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# Facteurs pronostiques et prédictifs dans le cancer du sein infiltrant

Séverine Lahaye Guiu Lahaye

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**UNIVERSITE DE BOURGOGNE – FRANCHE-COMTE**

**U.F.R DE MEDECINE**

**THESE**

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**Docteur de l'Université de Bourgogne – Franche-Comté**

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**Présentée et soutenue publiquement par**

**Séverine GUIU née LAHAYE**

**Le 16 Décembre 2015 à Dijon**

**FACTEURS PRONOSTIQUES ET PREDICTIFS DANS LE  
CANCER DU SEIN INFILTRANT**

**Directeur de thèse : Pr Franck BONNETAIN**

**JURY**

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Et à toute ma famille, ma belle-famille et mes amis pour leur soutien et leur amitié

## RESUME

Le traitement systémique adjuvant du cancer du sein infiltrant repose sur la chimiothérapie et l'hormonothérapie. Certains facteurs sont connus pour être pronostiques (âge, taille tumorale, statut ganglionnaire, grade tumoral, embolies vasculaires, statut des récepteurs hormonaux (RH) et de HER2) ou prédictifs de réponse aux traitements (RH et HER2) et influent sur nos décisions thérapeutiques. Cependant, certaines patientes récidivent malgré un traitement complet alors que d'autres vont recevoir un traitement qui aurait pu être évité de par leur bon pronostic « intrinsèque ». Nous avons cherché à identifier dans ce travail d'autres facteurs pronostiques et / ou prédictifs dans le cancer du sein infiltrant en situation néoadjuvante / adjuvante. Premièrement, nous montrons que le type histologique lobulaire, réputé pour être une histologie de cancer du sein de bon pronostic et peu chimiosensible, ne doit pas être un facteur décisionnel quant aux traitements systémiques. En situation adjuvante et concernant la chimiothérapie, la validité et l'utilité cliniques des tests génomiques nécessitent d'être évaluées spécifiquement dans ce sous-groupe. Ensuite, nous avons étudié la validité analytique, la validité clinique et l'utilité clinique de 2 classifications moléculaires des cancers du sein selon PAM50 et l'analyse immunohistochimique de biomarqueurs : récepteur œstrogène, HER2 et Ki67 avec un cut-off à 14%. Selon nos conclusions, il n'y a actuellement pas de données suffisamment robustes pour que ces 2 classifications modifient les décisions de traitement systémique. Nous avons mis en évidence un sous-groupe de tumeurs triples négatives exprimant le récepteur androgène et FOXA1 et se comportant comme des tumeurs lumineales. Enfin, nous avons montré sur une large série néoadjuvante que la réponse histologique complète est un critère substitutif de survie pour les tumeurs RH négatifs.

**MOTS-CLES :** cancer sein, lobulaire, classification moléculaire, PAM50, Ki-67, récepteur androgène, FOXA1, réponse histologique complète

## ABSTRACT

The adjuvant systemic treatment of invasive breast cancer is based on chemotherapy and endocrine therapy. Several prognostic factors (age, tumoral size, nodal status, tumoral grade, vascular embols, hormonal receptors (HR), HER2) and predictive factors of response to treatment (HR and HER2) are described and have an impact on our therapeutic decisions. However, recurrences are frequent after a complete treatment and patients could avoid such treatment because of the good “intrinsic” prognosis. In this work, we aimed to identify other prognostic and / or predictive factors for the invasive breast cancer in the neoadjuvant / adjuvant settings. Firstly, we showed that the lobular histology, considered as histology of good prognosis and low chemo sensitive, should not be a decisive factor regarding systemic therapy. In the adjuvant setting, regarding chemotherapy, clinical validity and utility of the genomic tests need to be specifically evaluated in this subgroup. Then, we studied analytical validity, clinical validity and clinical utility of 2 molecular classification of breast cancer: PAM50 and a panel of 3 biomarkers in immunohistochemistry (estrogen receptor, HER2 and Ki-67 with a cut-off of 14%). We concluded that the data were not strong enough and that the therapeutic decisions should not be influenced by these classifications. We identified a subgroup of triple negative breast cancer that express androgen receptor and FOXA1 and which behave like luminal tumors. At last, we showed in a large neoadjuvant population, that the pathological complete response is a surrogate marker of survival in RH negative tumors.

**KEY WORDS:** breast cancer, lobular, molecular classification, PAM50, Ki-67, androgen receptor, FOXA1, pathological complete response

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## ABREVIATIONS

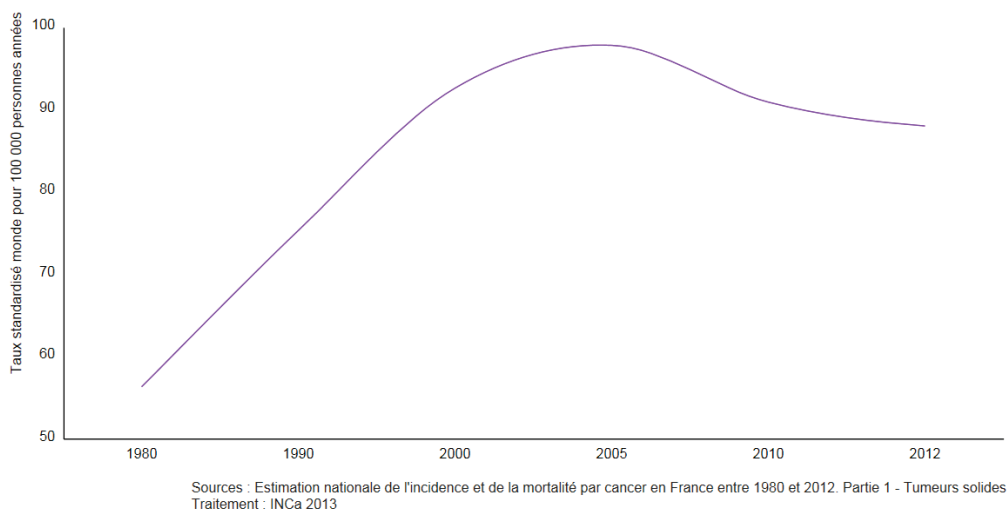
**ASCO** : American Society of Clinical Oncology  
**AJCC** : American Joint Committee on Cancer  
**BCI** : Breast Cancer Index  
**BRCA** : BReast Cancer  
**CAK** : carcinome adénoïde kystique  
**CAP** : Collège des Pathologistes Américains  
**CCI** : carcinome canalaire infiltrant  
**CCIS** : carcinome canalaire *in situ*  
**CGH** : hybridation génomique comparative  
**CK** : cytokératines  
**CLI** : carcinome lobulaire infiltrant  
**CLIS** : carcinome lobulaire *in situ*  
**CMF** : cyclophosphamide, méthotrexate, 5-fluorouracile  
**CTI** : cellules tumorales isolées  
**EGAPP** : Evaluation of Genomic Applications in Practice and Prevention  
**EGFR** : epidermal growth factor receptor  
**FOXA1** : forkhead box protein 1  
**GEP** : profil d'expression génomique  
**GS** : ganglion sentinelle  
**HER2** : human epidermal growth factor receptor 2  
**HIS** : hybridation *in situ*  
**IA** : inhibiteur d'aromatase  
**IHC** : immunohistochimie  
**IM** : index mitotique  
**IMC** : indice de masse corporelle  
**LAR** : luminal androgen receptor  
**LcT** : lymphocytes T  
**LVI** : embolies lymphovasculaires  
**MAP** : mitogen activated protein  
**MEC** : matrice extracellulaire  
**NST** : carcinome de type non spécifique  
**PAI-1** : inhibiteur 1 de l'activateur du plasminogène  
**PAM50** : prediction analysis of microarray  
**pCR** : réponse histologique complète  
**PD1** : programmed cell death protéine 1  
**PD-L1** : ligand de PD1  
**PI3K** : phosphoinositide 3-kinase  
**qPCR** : quantitative polymerase chain reaction  
**qRT-PCR** : quantitative reverse transcriptase – polymerase chain reaction  
**RA** : récepteur androgène  
**RE** : récepteur œstrogène  
**RH** : récepteurs hormonaux  
**RP** : récepteur progestérone

**RR** : risque relatif  
**RS** : recurrence score  
**SBR** : Scarff-Bloom-Richardson  
**SG** : survie globale  
**SSM** : survie sans maladie  
**SSR** : survie sans récurrence  
**SUV** : standard uptake value  
**TAC** : docetaxel, adriamycine, cyclophosphamide  
**TEP** : tomographie par émission de positons  
**THS** : traitement hormonal substitutif  
**TIL** : tumor infiltrating lymphocytes  
**TN** : triple négative  
**UICC** : International Union against Cancer  
**uPA** : urokinase activatrice du plasminogène  
**uPAR** : récepteur de l'urokinase activatrice du plasminogène  
**VEGF** : vascular endothelial growth factor  
**VEGFR** : vascular endothelial growth factor receptor

# GENERALITES

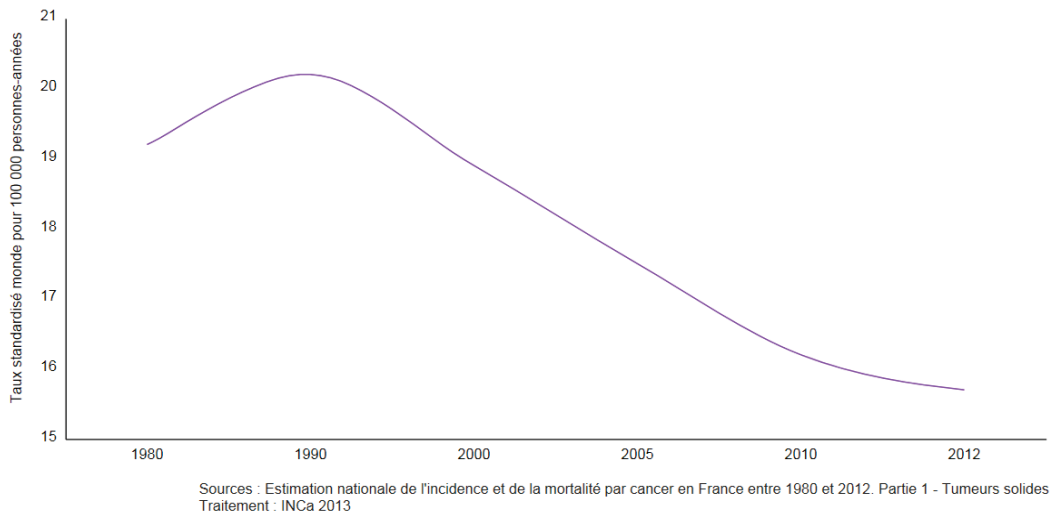
## I – EPIDEMIOLOGIE DU CANCER DU SEIN

Le cancer du sein est une pathologie extrêmement fréquente. En 2012, en France, plus de 48 700 nouveaux patients ont eu un diagnostic de cancer du sein (31,5% des cancers) et plus de 11 800 patients sont décédés d'un cancer du sein (18,8% des décès par cancer) [1]. Si l'incidence a augmenté entre 1980 et 2005 (taux d'incidence standardisé à la population mondiale : 56,3 pour 100 000 femmes en 1980 et 97,8 pour 100 000 femmes en 2005), elle tend à diminuer depuis (88,0 cas pour 100 000 femmes en 2012) (**Figure 1**). Ceci pourrait être expliqué par la diminution de la prescription de traitements hormonaux substitutifs (THS) chez les femmes ménopausées et une stabilisation des facteurs de risque.



**Figure 1 : Évolution de l'incidence (taux standardisé monde estimé) du cancer du sein de 1980 à 2012 [1]**

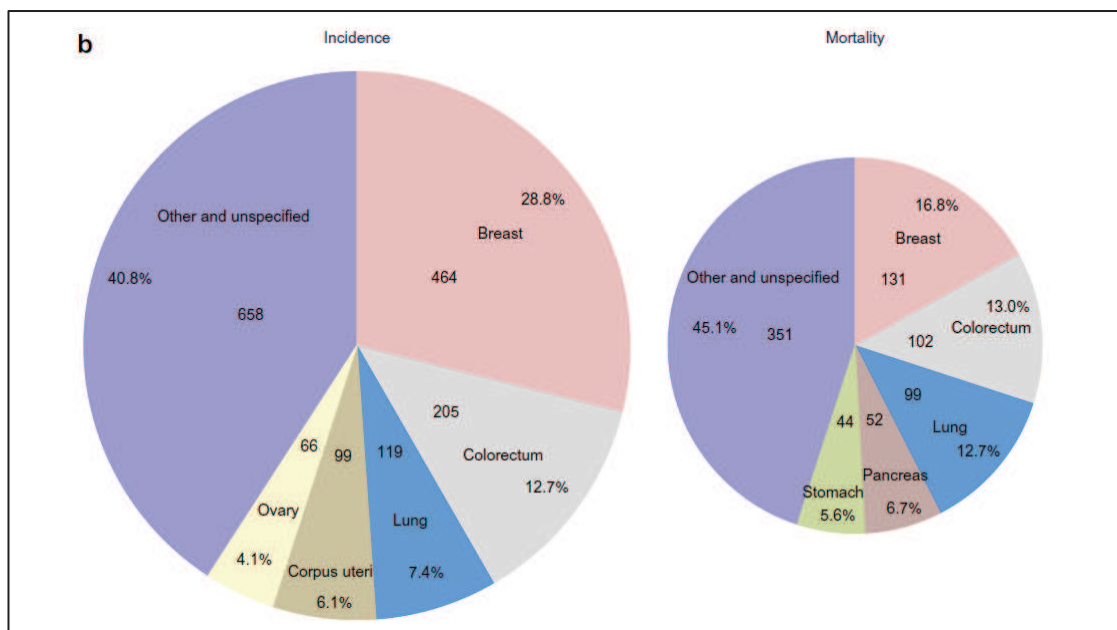
La mortalité liée au cancer du sein diminue depuis la fin des années 1990 (taux de mortalité standardisé monde en 2012 : 15,7 pour 100 000 femmes versus 19,4 pour 100 000 femmes en 1980) (**Figure 2**). Cette diminution est expliquée d'une part, par l'amélioration de la prise en charge thérapeutique, et d'autre part, par un diagnostic plus précoce lié au développement du dépistage en France.



**Figure 2 : Évolution de la mortalité (taux standardisé monde) par cancer du sein entre 1980 et 2012 [1]**

Malgré cela, le cancer du sein se situe en France au premier rang des cancers incidents chez la femme, loin devant le cancer colorectal et le cancer du poumon, en faisant un des enjeux majeurs en termes de santé publique.

Dans l'Union Européenne, le cancer du sein reste le cancer le plus fréquent avec 364 450 nouveaux cas en 2012 et la 3<sup>ème</sup> cause de décès par cancer (131 200) [2] (**Figures 3 & 4**).



**Figure 3 : Incidence et mortalité du cancer chez la femme en Europe en 2012 [2]**

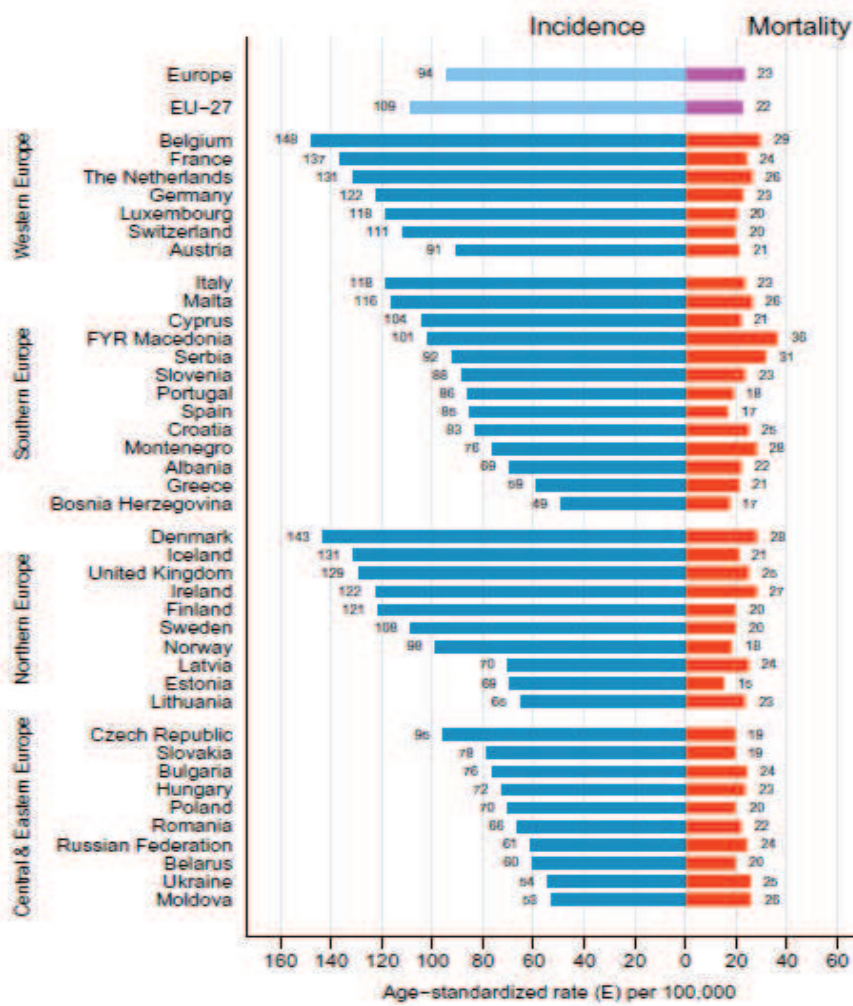
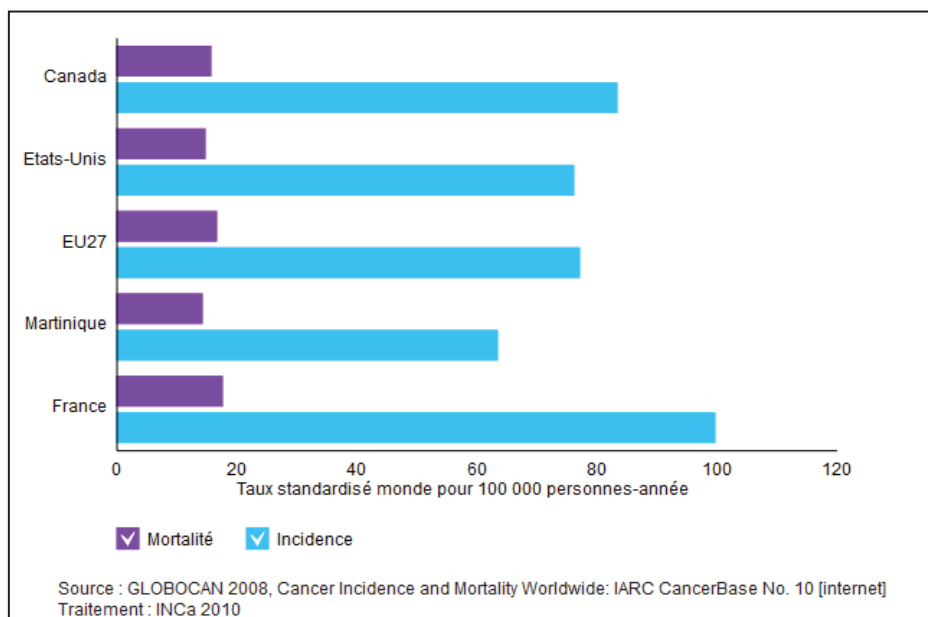


Figure 4 : Taux d'incidence et de mortalité standardisé sur l'âge du cancer du sein en Europe en 2012 [2]

Il s'agit également d'une pathologie très fréquente dans le reste du monde [3] (Figure 5).



**Figure 5 : Comparaison de la mortalité et de l'incidence estimées (taux standardisés monde) du cancer du sein entre le Canada, les Etats-Unis, l'Union Européenne, la Martinique, la France métropolitaine en 2008 [3]**

## II – FACTEURS DE RISQUE DU CANCER DU SEIN

Le cancer du sein est probablement d'origine multifactoriel (facteurs hormonaux, environnement, alimentation...), en dehors d'une origine génétique ne représentant que 5% de l'ensemble des cas. Le cancer du sein touche beaucoup plus les pays développés avec un mode de vie occidental (alimentation riche en graisses animales et protéines associée à un manque d'activité physique).

**\*L'âge :** l'incidence est en augmentation après 30 ans avec un pic entre 50 et 74 ans.

**\*Le sexe :** c'est une pathologie féminine dans 99% des cas.

### **\*Les facteurs hormonaux endogènes et les facteurs liés à la reproduction**

Les facteurs hormonaux associés à une augmentation du risque de cancer du sein sont une ménarche précoce (avant 12 ans), la nulliparité, une 1<sup>ère</sup> grossesse tardive (après 30 ans), l'absence d'allaitement, une ménopause tardive [4, 5]. Des taux sanguins élevés d'estradiol, de testostérone et d'androgène sont associés à un risque augmenté de cancer

du sein chez les femmes ménopausées, indépendamment des autres facteurs de risque [6]. Ceci est notamment observé pour les tumeurs récepteurs œstrogènes (RE) positifs [7]. Des taux élevés d'œstradiol et de progestérone sont également associés à un accroissement du risque chez les femmes non ménopausées [8].

#### **\*Les facteurs hormonaux exogènes**

La contraception orale par œstrogène et progestatif a longtemps été considérée comme à risque. Même si les données initiales suggéraient une augmentation modérée du risque relatif (RR) de cancer du sein chez les femmes utilisant une telle contraception en comparaison aux femmes naïves de contraception (RR = 1,24 en cours de traitement puis RR = 1,16 et RR = 1,07, respectivement 1-4 ans et 5-9 ans après l'arrêt) [9], une étude plus récente menée aux Etats-Unis n'a retrouvé aucun lien entre cancer du sein et contraception orale par œstro-progestatifs [10].

Un lien potentiel entre la prise d'un THS de la ménopause et la survenue d'un cancer du sein a été largement étudié. Pendant longtemps ce traitement a reposé uniquement sur des œstrogènes, puis par la suite, sur une association œstro-progestative, limitant ainsi le risque d'hyperplasie et de cancer de l'endomètre (liés à l'hyperœstrogénie). Il y a eu de nombreuses enquêtes d'observation, sources de nombreux biais : i) d'auto sélection : les patientes traitées par THS ont des caractéristiques différentes des autres et se préoccupent plus de leur santé, ii) de contre-indication : non prescription d'un THS en présence d'antécédents familiaux de cancer du sein, iii) de dépistage : réalisation plus fréquente de mammographies chez les femmes traitées. La 1<sup>ère</sup> méta-analyse publiée dans le Lancet en 1997 incluait 15 enquêtes de cohorte et 36 études cas-témoins [11]. Il s'agissait de femmes traitées en Europe et en Amérique du Nord, principalement par œstrogènes seuls. Le risque de cancer du sein invasif était plus élevé chez les utilisatrices d'un THS que les non-utilisatrices (RR = 1,14). Le risque augmentait avec la durée du THS, diminuait à l'arrêt de celui-ci et disparaissait presque complètement 5 années après son arrêt [11]. Une large enquête d'observation réalisée en Angleterre a évalué la survenue d'un cancer du sein chez 1 million de femmes dont la moitié recevait un THS [12]. En cours de traitement il existait un RR de 1,66 (IC95% 1,58 - 1,75), risque majoré en cas d'association avec les progestatifs (RR = 1,30 si œstrogènes seuls et RR = 2,00 si association), chez les femmes ayant un faible indice de masse corporelle (IMC) et si le traitement était débuté dans les 5 ans après la ménopause



[12, 13]. La 1<sup>ère</sup> étude randomisée était celle de Rossouw *et al.* L'essai WHI (Women's Health Initiative) a inclus aux Etats-Unis, de 1993 à 1998, 16 600 femmes de 50 à 79 ans, non hystérectomisées [14]. Dans cette étude réalisée en double aveugle, les femmes étaient randomisées entre placebo et THS à base d'œstrogènes d'origine équine et d'acétate de médroxyprogestérone (hormones différentes de celles utilisées en Europe). Elle a été stoppée prématurément car il a été constaté une augmentation significative du risque de cancer du sein (RR = 1,26, IC95% 1,00 - 1,59) mais également des pathologies coronariennes (29%), des accidents vasculaires cérébraux (41%) et un risque multiplié par deux de thrombose veineuse profonde avec le THS [14]. Le risque augmentait avec la durée du traitement. Actuellement il est recommandé de traiter uniquement les femmes ayant des symptômes liés à la carence ostrogénique, en particulier des troubles vasomoteurs, à dose minimale efficace et sur une période de temps restreinte. Un traitement par œstrogènes seuls ne peut être administré qu'aux femmes hystérectomisées. Le rapport bénéfice/risque d'un tel traitement doit être évalué régulièrement. Le cancer du sein est, bien entendu, une contre-indication absolue à la prescription d'un THS.

#### **\*Les facteurs environnementaux**

L'exposition à des radiations ionisantes pendant l'enfance ou l'adolescence [15], les facteurs alimentaires (graisses animales), la consommation d'alcool [16], le tabagisme, l'obésité (et notamment une prise de poids importante après la ménopause) [17] sont des facteurs de risque. La pratique d'une activité physique régulière diminue le risque de survenue d'un cancer du sein.

#### **\*Antécédents de lésions épithéliales prolifératives**

Le risque de développer un cancer du sein est plus élevé en présence de lésions d'hyperplasie canalaire simple (RR = 1,5), d'hyperplasie canalaire atypique (RR = 3 à 5) et de carcinome canalaire *in situ* (CCIS) (RR = 8 à 10) [18] ainsi qu'en cas de néoplasie lobulaire (hyperplasie lobulaire atypique, carcinome lobulaire *in situ* (CLIS)).

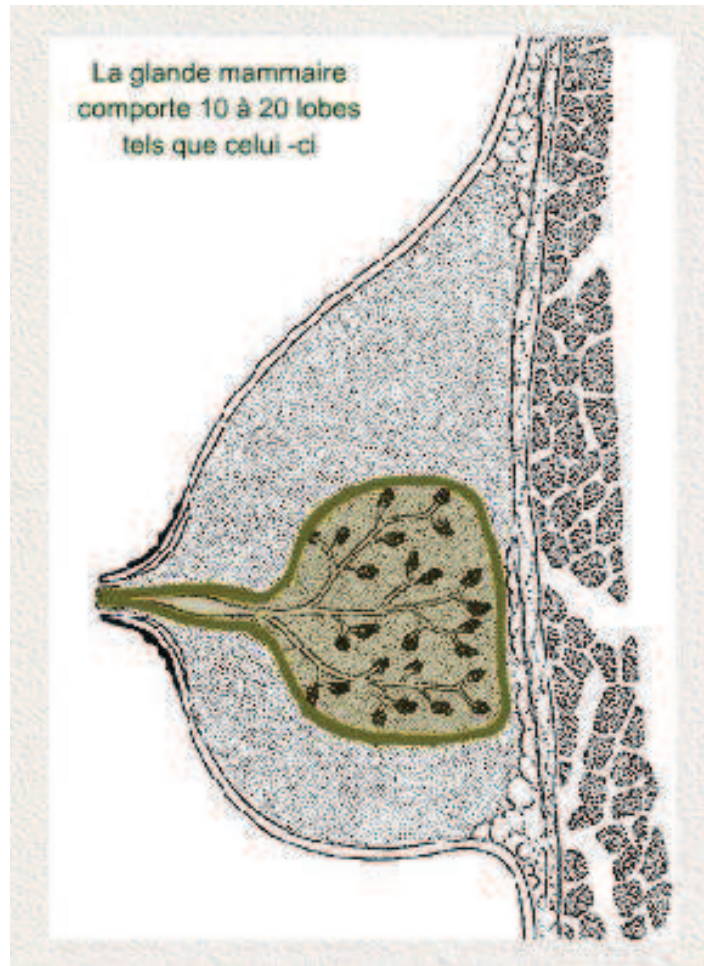
### **\*Histoire familiale et mutations génétiques**

Le RR de développer un cancer du sein pour toute forme de parenté est de 1,9 et l'excès de risque est plus marqué chez les femmes plus jeunes et quand la maladie touche un parent proche (mère, fille ou sœur), avant l'âge de 50 ans [19].

Par ailleurs certaines mutations génétiques sont susceptibles d'augmenter le risque de cancer du sein. Deux gènes *BRCA1* (*BR*east *C*ancer *1*) et *BRCA2* sont particulièrement impliqués. Ils sont à transmission autosomique dominante et sont impliqués dans la réparation des cassures double brin de l'ADN. Le risque cumulé de développer un cancer du sein est de 47 à 66% en cas de mutation *BRCA1*, et de 40 à 57% en cas de mutation *BRCA2* [20]. Le risque de cancer de l'ovaire à 70 ans est de 39% et de 11%, respectivement en cas de mutation *BRCA1* et *BRCA2* [21]. La mutation d'autres gènes peut également exposer à un risque accru de développer un cancer du sein ainsi que d'autres tumeurs (*P53* et syndrome de Li-Fraumeni, *PTEN* et syndrome de Cowden, *STK11* et syndrome de Peutz-Jeghers, *ATM* dans l'ataxie-télangiectasie et, plus récemment, a été découvert le gène *PALB2*).

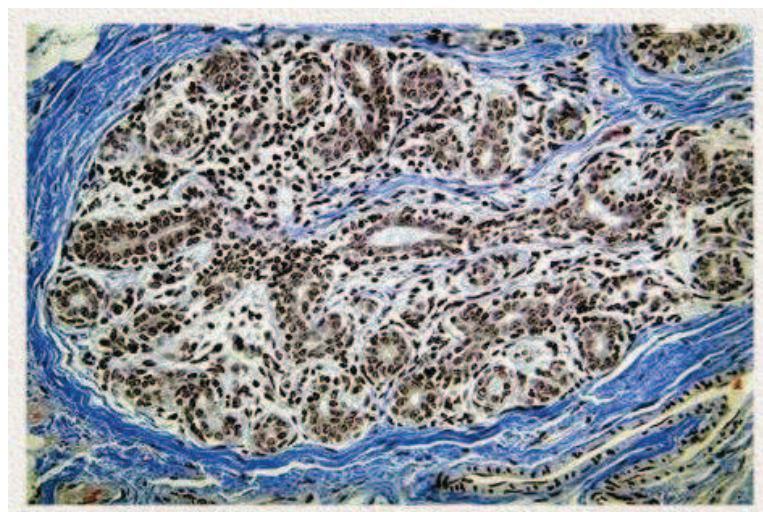
## **III – HISTOLOGIE DU TISSU MAMMAIRE**

Chacune des deux glandes mammaires est une glande exocrine, tubulo-alvéolaire, sécrétant le lait. On y trouve un système ramifié de canaux excréteurs terminés en cul-de-sac par des portions sécrétrices tubulo-alvéolaires. Chaque glande mammaire est constituée de 10 à 20 lobes drainés par des canaux galactophores collecteurs « lactifères » s'ouvrant individuellement à la peau au niveau du mamelon (**Figure 6**).



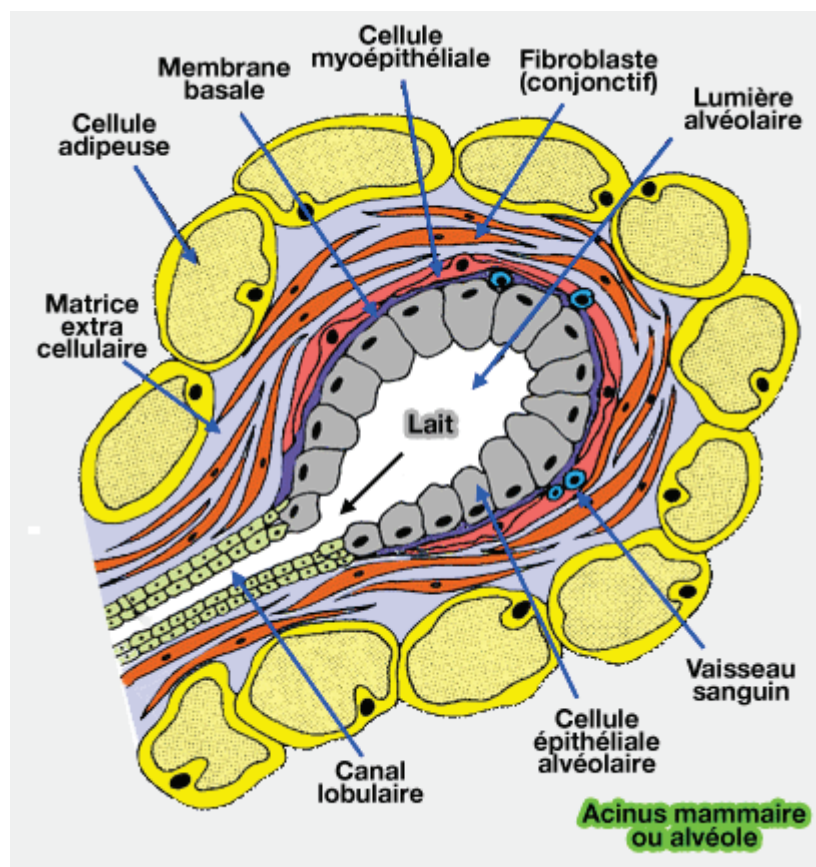
**Figure 6 : Organisation histologique du sein**

Chaque lobe est lui-même constitué de lobules, chaque lobule étant constitué de tubulo-alvéoles correspondant à la partie sécrétrice de la glande (**Figure 7**).



**Figure 7 : Lobule mammaire**

Les lobes sont séparés par un tissu conjonctif dense et entourés par un tissu adipeux abondant, de même les lobules sont entourés par un tissu conjonctif dense alors que le tissu conjonctif intralobulaire est lâche. Les canaux excréteurs (ou canaux galactophores) sont d'abord intralobulaires, puis interlobulaires (épithélium cubique avec présence de cellules myoépithéliales) et enfin interlobaires, à épithélium pavimenteux stratifié. Les tubulo-alvéoles de la glande mammaire au repos sont constituées de cellules disposées sur deux couches : sécrétrices, au contact de la lumière, et myoépithéliales, au pourtour (*Figure 8*).



**Figure 8 : Tubulo-alvéole mammaire**

Les cellules luminales qui bordent la lumière expriment les cytokératines CK8/18 et CK19. Les cellules myoépithéliales (ou basales), entourent les cellules luminales, et sont au contact de la membrane basale. Elles expriment les CK5/6, CK14, CK17 et des marqueurs musculaires lisses comme l'actine musculaire lisse et p63.

## IV – FACTEURS PRONOSTIQUES ET PREDICTIFS DU CANCER DU SEIN

### 1°) Définitions générales

\*Un **facteur pronostique** va permettre, en l'absence de traitement, de prédire l'évolution de la maladie en termes de risque de rechute et de décès (survie).

\*Un **facteur prédictif** va permettre de prédire l'évolution de la maladie sous traitement et d'orienter une décision thérapeutique (réponse, toxicité).

### 2°) Facteurs pronostiques « classiques » dans le cancer du sein

Plusieurs critères clinico-pathologiques sont utilisés pour déterminer si un cancer du sein est à priori de bon ou de mauvais pronostic, et déterminer si un traitement complémentaire systémique doit être administré (chimiothérapie, hormonothérapie), afin de diminuer le risque de récurrence et de décès.

- **Age**

Un âge  $\leq 40$  ans est associé à des taux de rechute et de mortalité plus élevés que ceux rapportés en population générale [22, 23]. En effet, les cancers du sein survenant chez des femmes très jeunes correspondent le plus souvent à des formes avancées au diagnostic et agressives [24].

- **Type histologique**

Le type histologique des carcinomes infiltrants a une valeur pronostique reconnue. Il est basé sur la morphologie au microscope optique de la tumeur. La classification OMS 2012 distingue une trentaine de types et sous-types histologiques.

#### 1. **Carcinome canalaire infiltrant (CCI) ou de type non spécifique (NST) (60 - 75%)**

Le CCI naît à la jonction canal - lobule dans la glande mammaire et ne présente aucune caractéristique de types histologiques définis [25]. Il s'agit donc d'une définition et

d'un diagnostic par exclusion, et d'un groupe hétérogène de tumeurs. Il peut être bilatéral dans 6 à 8% des cas, est associé à des embolies vasculaires dans 20 à 27% des cas, et une atteinte ganglionnaire axillaire dans 20 à 50% des cas. Il s'agit le plus souvent d'une masse spiculée (le plus souvent palpable ou découverte lors d'un dépistage mammographique). Il est fréquemment associé à des lésions de carcinome *in situ* (CIS) (70 - 80%). Le CCI peut être de grade Scarff Bloom et Richardson (SBR) I (29%), II (41%) ou III (30%) [26, 27].

## **2. Carcinome lobulaire infiltrant (CLI) (5 - 15%)**

Le CLI naît à la jonction terminale canal - lobule comme le CCI. Le CLI est composé de cellules non cohésives, se disposant isolément ou en files indiennes [25], E-cadhérine négative (80 - 100%). Il se présente souvent sous la forme d'une masse irrégulière, mal définie, de taille supérieure au CCI, car le diagnostic en imagerie est plus difficile, avec parfois seulement une asymétrie de densité à la mammographie entraînant un diagnostic plus tardif. L'IRM mammaire a donc un intérêt tout particulier dans ce type histologique. On observe plus fréquemment des cancers controlatéraux métachrones ou synchrones (RR = 1,5 à 1,8) et des tumeurs multicentriques (RR = 2 par rapport au CCI). Il existe souvent des lésions *in situ* associées : CLIS dans 60 à 90% des cas mais aussi néoplasie lobulaire ou CCIS. Des embolies vasculaires sont retrouvés dans 5 à 17% des cas, une atteinte ganglionnaire dans 40% des cas (parfois présence de cellules isolées dans les sinus, une immunohistochimie (IHC) avec un anticorps anti KL1 n'est pas recommandé systématiquement, mais peut être utile en cas de doute avec des histiocytes). Différents variants sont décrits : solide, alvéolaire, pléomorphique et tubulo-lobulaire [28]. Les CLI sont très fréquemment récepteurs hormonaux (RH) positifs, moins fréquemment HER2+, de grade SBR I ou II surtout (88%). Etant donné l'absence de tubes, le grade SBR est rarement de I (12%). Les sites métastatiques préférentiels sont différents des CCI : tube digestif, ovaires, utérus, méninges, peau, péritoine, plèvre, os. Il existe une augmentation de l'incidence, probablement liée à la prise de THS de la ménopause (RR sous THS = 2,7 pour le CLI et 1,5 pour le CCI).

## **3. Carcinomes mixtes canaux et lobulaires (2 - 5%)**

Au moins 10% de la surface tumorale est de type canalaire.

#### **4. Carcinome médullaire (< 1%)**

Cinq critères histologiques doivent être présents afin de porter le diagnostic de carcinome médullaire : la tumeur est limitée, le stroma est riche en lymphocytes ou « lymphoïde », l'architecture est de type syncytial (> 75%), il n'existe pas de glandes ni de lésions de CIS. Ces tumeurs sont fréquemment triple négative (TN) (RE-, RP-, HER2-) [28]. Elles sont de meilleur pronostic que les CCI de phénotype TN, à grade équivalent [29].

#### **5. Autres (15%)**

##### **a. Carcinomes infiltrants de bon pronostic (90% de survie à 10 ans, critères morphologiques stricts)**

##### **i. Carcinome tubuleux (2%)**

Il s'agit d'un carcinome très bien différencié caractérisé par des tubes et une stroma réaction fibreuse. Plus de 90% de la surface tumorale est composée de tubes avec une seule couche de cellules épithéliales, sans cellule myoépithéliale autour, ovoïdes, à contours anguleux et lumières ouvertes [25, 30]. Les noyaux sont de bas grade (SBR I), polarisés autour de la lumière, avec un pôle apical sécrétoire dans 30% des cas. Il est fréquemment associé à des lésions de CIS de bas grade nucléaire (> 50% des cas) ou de métaplasie cylindrique atypique. Il survient chez des patientes plus âgées (âge médian : 60 - 70 ans), et se traduit fréquemment sur la mammographie par une opacité à longs spicules. Sa taille moyenne est de 1 cm [30]. Son incidence est en augmentation, notamment grâce aux progrès du dépistage. Il s'agit de tumeurs hormonodépendantes : RE+ (98 - 100%), récepteur progestérone (RP) + (60 -70%) et HER2 négatives [30]. L'atteinte ganglionnaire est moins fréquente (0 - 18%) que pour les CCI et dépend de la taille (> 1cm) [31, 32]. Le taux de récurrence est faible et la récurrence surtout locale ; le pronostic est excellent même pour les tumeurs N+ (métastases à 10 ans : 0 à 1% pour le carcinome tubuleux et 6 à 10% pour le CCI de grade I ; SG à 10 ans : 94 à 99% pour le carcinome tubuleux et 88% pour le CCI de grade I [30, 33, 34]. Les principaux diagnostics différentiels sont la cicatrice radiaire, l'adénose sclérosante, le CCI bien différencié et le carcinome tubulo-lobulaire. Il existe une perte fréquente du chromosome 16q (78 - 86%), un gain du 1q (50 - 62%) et, à la différence du CCI,

une perte rare du 17p [35]. Selon leur profil d'expression génomique, ils sont classés parmi les tumeurs de type luminal A [36].

### ***ii. Carcinome mucineux (2%)***

Les carcinomes mucineux purs représentent environ 2% des carcinomes et 5% s'il existe une composante mucineuse associée à un CCI. Pour pouvoir porter le diagnostic de carcinome mucineux pur, la composante mucineuse doit représenter plus de 90% de la surface tumorale [37]. Ils surviennent chez des patients de plus de 55 ans [38] et, en mammographie, ils peuvent simuler une lésion bénigne. La taille moyenne est de 3 cm (< 1 à > 20 cm) [38] et l'aspect macroscopique correspond à une masse souple, gélatineuse, facilement reconnaissable. Les cellules tumorales forment des petits amas flottant au milieu de lacs de mucine, séparés par des septas fibreux contenant des vaisseaux sanguins. Les atypies nucléaires sont rares en cas de tumeur mucineuse pure, mais possibles [37] ; il existe souvent des lésions de CIS associées. Les carcinomes mucineux composés de larges amas cellulaires (hypercellulaires ou de type B) présentent fréquemment une différenciation neuroendocrine, comme en témoigne l'expression de chromogranine et de synaptophysine [39, 40] et sont de meilleur pronostic [41]. Le carcinome mucineux de type A, avec de grandes quantités de mucine extracellulaire, correspond au carcinome mucineux classique, non-endocrine. Les carcinomes mucineux sont fréquemment RH+ (RE+ : 92 - 100% , RP+ : 60%) [38], HER2 non amplifié [42]. L'atteinte ganglionnaire est présente dans 3 à 15% des cas en cas de tumeur mucineuse pure et, dans 33 à 46% des cas en cas de tumeurs mixtes. Le pronostic est favorable [38, 43, 44] avec des taux de survie à 10 ans compris entre 80% [45] et 100% [46]. En cas de composante canalaire infiltrante associée, le pronostic rejoint celui des formes canalaire. Selon leur profil d'expression génomique, ils sont classés parmi les tumeurs de type luminal A [36]. Ils représentent une entité moléculaire différente des carcinomes canalaire de type luminal A ; les carcinomes mucineux de type A sont distincts des carcinomes mucineux de type B (expression de gènes similaires aux carcinomes neuroendocrines) [47]. Ils présentent très peu d'instabilité génétique [48].

### ***iii. Carcinome adénoïde kystique (CAK) (< 0,1%)***

Histologiquement il s'agit d'une entité similaire aux tumeurs adénoïdes kystiques des glandes salivaires. Le CAK est formé par des cellules épithéliales et myoépithéliales



organisées selon une architecture tubulaire ou cribriforme. Les cellules basaloïdes sont positives pour la p63, l'actine musculaire lisse, la calponine [49-51] ainsi que les cytokératines 5/6 et 14. Les cellules luminales expriment les CK 7 et 8/18 [49]. Ce sont des tumeurs TN [49]. Il existe également une forte expression de KIT [49] sans mutation. Dans plus de 90% des cas, une translocation t(6 ;9) (MYB ; NFIB) est présente et on ne constate peu ou pas de gains ou pertes de chromosomes en hybridation génomique comparative (CGH) (environ 8% du génome remanié et pas de diminution d'expression de *BRCA1*) [52, 53]. L'âge moyen au diagnostic est de 64 ans [54, 55]. Il existe surtout un risque de récurrence locorégionale, un envahissement ganglionnaire est rare et le pronostic excellent (taux de survie à 5 et 10 ans de > 95% et 90%, respectivement) [54, 56].

