, Yung-Ming Jeng, et al. 2020. « B Cells Are Associated with Survival and Immunotherapy Response in Sarcoma ». *Nature* 577 (7791): 556-60. <https://doi.org/10.1038/s41586-019-1906-8>.
- Pikor, Natalia B., Jillian L. Astarita, Leslie Summers-Deluca, Georgina Galicia, Joy Qu, Lesley A. Ward, Susan Armstrong, et al. 2015. « Integration of Th17- and Lymphotoxin-Derived Signals Initiates Meningeal-Resident Stromal Cell Remodeling to Propagate Neuroinflammation ». *Immunity* 43 (6): 1160-73. <https://doi.org/10.1016/j.immuni.2015.11.010>.
- Posch, Florian, Karina Silina, Sebastian Leibl, Axel Mündlein, Holger Moch, Alexander Siebenhüner, Panagiotis Samaras, et al. 2018. « Maturation of Tertiary Lymphoid Structures and Recurrence of Stage II and III Colorectal Cancer ». *Oncoimmunology* 7 (2): e1378844. <https://doi.org/10.1080/2162402X.2017.1378844>.
- Prabhakaran, Sangeetha, Victoria T. Rizk, Zhenjun Ma, Chia-Ho Cheng, Anders E. Berglund, Dominico Coppola, Farah Khalil, James J. Mulé, et Hatem H. Soliman. 2017. « Evaluation of Invasive Breast Cancer Samples Using a 12-Chemokine Gene Expression Score: Correlation with Clinical Outcomes ». *Breast Cancer Research: BCR* 19 (1): 71. <https://doi.org/10.1186/s13058-017-0864-z>.
- Prat, Aleix, Maggie Chon U. Cheang, Miguel Martín, Joel S. Parker, Eva Carrasco, Rosalía Caballero, Scott Tyldesley, et al. 2013. « Prognostic Significance of Progesterone Receptor-Positive Tumor Cells within Immunohistochemically Defined Luminal A Breast Cancer ». *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 31 (2): 203-9. <https://doi.org/10.1200/JCO.2012.43.4134>.
- Prat, Aleix, Joel S. Parker, Olga Karginova, Cheng Fan, Chad Livasy, Jason I. Herschkowitz, Xiaping He, et Charles M. Perou. 2010. « Phenotypic and Molecular Characterization of the Claudin-Low Intrinsic Subtype of Breast Cancer ». *Breast Cancer Research: BCR* 12 (5): R68. <https://doi.org/10.1186/bcr2635>.
- Pruneri, Giancarlo, Andrea Vingiani, et Carsten Denkert. 2018. « Tumor Infiltrating Lymphocytes in Early Breast Cancer ». *Breast (Edinburgh, Scotland)* 37 (février): 207-14. <https://doi.org/10.1016/j.breast.2017.03.010>.
- Pylayeva-Gupta, Yuliya, Shipra Das, Jesse S. Handler, Cristina H. Hajdu, Maryaline Coffre, Sergei B. Korolov, et Dafna Bar-Sagi. 2016. « IL35-Producing B Cells Promote the Development of Pancreatic Neoplasia ». *Cancer Discovery* 6 (3): 247-55. <https://doi.org/10.1158/2159-8290.CD-15-0843>.
- Qin, Zhihai, Günther Richter, Thomas Schüler, Sabrina Ibe, Xuetao Cao, et Thomas Blankenstein. 1998. « B Cells Inhibit Induction of T Cell-Dependent Tumor Immunity ». *Nature Medicine* 4 (5): 627-30. <https://doi.org/10.1038/nm0598-627>.
- Randall, Troy D, Damian M Carragher, et Javier Rangel-Moreno. 2008. « Development of secondary lymphoid organs ». *Annual review of immunology* 26: 627-50. <https://doi.org/10.1146/annurev.immunol.26.021607.090257>.
- Rangel-Moreno, Javier, Damian M. Carragher, Maria de la Luz Garcia-Hernandez, Ji Young Hwang, Kim Kusser, Louise Hartson, Jay K. Kolls, Shabaana A. Khader, et Troy D. Randall. 2011. « The development of inducible Bronchus Associated Lymphoid Tissue (iBALT) is dependent on IL-17 ». *Nature immunology* 12 (7): 639-46. <https://doi.org/10.1038/ni.2053>.
- Roberts, Edward W., Miranda L. Broz, Mikhail Binnewies, Mark B. Headley, Amanda E. Nelson, Denise M. Wolf, Tsuneyasu Kaisho, Dusan Bogunovic, Nina Bhardwaj, et Matthew F. Krummel. 2016. « Critical Role for CD103(+)/CD141(+) Dendritic Cells Bearing CCR7 for Tumor Antigen Trafficking and Priming of T Cell Immunity in Melanoma ». *Cancer Cell* 30 (2): 324-36. <https://doi.org/10.1016/j.ccell.2016.06.003>.

- Rodriguez, Anthony B., J. David Peske, Amber N. Woods, Katie M. Leick, Ileana S. Mauldin, Max O. Meneveau, Samuel J. Young, et al. 2021. « Immune Mechanisms Orchestrate Tertiary Lymphoid Structures in Tumors via Cancer-Associated Fibroblasts ». *Cell Reports* 36 (3): 109422. <https://doi.org/10.1016/j.celrep.2021.109422>.
- Rosser, Elizabeth C., Kristine Oleinika, Silvia Tonon, Ronan Doyle, Anneleen Bosma, Natalie A. Carter, Kathryn A. Harris, Simon A. Jones, Nigel Klein, et Claudia Mauri. 2014. « Regulatory B Cells Are Induced by Gut Microbiota-Driven Interleukin-1 β and Interleukin-6 Production ». *Nature Medicine* 20 (11): 1334-39. <https://doi.org/10.1038/nm.3680>.
- Rossetti, Renata Ariza Marques, Noely Paula Cristina Lorenzi, Kaori Yokochi, Maria Beatriz Sartor de Faria Rosa, Luciana Benevides, Paulo Francisco Ramos Margarido, Edmund Chada Baracat, Jesus Paula Carvalho, Luisa Lina Villa, et Ana Paula Lepique. 2018. « B Lymphocytes Can Be Activated to Act as Antigen Presenting Cells to Promote Anti-Tumor Responses ». *PloS One* 13 (7): e0199034. <https://doi.org/10.1371/journal.pone.0199034>.
- Ruddle, Nancy H. 2016. « High Endothelial Venules and Lymphatic Vessels in Tertiary Lymphoid Organs: Characteristics, Functions, and Regulation ». *Frontiers in Immunology* 7 (novembre): 491. <https://doi.org/10.3389/fimmu.2016.00491>.
- Ruffin, Ayana T., Anthony R. Cillo, Tracy Tabib, Angen Liu, Sayali Onkar, Sheryl R. Kunning, Caleb Lampenfeld, et al. 2021. « B Cell Signatures and Tertiary Lymphoid Structures Contribute to Outcome in Head and Neck Squamous Cell Carcinoma ». *Nature Communications* 12 (1): 3349. <https://doi.org/10.1038/s41467-021-23355-x>.
- Safarzadeh, Elham, Shahryar Hashemzadeh, Pascal H. G. Duijf, Behzad Mansoori, Vahid Khaze, Ali Mohammadi, Tohid Kazemi, et al. 2019. « Circulating Myeloid-Derived Suppressor Cells: An Independent Prognostic Factor in Patients with Breast Cancer ». *Journal of Cellular Physiology* 234 (4): 3515-25. <https://doi.org/10.1002/jcp.26896>.
- Saint-Vis, B. de, J. Vincent, S. Vandenberghe, B. Vanbervliet, J. J. Pin, S. Aït-Yahia, S. Patel, et al. 1998. « A Novel Lysosome-Associated Membrane Glycoprotein, DC-LAMP, Induced upon DC Maturation, Is Transiently Expressed in MHC Class II Compartment ». *Immunity* 9 (3): 325-36. [https://doi.org/10.1016/s1074-7613\(00\)80615-9](https://doi.org/10.1016/s1074-7613(00)80615-9).
- Salgado, R., C. Denkert, S. Demaria, N. Sirtaine, F. Klauschen, G. Pruneri, S. Wienert, et al. 2015. « The Evaluation of Tumor-Infiltrating Lymphocytes (TILs) in Breast Cancer: Recommendations by an International TILs Working Group 2014 ». *Annals of Oncology: Official Journal of the European Society for Medical Oncology* 26 (2): 259-71. <https://doi.org/10.1093/annonc/mdu450>.
- Sautès-Fridman, Catherine, Florent Petitprez, Julien Calderaro, et Wolf Herman Fridman. 2019. « Tertiary Lymphoid Structures in the Era of Cancer Immunotherapy ». *Nature Reviews. Cancer* 19 (6): 307-25. <https://doi.org/10.1038/s41568-019-0144-6>.
- Sautès-Fridman, Catherine, Johanna Verneau, Cheng-Ming Sun, Marco Moreira, Tom Wei-Wu Chen, Maxime Meylan, Florent Petitprez, et Wolf Herman Fridman. 2020. « Tertiary Lymphoid Structures and B Cells: Clinical Impact and Therapeutic Modulation in Cancer ». *Seminars in Immunology* 48 (avril): 101406. <https://doi.org/10.1016/j.smim.2020.101406>.
- Schmidt, Marcus, Birte Hellwig, Seddik Hammad, Amnah Othman, Miriam Lohr, Zonglin Chen, Daniel Boehm, et al. 2012. « A Comprehensive Analysis of Human Gene Expression Profiles Identifies Stromal Immunoglobulin κ C as a Compatible Prognostic Marker in Human Solid Tumors ». *Clinical Cancer Research* 18 (9): 2695-2703. <https://doi.org/10.1158/1078-0432.CCR-11-2210>.
- Schrama, David, Heike Voigt, Andreas O. Eggert, Rong Xiang, He Zhou, Ton N. M. Schumacher, Mads H. Andersen, Per thor Straten, Ralph A. Reisfeld, et Jürgen C. Becker. 2008. « Immunological Tumor Destruction in a Murine Melanoma Model by Targeted L α Independent of Secondary Lymphoid Tissue ». *Cancer Immunology, Immunotherapy: CII* 57 (1): 85-95. <https://doi.org/10.1007/s00262-007-0352-x>.
- Shen, Hui, Xiang Wang, Zhe Shao, Ke Liu, Xiao-Yan Xia, Han-Zhong Zhang, Kai Song, Yong Song, et Zheng-Jun Shang. 2014. « Alterations of high endothelial venules in primary and metastatic

- tumors are correlated with lymph node metastasis of oral and pharyngeal carcinoma ». *Cancer Biology & Therapy* 15 (3): 342-49. <https://doi.org/10.4161/cbt.27328>.
- Shen, Ping, et Simon Fillatreau. 2015. « Antibody-Independent Functions of B Cells: A Focus on Cytokines ». *Nature Reviews Immunology* 15 (7): 441-51. <https://doi.org/10.1038/nri3857>.
- Shi, Jie-Yi, Qiang Gao, Zhi-Chao Wang, Jian Zhou, Xiao-Ying Wang, Zhi-Hui Min, Ying-Hong Shi, et al. 2013. « Margin-Infiltrating CD20+ B Cells Display an Atypical Memory Phenotype and Correlate with Favorable Prognosis in Hepatocellular Carcinoma ». *Clinical Cancer Research* 19 (21): 5994-6005. <https://doi.org/10.1158/1078-0432.CCR-12-3497>.
- Shi, Yang. 2021. « PLAN B for Immunotherapy: Promoting and Leveraging Anti-Tumor B Cell Immunity ». *Journal of Controlled Release: Official Journal of the Controlled Release Society* 339 (novembre): 156-63. <https://doi.org/10.1016/j.jconrel.2021.09.028>.
- Siliņa, Karīna, Alex Soltermann, Farkhondeh Movahedian Attar, Ruben Casanova, Zina M. Uckeley, Helen Thut, Muriel Wandres, et al. 2018. « Germinal Centers Determine the Prognostic Relevance of Tertiary Lymphoid Structures and Are Impaired by Corticosteroids in Lung Squamous Cell Carcinoma ». *Cancer Research* 78 (5): 1308-20. <https://doi.org/10.1158/0008-5472.CAN-17-1987>.
- Sjöberg, Elin, Magnus Frödin, John Lövrot, Artur Mezheyeuski, Martin Johansson, Ulrika Harmenberg, Lars Egevad, Per Sandström, et Arne Östman. 2018. « A Minority-Group of Renal Cell Cancer Patients with High Infiltration of CD20+B-Cells Is Associated with Poor Prognosis ». *British Journal of Cancer* 119 (7): 840-46. <https://doi.org/10.1038/s41416-018-0266-8>.
- Smith, Christof C., Sara R. Selitsky, Shengjie Chai, Paul M. Armistead, Benjamin G. Vincent, et Jonathan S. Serody. 2019. « Alternative Tumour-Specific Antigens ». *Nature Reviews Cancer* 19 (8): 465-78. <https://doi.org/10.1038/s41568-019-0162-4>.
- Sofopoulos, Michael, Sotirios P. Fortis, Christoforos K. Vaxevanis, Nectaria N. Sotiriadou, Niki Arnogiannaki, Alexandros Ardavanis, Dimitrios Vlachodimitropoulos, Sonia A. Perez, et Constantin N. Baxevanis. 2019. « The Prognostic Significance of Peritumoral Tertiary Lymphoid Structures in Breast Cancer ». *Cancer Immunology, Immunotherapy: CII* 68 (11): 1733-45. <https://doi.org/10.1007/s00262-019-02407-8>.
- Solinas, Cinzia, Soizic Garaud, Pushpamali De Silva, Anaïs Boisson, Gert Van den Eynden, Alexandre de Wind, Paolo Rizzo, et al. 2017. « Immune Checkpoint Molecules on Tumor-Infiltrating Lymphocytes and Their Association with Tertiary Lymphoid Structures in Human Breast Cancer ». *Frontiers in Immunology* 8: 1412. <https://doi.org/10.3389/fimmu.2017.01412>.
- Solinas, Cinzia, Diane Marcoux, Soizic Garaud, Joel Rodrigues Vitória, Gert Van den Eynden, Alexandre de Wind, Pushpamali De Silva, et al. 2019. « BRCA Gene Mutations Do Not Shape the Extent and Organization of Tumor Infiltrating Lymphocytes in Triple Negative Breast Cancer ». *Cancer Letters* 450 (mai): 88-97. <https://doi.org/10.1016/j.canlet.2019.02.027>.
- Solinas, G., G. Germano, A. Mantovani, et P. Allavena. 2009. « Tumor-Associated Macrophages (TAM) as Major Players of the Cancer-Related Inflammation ». *Journal of Leukocyte Biology* 86 (5): 1065-73. <https://doi.org/10.1189/jlb.0609385>.
- Somasundaram, Rajasekharan, Gao Zhang, Mizuho Fukunaga-Kalabis, Michela Perego, Clemens Krepler, Xiaowei Xu, Christine Wagner, et al. 2017. « Tumor-Associated B-Cells Induce Tumor Heterogeneity and Therapy Resistance ». *Nature Communications* 8 (1): 607. <https://doi.org/10.1038/s41467-017-00452-4>.
- Song, In Hye, Sun-Hee Heo, Won Seon Bang, Hye Seon Park, In Ah Park, Young-Ae Kim, Suk Young Park, Jin Roh, Gyungyub Gong, et Hee Jin Lee. 2017. « Predictive Value of Tertiary Lymphoid Structures Assessed by High Endothelial Venule Counts in the Neoadjuvant Setting of Triple-Negative Breast Cancer ». *Cancer Research and Treatment* 49 (2): 399-407. <https://doi.org/10.4143/crt.2016.215>.
- Stebegg, Marisa, Saumya D. Kumar, Alyssa Silva-Cayetano, Valter R. Fonseca, Michelle A. Linterman, et Luis Graca. 2018. « Regulation of the Germinal Center Response ». *Frontiers in Immunology* 9. <https://www.frontiersin.org/article/10.3389/fimmu.2018.02469>.