#### ***iv. Carcinome sécrétant juvénile (< 0,15%)***

Il s'agit d'un carcinome de bas grade avec une architecture solide, microkystique et tubulaire, composé de cellules produisant des sécrétions intra et extracellulaires (PAS+). L'antigène épithélial de membrane et la protéine S100 sont fréquemment exprimés, les RH, HER2 et p63 sont négatifs tandis que l'E-cadhérine, les CK8, 18, le CD117 et l'alpha muscle lisse peuvent être exprimés [57, 58]. Il est associé à une translocation t(12 ;15) créant un gène de fusion *ETV6/NTRK3* [57]. Il est décrit dans les 2 sexes et l'âge médian au diagnostic est de 25 ans (3 - 87 ans) [59]. Il est de très bon pronostic notamment chez les enfants et les jeunes adultes (< 20 ans) [59]. Les métastases à distance sont rares [58].

#### ***v. Carcinome cribriforme infiltrant (< 1%)***

Il s'agit le plus souvent d'une masse spiculée sur la mammographie avec microcalcifications, qui peut être multifocale (20%). Les RE sont toujours positifs et les RP dans 69% des cas, l'HER2 est négatif [28]. Le taux de survie globale (SG) à 10 ans est compris entre 90 et 100% [28].

### **b. Carcinomes de mauvais pronostic**

#### ***i. Carcinome micropapillaire (2%)***

Il est caractérisé par une inversion de la polarité des cellules, une rétraction des massifs et un stroma fibrovasculaire lâche [28]. Ce sont fréquemment des tumeurs de grade

SBR III (30 - 87% des cas) avec une atteinte ganglionnaire (72 - 95%) et / ou des embolies vasculaires (60 - 90%) [60-62]. Elles expriment le RE dans 61 à 100% des cas, le RP dans 46 à 83% des cas et possiblement HER2 (10 - 35%) [60, 63]. L'étude du profil d'expression génomique révèle que ces tumeurs sont classées parmi les tumeurs lumineales A ou lumineales B [36]. Les VEGF-C et VEGFR3 sont fortement exprimés [64]. Ces tumeurs constituent une entité moléculaire distincte (amplification *MYC*, *CCDN1* et *FGFR1* dans 33%, 8% et 17% des cas respectivement) [63].

#### ii. ***Carcinome métaplasique (1%)***

Il peut s'agir de tumeurs monophasiques (carcinome épidermoïde métaplasique ou carcinome à cellules fusiformes) ou de tumeurs biphasiques (carcinosarcome) avec une composante glandulaire et un contingent mésenchymateux (cellules fusiformes, tissus hétérologues : os, cartilage...) [25]. Ils sont de grade SBR II ou III (66%) avec une activité mitotique élevée, moins fréquemment N+ que les CCI (5 - 25%), avec plus de métastases à distance au diagnostic (principalement poumons et système nerveux central) [65]. Ils sont TN dans plus de 90% des cas [66-69], exprimant l'EGFR dans 76% des cas (dont 37% EGFR amplifié) [70], et expriment les marqueurs épithéliaux (CK14, CK5/6, AE1/AE3) et p63 [28]. Ces tumeurs présentent de nombreuses altérations génomiques, avec de fréquentes mutations de *PI3K* (47%) [71], de P53 [72] et une inactivation fréquente de la voie BRCA1 (63% de méthylation de *BRCA1*) [73]. Ils sont de moins bon pronostic que les autres cancers du sein TN avec une SG à 5 ans de 28 à 68% [66, 67, 74, 75].

- **Taille tumorale**

La taille de la tumeur est corrélée au risque d'envahissement ganglionnaire et à la survie [76, 77]. Après exérèse chirurgicale, toutes les lésions sont mesurées macroscopiquement et / ou microscopiquement. En macroscopie, la tumeur est mesurée dans ses trois dimensions. Lors de l'analyse histologique, la composante infiltrante est remesurée. En cas de discordance entre les 2 mesures, la plus grande sera retenue. La classification histopathologique « pT » de l'AJCC (American Joint Committee on Cancer) / UICC (International Union against Cancer) est déterminée après examen anatomopathologique des pièces chirurgicales (***Annexe***).

- **Statut ganglionnaire**

L'évaluation du statut ganglionnaire axillaire est indispensable chez tout patient pris en charge pour un cancer du sein localisé. Elle peut se faire par prélèvement du ganglion sentinelle (GS) en cas de tumeur de moins de 5 cm ou par curage ganglionnaire axillaire (comprenant un nombre minimum de 10 ganglions) en cas de tumeur plus importante et / ou multicentrique, en cas de suspicion d'atteinte clinique ou sur un sein déjà opéré ou traité. Le statut ganglionnaire est un facteur pronostique indépendant important dans le cancer du sein [76]. Il existe une corrélation entre la diminution de la survie sans maladie (SSM) et de la SG et le nombre de ganglions axillaires envahis [77, 78]. La classification pTNM de l'AJCC distingue d'ailleurs 4 sous-groupes en fonction du nombre de ganglions axillaires envahis (0, 1-3, 4-9,  $\geq 10$ ) (**Annexe**) [79, 80].

Pour certains oncologues, le seul fait d'avoir une atteinte ganglionnaire axillaire justifie l'administration d'une chimiothérapie adjuvante, quelle que soit la biologie tumorale et quel que soit le nombre de ganglions atteints. Dans la dernière conférence de consensus de St Gallen de 2015, il n'est pas recommandé de délivrer une chimiothérapie adjuvante en cas de tumeur de type « luminale A », considérée comme peu chimiosensible, sauf en cas d'envahissement ganglionnaire important ( $\geq 4$  N+) [81].

Il faut distinguer les macrométastases ganglionnaires (taille  $> 2$ mm), des micrométastases (taille  $> 0,2$ mm et  $\leq 2$ mm) et des nano métastases ( $\leq 0,2$ mm, cellules tumorales isolées (CTI) ou amas cellulaires). La mise en évidence des micrométastases ou de CTI est possible grâce aux analyses immunohistochimiques sur coupes sériées réalisées sur le ou les GS. L'indication d'une chimiothérapie adjuvante sur le seul facteur correspondant à une micrométastase (pN1mi) reste controversée. Par contre il n'est habituellement pas retenu d'indication de chimiothérapie adjuvante sur le seul facteur d'atteinte des ganglions par des CTI. Chez plus de 3 880 patientes avec une tumeur du sein opérable, N0 clinique, randomisées dans l'étude NSABP B-32 (prélèvement du GS plus curage axillaire *versus* prélèvement du GS seul), pour lesquelles un bloc tumoral était disponible et chez qui aucune métastase ganglionnaire n'avait été détectée initialement, une recherche de métastase ganglionnaire occulte a été réalisée à posteriori (par études immunohistochimiques) [82]. Des métastases occultes ont été détectées chez 15,9% des patientes : 11,1% avec des CTI, 4,4% avec des micrométastases et 0,4% avec des macrométastases. Les taux de survie estimés à 5 ans chez les patientes avec métastases

occultes étaient de 94,6% pour la SG, 86,4% pour la SSM et 89,7% pour la SSM à distance et de 95,8%, 89,2% et 92,5% respectivement pour les patientes sans métastases occultes [82], différences statistiquement significatives. Les taux estimés de survie à 5 ans parmi les patientes sans métastase occulte, avec CTI et avec micro ou macrométastase étaient respectivement de 98,4%, 97,8% et 96% [82].

Bien que l'absence d'atteinte ganglionnaire soit un facteur de bon pronostic, 10 à 30% des patients vont quand même développer des métastases à distance. Cela peut être dû à l'atteinte d'autres groupes ganglionnaires, comme la chaîne mammaire interne, non systématiquement prélevée par voie chirurgicale, ou du fait de la dissémination tumorale par voie hématogène, comme cela est fréquemment observé dans les tumeurs dites TN [83].

- **Grade histopronostique de Scarff Bloom et Richardson (SBR) modifié par Elston & Ellis**

Le grade SBR comprend 3 grades (I, II, III) et est obtenu par l'addition de 3 critères : architecture, atypies cytonucléaires et nombre de mitoses. Les 3 critères sont côtés en 1, 2 et 3.

- **Architecture** (appréciée sur l'ensemble de la tumeur)

1. La tumeur comprend majoritairement des tubes (> 75%)
2. Tumeur partiellement tubulaire (10 - 75%)
3. Aucun tube ou très peu (< 10%)

- **Atypies cytonucléaires** (partie de la tumeur comprenant le plus de pléomorphisme nucléaire)

1. Noyaux petits, réguliers et monomorphes
2. Atypies modérées
3. Noyaux pléomorphes avec atypies marquées

- **Nombre de mitoses** (contingent le plus prolifératif)

Le nombre de mitoses est compté sur 10 champs consécutifs, au grossissement X400.

1.  $\leq 10$  mitoses
2.  $11 \leq$  nombre de mitoses  $\leq 20$

### 3. $\geq 21$ mitoses

L'addition des 3 critères permet de déterminer le grade SBR :

**Grade I** : 3, 4 ou 5 (tumeur bien différenciée)

**Grade II** : 6 ou 7 (tumeur moyennement différenciée)

**Grade III** : 8 ou 9 (tumeur peu différenciée)

Plus le grade SBR est élevé, plus la tumeur est agressive et de moins bon pronostic.

- **Présence d'emboles tumoraux dans les vaisseaux lymphatiques et sanguins (LVI = lymphovascular invasion)**

Les emboles vasculaires sont définis par la présence de cellules tumorales dans les vaisseaux (sanguins et / ou lymphatiques) situés en périphérie du carcinome invasif (LVI péritumoraux) [28]. En 1999, la conférence de consensus du Collège des Pathologistes Américains (CAP) recommandait l'évaluation de la présence de LVI dans le tissu mammaire péritumoral, confirmant ainsi son rôle en tant que facteur pronostic. La présence de LVI a été intégrée dans les facteurs pronostiques lors de la conférence de St Gallen en 2005 chez les patientes N0 : en présence de LVI (15%), les patientes sont considérées comme à risque intermédiaire de récurrence, en l'absence de LVI, elles sont considérées comme étant à bas risque de récurrence [84]. La présence de LVI est donc un facteur pronostic indépendant, à la fois du risque de récurrence locale mais également à distance, et est prise en considération pour la décision du traitement adjuvant [85-87]. La présence, à la fois de LVI et d'un envahissement ganglionnaire, confère un plus mauvais pronostic qu'en présence d'un seul de ces 2 facteurs [88].

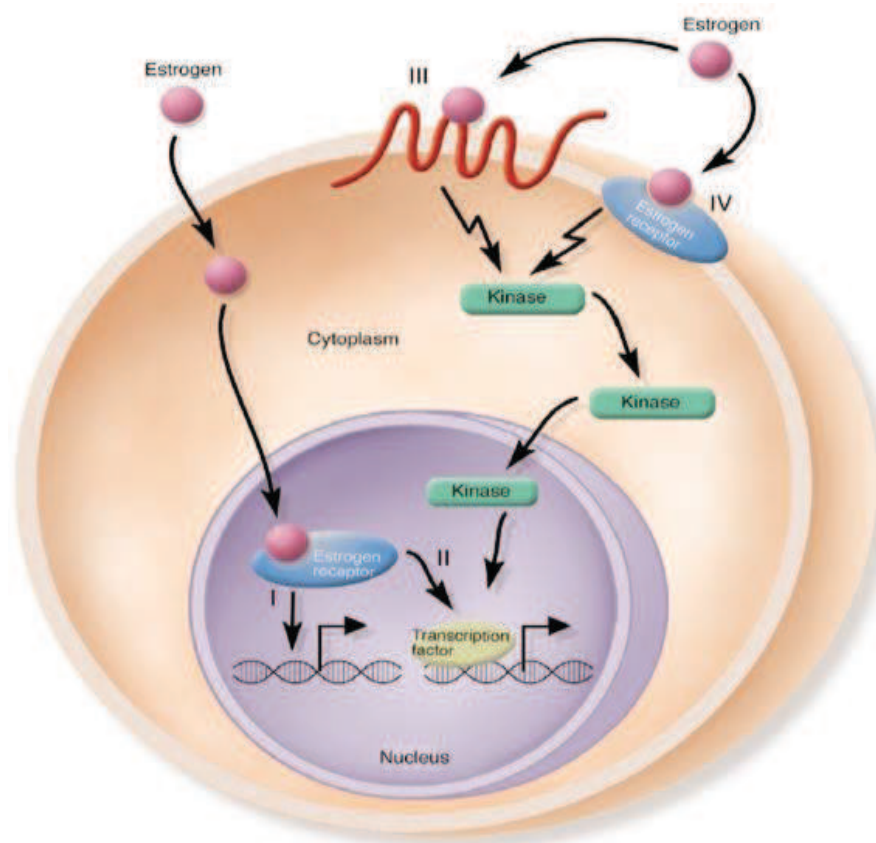
Il n'y a actuellement pas de consensus sur la façon de déterminer la présence de LVI. Nous ne savons pas s'il y a nécessité de distinguer l'invasion des vaisseaux lymphatiques de celle des vaisseaux sanguins, ni s'il faut déterminer l'extension de cette invasion. Dans la majorité des cas, la présence de LVI peut être facilement identifiée sur des coupes HES [89]. L'utilisation d'immunomarquages (CD-34, CD-31, D2-40) serait certainement plus fiable [88] et permettrait de s'affranchir du risque de faux positif, par artéfacts liés à la coupe des tissus et à leur fixation [90, 91]. Cependant, aucun marqueur n'est actuellement assez spécifique ni

sensible pour cela. En routine, il ne semble pas indispensable de déterminer le type de vaisseaux envahi puisque les 2 sont pronostiques [88].

- **Statut des récepteurs hormonaux (RH)**

Il existe 2 isoformes du RE : RE $\alpha$  et RE $\beta$ , encodés par 2 gènes distincts *ESR1* et *ESR2*, respectivement. RE $\alpha$  et RE $\beta$  sont impliqués dans de nombreux processus physiologiques, notamment la fonction de reproduction, mais ont aussi un rôle au niveau du système nerveux central, du foie, des vaisseaux sanguins, des tissus adipeux et osseux. Dans le cancer du sein, c'est le RE $\alpha$  qui est incriminé et qui est recherché en pratique clinique. Nous le dénommerons dans la suite de cet exposé « RE ».

Les œstrogènes diffusent librement à travers la membrane cellulaire et se lient au RE au niveau du noyau, entraînant un changement de conformation du récepteur, et induisant sa dimérisation. Le complexe ligand / récepteur se fixe au niveau des séquences régulatrices des gènes œstrogène-régulés pour moduler leur expression, agissant ainsi comme un facteur de transcription. La réponse cellulaire engendrée dépend des tissus où sont présents les RE, de l'activation de co-activateurs ou de co-répresseurs. Il s'agit de la voie d'action génomique la plus classique et la mieux connue (**Figure 9**). Les voies non-génomiques correspondent aux effets des RE sur les voies de signalisation intra-cellulaires, notamment calcium-dépendantes. Ces effets peuvent également se traduire sur la régulation de gènes (**Figure 9**).



**Figure 9. Mécanisme d'action du récepteur estrogène**

Jusqu'aux années 1990, la mesure du RE se faisait par des techniques de liaison avec des ligands radiomarqués ou par méthode immuno-enzymatique. Par la suite, la découverte de nouveaux anticorps monoclonaux a permis de quantifier l'expression du RE en IHC et sur des tissus inclus et fixés en paraffine. Seul le marquage nucléaire de la composante infiltrante est pris en compte. Si au moins 10% des cellules sont marquées, le récepteur est considéré, en France, comme positif. Le seuil est de 1% selon les recommandations de l'ASCO (American Society of Clinical Oncology) / CAP [92]. Environ 80% des cancers du sein sont hormono-dépendants. Cette positivité est un critère pronostique important, mais également prédictif négatif de réponse à l'hormonothérapie. En effet seules les patientes ayant une tumeur exprimant les RE peuvent avoir un bénéfice de l'hormonothérapie alors que les patientes ayant une tumeur RE- ne sont pas répondeuses à l'hormonothérapie [93]. Le pourcentage de cellules marquées est également un facteur pronostique et prédictif [92]. Au-delà du pourcentage de cellules marquées, l'intensité de fixation doit également être mentionnée dans les comptes rendus anatomopathologiques.

Chez les patientes (ménopausées ou non) ayant une tumeur RE+, le bénéfice du traitement par tamoxifène administré pendant 5 années en adjuvant est largement démontré et reconnu, grâce aux résultats de la méta-analyse réalisée sur données individuelles de l'Early Breast Cancer Trialists' Collaborative Group [94]. Le tamoxifène permet de diminuer le risque de récurrence de 39% et le risque de décès par cancer du sein de 30% à 15 ans, donc avec un bénéfice prolongé dans le temps, bien après l'arrêt du traitement (carry over effect). Le tamoxifène se fixe sur le RE et bloque la voie de signalisation œstrogène / RE et donc la prolifération des cellules tumorales.

Chez les femmes ménopausées, les inhibiteurs d'aromatase (IA) ont montré un bénéfice en termes de survie sans récurrence (SSR) et de SG les cinq 1<sup>ères</sup> années, que le traitement soit administré en relais du tamoxifène ou seul [95]. Les IA ont uniquement un bénéfice chez les patients ayant une tumeur RE+ [96]. Chez les femmes ménopausées, les œstrogènes sont principalement synthétisés dans le tissu adipeux périphérique par aromatisation des androgènes produits par les surrénales. Les IA bloquent cette conversion périphérique.

L'expression du RP est également évaluée en cas de cancer du sein. Le RE régule l'expression du RP, par conséquent la présence de RP indique que la voie du RE est fonctionnelle [97]. Le RP est activé par la fixation de la progestérone et est exprimé dans 60 à 70% des cancers du sein. Les patients ayant une tumeur RE+ / RP- auraient un moins bon pronostic que les tumeurs RE+ / RP+ [98, 99] et répondraient moins bien à l'hormonothérapie adjuvante [100]. L'existence réelle de tumeurs RE- / RP- est débattue.

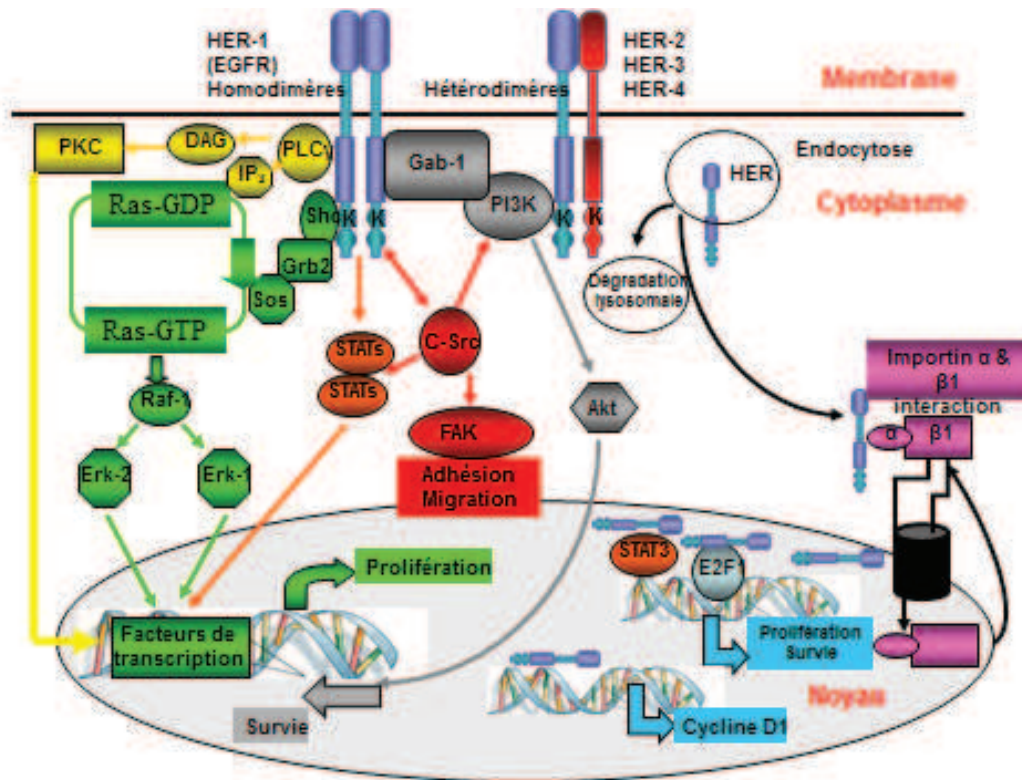
- **Statut HER2 (Human Epidermal Growth Factor 2)**

HER2 est une protéine transmembranaire de 185 kD, de type récepteur aux facteurs de croissance à activité tyrosine kinase. Le gène *HER2* ou *ERBB2*, codant pour la protéine HER2, est situé sur le bras long du chromosome 17 (17q21).

La famille des récepteurs de type HER est composée de 4 récepteurs tyrosine kinase : HER1 ou EGFR (Epidermal Growth Factor Receptor), HER2, HER3 et HER4. Cette famille joue un rôle important dans la croissance cellulaire normale et le développement tissulaire, et a pu être impliquée dans les processus de division, de différenciation, de survie, de motilité, d'adhérence cellulaire et d'angiogenèse [101]. En l'absence de ligand, les récepteurs sont

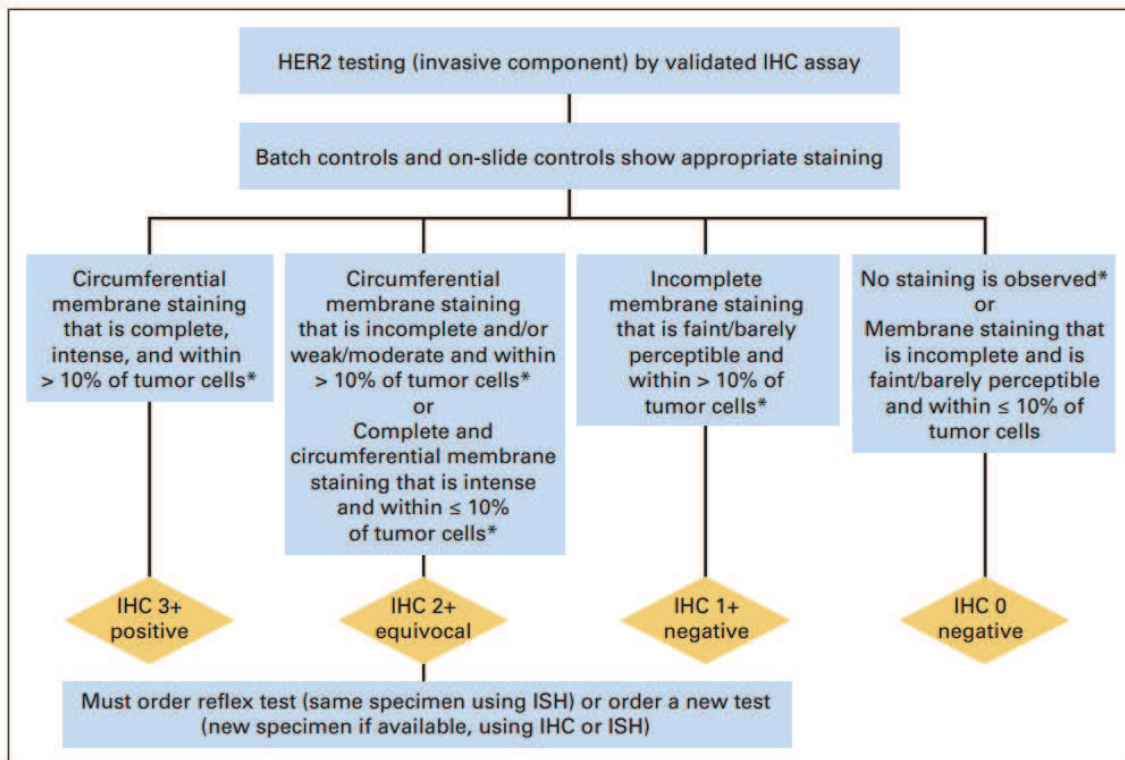


exprimés sous forme de monomères inactifs à la surface de la cellule. Lors de la fixation d'un ligand au niveau du domaine extracellulaire, les récepteurs de cette famille s'associent les uns aux autres pour former des homos- ou des hétéro-dimères. Une fois dimérisés, les récepteurs sont activés par le biais de la phosphorylation de certains résidus tyrosine kinase de leur partie intracellulaire. Cette autophosphorylation permet le recrutement de protéines possédant des motifs PTB (phospho-tyrosine binding) ou SH2 (Src homology domain de type 2). Les protéines recrutées dépendent des conditions physiologiques (développement embryonnaire, croissance, influence hormonale), pathologiques (stress, cancérogénèse...), du ligand impliqué mais également du type de dimères créés. Selon les conditions de dimérisation, les principales voies de signalisation activées seront la voie MAP (mitogen-activated protein) kinase (croissance, prolifération, différenciation et survie cellulaire) et la voie PI3K (phosphoinositide 3-kinase) (progression dans le cycle cellulaire, survie, anti-apoptose et invasion). HER2 n'a pas de ligand spécifique, et HER3 ne possède pas d'activité tyrosine kinase, les rendant ainsi dépendants et non autonomes en conditions physiologiques, et les faisant nommer par Yarden le « sourd et le muet » [102]. Lorsque HER2 est surexprimé à la surface de la cellule, des homodimères HER2 vont se former par rapprochement, de façon prioritaire, permettant alors son auto-phosphorylation. En cas d'hétérodimérisation avec HER3, la voie préférentiellement activée sera celle de PI3K / Akt, et avec HER1, celles des MAP kinases (activation de Ras). Ces signaux ont une durée de vie et une amplitude variables selon le type de combinaisons de dimères et de ligands et les conditions les ayant déclenchés. Les récepteurs sont ensuite internalisés par endocytose pour être dégradés ou recyclés à la surface cellulaire (**Figure 10**). Les hétérodimères avec HER2 sont caractérisés par une dissociation plus lente du ligand, une diminution du phénomène d'endocytose des récepteurs, une plus grande stabilité des dimères, un recyclage membranaire plus rapide et donc au final une activation prolongée des signaux de stimulation intracellulaire considérée comme plus puissante et plus intense [101, 103].



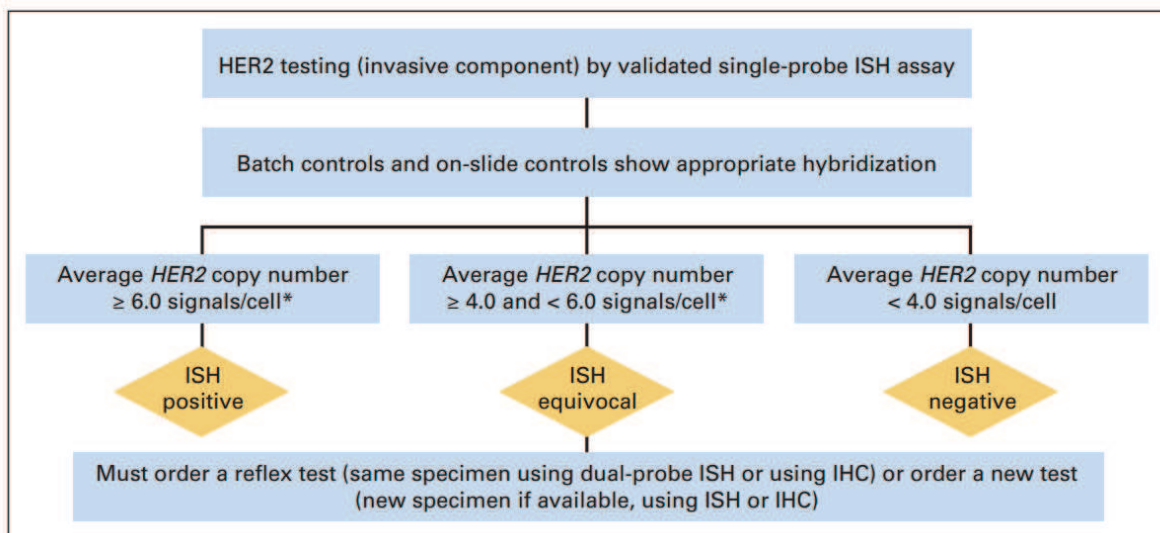
**Figure 10.** Récepteurs HER et voies de signalisation impliquées

Il existe une amplification et / ou une surexpression de HER2 dans environ 15% des cancers du sein infiltrant. Le statut HER2 est déterminé par IHC et / ou hybridation *in situ* (HIS) (chromogénique ou par fluorescence). Les dernières recommandations ASCO / CAP pour la détermination du statut HER2 sont résumées sur les **Figures 11 à 13** [104]. La surexpression et / ou l'amplification de HER2 sont associés à un pronostic défavorable, caractérisant des tumeurs avec un grade et un indice de prolifération élevés, une atteinte ganglionnaire plus fréquente, des RH négatifs dans 50% des cas et un risque de récurrence plus important [105-107].

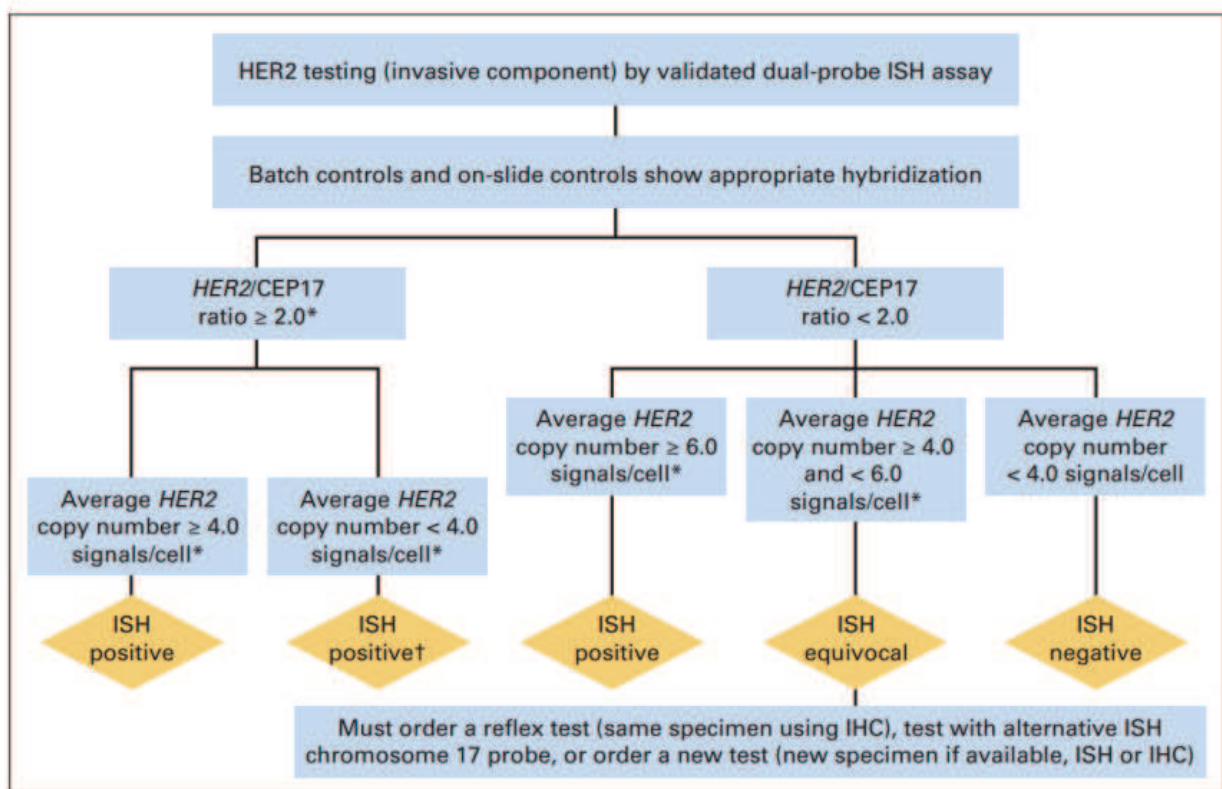


\*Évalué au microscope à faible grossissement

**Figure 11. Recommandations ASCO / CAP pour la détermination du statut HER2 en IHC [104]**



**Figure 12. Recommandations ASCO / CAP pour la détermination du statut HER2 en HIS (Hybridation *in situ*) [104]**



**Figure 13. Recommandations ASCO / CAP pour la conduite à tenir en cas de statut HER2 ambigu [104]**

Le statut HER2, indépendamment de son rôle pronostic, est un facteur prédictif du bénéfice clinique apporté par les thérapies anti-HER2 telles que le trastuzumab (Herceptin®, Roche), anticorps monoclonal humanisé qui se lie au domaine IV de la partie extracellulaire de HER2. Le trastuzumab a révolutionné le devenir des patientes ayant une tumeur HER2+ que ce soit en situation métastatique [108], adjuvante [109] ou néoadjuvante [110]. En cas de statut HER2 ambigu, malgré les techniques IHC et HIS, la décision de traiter ou non le patient avec du trastuzumab doit être prise en réunion de concertation pluridisciplinaire et dépend des autres caractéristiques anatomo-cliniques de la tumeur [104].

Les mécanismes d'action du trastuzumab semblent être multiples. En se liant à HER2, il diminuerait l'activation des voies MAP kinase [111] et PI3K / Akt [112], entraînant un arrêt du cycle cellulaire par augmentation de p27, conduisant à l'apoptose. Il diminuerait également le nombre de récepteurs HER2 disponibles à la surface de la cellule *via* une accélération de leur internalisation et de leur dégradation intracellulaire. Le trastuzumab, une fois lié à HER2, pourrait empêcher la formation de formes tronquées de la protéine en inhibant la protéolyse du domaine extracellulaire [113]. La portion Fc du trastuzumab pourrait permettre le recrutement d'effecteurs cellulaires *via* les différents récepteurs à la

portion Fc des immunoglobulines G (FcγRs) exprimés par les cellules immunitaires et conduisant à des mécanismes de cytotoxicité médiée : cytotoxicité cellulaire dépendante des anticorps [114], phagocytose. Il inhiberait également l'angiogenèse tumorale (diminution du taux de VEGF (Vascular Endothelial Growth factor)) mais aussi les processus de réparation des lésions d'ADN induites par la chimiothérapie et la radiothérapie [115]. Ses effets secondaires sont rares : réactions allergiques et toxicité cardiaque (du fait de la présence de récepteurs HER2 sur les cardiomyocytes) réversible à l'arrêt du traitement.

Malgré l'activité du trastuzumab, des résistances apparaissent parfois, et certaines patientes récidivent malgré un traitement de chimiothérapie et de trastuzumab adjuvant. D'autres thérapies anti-HER2 ont par conséquent été développées, et parmi elles, le pertuzumab (Perjeta®, Roche). Le pertuzumab, anticorps monoclonal entièrement humanisé de type IgG1, se lie au niveau du domaine II de la portion extracellulaire du récepteur HER2, domaine essentiel pour la dimérisation, à la différence du trastuzumab, qui se lie au domaine IV, qui ne semble pas directement réguler la dimérisation. Il inhibe ainsi plus spécifiquement la formation des hétérodimères HER2 - HER3, qui induisent le plus fort signal pour l'activation de la voie PI3K / Akt / mTOR. Le pertuzumab, en association au docetaxel et au trastuzumab, a permis d'augmenter la SSR et la SG des patientes ayant une tumeur HER2+ en 1<sup>ère</sup> ligne métastatique [116]. Les résultats de l'étude APHINITY qui évalue ce traitement en adjuvant sont attendus.

- **Ki-67**

Le Ki-67 est une protéine nucléaire présente dans la cellule à différentes phases du cycle cellulaire, mais pas en phase de quiescence G0. C'est donc un marqueur de prolifération cellulaire, mesuré en IHC. Le Ki-67 est associé de façon significative à la SSM en analyse multivariée, dans plusieurs essais randomisés et 2 méta-analyses [117], avec un hazard ratio d'environ 1,7 (IC95% 1,56 - 1,98) [118]. Dans la méta-analyse d'Azambuja *et al*, le Ki-67 a une valeur pronostic en analyse univariée pour la SSM et la SG [119]. Dans la méta-analyse de Stuart-Harris *et al*, un taux élevé de Ki-67 est associé à un mauvais pronostic (SSM et SG) en analyse multivariée [118]. Selon la grille de Simon (évaluation de l'utilité clinique d'un biomarqueur) et la publication de Luporsi *et al*, l'utilisation du Ki-67 comme facteur pronostic (SSM) chez les patients recevant un traitement adjuvant correspond à un niveau de preuve I-B [117]. Le niveau de preuve est plus faible en ce qui concerne sa valeur

prédictive de réponse à la chimiothérapie (II-B) [117]. Plusieurs études ont cependant montré un lien entre un taux élevé du Ki-67 avant traitement néoadjuvant (chimiothérapie ou hormonothérapie) et la réponse au traitement [117]. Après traitement néoadjuvant, le taux d'expression du Ki-67 a été décrit comme étant un facteur pronostic dans plusieurs études (quel que soit son taux avant traitement) [117].

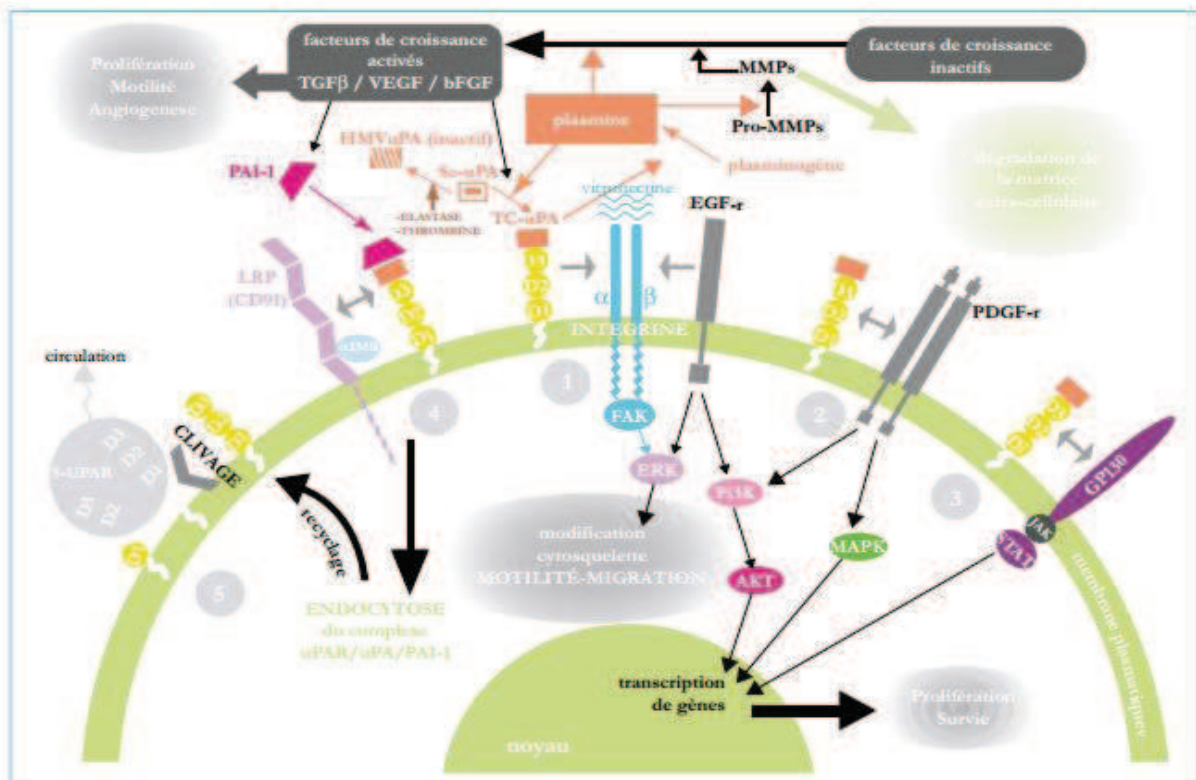
Le principal problème actuellement de l'utilisation du Ki-67 comme facteur pronostic est le choix du seuil au-delà duquel une tumeur est considérée comme étant de mauvais pronostic car très proliférative. Un seuil arbitraire de 14% a été retenu initialement dans la conférence de consensus de St Gallen en 2011 mais il ne reposait que sur une seule étude [120]. Ce seuil a été retenu pour distinguer les tumeurs lumineales A des tumeurs lumineales B, plus agressives, et nécessitant à priori un traitement de chimiothérapie. En effet, c'est dans la population des tumeurs RE+, HER2- et de grade II qu'un tel biomarqueur peut avoir le plus d'utilité quant à la décision thérapeutique. Dans la revue de Luporsi *et al*, les seuils choisis pour la décision de traitement adjuvant étaient, selon les études, compris entre 5% et 34% avec, pour une majorité des études, un seuil fixé à 10% ou 20% [117]. La plupart des cliniciens et anatomopathologistes s'accordent sur le fait qu'en-dessous d'un seuil de 10% une tumeur peut être considérée comme peu proliférative et qu'au-delà de 30% elle est agressive. Par la suite, un seuil de 20 - 29% a été proposé pour distinguer les tumeurs lumineales A des tumeurs lumineales B [121, 122]. Il est indispensable d'homogénéiser les méthodes de mesure (pré-analytique) que ce soit en pratique clinique ou pour les essais cliniques [123].

L'index mitotique (IM) est également un marqueur de prolifération cellulaire et est intégré au grade SBR dont nous avons déjà parlé. C'est un facteur pronostic majeur, les patients ayant une tumeur à fort IM ont plus de risque de récurrence [124] et de décéder de leur pathologie [118]. C'est également un facteur prédictif de réponse à la chimiothérapie adjuvante [125, 126] et néoadjuvante [127, 128]. Dans la revue de la littérature de Van Diest *et al*, il est le facteur pronostic le plus indépendant et le plus reproductible, avec un seuil fixé à 10 mitoses pour 10 champs [129]. Cependant la corrélation entre prolifération et le nombre de mitoses n'est pas strictement linéaire [130]. Par ailleurs des discordances sont parfois observées entre le Ki-67 et l'IM. Dans cette situation, rencontrée notamment en cas de tumeur RE+ / HER2-, de grade II, quel marqueur de prolifération privilégier ? Une étude

publiée en 2006 semblait montrer que le Ki-67 n'apportait pas de valeur pronostique additionnelle par rapport à l'IM [131]. En cas d'IM bas avec un Ki-67 élevé, les patientes avaient plutôt un bon pronostic [131]. Cependant dans une étude publiée récemment, les patientes ayant une tumeur avec un Ki-67 et un IM discordants semblent être de pronostic intermédiaire par rapport à celles avec un Ki-67 bas / IM bas ou un Ki-67 élevé / IM élevé [132]. Les 2 facteurs semblent donc être à prendre en considération en pratique clinique.

- **Urokinase activatrice du plasminogène (uPA) et inhibiteur 1 de l'activateur du plasminogène (PAI-1)**

UPA et PAI-1 sont des protéines impliquées dans l'hémostase et appartiennent au système fibrinolytique activateur du plasminogène. L'uPA est produite, outre dans les tumeurs, par les fibroblastes, les monocytes, les polynucléaires neutrophiles et les cellules épithéliales. Elle induit la conversion du plasminogène en plasmine, sa forme active, après fixation à son récepteur (uPAR). Au niveau tumoral, la plasmine va dégrader les composants de la matrice extracellulaire (MEC) (laminine, fibronectine...) et est capable d'activer les métalloprotéases, impliquées également dans la dégradation de la MEC. L'uPA participe ainsi à l'invasivité des cellules tumorales et donc au processus métastatique. PAI-1 soluble est l'inhibiteur majeur de l'uPA. En se fixant sur le complexe d'uPA avec son récepteur uPAR, il entraîne son endocytose, accélérant le turn-over de l'uPAR qui est ensuite recyclé à la surface cellulaire et peut ainsi de nouveau participer à l'activation du plasminogène [133] (*Figure 14*).



Au niveau extracellulaire : sc-uPA (single chain uPA) est une proenzyme de l'uPA, issue du *feed-back* positif de la plasmine, synthétisée à partir du plasminogène sous l'action de l'uPA. sc-uPA est activée en tc-uPA lors de sa fixation à son récepteur uPAR. La plasmine est l'initiation de la cascade des métalloprotéinases qui induisent la dégradation de la matrice extracellulaire et l'activation des facteurs de croissance, dont le TGFβ, qui active la synthèse de PAI-1. Ces mécanismes sont impliqués dans la motilité cellulaire, la prolifération et l'angiogenèse.

Au niveau cellulaire : uPAR est une molécule ancrée à la membrane plasmique qui est capable de fixer l'uPA par trois domaines de liaisons, laissant intacts les sites d'interaction avec les protéines transmembranaires impliquées dans la transduction des signaux extracellulaires.

- 1) Les intégrines transmembranaires jouent le rôle de transducteur du signal de la matrice extracellulaire vers la cellule initiant la voie ERK *via* la protéine associée FAK, ce qui modifie la motilité cellulaire. Elles sont aussi impliquées dans la voie des facteurs de croissance par les interactions avec les récepteurs tyrosines-kinases comme l'EGF-R. Le complexe tc-uPA/uPAR stabilise les intégrines, ce qui aboutit à une amplification des voies FAK et EGF-R.
- 2) Le complexe tc-uPA-uPAR lié au PDGF-R active la transcription de gènes de prolifération et de survie *via* les cascades de phosphorylation des Pi3 kinase et MAP kinases.
- 3) La phosphorylation de Stat, qui participe à la croissance vasculaire, est initiée par le complexe tc-uPA/uPAR en liaison avec la GP130 et les Janus kinases (Jak).
- 4) L'interaction du complexe uPAR/tc-uPA/PAI-1 avec la protéine LRP (CD91) conduit à l'internalisation de l'ensemble et au recyclage du complexe à la surface cellulaire. L'expression des molécules recyclées ne se fait pas au hasard sur la surface membranaire. Ainsi, dans les cellules endothéliales, cette voie activée par le VEGF, permet le recyclage du complexe sur le front d'adhésion des cellules orientant la migration de celle-ci.
- 5) Le clivage de l'uPAR conduit à la libération de formes libres, circulantes à deux ou trois domaines de liaisons, à l'uPA qui sont biologiquement actives.

Figure 14. L'interactome uPA / PAI-1 / uPAR [133]



Les biomarqueurs uPA / PAI-1 ont une valeur pronostique de la SSR à 10 ans chez les patients pN0 (niveau de preuve élevé selon le rapport de l'INCA de 2009 et confirmé dans le travail d'actualisation publié cette année, s'appuyant sur la grille de Hayes mais également sur la grille de Simon) [134]. Deux essais cliniques sont à l'origine de ces conclusions. Le 1<sup>er</sup>, « Chemo-N0 » incluait 556 patients sans atteinte ganglionnaire [135]. Lors de la 1<sup>ère</sup> analyse intermédiaire, après un suivi médian de 32 mois, les patients avec des faibles taux d'uPA et PAI-1 (N = 241) avaient un taux de récurrence à 3 ans moins important que les patients avec des taux élevés d'uPA et PAI-1 (N = 315) (6,7% *versus* 14,7%, RR = 2,8, p = 0,006) [135]. Ces données ont été confirmées lors de l'analyse à 10 ans : taux de récurrence de 12,9% *versus* 23% (HR = 1,84, p = 0,017) [136]. Ces données concordent avec celles d'une méta-analyse menée sur 8 377 patients (pN0 et pN+) en Europe [137]. Chez les patients pN0, les résultats à 10 ans montrent une différence entre le sous-groupe à risque le plus fort et le sous-groupe à risque le plus faible de 28,2% en termes de SG et de 34,5% en termes de SSR. Pour les patients pN+ ou ceux dont les tumeurs expriment fortement les RE, seul PAI-1 présenterait une valeur pronostique (niveau de preuve bas) [134].

Dans l'essai « Chemo-N0 » les patients avec des taux faibles d'uPA / PAI-1 ne recevaient pas de traitement systémique. Les patients avec des taux élevés étaient randomisés entre observation et chimiothérapie par CMF (cyclophosphamide, méthotrexate, 5-fluorouracile). Les taux de récurrence étaient de 21,3% (13,9 - 31,9%) dans le bras chimiothérapie *versus* 32,1% (22,9 - 43,8%) dans le bras observation (HR = 0,48, p = 0,019). Ces données ont permis de conférer à uPA / PAI-1 une valeur prédictive de réponse à la chimiothérapie à base de CMF. Cependant ce traitement n'est plus un standard actuellement. Lorsque la chimiothérapie est à base d'anthracyclines, uPA / PAI-1 présenteraient une valeur prédictive (niveau de preuve bas) [138].

UPA / PAI-1 sont mesurés par Elisa sur des échantillons protéiques tissulaires. Leur dosage nécessite du tissu frais congelé en assez grande quantité, limitant donc son usage aux tumeurs de plus de 1 cm. En France, ce test entre dans la nomenclature des actes de biologie médicale et est donc remboursé. Plusieurs centres réalisent actuellement cette analyse : les Centres Hospitaliers Universitaires de Grenoble, Marseille et Strasbourg ainsi que les Centres de Lutte Contre le Cancer de Montpellier, Nantes, Nice et Rouen.

- **Carcinome in situ**

Le CIS est une prolifération épithéliale maligne située dans la lumière des canaux et des lobules sans infiltration du parenchyme avoisinant. Soixante-dix pourcent des carcinomes infiltrants renferment une composante canalaire *in situ*. La présence d'un contingent de CIS extensif en périphérie de la tumeur est un facteur de risque de rechute locale. Le pourcentage du CIS par rapport au carcinome infiltrant doit être précisé dans le compte-rendu anatomopathologique, de même que le grade nucléaire de Holland ainsi que la présence ou l'absence de nécrose au sein du CIS.

- **Qualité des limites d'exérèse chirurgicale**

L'atteinte des berges d'exérèse chirurgicale augmente le risque de récurrence locale. Le degré d'atteinte (minime / focale / massive), la topographie et le type d'atteinte (infiltrant ou *in situ*) doivent être connus de même que la distance en mm entre la tumeur et la limite chirurgicale la plus proche. Une distance de 2 mm minimum est recommandée pour le CIS, les lésions infiltrantes ne doivent pas être au contact des berges pour être considérées en exérèse complète.

## **V – CLASSIFICATION MOLECULAIRE DES CANCERS DU SEIN**

Les analyses génomiques, grâce aux puces à ADN ou microarrays, permettent d'analyser simultanément l'expression de nombreux gènes et faire ainsi un « portrait » de chaque tumeur. L'analyse des résultats, grâce aux méthodes de *clustering*, permet de regrouper les tumeurs en fonction de leur profil d'expression génique. Perou *et al* ont été les premiers à subdiviser les cancers du sein en sous-groupes selon leur profil d'expression génique (analyses de clusters non supervisés). Ils ont d'abord identifié deux groupes principaux en fonction de l'expression des RE puis 4 groupes en comparaison au tissu normal : basal-like, HER2-enriched, luminal et normal-like [139] (**Figure 15**). D'autres travaux sur différentes plateformes de microarrays (Affymetrix®, Agilent®), menés sur des séries de cancers du sein indépendantes, avec des techniques de qPCR (quantitative polymerase chain reaction) ou de microarray sur tissu fixé et inclus en paraffine, ont validé ces sous-types

moléculaires [140-143]. Les sous-types luminal et basal-like sont les plus robustes. Au sein des tumeurs lumineuses, 2 sous-types ont été ensuite distingués : les tumeurs lumineuses A et les tumeurs lumineuses B [144]. Ces différents sous-types ont des pronostics différents, le type luminal, et notamment A, étant le plus favorable et le type basal-like étant de pronostic plus sombre.

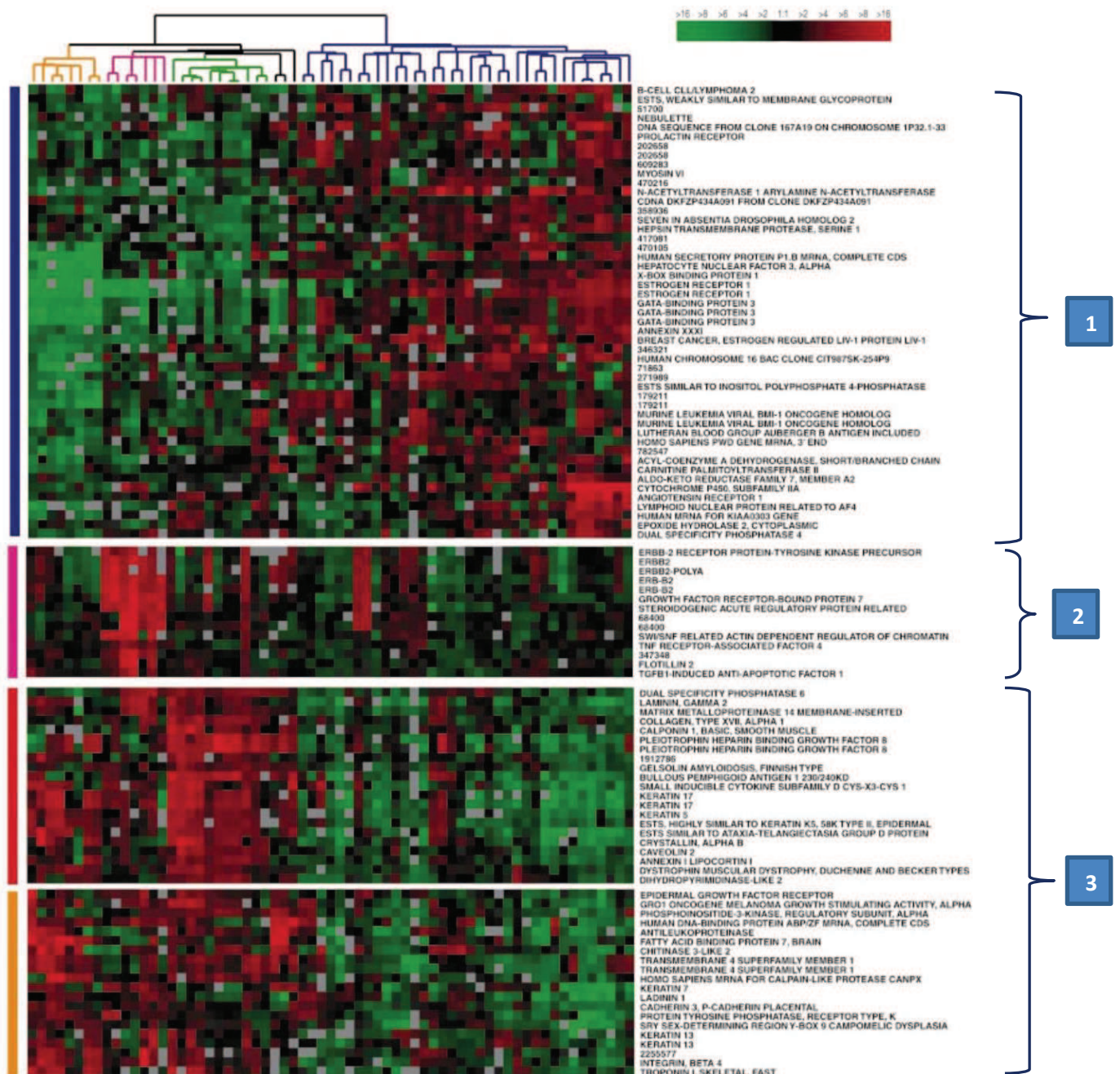


Figure 15. Classification moléculaire des cancers du sein (1. Cluster luminal / epithelial, 2. Cluster HER2, 3. Cluster basal epithelial)

Les tumeurs lumineuses sont classiquement RE+. Ce sont des tumeurs dont l'expression génique est proche de celle des cellules épithéliales lumineuses dont le profil IHC est caractérisé par l'expression de CK8/18 et CK19. Ce groupe comprend les tumeurs de faible grade et les formes histologiques particulières (mucineux, cribriforme, CCI de grades SBR I et II). Les tumeurs lumineuses A ont une expression élevée de RE et de gènes régulés par le RE (*GATA3*, *FOXA1*...), une faible expression de gènes liés à la prolifération, et une mutation de *P53* dans 13% des cas. Les tumeurs lumineuses B ont une expression plus faible

de RE, une expression moindre de gènes régulés par le RE, une expression élevée des gènes liés à la prolifération, une mutation de *P53* dans 66% des cas.

Les tumeurs basal-like sont caractérisées par l'expression de gènes identiques à ceux exprimés par des lignées de cellules myoépithéliales. En IHC, elles expriment les CK5/6, CK14, CK7 et l'actine musculaire lisse comme les cellules basales. Ce sous-groupe est le plus robuste dans les différentes études. Ces tumeurs ont un phénotype particulier et reproductible : RE-, HER2-, au moins un marqueur de cellules basales (CK5/6, EGFR, vimentine, actine musculaire lisse, c-KIT) [145, 146]. Elles sont souvent RP-, avec un taux élevé de mutation *P53* et un grade SBR III. Elles regroupent les carcinomes médullaires atypiques, les carcinomes métaplasiques, la plupart des carcinomes liés à une mutation de *BRCA1* et des CCI de grade SBR III. C'est le sous-groupe avec le pronostic le plus défavorable mais qui est associé au taux le plus élevé de réponse histologique complète (pCR) après chimiothérapie néoadjuvante à base d'anthracyclines [147].

Les tumeurs HER2 incluent toutes les tumeurs HER2 quel que soit l'expression des RH. Un progéniteur luminal serait à l'origine de ces tumeurs. Leur phénotype est HER2+, CK8/18/19+. Ce groupe comprend les tumeurs de type apocrine et des CCI de grades SBR II et III. Leur pronostic est moins bon que les tumeurs lumineales.

Le type normal-like correspond finalement à un artefact de dilution de l'ARN tumoral dans l'ARN de tissu mammaire normal.

## TRAVAIL DE THESE

Nous avons dans ce travail cherché à identifier de nouveaux facteurs pronostiques et / ou prédictifs dans le cancer du sein infiltrant.

Dans le 1<sup>er</sup> article, nous avons cherché à savoir si l'histologie de type lobulaire était en soi un facteur de bon pronostic et pouvait permettre de prédire la réponse au traitement.

Dans le 2<sup>ème</sup> article, nous avons cherché à savoir si la classification moléculaire des cancers du sein basée, soit sur PAM50, soit sur le Ki-67, avait une validité analytique, une validité et une utilité cliniques.

Dans le 3<sup>ème</sup> article, nous avons mis en évidence un sous-groupe de tumeurs TN exprimant le récepteur androgène (RA) et FOXA1 et se comportant comme des tumeurs de type luminal.

Dans le 4<sup>ème</sup> article, nous avons montré sur une large série néoadjuvante, que la réponse histologique complète est un critère substitutif de survie pour les tumeurs RH- (TN et RH- / HER2+).

Le 5<sup>ème</sup> article est une revue de la littérature sur les facteurs prédictifs de réponse aux thérapies anti-HER2 en situation néoadjuvante dans les cancers du sein HER2 positifs.

**Carcinome lobulaire infiltrant et ses  
variantes : est-ce que ce diagnostic histologique  
impacte le traitement systémique ?**

**(Article 1)**

**Invasive lobular breast cancer and its  
variants: How special are they for systemic  
therapy decisions?**

*S. Guiu, A. Wolfer, W. Jacot, P. Fumoleau, G. Romieu,  
F. Bonnetain, M. Fiche*

**Critical Reviews in Oncology Hematology**

## I – INTRODUCTION

La classification OMS 2012 des tumeurs du sein distingue, au-delà du CCI ou carcinome NST, une trentaine de types et sous-types histologiques spéciaux parmi lesquels le CLI est le plus fréquent (5-15%). Il est important d'individualiser ce type histologique spécial de par son incidence, ainsi que ses spécificités biologiques et cliniques. Son incidence a augmenté ces 20 dernières années, notamment chez les femmes de plus de 50 ans [148, 149]. Cette évolution est corrélée à l'utilisation de THS de la ménopause, notamment à base de progestérone [150, 151]. Soulignons qu'étant donné la grande incidence des cancers du sein dans le monde, l'incidence actuelle de ce sous-type particulier est comparable à celle des tumeurs cérébrales ou du myélome [152].

L'intérêt d'individualiser ce type histologique a parfois été remis en cause par certains auteurs, du fait des études génomiques distinguant les cancers du sein de bas grade (canaulaire ou lobulaire) et les cancers de haut grade présentant des anomalies récurrentes : gain du 1q et perte du 16q pour les bas grades, mutations de *P53* et gain du 17q pour les haut grades [153-155]. Cependant, certains arguments plaident en faveur de la distinction de ce type particulier : la grande fréquence de la perte de la E-cadhérine (molécule d'adhésion intercellulaire), non observée dans les autres cancers de bas grade, sa présentation clinique ainsi que des sites métastatiques préférentiels particuliers [28].

Des données contradictoires sont retrouvées dans la littérature concernant le pronostic spécifique des patients avec un CLI. Par ailleurs, certains auteurs suggèrent que l'efficacité de la chimiothérapie serait moindre dans ce sous-groupe [156-158]. L'utilisation de signatures génomiques pour aider à la décision de traitement systémique, et notamment l'addition d'une chimiothérapie à une hormonothérapie adjuvante, est rapportée par plusieurs auteurs [159]. Cependant, la plupart des corrélations entre les signatures moléculaires et le pronostic des patients sont dérivées d'études faites avec des CCI, et rarement des types histologiques spéciaux. Ainsi, pour les patients porteurs de CLI, la prise de décision concernant le traitement systémique en situations néoadjuvante et adjuvante représente un challenge pour l'oncologue : i) cela requiert d'extrapoler les données obtenues principalement chez des patients ayant un CCI, aux patients avec un CLI, un cancer du sein en partie biologiquement différent ; ii) étant donné l'hétérogénéité des CLI, des



considérations générales peuvent n'être pas ou peu pertinentes pour les patients ayant des variantes de CLI.

De plus, étant donné les travaux récents sur la compréhension de la biologie du cancer du sein, une évaluation pertinente de l'impact actuel de l'histologie de type lobulaire, nécessite de comparer les CLI avec des cancers du sein similaires d'un point de vue moléculaire (en particulier le sous-type luminal), plutôt qu'avec l'ensemble des CCI, comme cela a été fait par le passé.

Notre objectif dans cette étude est une revue exhaustive de la littérature sur i) la spécificité et l'hétérogénéité moléculaire du CLI ; ii) l'impact de cette histologie sur le pronostic et la prédiction de l'efficacité des traitements systémiques chez les patients avec un cancer du sein infiltrant.

## **II - ARTICLE**

## Invasive lobular breast cancer and its variants: How special are they for systemic therapy decisions?

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## Abstract

The WHO classification of breast tumors distinguishes, besides invasive breast cancer ‘of no special type’ (former invasive ductal carcinoma, representing 60–70% of all breast cancers), 30 special types, of which invasive lobular carcinoma (ILC) is the most common (5–15%). We review the literature on (i) the specificity and heterogeneity of ILC biology as documented by various analytical techniques, including the results of molecular testing for risk of recurrence; (ii) the impact of lobular histology on prediction of prognosis and effect of systemic therapies in patients. Though it is generally admitted that ILC has a better prognosis than IDC, is endocrine responsive, and responds poorly to chemotherapy, currently available data do not unanimously support these assumptions. This review demonstrates some lack of specific data and a need for improving clinical research design to allow oncologists to make informed systemic therapy decisions in patients with ILC. Importantly, future studies should compare various endpoints in ILC breast cancer patients among the group of hormonosensitive breast cancer.

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**Keywords:** Invasive breast cancer; Invasive lobular carcinoma; Pleomorphic invasive lobular carcinoma; Tubulo-lobular carcinoma; Prognosis; Predictive factor; Luminal breast cancer

## 1. Introduction

Breast cancer (BC) is a biologically heterogeneous disease encompassing subgroups associated with different epidemiology, natural history and response to systemic therapies [1]. Over the last decade, the BC community has shifted toward an understanding of this heterogeneity along the intrinsic subtypes generated in 2000 by unsupervised clustering of gene expression patterns generated by complementary DNA microarrays (e.g. luminal A and B, HER2-enriched, and basal-like) [2,3]. Current guidelines for systemic therapy decisions acknowledge four main subgroups of BC corresponding to these four main molecular classes that are determined by the presence/absence of targets for available therapeutic approaches. These targets are evaluated using morphological methods such as immunohistochemistry (IHC) for hormone receptors (HR), HER2 and Ki-67 protein expression, and *in situ* hybridization techniques (ISH) for HER2 gene amplification [3].

Histological types, based on BC morphology as observed under the optic microscope, represent a classical approach to describe BC heterogeneity. The 2012 WHO classification distinguishes, besides invasive BC ‘of no special type’ (former invasive ductal carcinoma (IDC), representing about 60–75% of all BC), 30 special types and subtypes, of which invasive lobular carcinoma (ILC) is the most common, representing 5–15% of all BC [1]. It has been long recognized that all BC, lobular and non-lobular, arise from the terminal duct lobular unit, thus the term lobular no longer designates its origin [4]. Most ILC express estrogen (ER) and/or progesterone (PR) receptors and thus belong to the luminal intrinsic molecular subgroup of BC [1].

The importance of individualizing this subgroup of BC lies in its incidence and its biological and clinical specificities. ILC incidence has increased over the past two decades

especially in women over 50 [5,6]. This increase has been correlated to the use of hormone replacement therapies, especially regimens containing progesterone [7,8]. It is important to stress that given the high overall incidence of BC worldwide, the current incidence of this most frequent subtype of BC is comparable to the one of brain tumors or myeloma [9].

The relevance of the individualization of the lobular subtype of BC has been challenged by some authors after genetic studies demonstrated common consistent abnormalities shared by *low grade* BC (ductal as well as lobular) (1q gain, 16q loss), as opposed to *high grade* BC (p53 mutations, 17q gains) [10–12]. The arguments in favor of conserving the lobular special type include the high incidence of loss of expression of the cell-cell adhesion molecule E-cadherin, not observed in other low grade BC, and the peculiarities of clinical presentation and metastatic pattern: peritoneum and retroperitoneum, hollow viscera, internal genital organs, leptomeninges (besides common bone metastases) [1,13–18].

Heterogeneity has long been recognized among *in situ* as well as invasive lobular BC. Classic-ILC is characterized by monotonous small, round, discohesive cells growing in linear strands (so called ‘indian files’) and forming target figures around ducts either preserved or involved by *in situ* lobular neoplasia (Fig. 1) [1]. The cytoplasm is pale or eosinophilic and may contain a small pink vacuole. The nuclear pleomorphism (e.g. nuclear size, shape, hyperchromatism, nucleoli) is low to moderate (Fig. 1). Mitotic figures are sparse. Host reaction varies from none to abundant fibrosis. ILC is commonly admixed with an *in situ* component that may be of lobular (lobular *in situ* carcinoma/lobular neoplasia) but also of low grade ductal type [11]. Morphological variants differ from the classic form either by their architectural pattern of growth (solid, alveolar, and trabecular variants) or by their cytology (pleomorphic, apocrine, histiocytoid, signet ring cell variants) [19–21]. Based on pattern of growth at low

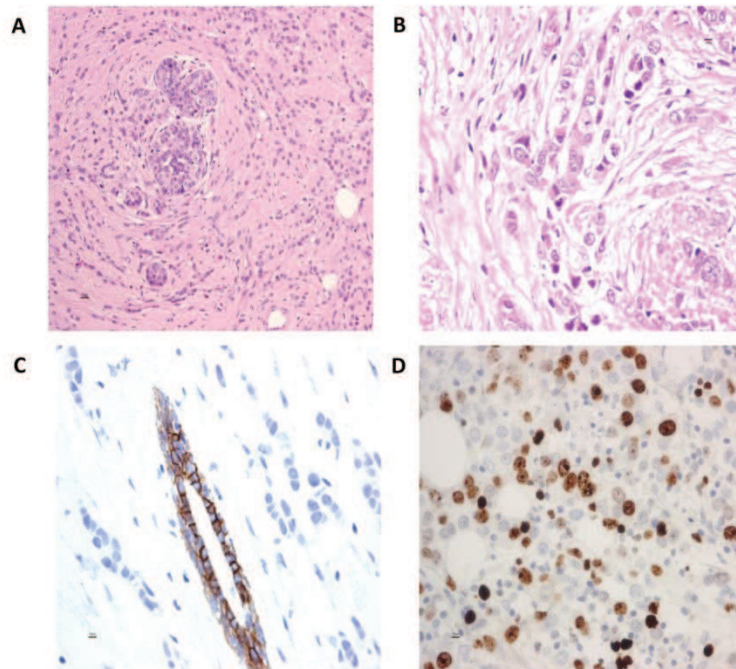


Fig. 1. Morphological aspects of classic and pleomorphic invasive lobular carcinoma. (A) Invasive lobular carcinoma, classic type. (B)–(D) Invasive pleomorphic lobular carcinoma: pleomorphic atypical nuclei (A); loss of E-cadherin expression (C) (E-cadherin is expressed in normal ductal cells); and Ki-67 immunostaining (D).

magnification, Tot also proposed to individualize diffuse ILC, estimated to represent 69% of cases, from uni- or multifocal classic ILC [22]. The most recent WHO classification of BC recognizes 4 histological ILC variants: solid, alveolar, pleomorphic, and tubulo-lobular ILC [1]. In the solid and the alveolar variants typical lobular cells grow in sheets or round aggregates [1]. The *tubulo-lobular* variant (tubulo-lobular carcinoma, TLC), first described by Fisher et al. in 1977 is characterized by a classic cytology and the association of small round tubules admixed with files and a diffuse and targetoid pattern of infiltration [20]. The *pleomorphic* variant (pleomorphic invasive lobular carcinoma, PILC), described by Dixon et al. in 1982, is characterized by tumor cells with an abundant, finely granular, eosinophilic cytoplasm, increased nuclear pleomorphism (large vesicular nuclei, prominent nucleoli) and/or increased mitotic count with a classic or trabecular or mixed architecture (Fig. 1) [21].

Conflicting data are found in the literature regarding the specific outcome of patients with ILC. Moreover, some evidence suggests that the effectiveness of cytotoxic chemotherapy is low in this subgroup [23–25]. The use of genomic signatures to assist systemic treatment decisions, and especially the decision of adding chemotherapy to endocrine therapy (ET), is supported by several authors

for BC in general [26]. However, most correlations between molecular signatures and patient outcome were derived from studies based on IDC, and rarely special types of BC. Making systemic therapy decisions for patients with ILC, either in the neoadjuvant or in the adjuvant setting is thus challenging for the oncologist: (i) it requires to extrapolate evidence obtained mostly in patients with IDC to patients with ILC, a biologically at least partly different BC; (ii) due to the heterogeneity of ILC some generic considerations about ILC may possibly be less or not relevant to some patients with variants of ILC.

Moreover, given the current understanding of BC biology, a relevant evaluation of the actual impact of lobular histology on various clinical endpoints would require to compare ILC within a matched molecular class of BC (as defined by microarray data, *i.e.* mostly the luminal subgroup) rather than with overall ductal BC, as was done in many studies in the past.

Our aim was to review the literature for available data on (i) the specificity and heterogeneity of ILC biology as documented by various analytical techniques during recent years, and (ii) the impact of lobular histology on prognosis and prediction of effectiveness of systemic therapies in patients with invasive BC.

## 2. Methods

### 2.1.1. For part I (specificities and heterogeneity of ILC biology)

After performing a MEDLINE search that proved ineffective, we started from the reference lists included in the 2012 WHO classification of breast tumors and two recent reviews [1,14,27]. In addition, for Section 3.1.7, we searched the MEDLINE database using the combination of the terms “invasive lobular carcinoma” and “genetics. We further selected papers by reading the abstracts applying the following criteria: English language, results relevant to our focus (biological specificity and heterogeneity of ILC), and human tumors. All together, we analyzed 87 papers published between 1989 and 2013.

### 2.1.2. For part II (impact of lobular histology on prognosis and treatment effectiveness)

We searched the MEDLINE database using the terms “invasive lobular carcinoma” as of February 2013. We considered papers in English language only and excluded those with mixed histology. Publications were classified in three categories: prognosis, response to chemotherapy, response to ET. Initial results were reviewed and cross-referenced to ensure that all eligible studies were captured. The same methodology was applied with the terms “pleomorphic invasive lobular carcinoma” and “tubulo-lobular carcinoma” (Fig. 2).

In order to evaluate the response of ILC to chemotherapy or ET, we have chosen to consider the response to neoadjuvant therapy as well as selecting some major randomized adjuvant trials. Indeed, neoadjuvant therapy offers a unique opportunity to determine the tumor’s *in vivo* drug sensitivity. Lobular tumors are often ER positive, and thus patients frequently received neoadjuvant ET. We also selected the adjuvant randomized trials with ET wherein all patients received ET, in order to analyze the outcome according to histology of patients who received a homogeneous treatment. For this, we searched the MEDLINE database using the terms “breast cancer and neoadjuvant endocrine therapy”, “breast cancer and adjuvant endocrine therapy”, “breast cancer and neoadjuvant chemotherapy” and considered only randomized trials (phase II and III). To evaluate the response to ET in the metastatic setting, we searched the MEDLINE database using the terms “metastatic breast cancer and endocrine therapy” and considered only phase III randomized trials.

To study the ability of histology to accurately predict response to chemotherapy or recurrence risk, we analyzed univariate and multivariate models that were reported. If more than one model was carried out on the same study to test different endpoints, we considered the one that was specified as the primary end-point except if several end points were pre-specified.

## 3. Results

### 3.1. Part I—Biology of ILC (a heterogeneous entity)

Invasive lobular carcinoma was first described by Foote and Stewart [28]. The morphological heterogeneity of this subgroup of BC was recognized and first classified as several histological variants in 1976 [14,19]. Over time, the use of various newly available techniques, including IHC, comparative genomic hybridization (CGH) and transcriptome/genome analysis further acknowledged the specificities and also the heterogeneity of ILC.

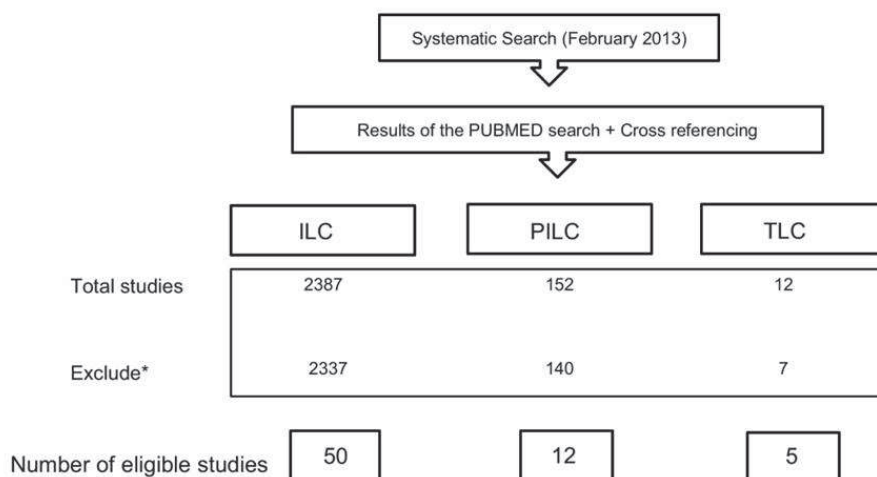
#### 3.1.1. Classic ILC and histological variants

In their study of 4987 cases, Rakha et al. identified 55% classic ILC and, respectively, 5%, 2% and 4% solid, alveolar, and tubulo-lobular variants, besides 34% lobular mixed cases [29]. Iorfida et al. classified 981 cases of pure ILC as 55.8%, 14.9%, 3.8% and 14.8% as classic, solid, trabecular, and mixed non-classic (including the PILC) variants [30]. Among the 860 ILC cases studied by Moeller Talman et al., 82% were pure ILC and 83% of these pure ILC cases were classic-ILC, while non-classic ILC cases were pleomorphic (34%), solid (17%), tubulo-lobular (10%), alveolar (6%), signet-ring cell (3%) or mixed (28%) variants [6].

**3.1.1.1. Outcome according to subtype.** In the study by Iorfida et al., including patients with an initial diagnosis of BC between 1994 and 2005, patients with classic-ILC showed longer disease-free survival (DFS) (Hazard Ratio [HR]=0.67, 95% confidence interval [CI] 0.50–0.90) and breast-cancer specific survival (BCSS) (HR=0.56, 95% CI 0.37–0.85) than patients with non-classic ILC [30]; overall survival (OS) was worse for patients with the solid and the mixed-non-classic variants (HR=2.44, 95% CI 1.39–4.29 and HR=1.99, 95% CI 1.12–3.53, respectively) [30]. A better prognosis for patients with classic ILC as compared to variants was also observed in previous studies [31,32]. A poorer prognosis for PILC in particular has been reported in several studies [33,34]. TLC was shown to be more often multifocal and associated with nodal involvement as compared with tubular carcinoma in 2 series including 90 and 10 pure tubular carcinomas and 17 and 19 TLC, respectively [35,36]. The 12-year actuarial survival rate was 100% for 23 cases of TLC in the study by Du Toit et al., better than for all other ILC variants [32].

#### 3.1.2. Histological grading of ILC

The prognostic utility of grading ILC has been controversial [37,38]. Several studies demonstrated, however, an independent prognostic value for histological grading in ILC as well as in non-lobular subgroups of BC [6,29,38,39]. The distribution of ILC among the three categories of histological



\* Title/abstract not relevant, systematic reviews, mixed histology

ILC = invasive lobular carcinoma; PILC = pleomorphic invasive lobular carcinoma; TLC = tubulo-lobular carcinoma

Fig. 2. Flowchart of eligible studies.

grade varies in the literature. Table 1 shows that the proportion of grade 3 varies from 0 to 16% in different series, while the proportion of grade 1 varies from 3% to 48% [6,29,30,32,39–42]. Rakha et al. classified 517 classic ILC cases as 12%, 76%, and 12% Nottingham/Elston and Ellis histological grade 1, 2, and 3, respectively [29]. Around 80% of these cases showed a '3+2+1' (no tubule formation: score 3; moderate degree of nuclear pleomorphism: score 2; and a low mitotic index: score 1) profile [29]. Significant differences in survival were observed in this study for patients with grade 1 vs grade 2, and grade 3 vs grade 2 ILC. Among the three criteria of grade, the mitotic count showed the strongest correlation with poor outcome [29]. In another study, in which 78% of classic ILC were similarly classified as grade 2, a

difference in survival was limited to grade 3 as compared to grade 1 or 2 [6].

There is a significant relationship between histological subtype and grade, as grade 3 tumors are overrepresented among non-classic variants (38% to 58.6%) [6,29,30]. Rakha et al. recently showed that grade 3 PILC, characterized by a '3+3+2 or 3' profile (tubules 3, nuclear pleomorphism 3, mitoses 2 or 3), had a worse prognosis than grade 2 ILC ('3+2+1'); while within grade 2 ILC, scores with either moderate or marked nuclear pleomorphism (e.g. '3+2+1' or '3+3+1'), were associated with a similar prognosis [43]. TLC is of grade 1 because of its characteristic tubular differentiation and had an excellent prognosis in one published series of 23 cases [32].

Table 1  
Distribution of ILC cases among three categories of histological grade.

References	Number of ILC cases	Histological Grade 1 (%)	Histological Grade 2 (%)	Histological Grade 3 (%)	Histological Grade ND (%)
Sastre-Garrau et al. [39]	726	48	43.5	3	5.5
Talman Moller et al. [6]	860	20	74	6	0
Rakha et al. [29]	517	12	76	12	0
Iorfida et al. [30]	981	35	52	13	0
Toikannen et al. [42]	217	34	54	12	0
Du Toit et al. [32]	171	20	64	16	0
Bane et al., 2005 [40]	50	20	66	14	0
Mathieu et al. [41]	38	3	97	0	0

ILC—invasive lobular carcinoma; ND—not determined.

### 3.1.3. Immunohistochemistry

Loss of *E-cadherin* expression on tumor cell membranes is a distinctive feature of ILC routinely used by pathologists to confirm the diagnosis of this histologic subtype [14,44]. However, this feature is lacking in about 13% of ILC while observed in up to 7% of non-lobular BC [45,46]. This loss results from the inactivation of the CDH1 gene at 16q22 by various different possible mechanisms including mutation of the CDH1 gene, a physical loss of this chromosomal region, or CDH1 promoter methylation [14]. Breast cancer genome sequencing analyses have confirmed that CDH1 gene mutation and down-regulation is a marker event of lobular BC [47]. It has been suggested that the loss of this cell adhesion protein may offer an explanation for the specific metastatic pattern of ILC [15–18]. Loss of E-cadherin expression in ILC cells is associated with abnormalities in catenin expression, resulting in a dysfunctional E-cadherin–catenin complex with consequences on cell-cell adhesion as well as inter-cellular and intracellular signaling [36,46,48,49]. Loss of E-cadherin is observed in all histological ILC variants, except for TLC [36,50,51].

The ER is expressed by ILC tumor cells in 85% to more than 96% of cases depending on the chosen cut-off value [17,30,40,52,53]. The PR is also frequently expressed. In a recent study of 417 pure ER+ (luminal) ILC cases, the proportions of different combinations of ER with PR and HER2 positivity were, respectively, 83.7% for ER+ PR+ HER2–, 10.1% for ER+ PR– HER2–, 4.8% for ER+ PR+ HER2+, and 1.4% for ER+ PR– HER2+ [54]. The rate of ER expression is high in TLC (100% of cases) [36,48], and variable in PILC where it can be as low as 20% of cases [55].

*HER2* protein overexpression or *HER2* gene amplification have been reported in <1% to 6.2% of all ILC cases [54,56–58] and in 2% to 31% of PILC [43,59,60]. The highest rates of *HER2* positivity (31%) have been reported in grade 3 PILC [59]. Among the 3401 patients with *HER2* positive BC included in the HERA trial (evaluating adjuvant trastuzumab), 5.5% [187] had ILC, including 55.3% ER+ and 44.7% ER– cases [61]. No difference in benefit from trastuzumab was observed in ILC as compared to IDC *HER2*+ BC patients in this study (that did not include mention of ILC variants) [61].

Few ILC cases exhibit a triple negative phenotype (1.5% in the study by Iorfida et al. [30]).

Another steroid hormone receptor frequently expressed in BC is the androgen receptor (AR). Interestingly, it might be more frequently expressed in ILC than in IDC as Riva et al. found expression of the AR in 87% of 38 consecutive ILC cases, as compared to 60% in IDC [62]. Similar results were found by Rakha et al. in 189 ILC cases [29].

### 3.1.4. CGH studies

Classic ILC demonstrates the type of copy number abnormalities shared by the “low grade” sub-group of BC *i.e.* frequent gains at 1q and losses at 16q together with overall less complex karyotypes than seen in non-lobular BC

[50,63–69]. Simpson et al. have proposed a ‘lobular genotype’ including gain of chromosome arms 1q and 16p, in addition to loss of 11q and 16q. They also showed that PILC shares many abnormalities with classic ILC as a result of a molecular evolution partly along the low grade pathway [50]. PILC however harbors additional genomic changes (*e.g.* *TP53* stabilization, amplifications of *HER2/TOP2A*, *MYC*, *MDM2* and 20q13) conferring to this variant a high grade phenotype [50]. These authors also suggested that PILC shares genotypic and phenotypic characteristics with BC in *BRCA2* mutation carriers [50].

### 3.1.5. Genome-wide transcription analyses

Several studies addressed the question of the specificities of the transcriptomic profile of ILC as compared to IDC [27,67,69–71].

Weigelt et al. found differences in expression of 5.8% of genes in ILC as compared to grade and molecular sub class-matched IDC [27]. The transcriptomic profile specific of ILC identified in this study included changes in expression of genes involved in cell adhesion, cell to cell signaling, and actin cytoskeleton signaling [27]. According to these authors, who studied 20 ILC cases including 11 classic forms and 9 pleomorphic variants, classic and pleomorphic ILC are remarkably similar at the transcriptome level and fall mainly into the molecular subgroup of the luminal type [27].

Three studies identified heterogeneity among ILC with a typical, homogeneous subgroup representing about 50% of cases, and an atypical subgroup with a less homogeneous profile more closely resembling IDC [67,70,71]. In the study by Bertucci et al., the typical subgroup profile consisted of less proliferation and more differentiation genes expressed together with few chromosomal changes (simplex type), whereas the atypical subgroup exhibited an opposite profile regarding the expression of proliferation and differentiation genes as well as greater chromosomal instability [67]. Weigelt et al., who compared the transcriptional profiles of ILC and IDC cases matched for molecular subgroups, did not find this heterogeneity [27].

### 3.1.6. Intrinsic molecular subtypes

Over the past decade, microarray-based gene expression studies have made it clear that BC comprises a heterogeneous group of diseases that have distinct molecular features. Based on hierarchical clustering of gene expression profiles of ~400 BC samples, 5 distinct ‘intrinsic’ subtypes have been identified: luminal A, luminal B, *HER2*-enriched, basal-like and normal-like [2,72–74]. This classification is of prognostic significance [72–74]. The BC cases included in these studies were overwhelmingly of ductal type. Some studies however included cases of ILC [2,27,72,73,75–84], but only few of them reported in which molecular subtype ILC were classified [27,73,80,82] (Table 2). Among 6 ILC classified as triple negative by IHC, 5 were classified as non-basal tumors and one was considered basal-like in the study by Bertucci et al. [75]. Most of the nine cases of ILC reported by Perreard et al.

Table 2  
Distribution of ILC cases among intrinsic molecular subtypes (by gene expression profiling and by immunohistochemistry).

Reference; number of ILC	Intrinsic molecular subtypes, gene expression profiling				
	Luminal A	Luminal B	HER2-enriched	Basal-like	Normal-like
Sorlie et al. [73]; n = 5	2	1	0	1	1
Kreike et al. [80]; n = 1	0	0	0	0	1
Nielsen et al. [82]; n = 61	32	21	4	1	3
Weigelt et al. [27]; n = 20	13	3	1	1	2
	Intrinsic molecular subtypes, Immunohistochemistry classification				
	Luminal A	Luminal B	HER2 positive	Triple negative	
Cheang et al. [86]; n = 197	133	64 (including 5 HER2+)	-	-	
Hugh et al. [87]; n = 286	90	183*	3	6	
Cancello et al. [89]; n = 164	102	59*	0	3	
Park et al. [88]; n = 27	23	1*	0	3	
Skarlos et al. [90]; n = 37	8	22*	2	5	
Iorrida et al. [30]; n = 981	342	476*	4	15	

ILC—invasive lobular carcinomas.

\* Luminal B = ER+ and/or PR+ and Ki-67 ≥ 14% or HER2-positive.

were classified as luminal or normal-like tumors (66% and 22%, respectively) but one was classified as HER2-enriched [83].

Weigelt et al. in their study of 20 ILC cases (11 classic, 9 PILC) found that 65% segregated into the luminal A (13/20, 8/11 classic, 5/9 pleomorphic), 10% into the luminal B (2/20, 1 classic, 1 pleomorphic). Of the remaining two classic ILC one fell into the normal subclass and one into the HER2-enriched and of the remaining 3 PILC one fell into the normal, one into the basal-like subclass and finally one PILC case was unclassifiable [27].

Using a semi-supervised clustering algorithm based on the analysis of gene expression data from 537 primary breast tumors, Guedj et al. identified six molecular subtypes: luminal A, luminal B, luminal C, basal-like, molecular apocrine and normal-like [78]. The distribution of these subgroups was determined by the combination of the expression of 3 large gene clusters organized around the (i) ER, (ii) AR, and (iii) cell cycle regulator genes. Most of the 28 ILC samples were classified as either normal-like ( $n = 15$ , 54%), or luminal (luminal A,  $n = 5$ , 17%; luminal B and luminal C, each  $n = 3$ , 11%) and the remaining 2 as basal or molecular apocrine (one in each category) [78].

In 2011, BC subtypes as defined by gene expression profiling were included in the 2011 St Gallen International Expert Consensus, and an approximation based on IHC surrogates (ER, PR, HER2, and Ki-67) was proposed using a Ki-67 cut-off of 14% for the distinction between luminal A and luminal B BC [85]. This IHC classification has been applied to a large series of ILC in two recent studies both using a 14% Ki-67 threshold as well as HER2 status to distinguish luminal A (ER/PR+, Ki-67 < 14% and HER2-) from luminal B (ER/PR+, Ki-67 > 14% and/or HER2+) [30,56]. In both studies, based on partly overlapping sample collections, 48.5% of 981 ER and/or PR positive (luminal) ILC cases were classified as luminal B on the basis of either high proliferation or HER2 overexpression or amplification [30]. The rate of luminal B-like cases was 35.9% within the classic ILC

subgroup and 64.1% within the mixed non-classic subgroup (that included the PILC cases) [30]. Other, smaller series reported the IHC classification of ILC cases [30,86–90] (Table 2).

The list of all the selected papers is presented in Supplementary data (Suppl. data 1).

### 3.1.7. Genomic tests

Several genomic tests are currently available for BC. Their aim is to provide prognostic information beyond that provided by classic clinico-pathological characteristics (nodal involvement, tumor size, histological grade, ER status). They were developed to assist physicians in determining if a patient would benefit from adjuvant chemotherapy, particularly in addition to ET in the case of ER+ disease in order to spare patients from unnecessary side effects in the absence of treatment benefit [91]. A majority of these tests have been developed from IDC samples. For this review we decided to analyze six genomic signatures and their relationship to ILC: Oncotype Dx™, MammaPrint®, Genomic Grade (GG), PAM50 (ROR-S), Breast Cancer Index and EndoPredict.