- Suematsu, Sachiko, et Takeshi Watanabe. 2004. « Generation of a Synthetic Lymphoid Tissue-like Organoid in Mice ». *Nature Biotechnology* 22 (12): 1539-45. <https://doi.org/10.1038/nbt1039>.
- Szpakowski, Sebastian, Xueguang Sun, José M. Lage, Andrew Dyer, Jill Rubinstein, Diane Kowalski, Clarence Sasaki, Jose Costa, et Paul M. Lizardi. 2009. « Loss of epigenetic silencing in tumors preferentially affects primate-specific retroelements ». *Gene* 448 (2): 151-67. <https://doi.org/10.1016/j.gene.2009.08.006>.
- Tadmor, Tamar, Yu Zhang, Hyun-Mi Cho, Eckhard R. Podack, et Joseph D. Rosenblatt. 2011. « The Absence of B Lymphocytes Reduces the Number and Function of T-Regulatory Cells and Enhances the Anti-Tumor Response in a Murine Tumor Model ». *Cancer Immunology, Immunotherapy: CII* 60 (5): 609-19. <https://doi.org/10.1007/s00262-011-0972-z>.
- Tamimi, Rulla M., David Cox, Peter Kraft, Graham A. Colditz, Susan E. Hankinson, et David J. Hunter. 2008. « Breast Cancer Susceptibility Loci and Mammographic Density ». *Breast Cancer Research: BCR* 10 (4): R66. <https://doi.org/10.1186/bcr2127>.
- Tanaka, Atsushi, et Shimon Sakaguchi. 2017. « Regulatory T Cells in Cancer Immunotherapy ». *Cell Research* 27 (1): 109-18. <https://doi.org/10.1038/cr.2016.151>.
- Tang, Jun, Daniel Ramis-Cabrer, Víctor Curull, Xuejie Wang, Mercé Mateu-Jiménez, Lara Pijuan, Xavier Duran, et al. 2020. « B Cells and Tertiary Lymphoid Structures Influence Survival in Lung Cancer Patients with Resectable Tumors ». *Cancers* 12 (9): E2644. <https://doi.org/10.3390/cancers12092644>.
- Tao, Huimin, Lin Lu, Yang Xia, Fu Dai, Yi Wang, Yangyi Bao, Steven K. Lundy, et al. 2015. « Antitumor effector B cells directly kill tumor cells via the Fas/FasL pathway and are regulated by IL-10 ». *European journal of immunology* 45 (4): 999-1009. <https://doi.org/10.1002/eji.201444625>.
- Taylor, Mark, Louise M Bolton, Peter Johnson, Tim Elliott, et Nick Murray. 2007. « Breast cancer is a promising target for vaccination using cancer-testis antigens known to elicit immune responses ». *Breast cancer research : BCR* 9 (4): R46. <https://doi.org/10.1186/bcr1749>.
- Thommen, D.S., V.H. Koelzer, P. Herzig, A. Roller, M. Trefny, S. Dimeloe, A. Kiialainen, et al. 2018. « A transcriptionally and functionally distinct PD-1+ CD8+ T cell pool with predictive potential in non-small cell lung cancer treated with PD-1 blockade ». *Nature medicine* 24 (7): 994-1004. <https://doi.org/10.1038/s41591-018-0057-z>.
- Tian, Chen, Chang Li, Yulan Zeng, Jinyan Liang, Qifan Yang, Feifei Gu, Yue Hu, et Li Liu. 2021. « Identification of CXCL13/CXCR5 Axis's Crucial and Complex Effect in Human Lung Adenocarcinoma ». *International Immunopharmacology* 94 (mai): 107416. <https://doi.org/10.1016/j.intimp.2021.107416>.
- Tokunaga, Ryuma, Wu Zhang, Madiha Naseem, Alberto Puccini, Martin D Berger, Shivani Soni, Michelle McSkane, Hideo Baba, et Heinz-Josef Lenz. 2018. « CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation - a target for novel cancer therapy ». *Cancer treatment reviews* 63 (février): 40-47. <https://doi.org/10.1016/j.ctrv.2017.11.007>.
- Tower, Helen, Meagan Ruppert, et Kara Britt. 2019. « The Immune Microenvironment of Breast Cancer Progression ». *Cancers* 11 (9): E1375. <https://doi.org/10.3390/cancers11091375>.
- Treilleux, Isabelle, Jean-Yves Blay, Nathalie Bendriss-Vermare, Isabelle Ray-Coquard, Thomas Bachelot, Jean-Paul Guastalla, Alain Bremond, et al. 2004. « Dendritic Cell Infiltration and Prognosis of Early Stage Breast Cancer ». *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* 10 (22): 7466-74. <https://doi.org/10.1158/1078-0432.CCR-04-0684>.
- Truxova, Iva, Lenka Kasikova, Michal Hensler, Petr Skapa, Jan Laco, Ladislav Pecen, Lucie Belicova, et al. 2018. « Mature Dendritic Cells Correlate with Favorable Immune Infiltrate and Improved Prognosis in Ovarian Carcinoma Patients ». *Journal for Immunotherapy of Cancer* 6 (1): 139. <https://doi.org/10.1186/s40425-018-0446-3>.
- Tsutsui, Shinichi, Kazuhiro Yasuda, Kosuke Suzuki, Kouichirou Tahara, Hidefumi Higashi, et Shoichi Era. 2005. « Macrophage Infiltration and Its Prognostic Implications in Breast Cancer: The

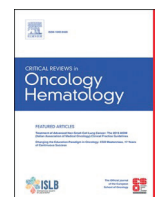
- Relationship with VEGF Expression and Microvessel Density ». *Oncology Reports* 14 (2): 425-31.
- Ukita. s. d. « Tertiary lymphoid structures induced by CXCL13-producing CD4+ T cells increase tumor infiltrating CD8+ T cells and B cells in ovarian cancer | bioRxiv ». Consulté le 7 mars 2022. <https://www.biorxiv.org/content/10.1101/2021.12.01.470493v1>.
- Vanhersecke, Lucile, Maxime Brunet, Jean-Philippe Guégan, Christophe Rey, Antoine Bougouin, Sophie Cousin, Sylvestre Le Moulec, et al. 2021. « Mature Tertiary Lymphoid Structures Predict Immune Checkpoint Inhibitor Efficacy in Solid Tumors Independently of PD-L1 Expression ». *Nature Cancer* 2 (8): 794-802. <https://doi.org/10.1038/s43018-021-00232-6>.
- Vella, Gerlanda, Sophie Guelfi, et Gabriele Bergers. 2021. « High Endothelial Venules: A Vascular Perspective on Tertiary Lymphoid Structures in Cancer ». *Frontiers in Immunology* 12 (août): 736670. <https://doi.org/10.3389/fimmu.2021.736670>.
- Vella, Laura A., Marcus Buggert, Sasikanth Manne, Ramin S. Herati, Ismail Sayin, Leticia Kuri-Cervantes, Irene Bukh Brody, et al. 2019. « T Follicular Helper Cells in Human Efferent Lymph Retain Lymphoid Characteristics ». *The Journal of Clinical Investigation* 129 (8): 3185-3200. <https://doi.org/10.1172/JCI125628>.
- Wang, H., M. Sang, C. Geng, F. Liu, L. Gu, et B. Shan. 2016. « MAGE-A Is Frequently Expressed in Triple Negative Breast Cancer and Associated with Epithelial-Mesenchymal Transition ». *Neoplasma* 63 (1): 44-56. https://doi.org/10.4149/neo_2016_006.
- Wang, Ke, Jianming Liu, et Jiansheng Li. 2018. « IL-35-producing B cells in gastric cancer patients ». *Medicine* 97 (19): e0710. <https://doi.org/10.1097/MD.00000000000010710>.
- Wang-Johanning, Feng, Laszlo Radvanyi, Kiera Rycaj, Joshua B. Plummer, Peisha Yan, K. Jagannadha Sastry, Chandrika J. Piyathilake, Kelly K. Hunt, et Gary L. Johanning. 2008. « Human Endogenous Retrovirus K Triggers an Antigen-Specific Immune Response in Breast Cancer Patients ». *Cancer research* 68 (14): 5869-77. <https://doi.org/10.1158/0008-5472.CAN-07-6838>.
- Weigelt, Britta, Felipe C. Geyer, et Jorge S. Reis-Filho. 2010. « Histological Types of Breast Cancer: How Special Are They? ». *Molecular Oncology* 4 (3): 192-208. <https://doi.org/10.1016/j.molonc.2010.04.004>.
- Weinstein, Aliyah M., Lu Chen, Emily A. Brzana, Prashanti R. Patil, Jennifer L. Taylor, Kellsye L. Fabian, Callen T. Wallace, et al. 2017. « Tbet and IL-36 γ Cooperate in Therapeutic DC-Mediated Promotion of Ectopic Lymphoid Organogenesis in the Tumor Microenvironment ». *Oncoimmunology* 6 (6): e1322238. <https://doi.org/10.1080/2162402X.2017.1322238>.
- Weinstein, Aliyah M., Nicolas A. Giraldo, Florent Petitprez, Catherine Julie, Laetitia Lacroix, Frédérique Peschard, Jean-François Emile, et al. 2019. « Association of IL-36 γ with Tertiary Lymphoid Structures and Inflammatory Immune Infiltrates in Human Colorectal Cancer ». *Cancer Immunology, Immunotherapy: CII* 68 (1): 109-20. <https://doi.org/10.1007/s00262-018-2259-0>.
- Werner, Franziska, Christine Wagner, Martin Simon, Katharina Glatz, Kirsten D. Mertz, Heinz Läubli, Johannes Griss, et Stephan N. Wagner. 2021. « A Standardized Analysis of Tertiary Lymphoid Structures in Human Melanoma: Disease Progression- and Tumor Site-Associated Changes With Germinal Center Alteration ». *Frontiers in Immunology* 12 (juin): 675146. <https://doi.org/10.3389/fimmu.2021.675146>.
- Wieland, Andreas, Mihir R. Patel, Maria A. Cardenas, Christiane S. Eberhardt, William H. Hudson, Rebecca C. Obeng, Christopher C. Griffith, et al. 2021. « Defining HPV-Specific B Cell Responses in Patients with Head and Neck Cancer ». *Nature* 597 (7875): 274-78. <https://doi.org/10.1038/s41586-020-2931-3>.
- Wilson, Eric, et Eugene C. Butcher. 2004. « CCL28 Controls Immunoglobulin (Ig)A Plasma Cell Accumulation in the Lactating Mammary Gland and IgA Antibody Transfer to the Neonate ». *The Journal of Experimental Medicine* 200 (6): 805-9. <https://doi.org/10.1084/jem.20041069>.
- Wouters, Maartje C. A., et Brad H. Nelson. 2018. « Prognostic Significance of Tumor-Infiltrating B Cells and Plasma Cells in Human Cancer ». *Clinical Cancer Research: An Official Journal of the*

- American Association for Cancer Research* 24 (24): 6125-35. <https://doi.org/10.1158/1078-0432.CCR-18-1481>.
- Xu, Wei, HyeMee Joo, Sandra Clayton, Melissa Dullaers, Marie-Cecile Herve, Derek Blankenship, Maria Teresa De La Morena, et al. 2012. « Macrophages induce differentiation of plasma cells through CXCL10/IP-10 ». *Journal of Experimental Medicine* 209 (10): 1813-23. <https://doi.org/10.1084/jem.20112142>.
- Yamaguchi, Kyoko, Mamoru Ito, Hirofumi Ohmura, Fumiyasu Hanamura, Michitaka Nakano, Kenji Tsuchihashi, Shuntaro Nagai, et al. 2020. « Helper T Cell-Dominant Tertiary Lymphoid Structures Are Associated with Disease Relapse of Advanced Colorectal Cancer ». *Oncoimmunology* 9 (1): 1724763. <https://doi.org/10.1080/2162402X.2020.1724763>.
- Yamamoto, Masahiro, et Kiyoshi Takeda. 2012. « Inhibition of ATF6 β -dependent host adaptive immune response by a Toxoplasma virulence factor ROP18 ». *Virulence* 3 (1): 77-80. <https://doi.org/10.4161/viru.3.1.18340>.
- Yersal, Özlem, Muhammed Ali Kaplan, Abdurrahman Işıkdoğan, Nuriye Özdemir, Mehmet Aliustaoğlu, Sabri Barutca, Halil İbrahim Erdoğan, et Nezi Meydan. 2019. « Molecular Subtypes Are Prognostic for N3 Breast Cancer Patients in the Modern Therapeutic Era ». *Molecular and Clinical Oncology* 10 (1): 180-84. <https://doi.org/10.3892/mco.2018.1771>.
- Zan, Hong, Andrea Cerutti, Patricia Dramitinos, Andrés Schaffer, et Paolo Casali. 1998. « CD40 Engagement Triggers Switching to IgA1 and IgA2 in Human B Cells Through Induction of Endogenous TGF- β : Evidence for TGF- β But Not IL-10-Dependent Direct $\Sigma\mu \rightarrow \Sigma\alpha$ and Sequential $\Sigma\mu \rightarrow \Sigma\gamma$, $\Sigma\gamma \rightarrow \Sigma\alpha$ DNA Recombination ». *The Journal of Immunology* 161 (10): 5217-25.
- Zhang, Yu, Richard Morgan, Chuan Chen, Yancheng Cai, Emily Clark, Wasif Noor Khan, Seung-Uon Shin, et al. 2016. « Mammary-tumor-educated B cells acquire LAP/TGF- β and PD-L1 expression and suppress anti-tumor immune responses ». *International Immunology* 28 (9): 423-33. <https://doi.org/10.1093/intimm/dxw007>.
- Zhang, Yue, Shaoqiang Cheng, Mingyan Zhang, Lina Zhen, Da Pang, Qingyuan Zhang, et Zhigao Li. 2013. « High-Infiltration of Tumor-Associated Macrophages Predicts Unfavorable Clinical Outcome for Node-Negative Breast Cancer ». *PloS One* 8 (9): e76147. <https://doi.org/10.1371/journal.pone.0076147>.
- Zhu, Wei, Claire Germain, Zheng Liu, Yinong Sebastian, Priyanka Devi, Samantha Knockaert, Philip Brohawn, et al. 2015. « A high density of tertiary lymphoid structure B cells in lung tumors is associated with increased CD4+ T cell receptor repertoire clonality ». *Oncoimmunology* 4 (12): e1051922. <https://doi.org/10.1080/2162402X.2015.1051922>.
- Zizzari, Ilaria Grazia, Alessandra Di Filippo, Andrea Botticelli, Lidia Strigari, Angelina Pernazza, Emma Rullo, Maria Gemma Pignataro, et al. 2022. « Circulating CD137+ T Cells Correlate with Improved Response to Anti-PD1 Immunotherapy in Patients with Cancer ». *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* 28 (5): 1027-37. <https://doi.org/10.1158/1078-0432.CCR-21-2918>.

ANNEXES

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European School of Oncology – Review

Neoadjuvant immune checkpoint inhibitors in cancer, current state of the art

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ABSTRACT

Immunotherapy has been a revolution in cancer management in the metastatic setting. This has led to a prompt evaluation of such therapies in earlier stages. This article discusses the still limited amount of data finding the rationale to assess such therapy in this setting and reviews preclinical and clinical data available. Overall, neoadjuvant immunotherapy is a promising approach for the treatment of cancers and the rationale supporting its use is strong. Neoadjuvant immunotherapy resulted, in the majority of clinical trials, in improved pathologic complete response rates with a favorable toxicity profile and no delay in surgery. Various regimens were effective: inhibitory immune check-point blockers (ICPB) alone, combination of PD-1 and CTLA-4 inhibitors, combination of chemotherapy (CT) and IICPB, phased CT and IICPB (either IICPB before CT or IICPB after CT). Yet the question whether neoadjuvant immunotherapy will benefit to patients in terms of disease-free and, ultimately, overall survival remains unknown.

1. Introduction

Neoadjuvant therapy refers to a systemic treatment administered prior to local treatment (surgery or radiotherapy) for any type of cancer. Neoadjuvant therapy usually takes the form of chemotherapy, but endocrine therapy or targeted therapies have also been described as alternatives in some cancers such as breast cancer (Cortazar et al., 2014; Spring et al., 2016). Neoadjuvant therapy has the purpose of downstaging tumor size allowing less extensive surgery or resectability in order to achieve local control, identifying sensitivity to systemic treatment, improve the pathological response rate and preventing early metastatic risk (Cortazar et al., 2014; Li et al., 2018; Petrelli et al., 2014).

Immunotherapy and especially inhibitory immune check-point blockers (ICPB) have drastically transformed the landscape of cancer. IICPB restore anti-tumor functions of T cells via blockade of negative regulatory signals such as PD1/PD-L1 and CTLA-4. Conceptually, CTLA4 blockade primarily acts at sites of priming in which CD28-positive costimulation is involved (e.g., tumor draining lymph nodes) whereas PD-1/PD-L1 blockade primarily acts in inflamed peripheral tissues (e.g., tumor). Checkpoint inhibition was shown to be associated with

significantly prolonged survival and long-lasting disease control for metastatic cancers. Consequently, IICPB emerged as front-line options for various cancers such as metastatic melanoma, lung cancer, renal clear cell carcinoma, bladder or urothelial cancers, Hodgkin's lymphoma and head and neck cancers. Currently, many trials are ongoing in the neoadjuvant setting due to the major impact of immunotherapy in the metastatic setting. Yet, few data are available in the literature on the rationale for using immunotherapy in the neoadjuvant setting and only preliminary efficacy results were published with no FDA approval yet.

The present review highlights available data on immunotherapy in the neoadjuvant setting, the rationale supporting the use of neoadjuvant immunotherapeutic approaches, the potential advantages and limits of this approach, available clinical data, and implications for future management of cancers.

2. Material and methods

2.1. Trial selection

In June 2019, all reports on immune checkpoint inhibitors in the

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neoadjuvant setting were identified. The research was performed using Text Words “Avelumab”, “Pembrolizumab”, “Ipilimumab”, “Tremelimumab”, “Atezolizumab”, “Durvalumab”, “Nivolumab”, “immunotherapy”, “CTLA-4 inhibitor”, “PD-1 inhibitor”, “PD-L1 inhibitor”, “neoadjuvant” and “preoperative”. Resources used for this research included the electronic database Pubmed, relevant articles retrieved from references, personal data and abstracts presented at the American Society of Clinical Oncology (ASCO), the American Association for Cancer Research (AACR), the European Society for Medical Oncology (ESMO) congresses and the San Antonio Breast Cancer Symposium. Included reports were published in English.

3. Rationale supporting the use of immunotherapeutic approaches in the early stages of cancer

3.1. Mechanistic arguments

From a mechanistic point of view, the availability of neoantigens for cross priming, the possibility of lymphatic migration of antigen-presenting cells (APC), the interaction between APC and naïve T cells in lymph nodes, the lymphatic recirculation of effector cells and the presence of tumor-infiltrating lymphocytes (TILs) are necessary to initiate an immune response. In case of lymphadenectomy or tumor resection, this immune response might be jeopardized (Melero et al., 2016). On the other hand, tumor cells can release immunosuppressive factors modulating immune cells to become tolerogenic. Tolerogenic APC and regulatory cells use lymphatics and blood flow respectively to migrate into the tumor and lymph nodes can act as a tumor-immune tolerogenic interface. These arguments are in favor of the use of IICPB in the neoadjuvant setting.

Moreover, until recently, IICPB were mostly evaluated in heavily pretreated patients or in patients with advanced disease, e.g. with an immune system already compromised by tumor progression (Bindea et al., 2013) and/or previous regimens of chemotherapy (Verma et al., 2016). It was shown in melanoma and renal cell carcinomas that the host immune response strikingly differ between earlier stages with micro metastatic disease and more advanced stages with measurable disease (Tarhini and Iqbal, 2010). For instance, healthy donors or patients free of disease following therapy tended to present tumor antigen specific mixed Th1/Th2 type or Th1-type polarized immune response while patients with advanced renal cell carcinoma or melanoma displayed increased tumor antigen-specific Th2-type polarization (Bindea et al., 2013; Tatsumi et al., 2002; Tatsumi et al., 2003).

Therefore, it may be more efficient to prescribe this type of anti-cancer drug earlier in the history of the disease when an anti-tumor immune response (Th1) can still be amplified/restored.

3.2. Impact of surgery on the immune system

Surgery may have an impact on the immune system. Indeed, in breast cancer patients, Péguillet and colleagues reported that the number of effector CD4+ blood T cells decreases ($P \leq 0.017$) after primary surgery. In addition, the total number of CD4+ blood T cells was not modified by adjuvant therapy but significantly increased during neoadjuvant chemotherapy (Péguillet et al., 2014). IICPB treatment aims to restore the functions of tumor effector T cells. Tumor removal will induce a decrease of intratumoral antigen-experienced effector T cells that can circulate between blood, tissue and secondary lymphoid organs. Therefore, prescribing IICPB after surgery may not be relevant.

3.3. Prognostic impact of PD-L1 expression

A meta-analysis of 61 studies showed that PD-L1 overexpression can predict a poor overall survival (OS) (HR = 1.58, 95 % CI = 1.38–1.81, $P < .000$) and disease- and progression-free survival (DFS/PFS) (HR = 1.72, 95 % CI = 1.26–2.33, $P = .001$) in various solid tumors (Wang

et al., 2017). PD-L1 expression was also shown to be associated with a higher risk of recurrence in surgically resected non-small cell lung cancer (Owonikoko et al., 2015), in human urothelial cancers (Nakanishi et al., 2007) and in hepatocellular carcinoma (Gao et al., 2009). Furthermore, multivariate analysis indicated that tumor-associated PD-L1 was a more significant prognostic factor than WHO grade for postoperative recurrence in human urothelial cancers (Wei et al., 2018).

As most tumors expressing immune co-inhibitory molecules such as PD-L1 exhibit a poor prognosis and a higher risk of relapse following surgery, neoadjuvant immunotherapeutic strategies could be beneficial in combination with surgery in order to decrease relapse risk.

3.4. Presumed efficacy

For metastatic disease, ORR is higher with immunotherapy compared to chemotherapy in various immunogenic cancers such as melanoma or lung cancer, which are the two cancers in which immunotherapy has first proven its efficacy. In lung cancer, in a phase III trial comparing pembrolizumab to platinum-based chemotherapy in front-line, ORRs for pembrolizumab and platinum-doublet chemotherapy were 45 % and 28 %, respectively and the time to response was equal in both arms and estimated at 2.2 months (Reck et al., 2016). In combination with standard first-line therapy (carboplatin and pemetrexed), pembrolizumab showed an ORR of 55 % vs 29 % with chemotherapy alone. In melanoma, dacarbazine (standard first-line chemotherapy until recently) was compared to nivolumab (Robert et al., 2015a) and to dacarbazine + ipilimumab (Robert et al., 2011). In the first trial, nivolumab achieved an ORR of 40 % versus 13.9 % with dacarbazine alone (Robert et al., 2015a). Complete response rate (CRR) was 7.6 % in the experimental arm versus 1.0 % in the comparative standard arm (Robert et al., 2015a). In the second trial, ipilimumab + dacarbazine resulted in an ORR of 15.2 % versus 10.3 % and a CRR of 1.6 % vs 0.8 % (Robert et al., 2011). In the phase III KEYNOTE-006 trial which compared pembrolizumab (anti-PD-1) to ipilimumab (anti-CTLA-4), ORR was 37 % vs 13 % respectively and CRR was 12 % vs 5% respectively (Robert et al., 2015b). In the CheckMate 067 trial, nivolumab + ipilimumab followed by nivolumab was compared to nivolumab alone and to ipilimumab alone. ORR were 58, 44, and 19 %, respectively. The CRR were 19, 16, and 5%, respectively (Larkin et al., 2015).

In these pivotal trials, immunotherapy resulted in an improvement of the ORR and the CRR suggesting an increased depth of response that could be interesting in the neoadjuvant setting for resectability and preservation of function. Yet, whether these results in the advanced setting can be extrapolated to the neoadjuvant setting is unknown.

3.5. Safety profile

Another potential advantage of IICPB in the neoadjuvant setting is its intrinsic safety profile. Neoadjuvant therapy-related adverse events may affect post-operative morbidity and/or mortality and/or delay surgery, thus jeopardizing surgery efficacy. As IICPB are generally well-tolerated, they are particularly interesting in the neoadjuvant setting. IICPB are associated with peculiar adverse events (AEs) commonly defined as immune-related AEs (irAEs). IrAEs are usually mild to moderate in severity and reversible when they are rapidly detected and when immunosuppressive and/or immunomodulatory strategies are promptly initiated (Puzanov et al., 2017). A meta-analysis of twenty randomized trials with 10,794 patients reported that treatment discontinuations and grade 3–5 (G3–5) AEs were less frequent for programmed-death-1 (PD-1) or PD-ligand-1 (PD-L1) inhibitors and cytotoxic-T-lymphocyte-associated-antigen-4 (CTLA-4) inhibitors than for standard cytotoxic chemotherapy (Man et al., 2018).

3.6. Preclinical data

In two mouse models of spontaneous metastatic mammary tumors

(orthotopic 4T1.2 and E0771 tumors)²⁴, Liu et al. demonstrated that the proportion of long-term mice survivors was more important in mice receiving neoadjuvant immunotherapy compared to adjuvant immunotherapy. Four different immunotherapies were assessed: complete depletion of regulatory T cells with an anti-CD25, anti-PD-1 alone and anti-PD-1 in combination with anti-CD137. Neoadjuvant immunotherapy-treated mice displayed significantly longer survival compared with those that received adjuvant immunotherapy. For instance, in the neoadjuvant T regulatory-depleted group (Treg), almost all mice (19/20) displayed long-term survival compared with the adjuvant Treg-depleted mice group in which only 5 out of 20 mice displayed long-term survival. In both 4T1.2 and E0771 tumor models, the efficacy of neoadjuvant therapy was dependent on interferon gamma (IFN γ) as there were no long-term survivors when it was neutralized. All three immune cell types, CD8, CD4, and NK cells, were also required. After neoadjuvant immunotherapy, an increase in tumor specific CD8 + T cells was detected. They displayed an effector/memory phenotype (CD44⁺ CD62L⁻), were proliferative and produced IFN γ . It is noteworthy that neoadjuvant immunotherapy given only 2 days before surgery still had a beneficial impact in this preclinical study.

Similarly, in the murine B16F10 melanoma model, neoadjuvant vaccination provided superior protection against tumor relapse following surgery compared with adjuvant vaccination (Grinshtein et al., 2009).

4. First clinical data

Efficacy results, tolerance and impact on surgery of the main clinical trials evaluating immune checkpoint inhibitors in the neoadjuvant setting are reported in Table 1.

In conclusion, neoadjuvant immunotherapy resulted, in the majority of clinical trials, in improved pathologic complete response rates with a favorable toxicity profile and no delay in surgery. Various regimens were effective: IICPB alone, combination of PD-1 and CTLA-4 inhibitors, combination of chemotherapy (CT) and IICPB, phased CT and IICPB (either IICPB before CT or IICPB after CT). The majority of novel trials evaluating neoadjuvant immunotherapy in solid cancers use pathological response as a surrogate endpoint for survival which is interesting because gives access to earlier results. Yet the question whether neoadjuvant immunotherapy will benefit to patients in terms of disease-free and, ultimately, overall survival remains unknown and we lack sufficiently validated association with OS in the era of immunotherapy. Concerns were raised from one study evaluating nivolumab monotherapy which was stopped prematurely due to rapid progression. Finally, two trials, in glioblastoma and melanoma, compared neoadjuvant IICPB versus neo and adjuvant IICPB and reported similar results suggesting that neoadjuvant may be more efficient than adjuvant immunotherapy which confirms preclinical data.