**3.1.7.1. Oncotype Dx™.** Nineteen articles were eligible (complete listing presented in Supplementary data 2) among which six reported the histological type in baseline BC characteristics [92–97]. The proportion of ILC in these studies ranged from 5 to 18%. In most studies, patients with ILC had a low or an intermediate recurrence score (RS) (97–100%, Table 3) [94–96]. In the study by Joh et al., that included 8% of ILC cases, no correlation was found between RS and histologic subtype [94]. Among the 40 patients (13% with ILC reported by Kelly et al., all had node negative disease, 24 (60%) were clinical stage I, 15 (37.5%) stage II and 1 (2.5%) stage IIIB; 28 (70%) had grade 2 tumors, 9 (22.5%) grade 1 and 3 (7.5%) grade 3 (pleomorphic) [95]. These ILC cases were classified as low (67.5%) or intermediate risk (32.5%) [95].



Table 3  
Distribution of ILC cases among genomic tests.

Genomic test	Tumors characteristics	Number of ILC (%)	Results		
			Low RS	Intermediate RS	High RS
Oncotype Dx, recurrence score (RS)	*Early stage ER+ cancer [94]	13 (8.4%)	10 (77%)	3 (23%)	0
	*HR+/HER2-, N0, stage I/II [95]	40 (13%)	27 (67.5%)	13 (32.5%)	0
	*ER+, N0 [96]	35 (11.7%)	21 (60%)	13 (37%)	1 (3%)
	*ER+, HER2-, N0 [97]	19 (18.3%)	6 (32%)	11 (58%)	2 (10%)
MammaPrint	*T1-4 N0 M0, <61 y [99]	47 (11%)	38 (81%)	–	9 (19%)
	*T1-2 N0, 55–70 y [102]	17 (11.5%)	17 (100%)	–	0
	*T1-3 N+ (1–3) [103]	15 (6.2%)	12 (80%)	–	3 (20%)
	*N+ (4 to 9) [104]	14 (8.1%)	11 (78.6%)	–	3 (21.4%)
	*stage II-III, neoadjuvant [105]	20 (12%)	8 (40%)	–	12 (60%)
	*pT1c-3, N0-3, G2-3, RH+, HER2-, ≥60 y [106]	9 (15%)	4 (44%)	–	5 (56%)
				Good prognosis	–
Genomic Grade	*pT1-2,N0,M0 [109]	17 (10.4%)	GG1	Equivocal	GG3
	*Early breast cancer [110]	166 (100%)	13 (76.5%)	0	4 (23.5%)
			106 (64%)	31 (19%)	29 (17%)

ILC—invasive lobular carcinomas; ER—estrogen receptor; HR—hormone receptor.

In the study by Allison et al., the majority of ILC cases in the test set had an intermediate RS and 2 cases (10%) had a high RS [97]. Histologic subtype was not significantly associated with RS category. In the validation set, the authors report one grade 3 PILC case with an intermediate RS [97].

**3.1.7.2. MammaPrint®.** Seventeen articles were eligible (complete listing presented as Supplementary data 3) and among them, eight reported the histological subtype in the baseline characteristics of studied BC [98–105]. The proportion of ILC cases ranged from 6.2 to 15% of analyzed samples. In all the studies but two [100,105], ILC presented mostly a good prognosis 70-gene signature (Table 3). In the study by Mook et al., 80% of patients with ILC had a good prognosis signature as compared to only 35.3% of patients with IDC [103]. The histological subtype was not included in the multivariate analysis of survival [103]. Among 173 patients with 4 to 9 positive lymph nodes, Saghachian et al. reported a low risk of recurrence in 78.6% of patients with ILC compared to 36.8% for IDC patients [104]. However, the histological subtype was not an independent predictive factor for survival, neither in the entire population nor in the luminal A subgroup [104]. Mook et al. selected 964 patients from previous studies with pT1 tumors (including 28% N+ and 71 ILC) [101]. In this population, a poor prognosis signature was associated with IDC histology ( $p < 0.001$ ) and histology was a significant predictor for distant metastasis free survival (DMFS) at 10 years in univariate analysis but not in multivariate analysis [101]. Knauer et al. identified, from a pooled database, 168 chemotherapy naive patients with T1-3 N0-1 HER2+ disease [106]. The cases presenting with a good prognosis 70-gene signature were more likely to have lobular histology (26% vs 4%,  $p = 0.002$ ). However, histology was not included in the multivariate model for survival [106].

In a series of 60 elderly (>60 y) patients with pT1c-pT3, grade 2–3, HR+ HER2– tumors, with 0–3 positive

lymph nodes, Hartmann et al. reported no difference in tumor or patient characteristics between the good- and the poor-prognosis signature group (44% patients with ILC and 59% with IDC had good-prognosis signature,  $p = 0.12$ ) [100].

Straver et al. reported the 70-gene profile of 167 stage II-III patients prior to neoadjuvant chemotherapy and correlated it to pathologic complete response (pCR) rates at the time of surgery [105]. In 20 patients with ILC, 40% had a good-prognosis signature as compared to 10.7% patients with IDC ( $p = 0.008$ ) [105]. None of the good-prognosis signature patients achieved a pCR. Among the 88 patients with ER+, HER2- disease, 3 (3.5%) achieved a pCR but no data regarding the histological subtype of these cases was reported [105].

Unfortunately, in all these studies, no information regarding the pathological characteristics of ILC with poor-prognosis signature was provided.

**3.1.7.3. Genomic grade.** Genomic grade (GG) is a 97-gene signature (mostly genes involved in proliferation and cell cycle control) which was initially designed to improve the discriminatory power of histological grading (HG) with the aim of eliminating the intermediate HG2 and classifying samples only into low grade GG1 or high grade GG3 tumors [107]. Nine articles were eligible (complete listing in Supplementary data 4) and the histological subtype was reported in the baseline characteristics in 3 of these studies [108–110]. Reyat et al. reported results for 17 ILC: 3 were HG1–GG1, 10 were HG2–GG1, 2 were HG2–GG3 and 2 HG3–GG3 [109] (Table 3). Metzger-Filho et al. retrospectively applied the MapQuant Dx™ assay (Ipsogen) to 166 frozen specimen of ILC from several centers, including 87% [144] classic ILC and 13% [22] PILC variant cases. Of these samples 20% were of HG1, 73% HG2, and 7% HG3 [110]. The assay assigned 64%, 19%, and 17% of these cases to GG1, equivocal, and GG3 categories, respectively. Within the HG2 subgroup, 78%

[94/120] of cases were classified as GG1 and 15% (18/120) as GG3, while 21.6% (26/120) of HG2 cases were classified as equivocal for GG. GG3 was associated with a poorer invasive disease-free survival:  $HR_{GG3 \text{ vs } GG1} = 5.6$  (2.1–15.3,  $p < 0.001$ ) and OS:  $HR_{GG3 \text{ vs } GG1} = 7.2$  (1.6–32.2,  $p = 0.01$ ). The three categories of GG were identified in both classic and pleomorphic subgroups of ILC. A GG3 or equivocal status was observed in 31% (45/144) of classic ILC and in 72.7% (16/22) of PILC. Correlation of GG with HG was partial [110]. These authors did not find a correlation between GG and Ki-67 (using a 14% cut-off) in ILC, nor did they observe a correlation between Ki-67 and outcome, unlike Colleoni et al. and Iorfida et al. [30,56].

**3.1.7.4. PAM50.** Seven articles were eligible (complete listing in Supplementary data 5) and among them, only one reported the histological subtype in the baseline characteristics [82]. PAM50 is a quantitative reverse-transcription polymerase chain reaction (qRT-PCR) assay based on a reduced gene set which identifies the major intrinsic biological subtypes of BC and generates risk-of-relapse (ROR) scores [111]. PAM50 was performed on 786 tumors, including 61 (7.8%) ILC [82]. All tumors were ER+ and patients were uniformly treated with adjuvant tamoxifen [82]. No information regarding the ROR-score among ILC cases was provided and histological subtype was not included in the multivariate analysis for disease-specific survival [82].

**3.1.7.5. Breast cancer index and EndoPredict.** Breast cancer index is a continuous risk prediction model that combines the ratio of expression levels of the HOXB13 and IL17BR genes and the 5-gene Molecular Grade Index, that assesses estrogen-mediated signaling and tumor grade, respectively [112]. EndoPredict (EP) is a mRNA-based qRT-PCR multi-gene score using 8 genes of interest and three normalization genes on formalin fixed paraffin embedded tissue; the combination of EP with tumor size and nodal status resulted in the EPclin [113]. Five and two relevant articles were, respectively, selected for these two signatures (complete listing in Supplementary data 5). None of these articles reported the histological subtypes of the tumors.

### 3.1.8. Latest molecular advances

Among the 510 breast tumor samples submitted to the multiplatform analysis approach used by the Cancer Genome Atlas Network working group, 36 were ILC (7%) [47]. This study confirmed that somatic mutations in the *PIK3CA* oncogene are frequent (39%) in luminal BC in general and are also found in ILC (5/36, 14%) [47]. Mutations in the *PIK3CA* oncogene had previously been reported in 11% to 53% of ILC primary tumors [114–116], more frequently affecting the helical domain (exon 9) than the kinase domain (exon 20) in this subtype of BC [115]. A recent study has shown a significant increase of these mutations in loco-regional recurrence (but not in distant metastases) of ILC as compared to matched primary tumors [117]. The TCGA analysis

further identified eight somatic mutations of the ERBB2 gene (HER2). Four out of these eight somatic variants were found to be enriched in ILC and three out of these four mutations occurred in the kinase domain likely leading to constitutive activation. The ILC cases harboring HER2 mutations were otherwise classified as luminal A in this study [47]. These *HER2* gene mutations have been shown to be targetable by anti-HER2 therapies [118]. While further studies are warranted to define the frequency of these events in a greater number of ILC tumor samples, these data suggest a potential benefit of new targeted therapeutic approaches in patients with ILC, especially in those with endocrine therapy resistant disease.

### 3.1.9. Conclusions

In summary, this review of the literature on ILC biology shows, despite some inter-study differences, a picture of ILC as a group of BC presenting with at least two facets (Tables 3 and 4). On the one hand 12 to 48% of ILC present histological grade I, a proportion that can go up to 64% when looking at GG. On the other hand, up to 40–48% of cases are classified as luminal B and associated with poorer outcome [30,56]. Finally 36% of cases are classified as either GG3 or equivocal for GG [110] and also associated with increased risk of relapse. In these subgroups of ILC with a significant risk of relapse, effective systemic therapies need to be identified to lower this risk. Thus, results of genomic tests to determine if patients with ILC would benefit from adjuvant chemotherapy may prove beneficial but need to be studied specifically in this subgroup of BC.

Few studies analyzing gene expression based prognostic assays have included patients with ILC and even fewer studies specified the different variants of ILC. The data obtained through these studies tend to confirm the heterogeneity of ILC at the molecular level. They point out a subgroup of BC with lobular histology that is associated with molecular profiles considered as indicative of high risk of disease recurrence. However this data is derived from studies conducted overwhelmingly in non-lobular BC and therefore needs to be interpreted with caution.

To conclude, in our opinion, further research into the clinical utility of these different genomic prognosis signatures for ILC is warranted. It would be particularly interesting to obtain data on histological type from studies with EndoPredict score, which could identify late relapse events.

## 3.2. Part II—Prognosis and systemic therapies in ILC

It is generally believed that ILC is a BC subtype with a better prognosis and rather indolent outcome as compared to IDC. It is also felt that as a hormone sensitive BC it responds poorly to cytotoxic chemotherapy. Hereunder we provide data from the literature supporting as well as contradicting these opinions. We will successively address the following questions:

- (1) What is the typical clinical course of ILC?

Table 4  
A practical approach to invasive lobular carcinomas biological heterogeneity.

Method, technique	Information	Higher risk of distant relapse	Main references
Morphology (Hemalun & Eosin stained sections)	Histological subtype: classic ILC, variants Histological grade (Elston and Ellis): Grade 2 most common	Pleomorphic or solid variants Grade 3 (3+3+2 or 3)	[30,33,34] [6,29,43]
Immunohistochemistry	ER, PR HER2  Ki-67	ER and PR negative Rare Up to 6.2% HER2 positive ILC Benefit from trastuzumab similar to HER2 positive ductal/NST breast cancer High* in around 40% ILC cases (*14% threshold in the literature)	[17,30] [56–58,61]  [30,56]

ILC—invasive lobular carcinomas; ER—estrogen receptor; PR—progesterone receptor; NST—no special type.

- (2) How chemoresponsive is ILC?  
(3) How effective is endocrine therapy in ILC?

### 3.2.1. What is the typical clinical course of ILC?

Several studies have concluded that patients with ILC had a favorable prognosis compared to patients with IDC [18,42,119–124], while others have found that their prognosis is similar [17,31,125–145], worse [56] or depending on follow-up duration [52,146].

**3.2.1.1. Better outcome for ILC than IDC patients.** Several studies reported a better outcome for patients with ILC compared to patients with IDC [18,42,119–124]. Results are summarized in Table 5, Part 1.

**3.2.1.2. Similar outcome for ILC compared to IDC.** Several retrospective studies have reported similar outcome between patients with ILC and IDC but with many variations in study design [17,125–139]: sample size, tumor stage, ethnic group, treatment, follow-up duration to name a few. Although patients with ILC had often tumors with more favorable biology than IDC (grade 1 or 2, ER+, HER2– tumors), their prognosis was similar to that of IDC patients [125,130,133,135,136]. Histological type is not an independent prognostic factor in these studies, but some clinico-pathological characteristics are frequently found as prognostic: age [17,130,133,135,136,138], tumor size [17,130,135,137,138], nodal involvement [17,130,135,137–139], TNM stage [133,136,139], HR status [17,135–137,139], tumor grade [135,137].

We summarized the results of studies with matched design in Table 5, Part 2.

**3.2.1.3. Worse outcome for ILC compared to IDC.** Patients with ILC had worse survival in the study by Colleoni et al. [56]. These authors reported the outcome of 7372 women with luminal BC (IHC-based classification); 5707 (76.2%) with IDC, 851 (11.4%) with ILC and the remainders with mixed tumors or other histological subtypes [56]. After a median follow-up of 5.8 years, they reported a worse DFS for patients with ILC, both overall (HR = 1.27, 95%CI 1.05–1.53,  $p=0.01$ ) and within the subgroup of luminal B

tumors (HR = 1.26, 95%CI 1.01–1.58,  $p=0.04$ ) [56]. Worse distant metastasis-free survival was also reported among all patients (HR = 1.48, 95%CI 1.16–1.88,  $p=0.002$ ), among the subgroup with luminal B tumors (HR = 1.46, 95%CI 1.11–1.92,  $p=0.007$ ) and among the subgroup with grade 1 tumors (HR = 2.83, 95%CI 1.16–6.91,  $p=0.002$ ). Patients with ILC also had significantly poorer OS (HR = 1.34, 95%CI 1.03–1.74,  $p=0.01$ ) [56]. The multivariate model was adjusted for luminal subtype, tumor size, nodal status, peritumoral lympho-vascular invasion and Ki-67 (20% cut-off) [56], but not for the type of adjuvant therapy (e.g. anthracycline-containing chemotherapy was proposed to 26.7% of patients with IDC and 18.6% of patients with ILC) [56]. In the study by Iorfida et al., based on partly similar cohorts of cases, patients with classic ILC showed longer DFS (HR = 0.67, 95%CI 0.50–0.90,  $p=0.007$ ) and BCSS (HR = 0.56, 95%CI 0.37–0.85,  $p=0.007$ ) than patients with non-classic ILC [30]. Among specific subtypes, OS was worse for patients with the solid (HR = 2.44, 95%CI 1.39–4.29) and the mixed non-classic subtypes (including PILC) (HR = 1.99, 95%CI 1.12–3.53) [30].

A poorer prognosis for PILC has also been reported in some studies [33,34,147,148] while others did not clearly demonstrate an impact of subtypes on patient survival (compared to classic ILC [149] or to IDC [150]).

### 3.2.1.4. Different outcome according to follow-up duration.

In two studies, the DFS and OS curves crossed with an initial favorable prognosis for ILC becoming unfavorable with longer follow up in this subgroup [52,146]. Indeed, in a large study of 15 adjuvant randomized trials (9,374 patients) with a long median follow-up (13 years), there was a statistically significant time-dependent effect of histological type with an early advantage in DFS for the ILC cohort, and an advantage for the IDC cohort after 6 years [146]. Similar observations were made for OS but after 10-year of follow-up [146]. Results remained significant when baseline factors (tumor size, grade, nodal involvement, vascular invasion, ER status, menopausal status and age) were included in multiple regression analyses [146]. Results within ER+ and ER– cohorts showed a similar pattern, although differences are more pronounced for the ER– cohort [146]. These conclusions remained unchanged when patients with

Table 5  
Comparison of outcome between patients with IDC and patients with ILC.

References	ILC, n	IDC, n	Primary objective	Median follow-up (years)	Systemic treatment	Univariate analysis/results	Multivariate analysis results (95% CI)	Comments
Part 1: Better outcome of ILC as compared to IDC								
Wasif et al. [124]	27 639	235 769	5 y BCSS	NR	NR	90% vs 88%, $p < 0.001$	HR = 0.86 (0.80–0.92), $p < 0.001$	Pts matched for stage Similar results whatever the stage
Du Toit et al. [32]	171	342	OS	5.3	No treatment	<0.05	NR	Pts matched for tumor size, nodal involvement, grade, age No difference in terms of HR status
Silverstein et al. [123]	161	1138	–7 y DFS –7 y BCSS	6.6	No treatment	–74% vs 63%, $p < 0.03$ –83% vs 77%, $p < 0.04$	NA	Pts with ILC were older, had larger tumors, with lower nuclear grade and more ER+ status
Bharat et al. [122]	480	3595 (261 mixed)	10 y OS	NR	NR	68% vs 61%, $p = 0.02$	NR	No difference in terms of DFS
Dian et al. [121]	411	1647	OS	4.7	No treatment	HR = 0.73 (0.54–0.98), $p = 0.032$	$p = 0.046$	
Li et al. [119]	19 383	132 284	OS	ILC: 6.4 IDC: 7.0	NR	NR	HR = 0.89 (0.86–0.91) between 1974 and 1998	Data from nine cancer registries, women aged 50 to 79 y, no data regarding HR status 1974–1988: HR = 0.92 (0.86–0.98) 1989–1993: HR = 0.81 (0.76–0.86) 1994–1998: HR = 0.74 (0.67–0.82) Data from The Netherlands cancer registry
Louwman et al. [120]	13 792	2005	Relative 5 y survival rate	NR	43% of pts	NR	RER = 0.82 (0.7–0.9)	
Toikkanen et al. [42]	217	1121	OS	17	8.7% of pts	5 y: 78% vs 63%, $p = 0.001$ 30y: 50% vs 37%, $p = 0.001$	$p = 0.98$	Similar results after exclusion of pts which received adjuvant treatment
Part 2: Similar outcome of ILC as compared to IDC (studies with matched design only)								
Viale et al. [145]	301	301	DFS OS	7.3		DFS: $p = 0.32$ OS: $p = 0.83$	DFS: HR = 1.27 (0.82–1.96) OS: HR = 1.03 (0.49–2.17)	Pts matched for year of surgery, age, menopausal status, tumor size, nodal involvement, HR status and if possible tumor grade 94.9% HR+ Grade 1 or 2 tumors only Pts with ILC had more grade 1 tumors with a low KI-67 index

Table 5 (Continued)

References	ILC, n	IDC, n	Primary objective	Median follow-up (years)	Systemic treatment	Univariate analysis/results	Multivariate analysis results (95%CI)	Comments
Ni Mhuircheartaigh et al. [144]	211	422	OS	NR	Tamoxifen 5 years if ER+	$p=0.93$	NA	Pts matched for age at diagnosis and stage Pts with IDC had more grade 3 (39% vs 18%) and ER-(32% vs 15%) tumors Similar results after stratification by tumor grade but survival was improved in ER+ IDC compared to ER+ ILC ( $p=0.01$ )
Korhonen et al. [143]	295	295	5 y DFS 5 y OS	5.1	Pts with ILC received less treatment (33% vs 44%, $p=0.01$ )	5 y DFS: 75% vs 75%, NS 5 y OS: 80% vs 77%, NS	NR	Pts matched for year of diagnosis and age Pts with ILC had more ER+ tumors (92% vs 77%), low proliferation rate (59% vs 37%) and HER2- tumors (96% vs 78%), $p<0.001$
Fondrinier et al. [142]	188	188	5 y DFS 5 y OS	NR	No difference in terms of treatment	5 y DFS: 69.9% vs 66.9%, NS 5 y OS: 83.2% vs 75.8%, NS	NR	Pts matched for year of diagnosis, age, menopausal status, TNM stage No difference in terms of HR status
Di Costanzo et al. [31]	140	140	DFS	NR	No treatment	Stage II, NS	NR	Pts matched for age, menopausal status, tumor size and nodal involvement Higher DFs of pts with stage I ILC compared to stage I IDC ( $p=0.02$ )

IDC—invasive ductal carcinoma; ILC—invasive lobular carcinoma; pts—patients; HR—hormone receptor; ER—estrogen receptor; NR—not reported; NA—not applicable; BCSS—breast cancer specific survival; OS—overall survival; DFS—disease-free survival; NS—no significant; RER—relative excess risk of death.

Table 6  
Comparison of prognosis between ILC and IDC.

Prognosis (comparison ILC and IDC)	Number of studies/endpoint		
	OS	BCSS	DFS
Better outcome of ILC	5	2	4
Similar outcome of ILC	17	2	–
Worse outcome of ILC	1	1	
According to follow-up duration	1		

ILC—invasive lobular carcinoma; IDC—invasive ductal carcinoma; OS—overall survival; BCSS—breast cancer specific survival; DFS—disease-free survival.

lobular ER– (17.3%), lobular with vascular invasion (19.1%) or lobular ER– with vascular invasion tumors were excluded [146].

A large consecutive series of primary invasive BC including 415 (8%) patients with ILC with a long follow-up (up to 25 years) was reported by Rakha et al. [52]. A trend toward better survival for ILC patients was observed during the initial years of follow-up (10 years), then the two survival curves converged and finally ILC patients showed a continued decline while IDC patients showed a plateau [52]. Similar patterns were observed in both ER+ and ER– subgroups. Tumor type was independently associated with survival in a multivariate analysis (HR = 1.16, 95%CI 1.01–1.3,  $p = 0.034$ ) [52]. In this study, 37% of ILC and 38% of IDC patients received ET. The authors suggest that ILC showed a better response to adjuvant ET and therefore a trend of improvement of survival compared with matched IDC patients in this subgroup (Log Rank [LR] = 3.3,  $p = 0.071$ ) [52]. In the subgroup of patients with moderate Nottingham Prognostic Index (NPI) who did not receive ET, they reported a significantly worse BCSS (LR = 7.6,  $p = 0.006$ ) and distant metastasis free survival (DMFS) (LR = 5.8,  $p = 0.02$ ) for patients with ILC [52]. On the contrary, in the subgroup with moderate NPI who received ET, a significantly better DMFS (LR = 4.7,  $p = 0.03$ ) was observed for patients with ILC compared to patients with IDC [52]. The BCSS was also better in the ILC group overall, but this was not statistically significant. However, the number of patients with ILC in the different subgroups was relatively small. Using a multivariate analysis, the authors showed a significant interaction test between histologic type and ET [52].

**3.2.1.5. Discussion/conclusions.** More studies reported a similar outcome for patients with ILC compared to patients with IDC than better or worse outcome (Table 6). However, the variability of the results might be due to relatively small sample sizes and/or different prognostic factors such as tumor size, nodal involvement, tumor grade, ER status or adjuvant therapy. Consequently, some studies have used a matched design to allow for imbalances of prognostic factors other than histology and to account for later diagnosis of ILC [18,31,124,141–145]. Among them, two studies with patients matched for stage or stage, grade and age concluded to a better prognosis of patients with ILC [18,124]. In five other studies

Table 7  
Evaluable multivariate models for prognosis.

Number of unique patients	
ILC	108 724
IDC	864 198
Number of multivariate models	27
Histological subtype is significant ( $p < 0.05$ )	
ILC < IDC	4
ILC > IDC	4
Adjustment factors (%)	
Age	78%
Tumor size	74%
Nodal status	78%
Stage	26%
Tumor grade	63%
ER/HR status	67%
HER2 status	7%
LVI	22%
Ki-67	11%
Menopausal status	11%
Multifocality	11%
Family history	11%
Adjuvant systemic therapy	15%
Type of surgery	11%
Chemotherapy	22%
Endocrine therapy	26%
Radiotherapy	19%
Others*	<10%

ILC—invasive lobular carcinoma; IDC—invasive ductal carcinoma; ER—estrogen receptor; HR—hormone receptor; LVI—lympho-vascular invasion.

\* Luminal subtype A/B, race, residual tumor, margins, S-phase, year of diagnosis, SEER historic, SEER registry, mitotic count, presence of tumor necrosis.

with this design, patients with ILC and IDC had similar outcome [31,142–145]. However, only one of these studies took into account HR status and none included tumor grade into the eligibility criteria. Unfortunately, no study incorporated in its design all the major clinic-pathological characteristics (stage, grade, age, HR status).

Results of multivariate analyses are interesting to determine if histological type is an independent prognostic factor. The results of the multivariate analyses included in all the studies are summarized in Table 7, as well as the other adjustment factors. Finally, there are as many models for a better outcome for ILC patients as for IDC patients. However, only few multivariate analyses provide significant results (8/27, 30%). Moreover, only two (of 34) studies compared luminal ILC to luminal IDC and these results are conflicting, showing either worse [56] or similar prognosis of luminal ILC compared to luminal IDC [145].

All these studies were retrospective. Lobular tumors are often ER+ and consequently patients with ILC often received adjuvant ET. Consequently, we searched for prospective randomized adjuvant trials with ET and looked at the prognostic value of histology within these cohorts. We identified 14 phase III randomized trials with adjuvant ET: comparisons of 5 years of tamoxifen vs 5 years of letrozole ( $n = 1$ ) or anastrozole ( $n = 2$ ); sequencing treatments ( $n = 7$ ); extended ET ( $n = 3$ ); comparison between 5 years of exemestane and

anastrozole ( $n = 1$ ) (complete listing in Supplementary data 6). Thirteen of these trials randomized post-menopausal women. Unfortunately, only 2 (14%) studies reported the percentage of patients with ILC at baseline (4.4% and 14.2%) [151,152] and among them, histology was not used as a stratification factor and was not evaluated for prognosis.

Finally, in our opinion, available data do not allow to conclude if patients with ILC have a similar or different prognosis compared to patients with IDC. This point should be addressed through a prospective study with histological type as a stratification factor. This prospective study would be more interesting in luminal HER2-negative tumors exclusively, given that HR and HER2 status are major independent prognostic factors in BC.

Moreover, we found few data regarding the prognosis of different variants of ILC and these data were retrospective.

### 3.2.2. How chemoresponsive is ILC?

Neoadjuvant chemotherapy (NAC) in BC treatment is now recognized as a standard of care to increase breast conservative surgery (BCS) [153,154]. Its utility is also documented in inflammatory or locally advanced tumors [155]. Long term results of NAC are equivalent to those obtained with adjuvant chemotherapy if locoregional treatments (surgery and radiotherapy) are fully applied [153,154]. Moreover, NAC offers an opportunity to assess *in vivo* tumor chemosensitivity and biological markers. Several large studies have validated pCR as a surrogate marker of survival in certain but not all BC subtypes [156,157]. ILC is usually reported as being less chemosensitive than IDC.

**3.2.2.1. Retrospective studies.** The aim of several retrospective studies was to especially compare pCR rates after NAC between IDC and ILC [23–25,41,158–163] (Supplementary Table 1). In all of them, pCR rates were significantly higher in IDC (9% to 28%) than in ILC (0% to 11%). The level of expression of ER and PR is consistently reported as correlated with the probability of response to NAC [164] and ILC are often ER and/or PR+. In two studies with anthracycline- and/or taxane-based NAC, the pCR rates were compared in patients with ER+ disease with contradictory results [25,81] (Supplementary Table 1). Delpuch et al. reported a significantly higher pCR rate in IDC patients (14%) than in ILC patients (3.5%) [25], whereas pCR rates were similar in the study of Lips et al. (4.2% and 4.3%, respectively) [81]. Despite lower pCR rates, ILC have often a better prognosis in these studies. The relapse-free survival (RFS) and OS were significantly better for ILC patients than IDC patients in two studies (5-years RFS: 87% vs 66%,  $p = 0.004$  [24] and 76.5% vs 61%,  $p = 0.006$  [161]; 5-years OS: 93% vs 70%,  $p = 0.001$  [24] and 92% vs 79%,  $p = 0.02$  [161]). However, histology was not an independent prognostic factor in multivariate analysis [161] (Supplementary Table 2).

NAC in lobular tumors does not seem to improve the BCS rate [165]. This is often due to the diffuse growth pattern of ILC and the difficulty to delineate adequately the

residual tumor after neoadjuvant therapy. Indeed, BCS rates are lower for patients with ILC compared to patients with IDC (Supplementary Table 1).

Joh et al. reported the pathological response of 30 patients with pure ILC (including 7 PILC) who received NAC (53.3% had combination anthracycline- and taxane-based chemotherapy) [166]. All tumors were HR+ and had clinical stage II/III disease. There was no pCR and only one patient (3.3%) was converted from mastectomy to BCS [166].

**3.2.2.2. Randomized trials.** We identified 46 NAC randomized trials; 29 were phase III trials, 16 were randomized phase II studies and 1 was an exploratory retrospective analysis of the AZURE study (complete listing in Supplementary data 7). Among these studies, histology was not used as a stratification factor. In 23 (50%) of them, the proportion of patients with ILC was reported among the baseline characteristics and ranged from 2.3% to 20.2%.

The pCR rate according to histology was reported in nine studies, with different definitions [167–175] (Table 8). In all of them, pCR rates were higher in IDC (5.9% to 26.3%) than in ILC (0% to 14.3%). However, these results were statistically significant in only four studies [169,172–174]. In multivariate analysis, histology was an independent predictive factor of pCR in only two studies [172,174].

Von Minckwitz et al. reported the prognostic relevance of pCR in various intrinsic subtypes from a pooled analysis of seven anthracycline-taxane-based NAC German trials (including the GEPARTRIO [170–172] and GEPARQUINTO [175] trials) including 6377 patients [156]. Eight hundred and forty-four (13.6%) patients had ILC. Only 51 (6%) with ILC had a pCR (ypT0 ypN0) compared to 800 (16.1%) patients with IDC ( $p < 0.001$ ) [156]. In the ILC group, patients with HR+, triple-negative and HER2+ tumors had pCR rates of 5%, 18.3% and 9.2%, respectively [176]. One of 35 patients with grade 1 and HR+ ILC had a pCR [176]. Predictors of pCR for ILC were age, grade and HR status. In this study, pCR was predictive for neither DFS nor OS in subgroups associated with lower proliferation: lobular type, grade 1 and positive ER or PR status [156]. In contrast, pCR was predictive for DFS and OS in ductal or other histological types of grade 2 or 3 and negative ER status [156].

**3.2.2.3. Discussion/conclusions.** We found no study reporting a significantly higher pCR rate in ILC patients compared to IDC patients. However, very few of the aforementioned studies have incorporated multivariate analyses to test histological subtype as an independent predictive factor of response. Histology was an independent negative predictive factor of pCR in only two out of the nine reported multivariate models, after adjustment for other factors (Table 9). No study reported the histological type as an independent predictive factor of survival after NAC (Table 9).

Table 8  
Comparison of pCR rates between ILC and IDC/other histology patients (randomized studies).

Tumor staging	NAC	ILC, n	IDC/others, n	ILC, pCR	IDC/others, pCR	Univariate analysis	Multivariate analysis (95%CI)	Comments	Reference
T2-3, N0-1	4 AP vs 4 AC	25	164	4% <sup>a</sup>	8.4% <sup>a</sup>	NR	NR	NA	[167]
Stage II-III	ED 3 vs 6 cycles	7	38	14.3% <sup>b</sup>	26.3% <sup>b</sup>	NR	NR	NA	[168]
T2-T4, N0-N+	4 EC → 4 P vs 3 E <sub>dd</sub> → 3 P <sub>dd</sub> → 3 CMF	75	395	6.7% <sup>a</sup>	19.8% <sup>a</sup>	OR = 3.45, 95%CI 1.35–8.82, <i>p</i> = 0.01	OR = 1.75 (0.58–5.28), <i>p</i> = 0.3		[169]
T1-T4, N0-N+	2 TAC → if responders 4 TAC if no-responders 4 TAC vs 4 NX	43	242	NR	NR	<sup>c</sup> OR = 1.95%CI 0.5–2.1, <i>p</i> = 0.9	OR = 0.9 (0.2–3.1), <i>p</i> = 0.8	Sonographic response to TACX2, clinical tumor size, HR status were predictive factors of pCR	[170]
T1-T4, N0-N+	6 TAC vs 2 TAC → 4 NX	92	526	4.3% <sup>b</sup>	5.9% <sup>b</sup>	OR = 1.38 95%CI 0.48–4.0, <i>p</i> = 0.56	OR = 1.31 (0.16–10.88), <i>p</i> = 0.8	Tumor size and HR status were predictive factors of pCR	[171]
T1-T4, N0-N+	6 TAC vs 8 TAC	177	1090	10.2% <sup>b</sup>	24% <sup>b</sup>	OR = 2.78 95%CI 1.68–4.61, <i>p</i> = 0.001	OR = 2.54 (1.20–5.36), <i>p</i> = 0.014	Age, tumor grade and HR status were also predictive factors of pCR	[172]
T2-T4, HER2-, HR- or HR+ and N+	EC → D ± bevacizumab	208	1543	NR	NR	<sup>d</sup> OR = 0.31 (lobular), 95%CI 0.17–0.54, <i>p</i> < 0.001	OR = 0.75 (0.41–1.37), <i>p</i> = 0.3	Treatment arm, clinical tumor size, grade and HR status were independent predictive factors of pCR	[173]
T2-T4, N0-N+	4 TC ± trastuzumab if HER2+ ( <i>n</i> = 30)	9	58	0% <sup>a</sup>	19% <sup>a</sup>	0.01	OR = 8.47 (1.2–22.5), <i>p</i> = 0.022	Histology was the most independent significant factor associated with pCR, among age, HR status, tumor size, TNBC status	[174]
HER2+ and cT3/4c-d or cT2cN+ or cT1pN+ or HR-	4 EC → 4 D + lapatinib or trastuzumab	17	549	NR	NR	<sup>e</sup> OR = 1.53 95%CI 0.56–4.19, <i>p</i> = 0.41	OR = 1.63 (0.57–4.67), <i>p</i> = 0.36	HR status and treatment arm were independent predictive factors of pCR	[175]

pCR—pathological complete response/pCR<sup>a</sup>—defined as no invasive residual disease in the breast and axilla/pCR<sup>b</sup>—defined as no invasive residual disease in the breast only/pCR<sup>c</sup>—defined as no invasive or non-invasive residual disease in breast and axilla/pCR<sup>d</sup>—defined as no invasive or non-invasive residual disease in breast only/pCR<sup>e</sup>—defined as no invasive or non-invasive residual disease in breast and axilla or no residual tumor in breast but involved lymph nodes.

ILC—invasive lobular carcinoma; IDC—invasive ductal carcinoma; NAC—neoadjuvant chemotherapy; NR—not reported; NA—not applicable.

AP—doxorubicin + paclitaxel; AC—doxorubicin + cyclophosphamide; ED—epirubicin—doctaxel; EC—epirubicin + cyclophosphamide; P—paclitaxel; E<sub>dd</sub>—epirubicin dose-dense; P<sub>dd</sub>—paclitaxel dose-dense; CMF—cyclophosphamide + methotrexate + 5-fluorouracil; TAC—doctaxel + doxorubicin + cyclophosphamide; NX—vinorelbine + capecitabine; D—doctaxel; TC—doctaxel + carboplatin.



Table 9  
Multivariate models that incorporate histology for predicting pCR and survival after neoadjuvant chemotherapy.

	pCR	Survival
Number of unique patients	7376	1317
Number of multivariate models	9	3
Histological subtype is significant ( $p < 0.05$ )	2	0
Adjustment factors (%)		
Age	100	100
Baseline T stage	100	100
Baseline N stage	78	0
Nuclear grade	89	100
HR status	89	100
HER2 status	44	–
Neoadjuvant chemotherapy	78	33
Clinical response	11	33
Surgical treatment	11	33
Pathological response		33
Pathological nodal status		100
Others <sup>a</sup>		–

pCR—pathological complete response; T—clinical tumor size; N—clinical nodal involvement; HR—hormone receptor.

<sup>a</sup> Menopausal status, multifocality, sonographic response.

In addition, the BCS rates were higher in patients with IDC compared to ILC. Nevertheless, NAC allowed a significant percentage of patients with ILC to have BCS.

Thus, in our opinion all these data do not support a policy of withholding NAC only on the basis of lobular histology. It is important to consider other predictive factors, such as tumor grade and ER status. Moreover, in ILC, pCR does not seem to be a suitable surrogate marker of survival, in contrast to certain subtypes of IDC.

**3.2.2.4. Trastuzumab.** Among a recent series including 981 ILC tumors, 4.3% of them were HER2+ and this characteristic was an independent prognostic factor associated with worse survival outcomes [30]. In the HERA trial, patients with HER2+ BC were randomized between observation and 1 year of adjuvant trastuzumab. Of the 3,401 women included in this study, 5.5% and 94.5% had ILC and IDC, respectively [61]. The trend was for patients with IDC to have fewer positive nodes and smaller tumor size. Histological grade 3 was more common among IDC than ILC patients. High Allred scores were more common in ILC for both ER and PR and a trend toward decreased HER2 copy number was observed in the ILC group [61]. After a median follow-up of 4 years, no evidence of a difference in magnitude of trastuzumab benefit was observed between the IDC and ILC subgroups. The DFS Hazard Ratios comparing 1 year of trastuzumab vs observation were 0.63 for ILC (95%CI, 0.34–1.15) and 0.77 for IDC (95%CI, 0.67–0.89;  $p$  for interaction  $n = 0.49$ ). This study however included no central pathology review for BC histologic subtypes [61].

Thus, patients with HER2+ ILC should be offered 1 year of adjuvant trastuzumab, like patients with HER2+ IDC.

### 3.2.3. How effective is endocrine therapy in ILC?

There are few reports on the relationship between histological subtypes and ET response.

**3.2.3.1. Benefit of adjuvant tamoxifen.** The old studies that validated the use of adjuvant tamoxifen in early BC provided no information regarding histology.

Jirstrom et al. investigated the effect of adjuvant tamoxifen according to histological subtype in 454 premenopausal patients with stage II disease included in a randomized trial irrespective of HR status (2 years of tamoxifen vs no treatment) [177]. Patients with ILC had more frequently ER+ tumors (76.7% vs 66.9% of patients with IDC,  $p = 0.01$ ) [177]. In ER+ tumors, tamoxifen was associated with a significantly increased relapse-free survival ( $p = 0.033$ ) for patients with IDC, whereas there was no significant difference for patients with ILC [177]. However, in multivariate interaction analyses, no significant difference was seen in terms of tamoxifen benefit between IDC and ILC patients. Moreover, the number of ILC was very small in this survival study ( $n = 33$ ) [177].

**3.2.3.2. Benefit of adjuvant letrozole.** The effectiveness of letrozole compared to tamoxifen was investigated in 2923 post-menopausal women with ER+ early BC included in the monotherapy arms of the BIG 1–98 trial (centrally-reviewed histology data) [178]. Three-hundred and twenty-four women (11.1%) had ILC (59% luminal A, 22% luminal B) and 2599 patients had IDC (44% luminal A, 36% luminal B). The 5-year DFS estimates were 88% and 84% in the letrozole and tamoxifen arms, respectively, in IDC; 89% and 76% in ILC [178]. The 5-year OS estimates were 94% and 92% in the letrozole and tamoxifen arms, respectively, in IDC; 96% and 86% in ILC [178]. In multivariate models for DFS, significant interaction between treatment and histology was observed (ILC or IDC) ( $p = 0.01$ ) and almost significant interaction for OS ( $p = 0.053$ ) [178]. The DFS hazard ratios for letrozole vs tamoxifen were 0.51 (95% CI, 0.33–0.79) for ILC luminal A, 0.35 (95% CI, 0.21–0.56) for ILC luminal B, 0.93 (95% CI, 0.74–1.17) for IDC luminal A and 0.64 (95% CI, 0.52–0.78) for IDC luminal B [178]. These data suggest that adjuvant letrozole is more effective for luminal B and lobular histology tumors.

**3.2.3.3. Response to neoadjuvant endocrine therapy.** We selected nine randomized neoadjuvant ET studies, including combinations with everolimus (1), gefitinib (2), and celecoxib (1) (complete listing in Supplementary data 8). Three were phase III trials and 6 were randomized phase II studies. Among these studies, histology was not used as a stratification factor. In 4/9, the proportion of patients with ILC was reported among the baseline characteristics and values ranged from 3% to 17.3%. Chow et al. mentioned the histology of the tumors at randomization, but no patients with ILC were included in this study [179].

Dixon et al. reported a very good rate of breast conservation in 61 post-menopausal women with ER+, large operable

or locally advanced ILC who received neoadjuvant letrozole for  $\geq 3$  months [180]. Forty patients with 41 cancers underwent surgery at 3 months or when the cancer was considered operable: the rate of BCS was 81% [180]. There were no pCR [180]. Barnadas et al. reported the efficacy of 6 months of neoadjuvant exemestane in 55 post-menopausal women with ER+ disease [181]. Pathological complete response was observed in only one patient and it was a lobular carcinoma [181].

**3.2.3.4. Response to endocrine therapy in the metastatic setting.** We selected 16 phase III randomized trials with ET in the metastatic setting (complete listing in Supplementary data 9). Eleven studies were in the first line setting while in the five other studies patients had received prior therapy for their metastatic disease. The number of patients with ILC at inclusion was reported in only one study (8.1%) but outcome according to histology was not analyzed [182].

In a retrospective study, Smith et al. reported similar responses in 264 patients with IDC and 33 patients with ILC who received ET (tamoxifen, oophorectomy) for advanced BC [183]. There were 92 (35%) responses among the IDC patients and 9 (27%) among the ILC patients whereas 67% of IDC patients tested had HR+ tumors compared to 90% for ILC ( $p < 0.001$ ) [183]. There was no survival difference between the two groups [183].

Resistance to ET in BC is associated with activation of the mTOR signaling pathway and in two randomized studies, the addition of everolimus (mTOR inhibitor) to ET improved PFS [184,185]. Unfortunately, no data regarding histology was provided in these trials.

**3.2.3.5. Conclusions.** Few studies have reported the efficacy of ET in ILC compared to IDC. Letrozole, in the adjuvant setting, seems to be more effective than tamoxifen in ILC and possibly even more so than in IDC, however further prospective studies, based on this criterion, are essential in order to draw more definitive conclusions.

#### 4. Discussion/conclusion

This review supports the biological specificity and heterogeneity of ILC, at the morphological, gene copy number, and molecular levels. Up to 40–48% of ILC are classified as luminal B and 36% as either GG3 or equivocal for GG. Currently, criteria/tests that have been related to and may help identifying ILC with a higher risk of relapse include histological variants (pleomorphic and solid), histological grade 3, and high cellular proliferation as determined by immunohistochemistry for Ki-67 expression (Table 4). Molecular tests still have to be validated for prediction of relapse in ILC, as they were developed in cohorts including mainly IDC. Tests addressing the risk of late relapse, like EndoPredict, seem likely to prove of particular utility in ILC (given the frequently protracted course of the disease).

Whereas it is generally believed that ILC has a rather indolent outcome compared to IDC, more data are in favor of at least a similar prognosis. Poor outcome is observed in some sub-groups of ILC characterized either by their biology or/and by advanced stage at diagnosis. Whereas it was not within the intended span of this review to focus on these important dimensions of ILC specificities, it has been long recognized that early clinical as well as radiological diagnosis of ILC is hampered by difficulties generally considered to be related to the peculiar growth pattern of this subtype, e.g. in single cells and or diffuse, often infiltrating the breast in between preserved normal ductal structures and with no clearly defined mass. These difficulties are considered to be the main cause for ILC being overall diagnosed at a more advanced staged compared to IDC.