5. Neoadjuvant immunotherapy as a platform for identification of efficacy and resistance biomarkers and for drug development

IICPB are not effective in all cancers nor on all patients, even with immunogenic tumors (Topalian et al., 2016). Predictive biomarkers of efficacy are urgently needed. Neoadjuvant immunotherapy offers the possibility of immune cells monitoring and identification of underlying response or resistance mechanisms given the access to blood, to tumor and even to fecal samples prior and post therapy. Understanding resistance mechanisms will help us design next generation trials. Characteristics of the immune system evaluated in neoadjuvant trials testing IICPB and predictive factors of efficacy and toxicity are reported in Table 2.

6. Limits

6.1. Atypical and unconventional responses

In most studies, tumor response is evaluated at week 8 or 12, yet pseudo progression on immunotherapy can occur at this point (Wolchok et al., 2009). Activation/restimulation of T cell mediated immunity may initially delay tumor regression and appearance of new lesions along with progression of existent lesions may precede objective tumor response. Pseudo progression can occur early (≤ 12 weeks) but can also be delayed (> 12 weeks)⁵⁰. This might have an impact on the time of surgery as one of the objectives of neoadjuvant therapy is to render operable an otherwise inoperable disease. Hyper progression disease, defined as a rapid progression after initiation of anti-PD1 and anti-PD-L1, was also reported (Champiat et al., 2017; Saada-Bouzid et al., 2017). Atypical responses are not so rare and approximately 15 % of patients experience such responses (Hodi et al., 2016). Moreover, the expression of antigens by tumor cells is not homogeneous and immunologic heterogeneity within the tumor was described (Miller, 1982). Intratumor heterogeneity influenced immune evasion (Caswell and Swanton, 2017). This principle was first shown in patients presenting responding lesions and progressive lesions at the same time under high-dose IL-2 therapy (Topalian, 2014). Responding lesions showed brisk CD8 + T cells infiltration while progressive lesions showed poor lymphocyte infiltration. Unconventional dissociated responses were reported in patients treated with anti-PD1, anti-PDL1 and anti-CTLA-4. In a French retrospective analysis of all consecutive patients treated with anti-PD-1 or anti-PD-L1 Abs for advanced NSCLC, dissociated responses occurred in 8% of cases (Tazdait et al., 2018). Atypical and unconventional responses are limiting factors in the prescription of immunotherapy in the neoadjuvant setting.

6.2. Stable disease is a marker of IICPB efficacy

Through its direct cytotoxic effect, chemotherapy yields measurable effects within a few weeks. This phenomenon is often expressed as partial responses defined as a significant decrease in the size of the tumor. In contrast, stable disease is often transient and not reflective of the true benefit. On the contrary, for immunotherapy, stable disease was described as a surrogate endpoint for improved survival outcome (Hughes et al., 2015; Tsujino et al., 2009). Some IICPB were shown to improve OS without improving PFS (Hodi et al., 2010). Durable stable disease is even one of the four immune-related response patterns (Wolchok et al., 2009). Consequently, stable disease is associated with a true clinical benefit for patients, however, this clinical benefit may not be sufficient when the objective is to render an inoperable tumor operable.

6.3. Primary resistant disease

Interesting results in terms of duration of responses and response rates with IICPB were reported in inflamed tumors. Yet, some patients still present primary resistance to IICPB. In this context, combination with chemotherapy might be interesting as it has been shown to further increase the ORR in lung cancer (Zhou et al., 2019). Furthermore, immune-desert and immune-excluded tumors exhibit primary resistant to currently available immune checkpoint inhibitors (Chen and Mellman, 2017),⁶². This indicates that selection of patients is mandatory in the neoadjuvant setting in order to give our patients the best opportunity of treatment. Microsatellite instability, TMB or PD-L1 expression could be used.

6.4. Adverse events

Another important consideration to take into account is the potential risk of serious immune-related adverse events in the first few cycles of

Table 1
Efficacy and safety results of the main clinical trials evaluating immune checkpoint inhibitors in the neoadjuvant setting.

Name of the trial or first author	Phase	Primitive tumor type	Number of patients	Type of therapy evaluated	Efficacy	Toxicity	Surgical outcomes
I-SPY2 (Nanda et al., 2017)	Adaptively randomized phase II	≥ T2 HER2 negative breast cancer	69	Pembrolizumab + chemotherapy	↑ pCR rate: TNBC (pCR 60 % vs 20 %) HR+/HER2- (34 % vs 13 %)	Adrenal insufficiencies (n = 6/69)	NA
KEYNOTE-173 (Schmid et al., 2019)	Phase Ib	TNBC	20	Pembrolizumab + chemotherapy	ypT0 ypN0 pCR rate: 50%–80%	1 grade 2/3 alanine aminotransferase elevation	NA
KEYNOTE-522 (Schmid et al., 2018)	Phase III	TNBC	602	Pembrolizumab + chemotherapy	pCR rate 64.8 % with the pembrolizumab/ chemotherapy regimen compared to 51.2 % (standard arm) Benefit more important in lymph node positive TNBC compared to lymph node negative TNBC	grade 3 or higher treatment-related adverse event (AE) rates: 78.0 % - experimental arm- compared to 73.0 % - standard arm. Incidence of death: 0.4 % versus 0.3 %.	NA
GeparNuevo (Loibl et al., 2019)	Randomized phase II	TNBC	31	Durvalumab + chemotherapy (CT)	Patients who started durvalumab before chemotherapy + durvalumab presented more pCR (OR = 2.22 [95 %CI 1.06–4.64]. This phased administration of IICPB called “window of opportunity” resulted in a higher pCR rate 61.0 % compared to 41.4 % of the patients in the control arm (p = 0.052)	Treatment delay in 54.8 % of patients receiving the combination vs 40.0–67.7% patients treated with chemotherapy alone	NA
NeoTRIPaPDL1 (Antonio, 2020)	Phase III	TNBC	280	Atezolizumab + CT	pCR rate 43.5 % vs. 40.8 % not statistically significant	Immune-mediated adverse events of any grade: 8% >G3 infusion reactions: 1.4 %	NA
McArthur HL (McArthur et al., 2016)	Pilot study	Breast cancer	19	Single dose ipilimumab +/- cryoablation	NA	Grade III unrelated rash in one patient	No delay in surgery
Tarhini AA (Tarhini et al., 2014)	NA	Melanoma	35	Ipilimumab	ORR was 9% (2 complete responses and 1 partial response)	Grade 3 diarrhea/colitis (5; 14 %), hepatitis (2; 6%), rash (1; 3%), elevated lipase (3; 9%)	NA
OpACIN (Rozeman et al., 2017)	Two-arm phase Ib feasibility trial	Melanoma	20 (10 in the neoadjuvant arm)	Ipilimumab + Nivolumab	ORR 80 % pCR 30 % Estimated 30 months relapse-free survival rates were 80 % for the neoadjuvant arm and 60 % for the adjuvant arm and 30 months OS rates were 90 % and 67 %, respectively	G3 elevated lipase (n = 8), G3 diarrhea and colitis (n = 6) No surgery related AEs attributed to immunotherapy	No delay in surgery
AMARIA RN (Amaria et al., 2018)	Phase II	Melanoma	23	Ipilimumab + Nivolumab	ORR 73 % pCR 45 %	G3 irAEs 73 %	Rapid disease progression for 17 % of patients in the nivolumab monotherapy arm
Tarhini AA (Tarhini et al., 2018)	Randomized trial	Melanoma	30	Ipilimumab (3 or 10 mg/kg) + high-dose interferon	pCR 35 % (95CI (Reck et al., 2016; Robert et al., 2015a; Robert et al., 2011; Robert et al., 2015b; Larkin et al., 2015; Puzanov et al., 2017; Man et al., 2018; Liu et al., 2016; Grinshtein et al., 2009; Nanda et al., 2017; Schmid et al., 2019; Schmid et al., 2018; Loibl et al., 2019; Antonio, 2020; McArthur et al., 2016; Tarhini et al., 2014; Rozeman et al.,	One toxicity-related drug withdrawal. Grade ≥3 elevated transaminases (n = 6, 20.0 %). Grade ≥3 rash (n = 7, 23.0 %) Toxicities more frequent at 10 mg/kg	NA

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Table 1 (continued)

Name of the trial or first author	Phase	Primitive tumor type	Number of patients	Type of therapy evaluated	Efficacy	Toxicity	Surgical outcomes
TOP1201 ipi (Yang et al., 2018)	Phase II	Non-small cell lung cancer (NSCLC)	24	Ipilimumab + chemotherapy	2017; Amaria et al., 2018; Tarhini et al., 2018; Yang et al., 2018; Forde et al., 2018; Rusch et al., 2018; Uppaluri et al., 2017; Wise-Draper et al., 2018; Gao et al., 2017; Carthon et al., 2010; Necchi et al., 2018; Powles et al., 2019; Grootsholten et al., 2018; Gao et al., 2018; Cloughesy et al., 2019; Topalian et al., 2016; Wolchok et al., 2009; Hodi et al., 2016; Champiat et al., 2017; Saâda-Bouid et al., 2017; Miller, 1982; Caswell and Swanton, 2017; Topalian, 2014; Tazdait et al., 2018)) ORR (n = 14, 58 %)	Ipilimumab related AEs: grade 2 pneumonitis (n = 1, 4%), grade 3 adrenal insufficiency (n = 4, 17 %), diarrhea/colitis 8 (grade 1 or 2: n = 6, 25 %; grade 3: n = 3, 13 %).	Prolonged air leak (n = 2, 15 %) Urinary tract infection (n = 2, 15 %) No apparent increased occurrence of adverse surgical outcomes compared to historical cohort. No delay in surgery
NCT02259621 (Forde et al., 2018)	Phase II	NSCLC	22	Nivolumab (2 doses, 4 weeks and 2 weeks prior to surgery)	ORR 10 % (n = 2)pCR 43.0 % 95IC (Liu et al., 2016; Grinshtein et al., 2009; Nanda et al., 2017; Schmid et al., 2019; Schmid et al., 2018; Loibl et al., 2019; Antonio, 2020; McArthur et al., 2016; Tarhini et al., 2014; Rozeman et al., 2017; Amaria et al., 2018; Tarhini et al., 2018; Yang et al., 2018; Forde et al., 2018; Rusch et al., 2018; Uppaluri et al., 2017; Wise-Draper et al., 2018; Gao et al., 2017; Carthon et al., 2010; Necchi et al., 2018; Powles et al., 2019; Grootsholten et al., 2018; Gao et al., 2018; Cloughesy et al., 2019; Topalian et al., 2016; Wolchok et al., 2009; Hodi et al., 2016; Champiat et al., 2017; Saâda-Bouid et al., 2017; Miller, 1982; Caswell and Swanton, 2017; Topalian, 2014; Tazdait et al., 2018; Hughes et al., 2015; Tsujino et al., 2009; Hodi et al., 2010; Zhou et al., 2019; Chen and Mellman, 2017; Sharma et al., 2017; Topalian et al., 2012) Discrepancy between ORR and pCR suggests	Pneumonia grade≥3 (n = 1,5%)	

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Table 1 (continued)

Name of the trial or first author	Phase	Primitive tumor type	Number of patients	Type of therapy evaluated	Efficacy	Toxicity	Surgical outcomes
LCMC3 (Rusch et al., 2018)	Phase II	NSCLC	21	Atezolizumab	that some patients may derive benefit from immunotherapy that is not captured radiographically Major pathologic response: 21 % (95 % CI 6–46)	Gr 1 pyrexia, Gr 2 dyspnea for 2 patients	No major delay in surgery
NCT02296684 (Uppaluri et al., 2017)	Phase II	HPV negative HNSCC	25	Pembrolizumab	pCR 42.0 % (n = 10)	No serious drug-related AEs	No unexpected surgery delays or complications
NCT02641093 (Wise-Draper et al., 2018)	Phase II	HNSCC	28	One dose Pembrolizumab 200mg	Major pathologic response (>70 %): 32 %	NA	NA
NCT01194271 (Gao et al., 2017)	Phase II	Prostate	19	Ipilimumab + androgen deprivation therapy (ADT)	No pCR	NA	NA
Carthon BC (Carthon et al., 2010)	Controlled clinical trial	Bladder	12 (6 patients at 3 mg/kg/dose with results on immune monitoring and 6 at 10 mg/kg/dose)	Ipilimumab	pCR 33.3 % (n = 4/12)	Treatment completion rate (n = 11/12) Reason for not receiving second dose = diarrhea grade 3.	No significant surgical delays due to irAEs in the 3 mg/kg/dose cohort Surgical delays (n = 3, grade 2/3 diarrhea)
PURE-01 (Necchi et al., 2018)	Phase II	Muscle invasive urothelial bladder cancer	50	Pembrolizumab	Downstaging to pT0 (42 %; 95 % CI, 28.2%–56.8%).	One discontinuation of pembrolizumab (grade 3 transaminase increase - 2%)	All patients underwent radical cystectomy
ABACUS (Powles et al., 2019)	Phase II	Muscle invasive urothelial bladder cancer	95	Atezolizumab	pCR rate 31 % (95 % CI: 21–41 %)	No new safety signals	Grade I to II surgical complications (Clavien Dindo classification): 39 of 87 (45 %) No delays in surgery
Grootscholten C (Grootscholten et al., 2018)	Phase II	Colon cancer	14 (mismatch repair proficient (pMMR) (n = 8) or mismatch repair deficient (dMMR) (n = 7)	Ipilimumab + Nivolumab	Major pathological responses (<5% viable tumor cells) 100 % (n = 7/7) CR 57 % (n = 4/7)	Well tolerated	No delays in surgery
NCT02210117 (Gao et al., 2018)	Phase I	Kidney cancer	105	Nivolumab (Nivo) Nivo + Bevacizumab (Bev) Nivo + Ipilimumab (Ipi) Nivolumab	ORR: 55 % nivo, 44 % nivo + bev, 43 % nivo + ipi	> Grade 3: 38 % for nivo, 42 % for nivo + bev (including 18 % hypertension), and 47 % for nivo + ipi	NA
Neo-nivo (Cloughesy et al., 2019)	Phase II	Glioblastoma	35 (16 in the neoadjuvant group and 19 in the adjuvant group)	Nivolumab	Benefit for the neoadjuvant group compared to the adjuvant group: • Increased OS: HR 0.39 (95 %CI 0.17–0.94; P = 0.04). • Increased PFS: (HR 0.43; 95 % CI 0.20–0.90; P = 0.03)	One grade 3 pneumonitis. One grade 4 elevation in alanine aminotransferase.	No delays in surgery

neoadjuvant immunotherapy that could prevent or delay surgery. For this type of therapy, severe and even life-threatening AEs were described with treatment-related deaths occurring in up to 2% of patients (Topalian et al., 2012). Yet, consensus recommendations on how to detect and treat irAEs have recently been published by the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group and will most likely reduce the rate of serious AEs (Puzanov et al., 2017). Furthermore, the impact on the tumor microenvironment and the hemostatic effects after immunotherapy are unknown. The normal wound-healing process involves an inflammation phase with neutrophils and lymphocytes infiltration and recruitment of monocytes that will differentiate into macrophage (Guo and Dipietro, 2010). Immunotherapy might jeopardize wound-healing and therefore delay adjuvant therapy (if needed) impacting negatively the prognostic of the patient.