The risk of relapse in patients with lobular invasive BC being significant, it implies that effective systemic therapies need to be identified to lower this risk. In our opinion based on this review:

- The poor response of ILC to cytotoxic chemotherapy compared to non-lobular BC does not support a policy of withholding NAC only on the basis of lobular histology. Moreover, in ILC, pCR does not seem to be a suitable surrogate marker of survival, in contrast to certain subtypes of IDC.
- In the adjuvant setting:
  - Regarding chemotherapy, the clinical validity and utility of genomic tests in lobular BC have to be assessed.
  - Adjuvant letrozole seems to be more effective than tamoxifen in ILC; however further prospective studies taking into account lobular histology are needed in order to draw more definitive conclusions.
  - The interest of extended adjuvant endocrine therapy in ILC, in view of later relapse compared to IDC, has to be evaluated.

This review thus points to a current lack of available evidence to support informed systemic therapy decisions in patients with a lobular BC as compared to other hormonosensitive BC. It points out the need for studies/trials designs aimed at better identifying the specificities of lobular histology, including ILC biological heterogeneity, in relation to response of this special type to new therapeutic approaches and potential predictive markers in BC. It will be of particular interest to pay attention to the specificities of ILC within studies addressing the molecular basis of resistance to hormonal therapy and the future pharmacologic possibilities to circumvent this resistance.

#### Conflict of interest statement

The authors declared no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.critrevonc.2014.07.003>.

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### III – DISCUSSION

Cette revue de la littérature met en évidence la spécificité et l'hétérogénéité biologiques des CLI, d'un point de vue morphologique et moléculaire. Quarante à quarante-huit pourcent des CLI sont classés parmi les tumeurs luminales B, et 36% sont de grade génomique 3 ou équivoque. Actuellement, les critères qui peuvent aider à identifier un CLI de haut risque de récurrence sont : les variants de type pléomorphe et solide, un grade SBR III et une forte prolifération cellulaire (expression du Ki-67 en IHC). L'utilisation de signatures génomiques nécessite une validation dans ce sous-type spécifique, étant donné qu'elles ont été développées principalement chez des patients ayant des CCI. De plus, leur utilisation même pour les CCI, n'est encore pas validée. Les signatures évaluant le risque de rechute tardive, comme EndoPredict, pourraient être particulièrement adaptés dans ce sous-groupe, du fait de leur profil évolutif particulier.

Alors que les CLI sont souvent considérés comme des tumeurs plus indolentes que les CCI, la majorité des données est en faveur d'un pronostic comparable à celui des CCI. Un mauvais pronostic est observé dans certains sous-groupes de CLI, caractérisés par une biologie agressive et / ou un stade avancé de la maladie. En effet, les CLI sont de diagnostic clinique et / ou radiologique difficiles de par leur morphologie (cellules en files indiennes, absence de masse palpable), aboutissant à un diagnostic souvent plus tardif que les CCI.

Le risque de récurrence en cas de CLI étant significatif, cela implique de pouvoir déterminer quels sont les patients qui vont bénéficier de traitements systémiques. En se basant sur cette revue de la littérature, nous pouvons conclure que :

- Les taux de réponse moindres à la chimiothérapie néoadjuvante en cas de CLI, en comparaison aux autres cancers du sein, ne permettent pas de se soustraire à ce traitement uniquement sur l'histologie de type lobulaire. De plus, dans les CLI, la pCR ne semble pas être un bon critère substitutif de la survie, contrairement à certains sous-types de CCI.
- En situation adjuvante :
  - Concernant la chimiothérapie, la validité et l'utilité cliniques des tests génomiques nécessitent d'être évaluées spécifiquement dans les CLI.

- Le letrozole semble être plus efficace que le tamoxifène en cas de CLI, cependant des études prospectives prenant en compte l'histologie lobulaire sont nécessaires pour pouvoir établir des conclusions définitives.
- L'intérêt d'une prolongation de l'hormonothérapie adjuvante dans le CLI, étant donné le risque de récurrence tardive, nécessite d'être spécifiquement évaluée.

Cette revue souligne, par conséquent, le manque crucial de données spécifiques sur le CLI permettant d'aider à la décision thérapeutique. Cela souligne la nécessité de mener des études spécifiques dans ce type histologique particulier.

**La classification moléculaire  
des cancers du sein : comment la définir ?  
Conférence IMPAKT 2012  
(Article 2)**

**Molecular subclasses of breast cancer: how do  
we define them? The IMPAKT 2012 Working  
Group Statement**

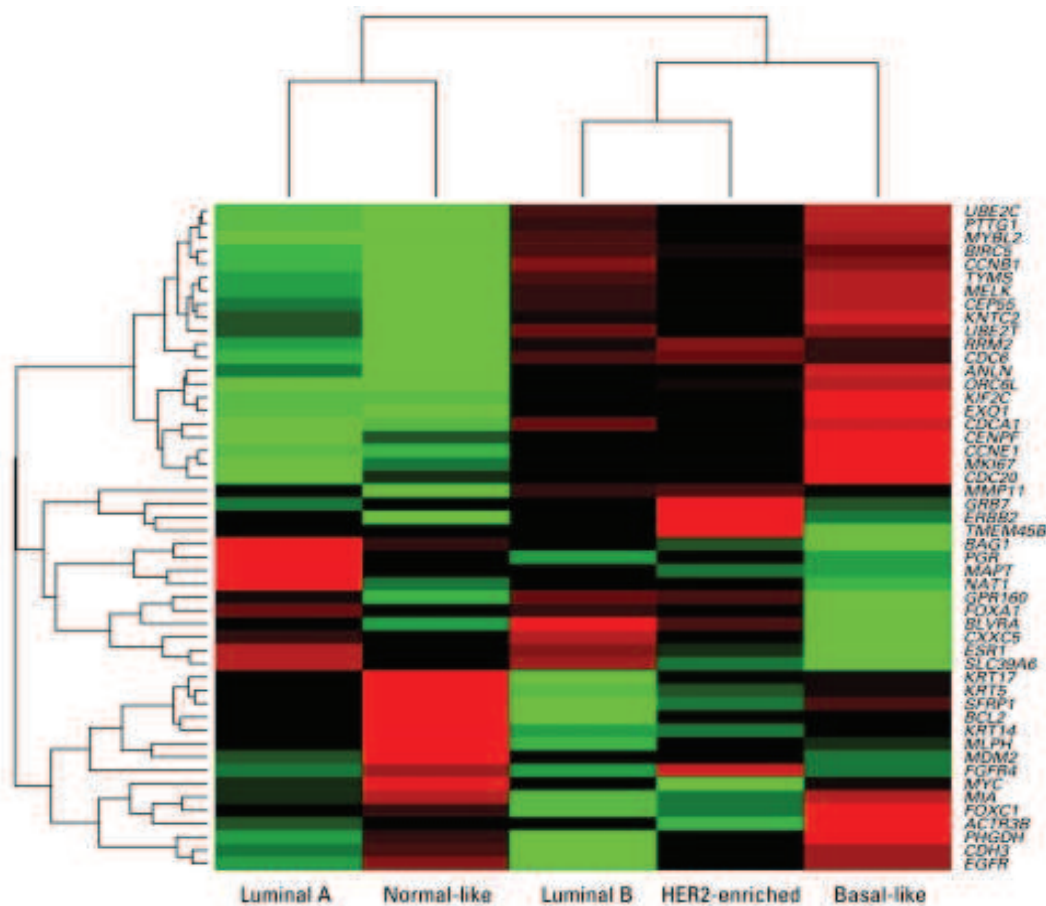
*S. Guiu, S. Michiels, F. André, J. Cortes, C. Denkert, A. Di Leo, B. T.  
Hennessy, T. Sorlie, C. Sotiriou, N. Turner, M. Van de Vijver, G. Viale,  
S. Loi, J. S. Reis-Filho*

**Annals of Oncology**

## I – INTRODUCTION

Pendant des décennies, la classification des cancers du sein a été basée uniquement sur l'aspect morphologique en microscopie optique des tumeurs. La classification OMS 2012 reconnaît une trentaine de types histologiques distincts [28], cependant les critères diagnostiques pour la caractérisation de chaque entité sont en partie subjectifs, et l'information sur le type histologique a finalement un impact limité sur la décision thérapeutique, sauf cas exceptionnels (carcinome tubuleux, adénoïde kystique... comme nous l'avons évoqué en introduction). Par conséquent, la stratification actuelle des cancers du sein en sous-groupes ayant un impact clinique est basée sur des facteurs clinico-pathologiques pronostiques incluant le grade histologique, la présence de métastases ganglionnaires et la présence d'embolies vasculaires péri-tumorales. De plus, des biomarqueurs prédictifs, comme l'expression du RE, du RP et le statut HER2 sont reconnus d'utilité clinique [92, 94, 104, 109].

Il y a 15 ans, l'étude d'expression de gènes en microarray a mis sur le devant de la scène le fait que le cancer du sein était une maladie hétérogène d'un point de vue moléculaire, avec un impact tant sur le plan de la recherche qu'en pratique clinique [139]. Quatre grands sous-groupes moléculaires ont été initialement identifiés : basal-like, HER2-enriched, luminal A et luminal B [144]. Ces groupes ont des pronostics différents [141, 144, 160]. Étant donné les limites pratiques de l'analyse de clusters non supervisée pour la classification d'un seul échantillon tumoral, une technique a été développée appelée SSP (Single Sample Predictor). Cette méthode permet de déterminer à quel sous-groupe appartient une tumeur grâce à l'analyse de son profil d'expression génique [141]. Cette méthode a par la suite été affinée [160, 161] pour aboutir à la construction de PAM50 (Prediction Analysis of Microarray), basé sur l'expression de 50 gènes (**Figure 16**) [161]. Cette technique est réalisable par qRT-PCR (quantitative reverse transcriptase - polymerase chain reaction), sur ARN extrait d'échantillons inclus et fixés en paraffine, contrairement aux méthodes initiales nécessitant du tissu tumoral congelé. En parallèle, un modèle pronostique appelé ROR (Risk Of Relapse) Score (ROR-S) a été développé, prédisant le risque de récurrence à 10 ans [161]. PAM50 est commercialisé sous le nom de Breast cancer Intrinsic Classifier™, laboratoires Arup.



**Figure 16. Prediction Analysis of Microarray (PAM) 50 [161]**

Le coût, la complexité et la nécessité initialement de tissu tumoral congelé pour le profil d'expression génomique (GEP), ont limité son utilisation en pratique clinique et ont mené au développement de critères substitutifs en IHC, pour l'identification des sous-types moléculaires [162, 163]. Bien que dans la classification initiale, il y ait une grande concordance entre les tumeurs HER2-enriched en GEP et les tumeurs HER2 positives en IHC / HIS, dans les versions suivantes, cette concordance est plus limitée : 31 à 59% des tumeurs HER2+ en IHC / HIS sont classées dans un autre sous-groupe « intrinsèque » que HER2-enriched [161, 164]. La majorité des tumeurs basal-like ont un phénotype TN, mais 1 à 3% de tumeurs RE+ ont été décrites comme appartenant au sous-groupe basal-like [139, 141, 144]. Selon le niveau d'expression des gènes de prolifération (incluant le *Ki-67*), les tumeurs luminales sont classées en 2 groupes : A et B. Cheang *et al* ont établi, en comparant les données GEP et IHC, qu'un cut-off de 14% pour le *Ki-67* permettait de distinguer les tumeurs luminales A des tumeurs luminales B (sensibilité de 72% et spécificité de 77%) [165].

En 2011, les sous-groupes moléculaires définis par GEP ont été intégrés dans la conférence de consensus de St Gallen, et une corrélation basée sur les marqueurs IHC a été proposée, bien que les données aient été peu nombreuses [120] :

- *Luminal A* : RE et / ou RP+, HER2-, Ki-67 < 14%
- *Luminal B, HER2 négatif* : RE et / ou RP+, HER2-, Ki-67 ≥ 14%
- *Luminal B, HER2 positif* : RE et / ou RP+, HER2+, quel que soit le Ki-67
- *HER2-enriched* : RE et RP-, HER2+
- *Basal-like* : RE-, RP-, HER2-

Etant donné l'importance de la classification des cancers du sein en sous-groupes utiles cliniquement pour les décisions thérapeutiques, et les controverses autour des définitions moléculaires par GEP et en IHC proposées par St Gallen, un panel d'experts a été réuni en amont de la conférence IMPAKT en 2012 pour évaluer l'utilité clinique 1°) des sous-types moléculaires intrinsèques (déterminés par PAM50) [120, 161] et représentant la liste des gènes « intrinsèques » décrite initialement par Perou *et al*, 2°) la définition immunohistochimique des sous-types moléculaires basée sur les recommandations de St Gallen, pour déterminer la classification la plus appropriée pour la pratique clinique.

Cette évaluation s'est basée sur un processus Delphi :

- Sélection de membres experts dans le cancer du sein et / ou le GEP (*S. Michiels, F. André, J. Cortes, C. Denkert, A. Di Leo, B.T. Hennessy, T. Sorlie, C. Sotiriou, N. Turner, M. Van de Vijver, G. Viale, S. Loi, JS. Reis-Filho*) et un doctorant (*S. Guiu*)
- Après un 1<sup>er</sup> meeting et une conférence téléphonique, revue de la littérature (*S. Guiu*) supervisée par un statisticien (*S. Michiels*) et les leaders du groupe de travail (*S. Loi et JS. Reis-Filho*) avec évaluation de chacun des items EGAPP (Evaluation of Genomic Applications in Practice and Prevention) [166] par article
- Les membres du panel ont ensuite revu toute la littérature disponible et répondu à un questionnaire de façon indépendante sur la validité analytique, la validité clinique et l'utilité clinique de chacune des classifications
- Les réponses ont été recueillies, résumées puis soumises à l'ensemble des membres (*S. Guiu*)
- Les résultats ont été analysés lors de conférences téléphoniques et un consensus atteint lors du 2<sup>ème</sup> meeting (février 2012).

La **validité analytique** d'un test est la capacité d'un test à mesurer de façon exacte et sûre le génotype d'intérêt, l'expression d'ARNm par les cellules tumorales, aussi bien que la reproductibilité d'analyse, la robustesse (par exemple, résistance à des changements mineurs dans des variables analytiques ou pré-analytiques) et le contrôle de qualité.

La **validité clinique** est la capacité d'un test à identifier ou prédire de façon exacte et sûre un objectif de survie pertinent dans le cancer du sein, 5 à 10 ans après la chirurgie.

L'**utilité clinique** est :

- La preuve qu'utiliser un test pour guider la prise en charge des patients avec un cancer du sein localisé va améliorer de façon significative la survie.
- Evaluée en étudiant la balance bénéfices (effets secondaires de la chimiothérapie évités chez les femmes à faible risque) / risques (récidive de cancer qui pourrait être évitée avec la chimiothérapie).

Le nombre d'articles éligibles pour les 2 classifications figure dans le tableau ci-dessous (*Figure 17*).

<b>Sous classe moléculaire</b>	<b>Articles, <i>n</i></b>
<b>Classification IHC</b>	<b>13 (/330)</b>
<b>Luminal A/B basé sur le Ki67</b>	<b>11 (8 cut-off 14%)</b>
<b>PAM50</b>	<b>36 (/122)</b>

*Figure 17.* Articles éligibles pour l'évaluation des classifications du cancer du sein

Le nombre d'articles et les critères étudiés pour la validité analytique de PAM50 et du Ki-67 sont résumés dans la **Figure 18**. Pour les RH et le statut HER2, nous avons basé nos conclusions sur les recommandations ASCO / CAP.

Critères	Ki67 14%	PAM50 (qRT-PCR)
Nombre d'articles	8	2
Reproductibilité	2	0
Tests en aveugle	0	1
Echecs et résultats incertains reportés	6	2

**Figure 18.** Validité analytique de PAM50 et Ki-67

Le nombre d'articles étudiés pour la validité et l'utilité cliniques de PAM50 et du Ki-67 sont résumés dans la **Figure 19**.



Classifications	Articles	Patients	Nombre d'essais randomisés
IHC (Ki67 14%)	8	13 085	3
PAM50 (qRT-PCR)	2	1 262	1
PAM50 (autre plateforme)	10	8 167	1

*Figure 19. Validité et utilité cliniques de PAM50 et Ki-67*

## II - ARTICLE

## Molecular subclasses of breast cancer: how do we define them? The IMPAKT 2012 Working Group Statement<sup>†</sup>

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The 2012 IMPAKT task force investigated the medical usefulness of current methods for the classification of breast cancer into the 'intrinsic' molecular subtypes (luminal A, luminal B, basal-like and HER2). A panel of breast cancer and/or gene expression profiling experts evaluated the analytical validity, clinical validity and clinical utility of two approaches for molecular subtyping of breast cancer: the prediction analysis of microarray (PAM)50 assay and an immunohistochemical (IHC) surrogate panel including oestrogen receptor (ER), HER2 and Ki67. The panel found the currently available evidence on the analytical validity and clinical utility of Ki67 based on a 14% cut-off and PAM50 to be inadequate. The majority of the working group members found the available evidence on the analytical validity, clinical validity and clinical utility of ER/HER2 to be convincing. The panel concluded that breast cancer classification into molecular subtypes based on the IHC assessment of ER, HER2 and Ki67 with a 14% cut-off and on the PAM50 test does not provide sufficiently robust information to modify systemic treatment decisions, and recommended the use IHC for ER and HER2 for the identification of clinically relevant subtypes of breast cancers. Methods for breast cancer classification into molecular subtypes should, however, be incorporated into clinical trial design.

**Key words:** breast cancer, Ki-67, molecular classification, molecular subclasses, PAM50

### introduction

For many decades, the classical breast cancer classification systems were solely based on the histological appearances of breast cancers. Numerous classification systems with limited agreement have been developed [1]. The 2003 World Health Organisation classification recognises 18 distinct histological types of invasive breast cancer [1, 2]; however the diagnostic criteria for the characterisation of each entity are rather subjective, and information on histological subtype has a limited impact on therapeutic decision making. In fact, the

current stratification of breast cancers into clinically meaningful subgroups is based on prognostic clinicopathological parameters other than type, including histological grade, presence of lymph node metastasis and lympho-vascular invasion. Furthermore, predictive biomarkers, such as expression of oestrogen receptor (ER), progesterone receptor (PR), and the assessment of HER2 status, have proven to be clinically useful [3–6].

Over the past decade, microarray-based gene expression studies brought to the forefront of breast cancer research and clinical practice the fact that breast cancer comprises a heterogeneous group of diseases that have different distinct molecular features. Based on hierarchical clustering, Perou et al. [7] initially identified four breast cancer 'intrinsic' subtypes (basal-like, HER2-enriched, luminal and normal breast-like), which were shown to display gene expression patterns. Subsequent studies have led to the sub-stratification of luminal breast cancers into luminal A and luminal B, and shown that this classification system is of prognostic

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<sup>†</sup>These recommendations were presented at the IMPAKT 2012 Breast cancer conference in Brussels, Belgium.

significance [8–10]. Furthermore, additional class discovery studies with larger datasets resulted in the identification of additional molecular subtypes, including interferon-rich, claudin-low and molecular apocrine [8, 11–16]. Owing to the limitations of hierarchical clustering for the classification of individual samples, the proponents of the microarray-based breast cancer classification developed single sample predictors (SSPs), which enable the subtyping of a single tumour based on microarray gene expression profiling (GEP) [10]. The SSP has been further refined [8, 12] and a classifier using 50 genes was developed to identify the four major intrinsic subtypes, namely luminal A, luminal B, HER2-enriched and basal-like (Table 1) and named prediction analysis of microarray (PAM) 50 [12]. This classifier was subsequently converted into a quantitative real-time PCR (qRT-PCR) and can be carried out with RNA extracted from formalin-fixed paraffin-embedded (FFPE) samples, thereby making it applicable on archival material. In conjunction with the development of the qRT-PCR version of the PAM50 assay, a prognostic model named risk of relapse score (ROR-S), was devised based on the molecular subtypes [12]. An assay in development by NanoString Technologies, based on the PAM50 gene expression signature, provides a subtype classification as well as a prognostic score (referred to as the ROR-S) that predicts the probability of cancer recurrence over 10 years and is treated by the other IMPAKT task force (a companion manuscript concurrently submitted to *Annals of Oncology*). This PAM50 Breast Cancer Intrinsic Classifier™ is currently commercially available from ARUP laboratories ([www.aruplab.com](http://www.aruplab.com)).

The cost, complexity and initial requirement of fresh frozen tissues for GEP have limited its use in clinical practice and led to the development of immuno-histochemical (IHC) surrogate definitions for the identification of the molecular subtypes of breast cancer (Table 1), given the similarities of the molecular subtypes as defined by GEP. Although in the original GEP-defined molecular classification, there was a great degree of agreement between HER2-enriched tumours as defined by GEP and HER2-positive tumours as defined by IHC and *in situ* hybridisation (ISH), in later versions of the classification, the concordance between the two subtypes is more limited. Up to 31%–59% of cases with HER2 positivity as defined by IHC and/or ISH are classified as an ‘intrinsic’ subtype other than HER2 enriched [12, 13, 17, 18]. The majority of basal-like breast cancers (~80%) have been shown to be of triple-negative phenotype (i.e. negative for ER, PR and HER2), however between 1%–3% of ER positive tumours have been shown to display a basal-like phenotype [7, 9, 10]. Luminal breast cancers are characterised by the expression of ER-associated genes [7] and can be sub-stratified in at least two groups based on the expression levels of proliferation-related genes, including MKI67, the transcript of the proliferation marker Ki67. Cheang et al. [19] established a Ki67 cut-off of 14% to distinguish luminal B from luminal A breast cancers by comparing GEP and IHC data; this cut-off was shown to have a sensitivity of 72% and a specificity of 77% to identify luminal B tumours [19].

In 2011, the subtypes as defined by GEP (i.e. luminal A, luminal B, HER2-enriched and basal-like) were included in the 2011 St Gallen International Expert Consensus (Table 1), and

**Table 1.** Molecular subtypes of breast cancer

Intrinsic subtypes (GEP)	IHC classification (St Gallen)	Agreement IHC/GEP
Luminal A	‘Luminal A’ ER and/or PR positive HER2 negative Ki-67 <14%	73%–100%
Luminal B	‘Luminal B (HER2 negative)’ ER and/or PR positive HER2 negative Ki-67 ≥14% ‘Luminal B (HER2 positive)’ ER and/or PR positive Any Ki-67 HER2 over-expressed or amplified	73%–100%
HER2-enriched	‘HER2 positive (non-luminal)’ HER2 over-expressed or amplified ER and PR absent	41%–69%
Basal-like	‘Triple negative’ ER and PR absent HER2 negative	80%

GEP, gene expression profiling; IHC, immuno-histochemical; ER, oestrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

an approximation based on IHC surrogates was proposed [20]. The assessment of Ki67 and a 14% cut-off for the distinction between luminal A and luminal B were recommended by St Gallen, despite the limited evidence available in support of their use in clinical practice. In addition, discrepancies between GEP- and IHC-defined subtypes also have an impact on the translatability of the subtypes in clinical practice.

Given the importance of the sub-classification of breast cancers into clinically useful groups and the controversies surrounding the GEP- and IHC-defined breast cancer taxonomy, we sought to evaluate the medical usefulness of (i) the ‘intrinsic’ molecular subtypes (determined by PAM50 assay [12] and representing the ‘intrinsic’ gene list initially described by Perou et al.) and (ii) the IHC definition of molecular subtypes, based on the St Gallen Consensus recommendation [20], to determine the most appropriate molecular subtype’s classification for today’s daily practice.

## methods

### molecular subtypes

The IHC definition of molecular subtypes is based on the St Gallen Consensus 2011 [20]: this approach uses IHC definition of ER and PR, the detection of HER2 IHC overexpression and/or *HER2* gene amplification as defined by ISH and Ki-67 labelling index, with a pre-defined cut-off 14% [19]. For ER and HER2 international guidelines have already been published [5, 6], while the efforts to standardise Ki-67 have not been completed as yet [21]. Therefore, for this work, we evaluated ER/HER2 and Ki-67 separately. We did not assess

Table 2. PUBMED search

Search terms immuno-histochemical classification	1) BREAST NEOPLASMS AND 2) Immuno-histochemical OR immunohistochemistry OR immunocytochemistry OR immunochemistry AND 3) Molecular subtypes OR molecular subtype intrinsic subtypes OR intrinsic subtype OR 'luminal A' OR 'luminal B' OR PAM50 OR molecular classes
Search terms PAM50	1) BREAST NEOPLASM OR BREAST NEOPLASMS OR BREAST CANCER AND 2) Molecular portraits OR tumor subtypes OR tumor subtyping OR molecular characterization OR molecular subtyping OR molecular subtyping OR tumor subclasses OR molecular subtypes OR molecular subtype OR intrinsic molecular subtypes OR intrinsic molecular subtype OR INTRINSIC SUBTYPE OR INTRINSIC SUBTYPES OR INTRINSIC GENE LIST OR INTRINSIC GENE LISTS OR PAM50 OR PAM50 intrinsic subtyping OR PAM50-based intrinsic subtype AND 3) Profiling OR SINGLE SAMPLE PREDICTOR OR SINGLE SAMPLE PREDICTORS OR 50-GENE OR 50-GENE SUBTYPE PREDICTOR OR subtype predictor OR gene predictor OR hierarchical clustering OR hierarchical cluster OR microarrays OR gene expression profiling OR microarray profiling OR transcriptome OR gene expression arrays OR gene expression microarrays OR transcriptome OR microarrays OR RT-PCR OR reverse transcription polymerase chain reaction OR real-time polymerase chain reaction OR qRT-PCR
Limits (exclusion)	1) EDITORIAL 2) NEWS 3) CASE REPORTS 4) <i>IN VITRO</i> 5) ANIMALS
Language	ENGLISH only
Period	2000 to February 2012

the IHC4 test [22], which also assesses ER, PR, HER2 and Ki67, as this test does not ascribe cancers into molecular subtypes and was considered to be beyond the remit of this taskforce. PAM50 representing the 'intrinsic' gene list [12] is currently under development by NanoString Technologies with a quantitative reverse transcription polymerase chain reaction (qRT-PCR) carried out on FFPE. Table 1 summarises the two molecular classifications, which were evaluated.

#### literature search

On February 2012, we searched in the MEDLINE database using the terms summarised in Table 2. Initial results were reviewed and cross-referencing was carried out among the identified studies to ensure that all eligible studies were captured. The main eligibility criterion was studies on the breast cancer molecular classification applied in the clinical setting. We deemed some studies as not eligible for this evaluation, namely (i) studies without distinction between luminal A and luminal B tumours; and (ii) studies where luminal B tumours were defined exclusively ER and/ or PR positive and HER2 positive as defined by IHC/fluorescence ISH.

#### evaluation method and procedure

This project was undertaken by a working group composed of oncologists, pathologists, scientists and biostatistician with expertise in the field of breast cancer and/or GEP. The working group adopted a Delphi process which was coordinated by a medical oncology doctorate scholar. The Delphi process is a structured communication technique; joining an expert panel to answer to a pre-defined question. The experts answer panel questionnaires blinded to the responses provided by other

members of the group in at least two rounds. After each round, a facilitator provides a summary of the experts' forecasts from the previous round as well as the reasons they provided for their judgments. Thus, experts are encouraged to revise their earlier answers in light of the replies of other members. Finally, the process is stopped after a consensus is reached.

To assess the quality of the studies, evaluation was carried out based on the general principles of the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) initiative [23]. This initiative provided rigorous evidence-based criteria for evaluating genetic tests and other genomic applications for clinical and public health practice. The EGAPP initiative was not specifically designed to evaluate genomic applications in the oncologic field.

Each study (where appropriate) was evaluated for:

- (i) The test's ability to accurately and reliably measure the molecular phenotype of interest, the expression of mRNA by breast cancer tumour cells, as well as assay reproducibility, robustness (e.g. resistance to small changes in pre-analytical or analytical variables), and quality control (analytical validity);
- (ii) The test's ability to identify accurately and reliably or predict a relevant breast cancer survival end point 5–10 years after surgery (clinical validity);
- (iii) The evidence that using a test to guide management in patients with diagnosed early-stage breast cancer will significantly improve health-related outcomes. It was assessed by investigating the balance of benefits (reduced adverse events due to low risk women avoiding chemotherapy) and harms (cancer recurrence that might have been prevented) (clinical utility).

**Table 3.** Parameters used to evaluate the eligible studies

Analytical validity	Clinical validity	Clinical utility
Data source	Data source	Data source
<ul style="list-style-type: none"> <li>• Number of samples</li> <li>• Source of samples</li> </ul>	<ul style="list-style-type: none"> <li>• Cohort studies</li> <li>• Case-control studies</li> <li>• Case series</li> </ul>	<ul style="list-style-type: none"> <li>• Meta-analysis</li> <li>• Randomised trials</li> <li>• Case-control studies</li> <li>• Case series</li> </ul>
Reproducibility	Eligibility criteria	End points
<ul style="list-style-type: none"> <li>• Intra-laboratory validation</li> <li>• Inter-laboratory validation</li> <li>• Effect of time</li> </ul>		<ul style="list-style-type: none"> <li>• Primary</li> <li>• Secondary</li> </ul>
Blinded testing	Sample size and demographics	Data collection
		<ul style="list-style-type: none"> <li>• Prospective</li> <li>• Retrospective</li> </ul>
Specimen	Point estimates of prognostic value	Treatment used
<ul style="list-style-type: none"> <li>• FFPE</li> <li>• Frozen tissue</li> <li>• Fresh tissue</li> </ul>	<ul style="list-style-type: none"> <li>• Sensitivity</li> <li>• Specificity</li> <li>• Hazard ratio</li> </ul>	
Report of test failures	Study population	Randomisation
Report of indeterminate results	Power calculation	Independence of the test
		<ul style="list-style-type: none"> <li>• Multivariate model</li> <li>• Comparison with current standards</li> </ul>

FFPE, formalin-fixed paraffin embedded.

Different parameters were used in the evaluation of analytical validity, clinical validity and clinical utility (Table 3). To evaluate the ability of a molecular classification to predict recurrence risk accurately, we evaluated univariate and multivariate survival models that were reported for each classification. If more than one model was carried out on the same dataset to test different end points, we considered the one that was specified as the primary end point except if several end points were pre-specified. If more than one multivariate model was reported in the same paper but on different datasets, both models were considered.

The members of the working group were asked to review and grade all the available literature blinded to the score provided by other experts. Grading was based on the criteria provided in Table 3. Each member was asked to provide a qualitative evaluation of the evidence in support of the analytical validity, clinical validity and clinical utility for each molecular classification. Qualitative scores ranged from inadequate, adequate to convincing (Table 4). Next, all responses are gathered, tabulated, and shared with the task force members for discussion to reach a final consensus.

**results**

Following the literature search criteria employed, 13 articles were deemed eligible and evaluated for IHC classification,

including 11 with distinction between luminal A/B based on Ki-67 and only 8 with a cut-off of 14% (Figure 1). Thirty-six publications were evaluated for PAM50 with only two studies using the PAM50 NanoString test (Figure 1). A complete publication list of all articles reviewed and evaluated is provided in Supplement 1.

**analytical validity**

Details on some of the parameters related to the assessment of analytical validity of the tests were reported in eight and two papers on the IHC and GEP molecular classification of breast cancers (Table 5). The source of tissue samples was mainly from retrospective studies, which were variable in size.

**IHC classification**

Eight studies provided analytical validity information for IHC classification based on Ki67 14%. Among 144 patients with luminal A (*n* = 84) or B (*n* = 60) tumours (PAM50) and ER +/HER2- (IHC), Cheang et al. [19] determined a Ki-67 cut-off of 14% to distinguish luminal A and B tumors with a sensitivity of 72% (95% CI 59% to 82%) and a specificity of 77% (95% CI 67% to 85%). When 17 borderline tumours were excluded (difference between Spearman rank correlation coefficients for luminal A and B centroids < 0.1), the same cut-off was found with a sensitivity of 77% (95% CI 64% to 87%) and a specificity of 78% (95% CI 68% to 87%). Independent validation of the 14% cut-off for the sub-stratification of ER-positive breast cancers into luminal A and luminal B has yet to be carried out.

It should be noted that assessment of IHC markers was carried out by a central laboratory in only four studies [24–27]. An independent review by two pathologists was reported in only two publications, although no agreement rate was provided [26, 28]. IHC staining was carried out on tissue microarrays slides in 50% of the studies, while 50% was applied on full-face slides. Six retrospective studies reported a sampling failure rate for IHC biomarkers of 2%–11.5% (insufficient tumour material or fixation with a fixative other than buffered formalin) [19, 24–27, 29].

**PAM50**

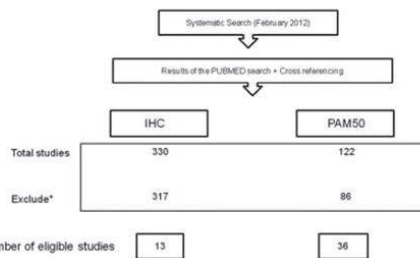
The ‘intrinsic’ molecular subtypes have been initially described by Perou et al. [7, 9] through hierarchical clustering. In 2003, Sorlie et al. [10] reported these distinct subtypes (mainly luminal and basal-like tumours) with distinct outcomes in three independent datasets comprising different patient populations whose GEP was determined by using different microarray platforms (extended Norway/Stanford cohort, *n* = 122; Van’t Veer et al., *n* = 97 and West et al., *n* = 49). The overlapping ‘intrinsic’ genes of these three cohorts were used to develop a breast cancer class predictor using PAM with an agreement ranging from 79% to 89% when compared with the results of the hierarchical clustering applied for Van’t Veer and West datasets, respectively. Of note, some genes used as predictors were used to define the test set groups. Several studies (with <100 tumour samples) subsequently reported on similar classifications (where luminal and basal-like tumors were the most robust and distinct subtypes), including distinct

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**Table 4.** Criteria used to assess the quality of evidence for the analytical validity, clinical validity and clinical utility (EGAPP criteria)

	Analytical validity	Clinical validity	Clinical utility
Convincing	Studies that provide confident estimates of analytic sensitivity and specificity using intended sample types from representative populations  Collaborative study using a large panel of well-characterized samples or well-designed peer-reviewed study (inter-laboratory comparison and validation study) that is generalisable and has an appropriate number and distribution of challenges	Well-designed and conducted studies in representative population(s) that measure the strength of association between a genotype and a specific and well-defined disease  High-quality well-designed longitudinal cohort study or systematic review/meta-analysis of well-designed longitudinal cohort studies with homogeneity	Well-designed and conducted studies in representative population(s) that assess specified health outcomes  Systematic review/meta-analysis of randomised, controlled trials showing consistency in results or at least one large randomised, controlled trial
Adequate	Two or more collaborative studies using a large panel of well-characterized samples or well-designed peer-reviewed studies (inter-laboratory comparisons and validation studies) are consistent but not generalisable or that lack the appropriate number and/or distribution of challenges	Systematic review of lower quality studies or review of well-designed longitudinal cohort or case-control studies with heterogeneity	Systematic review of randomised, controlled trials showing heterogeneity or controlled trial(s) without randomisation or systematic review of cohort or case-control studies with consistent results
Inadequate	Lower quality studies or combinations of higher quality studies that show important unexplained inconsistencies	Single well-designed cohort or case-control study or case series or non-consecutive cases	Systematic review of cohort, case-control studies or controlled trials without randomisation showing heterogeneity or single cohort or case-control study or case series

EGAPP, Evaluation of Genomic Applications in Practice and Prevention.



\* Title/abstract not relevant, systematic reviews

**Figure 1.** Flowchart of eligible studies.

ethnic populations and inflammatory breast cancers [30–35]. In a larger population-based Swedish cohort ( $n = 412$ ), Calza et al. [36] described a concordance rate of 77.5% between the centroid prediction (using Norway/Stanford data as the training set) and the k-means clustering carried out internally within the Swedish cohort (the highest rates of discordant assignments were between luminal A and B and luminal B and HER2-enriched subtypes). In addition, Perreard et al. [37] showed that a minimised 'intrinsic' gene set could be used in a qRT-PCR assay to recapitulate the microarray classification with a concordance rate of 93%. Parker et al. [12] applied an expanded 'intrinsic' gene set of 1900 common genes in four

**Table 5.** Publications that provided information on analytical validity

	Ki-67 (14%)	PAM50 (qRT-PCR)
No. of papers	8	2
Reproducibility	2	0
Blinded testing	0	1
Reporting test failures/indeterminate results	6	2

qRT-PCR, quantitative reverse-transcriptase-polymerase chain reaction.

previous studies [8–10, 37] to the study of 122 breast cancers, and developed a 50-gene subtype predictor (minimised gene set using the qRT-PCR data for genes that passed FFPE performance criteria [38]).

Several publications have investigated the limitations of the use of SSPs for the classification of breast cancers into the 'intrinsic' subtype classes. PAM50 was compared with two others SSPs (Sorlie et al., 2003 and Hu et al., 2006) in a combined analysis that assessed the performance of SSPs in four datasets ( $n = 832$  patients) [17]. The three SSPs were shown not to assign consistently the same patients into the molecular subtypes (fair-to-substantial agreement between every pair of SSPs in each cohort was recorded,  $\kappa = 0.238–0.740$ ). Nevertheless, all SSPs identified molecular subtypes with similar but not equivalent survival. Molecular classification with each SSP remained an independent prognostic factor in multivariate analysis. Only the proportion

of basal-like tumours was similar between the three SSPs (agreement,  $\kappa > 0.812$ ). Mackay et al. [39] evaluated the interobserver reproducibility between five researchers to assign a molecular subtype in three publicly available datasets ( $n = 779$ ) using five distinct 'intrinsic' gene lists [39]. Substantial interobserver agreement was consistently observed in all datasets for four molecular subtypes (luminal, basal, HER2-enriched and normal-like) for 70.8%–76.1% of the samples ( $\kappa$  scores from 0.635 to 0.701). Only basal-like and HER2-enriched molecular subtypes were reproducibly identified with an almost perfect agreement ( $\kappa \geq 0.81$ ). Haibe-Kains et al. [40] compared the three SSPs in 36 publicly available datasets ( $n = 5715$ ) and tested their robustness using the 'prediction strength statistic'. PAM50 had low prediction strength of 0.59, 0.36 and 0.25 for 3, 4 and 5 subtypes, respectively. In addition, the three SSPs were fair to moderate concordance with each other (58%–68%;  $\kappa = 0.45$ –0.58). Elloumi et al. [41] highlighted that genomic classification by PAM50 could be altered by normal tissue contamination (typically 30%–50% in samples) and caused misclassification of a given tumour, mainly from more aggressive to less aggressive subtypes as the content of normal/non-neoplastic cells increased.

Limited information is available on the analytic validity of the PAM50 NanoString test. Nielsen et al. [42] reported a failure rate of 21% in a large retrospective study involving 991 patients, which was mainly due to RNA of suboptimal quality. No details of intra- or inter-laboratory comparisons were reported in this study. Sixty-seven percent of samples were obtained for qRT-PCR in a retrospective study conducted from the NCICTG MA.5 randomised trial. No further explanation for the exclusion of 33% of samples was provided in this study [43].

**clinical validity/utility**

Tumour stage and treatment modalities were heterogeneous in the patient population included in studies investigating the IHC and GEP breast cancer molecular classification.

Table 6 summarises the multivariate survival models developed for each molecular classification. A total of 15 multivariate models were evaluated across the two molecular classifications. In 10 models (66%), the molecular classification was significantly associated ( $P < 0.05$ ) with prognosis, but in almost no cases, an appropriate likelihood ratio test was applied. End points were disease-free survival ( $n = 4$ ), loco-regional free survival ( $n = 3$ ), breast cancer-specific survival ( $n = 3$ ), overall survival (OS;  $n = 2$ ), relapse-free survival ( $n = 2$ ) and distant metastasis-free survival ( $n = 1$ ). There was strong variability between the factors included in the multivariate models.

**IHC classification**

In seven of the eight studies, the source of information was retrospective in nature. In two of seven studies, the IHC biomarkers were carried out on retrospectively collected samples from patients that were enrolled in a prospective randomised trial [24, 27]. Cheang et al. [19] tested his 14% Ki-67cut-off in a retrospective analysis of an independent

**Table 6.** Evaluable multivariate models

	IHC classification	PAM50 NanoString test
Number of unique patients	13 085	1262
Number of multivariate models	13	2
Adequate documentations of multivariable regressions		
Molecular classification is significant ( $P < 0.05$ )	9	1 <sup>a</sup>
Added value demonstrated using the likelihood ratio test	0	1
Adjustment factors (%)		
Histological subtype	0	0
Tumour size	92	100
Nodal status	62	100
Histological grade	62	50
ER	0	50
HER2	8	50
Ki-67	8	0
Age	85	100
Lympho-vascular invasion	77	50
Treatment	38	50

<sup>a</sup>In one extra model, an interaction between PAM50 and treatment was evaluated.

population of 2598 HR+ patients with different adjuvant modalities (no treatment, tamoxifen alone, tamoxifen and chemotherapy with anthracyclines or CMF). Luminal B (33%) and luminal-HER2-positive (8%) breast cancers were statistically significantly associated with poor 10-years recurrence-free and disease-specific survival in all adjuvant systemic treatment categories, and similar results were seen in lymph node-positive or lymph node-negative patients treated with tamoxifen alone. Similarly, Hugh et al. [24] analysed 1326 patients with lymph node-positive disease, constituting 91% of those who were previously treated in the BCIRG001 randomised trial and who all received adjuvant chemotherapy (TAC versus FAC), and observed a significantly longer 3-year disease-free survival for patients with luminal A breast cancers ( $n = 212$ ) compared with those with luminal B tumours ( $n = 808$ ). A retrospective study of 1006 non-consecutive Korean patients, including 53% luminal A and 22% luminal B tumours, reported a significantly better 5-year disease-free and OS for patients with luminal A cancers as compared with those with luminal B disease [26]. No adjustment for the type of adjuvant treatment was provided in that publication [26]. Five-year OS and disease-free survival were significantly better in patients with luminal A cancers from another study conducted from 50% ( $n = 298$ ) of patients with high-risk breast cancer included in the phase III HE10/97 randomised trial [27].

Risks of local and regional relapse was significantly higher in patients with HER2, basal-like and luminal B tumours than in those with luminal A breast cancers after mastectomy ( $n = 2085$  patients) [25]. After breast conserving surgery and adjuvant radiotherapy, risk of regional recurrence was only higher in patients with HER2 and basal-like disease on a multivariate survival analysis [25]. In 1691 consecutive women with small invasive node-negative breast tumour, higher risk of loco-regional relapse was only found for HER2 and basal-like

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tumours [28]. A consistent finding in multiple studies [24, 26, 27] was a significantly poorer outcome for HER2-positive and basal-like tumours when compared with luminal A cancers, even in small breast cancers (size  $\leq 1$  cm), which are often considered as of good prognosis [28].

One study investigated the predictive value of the IHC classification in determining patients who would benefit taxanes [24]. Hugh et al. [24] reported that TAC was superior to FAC only in patients with luminal B tumours, even if combined chemo-endocrine therapy containing tamoxifen was administered. However, no interaction test between IHC classification and TAC versus FAC was provided in this study. The 3-year disease-free survival rates were 89.4% and 82%, respectively (HR = 0.71; 95% CI 0.53–0.95;  $P = 0.02$ , multivariate model). An exploratory analysis conducted by Canello et al. [29] in 199 young patients (<35 years) with luminal B breast cancers reported a statistically shorter disease-free survival when received tamoxifen or LH-RH analogue alone versus the combination of the two drugs.

It should be noted that in all studies, no patients received adjuvant trastuzumab, as they preceded the approval of this humanised monoclonal antibody for the management of patients with early-stage breast cancer. Table 6 summarises the design of the different multivariate survival models included in the studies evaluated ( $n = 13$ ).

#### PAM50

Two studies using the PAM50 SSP found that molecular subtypes were prognostic in two large, N0, untreated cohorts of patients ( $n = 761$  [12] and  $n = 1260$  [40]). These data were confirmed in a multivariate survival model adjusted by tumour size, node status, grade and ER status in one of the studies [12]. The same study reported a predictive value of the 'intrinsic' subtypes in 133 patients treated with neoadjuvant chemotherapy in a multivariate model (with or without histological grade) [12].

In 120 patients treated with neoadjuvant chemotherapy, the authors described higher pCR rates for basal-like and HER2-enriched tumours but molecular subtypes added no predictive value to ER and HER2 status in multivariate model [44].