Finally, there is also a threat of prolonged toxicity, particularly affecting the lung with interstitial inflammation and resulting difficulties for subsequent surgery in the neoadjuvant setting with immunotherapy which could preclude curative resection for lung cancer. This is different from the, -although more frequent-, but often relatively transient toxicity of neoadjuvant chemotherapy.

6.5. Window of opportunity studies versus neoadjuvant trials

Window of opportunity studies differ from neoadjuvant trials in that no therapeutic effect is expected. Window of opportunities are trials in which patients receive the investigational compound between their cancer diagnosis and standard of care treatment (K, K. and Di, 2012). Tumor biopsies before and after the investigational treatment are

Table 2
Immune monitoring and predictive biomarkers of efficacy and toxicity in neoadjuvant trials evaluating immune checkpoint inhibitors.

Name of the trial or first author	Immune monitoring	Biomarkers of response	Biomarkers of toxicity
I-SPY2 (Nanda et al., 2017)	NA	NA	NA
KEYNOTE-173 (Schmid et al., 2019)	NA	NA	NA
KEYNOTE-522 (Schmid et al., 2018)	NA	No effect of PD-L1 PD-L1+: pCR 68.9 % vs 54.9 % PD-L1-: pCR 45.3 % vs 30.3 %	NA
GeparNuevo (Loibl et al., 2019)	NA	NA	NA
NeoTRIPaPDL1 (Antonio, 2020)	NA	PD-L1–positive status according to immunohistochemistry (P < .0001)	NA
McArthur HL (McArthur et al., 2016)	Sustained peripheral elevations in: -Th1-type cytokines -activated (ICOS+) and proliferating (Ki67+) CD4+ and CD8 + T cells And high ratio of Ki67+ effector T cells/regulatory T cells within tumor	NA	
Tarhini AA (Tarhini et al., 2014)	Linear combination of 2 regulatory cytokines at baseline [TGF-β1 (ρ = 0.19) and IL-10 (ρ = -0.34)] significantly associated with PFS (HR 2.66; p = 0.035).	<ul style="list-style-type: none"> • Significant increase in circulating regulatory T cells • Significant increase in CD8⁺ T cell • Increased tumor infiltration by fully activated (CD69⁺) CD3⁺/CD4⁺ and CD3⁺/CD8⁺ T cells with evidence of induction/potential of memory T cells (CD45RO⁺) • Significant decrease in circulating MDSC Lin1-/HLA-DR-/CD33⁺/CD11b⁺ • a 22 immune active and proinflammatory tumor microenvironment gene signature • Cytokine expression profile (IL-1β, VEGF, G-CSF, HGF, IL-13, IL-17, GM-CSF, MCP-1, IL-5, IL-7, IL-4, IL-10, IFN-γ, IL-8 and IL-2) from patients PBMCs (peripheral blood mononuclear cells) in response to NY-ESO-1 	IL-17 at baseline (p = 0.02) correlated with the incidence of grade 3 diarrhea/colitis
OpACIN (Rozeman et al., 2017)	NA	Baseline PD-L1 and β2 microglobulin (absolute protein counts) B cells within Tertiary Lymphoid Structures (TLS) ⁴⁹	NA
AMARIA RN (Amaria et al., 2018)	NA	Higher TMB (trend) Higher CD8 + T-cell infiltrate, tumor cell PD-L1 expression, and expression of lymphoid markers (Granzyme B, CD4, FoxP3, CD20, and PD-1) Expression of CD45RO, β2-microglobulin, T cell markers (CD3, CD8), B cell markers (CD19, CD20), cell proliferation (assessed by Ki67) within CD45+ cell Higher clonality of TCR B cells within TLS ⁴⁹	NA
Tarhini AA (Tarhini et al., 2018)	NA	NA	NA
TOP1201 ipi (Yang et al., 2018)	<ul style="list-style-type: none"> • Significantly increased frequencies of highly activated T cells in the peripheral circulation • Significantly increase of CD4+ and CD8+ cells expressing ICOS, HLA-DR, CTLA-4 and PD-1 • Higher frequencies of activated TILs in resected tumors compared to PBMCs 	NA	NA
NCT02259621 (Forde et al., 2018)	NA	<ul style="list-style-type: none"> • Higher mutational and neoantigen burden • Mutation associated neoantigen specific TCR increase in peripheral blood • Responses in PD-L1-negative tumors 	NA
LCMC3 (Rusch et al., 2018)	NA	NA	NA
NCT02296684 (Uppaluri et al., 2017)	NA	<ul style="list-style-type: none"> • PDL1, CD8, CD8/PD1 and CD4 in baseline biopsies • Serum secreted cytokines • Tumor mutational burden 	NA
NCT02641093 (Wise-Draper et al., 2018)	NA	Immune cell infiltration Increased PD-L1 and PD-L2	NA
NCT01194271 (Gao et al., 2017)	<ul style="list-style-type: none"> • Increase in CD4+ and CD8 + T cells, including PD-1+ and ICOS + subsets, increase in CD45RO+, granzyme-B (GrB)+, and CD68+ cells • Significantly greater immune cell infiltration in prostate tumors compared to ADT alone group • Significantly higher PD-L1 expression on CD4 + T cells, CD8 + T cells, and CD68+ macrophages after treatment • Increase in CD4+ PD-L1+ T cells from 0.2 to 0.7 %, CD8 PDL1+ (4.4–21.3%) and CD68 PD-L1+ (from 2.5–25%) 	NA	NA

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Table 2 (continued)

Name of the trial or first author	Immune monitoring	Biomarkers of response	Biomarkers of toxicity
Carthon BC (Carthon et al., 2010)	<ul style="list-style-type: none"> Significantly higher expression of V-domain Ig-containing Suppressor of T-cell Activation (VISTA) on CD4 (0.0%–4%), CD8 (0.0–7.0%) and CD68 macrophages (7–31%) Significant changes in the expression of a total of 690 genes (increased PD-L1 and VISTA expression) Significantly greater proportion of CD68+ macrophages with PD-L1 and VISTA expression in post-treatment prostate tumors in comparison to historical melanomas increase in the frequency of PD-L1+ and VISTA + macrophages with expression of CD163 and ARG1, suggesting an M2-like phenotype and function ICOS expression in peripheral blood and tumor CD4 T cells is increased IFNγ-Producing CD4+ICOShi Effector T Cells That Recognize the Tumor Antigen NY-ESO-1 in the Peripheral Blood of Anti-CTLA-4 Treated Patients FOXP3 Expression Is Lower in CD4 T Cells in Tumor Tissues IFNγ Expression Is Increased in Tumor Tissues The Ratio of CD4+ICOShi Effector to CD4+FOXP3+ Regulatory T Cells Is Increased Higher frequency of CD4+ICOShi T cells and IFN-γ mRNA levels in nonmalignant prostate tissues and incidental prostate tumor tissues removed at the time of radical cystoprostatectomy 	<ul style="list-style-type: none"> Increased frequency of CD4+ICOShi T cells, sustained over a period of 12 weeks of therapy 	NA
PURE-01 (Necchi et al., 2018)	NA	<ul style="list-style-type: none"> PD-L1 CPS \geq 10 %: pT0 after RC in 54.3 % of patients with PD-L1 CPS \geq 10 % (n = 35), vs 13.3 % in those with CPS < 10 % (n = 15). Tumor mutation burden (TMB) - cutoff at 15 mutations/Mb. 	NA
ABACUS (Powles et al., 2019)	<ul style="list-style-type: none"> 5 patients changed from an excluded to an inflamed phenotype and 4 patients changed from an inflamed to an excluded phenotype. 	<ul style="list-style-type: none"> High presence of intraepithelial CD8+ cells (pCR rate of 40 % (95 % CI: 26–57 %) compared to a rate of 20 % (95 % CI:9–35 %) with absence of CD8 (P < 0.05) A predefined eight-gene cytotoxic T cell transcriptional signature (tGE8) (P < 0.01) Expression of dually stained cells for CD8 and GZMB (14 out of 16 patients, 87 % in responding patients versus 3 out of 10 in non responding patients, 30 %; P < 0.05) Low FAP (Fibroblast Activation Protein) expression (P < 0.01) 	NA
Grootscholten C (Grootscholten et al., 2018)	<ul style="list-style-type: none"> T-cell infiltration, particularly CD8 + T-cells 	Post-treatment IFN γ gene signatures Deficiency in the mismatch repair system	NA
NCT02210117 (Gao et al., 2018)	NA	<ul style="list-style-type: none"> Tumor infiltrating CD8 T cells correlate with clinical responses to nivo or nivo + bev, but not to nivo + ipi Tumor IFN pathway gene expression B cells within TLS⁴⁹ PD-L1 status, tumor mutation or mutation burden, neoantigens did not correlate with response 	NA
Neo-nivo (Cloughesy et al., 2019)	<ul style="list-style-type: none"> Upregulation of T cell- and interferon-γ-related gene expression Downregulation of cell-cycle-related gene expression within the tumor Focal induction of programmed death-ligand 1 in the tumor microenvironment Enhanced clonal expansion of T cells Decreased PD-1 expression on peripheral blood T cells Decreasing monocytic population 	<ul style="list-style-type: none"> Standardized baseline peripheral T cell receptor clonality (hazard ratio of 1.48 for each standard deviation increase of 1, P = 0.12). Cell-cycle-related gene set variation analysis enrichment score (R2 = 0.57). 	NA

collected for translational research. These trials have the potential to reveal pharmacodynamics effect of a therapeutic compound and to assess predictive biomarkers of efficacy or resistance. These trials could therefore be more efficient than neoadjuvant studies in selecting patients who might benefit from a given therapy.

6.6. Adjuvant versus neoadjuvant trials

Currently, immunotherapy is being tested in various settings in cancer and sometimes without a global strategy. For example, in melanoma, neoadjuvant IICPB versus neo and adjuvant IICPB reported similar results suggesting that neoadjuvant may be more efficient than

adjuvant immunotherapy which confirms preclinical data (Rozeaman et al., 2017). However, at present, the study of neoadjuvant immunotherapy for melanoma is in the exploratory stage with no mature research result. On the other hand, adjuvant immunotherapy has already been associated with improved results for nivolumab (Weber et al., 2017) and pembrolizumab (Eggermont et al., 2018) and are approved by the US Food and Drug Administration (FDA) for this indication. The positive results of these large phase III trials with adjuvant immunotherapy might be an obstacle for many ongoing neoadjuvant immunotherapy trials, particularly in earlier stages, as has happened with neoadjuvant chemotherapy after adjuvant treatment became standard of care.

6.7. Uncertainty about timing of neoadjuvant immunotherapy

Using orthotopic 4T1.2 and E0771 mouse models of spontaneously metastatic mammary cancer, Liu et al. demonstrated that a short duration (4–5 days) between first administration of neoadjuvant immunotherapy and resection of the primary tumor was necessary for optimal efficacy, while extending this duration (10 days) abrogated immunotherapy efficacy. However, efficacy was also lost if neoadjuvant immunotherapy was given too close to surgery (2 days). Interestingly, an additional 4 adjuvant doses of treatment following a standard 2 doses of neoadjuvant immunotherapy, did not significantly improve overall tumor-free survival regardless of the combination treatment (anti-PD-1+anti-CD137 or anti-CTLA4+anti-PD-1). Furthermore, biochemical immune-related adverse events (irAEs) increased in tumor-bearing mice that received the additional adjuvant immunotherapy suggesting that shorter doses of neoadjuvant immunotherapy scheduled close to the time of surgery may optimize effective anti-tumor immunity and reduce severe irAEs (Liu et al., 2019).

Usually, immunotherapy is either repeatedly given alone or in combination with chemotherapy. However, contradictory results were reported in human studies. In breast cancer, interesting results were reported when immunotherapy (durvalumab) was started before chemotherapy (Loibl et al., 2019). In non-small-cell lung cancer, Lynch and colleagues found that phased ipilimumab plus paclitaxel and carboplatin improved immune-related PFS and PFS but not concurrent ipilimumab plus paclitaxel and carboplatin in a phase II study (Lynch et al., 2012), but the addition of phased ipilimumab to first-line chemotherapy did not prolong OS compared with chemotherapy alone in patients with advanced squamous NSCLC in the phase III study (Govindan et al., 2017). In head and neck squamous cell carcinoma (HNSCC), neoadjuvant pembrolizumab monotherapy resulted in a high pCR in two phase II trials (Tarhini et al., 2018; Yang et al., 2018) even with only one dose of 200 mg (Wise-Draper et al., 2018). Taken together, these data suggest that the optimal timing administration (before, concomitant or after chemotherapy) is not completely understood. Moreover, the efficacy of immunotherapy could be prolonged and only one exposure could be sufficient.

7. Conclusion and implications for future management of cancers

Immunotherapy has been a revolution in cancer management with long-term survivors in the metastatic setting in diseases such as melanomas, for which prognosis was poor a few years ago. This has led to a prompt evaluation of such therapies in earlier stages of cancer with the objective to cure patients. This article discussed the still limited amount of data finding the rationale to assess such therapy in this setting and reviewed the preclinical and clinical data available.

Overall, neoadjuvant immunotherapy is a promising approach for the treatment of cancers and the rationale supporting its use is strong. However, durable responses were reported only in a minority of patients. In order to understand why immune therapies work or fail, and how they can be improved to reach their hoped-for potential as a broadly transformative treatment for cancer, the best way is to assess those drugs in the neoadjuvant setting where we have access to samples pre- and post-treatment. T-cell-Inflamed tumors usually respond well to IICPB. In this regard, inflamed tumors are an appropriate model for IICPB monotherapy in the neoadjuvant setting in order to increase OS (Chen and Mellman, 2017). For immune desert or excluded tumors, the neoadjuvant setting is a good option to test new combinations (Chen and Mellman, 2013) (with chemotherapy, with radiotherapy, with targeted therapies,...) or novel immuno-oncology drugs (Chen and Mellman, 2017). In addition to anti-PD-1, anti-PD-L1 and anti-CTLA-4, novel immunologic therapeutic approaches being developed in the neoadjuvant setting that could be of interest in immune desert and excluded tumors are represented by toll-like receptor 8 agonist (ClinicalTrials.gov

Identifier: NCT02124850), and oncolytic viruses for example (ClinicalTrials.gov Identifier: NCT03259425). Moreover, bispecific antibodies, cancer vaccines and adoptive T-cell therapy are emerging therapies in immune-oncology that could be relevant in the neoadjuvant setting. In this context, cancer vaccine sipuleucel-T was evaluated in prostate cancer. Forty-two patients with untreated localized prostate cancer were treated on an open-label phase II study prior to planned radical prostatectomy (RP) (Fong et al., 2014). However, downstaging was not observed at the time of RP relative to baseline. The authors described a greater than three-fold increase in infiltrating CD3(+), CD4 (+) FOXP3(-), and CD8(+) T cells in the radical prostatectomy tissues compared with the pretreatment biopsy ($P < .001$). This level of T cell infiltration was observed at the tumor interface. The majority of infiltrating T cells were PD-1(+) and Ki-67(+) in favor of activated T cells.

Moreover, the timing of administration of immunotherapy is not well understood. Whether, IICPB present persistent immunomodulatory effects or prolonged exposure is necessary is unknown. Unlike vaccines, IICPBs are passively administered antibodies with uncertainty and variability in their ability to engage the adaptive immune system. It is unknown whether ongoing therapy is truly superior to limited treatment of a defined duration or to the use of a maintenance regimen with less frequent administration. Finally whether we need to administer neoadjuvant immunotherapy or adjuvant immunotherapy or both and how to combine immunotherapy with other therapies either concurrently or sequentially are not known. To this day, we do not know the best way to combine IICPB, a challenge for future clinical trials, indeed.

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Declaration of Competing Interest

The other authors declare no conflict of interest.

References

- Amaria, R.N., et al., 2018. Neoadjuvant immune checkpoint blockade in high-risk resectable melanoma. *Nat. Med.* 24, 1649–1654.
- Antonio, S., 2020. Combining Atezolizumab With Neoadjuvant Chemotherapy Does Not Improve Pathologic Complete Response Rates for Patients With Triple-Negative Breast Cancer, p. 3.
- Bindea, G., et al., 2013. Spatiotemporal Dynamics of Intratumoral Immune Cells Reveal the Immune Landscape in Human Cancer. *Immunity* 39, 782–795.
- Carthon, B.C., et al., 2010. Preoperative CTLA-4 blockade: tolerability and immune monitoring in the setting of a presurgical clinical trial. *Clin. Cancer Res.* 16, 2861–2871.
- Caswell, D.R., Swanton, C., 2017. The role of tumour heterogeneity and clonal cooperativity in metastasis, immune evasion and clinical outcome. *BMC Med.* 15, 133.
- Champiat, S., et al., 2017. Hyperprogressive Disease Is a New Pattern of Progression in Cancer Patients Treated by Anti-PD-1/PD-L1. *Clin. Cancer Res.* 23, 1920–1928.
- Chen, D.S., Mellman, I., 2017. Elements of cancer immunity and the cancer-immune set point. *Nature* 541, 321–330.
- Chen, D.S., Mellman, I., 2013. Oncology meets immunology: the cancer-immunity cycle. *Immunity* 39, 1–10.
- Cloughesy, T.F., et al., 2019. Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. *Nat. Med.* 25, 477–486.
- Cortazar, P., et al., 2014. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet* 384, 164–172.
- Eggermont, A.M.M., et al., 2018. Adjuvant Pembrolizumab versus Placebo in Resected Stage III Melanoma. *N. Engl. J. Med.* 378, 1789–1801.
- Fong, L., et al., 2014. Activated lymphocyte recruitment into the tumor microenvironment following preoperative sipuleucel-T for localized prostate cancer. *J. Natl. Cancer Inst.* 106.

- Forde, P.M., et al., 2018. Neoadjuvant PD-1 Blockade in Resectable Lung Cancer. *N. Engl. J. Med.* 378, 1976–1986.
- Gao, Q., et al., 2009. Overexpression of PD-L1 significantly associates with tumor aggressiveness and postoperative recurrence in human hepatocellular carcinoma. *Clin. Cancer Res.* 15, 971–979.
- Gao, J., et al., 2017. VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy in patients with prostate cancer. *Nat. Med.* 23, 551–555.
- Gao, J., et al., 2018. A pilot randomized study evaluating nivolumab (nivo) or nivo + bevacizumab (bev) or nivo + ipilimumab (ipi) in patients with metastatic renal cell carcinoma (MRCC) eligible for cytoreductive nephrectomy (CN), metastasectomy (MS) or post-treatment biopsy (Bx). *JCO* 36, 4520–4520.
- Govindan, R., et al., 2017. Phase III Trial of Ipilimumab Combined With Paclitaxel and Carboplatin in Advanced Squamous Non–Small-Cell Lung Cancer. *JCO* 35, 3449–3457.
- Grinshtein, N., Bridle, B., Wan, Y., Bramson, J.L., 2009. Neoadjuvant Vaccination Provides Superior Protection against Tumor Relapse following Surgery Compared with Adjuvant Vaccination. *Cancer Res.* 69, 3979–3985.
- Grootscholten, C., Voest, E.E., Chalabi, M., et al., 2018. LBA37_PRNeO adjuvant ipilimumab plus nivolumab in early stage colon cancer. *Ann. Oncol.* 29.
- Guo, S., Dipietro, L.A., 2010. Factors affecting wound healing. *J. Dent. Res.* 89, 219–229.
- Hodi, F.S., et al., 2016. Evaluation of Immune-Related Response Criteria and RECIST v1.1 in Patients With Advanced Melanoma Treated With Pembrolizumab. *J. Clin. Oncol.* 34, 1510–1517.
- Hodi, F.S., et al., 2010. Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* 363, 711–723.
- Hughes, T., et al., 2015. The prognostic significance of stable disease following high-dose interleukin-2 (IL-2) treatment in patients with metastatic melanoma and renal cell carcinoma. *Cancer Immunol. Immunother.* 64, 459–465.
- K, K, Di, H., 2012. Cracking open window of opportunity trials. *J. Clin. Oncol.* 30, 2573–2575.
- Larkin, J., et al., 2015. Combined Nivolumab and Ipilimumab or Monotherapy in Previously Untreated Melanoma. *N. Engl. J. Med.* 373, 23–34.
- Li, Z., et al., 2018. Correlation of pathological complete response with survival after neoadjuvant chemotherapy in gastric or gastroesophageal junction cancer treated with radical surgery: A meta-analysis. *PLoS One* 13, e0189294.
- Liu, J., et al., 2016. Improved Efficacy of Neoadjuvant Compared to Adjuvant Immunotherapy to Eradicate Metastatic Disease. *Cancer Discov.* 6, 1382–1399.
- Liu, J., et al., 2019. Timing of neoadjuvant immunotherapy in relation to surgery is crucial for outcome. *Oncoimmunology* 8.
- Loibl, S., et al., 2019. A randomised phase II study investigating durvalumab in addition to an anthracycline taxane-based neoadjuvant therapy in early triple-negative breast cancer: clinical results and biomarker analysis of GeparNuevo study. *Ann. Oncol.* 30, 1279–1288.
- Lynch, T.J., et al., 2012. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. *J. Clin. Oncol.* 30, 2046–2054.
- Man, J., Ritchie, G., Links, M., Lord, S., Lee, C.K., 2018. Treatment-related toxicities of immune checkpoint inhibitors in advanced cancers: a meta-analysis. *Asia. J. Clin. Oncol.* 14, 141–152.
- McArthur, H.L., et al., 2016. A Pilot Study of Preoperative Single-Dose Ipilimumab and/or Cryoablation in Women with Early-Stage Breast Cancer with Comprehensive Immune Profiling. *Clin. Cancer Res.* 22, 5729–5737.
- Melero, I., Berraondo, P., Rodríguez-Ruiz, M.E., Pérez-Gracia, J.L., 2016. Making the Most of Cancer Surgery with Neoadjuvant Immunotherapy. *Cancer Discov.* 6, 1312–1314.
- Miller, F.R., 1982. Intratumor immunologic heterogeneity. *Cancer Metastasis Rev.* 1, 319–334.
- Nakanishi, J., et al., 2007. Overexpression of B7-H1 (PD-L1) significantly associates with tumor grade and postoperative prognosis in human urothelial cancers. *Cancer Immunol. Immunother.* 56, 1173–1182.
- Nanda, R., et al., 2017. Pembrolizumab plus standard neoadjuvant therapy for high-risk breast cancer (BC): Results from I-SPY 2. *JCO* 35, 506–506.
- Necchi, A., et al., 2018. Pembrolizumab as Neoadjuvant Therapy Before Radical Cystectomy in Patients With Muscle-Invasive Urothelial Bladder Carcinoma (PURE-01): An Open-Label, Single-Arm, Phase II Study. *J. Clin. Oncol.*, JCO1801148 <https://doi.org/10.1200/JCO.18.01148>.
- Owonikoko, T.K., et al., 2015. PD-L1, PD-1, and CTLA-4 as prognostic biomarkers in resected non-small cell lung cancer. *JCO* 33, 7551–7551.
- Péguillet, I., et al., 2014. High numbers of differentiated effector CD4 T cells are found in patients with cancer and correlate with clinical response after neoadjuvant therapy of breast cancer. *Cancer Res.* 74, 2204–2216.
- Petrelli, F., et al., 2014. Correlation of pathological complete response with survival after neoadjuvant chemotherapy in bladder cancer treated with cystectomy: a meta-analysis. *Eur. Urol.* 65, 350–357.
- Powles, T., et al., 2019. Clinical efficacy and biomarker analysis of neoadjuvant atezolizumab in operable urothelial carcinoma in the ABACUS trial. *Nat. Med.* 25, 1706–1714.
- Puzanov, I., et al., 2017. Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. *J. Immunother. Cancer* 5, 95.
- Reck, M., et al., 2016. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N. Engl. J. Med.* 375, 1823–1833.
- Robert, C., et al., 2011. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N. Engl. J. Med.* 364, 2517–2526.
- Robert, C., et al., 2015a. Nivolumab in previously untreated melanoma without BRAF mutation. *N. Engl. J. Med.* 372, 320–330.
- Robert, C., et al., 2015b. Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N. Engl. J. Med.* 372, 2521–2532.
- Rozeman, E.A., et al., 2017. Neoadjuvant ipilimumab + nivolumab (IPI+NIVO) in palpable stage III melanoma: Updated data from the OpACIN trial and first immunological analyses. *JCO* 35, 9586–9586.
- Rusch, V.W., et al., 2018. Neoadjuvant atezolizumab in resectable non-small cell lung cancer (NSCLC): Initial results from a multicenter study (LCMC3). *JCO* 36, 8541–8541.
- Saàda-Bouزيد, E., et al., 2017. Hyperprogression during anti-PD-1/PD-L1 therapy in patients with recurrent and/or metastatic head and neck squamous cell carcinoma. *Ann. Oncol.* 28, 1605–1611.
- Schmid, P., et al., 2019. Abstract PD5-01: KEYNOTE-173: Phase 1b multicohort study of pembrolizumab (Pembro) in combination with chemotherapy as neoadjuvant treatment for triple-negative breast cancer (TNBC). *Cancer Res.* 79, PD5-PD5-01.
- Schmid, P., et al., 2018. KEYNOTE-522: Phase III study of pembrolizumab (pembro) + chemotherapy (chemo) vs placebo + chemo as neoadjuvant therapy followed by pembro vs placebo as adjuvant therapy for triple-negative breast cancer (TNBC). *JCO* 36, TPS602–TPS602.
- Sharma, P., Hu-Lieskovan, S., Wargo, J.A., Ribas, A., 2017. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell* 168, 707–723.
- Spring, L.M., et al., 2016. Neoadjuvant Endocrine Therapy for Estrogen Receptor-Positive Breast Cancer: A Systematic Review and Meta-analysis. *JAMA Oncol.* 2, 1477–1486.
- Tarhini, A.A., Iqbal, F., 2010. CTLA-4 blockade: therapeutic potential in cancer treatments. *Onco Targ. Ther.* 3, 15–25.
- Tarhini, A.A., et al., 2014. Immune monitoring of the circulation and the tumor microenvironment in patients with regionally advanced melanoma receiving neoadjuvant ipilimumab. *PLoS One* 9, e87705.
- Tarhini, A., et al., 2018. Neoadjuvant ipilimumab (3 mg/kg or 10 mg/kg) and high dose IFN- α 2b in locally/regionally advanced melanoma: safety, efficacy and impact on T-cell repertoire. *J. Immunother. Cancer* 6.
- Tatsumi, T., et al., 2002. Disease-associated Bias in T Helper Type 1 (Th1)/Th2 CD4+ T cell responses against MAGE-6 in HLA-DRB10401+ patients with renal cell carcinoma or melanoma. *J. Exp. Med.* 196, 619–628.
- Tatsumi, T., et al., 2003. Disease stage variation in CD4+ and CD8+ T-Cell reactivity to the receptor tyrosine kinase EphA2 in patients with renal cell carcinoma. *Cancer Res.* 63, 4481–4489.
- Tazdait, M., et al., 2018. Patterns of responses in metastatic NSCLC during PD-1 or PDL-1 inhibitor therapy: Comparison of RECIST 1.1, irRECIST and iRECIST criteria. *Eur. J. Cancer* 88, 38–47.
- Topalian, S.L., 2014. Immunologic heterogeneity of cancer: determinants of response and resistance to immunotherapy. [Abstract]. In: Proceedings of the AACR Precision Medicine Series: Drug Sensitivity and Resistance: Improving Cancer Therapy; Jun 18-21. Orlando, FL. Philadelphia (PA). AACR; Clin Cancer Res 2015;21(4 Suppl): Abstract nr IA01.
- Topalian, S.L., Taube, J.M., Anders, R.A., Pardoll, D.M., 2016. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. *Nat. Rev. Cancer* 16, 275–287.
- Topalian, S.L., et al., 2012. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* 366, 2443–2454.
- Tsujiino, K., et al., 2009. Response rate is associated with prolonged survival in patients with advanced non-small cell lung cancer treated with gefitinib or erlotinib. *J. Thorac. Oncol.* 4, 994–1001.
- Uppaluri, R., et al., 2017. Neoadjuvant pembrolizumab in surgically resectable, locally advanced HPV negative head and neck squamous cell carcinoma (HNSCC). *JCO* 35, 6012–6012.
- Verma, R., et al., 2016. Lymphocyte depletion and repopulation after chemotherapy for primary breast cancer. *Breast Cancer Res.* 18, 10.
- Wang, Q., Liu, F., Liu, L., 2017. Prognostic significance of PD-L1 in solid tumor. *Medicine (Baltimore)* 96.
- Weber, J., et al., 2017. Adjuvant Nivolumab versus Ipilimumab in Resected Stage III or IV Melanoma. *N. Engl. J. Med.* 377, 1824–1835.
- Wei, S.C., Duffy, C.R., Allison, J.P., 2018. Fundamental mechanisms of immune checkpoint blockade therapy. *Cancer Discov.* 8, 1069–1086.
- Wise-Draper, T.M., et al., 2018. Phase II multi-site investigation of neoadjuvant pembrolizumab and adjuvant concurrent radiation and pembrolizumab with or without cisplatin in resected head and neck squamous cell carcinoma. *JCO* 36, 6017–6017.
- Wolchok, J.D., et al., 2009. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin. Cancer Res.* 15, 7412–7420.
- Yang, C.-F.J., et al., 2018. Surgical Outcomes After Neoadjuvant Chemotherapy and Ipilimumab for Non-Small Cell Lung Cancer. *Ann. Thorac. Surg.* 105, 924–929.
- Zhou, Y., et al., 2019. First-line treatment for patients with advanced non-small cell lung carcinoma and high PD-L1 expression: pembrolizumab or pembrolizumab plus chemotherapy. *J. Immunother. Cancer* 7.