PAM50 NanoString test was evaluated in two studies, including >1200 patients [42, 43]. The first study included both lymph node-positive (65%) and negative ER+ breast cancers from patients uniformly treated with only adjuvant tamoxifen [42]. It should be noted that his cohort was the same as the one partly described by Cheang et al. [19]. Luminal A breast cancers assigned by the PAM50 assay had significantly better 5 and 10-years disease-specific survival than luminal B, HER2-enriched or basal-like breast cancers. In multivariate model, the 'intrinsic' subtypes remained independent prognostic factor, as did tumour size and node status, particularly during the first 5 years. A model of IHC subtype (incorporating data on Ki-67 and HER2) and tumour size (IHC-T) was constructed following the same process used for development of ROR scores [42]. This model failed to show an added prognostic value using c-index to Adjuvant! Online (AOL) in node-negative or node-positive disease [42].

The second study included retrospectively samples of 476 of 716 pre-menopausal patients with node-positive breast cancer who were randomised between anthracycline (CEF) versus non-anthracycline (CMF) adjuvant chemotherapy in the NCIC.CTG MA.5 trial [43]. 'Intrinsic' subtypes were significantly associated with 5-years relapse-free and OS in the entire cohort uniformly treated with chemotherapy. Multivariate analyses were used with treatments, intrinsic subtypes (PAM50) and their interaction as covariates, and adjusted for age, number of positive lymph nodes, ER level, type of surgery and tumour size. A benefit in relapse-free survival and OS with anthracycline regimen was seen only for HER2-enriched tumours ( $P = 0.03$ , interaction test), but this differential benefit did not appear different from the one using the FISH/IHC-based HER2 status. A non-significant statistical trend was observed in patients treated with an anthracycline-based regimen, where patients with luminal B cancers appeared to have an improved survival, whereas in patients with basal-like and luminal A disease, anthracycline-based therapy was associated with a numerical short survival.

Table 6 summarises the different multivariate survival models included in the studies evaluated ( $n = 2$ ).

#### Limitations of this study

It should be noted that the analyses of analytical validity, clinical validity and clinical utility provided in this study have limitations that are applicable to exercises of this nature. First, new data may have emerged between the literature review and the publication of the results, which could have had an impact on the interpretation of the levels of analytical and clinical validity and clinical utility of the tests investigated. Second, the analyses of analytical validity, clinical validity and clinical utility were carried out using the EGAPP criteria; it is possible that if other sets of rules had been employed, slightly different conclusions would have been rendered. Third, although the task force was composed of breast cancer and/or GEP experts, we cannot rule out the possibility that a different panel of experts with different expertise would have come to slightly different conclusions.

#### IMPAKT 2012 Working Group Statement

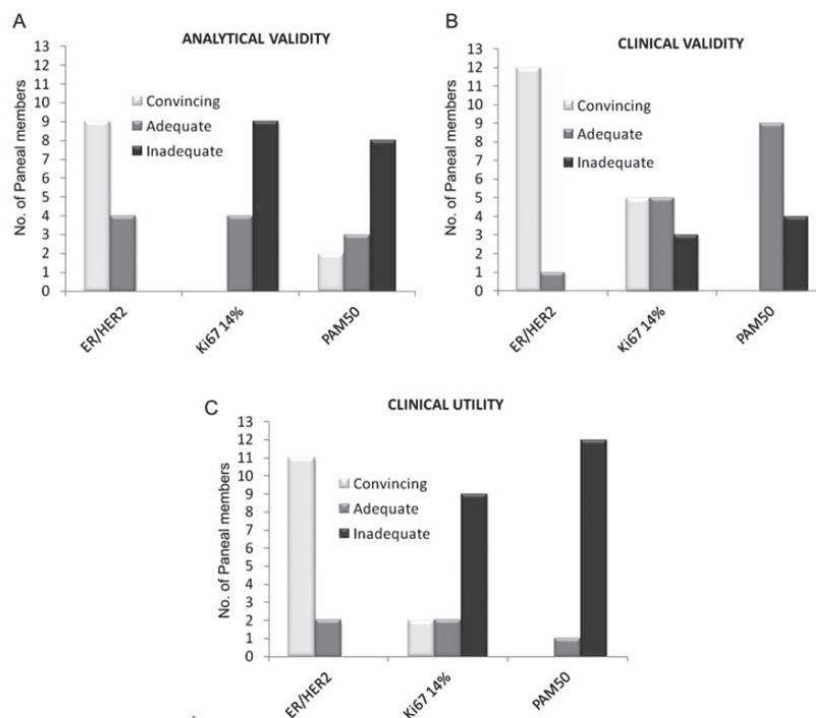
##### analytical validity

According to the EGAPP criteria, the majority of the working group members found the available evidence on the analytical validity of ER/HER2 to be convincing (Figure 2A). The panel found the available evidence on the analytical validity of Ki-67 and PAM50 to be inadequate and the panel acknowledges that further data are required.

##### clinical validity

According to the EGAPP criteria, the majority of the working group members found the available evidence on the clinical validity of ER/HER2 to be convincing and on the clinical validity of Ki-67 and PAM50 to be adequate (Figure 2B). The panel found that currently molecular subtypes should be defined based on pathological assessment of ER and HER2 (IHC  $\pm$  ISH) which have already largely demonstrated their





**Figure 2.** Panel assessment of the evidence on analytical validity, clinical validity and clinical utility of PAM50 and an immuno-histochemical surrogate panel for the identification of the molecular subtypes of breast cancer, according to the EGAPP criteria. (A) Quality of evidence for analytical validity (answers of the panel); (B) quality of evidence for clinical validity (answers of the panel) and (C) quality of evidence for clinical utility (answers of the panel).

prognostic and predictive values (i.e. HER2 as defined by HER2 IHC overexpression and/ or HER2 gene amplification; luminal as defined by HER2 negativity and ER and/or PR expression; basal-like as defined by triple-negative IHC phenotype). Neither Ki-67 nor PAM50 reached this level of evidence, however both approaches were shown to lead to the identification of subsets of ER-positive breast cancers that have distinct outcomes, and may consist in promising ways to distinguish luminal A from luminal B tumours. It was a consensus in the panel that further data on the clinical validity of the tests are still required. These include the development of standardised test (particularly for Ki-67) [21] and the evaluation of both Ki67 and PAM50 in a larger number of patients from randomised prospective trials.

**clinical utility**

The majority of the working group members found the available evidence on the clinical utility of ER/HER2 to be convincing. The panel found the available evidence on the clinical utility of Ki67 and PAM50 to be inadequate (Figure 2C). For current clinical practice, the panel recommends to use IHC for ER and HER2 for the

identification of clinically relevant subgroups of breast cancers, however no convincing data for the use of Ki67 with a cut-off of 14% for the subdivision of luminal tumours into luminal A and luminal B. The panel does not support the use of PAM50 for current clinical decisions in regards to systemic therapy, especially in cases of discordance with IHC. For example, a HER2-enriched tumour that is not HER2 positive by IHC or FISH should be managed as HER2 negative, as there is no evidence that these patients would benefit from anti-HER2 therapies. In a patient with an ER-positive/HER2-negative with high-risk clinical features breast cancer that is classified as luminal A by PAM50, chemotherapy should not be ruled out for consideration of systemic chemotherapy in the adjuvant setting.

Although patients with luminal B breast cancers are often described as having a poorer outcome than those with luminal A tumours, the panel does not support the notion that luminal A and B should be used to inform clinical decision in regards to the use of adjuvant chemotherapy due to the absence of convincing data on clinical utility both in lymph node-positive or -negative disease (for Ki67 and PAM50). To date, the group believes that decisions on assigning chemotherapy should be made using the clinically available tools until more robust data

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on the value of molecular subtyping of breast cancers are available.

All the members of the working group agreed on the need to incorporate the molecular subtypes based on PAM50 or other molecular models in the design of clinical trials. Ideally tests that could be carried out using FFPE samples would be preferable in the context of clinical trials. PAM50 and IHC data could be accrued concurrently to build more comprehensive databases of cases with both types of data. To provide information on clinical utility, Ki67 expression should be subjected to a central review.

### conclusions and future directions

Despite the progress made over the past 12 years on the understanding of the molecular heterogeneity of breast cancers, the classification into molecular subtypes based on the IHC assessment of ER, PR, HER2 and Ki67 with a single 14% cut-off or PAM50 NanoString GEP test does not provide sufficiently robust information to modify treatment decisions based on their results. In addition, discrepant results between molecular subtypes defined by PAM50 on one hand and by IHC on the other hand, are frequently reported. In these cases, there is no evidence to support the definition of one subtype classification as having a higher predictive ability. Currently, clinical variables including tumour size, nodal status, histological grade, ER, PR and HER2 status remain the current 'gold standard' for systemic therapy decision making. Further studies addressing the clinical utility of the IHC and PAM50 classification and investigating the optimal therapy for patients with discrepant results are warranted.

The breast cancer taxonomy including the five 'intrinsic' molecular subtypes (luminal A, luminal B, basal-like, HER2-enriched and normal-like) is a working model, and that additional molecular subtypes with distinct repertoires of molecular aberrations and clinical behaviour will be identified. In fact, recent studies have led to the identification of claudin-low [11] and molecular apocrine subtypes [14], whose clinical and biological significance remain to be fully elucidated. Furthermore, unsupervised analysis of triple-negative breast cancers led to the identification of six distinct subtypes displaying unique gene expression and ontologies, including two basal-like, an immunomodulatory, a mesenchymal, a mesenchymal stem-like and a luminal androgen receptor subtypes [45]. Taken together, these observations demonstrate that the final molecular taxonomy of breast cancer is likely to be more complex than initially envisaged [7]. With the development of high-throughput technologies that allow for the complete characterisation of the repertoire of somatic genetic aberrations and the complete transcriptomic features of human cancers, it is likely that the molecular taxonomy of breast cancers will evolve further.

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### disclosures

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- All remaining authors have declared no conflicts of interest.

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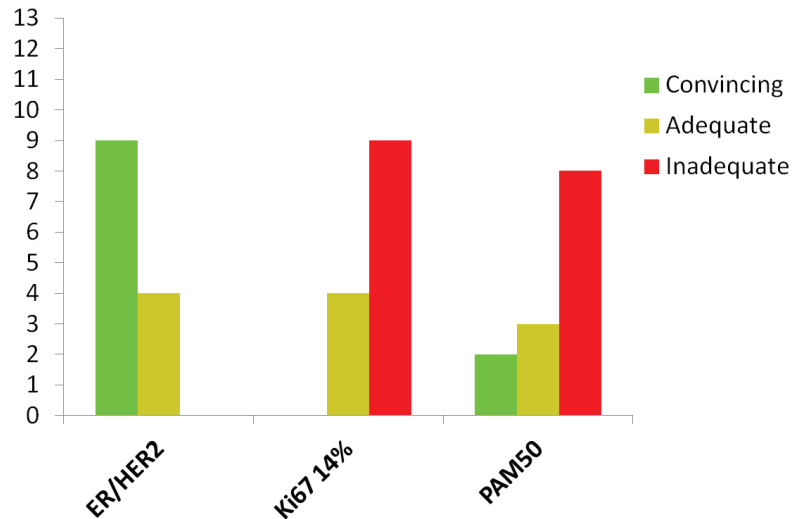
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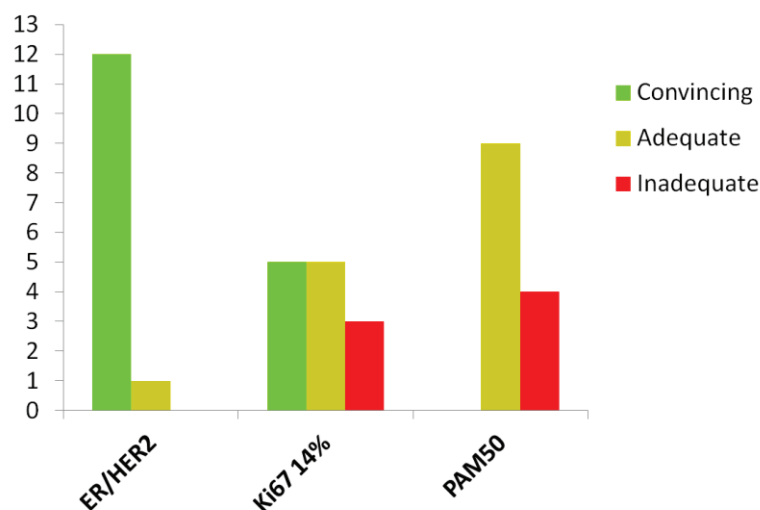
### III – DISCUSSION

#### 1°) Validité analytique



Selon les critères EGAPP, la majorité du panel considère que les données sur la validité analytique de RE / HER2 sont convaincantes, et que celles pour le Ki-67 et PAM50 sont inadéquates. Le panel précise que des données supplémentaires sont nécessaires.

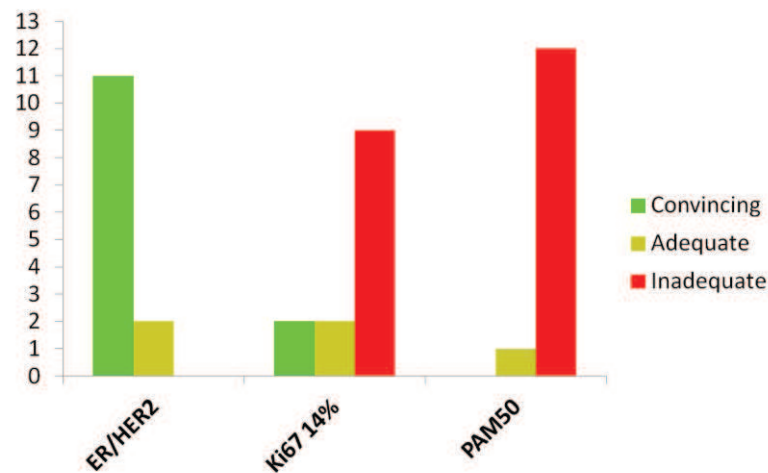
#### 2°) Validité clinique



Selon les critères EGAPP, la majorité du panel trouve que les données sur la validité clinique de RE / HER2 sont convaincantes et que celles pour le Ki-67 et PAM50 sont

adéquates. Le panel considère que les sous-types moléculaires devraient être définis sur la base de l'évaluation de RE et HER2 (IHC ± HIS) qui ont largement démontré leurs valeurs pronostiques et prédictives. Ni le Ki-67, ni PAM50, n'atteignent ce niveau de preuve, cependant les 2 approches permettent de distinguer 2 groupes de tumeurs RE+ qui ont un pronostic différent, et peuvent être des pistes intéressantes pour distinguer les tumeurs lumineales A des tumeurs lumineales B. La nécessité d'avoir plus de données sur la validité clinique des 2 tests est reconnue par l'ensemble du panel. Cela inclue le développement de tests standardisés (en particulier pour le Ki-67) [123] et l'évaluation à la fois du Ki-67 et de PAM50 sur un grand nombre de patients à partir d'études prospectives randomisées.

### 3°) Utilité clinique



La majorité du panel considère que l'utilité clinique de RE et HER2 est convaincante. Le panel considère que l'utilité clinique du Ki-67 et de PAM50 est inadéquate. Pour la pratique clinique, le panel recommande d'utiliser RE et HER2 pour l'identification de sous-groupes cliniquement pertinents, cependant il n'y a pas de données convaincantes pour l'utilisation du Ki-67 avec un cut-off à 14% pour la distinction des tumeurs lumineales A et B. Le panel ne recommande pas l'utilisation de PAM50 pour les décisions de traitement systémique, notamment en cas de discordance avec l'IHC. Par exemple, une tumeur HER2-enriched qui n'a pas de surexpression ou d'amplification de HER2 en IHC / HIS doit être traitée comme une tumeur HER2 négative, il n'y a aucune preuve que ce patient bénéficie d'une thérapie anti-HER2. Pour un patient avec une tumeur RE+ / HER2-, avec des facteurs

de risque clinico-pathologiques de récurrence importants, et classée luminal A par PAM50, la chimiothérapie ne doit pas être exclue totalement sur cette seule donnée.

Bien que les patients ayant une tumeur luminale B sont souvent décrits comme ayant un moins bon pronostic que les patients avec une tumeur luminale A, le panel ne soutient pas le fait que cette distinction A / B doive être faite pour prendre des décisions sur la chimiothérapie adjuvante, du fait de l'absence de données convaincantes sur son utilité clinique, à la fois en cas de tumeur NO ou N+ (Ki-67 et PAM50). Le panel estime que la décision de chimiothérapie adjuvante doit être exclusivement prise sur les données clinico-pathologiques habituelles, en attendant des données plus robustes.

Tous les membres du panel sont d'accord sur la nécessité d'intégrer les sous-types moléculaires définis par PAM50 ou d'autres modèles moléculaires dans le design des essais cliniques, en privilégiant ceux applicables sur des échantillons tumoraux inclus et fixés en paraffine. Les données sur PAM50 et l'IHC devraient être implémentées en parallèle pour pouvoir les comparer. Pour avoir la preuve de son utilité clinique, le Ki-67 devrait être soumis à relecture centralisée.

Cette étude a certaines limites : la revue de la littérature a été réalisée jusqu'en février 2012, les conclusions auraient peut-être été différentes en utilisant d'autres critères que les critères EGAPP ou en faisant intervenir d'autres experts.

**Co-expression du récepteur androgène et de  
FOXA1 dans les cancers du sein triple négatif :  
étude ancillaire de l'essai PACS08  
(Article 3)**

**Co-expression of androgen receptor and FOXA1  
in nonmetastatic triple-negative breast cancer:  
ancillary study from PACS08 trial**

*S. Guiu, C. Charon-Barra, D. Vernerey, P. Fumoleau, M. Campone,  
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**Future Oncology**

## I – INTRODUCTION

Les cancers du sein TN représentent environ 15% de l'ensemble des cancers du sein et sont caractérisés par l'absence d'expression des RE et RP ainsi que l'absence de surexpression et / ou d'amplification de HER2. Ce sont généralement des tumeurs agressives, de haut grade, touchant des femmes jeunes associées à un taux de récurrence plus important, malgré une fréquente chimiosensibilité initiale [167, 168]. L'absence de thérapie ciblée validée actuellement fait de ce sous-type particulier un véritable challenge thérapeutique.

Les tumeurs basal-like, définies par le profil d'expression génomique intrinsèque selon Perou *et al*, correspondent le plus souvent aux tumeurs dites TN mais la concordance IHC / analyse génomique n'est pas parfaite avec 20 à 30% de cas discordants, comme nous l'avons déjà souligné [169]. Afin d'améliorer la définition des tumeurs basal-like en IHC, une nouvelle définition a ainsi été proposée incluant l'expression des cytokératines 5/6 et / ou de l'EGFR [145, 170].

Il existe une grande hétérogénéité biologique au sein de ces tumeurs TN qui ne sont pas toutes « basales ». Lehmann *et al*, grâce à l'analyse du profil d'expression génomique de 587 tumeurs TN, a identifié 6 sous-types distincts : basal-like 1, basal-like 2, immunomodulatory, mesenchymal-like, mesenchymal stem-like, luminal androgen receptor (LAR) [171]. Les tumeurs LAR ont un faible taux de pCR après chimiothérapie néoadjuvante conventionnelle, mais semblent paradoxalement être de meilleur pronostic [172]. Elles semblent correspondre aux tumeurs dites moléculaires apocrines définies par Farmer *et al* [173] et Doane *et al* [174] auparavant, avec les caractéristiques suivantes : tumeurs RE négatif, RA positif, profil d'expression génomique proche des tumeurs luminales RE positif (dont *FOXA1+*) et une perte des cytokératines basales (CK5/6). Elles représentent 8 à 12% des cancers du sein [171, 173, 174]. Les lignées cellulaires LAR sont sensibles aux antagonistes du RA [171, 175].

Le RA est un membre de la famille des récepteurs nucléaires et un facteur de transcription ligand-dépendant. Il est fréquemment exprimé dans les cancers du sein RE+ (70 - 90%) mais moins souvent dans les cancers du sein TN, avec un taux d'expression variable selon les études, compris entre 8 et 53% [176, 177]. Cette variabilité est probablement liée à



l'hétérogénéité des méthodes d'évaluation et des seuils définis pour la positivité [176]. Les tumeurs TN exprimant le RA seraient moins à risque de récurrence selon une méta-analyse récente, sans impact sur la SG [178]. Ce biomarqueur est également une cible thérapeutique potentielle : le bicalutamide (antagoniste non stéroïdien du RA) a déjà montré un bénéfice clinique de 19% chez 50 patients RE-, RA+ [179]. D'autres molécules sont en cours d'étude, comme l'enzalutamide, l'orterone ou l'enobosarm.

FOXA (Forkhead box protein A) est une famille de régulateurs transcriptionnels. Les protéines FOXA se lient à l'ADN et induisent un réarrangement nucléosomal qui facilite la liaison d'autres facteurs de transcription comme les RH [180]. FOXA1 (ou HNF3 $\alpha$ ) et RE $\alpha$  sont co-exprimés sur les cellules épithéliales luminales dans la glande mammaire en développement [181]. FOXA1 est nécessaire à l'expression du RE $\alpha$  au niveau de l'épithélium mammaire normal et semble requis pour le développement des canaux mammaires [181]. Il a également un rôle dans la pathogénèse car est essentiel pour l'expression de 50% des gènes liés au RE [182, 183]. Le gène *FOXA1* est normalement exprimé par les tumeurs luminales, exprimant le RE [184]. La présence d'une surexpression de la protéine FOXA1 en IHC est, dans ces tumeurs, décrite comme facteur de bon pronostic [185]. Dans les tumeurs moléculaires apocrines, FOXA1 pourrait diriger le RA sur des sites normalement occupés par le RE dans les tumeurs luminales, induisant ainsi la transcription de facteurs stimulant la prolifération cellulaire [186]. Pour être actif, le RA doit interagir avec l'ADN au niveau des HRE (hormone response elements). Des études précliniques suggèrent que dans ces tumeurs apocrines, FOXA1 est nécessaire à l'interaction du RA avec la chromatine, comme pour le RE dans les tumeurs luminales [186].

Le coût et la complexité de l'utilisation des profils d'expression génique pour l'identification des différents sous-types moléculaires, ont mené au développement de marqueurs de substitution en IHC, comme nous l'avons déjà évoqué (RE, RP, HER2 et Ki-67) [121]. Etant donné l'existence d'options thérapeutiques potentielles dans ce sous-groupe de tumeurs moléculaires apocrines, il est indispensable de pouvoir les identifier facilement en pratique clinique. Dans cette étude nous avons évalué l'expression en IHC (sur Tissue Microarrays) du RA et de FOXA1 sur des tumeurs TN (RE et RP <10%, HER2-) provenant de patientes incluses dans l'étude adjuvante multicentrique d'UNICANCER, PACS08. Nous avons étudié les profils cliniques et histologiques de ces tumeurs TN en fonction de l'expression à la fois du RA et de FOXA1.

**II- ARTICLE**

## RESEARCH ARTICLE

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# Coexpression of androgen receptor and FOXA1 in nonmetastatic triple-negative breast cancer: ancillary study from PACS08 trial

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**Aim:** Microarray studies identified a subgroup of molecular apocrine tumors (estrogen receptor [ER] negative/androgen receptor [AR] positive) that express luminal genes including *FOXA1*. *FOXA1* may direct AR to sites normally occupied by ER in luminal tumors, inducing an estrogen-like gene program that stimulated proliferation. **Materials & methods:** Expression of AR and *FOXA1* was evaluated by immunohistochemistry in 592 patients with nonmetastatic triple-negative breast cancer (TNBC). **Results:** Coexpression of AR and *FOXA1* was found in 15.2% of patients. These tumors were more frequently lobular, found in older patients and exhibited a lower nuclear grade and a greater degree of node involvement. They less often exhibited lymphocytic infiltrate, pushing margins, syncytial architecture, central fibrosis or necrosis. **Conclusion:** TNBC with coexpression of AR and *FOXA1* seems to behave like luminal tumors with a morphological profile distinct from other TNBC. These biomarkers could be useful to identify a subgroup of TNBC and could have future therapeutic implications.

Triple-negative breast cancers (TNBCs) represent 10–20% of all breast cancers (BCs) and are characterized by the lack of estrogen receptor (ER) and progesterone receptor (PR) expression as well as HER2 overexpression and/or amplification. Patients with TNBC have a poor prognosis despite the association of surgery, adjuvant chemotherapy and radiotherapy. This is mainly due to the heterogeneity of the disease and the absence of well-defined molecular targets [1,2]. They are thus a therapeutic challenge.

Based on hierarchical clustering, five ‘intrinsic’ molecular BC subtypes were initially described (luminal A, luminal B, basal-like, HER2-enriched and normal breast-like) with prognostic significance [3–6]. Luminal and basal-like tumors were the most robust subgroups. Furthermore, additional class discovery studies with larger data sets resulted in the identification of other molecular subtypes, including molecular apocrine tumors [7,8]. These tumors are ER negative, but express genes that are normally expressed in ER-positive luminal tumors like androgen receptor (AR) [7,8]. AR

## KEYWORDS

- androgen receptor
- FOXA1 • molecular apocrine tumors
- triple-negative breast cancer

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is a member of the nuclear receptor family and is a ligand-dependent transcription factor. AR is frequently expressed in ER+ BC (70–90%) but it is less frequent in TNBC, and extremely variable across studies (8–53%) probably due to the heterogeneity of both assays for AR evaluation and chosen cutoffs for positivity heterogeneity [9]. Recent studies suggested an antitumoral effect of bicalutamide, a nonsteroidal AR antagonist, in AR+/ER- BC and luminal AR (LAR) subtype [2,10].

FOXA is a family of transcriptional regulators. FOXA proteins bind DNA and induce a nucleosomal rearrangement that facilitates binding of other transcriptional regulators (e.g., sex steroid hormone nuclear receptor) [11]. FOXA1 (also known as HNF3 $\alpha$ ) and ER $\alpha$  are coexpressed in luminal epithelial cells in the developing mammary gland [12]. FOXA1 is necessary for the expression of ER $\alpha$  in the normal mammary epithelium and seems to be required for mammary ductal development [12]. Moreover, FOXA1 plays a critical role in pathogenesis: it is essential for the expression of 50% of ER-related genes [13,14]. FOXA1 is expressed in molecular apocrine tumors and preclinical data suggested that in these tumors FOXA1 may direct AR to sites normally occupied by ER in luminal tumors, inducing an estrogen-like gene program that stimulates proliferation [15].

The cost and complexity of gene-expression (GE) profiling for the identification of molecular subtypes have limited its use in clinical practice and led to the development of immunohistochemical surrogate definitions, based on ER, PR, HER2 and Ki-67 [16]. Due to the potential new therapeutic options in the subgroup of molecular apocrine or LAR tumors, it is essential to identify them easily in daily practice. In a prospective collection of nonmetastatic TN breast tumors from patients enrolled in the UNICANCER PACS08 adjuvant multicenter trial, we therefore aimed to specify clinical and histological profiles associated with AR and FOXA1 expression by immunohistochemistry (IHC).

## Materials & methods

### • Patients

This biomarker study is ancillary to the PACS08 trial, which, between October 2007 and September 2010, included 762 patients with BC. These patients had unilateral, localized, poor prognosis BC defined as TNBC (ER, PR and HER2 negative) whatever the lymph node

status (n = 592) or HER2 and PR-negative tumors with positive lymph nodes (n = 170). This adjuvant, open-label, multicenter Phase III study compared three cycles of FEC100 (fluorouracil 500 mg/m<sup>2</sup>, epirubicin 100 mg/m<sup>2</sup>, cyclophosphamide 500 mg/m<sup>2</sup>) followed by three cycles of docetaxel (100 mg/m<sup>2</sup>) every 3 weeks with three cycles of FEC100 followed by three cycles of ixabepilone (Ixemptra®, Bristol-Myers Squibb [BMS], NY, USA; 40 mg/m<sup>2</sup>) every 3 weeks. The primary end point was disease-free survival (DFS) at 5 years. A total of 2500 patients were supposed to have been included, but the trial was stopped prematurely following the BMS announcement in August 2010 that it would stop ixabepilone development in an adjuvant setting. All of the patients included were followed according to the protocol.

Prior to inclusion, ER, PR and HER2 status were centrally assessed in expert centers by pathologists with considerable expertise in BC. Hormone receptor negativity was defined as ER and PR <10% by IHC. Indeed, the UNICANCER-PACS08 trial is a French study and in France, the cutoff used to define ER and PR negativity is less than 10%. This threshold was part of the inclusion criteria for the trial. This is different of the American Society of Clinical Oncology/College of American Pathologists guidelines' cutoff: ER and PR negativity are defined as ER and PR <1%. HER2 negativity was defined as IHC 0/1+ or IHC 2+ and negative fluorescent/chromogenic hybridization *in situ*.

A tumor block representative of the primary tumor was collected for the 762 enrolled patients. All samples were obtained from operated tumors before any systemic treatment. Patients provided written informed consent for research use. The study was approved by a French Ethic Committee (CPP Ouest V, CHU Pontchaillou, Rennes, France; reference number: 07/09-626).

In parallel to this ancillary study, 745 cases (97.8%) were reviewed by a board of expert breast pathologists during a 1 week working session. Difficult cases were examined under a multihead microscope and discussed. The following pathological features were determined: histological type, histological grade according to Elston and Ellis, mitotic count, lympho-vascular invasion, lymphocytic infiltration, pushing margins, syncytial architecture, central fibrosis, necrosis and the presence of *in situ* ductal and/or lobular carcinoma.

- **Tissue microarray**

- **& immunohistochemistry**

The 762 tumors were used to build the 14 tissue microarray blocks (TMAs). The description of creation of tissue microarray is in the **Supplementary Material** (see online at [www.futuremedicine.com/doi/suppl/10.2217/fon.15.102](http://www.futuremedicine.com/doi/suppl/10.2217/fon.15.102)). IHC was performed with an indirect immunoperoxidase method using the Benchmark XT from Ventana. Two antibodies directed against AR (a rabbit monoclonal primary antibody, clone SP107, prediluted, Ventana) [17,18] and FOXA1 (an affinity purified goat polyclonal antibody, HNF-3 $\alpha$ / $\beta$  (C-20), Santa Cruz biotechnology, dilution 1/200e) [19–22] were tested on the 14 TMAs.

- **Analysis / interpretation**

The 28 slides (14 for each antibody) were digitized at  $\times 40$  magnification using the Hamamatsu Nanosizer scanner. The virtual TMA sections were analyzed independently by two observers both blinded to the clinicopathological characteristics: a pathologist (C Charon-Barra) and an oncologist who was trained in immunohistological and signal quantification analyses (S Guiu). In cases of disagreement after comparing the results, images were viewed by a second pathologist (L Arnould) and the final decision was made by consensus. Nuclear staining in the tumor area was evaluated for each patient. A tumor was considered positive when at least 10% of the tumor cells were stained. In the majority of the studies regarding AR in TNBC, the criteria for AR positivity is the percentage of stained cells. Moreover, a cutoff of 10% of stained cells is most widely used [9]. TMAs have known limitation in terms of assessing intratumoral heterogeneity in contrast to whole section analysis. Consequently, results from the triplicate cores were averaged to minimize this. The healthy glands were noted positive or not.

- **Statistical analyses**

Kappa coefficients were used to assess the agreement between the two observers for AR and FOXA1 expression in the whole population of the trial.

Descriptive analyses were performed exclusively in the triple-negative population (Figure 1). We provided means and standard deviations for descriptive analyses of continuous variables. Means and proportions were compared using Student's *t*-test (or one-way ANOVA) and the chi-square test (or

Fisher's exact test, if appropriate). Associations between clinicopathological characteristics and the AR+/FOXA1+ phenotype in TNBC were further examined using univariate and multivariate unconditional logistic regression. All variables with a *p*-value of 0.20 or less in the univariate analysis were then included in the multivariate analysis. The internal validity of this model was explored using bootstrapping (1000 replications). All tests were two-sided, and *p*-values less than 0.05 were considered statistically significant.

The analyses were done using SAS software, version 9.3 (SAS Institute).

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## Results

- **AR expression**

The evaluation of AR expression was highly reproducible in the whole population of the trial with interobserver Kappa coefficients of 0.96 (95% CI: 0.94–0.98).

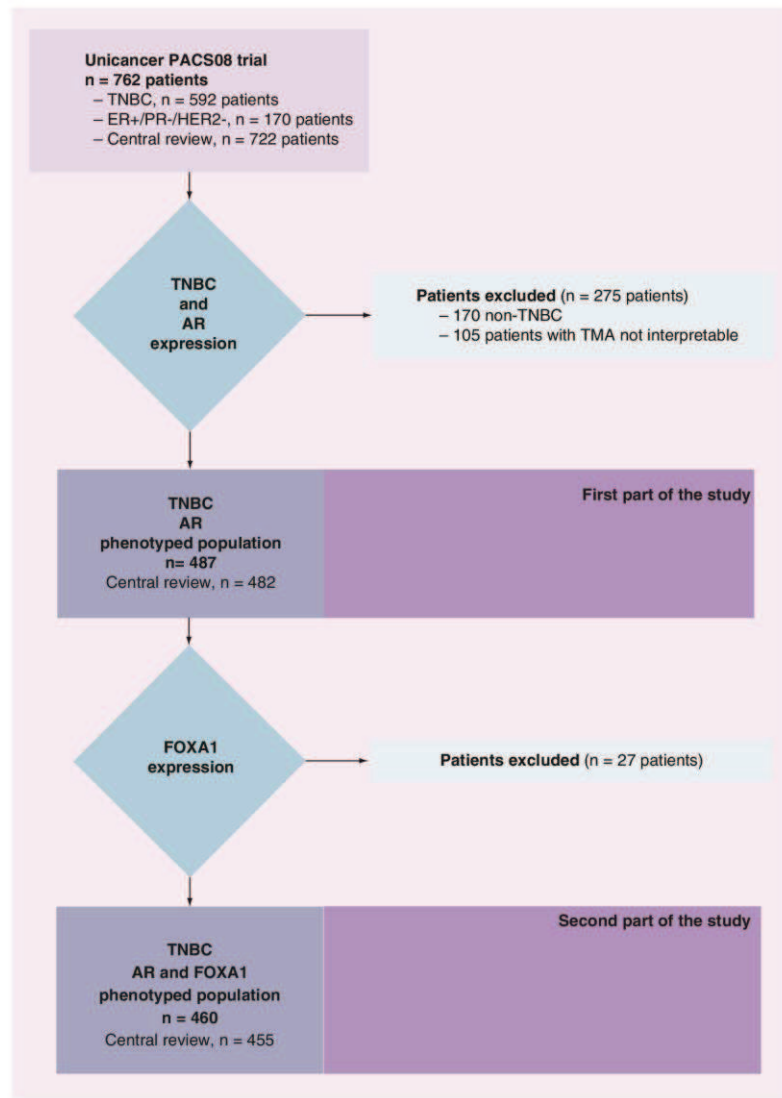
AR expression was available in 487 (82.3%) TNBCs (Figure 1), 126 (25.9%) of which were AR+ tumors. Illustrations of positive and negative AR nuclear staining are shown in Figure 2 (magnification  $\times 10$ ). There was no significant difference for the main clinical and pathological characteristics (age at diagnosis, grade, nodal involvement, pathological type and size) between patients with available AR expression and patients without available IHC (Supplementary Table 1).

Table 1 reports clinical and tumor characteristics of the whole population of TNBC and of patients with AR- and AR+ TNBCs. Patients with AR+ tumors were significantly older ( $p < 0.0002$ ) than patients with AR- tumors. They exhibited tumors with significantly lower grades (grade 1–2: 22 vs 6%;  $p < 0.0001$ ) confirmed by the central review, and less frequent ductal histology ( $p = 0.004$ ), lymphocytic infiltrate ( $p = 0.03$ ), pushing margins ( $p = 0.0009$ ), central fibrosis ( $p = 0.004$ ) and necrosis ( $p < 0.0001$ ), but more frequent DCIS ( $p = 0.05$ ) than AR- TNBC.

- **Coexpression of AR and FOXA1**

The evaluation of FOXA1 expression was highly reproducible in the whole population of the trial with interobserver Kappa coefficients of 0.90 (95% CI: 0.86–0.94). Illustrations of positive and negative FOXA1 nuclear staining are shown in Figure 2 (magnification  $\times 10$ ).

In TNBC, both AR and FOXA1 expressions were available in 460 (77.7%) cases (Figure 1). There was no significant difference for the main clinical and pathological characteristics



**Figure 1.** Flow chart of the study. Of 762 patients included in the UNICANCER-PACS08 trial, 592 had TNBC. Among them, 487 had AR expression by immunohistochemistry available on their tumor. Among patients with AR expression available, 460 had also FOXA1 expression by immunohistochemistry available.

AR: Androgen receptor; TMA: Tissue microarray block; TNBC: Triple-negative breast cancer.

between patients with available AR and FOXA1 expression and patients without available IHC (Supplementary Table 2).

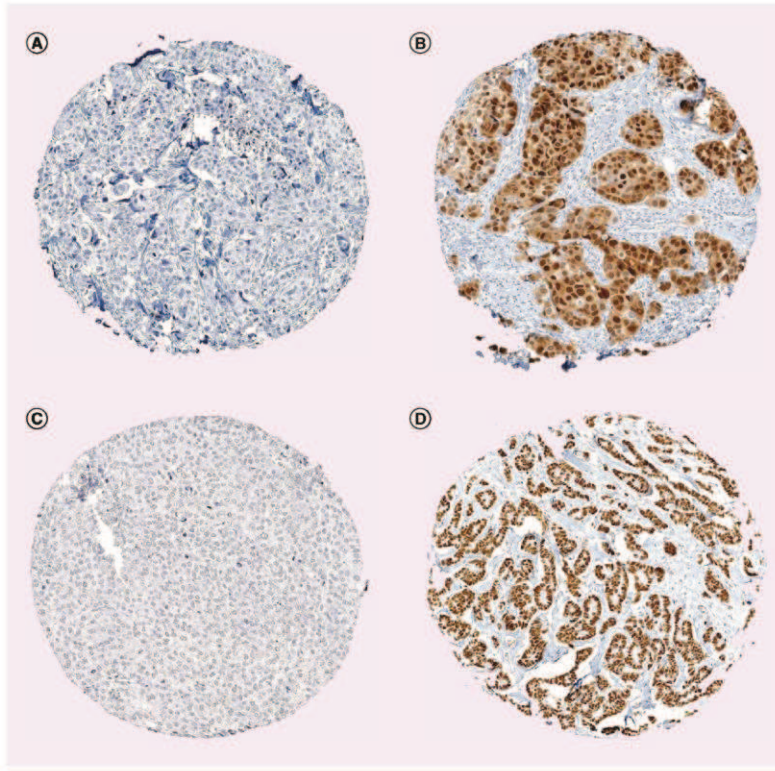
Three hundred and thirty-five patients (72.8%) had AR-/FOXA1- tumors and 70 (15.2%) had AR+/FOXA1+ tumors. Several

tumors had a discrepant status regarding both AR and FOXA1 expressions (10.5% AR+/FOXA1-, 1.5% AR-/FOXA1+). Table 2 reports the clinical and tumor characteristics of the TNBC according to both AR and FOXA1 expression.

In univariate unconditional logistic regression analysis, AR+/FOXA1+ tumors were more frequently found in older patients (OR: 1.06; 95% CI: 1.031–1.090;  $p < 0.0001$ ), were more frequently of lobular histology (OR: 13.432; 95% CI: 3.388–75.548;  $p = 0.006$ ), with a lower grade (OR: 0.106; 95% CI: 0.054–0.207;  $p < 0.0001$ ) and more node involvement (OR: 1.671; 95% CI: 0.996–2.805;  $p = 0.05$ ) with capsular rupture (OR: 2.274; 95% CI:

1.209–4.279;  $p = 0.01$ ) than other subgroups (Table 3). They were also less likely to exhibit lymphocytic infiltrate ( $p = 0.009$ ), pushing margins ( $p < 0.0001$ ), syncytial architecture (OR: 0.132; 95% CI: 0.047–0.372;  $p = 0.0001$ ), central fibrosis (OR: 0.446; 95% CI: 0.239–0.832;  $p = 0.01$ ) and necrosis (OR: 0.293; 95% CI: 0.169–0.509;  $p < 0.0001$ ), but more likely to have an *in situ* component (OR: 2.735; 95% CI: 1.557–4.805;  $p = 0.0005$ ) than were other TN tumors (Table 3).

In multivariate unconditional logistic regression analysis, age at diagnosis (OR: 1.059; 95% CI: 1.022–1.098;  $p = 0.002$ ), grade (OR: 0.222; 95% CI: 0.098–0.505;  $p = 0.001$ ), syncytial architecture (OR: 0.109; 95% CI:



**Figure 2.** Illustrations of triple-negative breast tumor samples with negative and positive nuclear immunostaining for androgen receptor or FOXA1 (magnification  $\times 10$ ). (A) Androgen receptor-negative triple-negative breast cancer. (B) Androgen receptor-positive triple-negative breast cancer with 90% of stained tumor cells. (C) FOXA1-negative triple-negative breast cancer. (D) FOXA1-positive triple-negative breast cancer with 95% of stained cells.

Table 1. Clinical and pathological characteristics of all patients with triple-negative breast cancer and according to androgen receptor status.				
Patient characteristics	Overall TNBC (n = 487), n (%)	AR- (n = 361), n (%)	AR+ (n = 126), n (%)	p-value
Age at diagnosis (years), number of patients with available data (mean ± standard deviation)	487 (51.5 ± 10.3)	361 (50.5 ± 10.3)	126 (54.5 ± 9.9)	<0.0002
Menopausal status:	483	358	125	
– Premenopausal	230 (48)	187 (52)	43 (34)	
– Postmenopausal	253 (52)	171 (52)	82 (55)	<0.0001
Clinical tumor size:	358	265	93	
– ≤2 cm	163 (46)	121 (46)	42 (45)	
– >2 cm	195 (54)	144 (54)	51 (55)	1
Pathological tumor size (pT):	487	361	26	
– pT1	149 (31)	107 (30)	42 (33)	
– pT2–4	338 (69)	254 (70)	84 (67)	0.4
Pathological nodal involvement (pN):	486	360	126	
– pN0	250 (51)	191 (53)	59 (47)	
– pN+	236 (49)	169 (47)	67 (53)	0.2
Vascular emboli	455 164 (36)	337 115 (34)	118 49 (42)	0.2
Capsular rupture	442 71 (16)	332 50 (15)	110 21 (19)	0.3
Pathological type:	451	329	122	
– Ductal	439 (97)	323 (98)	116 (95)	
– Lobular	12 (3)	6 (2)	6 (5)	0.09
Pathological type <sup>1</sup> :	482	358	124	
– Ductal	351 (73)	271 (76)	80 (65)	
– Lobular	6 (1)	2 (0.5)	4 (3)	
– Medullary	14 (3)	11 (3)	3 (2)	
– Metaplastic	26 (5.5)	21 (6)	5 (4)	
– Mixed	6 (1)	6 (2)	0	
– Apocrine	19 (4)	2 (0.5)	17 (14)	
– Other	53 (11)	41 (11.5)	12 (10)	
– Unknown	7 (1.5)	4 (0.5)	3 (2)	<0.0001
Multifocality:	487	361	126	
– Yes	73 (15)	51 (14)	22 (17)	
– No	414 (85)	310 (86)	104 (83)	0.4
Grade:	487	361	126	
– Grade 1	1 (1)	0	1 (1)	
– Grade 2	47 (10)	20 (6)	27 (21)	
– Grade 3	439 (89)	341 (94)	98 (78)	<0.0001
Elston and Ellis grade <sup>1</sup> :	482	358	24	
– Grade 1	4 (1)	2 (1)	2 (2)	
– Grade 2	45 (9)	18 (5)	27 (22)	
– Grade 3	377 (78)	295 (82)	82 (66)	
– Unknown	56 (12)	43 (12)	13 (10)	<0.0001
Peritumoral emboli <sup>1</sup> :	482	358	124	
– Yes	127 (26)	90 (25)	37 (30)	
– No	333 (69)	254 (71)	79 (64)	
– Unknown	22 (5)	14 (4)	8 (6)	0.25
Lymphocytic infiltrate <sup>1</sup> :	480	356	124	
– Absent	46 (10)	26 (8)	20 (16)	
– 1+	186 (39)	140 (39)	46 (37)	
– 2+	168 (35)	126 (35)	42 (34)	

<sup>1</sup>Data of central review.

AR: Androgen receptor; DCIS: Ductal carcinoma *in situ*; LCIS: Lobular carcinoma *in situ*; TNBC: Triple-negative breast cancer.



**Table 1. Clinical and pathological characteristics of all patients with triple-negative breast cancer and according to androgen receptor status (cont.).**

Patient characteristics	Overall TNBC (n = 487), n (%)	AR- (n = 361), n (%)	AR+ (n = 126), n (%)	p-value
Lymphocytic infiltrate <sup>†</sup> (cont.):	480	356	124	
– 3+	68 (14)	56 (16)	12 (10)	
– Unknown	12 (2)	8 (2)	4 (3)	0.03
Pushing margins <sup>†</sup> :	480	356	124	
– No	173 (36)	110 (31)	63 (51)	
– <50%	116 (24)	93 (26)	23 (18)	
– ≥50%	173 (36)	140 (39)	33 (27)	
– Unknown	18 (4)	13 (4)	5 (4)	0.0009
Syncytial architecture <sup>†</sup> :	482	358	124	
– No	325 (68)	231 (65)	94 (76)	
– ≤75%	84 (17)	69 (19)	15 (12)	
– >75%	50 (10)	41 (11)	9 (7)	
– Unknown	23 (5)	17 (5)	6 (5)	0.11
Central fibrosis <sup>†</sup> :	482	358	124	
– No	305 (63)	212 (59)	93 (75)	
– Yes	164 (34)	137 (38)	27 (22)	
– Unknown	13 (3)	9 (3)	4 (3)	0.004
Necrosis <sup>†</sup> :	482	358	124	
– No	210 (44)	132 (37)	78 (63)	
– Yes	262 (54)	218 (61)	44 (35)	
– Unknown	10 (2)	8 (2)	2 (2)	<0.0001
DCIS associated <sup>†</sup> :	481	358	123	
– No	291 (60)	226 (63)	65 (53)	
– Yes	181 (38)	124 (35)	57 (46)	
– Unknown	9 (2)	8 (2)	1 (1)	0.05
Grade of DCIS <sup>†</sup> :	181	124	57	
– Low	1 (1)	1 (1)	0	
– Intermediate	24 (13)	12 (9)	12 (21)	
– High	153 (84)	109 (88)	44 (77)	
– Unknown	3 (2)	2 (2)	1 (2)	0.13
LCIS associated <sup>†</sup> :	478	356	122	
– No	466 (97)	349 (98)	117 (96)	
– Yes	4 (1)	2 (1)	2 (2)	
– Unknown	8 (2)	5 (1)	3 (2)	0.3

<sup>†</sup>Data of central review.  
AR: Androgen receptor; DCIS: Ductal carcinoma *in situ*; LCIS: Lobular carcinoma *in situ*; TNBC: Triple-negative breast cancer.

0.025–0.467;  $p = 0.003$ ) and central fibrosis (OR: 0.343; 95% CI: 0.153–0.769;  $p = 0.009$ ) were the only independent factors associated with the AR+/FOXA1+ phenotype (Table 4). Internal validation using bootstrapping confirmed these results. The 95% adjusted bootstrap percentile OR intervals were the following: age at diagnosis (OR: 1.059; 95% CI: 1.02–1.10), grade (OR: 0.222; 95% CI: 0.09–0.57), syncytial architecture (OR: 0.109; 95% CI:  $1.99 \times 10^{-6}$ –0.43) and central fibrosis (OR: 0.343; 95% CI: 0.16–0.75). The lobular histology is not an independent factor associated with the AR+/FOXA1+ phenotype in the multivariate analysis, indicating

that the results of the univariate analysis must be taken with caution.

## Discussion

Among TNBCs, AR expression is extremely variable across studies (8–53%), probably due to their retrospective nature, patient selection (primary tumor vs metastases) and the heterogeneity of both assays for AR evaluation and chosen cutoffs for positivity [9]. In our study, the rate was almost 26%. We found only two studies, with smaller sample sizes, that used the same cutoffs as ours for AR, ER, PR and HER2. The rates for AR positivity were 13.5

Patient characteristics	AR+/FOXA1+ (n = 70), n (%)	AR+/FOXA1- (n = 48), n (%)	AR-/FOXA1+ (n = 7), n (%)	AR-/FOXA1- (n = 335), n (%)	p-value
Age at diagnosis (years), number of patients with available data (mean $\pm$ standard deviation)	70 (56.4 $\pm$ 8.6)	48 (51.6 $\pm$ 11.3)	7 (46.6 $\pm$ 5.1)	335 (50.8 $\pm$ 10.2)	0.0002
Menopausal status:	69	48	7	332	<0.0001
– Premenopausal	17 (25)	23 (48)	6 (86)	171 (52)	
– Postmenopausal	52 (75)	25 (52)	1 (14)	161 (48)	
Clinical tumor size:	52	37	6	247	0.6
– $\leq$ 2 cm	23 (44)	17 (46)	1 (17)	115 (47)	
– >2 cm	29 (56)	20 (54)	5 (83)	132 (53)	
Pathological tumor size (pT):	70	48	7	335	0.7
– pT1	20 (29)	18 (38)	2 (29)	100 (30)	
– pT2–4	50 (71)	30 (62)	5 (71)	235 (7)	
Pathological nodal involvement (pN):	70	48	7	334	<0.0001
– pN0	28 (40)	26 (54)	4 (57)	175 (52)	
– pN+	42 (60)	22 (46)	3 (43)	159 (48)	
Number of patients with available data, number of patients with vascular emboli (%)	66, 33 (50)	45, 14 (31)	7, 1 (14)	313, 107 (34)	0.001
Number of patients with available data, number of patients with capsular rupture (%)	62, 17 (27)	43, 4 (9)	60	309, 47 (15)	0.048
Pathological type:	68	48	7	303	0.023
– Ductal	63 (93)	47 (98)	6 (86)	298 (98)	
– Lobular	5 (7)	1 (2)	1 (14)	5 (2)	
Pathological type <sup>1</sup> :	69	47	7	332	NA
– Ductal	43 (63)	34 (73)	4 (57%)	251 (75)	
– Lobular	4 (6)	0	0	2 (1)	
– Mucinous	1 (1)	0	0	0	
– Tubular	0	0	0	0	
– Medullary	0	3 (6)	0	11 (3)	
– Metaplastic	2 (3)	1 (2)	0	21 (6)	
– Mixed	0	0	0	5 (2)	
– Other	18 (26)	9 (19)	2 (29)	40 (12)	
– Unknown	1 (1)	0	1 (14)	2 (1)	
Multifocality:	70	48	7	335	0.23
– Yes	15 (21)	5 (10)	0	49 (15)	
– No	55 (79)	43 (90)	7 (100)	286 (85)	
Grade:	70	48	7	335	<0.0001
– Grade 1	0	0	0	0	
– Grade 2	23 (33)	4 (8)	1 (14)	17 (5)	
– Grade 3	47 (67)	44 (92)	6 (86)	318 (95)	
Elston and Ellis grade <sup>1</sup> :	69	47	7	332	NA
– Grade 1	1 (1)	1 (2)	0	2 (1)	
– Grade 2	23 (33)	3 (6)	0	16 (4)	
– Grade 3	37 (54)	40 (86)	6 (86)	275 (83)	
– Unknown	8 (12)	3 (6)	1 (14)	39 (12)	
Peritumoral emboli <sup>1</sup> :	69	47	7	332	NA
– Yes	20 (29)	16 (34)	2 (29)	81 (24)	
– No	46 (67)	30 (64)	5 (71)	238 (72)	
– Unknown	3 (4)	1 (2)	0	13 (4)	

<sup>1</sup>Data of central review.

AR: Androgen receptor; DCIS: Ductal carcinoma *in situ*; LCIS: Lobular carcinoma *in situ*; NA: Not applicable.

**Table 2. Clinical and pathological characteristics of patients with triple-negative breast cancer according to both androgen receptor and FOXA1 status: comparison of AR+/FOXA1+, AR+/FOXA1-, AR-/FOXA1+ and AR-/FOXA1- subgroups (cont.).**

Patient characteristics	AR+/FOXA1+ (n = 70), n (%)	AR+/FOXA1- (n = 48), n (%)	AR-/FOXA1+ (n = 7), n (%)	AR-/FOXA1- (n = 335), n (%)	p-value
Lymphocytic infiltrate <sup>†</sup> :	69	47	7	331	NA
– Absent	14 (20)	4 (9)	2 (29)	24 (8)	
– 1+	29 (42)	16 (34)	2 (29)	131 (40)	
– 2+	21 (31)	19 (40)	2 (29)	117 (35)	
– 3+	5 (7)	6 (13)	1 (13)	51 (15)	
– Unknown	0	2 (4)	0	8 (2)	
Pushing margins <sup>†</sup> :	69	47	7	330	NA
– No	45 (66)	17 (37)	1 (14)	100 (30)	
– <50%	11 (16)	10 (21)	1 (14)	91 (28)	
– ≥50%	12 (17)	19 (40)	5 (72)	127 (38)	
– Unknown	1 (1)	1 (2)	0	12 (4)	
Syncytial architecture <sup>†</sup> :	69	47	7	332	NA
– No	62 (90)	29 (62)	4 (57)	215 (65)	
– ≤75%	2 (3)	12 (26)	3 (43)	62 (19)	
– >75%	2 (3)	5 (11)	0	39 (12)	
– Unknown	3 (4)	1 (2)	0	16 (5)	
Central fibrosis <sup>†</sup> :	69	47	7	332	<0.0001
– No	54 (78)	34 (73)	5 (71)	200 (60)	
– Yes	14 (20)	12 (25)	2 (29)	125 (38)	
– Unknown	1 (2)	1 (2)	0	7 (2)	
Necrosis <sup>†</sup> :	69	47	7	332	<0.0001
– No	48 (70)	26 (55)	4 (57)	122 (37)	
– Yes	21 (30)	21 (45)	3 (43)	203 (61)	
– Unknown	0	0	0	7 (2)	
DCIS associated <sup>†</sup> :	69	47	7	332	<0.0001
– No	31 (45)	32 (68)	4 (57)	212 (64)	
– Yes	38 (55)	15 (32)	3 (43)	113 (34)	
– Unknown	0	0	0	7 (2)	
Grade of DCIS <sup>†</sup> :	38	15	3	113	<0.0001
– Low	0	0	0	1	
– Intermediate	7 (18)	4 (27)	1 (33)	11 (9)	
– High	30 (79)	11 (73)	2 (67)	100 (89)	
– Unknown	1 (3)	0	0	1 (1)	
LCIS associated <sup>†</sup> :	68	47	7	330	<0.0001
– No	65 (96)	46 (98)	7 (100)	324 (98)	
– Yes	2 (3)	0	0	2 (1)	
– Unknown	1 (1)	1 (2)	0	4 (1)	

<sup>†</sup>Data of central review.  
AR: Androgen receptor; DCIS: Ductal carcinoma *in situ*; LCIS: Lobular carcinoma *in situ*; NA: Not applicable.

and 33% [23,24]. To our knowledge, the largest study is the retrospective study of Thicke *et al.*: 38% of 699 TNBCs were AR+ with a cutoff of 1% for AR, ER and PR. Their results were concordant with ours (20.5% of AR positive tumors) if they considered a cutoff of 10% for AR positivity, like in our study [25]. If we considered a cutoff of 1% for AR positivity, 33% of TNBC were AR+ in our study (data not shown).

Our results suggest that AR+ TNBC are distinct from the other TNBC and have clinicopathological characteristics similar to those of luminal tumors. The majority of TNBC are usually histological grade 3 [26]. AR+ tumors had significantly lower nuclear grades than AR- tumors. These results are concordant with other studies, both in ER- [24,27–29] and TNBC [23,25,30–33]. These studies found an inverse correlation between AR positivity

**Table 3. Comparison of AR+/FOXA1+ tumors with the other triple negative tumors: univariate logistic regression for factors associated with AR+/FOXA1+ phenotype.**

Characteristics	Number of patients/ number of cases	Univariate analysis	
		Odds ratio (95% CI)	p-value
Age at diagnosis (years)	460/70	1.060 (1.031–1.090)	<0.0001
Clinical tumor size:			0.83
– ≤2 cm	156/23	1	
– >2 cm	186/29	1.068 (0.590–1.935)	
Pathological tumor size (pT):			0.71
– pT1	140/20	1	
– pT2–4	320/50	1.111 (0.634–1.948)	
Pathological nodal involvement (pN):			0.05
– pN0	233/28	1	
– pN+	226/42	1.671 (0.996–2.805)	
Capsular rupture:			0.01
– No	352/45	1	
– Yes	68/17	2.274 (1.209–4.278)	
Pathological type <sup>†</sup> :			0.006
– Ductal	332/43	1	
– Lobular	6/4	13.432 (2.388–75.548)	
– Other	117/22	1.556 (0.886–2.735)	
Multifocality:			0.06
– No	405/57	1	
– Yes	55/13	1.890 (0.955–3.738)	
Elston and Ellis grade <sup>†</sup> :			<0.0001
– Grade 1–2	46/24	1	
– Grade 3	358/37	0.106 (0.054–0.207)	
– Unknown	51/8	0.171 (0.066–0.441)	
Peritumoral emboli <sup>†</sup> :			0.53
– Yes	319/46	1	
– No	119/20	0.834 (0.470–1.479)	
Lymphocytic infiltrate <sup>†</sup> :			0.009
– Absent	44/14	1	
– 1+	178/29	0.417 (0.197–0.882)	
– 2+	159/21	0.326 (0.149–0.714)	
– 3+	63/5	0.185 (0.061–0.562)	
Pushing margins <sup>†</sup> :			<0.0001
– No	163/45	1	
– <50%	113/11	0.283 (0.139–0.575)	
– ≥50%	163/12	0.208 (0.106–0.412)	
Syncytial architecture <sup>†</sup> :			0.0001
– No	310/62	1	
– Yes	125/4	0.132 (0.047–0.372)	
Central fibrosis <sup>†</sup> :			0.01
– No	293/54	1	
– Yes	153/14	0.446 (0.239–0.832)	
Necrosis <sup>†</sup> :			<0.0001
– No	200/48	1	
– Yes	248/1	0.293 (0.169–0.509)	
<i>In situ</i> component:			0.0005
– No	221/20	1	
– Yes	215/46	2.735 (1.557–4.805)	

<sup>†</sup>Data of central review.

and different proliferation markers: grade [24–25,28–32], mitotic score [23,25,32] or Ki67 expression [24,27,32]. In the study of Lehmann, Ki-67 expression was higher in basal-like tumors than in the other subtypes, including LAR tumors [2]. TNBC more frequently affects younger patients (<50 years) [26]. In our study, AR+ tumors appeared more often in older women and in patients with postmenopausal status. Agoff *et al.* reported similar results in 69 patients with ER- tumors [28]. The median age of the 26 patients enrolled in the bicalutamide Phase II trial was 66 years [10]. Age at diagnosis was significantly greater in the LAR subtype [2]. The authors suggested that the use of hormone replacement therapy could be responsible for the higher incidence of LAR tumors in postmenopausal patients [2]. Indeed, synthetic progestins included in hormone replacement therapy have been shown to disrupt AR signaling and could therefore increase the risk of developing BC [34]. Another explanation could be the more indolent profile of these AR+ tumors among TNBC, thereby appearing in older patients.

In our study, AR+ TNBC less frequently exhibited ductal histology (65%) than did AR- tumors (76%). Riva *et al.* showed a high AR positivity rate in invasive lobular carcinomas, whatever the ER status [35]. Conversely, no difference regarding AR expression was found according to histological type in other series; however, the results were limited by the small samples size [28,32]. AR is frequently expressed (70–100%) in carcinomas with apocrine differentiation (abundant eosinophilic cytoplasm, prominent nuclei), including ER- tumors [25,29,36–37]. In our study, 89.5% of tumors with apocrine features were AR+. Morphologically, basal-like BCs are characterized by, among other things, a syncytial growth pattern, the presence of central necrotic zones, pushing borders and lymphocytic infiltrate [26]. AR+ tumors less often exhibited lymphocytic infiltrate, pushing margins, central fibrosis and necrosis than did AR- TNBC. Like Thike *et al.* [25], Putti *et al.* showed a significant correlation between the presence of pushing margins and AR negativity in 291 consecutive ER- BCs [38]. Furthermore, necrosis correlated with AR negativity [38].

Unlike other series with smaller sample sizes, we did not find an association between AR expression and tumor size [28], clinical stage [32] or node involvement [31,39].

Modulation of AR can stimulate or inhibit BC cell growth, depending on the cell line model

used [40,41]. Most ER+ BC are also AR+ [24,42]. In this context, AR may interfere with ER signaling and has an antiproliferative effect, leading to a better prognosis in patients with ER+/AR+ BC [24,43]. However, the prognostic value of AR expression in TNBC is still a matter of debate. In some studies, better DFS and/or overall survival (OS) was observed when tumors expressed AR [25,30–31,39,44–45], and in one study only in the subgroup of nonbasal TNBC [33]. This was possibly due to their more favorable characteristics resulting in a more indolent subgroup of tumors. However, in other studies, there was no impact of this biomarker on the outcome of patients [2,24,32,42,46]. In a recent meta-analysis, the expression of AR in TNBC could predict DFS benefit (HR: 0.40; 95% CI: 0.23–0.69), but not OS benefit [47]. In our study, survival data are not available so far to report the prognostic value of this marker.

Several genomic studies support the existence of a subgroup of ER tumors characterized by a hormonally regulated transcriptional program and response to androgen, called molecular apocrine tumors [7–8,48]. Indeed, 8–24% of BCs share some gene clusters with luminal tumors, others with basal tumors and express AR [7,8]. The MDA-MB-453 cell line, corresponding to this molecular phenotype, demonstrated a proliferative response to androgen in an AR-dependent and an ER-independent manner; the induced transcriptional program significantly overlapped that induced by estrogen in ER+ BC [8]. Thirty to sixty-nine percent of ER-/AR+ BC present HER2 overexpression or amplification [7–8,24,28].

**Table 4. Comparison of AR+/FOXA1+ tumors with the other triple negative tumors: multivariate logistic regression for factors associated with AR+/FOXA1+ phenotype.**

Characteristics	Multivariate analysis	
	Odds ratio (95% CI)	p-value
Age at diagnosis	1.059 (1.022–1.098)	0.002
Elston and Ellis grade <sup>†</sup> :		
– Grade 1–2	1	0.001
– Grade 3	0.222 (0.098–0.505)	
– Unknown	0.382 (0.123–1.192)	
Syncytial architecture <sup>†</sup> :		0.003
– No	1	
– Yes	0.109 (0.025–0.467)	
Central fibrosis <sup>†</sup> :		0.009
– No	1	
– Yes	0.343 (0.153–0.769)	

<sup>†</sup>Data of central review.

A functional cross-talk between AR and HER2 signaling in molecular apocrine cells was described, which modulates cell proliferation and expression of steroid-response genes [49], probably via *FOXA1* [50]. However, a particular subgroup of ER-/AR+ BC was also described independently of HER2 status [2]. Lehmann *et al.* distinguished seven subtypes displaying unique GE and ontologies among 587 TNBCs, including two basal-like, immunomodulatory, mesenchymal, mesenchymal stem-like, unstable and LAR (11%) subtypes [2]. LAR tumors highly expressed AR mRNA, AR targets and coactivators, and displayed luminal GE patterns with *FOXA1* among the most highly expressed genes [2]. Moreover, the TNBC subtype was an independent predictor of pathological complete response in 130 patients who received neoadjuvant chemotherapy (anthracycline and/or taxane regimens) [51]. Patients with LAR tumors (15.4%) had the second lowest pathological complete response rate (10%) but the best OS rate, although the difference was not significant [51]. In three cell lines representative of the LAR subtype (including MDA-MB-453), there was a significant reduction in tumor growth after knockdown of AR expression, indicating that AR is in part responsible for tumor cell viability/survival [2]. Moreover, LAR cell lines were sensitive to bicalutamide [2]. The efficacy of AR blockade was recently confirmed by a proof-of-concept Phase II trial that included women with ER-/PR- and AR+ metastatic BC [10]. On 26 evaluable patients, bicalutamide gave a 19% clinical benefit rate without major toxicities [10]. Other drugs are under investigation (enzalutamide, NCT01889238; abiraterone, NCT01842321).

TNBC, defined by IHC/FISH, is composed of all the intrinsic molecular subtypes, the basal-like subtype being the predominant one. However, TNBC and basal-like definitions should not be considered synonymous due to a high discordance rate (~25%) [52]. Lehmann *et al.* correlated LAR tumor profiles with the intrinsic gene set centroids of the five molecular subtypes [6] and showed that the majority (88%) were classified as either luminal A or B and none as basal-like, supporting the luminal origin of this subtype [2]. Using PAM50 GE analysis, Masuda *et al.* showed that most LAR tumors belonged to the non-basal-like group [51].

In our study, we aimed to identify by immunohistochemistry the LAR subtype, in addition to ER/PR/HER2 and AR biomarkers. Using GE analysis, *FOXA1* is specifically expressed in luminal tumors [3] and the protein FOXA1

is frequently associated with ER expression [19–22,53]. Some studies have shown that FOXA1 can act either as a growth stimulator or as a repressor in BC [11]. FOXA1 has been shown to be present at many ER binding regions in BC cell lines [13–14,54]. It is essential for ER binding to chromatin and transcriptional activity [55] and is required for the optimal expression of nearly 50% of ER $\alpha$ -regulated genes [13,14]. However, FOXA1 may have a growth inhibitory activity in upregulating the expression of the BRCA1-associated cell-cycle inhibitor p27 and promoting the expression of the cell adhesion molecule E-cadherin, thus reducing the motility and invasion of BC cells [56,57]. These data and others suggest a better outcome in patients with ER+/FOXA1+ tumors [21,58–59].

FOXA1 expression was also reported in ER-BCs in several clinical studies and rates varied between 22 and 50% [19–20,22,53,60–61]. Two studies reported a significant association between FOXA1 and AR protein expression [53,61]. No association between FOXA1 expression and clinicopathological features was found [19,53]. FOXA1 had no prognostic value [19,61]. In this ER negative context, using the MDA-MB-453 cell line, Robinson *et al.* suggested that AR may be able to utilize estrogen response element like motifs to bind to DNA and drive the transcription of genes that influence cell growth in an ER-independent manner [15]. FOX motifs are enriched at the center of AR binding regions, implying potential cooperation between AR and FOX proteins in mediating AR binding. 98% of all AR binding events overlap a FOXA1 binding region, which is greater than the 50% overlap between ER and FOXA1, suggesting that all AR binding events in molecular apocrine BC cells may be mediated by FOXA1 [15]. They showed that AR functionality is dependent on FOXA1: FOXA1 is required for AR to bind chromatin, for AR transcriptional activity and for cell growth [15]. To our knowledge, our study is the first to report coexpression of AR and FOXA1 in patients with TNBC. About 15% of TNBC were AR+/FOXA1+ tumors. This result is concordant with the literature in which 11–15.4% of TNBC are of the LAR subtype, when assessed by expression profiling [2,51]. If we considered a cutoff of 1% for AR and FOXA1 positivity, 17.6% of TNBC were AR+/FOXA1+ in our study (data not shown). Only 12% of TNBC had discordant AR and FOXA1 expression. Forty-eight patients (10.4%) had AR+/FOXA1- tumors.

Compared with patients with AR+/FOXA1- tumors, patients with AR+/FOXA1+ tumors were significantly older, had tumors of lower grade, with less pushing margins, less syncytial architecture and more associated ductal carcinoma *in situ* (Supplementary Table 3). These data suggest the necessity of these two biomarkers (AR and FOXA1) to identify a specific subgroup of TNBC.

Furthermore, Bernardo *et al.* suggested an ER-independent luminal-specific function for FOXA1 in maintaining the highly differentiated characteristics of luminal BC cells through transcriptional regulation of both luminal and basal genes *in vitro* [60]. All examined BC cell lines molecularly defined as luminal (including those lacking ER) expressed FOXA1. Several luminal ER-/HER2- BC cell lines expressed FOXA1 [60]. In contrast, BC cell lines representing the basal subtype did not express FOXA1. Silencing

*FOXA1* in luminal BC cell lines decreased the expression of several luminal genes, increased multiple basal genes and increased cellular aggressiveness (migration, invasion) [60]. These results are concordant with ours, where AR+/FOXA1+ TNBC seem to behave like luminal tumors (older patients, lower nuclear grade). These results also suggest that FOXA1 could be a potential therapeutic target. Indeed, inducing or increasing FOXA1 expression in basal BC may induce luminal differentiation and a less aggressive phenotype. These results need further consideration and we will evaluate the prognostic value of AR/FOXA1 coexpression in the future.

#### Conclusion & future perspective

In this large series, 25.9% of TNBC were AR+ and had distinct clinicopathological characteristics. TNBC with coexpression of AR and

## EXECUTIVE SUMMARY

### Androgen receptor and FOXA1 in triple-negative breast cancer

- Androgen receptor (AR) is frequently expressed in estrogen receptor positive breast cancer (70–90%) but it is less frequent in triple-negative breast cancer (TNBC) and extremely variable across studies (8–53%) probably due to their heterogeneity.
- Microarray studies identified a subgroup of TNBC called molecular apocrine breast cancer or luminal AR subtype that expressed AR and luminal genes including *FOXA1*, a transcriptional regulator. FOXA1 may direct AR to sites normally occupied by estrogen receptor in luminal tumors, inducing an estrogen-like gene program that stimulated proliferation.

### Clinical setting

- This biomarker study is ancillary to the UNICANCER-PACS08 adjuvant multicenter trial which, between October 2007 and September 2010, included 592 patients with nonmetastatic TNBC. Expression of AR and FOXA1 was evaluated by immunohistochemistry in this homogeneous population. We aimed to specify clinical and histological profiles associated with AR and FOXA1 coexpression.

### Coexpression of AR & FOXA1 identified a subgroup of TNBC with a distinct clinical, morphological & histopathological profile

- In this series, 25.9% of TNBC were AR+. Coexpression of AR and FOXA1 was found in 15.2% of patients. These tumors seem to behave like luminal tumors: they were more frequently lobular, found in older patients and exhibited a lower nuclear grade and a greater degree of node involvement. They less often exhibited lymphocytic infiltrate, pushing margins, syncytial architecture, central fibrosis or necrosis.
- This result is concordant with the literature in which 11–15.4% of TNBC are of the luminal AR subtype, when assessed by expression profiling.
- These biomarkers could be useful to identify easily in daily practice a subgroup of TNBC.

### Limitations of the study & future perspective

- The prognostic value of the coexpression of AR and FOXA1 need to be investigated.
- The prognostic value of the AR expression level needs to be investigated in TNBC.
- Anti-androgen therapies are currently under investigation in AR+ TNBC. FOXA1 could be another potential therapeutic target.

FOXA1 (15.2%) seems to behave like luminal tumors with a morphological profile distinct from other TNBC. These biomarkers could be useful to identify a subgroup of TNBC and could have future therapeutic implications: antiandrogen therapies are currently under investigation; FOXA1 could be another potential therapeutic target.

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#### Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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### III - DISCUSSION

Dans cette étude, 25,9% des tumeurs TN exprimaient le RA. Dans une autre large série publiée, le taux de tumeurs RA+ était de 20,5% avec les mêmes seuils de positivité en IHC (10% pour RA) [187].

La co-expression de RA et FOXA1 était retrouvée chez 15,2% des patients. Ces tumeurs RA+ / FOXA1+ étaient plus fréquentes chez les patientes plus âgées, étaient moins prolifératives et avaient des caractéristiques morphologiques proches des tumeurs luminales (histologie lobulaire plus fréquente, moins d'infiltrat lymphocytaire, d'architecture syncytiale, de fibrose centrale, de nécrose) par rapport aux autres tumeurs TN (RA- / FOXA1-, RA-/ FOXA1+, RA+ / FOXA1-). Ces résultats sont cohérents avec ceux de la littérature où 11 à 15,4% des cancers du sein TN sont classés comme étant de profil luminal A en génomique [171, 172]. Ces biomarqueurs pourraient donc permettre d'identifier facilement ce sous-groupe particulier de tumeurs TN. Par ailleurs, peu de cas discordants ont été rapportés dans cette étude : 10,5% de tumeurs RA+ / FOXA1- et 1,5% de tumeurs RA- / FOXA1+. Lorsque l'on compare spécifiquement les tumeurs RA+ / FOXA1+ avec les tumeurs RA+/FOXA1-, les caractéristiques morphologiques et histologiques décrites précédemment sont retrouvées.

Une étude préclinique suggère que FOXA1 maintiendrait l'expression de gènes luminaux, indépendamment de la voie RE [188]. Dans cette étude, toutes les lignées cellulaires de profil génomique luminal (y compris les RE-) expriment FOXA1. A l'inverse, les lignées cellulaires représentant le sous-type basal n'expriment pas FOXA1 [188]. En supprimant le gène *FOXA1* dans les lignées cellulaires luminales, on provoque une diminution de l'expression de gènes luminaux et une augmentation de gènes de type basal, ainsi que l'agressivité tumorale (capacités augmentées de migration et d'invasion) [188]. FOXA1 pourrait ainsi être une cible thérapeutique potentielle. En effet, induire ou augmenter son expression dans les tumeurs basales pourrait favoriser une différenciation en tumeur de type luminale, moins agressive.

L'impact pronostique potentiel, à la fois de la co-expression RA et FOXA1, mais aussi du niveau d'expression du RA, nécessite d'être évalué dans les cancers du sein TN.

**Réponse pathologique complète et survie après  
chimiothérapie néoadjuvante : étude  
monocentrique sur 30 ans  
(Article 4)**

**Pathological response and survival after  
neoadjuvant therapy for breast cancer:  
A 30-year study**

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**The Breast**

## I – INTRODUCTION

La chimiothérapie néoadjuvante est le traitement de choix pour les patients avec un cancer du sein localement avancé [189], avec comme objectif de réduire la taille tumorale et par conséquent, d'augmenter le taux de chirurgie conservatrice chez les patients sélectionnés avec une maladie opérable [189]. La chimiothérapie néoadjuvante est équivalente à la chimiothérapie adjuvante en termes de SSR et de SG, à condition qu'un traitement locorégional optimal (chirurgie et radiothérapie) soit réalisé [190].

Comme nous l'avons déjà souligné, le trastuzumab permet d'améliorer le pronostic des patients avec une tumeur HER2+ en adjuvant, et permet d'augmenter le taux de pCR en situation néoadjuvante [110]. Bien que la pCR soit souvent considérée comme un critère substitutif de la survie après chimiothérapie néoadjuvante, ce facteur reste controversé dans certains sous-types de cancer du sein [191, 192].

Nous avons mené sur 30 ans (entre 1978 et 2008), une étude exhaustive de l'ensemble des patientes prises en charge au Centre Georges-François Leclerc et ayant reçu une chimiothérapie néoadjuvante pour un cancer du sein localisé (N = 516). Leur devenir a été évalué selon le statut RH et HER2 de leur tumeur, avec un long suivi. Nous avons également étudié la valeur pronostique de la pCR selon différents sous-types de cancer du sein, ainsi que l'apport du trastuzumab en situation néoadjuvante en cas de tumeur HER2+.

Les critères d'inclusion des patientes étaient les suivants :

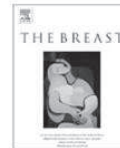
- CCI du sein histologiquement prouvé
- Tumeur unilatérale et non inflammatoire
- Statuts RH et HER2 disponibles
- Patients ayant reçu au moins un cycle de chimiothérapie
- Réponse histologique après chimiothérapie néoadjuvante connue et évaluée selon la classification de Chevallier (pCR = absence de carcinome invasif à la fois dans le sein et les ganglions lymphatiques, sans (grade 1) ou avec (grade 2) du CIS).

Les différents sous-groupes ont été définis comme suit (étude des RH par radioligand de 1978 à 1988, par IHC après 1988 ; étude de HER2 par IHC + /- HIS si HER2 ++):

- RH+ / HER2- (53%)

- RH+ / HER2+ (19%)
- RH- / HER2+ (10%)
- RH- / HER2- = triple négatif (18%)

## **II - ARTICLE**



## Original article

## Pathological response and survival after neoadjuvant therapy for breast cancer: A 30-year study

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## ABSTRACT

**Purpose of the research:** HER2-positive and triple-negative breast cancer (TNBC) still have a poor prognosis. Pathological complete response (pCR) is usually considered a surrogate marker for outcome. The aim of this study was to reconsider these parameters on a large population after a long follow-up. 348 patients with unilateral breast cancer who received neoadjuvant treatment at our institution over 30 years were included.

**Results:** Patients were classified according to hormonal receptors (HR) and HER2. Median follow-up was 7 years. pCR was significantly lower in HR+/HER2- tumors ( $P < 0.0001$ ). The 7-year OS rates were 76.1% (HR+/HER2-), 60.1% (TNBC), 72.4% (HR+/HER2+), and 49.9% (HR-/HER2+). Disease-free survival (DFS) and OS differed significantly according to pCR. Among HER2+ patients, pCR rate, DFS and OS were greater with trastuzumab.

**Conclusions:** TNBC and HR-/HER2+ tumors have the worst outcome. pCR remains a significant prognostic factor. Trastuzumab strongly improves pCR and survival in HER2+ tumors.

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## Introduction

Breast cancer is a highly heterogeneous disease and prognosis varies according to parameters such as tumor size, tumor grade, lymph-node involvement, and molecular markers. Among these, hormonal receptors (estrogen receptor (ER), progesterone receptor (PR)), and human epidermal growth factor receptor 2 (HER2) are decisive markers when selecting drug therapies. Although the prognosis of patients with local breast cancer has improved considerably during the last 30 years because of the development of new drugs (taxanes, trastuzumab), triple-negative breast cancer (TNBC) and HER2 over-expressed breast (HER2+) cancer have poor outcomes.<sup>1,2</sup>

Systemic neoadjuvant therapy is the treatment of choice for women with locally advanced breast cancer.<sup>3</sup> It aims to reduce

tumor size and, therefore, may facilitate breast-conserving surgery in selected patients with operable disease.<sup>3</sup> Neoadjuvant therapy is equivalent to adjuvant therapy in terms of overall survival and disease progression, provided that adequate loco-regional therapy (surgery and radiotherapy) is performed.<sup>4</sup>

In adjuvant studies, trastuzumab (Herceptin<sup>®</sup>, Roche) given to patients with a HER2+ tumor decreased the risk of relapse by 50% and the risk of death by 30% after a long median follow-up of ~5 years.<sup>5–9</sup> Trastuzumab, in association with neoadjuvant chemotherapy, can increase the rate of pathological complete response (pCR) in HER2+ tumors.<sup>10–13</sup>

Although pCR is often considered a surrogate marker for outcome after neoadjuvant chemotherapy, this factor remains controversial for some subtypes of breast cancer<sup>14,15</sup> and for some new targeted therapies. Moreover, no study has investigated surrogacy in this setting.

We have led a 30-year exhaustive study on a population of patients with non-metastatic breast cancer treated with neoadjuvant therapy. The outcomes of these patients were assessed according to both the hormonal receptor (HR) and HER2 status during a long follow-up. We have also studied the prognostic value

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of pCR with regards to the subtype of breast cancer as well as the contribution of trastuzumab in treating the HER2+ subgroup.

## Materials and methods

### Patients

A total of 516 consecutive patients, diagnosed with non-metastatic breast cancer and who had received neoadjuvant chemotherapy between 1978 and 2008 at Georges-François Leclerc Cancer Center (Dijon, France), were recorded in the neoadjuvant database.

All patients who had the following criteria were included in this study: (1) histologically confirmed ductal breast carcinoma, (2) a unilateral and non-inflammatory tumor, (3) status of both HR and HER2 were available, (4) patients had received at least one cycle of chemotherapy, and (5) a known pathological stage after neoadjuvant therapy. Patients with a history of breast cancer were excluded. Clinical tumor size was determined on the basis of physical examinations and imaging tests, including mammograms.

This data analysis was approved by our institutional review board.

### Pathology assessment

From 1978 to 1988, ER and PR status were assessed by a radioligand assay using a quality-control program proposed by the European Organisation for Research and Treatment of Cancer. The cut-off point was 10 fmol/mg. From 1988 to 2008, ER and PR status were assessed by immunohistochemistry (IHC), with different antibody, detection kits and automata. IHC quality was always supervised by several quality-assurance programs (Groupe d'Etude des Facteurs Pronostiques Immunohistochimiques dans le Cancer du Sein "GEPPICS", United Kingdom National External Quality Assessment Service "UK NEQAS"). The cut-off points for both ER and PR positivity were  $\geq 10\%$  positive nuclear-stained tumor cells.

HER2 status was assessed by IHC according to the Herceptin scoring system, and by FISH (fluorescent *in situ* hybridization) for ambiguous results (2+). The antibodies and the probes were different during the different periods but, as for HR, the techniques were evaluated by quality-assurance programs (GEPPICS, UK NEQAS). HER2+ was defined by either HER2 gene amplification (FISH) or protein overexpression (3+) in IHC. In 2010, all tumors for which HER2 status had not been established, were tested by IHC  $\pm$  FISH. Tumors negative for ER, PR, and HER2 were classified as TNBC. Tumors with ER and/or PR+ were classified as HR+.

Histological grading was carried out using the Nottingham-combined histological (Elston–Ellis modification of Scarff–Bloom–Richardson (SBR)) grading system.<sup>16</sup>

PCR was determined by microscopic examination of the excised tumor and lymph nodes after completion of chemotherapy, and was assessed according to Chevaliers' classification<sup>17</sup>: no evidence of carcinoma in either the breast or lymph nodes, without (grade 1) or with (grade 2) *in situ* carcinoma, was considered as pCR.

### Neoadjuvant chemotherapy

The neoadjuvant chemotherapy regimens varied over the 30 years. The type of treatment can be broadly classified as either anthracycline-based therapy (epirubicin or doxorubicin) or as antimetabolic based-therapy (including taxanes and vinca-alkaloids). A sequential combination of anthracycline and taxane was also sometimes administered.

Since 2006 (date of marketing authorization), patients with a HER2+ tumor have systematically received trastuzumab

(Herceptin®) in neoadjuvant and/or adjuvant settings. Before 2006, some patients received trastuzumab.

### Statistical analyses

Qualitative variables were described using frequency and percentages. Chi-squared and Fisher's exact tests were used to compare patient or tumor characteristics according to HR and HER2 status.

Univariate and multivariate logistic regressions were used to determine the predictive factors for pCR. Parameters assessed were the period of diagnosis, age at the time of diagnosis, tumor size, nodal status, nuclear grade (SBR), HR, and HER2 status. Because of the low number of T1 and T4 tumors, these variables were combined with data from T2 and T3 tumors, respectively. Because of the use of new chemotherapy drugs (such as taxanes) and targeted therapies during the last 10 years, two periods of diagnosis were distinguished: 1976–1998 and 1999–2008. All the parameters were included in multivariate analyses, except for neoadjuvant treatment, because of its heterogeneity and the collinearity between the HER2 status and the type of neoadjuvant regimen.

Median follow-up was calculated using the reverse Kaplan–Meier method. Disease-free survival (DFS) was defined as the date of the first histology to the date of the first recurrence of breast cancer at any site, or death from any cause, or the date of a second cancer. Surviving patients without recurrence were censored at the last follow-up. Overall survival (OS) was defined as the date of the first histology to death from any cause. Survival distributions were estimated using the Kaplan–Meier method and compared using log-rank statistics to describe the median or rate at specific time endpoints, with a 95% CI.

The 7-year DFS or OS rates, according to HR/HER2 status, pCR or no pCR, both pCR and HR status, and neoadjuvant trastuzumab (among HER2+), were recorded.

Univariate and multivariate Cox's proportional hazard models were fitted to test for an association between classical prognostic variables, both HR and HER2 status, pCR, and DFS or OS. Variables were included in the multivariate analyses if the univariate *P*-value was  $\leq 0.15$ . The Akaike information criterion (AIC) was computed for goodness-of-fit for the multivariate models, Harrell's C-statistic was used on each variable (a Harrell's C index of 0.5 indicates no predictive discrimination, and a Harrell's C index of 1.0 indicates perfect separation of patients) and on final multivariate Cox's models. The multivariate models were internally validated using bootstrapping (400 replications). *P*-values were two-tailed and considered significant when  $< 0.05$ . All analyses were performed using Stata V11 software (StataCorp LP, College Station, TX).

## Results

### Patients' characteristics

A total of 348 patients met the eligibility criteria. Among these, 186 (53%) were designated as having a HR+/HER2– tumor, 61 (18%) a TNBC, 66 (19%) a HR+/HER2+ tumor, and 35 (10%) a HR–/HER2+ tumor. Table 1 lists the patients' and tumor characteristics according to both HR and HER2 status. Patients with TNBC were younger other patients ( $P = 0.038$ ). TNBC and HR–/HER2+ tumors had a higher histological grade than HR+ tumors ( $P < 0.0001$ ).

Table 2 lists the types of neoadjuvant treatments that were administered. Fifty-four (53.5%) patients with a HER2+ tumor received neoadjuvant trastuzumab (40 HR+, 14 HR–).

Forty-seven percent of patients underwent conservative surgery. Thirty-three (9.5%) patients received adjuvant

**Table 1**  
Patient and tumor characteristics according to HR and HER2 status.

Characteristic	Total (N = 348)	HR+/HER2- (N = 186)	TNBC (N = 61)	HR+/HER2+ (N = 66)	HR-/HER2+ (N = 35)	P
Median age at diagnosis [range]	48.6 [24.1–79.8]	49.9 [30.5–79.8]	46.4 [24.1–70.3]	48.3 [25.4–75.3]	48.3 [30.1–69.7]	0.038
≤50 years (%)	198 (56.9)	93 (50)	42 (68.9)	41 (62.1)	22 (62.9)	
>50 years (%)	150 (43.1)	93 (50)	19 (31.2)	25 (37.9)	13 (37.1)	
Period of diagnosis						0.066 <sup>a</sup>
1976–1988	26 (7.5)	18 (9.7)	5 (8.2)	2 (3)	1 (2.9)	
1989–1998	117 (33.6)	68 (36.6)	22 (36.1)	14 (21.2)	13 (37.1)	
1999–2008	205 (58.9)	100 (53.8)	34 (55.7)	50 (75.8)	21 (60)	
Breast and/or ovary neoplastic heredity						0.041
No	210 (70.5)	118 (76.6)	34 (64.2)	35 (58.3)	23 (74.2)	
Yes	88 (29.5)	36 (23.4)	19 (35.8)	25 (41.7)	8 (25.8)	
Unknown	50	32	8	6	4	
Tumor stage						0.008
T1	16 (4.8)	7 (3.9)	3 (5.1)	5 (7.9)	1 (3)	
T2	198 (58.8)	107 (58.8)	28 (47.5)	40 (63.5)	23 (69.7)	
T3	86 (25.5)	38 (20.9)	23 (39)	16 (25.4)	9 (27.3)	
T4	37 (11)	30 (16.5)	5 (8.5)	2 (3.2)	0	
Unknown	11	4	2	3	2	
Nodal status						0.628
N0	121 (35.1)	67 (36.2)	23 (38.3)	22 (33.9)	9 (25.7)	
N+	224 (64.9)	118 (63.8)	37 (61.7)	43 (66.2)	26 (74.3)	
Unknown	3	1	1	1	0	
Tumor grade						<0.0001 <sup>a</sup>
SBR1	28 (8.4)	21 (11.7)	0	6 (9.4)	1 (3)	
SBR2	168 (50.6)	112 (62.2)	15 (27.3)	30 (46.9)	11 (33.3)	
SBR3	136 (41)	47 (26.1)	40 (72.7)	28 (43.8)	21 (63.6)	
Unknown	16	6	6	2	2	
Neoadjuvant chemotherapy						<0.0001
Anthracyclines						
Yes	280 (80.5)	176 (94.6)	60 (98.4)	25 (37.9)	19 (54.3)	
No	68 (19.5)	10 (5.4)	1 (1.6)	41 (62.1)	16 (45.7)	
Anti-mitotics						<0.0001
Yes	168 (48.3)	73 (39.3)	26 (42.6)	49 (74.2)	20 (57.1)	
No	180 (51.7)	113 (60.8)	35 (57.4)	17 (25.8)	15 (42.9)	
Trastuzumab						<0.0001
Yes	62 (17.8)	4 (2.1)	0	43 (65.1)	15 (42.9)	
No	286 (82.2)	182 (97.9)	61 (100)	23 (34.9)	20 (57.1)	
Other						0.496 <sup>a</sup>
Yes	7 (2)	6 (3.2)	1 (1.6)	0	0	
No	341 (98)	180 (96.8)	60 (98.4)	66 (100)	35 (100)	
pCR						<0.0001 <sup>a</sup>
Yes	54 (15.5)	8 (4.3)	14 (23)	19 (28.8)	13 (37.1)	
No	294 (84.5)	178 (95.7)	47 (77)	47 (71.2)	22 (62.9)	
Surgical therapy						0.049
BC	163 (46.8)	75 (40.3)	36 (59)	35 (53)	17 (48.6)	
Mastectomy	185 (53.2)	111 (59.7)	25 (41)	31 (47)	18 (51.4)	
Adjuvant radiation						0.662 <sup>a</sup>
Yes	343 (98.9)	181 (97.8)	61 (100)	66 (100)	35 (100)	
No	5 (1.1)	5 (2.2)	0	0	0	
Adjuvant chemotherapy (including trastuzumab)						<0.0001
Yes	145 (41.7)	59 (31.8)	22 (36.1)	41 (62.1)	23 (65.7)	
No	203 (58.3)	127 (68.2)	39 (63.9)	25 (37.9)	12 (34.3)	
Adjuvant hormone therapy						<0.0001
Yes	215 (62.1)	157 (85.3)	4 (6.6)	52 (78.8)	2 (5.7)	
No	133 (37.9)	29 (14.7)	57 (93.4)	14 (21.2)	33 (64.3)	

SBR = Scarff–Bloom–Richardson; pCR = pathologic complete response; BC = breast conservative surgery.  
<sup>a</sup> Fisher's exact test.

anthracycline, 37 (10.6%) received anti-mitotics, and one patient received a further adjuvant regimen. Information on the type of adjuvant chemotherapy was not available for 32 (9.2%) patients. Forty-five (44.5%) of the 101 patients with a HER2+ tumor received adjuvant trastuzumab. More than 98% of patients received adjuvant

radiotherapy. Ninety-seven percent (N = 246) of patients with HR + disease received adjuvant endocrine therapy that included either tamoxifen (N = 126), aromatase inhibitors (N = 59), sequential treatment with tamoxifen and aromatase inhibitors (N = 24), or castration (medical or surgical) (N = 37).