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Original article

IgD multiple myeloma: Clinical, biological features and prognostic value of the serum free light chain assay



Myélome multiple à IgD : caractéristiques clinicobiologiques et intérêt pronostique du dosage sérique des chaînes légères libres

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ABSTRACT

IgD multiple myeloma (MM) is a rare subtype of myeloma, it affects less than 2% of patients with MM. To evaluate the clinical and prognostic attributes of serum free light chains (sFLCs) analysis, we examined 17 cases of IgD MM. From 1998 to 2012, we obtained 1250 monoclonal gammopathies including 590 multiple myeloma and 17 patients had IgD MM. With preponderance of men patients with a mean age at diagnosis of: 59 ± 12 years. Patients with IgD MM have a short survival (Median survival = 9 months). The presenting features included: bone pain (75%), lymphadenopathy (16%), hepatomegaly (25%), splenomegaly (8%), associated AL amyloidosis (6%), renal impairment function (82%), infections (47%), hypercalcemia (37%) and anemia (93%). Serum electrophoresis showed a subtle M-spike (Mean = 13.22 ± 10 g/L) in all patients associated to a hypogammaglobulinemia. There was an over-representation of Lambda light chain (65%); high serum β_2 -microglobulin in 91% and Bence Jones proteinuria was identified in 71%. The median rate of sFLCs κ was 19.05 mg/L and 296.75 mg/L for sFLCs λ . sFLCR was abnormal in 93% of patients and it showed concordance between baseline sFLCR and the survival ($P = 0.034$). The contribution of FLC assay is crucial for the prognosis of patients with IgD MM.

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R É S U M É

Le myélome à IgD est une entité rare du myélome multiple (MM), il représente moins de 2 % des cas de MM. L'objectif de cette étude est d'évaluer l'intérêt diagnostique et pronostique du dosage des chaînes légères libres sériques (CLL) dans MM à IgD. Sur une période de 14 ans (1998 à 2012), nous avons recruté 590 MM (sur 1250 gammopathies monoclonales) dont 17 (2,88 %) MM à IgD. Dans notre série MM à IgD, on note une prédominance masculine, un âge moyen au moment du diagnostic de 59 ± 12 ans et une espérance de vie réduite (médiane de survie = 9 mois). Les caractéristiques cliniques à l'inclusion sont : douleurs osseuses (75 %), adénopathies (16 %), hépatomégalie (25 %), splénomégalie (8 %), amylose AL (6 %), insuffisance rénale (82 %), infections (47 %), hypercalcémie (37 %) et anémie (93 %). L'électrophorèse sérique a montré un composant monoclonal chez tous les patients associées à une hypogammaglobulinémie (Concentration moyenne = $13,22 \pm 10$ g/L). Par ailleurs, on note une prédominance des chaînes légères lambda (65 %) ; un taux sérique élevé de la β_2 -microglobuline dans 91 % des cas et la protéine de Bence Jones a été identifiée dans 71 % des cas. Le taux sérique médian de CLL κ était de 19,05 mg/L et de 296,75 mg/L pour CLL λ . Le rapport rCLL était anormal chez 93 % des patients avec une corrélation de ce rapport avec la survie ($p = 0,034$). Le dosage des chaînes légères libres (CLL) représente un bon marqueur pronostic des patients atteints de MM IgD.

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1. Introduction

Multiple myeloma (MM) is a hematologic malignancy characterized by monoclonal proliferation of plasma cells. It accounts for approximately 10% of all hematologic cancers [1,2]. There are MM with all isotypes of heavy chains and all types of light chains as well as non-secreting MM.

IgD multiple myeloma represents less than 2% of all myelomas [3–5]. It affects relatively a young population and is characterized by their poor prognosis, small peaks or their absence on electrophoresis, the predominance of lambda light chain and a frequent occurrence of renal failure [3,6]. Hypercalcemia and association with AL amyloidosis are more encountered in IgD MM than in other myelomas [7]. Due to the rarity of this disease, few articles are found in the literature.

Free light chains (FLC) are present in the serum and the urine of many patients with B-cell proliferative disorders including MM. Sensitive, automated immunoassays, which specifically measure FLC in serum, have become recently available [8,9]. The importance of the serum free light chains ratio (sFLCR) has been widely proven in: diagnosis of light chain multiple myeloma and non-secreting MM, monitoring of light chain MM, non-secreting MM and MM with whole Ig including IgD MM and as an independent prognostic factor in monoclonal gammopathy of undetermined significance (MGUS), smoldering myeloma and symptomatic MM [10].

The aim of our study is the presentation of clinical and biological characteristics of 17 cases of IgD multiple myeloma and the determination of the prognostic value of the serum free light chain assay.

2. Patients and methods

During 14 years of activity at the Immunochimistry Unit at the central laboratory of the Teaching Hospital Beni Messous, Algiers, Algeria, we diagnosed 1250 monoclonal gammopathies including 590 multiple myelomas. Seventeen patients out of these cases had IgD MM. These patients, newly diagnosed myelomatosis, were mainly from clinical divisions of our hospital: hematology (8/17), nephrology (6/17), rheumatology (1/17), internal medicine (1/17) and a last one from a private laboratory.

The serum analysis was performed on non-hemolyzed serum samples stored at 4°. For each serum, we made total protein assay by colorimetric method. Urinalysis was performed on urine collected for 24 hours. Diuresis and proteinuria were performed with colorimetric method using red of pyrogallol.

As recommended by a consensus conference [11], monoclonal gammopathies were detected by a joint analysis of serum and urine electrophoresis and the monoclonal component was identified by an immunoprecipitation technique. In most cases, sera and concentrated urines were tested by electrophoresis and immunofixation on agarose gel “Hydrasys” (Sebia) or “SAS3” (Helena) or capillary electrophoresis on “Capillarys” (Sebia). Antisera against IgG, IgA, IgM, IgD, IgE, Kappa and lambda were employed to type the monoclonal peak. sFLC measurement was performed by using a nephelometric technique on “IMMAGE-Beckman coulter”. Reagents “Freelite” (The Binding Site), which are antibodies specifically recognizing free light chains, were used. The latter set the constant domain at their epitopes normally masked when combined with the heavy chain immunoglobulin. To reduce the risk of non-detection of antigen excess, all samples whose concentrations were found near the upper limit of detection at a given dilution were manually diluted and reanalyzed.

Normal range of sFLC are those proposed by the manufacturer obtained from Katzmann et al. [12] work which are for sFLC κ = 3.3 to 19.4 mg/L, for sFLC λ = 5.7 to 26.3 mg/L and the ratio sFLC κ/λ = 0.26 to 1.65 (Renal reference range sFLCR = 0.3 to 3.3) [13].

Serum free light chain analysis has prognostic significance. In 2008, Snozek et al. [14] incorporated abnormal sFLC in the International Staging System (ISS) risk factors to create a risk stratification model. On the basis of the distribution of values, a cutpoint κ/λ FLC ratio of < 0.03 or > 32 was selected for further analysis. The same cut off was applied to our study.

For the statistical analysis, calculations were performed using SPSS 17.0. Survival was defined as the time from initial diagnosis to the death or last follow-up (For patients who were lost to follow-up) and was estimated according to the Kaplan–Meier method; survival curves were compared using the log-rank test. The prognostic ability of potential risk factors was evaluated in univariate model using Cox proportional hazards regression analysis. All statistical tests were two-sided, and *P*-values of less than 0.05 were considered to be significant.

3. Results

Patients characteristics: in this retrospective study, we report a series of 17 cases of IgD multiple myeloma. Some clinical and biological data on several cases were not recorded. Our series consisted of 12 men (70%) and 5 women (30%) aged from 24 to 89 years (Mean, 59 years; median, 60 years) at diagnosis. Two patients had an age lower than 40 years.

Clinical and biological characteristics of our patients are summarized in Tables 1 and 2.

For the 8 on 14 patients with a renal failure, the renal symptom allows the discovery of the disease.

All patients had confirmed plasmacytosis but we were not able to obtain the percentage and the morphology of plasma cells for 5 patients. Plasma cells were dysmorphic in 9 patients (75%); one of them had 5% plasma cells at diagnosis.

Seven patients had a thrombocytopenia and one patient had pancytopenia (8%).

For many patients, the duration of the disease was difficult to establish since no detailed information had been provided. However, 12 of 17 patients died (2 patients were lost to follow-up) and 3 patients are still alive. The median duration of survival in our series was 9 months. The proportion of patients alive at 1 year and 2 years after diagnosis was 33% and 12%, respectively. There were no statistically significant differences in mean survival between patients with λ et κ light chain (*P* = 0.39).

According to Durie and Salmon staging [15] all patients were in stage III; 3 patients in IIIA and 14 patients in IIIB. On 14 patients, 13 patients (93%) had an sFLCR disrupted with the most disturbed one for κ light chain is 2400 and for λ light chain is 0.00097. The patient with a normal ratio has normal serum free light chains concentrations. The median rate of sFLCs κ was 19.05 mg/L and 296.75 mg/L for sFLCs λ .

Overall survival was significantly inferior in patients with an sFLCR < 0.03 or > 32 (*n* = 9) compared to those with an sFLCR

Table 1

Comparison of patient characteristics at diagnostic of our series with Jancelewicz et al. and Bladé et al. studies.

	Our study	Jancelewicz et al. [6]	Bladé et al. [3]
Number of patients	17	133	53
Men/Women	2.4: 1	3: 1	1.65: 1
Median age [interval] (years)	60 [24; 89]	56 [28; 86]	60 [19; 86]
Bone pain	12/16 (75%)	64/89 (72%)	38/53 (72%)
Hepatomegaly	3/12 (25%)	36/64 (55%)	7/53 (13%)
Splenomegaly	1/12 (8%)	–	3/53 (6%)
Lymphadenopathy	2/12 (16.67%)	–	5/53 (9%)
Renal failure	14/17 (82%)	–	18/53 (33%)
Infections (Pulmonary)	8/17 (47%)	–	2/53 (4%)
AL amyloidosis (Atteinte cardiaque)	1/17 (6%)	44%	19/53 (27%)
Plasma cell leukemia	3/17 (17.6%)	–	12%
Bone lesions (Radiographs)	8/10 (80%)	–	–
Plasmacytosis	9/12 (75%)	–	45% of patients > 40%
Anemia (<12 g/dL)	15/16 (94%)	38/44 (84%)	33/53 (63%)
Hb (Mean, interval)	8 [5.2–14]	–	–
Hypercalcemia	6/16 (37%)	30%	11/53 (22%)
Calcemia	105 [77–165]	–	–
ISS ^a	Stade I : 1/13 (7 %) Stade II : 1/13 (7 %) Stade III : 11/13 (84 %)	–	–
Accelerated ESR	11/11 (100%)	–	–
Survival (months)	9	9	21

ISS: International staging system; ESR: Erythrocyte Sedimentation Rate.

^a ISS including β 2m and albumin. Available in only 13 patients.

Table 2
Comparison of Immunochemical data in our study with Jancelewicz et al. and Bladé et al. studies.

	Our study	Jancelewicz et al. [6]	Bladé et al. [3]
Total serum protein (g/L)			
Median	68	74	67
Interval	[43–94]	[46; 103]	[83; 89]
Monoclonal component (g/L)			
Median	9.42	17	10
Interval	[4–43]	[3; 43]	[5; 30]
Number of peaks			
1 peak	13/17 (77%)	74/97 (76%)	26/47 (55%)
2 or 3 peaks	4/17 (23%)	11/97 (11%)	2/47 (4%)
0 peak	0/17 (0%)	12/97 (12%)	19/47 (40%)
Position of the peak			
Alpha2	1/20 (5%)	69% (59/85)	-
Beta	3/20 (15%)	29% (25/85)	-
Gamma	16/20 (80%)	1% (1/85)	-
Type of light chain			
Kappa	6/17 (35%)	13/128 (10%)	20/53 (38%)
Lambda	11/17 (65%)	115/128 (90%)	33/53 (60%)
Hypogammaglobulinemia			
BJP	12/17 (71%)	29/41 (71%)	51/53 (96%)
β2m			
Median	45	-	3.9
Interval	[1.6–87]	-	[1.5–35]
FLCs assay			
FLC κ mg/L	19.05	-	-
FLC λ mg/L	297	-	-
Disturbed FLCR (0.26–1.65)	12/14 (86%)	-	-

BJP: Bence Jones Protein; β 2m: β 2 microglobulin; FLC assay: free light chains assay; FLCR: free light chains ratio.

between 0.03 and 32 ($n = 5$), with median survival of 3 months versus 12 months, respectively ($P = 0.03$) (Fig. 1).

4. Discussion

Among 590 MM diagnosed in our laboratory, 17 patients had IgD MM (2.9%). These results correspond well with those reported in the literature [3–6]. Due to the rarity of IgD myeloma, all series known in the literature report a small number of patients except Jancelewicz et al. study [6], which is the largest review of IgD MM cases, reported.

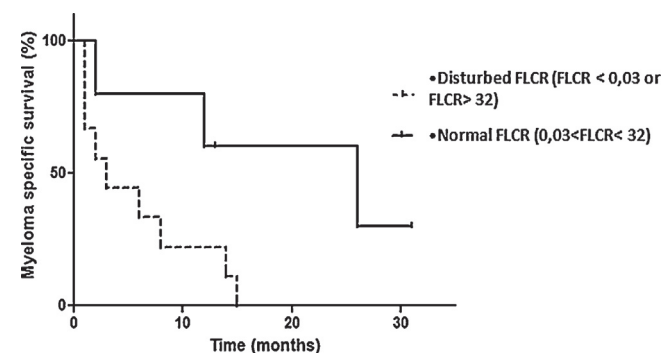


Fig. 1. Overall survival based on disturbed FLCR (FLCR < 0.03 or FLCR > 32) and normal FLCR (0.03 < FLCR < 32). Kaplan-Meier analysis of survival in patients with disturbed FLCR ($n = 9$) and normal FLCR ($n = 5$) shows a significant difference (P -value = 0.03).

Many authors consider IgD multiple myeloma as a variant of multiple myeloma, but with more aggressive clinical subjects, younger age at diagnosis, higher frequency of extramedullary manifestations, association with AL amyloidosis and renal failure and a poor prognosis. However, according to Fahey et al. [5], the clinical and median survival of IgD MM is not very different from MM of other isotypes.

The median age at diagnosis is 60 years and average age is 59, this result was similar in different studies [3,6,16]. These average ages are lower than those found in patients with IgG multiple myeloma (62 years) and IgA (64 years) but are comparable to light chain multiple myeloma (56 years) [Mayo Clinic series].

The clinical and biological characteristics of our patients are similar to other studies (Tables 1 and 2). However, it seems that IgD MM is more aggressive than MM with other isotypes when compared to Mayo Clinic series.

The majority of patients complained of fatigue, weight loss and pallor. But the most encountered symptom is bone pain (75%) approaching the results of many authors [16,17].

In 1970, the first case of IgD myeloma associated with AL amyloidosis was reported [18]. In our series, one patient had a cardiac AL amyloidosis (6%). According to Gertz et al. [7], 1.3% of patients with AL amyloidosis had IgD multiple myeloma, most of them were cardiac type (45%). Concerning the Mayo Clinic series, while AL amyloidosis is found in 5% of IgG myeloma, 2% of IgA myeloma and 13% of myeloma with light chains, there are found in 19% of IgD myeloma [3]. The low proportion found in our series could be explained by the difficulties encountered for the diagnosis of AL amyloidosis in our country.

Eighty-two percent of patients were suffering from renal failure with high blood urea and creatinine in 69% of cases. Our results are superior to those of Bladé et al. [3] and Ashutosh et al. [17]. It's probably due to the delay in diagnosis.

In all, 17.6% of myelomas have a high rate of plasma cells circulating in the blood. Shimamoto et al. [19] found plasma cells circulating in the blood in 14% of cases, however, according to Pruzanski and Rother [20], only 2 patients among 50 patients (4%) had developed a plasma cell leukemia.

On serum protein electrophoresis, all our patients had monoclonal components and 4 out of them had two peaks (23%). The study of LeQuellec et al. [21] revealed that there were monoclonal peaks in 81% of cases. The predominance of lambda light chains that we found has been confirmed by several studies [3,6,19,22]. This could be explained by preferential rearrangements of genes of heavy chains δ with light chains λ .

The concentrations of monoclonal peaks (Median = 9.42) are lower than those found in IgG (median: 35 g/L) and IgA (median: 32 g/L) multiple myeloma. This is probably due to the small amount of physiological IgD.

The search of Bence Jones proteinuria (BJP) revealed 71% of positivity. According to the literature, BJP is almost constant in IgD MM [3,21]. In IgG multiple myeloma, the BJP is only present in 35% of cases and 20% in IgA MM [23] (Table 2).

Sixty seven percent of patients died in less than 1 year from the diagnosis with a median of 9 months (Poor prognosis). However, in most studies, the median duration of survival ranges is from 12 to 17 months [3]. Bladé et al. [3] and Kuliszewicz-Janus et al. [24] reported a better overall survival (21 months and 36 months, respectively). Thus, the survival of our patients is definitely shorter than patients with IgG (49 months), IgA (40 months) and light chain (35 months) MM [25]. This short duration is probably due to its later recognition because of the small M-spike. Establishing the diagnosis of IgD MM is also difficult because of the variety of non-specific initial symptoms. It is important

to know that neurological, renal, cardiac and rheumatologic symptoms can be observed in IgD MM patients because of the high frequency of amyloidosis and renal failure.

Complete remission was achieved in one case after autologous stem cells transplant (ASCT). In one study of 11 patients with MM after ASCT, 2 had a complete remission and 9 had a good response, with a mean overall survival of 5 years [26].

There is no statistically significant difference in survival between patients with λ and κ light chains ($P = 0.39$, median, 6 months vs. 17 months). Bladé et al. [3] confirmed our results ($P = .99$, median, 20 vs. 29). Also, there is a lack of correlation between the concentrations of $\beta 2$ microglobulin and the overall survival ($P = 0.078$).

Serum free light chains assay (sFLCs) were made in 14 patients. In our series, 93% of patients (13/14) had higher levels of the sFLC with a disturbed sFLCR. One patient had a normal rate of sFLCs with normal sFLCR ($= 0.979$) at diagnosis, which was maintained for 15 months. The median rate of sFLC κ was 19.05 mg/L and 296.75 mg/L for FLCs λ . These concentrations are much higher than those obtained by Kyrtonis et al. [27] on 94 MM (67% IgG, 18% IgA, 13% light chains and 2% IgD) were 3.57 mg/L for sFLCs κ and 45.09 mg/L for λ sFLCs. According to the study of Mead et al. [28], the sFLC concentrations in intact immunoglobulin MM patients producing monoclonal IgG and IgA were similar while patients with IgD MM had considerably higher FLC concentrations which is comparable to those seen in light chain MM [29].

Several studies have shown that baseline sFLC has a prognostic value for survival in patients with newly diagnosed active myeloma [13,27,30]. Kyrtonis et al. [27] and Snozek et al. [13] have indicated that sFLCR is independent from sAlbumin and s $\beta 2m$ when incorporated into the ISS. According to Snozek et al. [13] abnormal sFLCR (sFLCR > 32 or < 0.03) was introduced in the ISS as a prognostic factor of MM as well as low sAlbumin (< 35 g/L) and high s $\beta 2m$ (> 3.5 mg/L). Patients with 0, 1, 2 or 3 adverse risk factor had significantly different overall survival with median survival times of 51, 39, 30 and 22 months, respectively ($P < 0.001$). Our series have shown that baseline sFLCR < 0.03 or > 32 had inferior overall survival compared to those with an sFLCR between 0.03–32 with a median survival of 3 months versus 12 months respectively ($P = 0.034$). Snozek et al. [13] obtained a significant difference in overall survival between patients who had baseline sFLCR < 0.03 or > 32 and those whose baseline sFLCR was between 0.03–32 (39 months versus 30 months). We noticed that none of our patients has an overall survival corresponding to the different stages of the current ISS with a lower survival for each of them.

Also, one patient with IgD MM associated with cardiac AL amyloidosis had a lower survival (6 months) even if he had only 1 risk factor. However, according to Gertz et al. [7], there is no significant difference between the survival of patients with IgD multiple myeloma and those with IgD multiple myeloma associated with AL amyloidosis.

5. Conclusion

In summary, IgD MM have a very aggressive course with poor prognosis compared to other subtypes of MM. The clinical features and prognosis of these patients differ from those that characterize patients with other immunoglobulin MM subtypes.

Despite the small number of patients with IgD MM in our series, we can conclude that sFLC assay is an essential test for clinicians dealing with active myeloma concerning the assessment of prognosis at the diagnosis.

However, IgD MM is a separate entity with specific features, this is why, it would be interesting if a multi-center study can assess

median survival times of this recent ISS for patients with IgD MM and IgD MM associated with AL amyloidosis.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References

- [1] Kyle RA, Rajkumar SV. Multiple myeloma. *N Engl J Med* 2004;351:1860–73.
- [2] Rajkumar SV, Kyle RA. Multiple myeloma: diagnosis and treatment. *Mayo Clin Proc* 2005;80:1371–82.
- [3] Bladé J, Lust JA, Kyle RA. Immunoglobulin D multiple myeloma presenting features response to therapy, and survival in a series of 53 cases. *J Clin Oncol* 1994;12:2398–404.
- [4] Fine JM. Deux cas de myelome avec immunoglobuline IgD a character monoclonal. *Rev Fr Etudes Clin Biol* 1969;14:1018–24.
- [5] Fahey JL, Carbone PP, Rowe DS, et al. Plasma cell myeloma with D-myeloma protein (IgD myeloma). *Am J Med* 1968;45:373–80.
- [6] Jancelewicz Z, Takatsuki K, Sugai S, Pruzanski W. IgD multiple myeloma. Review of 133 cases. *Arch Intern Med* 1975;135:87–93.
- [7] Gertz MA, Buadi FK, Hayman SR, Dingli D, Dispenzieri A, Greipp PR, et al. Immunoglobulin D amyloidosis: a distinct entity. *Blood* 2012;119(1):44–8.
- [8] Bradwell AR, Carr-Smith HD, Mead GP, Tang LX, et al. Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. *Clin Chem* 2001;47:673–80.
- [9] Bradwell AR, Carr-Smith HD, Mead GP, Drayton MT. Serum free light chain immunoassays and their clinical application. *Clin Appl Immunol Rev* 2002;3:17–33.
- [10] Dispenzieri A, Kyle R, Merlini G, Miguel JS, Ludwig H, Hajek R, et al. International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. *Leukemia* 2009;23(2):215–24.
- [11] Keren DF, Alexanian R, Goeken JA, Gorevic PD, Kyle RA, Tomar RH. Guidelines for clinical and laboratory evaluation patients with monoclonal gammopathies. *Arch Pathol Lab Med* 1999;123:106–7.
- [12] Katzmann JA, Clark RJ, Abraham RS, Bryant S, Lymp JF, Bradwell AR, et al. Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains. *Clin Chem* 2002;48:1437–44.
- [13] Hutchison CA, et al. Serum free light chain measurement aids the diagnosis of myeloma in patients with severe renal failure. *BMC Nephrol* 2008;9:11.
- [14] Snozek CLH, Katzmann JA, Kyle RA, Dispenzieri A, et al. Prognostic value of the serum free light chain ratio in newly diagnosed myeloma: proposed incorporation into the international staging system. *Leukemia* 2008;22(10):1933–7. <http://dx.doi.org/10.1038/leu.2008.171>.
- [15] Durie BG, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features response to treatment, and survival. *Cancer* 1975;36:842–54.
- [16] Pisani F, Petrucci MT, Giannarelli D, Bongarzone V, et al. IgD multiple myeloma a descriptive report of 17 cases: survival and response to therapy. *J Exp Clin Cancer Res* 2012;31:17.
- [17] Wechalekar A, Amato D, Chen C, Stewart AK, Reece D. IgD multiple myeloma—a clinical profile and outcome with chemotherapy and autologous stem cell transplantation. *Ann Hematol* 2005;84(2):115–7.
- [18] Friman C, Tornroth T, Wegelius O. IgD myeloma associated with multiple extramedullary amyloid containing tumors and amyloid casts in the renal tubules. *Ann Clin Res* 1970;2(2):161–6.
- [19] Shimamoto Y, Anany Y, Yamaguchi M. A new risk grouping for IgD myeloma based on analysis of 165 Japanese patients. *Eur J Haematol* 1991;47:262–7.
- [20] Pruzanski W, Rother I. IgD plasma cell neoplasia: clinical manifestations and characteristic features. *Can Med Assoc J* 1970;102:1061–5.
- [21] LeQuellec A, Bataille R, Levy-Robinet M, Sany J, Cjurana AJ. Immunoglobulin D multiple myeloma. A retrospective study in the Languedoc region. *Presse Med* 1989;18:1110–3.
- [22] Fibbe WE, Jansen J. Prognostic factors in IgD myeloma: a study of 21 cases. *Scand J Haematol* 1984;33(5):471–5.
- [23] Alcalay M, Gombert J, Frocrain C, Bontoux D. Le myélome à IgD, à propos d'un cas. *Sem Hop* 1975;51:1227–34.
- [24] Kuliszkiwicz-Janus M, Zimny A, Sokolska V, Sasladek M, Kuliczowski K. Immunoglobulin D myeloma—Problems with diagnosing and staging (own experience and literature review). *Leuk Lymphoma* 2005;46(7):1029–37.
- [25] Greipp PR, San Miguel J, Durie BG, Crowley JJ, et al. International staging system for multiple myeloma. *J Clin Oncol* 2005;23:3412–20.
- [26] Wechalekar A, Amato D, Chen C, Stewart AK, Reece D. IgD multiple myeloma—a clinical profile and outcome with chemotherapy and autologous stem cell transplantation. *Ann Hematol* 2005;84:115–7.

- [27] Kyrtonis M-C, Vassilakopoulos TP, Kafasi N, Sachanas S, et al. Prognostic value of serum free light chain ratio at diagnosis in multiple myeloma. *Br J Haematol* 2007;137:240–3 [Blackwell Publishing Ltd].
- [28] Mead GP, Carr-Smith HD, Drayson MT, Morgan GJ, et al. Serum free light chains for monitoring multiple myeloma. *Br J Haematol* 2004;126:348–54 [Blackwell Publishing Ltd].
- [29] Bradwell AR, Carr-Smith HD, Mead GP, et al. Serum test for assessment of patients with Bence Jones myeloma. *Lancet* 2003;361:489–91.
- [30] van Rhee F, Bolejack V, Hollmig K, Pineda-Roman M, et al. High serum-free light chain levels and their rapid reduction in response to therapy define an aggressive multiple myeloma subtype with poor prognosis. *Blood* 2007;110:827–32.