**Table 2**  
Type of neoadjuvant therapy according to HR and HER2 status.

	HR+/-HER2- N = 186	TNBC N = 61	HR+/HER2+ N = 66	HR-/HER2+ N = 35	Total N = 348
Anthracyclines	107 (57.5%)	34 (55.7%)	16 (24.2%)	14 (40%)	171 (49.1%)
Anthracyclines + anti-mitotics	68 (36.6%)	26 (42.6%)	7 (10.6%)	5 (14.3%)	106 (30.5%)
Trastuzumab + anti-mitotics	3 (1.6%)	0 (0%)	40 (60.6%)	14 (40%)	57 (16.4%)
Other	8 (4.3%)	1 (1.6%)	3 (4.6%)	2 (5.7%)	14 (4%)



Response according to HR and HER2 status

In the overall population, according to Chevallier's classification, 54 (15.5%) of patients had a pCR whereas 294 (84.5%) did not have a pCR.

In univariate logistic analysis, the period of diagnosis ( $P < 0.0001$ ), tumor size ( $P = 0.01$ ), histological tumor grade ( $P = 0.01$ ), and HR/HER2 status ( $P < 0.001$ ) were associated with pCR. In multivariate logistic regression (MLR), the period of diagnosis and HR/HER2 status were independently associated with pCR. Internal validation using bootstrapping confirmed these results (Table 3).

PCR was significantly higher in TNBC (23%; OR = 6.34, CI: 2.24–17.95) and HER2+ tumors whatever the HR status (i.e., 28.8% for HR+ [OR = 6.69, CI: 2.64–16.98] and 37.1% for HR- [OR = 8.52, CI: 2.86–25.37]) compared to HR+/HER2- tumors (4.3%;  $P < 0.0001$ ; Tables 1 and 3). PCR was significantly greater in the last 10 years of the study (OR = 3.35, CI: 1.45–7.71,  $P = 0.004$ ).

Among the HER2+ tumors, increased pCR rate was observed in patients who received neoadjuvant trastuzumab (43.1 vs. 16.3%; OR = 3.9, CI: 1.49–10.2,  $P = 0.006$ ). Neoadjuvant trastuzumab was mainly administered with anti-mitotics (mainly taxanes); few patients received neoadjuvant anthracyclines and trastuzumab in

this study. Because of the small sample sizes in these categories, we were not able to draw conclusions regarding the relevance of anthracyclines/anti-mitotics + trastuzumab vs. only anti-mitotics + trastuzumab in a neoadjuvant setting.

Among the TNBC, pCR rates were not statistically different regarding the type of neoadjuvant treatment: 28.5% with anthracyclines and anti-mitotics vs. 15% with anthracycline alone (OR = 0.45, CI: 0.12–1.66,  $P = 0.23$ ).

Disease-free survival according to HR and HER2 status

The median follow-up time was 7 years (range: 0.49–29.8). Local or regional recurrence occurred in 15 (4.3%) patients. Metastatic recurrence (alone or with loco-regional recurrence) occurred in 114 (32.7%) patients. Twenty-one (6.0%) patients died without a diagnosis of recurring breast cancer, three patients had a second cancer, and one patient had a contralateral breast cancer.

Median DFS was 7.6 years (95% CI = 6.2–9.6 years). The 7-year DFS rates were 60.6% (95%CI: 45.2–72.9%) for patients with a HR+/HER2+ tumor, 54.4% (95%CI: 45.8–62.2%) for HR+/HER2-, 46.5% (95%CI: 30.8–60.7%) for TNBC and 44% (95%CI: 26.0–60.7%) for HR-/HER2+. There was a trend toward a higher DFS rate for

**Table 3**  
Univariate and multivariate logistic regression of predictive factors for pathological complete response (pCR).

	pCR Yes/No N = 348	Univariate analysis			pCR Yes/No N = 318	Multivariate analysis			Bootstrapping <sup>a</sup>	
		OR	95% CI	P		OR	95%CI	P	95%CI	P
<i>Age at diagnosis</i>				0.497				0.503		0.522
≤50years	33/165	1	–		28/152	1	–		–	
>50years	21/129	0.81	[0.45–1.47]		20/118	1.27	[0.63–2.59]		[0.61–2.68]	
<i>Period of diagnosis</i>				<0.0001				0.004		0.015
1976–1998	10/133	1	–		9/123	1	–		–	
1999–2008	44/161	3.63	[1.76–7.5]		39/147	3.35	[1.45–7.71]		[1.26–8.89]	
<i>Tumor size</i>				0.015				0.11		0.155
T1/T2	41/173	1	–		37/166	1	–		–	
T3/T4	11/112	0.41	[0.2–0.84]		11/104	0.52	[0.23–1.16]		[0.21–1.28]	
Unknown	2/9									
<i>Nodal status</i>				0.771				0.659		0.701
N0	18/103	1	–		18/98	1	–		–	
N+	36/188	1.1	[0.59–2.03]		30/172	1.18	[0.57–2.42]		[0.51–2.69]	
Unknown	0/3									
<i>Tumor grade</i>				0.011				0.139		0.302
SBR1	1/27	1	–		1/26	1	–		–	
SBR2	19/149	3.44	[0.44–26.8]		18/144	2.56	[0.3–21.7]		[2–2,482,449]	
SBR3	30/106	7.64	[1–58.57]		29/100	4.71	[0.55–40.11]		[0–4,367,043]	
Unknown	4/12									
<i>HR/HER2 status</i>				<0.001				<0.0001		0.0002
HR+/HER2-	8/178	1	–		8/167	1	–		–	
TNBC	14/47	6.63	[2.62–16.73]		13/39	6.34	[2.24–17.95]		[2.05–19.64]	
HR+/HER2+	19/47	8.99	[3.71–21.82]		17/43	6.69	[2.64–16.98]		[2.43–18.44]	
HR-/HER2+	13/22	13.15	[4.91–35.24]		10/21	8.52	[2.86–25.37]		[2.8–25.95]	
<i>HR status</i>				<0.001						
HR-	27/69	1	–							
HR+	27/225	0.31	[0.17–0.56]							
<i>HER2 status</i>				<0.001						
HER2-	22/225	1	–							
HER2+	32/69	4.74	[2.59–8.7]							
<i>Neoadjuvant therapy</i>				<0.001						
<i>Anthracycline</i>				<0.001						
No	24/44	1	–							
Yes	30/250	0.22	[0.12–0.41]							
<i>Anti-mitotics</i>				<0.042						
No	21/159	1	–							
Yes	33/135	1.85	[1.02–3.35]							
<i>Trastuzumab</i>				<0.001						
No	28/258	1	–							
Yes	26/36	6.65	[3.52–12.59]							
AIC	236.0645									

pCR = pathologic complete response; N = number; OR = odds ratio; CI = confidence interval; SBR = Scarff–Bloom–Richardson; AIC = Akaike information criterion.  
<sup>a</sup> 400 replications.

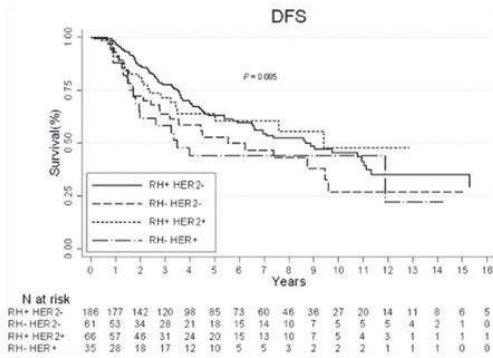


Fig. 1. Disease-free survival (DFS) according to HR and HER2 status.

HR+/HER2+ but this did not reach statistical significance ( $P = 0.085$ , log-rank test) (Fig. 1).

DFS differed significantly according to pCR rate ( $P = 0.02$ , log-rank test), with a 7-year DFS rate of 72.3% (95%CI: 55.2–83.8%) if pCR was observed, whereas this was only 49.3% (95%CI: 42.7–55.8%) in the contrary case. In the HR- subgroup, DFS was

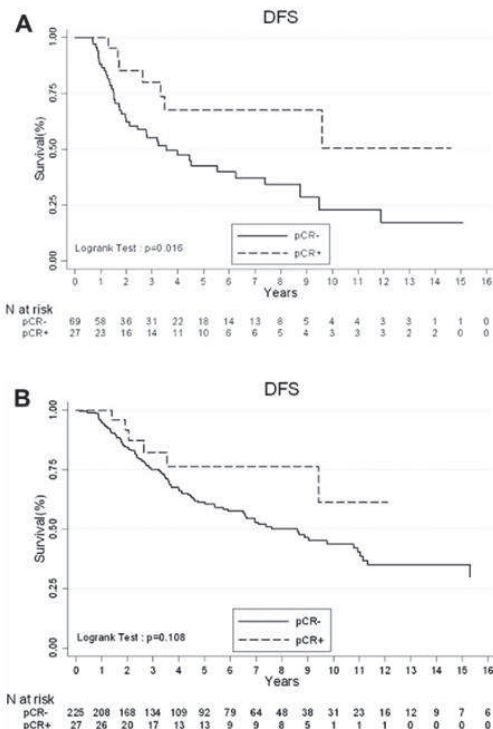


Fig. 2. Disease-free survival (DFS) according to pCR rate in negative hormone-receptor (HR) tumors (A) or positive HR tumors (B).

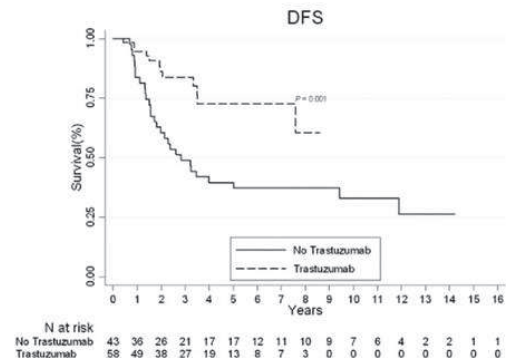


Fig. 3. Disease-free survival (DFS) according to neoadjuvant treatment in the HER2+ subgroup.

correlated with pCR ( $P = 0.01$ ) (Fig. 2A) but not in the HR + subgroup ( $P = 0.1$ ) (Fig. 2B).

In the HER2+ subgroups ( $N = 101$ ), whatever the HR status, the 7-year DFS rates were 72.5% (95%CI: 54.4–84.4%) and 37.2% (95%CI: 23.1–51.3%) in patients who received or did not receive trastuzumab, respectively (Fig. 3).

In univariate Cox's analysis, the period of diagnosis ( $P = 0.023$ ), nodal status ( $P = 0.048$ ), HR status ( $P = 0.013$ ), pCR ( $P = 0.023$ ), and post-operative nodal status ( $P = 0.003$ ) were significantly associated with DFS. In MLR, only HR/HER2 status ( $P = 0.001$ ) and postoperative nodal status ( $P = 0.021$ ) were independently associated with DFS (results confirmed by bootstrapping).

Overall survival according to HR and HER2 status

During follow-up, 104 (29.9%) patients died. The 7-year OS rates were 76.1% (95%CI: 68.0–82.4%) for HR+/HER2-, 72.4% (95%CI: 55.3–83.8%) for HR+/HER2+, 60.1% (95%CI: 43.7–73.0%) for TNBC, and 49.9% (95%CI: 26.9–69.1%) for HR-/HER2+. These differences tended toward significance ( $P = 0.08$ , log-rank test) (Fig. 4).

OS differed significantly according to pCR rate ( $P = 0.003$ , log-rank test) and both pCR/HR status ( $P = 0.0001$ , log-rank test). In the HR- subgroup, pCR was strongly correlated with OS ( $P = 0.008$ ). In the HR + subgroup, this correlation was also

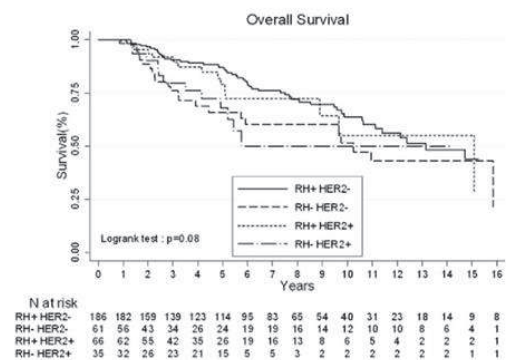


Fig. 4. Overall survival (OS) according to HR and HER2 status.

statistically significant ( $P = 0.02$ ), but may not be relevant because of the small number of events.

In Cox's univariate analysis, tumor size ( $P = 0.013$ ), HR status ( $P = 0.012$ ), pCR ( $P = 0.006$ ), postoperative nodal status ( $P < 0.0001$ ), and type of surgery ( $P = 0.002$ ) were significantly associated with OS. In MLR, only HR/HER2 status ( $P = 0.0001$ ) and post-operative nodal status ( $P = 0.001$ ) were independently

associated with OS, and were confirmed as prognostic factors by bootstrapping (Table 4).

Among the patients with a HER2+ tumor who had not received trastuzumab, the prognosis was poor. Indeed, the 7-year OS rate was 48.6% (95%CI: 32.4–62.9%) in this population, whereas it was 83.6% (CI: 55.9–94.6%) after neoadjuvant trastuzumab based-therapy.

**Table 4**  
Univariate and multivariate Cox's analysis of predictive factors for overall survival (OS).

	Death Yes/No N = 348	Univariate analysis			Death Yes/No N = 337	Multivariate analysis			Bootstrapping*	
		HR	95% CI	P		HR	95%CI	P	95%CI	P
<i>Age at diagnosis</i>				0.131				0.119		0.156
<50years	56/142	1	–		56/137	1	–			
>50years	48/102	1.35	[0.91–1.99]		47/97	1.38	[0.92–2.05]		[0.89–2.14]	
<i>Period of diagnosis</i>				0.051				0.701		0.727
1976–1988	19/7	1	–		19/7	1	–			
1989–1998	56/61	0.77	[0.45–1.32]		56/61	0.91	[0.52–1.59]		[0.46–1.8]	
1999–2008	29/176	0.48	[0.26–0.9]		28/166	0.77	[0.4–1.48]		[0.37–1.61]	
<i>Tumor size</i>				0.013				0.083		0.085
T1/T2	51/163	1	–		51/163	1	–			
T3/T4	52/71	1.64	[1.11–2.42]		52/71	1.43	[0.95–2.14]		[0.95–2.14]	
Unknown	2/9									
<i>Nodal status</i>				0.052						
N0	29/92	1	–							
N+	74/150	1.53	[1–2.35]							
Unknown	1/2									
<i>Tumor grade</i>				0.527						
SBR1	7/21	1	–							
SBR2	52/116	1.48	[0.67–3.27]							
SBR3	15/51	1.59	[0.71–3.58]							
Unknown	6/10									
<i>HR/HER2 status</i>				0.087				0.0001		0.0007
HR+/HER2–	54/132	1	–		54/128	1	–			
TNBC	23/38	1.66	[1.02–2.72]		23/36	2.6	[1.56–4.35]		[1.49–4.55]	
HR+/HER2+	15/51	1.13	[0.64–2.01]		14/49	1.53	[0.84–2.81]		[0.76–3.09]	
HR–/HER2+	12/23	1.87	[1–3.52]		12/21	3.53	[1.81–6.87]		[1.57–7.94]	
<i>HR status</i>				0.012						
HR–	35/61	1	–							
HR+	69/183	0.59	[0.39–0.89]							
<i>HER2 status</i>				0.405						
HER2–	77/170	1	–							
HER2+	27/74	1.21	[0.77–1.88]							
<i>Neoadjuvant therapy</i>				0.053						
<i>Anthracycline</i>										
No	8/60	1	–							
Yes	96/184	2.05	[0.99–4.24]							
<i>Ant-mitotics</i>				0.344						
No	54/126	1	–							
Yes	50/118	1.2	[0.82–1.77]							
<i>Trastuzumab</i>				0.016						
No	101/185	1	–							
Yes	3/59	0.24	[0.08–0.77]							
<i>pCR</i>				0.006				0.094		0.854
pCR–	100/194	1	–		99/186	1	–			
pCR+	4/50	0.24	[0.09–0.66]		4/48	0.39	[0.13–1.17]		[0.887–61]	
<i>pN</i>				<0.0001				0.001		0.001
pN0	22/128	1	–		21/124	1	–			
pN+	82/116	2.5	[1.56–4.01]		82/110	2.61	[1.51–4.53]		[1.47–4.65]	
<i>Surgical therapy</i>				0.002						
Mastectomy	71/114	1	–							
Breast conserving	33/130	0.51	[0.34–0.078]							
<i>Adjuvant radiation</i>				0.153						
No	2/2	1	–							
Yes	102/242	0.36	[0.09–1.46]							
<i>Adjuvant chemotherapy</i>				0.497						
No	56/145	1	–							
Yes	48/99	1.15	[0.77–1.7]							
<i>Adjuvant hormone therapy (HR+ only)</i>				0.352						
No	16/25	1	–							
Yes	52/157	1.32	[0.74–2.37]							
AIC	996.89									
Harrell's C statistic	0.7169									

\* Signifies 400 replications.

## Discussion

To the best of our knowledge, this study is the first to report the outcome of patients after neoadjuvant therapy according to both HR and HER2 status after such a long follow-up. Our results are in accordance with the well-known "hormone-receptor negative paradox", which suggests that, despite good responses to neoadjuvant chemotherapy, HER2 and basal breast cancers result in poor overall survival rates.<sup>1,18,19</sup>

A molecular classification of breast cancer based on gene-expression profiles was described by Perou et al.<sup>20</sup> Luminal, basal-like, normal-like, and erbB2+ subgroups were identified and were shown to have different prognoses: basal-like (mostly HR negative) and erbB2+ (mostly HER2 overexpressed/amplified and ER-) had the worst outcomes with the shortest DFS and OS rates.<sup>20–23</sup> Among ER+ tumors, two major subtypes are distinguished (luminal A and luminal B). They are biologically distinct in that luminal A tumors tend to have a higher expression of ER-related genes, and lower expression of proliferative genes than luminal B, as well as a better prognosis.<sup>21,22,24</sup>

For practical reasons, in clinical routine practice, the available tests for ER, PR (IHC), and HER2 status (IHC and/or FISH) are used to approximate breast-cancer subtypes as follows: luminal A (ER+ and/or PR+, and HER2-), luminal B (ER+ and/or PR+, and HER2+), HER2 (ER- and PR-, and HER2+), and basal (ER- and PR-, and HER2-).<sup>24,25</sup> We confirm in this present study, that HR status is a major predictive factor for overall survival and may be stronger than IHC HER2 status. Indeed, HR+/HER2- and HR+/HER2+ subgroups have higher overall survival rates than HR-, with 76.1 and 72.4% of patients alive at 7 years, respectively, after a 7-year median follow-up. In addition, it must be underlined that only 65% of patients received trastuzumab in the HR+/HER2+ subgroup. These results are consistent with previously published data.<sup>18,26</sup>

In this study, pCR rate was dramatically higher in TNBC (23%) and HER2+ tumors (28.8 and 37.1%, according to HR+ or HR-, respectively) than HR+/HER2- (4.3%). Our pCR rate for TNBC is similar to that described in other studies (22–30%) despite the different regimens of chemotherapy that patients received.<sup>1,18</sup> A higher pCR rate was observed in HER2+ tumors in the HR- subgroup (37.1%) compared to the HR+ subgroup (28.8%). Comparable results, whatever the HER2 status, have been often described in neoadjuvant studies.<sup>3,27–30</sup> It should be noted that, in our study, a higher proportion of patients (60%) received neoadjuvant trastuzumab in the HR+/HER2+ subgroup compared to the HR-/HER2+ subgroup (40%), thereby underestimating pCR rate if all patients had received neoadjuvant trastuzumab. Indeed, according to several randomized trials, the addition of trastuzumab to different regimens of chemotherapy for patients with HER2+ breast cancer increases pCR rate.<sup>10–13</sup> De Ronde et al.<sup>19</sup> suggest that, for luminal<sub>IHC</sub> and triple-negative<sub>IHC</sub> tumors, further subdivision into molecular subgroups does not offer a clear advantage in neoadjuvant treatment, although an mRNA expression-based test may predict the response to trastuzumab-based therapy for HER2+<sub>IHC</sub> tumors. Indeed, the authors studied concordance between IHC and molecular (mRNA) breast-cancer subtypes and their prediction of response to neoadjuvant chemotherapy. In their study, all patients with a HER2+ tumor received neoadjuvant trastuzumab. A high concordance was shown between these two classifications except for the HER2+ group: 60% of HER2+<sub>IHC</sub> tumors were not classified as HER2+<sub>mRNA</sub>, and the HER2+<sub>IHC</sub>/Luminal<sub>mRNA</sub> group had a low response rate to combined trastuzumab chemotherapy, with a pCR rate of 8% compared to 54% in the HER2+<sub>mRNA</sub> group (21% in the HER2+/ER+ group (IHC)). These results suggest an intrinsic resistance of HER2-amplified Luminal<sub>mRNA</sub> tumors to trastuzumab-based chemotherapy regimens.<sup>19</sup>

pCR is often considered a surrogate marker for outcome after neoadjuvant chemotherapy with a more favorable prognosis.<sup>31–35</sup> In our study we did not formally investigate surrogacy; however, after a long follow-up, pCR was a strong predictive factor for DFS ( $P = 0.02$ ) and OS ( $P = 0.003$ ). However, pCR only correlated with DFS for HR- tumors ( $P = 0.01$ ) and not for HR+ tumors ( $P = 0.1$ ). These results are in line with those of Von Minckwitz et al.<sup>14</sup> who showed a strong correlation between pCR and DFS in TNBC and HR-/HER2+, but not in luminal A-like or HR+/HER2+ tumors. Their study included 6377 patients from seven German neoadjuvant trials that used an anthracyclines + taxane regimen with or without trastuzumab.<sup>14</sup> These data suggest that obtaining a pCR could be challenging, particularly for TNBC and HR-/HER2+ tumors.

HER2+ patients mainly received neoadjuvant trastuzumab combined with taxanes because of the two ongoing phase-II clinical trials at our center (TAXHER<sup>36</sup> and GETN(A)-1<sup>37</sup>) during the time of this study. Our team has already published the survival data from these two multicentric neoadjuvant trials (135 patients) after a median follow-up of 48 months: DFS and OS rates were 73.2% (CI: 63.7–80.5%) and 91.9% (CI: 84.2–95.9%), respectively.<sup>38</sup> Despite anthracycline-free regimens we found, in the present study, 7-year DFS and OS rates of 72.5% (CI: 54.4–84.4%) and 83.6% (CI: 55.9–94.6%), respectively. These survival rates compare favorably with several other studies that have evaluated neoadjuvant trastuzumab combined with cytotoxic therapy, but over shorter follow-up times.<sup>10–12,39</sup> Thus, this schedule could be proposed to patients with contraindications for anthracyclines or because of the patient's or physician's preference for a taxane-only schedule.

In conclusion, we confirm in this exhaustive neoadjuvant study, the worse outcome of patients with TNBC and HR-/HER2+ tumors compared to those with HR+ tumors after a long median follow-up. We support that pCR is a strong prognostic factor, particularly for patients with HR- tumors, and confirm the major impact of trastuzumab in patients with HER2+ tumors.

## Ethical approval

This study was approved by our institutional review board.

## Conflict of interest statement

None of the authors has any conflicts of interest to declare regarding this study.

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### III - DISCUSSION

A notre connaissance, et au moment de la publication, cette étude était la première à rapporter le devenir des patients après chimiothérapie néoadjuvante, selon les statuts RH et HER2 avec un si long suivi (suivi médian de 7 ans). Nos résultats sont concordants avec le paradoxe bien connu des tumeurs RH- qui suggère que, malgré une bonne chimiosensibilité initiale, les patients avec une tumeur HER2+ ou TN ont de moins bonnes survies [163, 168, 193]. En effet, dans notre étude, les taux de pCR étaient significativement plus importants en cas de tumeur TN (23%) et HER2+, quel que soit le statut des RH (28,8% si RH+ et 37,1% si RH-), en comparaison aux tumeurs RH+ / HER2- (4,3%) ( $p < 0,0001$ ). Les taux de SSM à 7 ans étaient de 60,6% en cas de tumeur RH+ / HER2+, 54,4% pour les RH+ / HER2-, 46,5% pour les TN et 44% pour les RH- / HER2+. Les taux de SG à 7 ans étaient de 72,4% en cas de tumeur RH+ / HER2+, 76,1% pour les RH+ / HER2-, 60,1% pour les TN et 49,9% pour les RH- / HER2+. Ces résultats confirment que le statut des RH est un facteur prédictif majeur de SG, et pourrait même être plus important que le statut HER2 [193, 194]. Il faut par ailleurs souligner que seuls 65% des patients ayant une tumeur RH+ / HER2+ ont reçu du trastuzumab.

La pCR est souvent considérée comme un critère substitutif de la survie après traitement néoadjuvant [195-199]. Dans notre étude, après un long suivi, la pCR est un fort facteur prédictif de SSM (SSM à 7 ans de 72,3% *versus* 49,3%, respectivement en présence ou en l'absence de pCR,  $p = 0,02$ ) et de SG ( $p = 0,003$ ). Cependant, la pCR est corrélée à la SSM uniquement en cas de tumeurs RH- (quel que soit le statut HER2). Ces résultats sont concordants avec ceux de Von Minckwitz *et al* incluant plus de 6 000 patients de 7 essais néoadjuvants recevant un régime à base de taxane et anthracycline + /- trastuzumab [192]. Ces données suggèrent que l'obtention d'une pCR est un challenge important en cas de tumeur TN ou RH- / HER2+.

Le taux de pCR est significativement augmenté en cas de traitement par trastuzumab en association à la chimiothérapie (43,1% *versus* 16,3%,  $p = 0,006$ ), de même que la SSM et la SG quel que soit le statut des RH (taux de SSM à 7 ans de 72,5% *versus* 37,2% et taux de SG à 7 ans de 83,6% *versus* 48,6% si addition de trastuzumab *versus* pas de traitement ciblé anti-HER2, respectivement). Le plus souvent les patients ont reçu uniquement des taxanes en néoadjuvant dans cette étude, et très peu d'anthracyclines, étant donné que la majorité

étaient incluses dans 2 études en cours dans l'établissement durant cette période (TAXHER et GETN(A-)1 [200, 201]). Du fait du faible nombre de patients dans cette dernière situation, nous ne pouvons tirer aucune conclusion pertinente sur une différence de pCR ou de survie en cas de traitement par anthracyclines puis taxane-trastuzumab *versus* taxane-trastuzumab seuls.

En conclusion, nous confirmons dans cette large population, le mauvais pronostic des patients ayant un cancer du sein TN ou RH- / HER2+ par comparaison à ceux ayant une tumeur RH+, après un long suivi médian. Nous soutenons l'hypothèse que la pCR est un facteur pronostique fort, notamment en cas de tumeur RH-, et confirmons l'impact majeur du trastuzumab chez les patients ayant une tumeur HER2+.

**Facteurs prédictifs de réponse chez les patients  
ayant un cancer du sein HER2 positif recevant un  
traitement néoadjuvant**

**(Article 5)**

**Predictive Factors of Response in HER2-Positive  
Breast Cancer Treated by Neoadjuvant Therapy**

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**Journal of Oncology**



## Review Article

# Predictive Factors of Response in HER2-Positive Breast Cancer Treated by Neoadjuvant Therapy

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Since 2005, major progresses have been made in the neoadjuvant treatment of HER2-positive breast cancer. Trastuzumab introduction associated with chemotherapy has been the first major step leading to the improvement of the complete pathological response rate and, like in the adjuvant studies, better survivals. Dual HER2 blockade has been the next step and trastuzumab is associated now with other anti-HER2 therapies like lapatinib or pertuzumab, the latter being much more easy to use in combination with chemotherapy. Additional knowledge is necessary to better define within the HER2 tumor subgroup which patients could benefit more from targeted therapies. Different biomarkers have been studied to predict the response after anti-HER2 neoadjuvant therapies but until now none has been validated.

## 1. Introduction

Neoadjuvant chemotherapy in breast cancer treatment is now recognized as a standard care to increase conservative surgery [1, 2]. Its utility is also documented in inflammatory or locally advanced tumors [3]. Long-term results of neoadjuvant chemotherapy are equivalent to those obtained with adjuvant chemotherapy if locoregional treatments are fully applied [1, 2].

In HER2-positive breast cancer, randomized studies with and without anthracyclines have demonstrated the essential role of anti-HER2 therapies in obtaining increased pathological complete response rates and good long-term results.

## 2. Neoadjuvant Anti-HER2 Therapies and Their Impact on the Pathological Complete Response Rate

**2.1. Trastuzumab.** Several randomized trials have evaluated, in the neoadjuvant setting, the role of trastuzumab (Herceptin, Roche laboratory), a recombinant humanized

monoclonal antibody that targets HER2 receptor. The first randomized trial in patients with operable noninflammatory disease was stopped early when the pathological complete response (pCR) rate in the trastuzumab group was more than twice as high as that of the control group (65% versus 26%) [4, 5]. The pCR rates in the following studies varied between 26% and 40% in the trastuzumab arms [6–9]. These differences can be explained by various inclusion criteria, different type, and different duration of the regimens. Nonetheless, all the studies showed a higher pCR rate when trastuzumab was combined with chemotherapy compared to chemotherapy alone (Table 1). In the ABCSG-24 study, 536 patients were randomized to receive either 6 cycles of EDC (epirubicin, docetaxel, and capecitabine) or 6 cycles of ED (epirubicin, Docetaxel) [7]. Patients with HER2-positive tumors were also randomized to receive trastuzumab or not. In the 512 eligible patients for efficacy, the pCR rate was significantly higher after neoadjuvant EDC (23.8% versus 15.2%,  $P=0.036$ ). In the HER2-positive subgroup ( $n=90$ ), the addition of trastuzumab to the chemotherapy increased the pCR rate (40% versus 26.7%) but this result was not statistically significant ( $P=0.37$ ). Authors suggested it might

TABLE I: Randomized trials with neoadjuvant trastuzumab.

Study (ref.)	No. of patients	Clinical stage	Neoadjuvant chemotherapy	pCR rate (%)	cCR (%)	Breast-conserving surgery rate (%)
Buzdar et al. [4, 5], phase III	42	II-III	P (4 c) → FEC (4 c)	*Trastuzumab: 65% *No trastuzumab: 26%	Trastuzumab: 87% No trastuzumab: 47%	Trastuzumab: 53% No trastuzumab: 56.5%
H2269s [6], phase II	30	II-III	Doc + carboplatin (4 c)	†Trastuzumab: 40% †No trastuzumab: 7.1%	NR	NR
ABCSG-24 [7], phase III	90	T1-4 (except T4d), any N	ED ± Cap (6 c)	*Trastuzumab: 40% *No trastuzumab: 26.7%	NR	Trastuzumab: 69% No trastuzumab: 79%
NOAH [8], phase III	235	T3N1, T4, or any T N2-3	Doxo + P (3 c) → P (4c) → CMF (3 c)	*Trastuzumab: 38% *No trastuzumab: 19%	Trastuzumab: 87% No trastuzumab: 74%	‡Trastuzumab: 23% ‡No trastuzumab: 13%
REMGUS 2 [9], phase II	120	II-III	EC (4 c) → Doc (4 c)	*Trastuzumab: 26% *No trastuzumab: 19%	Trastuzumab: 34% No trastuzumab: 22%	Trastuzumab: 47% No trastuzumab: 47%

pCR: pathologic complete response; cCR: clinical complete response; NR: not reported; P: paclitaxel; FEC: 5-fluorouracil + epirubicin + cyclophosphamide; Doc: docetaxel; ED: epirubicin + docetaxel; Cap: capecitabine; Doxo: doxorubicin; CMF: cyclophosphamide + methotrexate + 5-fluoro-uracile; EC: epirubicin + cyclophosphamide.

pCR: \*no invasive residual tumor in breast and axilla; †no invasive residual tumor in breast only; ‡definition not reported.

‡[11].

be due to the unexpectedly high rate of pCR achieved in the ED/EDC group (the sample size had been calculated to detect a difference in pCR rate of 20% after ED or EDC versus 50% after ED or EDC plus trastuzumab; power = 80%,  $P < 0.05$ ). The lowest pCR rate is reported by Pierga et al. [9]. In this study, trastuzumab was introduced later after anthracyclines and concomitantly with docetaxel while in the other studies, trastuzumab was given upfront for a longer period of time [4, 7, 8].

In the GeparQuattro study, 445 patients with HER2-positive tumors were scheduled to receive 4 cycles of epirubicin plus cyclophosphamide and then were randomly assigned to either 4 cycles of docetaxel or 4 cycles of docetaxel plus capecitabine or 4 cycles of docetaxel followed by 4 cycles of capecitabine [10]. All patients received trastuzumab concomitantly to chemotherapy. Forty percent of patients showed no invasive residual tumor in the breast and had no histologic nodal involvement at surgery (ypT0/is, ypN0). The pCR rates were similar between the 3 groups and the overall breast conservation rate was 63.1% [10].

**2.2. Lapatinib.** Despite improvement of pCR rates, all patients do not equally benefit from trastuzumab therapy. Indeed multiple pathways can contribute to acquired or intrinsic resistance to trastuzumab [21, 22]. Lapatinib (Tyverb, GSK laboratory), a small molecule inhibitor of the tyrosine kinase activity of EGFR and HER2, was approved for use in combination with capecitabine for the treatment of HER2-positive metastatic breast cancer patients who have progressed on trastuzumab [23, 24].

Trastuzumab and lapatinib have well-characterized synergistic interaction in HER2-positive breast cancer models [25–27], due to partly nonoverlapping mechanisms of action. Indeed, trastuzumab inhibits ligand-independent HER2 and HER3 signaling and extracellular domain cleavage, triggers antibody-dependent cellular cytotoxicity (ADCC), and involves a reduction of angiogenesis and a decrease of DNA repair [21]. Lapatinib blocks ligand-induced heterodimer

signaling and prevents signaling via a frequently expressed truncated version of the HER2 receptor (p95) that could render cells resistant to trastuzumab.

Lapatinib leads also to an accumulation of HER2 at the surface of the cell, enhancing trastuzumab-dependent ADCC [27, 28]. These data are in favor of a dual HER2 blockade. In heavily pretreated patients, the combination of trastuzumab and lapatinib significantly prolonged progression-free and overall survivals as compared with lapatinib alone [29, 30].

Table 2 summarizes the randomized neoadjuvant studies with lapatinib versus trastuzumab or lapatinib versus trastuzumab versus lapatinib plus trastuzumab. The pCR rates reported here correspond to the absence of invasive carcinoma both in breast and axilla.

The direct comparison in the GeparQuinto trial showed that pCR rate with chemotherapy and lapatinib (30%) was significantly lower than that with chemotherapy and trastuzumab (44%) [13]. The results are similar in the randomized phase 2 GEICAM 2006-14 study with a pCR rate in the trastuzumab group twice higher than that in the lapatinib group, with the same chemotherapy regimen as in the GeparQuinto trial [17]. Surprisingly in this study, the breast-conserving surgery rates were similar in the 2 arms. The inferiority of the lapatinib compared to trastuzumab has already been highlighted in the interim analysis of the MA.31/EGF108919 trial (metastatic first line) with a shorter progression-free survival in the lapatinib arm (8.8 versus 11.4 months) [31]. Moreover, the Independent data monitoring committee of the ALTTO study (a phase III four-arm study of adjuvant lapatinib, trastuzumab, their sequence, and their combination) indicated that the lapatinib alone arm was unlikely to meet the prespecified criteria to demonstrate noninferiority to trastuzumab alone with respect to disease-free survival and patients assigned to this arm were offered to discontinue lapatinib and to potentially receive trastuzumab [32].

In the NeoALTTO study, pCR rate was significantly higher in the group given lapatinib and trastuzumab (47%)

TABLE 2: Randomized trials with neoadjuvant lapatinib versus trastuzumab or lapatinib versus trastuzumab plus lapatinib plus trastuzumab.

Study (ref.)	No. of pts	Clinical stage	Neoadjuvant chemotherapy	Neoadjuvant anti-HER2 therapy	pCR rate (%)	cCR (%)	Breast-conserving surgery rate (%)
NeoALTO [12], phase III	455	T > 2 cm, any N	Weekly P (12 w)	(i) Trastuzumab 4 → 2 mg/kg/w (ii) Lapatinib 1500 mg/d (iii) Lapatinib 1000 mg/d + trastuzumab 2 mg/kg/w	28%	NR	39% 43% 41%
GeparQuinto [13], phase III	615	cT3/4a-d or HR- or cT2N+ HR+ or cT1pN <sub>SLN+</sub> HR+	EC (4 c) → Doc (4 c)	(i) Trastuzumab 8 → 6 mg/kg/3 w (ii) Lapatinib 1250 mg/d (adjustment to 1000 mg/d)	44% 30%	33% 28.5%	64% 59%
CHER-LOB [14], phase II	121	II-IIIa	Weekly P (12 w) → FEC (4 c)	(i) Trastuzumab 4 → 2 mg/kg/w (ii) Lapatinib 1500 mg/d (iii) Lapatinib 1000 mg/d + trastuzumab 2 mg/kg/w	25% 26% 47%	NR	67% 58% 69%
NSABP B-41 [15], phase III	464	T > 2 cm, any N	AC (4 c) → P (D1, 8, 15, 28; 4 c)	(i) Trastuzumab 4 → 2 mg/kg/w (ii) Lapatinib: 1250 mg/d (iii) Lapatinib 750 mg/d + trastuzumab 2 mg/kg/w	49% 47% 60%	82% 70% 77%	54% 46% 50%
Holmes et al. [16], phase II	100	II-III	FEC75 (4 c) → weekly P (12 w)	(i) Trastuzumab 4 → 2 mg/kg/w (ii) Lapatinib 1250 mg/d (iii) Lapatinib 750 mg/d + trastuzumab 2 mg/kg	54% 45% 74%	NR	NR
GEICAM 2006-14 [17], phase II	99	T > 2 cm or N+ any T	EC (4 c) → Doc (4 c)	(i) Trastuzumab: 8 → 6 mg/kg/3 w (ii) Lapatinib: 1250 mg/d	48% 24%	NR	58% 58%

pCR: pathological complete response; cCR: clinical complete response; NR: not reported; HR: hormonal receptors; EC: Epirubicin + Cyclophosphamide; Doc: Docetaxel; FEC: 5-fluoro-uracil-epirubicin-cyclophosphamide; AC: adriamycin-cyclophosphamide.

than in the group given trastuzumab alone (28%) [12]. The results were similar in the noncomparative, randomized phase 2 CHER-LOB study with an anthracycline- and taxane-based chemotherapy [14]. Pathological complete response rates were not significantly different between the lapatinib alone arm and the trastuzumab alone arm in these two studies. In the NSABP B-41 study, although the pCR rate in the dual-blockade arm was 60%, the difference was not statistically significant [15]. It should be noted that only 63% of patients completed the treatment in this dual-blockade arm (essentially due to digestive toxicity), compared to 78% and 68% in the trastuzumab and lapatinib arms, respectively ( $P = 0.01$ ) [15].

**2.3. Pertuzumab.** Pertuzumab (Perjeta, Roche Laboratory) is a humanized monoclonal antibody directed at the dimerization domain of HER2. Pertuzumab and trastuzumab have complementary mechanisms of action due to their distinct binding sites. Pertuzumab inhibits ligand-dependent signaling particularly between HER2 and HER3 which is known to activate a proliferation pathway [33]. Pertuzumab in association with trastuzumab and docetaxel as compared with trastuzumab plus docetaxel significantly prolonged progression-free survival when used as first line in the metastatic setting (18.5 versus 12.4 months,  $P < 0.001$ ) [34]. NeoSphere is a proof-of-concept phase II study with 4 neoadjuvant arms: docetaxel plus trastuzumab (arm A), docetaxel plus trastuzumab plus pertuzumab (arm B), trastuzumab plus pertuzumab without chemotherapy (arm C), and docetaxel plus pertuzumab (arm D, added after a protocol amendment and hence not formally compared to arm A) [18]. Three hundred ninety two patients (of 417 eligible) underwent surgery. Thirty two percent of patients had locally advanced breast cancer and 7% an inflammatory tumor. The pCR rates (defined by no invasive carcinoma in the breast) were 29% (arm A) versus 45.8% (arm B),  $P = 0.0141$ ; 16.8% (arm C) and 24% (arm D). The superiority of the pertuzumab-trastuzumab arm either in first-line metastatic setting or in the neoadjuvant setting leads to the initiation of the ongoing adjuvant trial with pertuzumab (NCT01358877).

### 3. Breast-Conserving Surgery (BCS) after Neoadjuvant Anti-HER2 Therapies

In a recent meta-analysis, Valachis et al. studied the overall effect of neoadjuvant trastuzumab on BCS [35]. Breast-conserving surgery is scarcely described even in the randomized studies and the number of patients who underwent BCS was available in only four trials [35]. They did not find any difference in terms of BCS between the treatment arms (with or without trastuzumab) (OR: 0.98, 95% CI: 0.80–1.19,  $P = 0.82$ ) [35]. This could partially be explained by the fact that data from only 280 patients was available for analysis. Moreover inclusion criteria varied among studies and one study included T4 tumors [7].

In another meta-analysis, Valachis et al. reported the effect of lapatinib versus trastuzumab or the combination (lapatinib plus trastuzumab) versus trastuzumab on the BCS

rate [36]. They found no difference in terms of BCS between the treatment arms [36]. Once again, the number of studies and patients is relatively small and can affect the power of the meta-analysis to reveal statistically significant results (five trials and 1442 patients for trastuzumab versus lapatinib; 3 trials and 734 patients for trastuzumab versus combination) [36].

Moreover, other criteria can influence the BCS rate and must be taken into account like the tumor size, the presence of in situ carcinoma, the multifocality of the lesion, and the preference of the patient. Unfortunately, these data were not reported in the different studies.

### 4. Data of Survival after Neoadjuvant Anti-HER2 Therapies

Most of the studies in this review did not report data of survival. In the NOAH trial, the primary end point was the event-free survival (EFS) [8]. Adjuvant trastuzumab was administered only in patients randomized to receive trastuzumab preoperatively for one year (excepted for 7% of patients). There was a 41% reduction in risk of recurrence, progression, or death with addition of trastuzumab. The 3-year EFS rates were 71% with trastuzumab versus 56% without trastuzumab (HR = 0.59, 95% CI: 0.38–0.90,  $P = 0.013$ ), although 19 (16%) patients with HER2-positive disease assigned to chemotherapy alone crossed over to receive adjuvant trastuzumab [8]. Benefits of trastuzumab were seen in all subgroups tested, including patients with inflammatory disease. The 3-year overall survival rates were 87% with trastuzumab versus 79% without trastuzumab ( $P = 0.114$ ) [8].

Buzdar et al. reported a better disease-free survival (DFS) among patients randomized to chemotherapy plus trastuzumab compared to patients with chemotherapy alone ( $P = 0.041$ ) after a median followup of 36.1 months [5]. DFS was a secondary end point in this study and patients did not receive adjuvant trastuzumab. Among the chemotherapy alone group, the 1-year DFS rate was 94.7% (95% CI: 85.2–100%) and the 3-year DFS rate was 85.3% (95% CI: 67.6–100). There has been no recurrent disease in the patients randomized to chemotherapy plus trastuzumab and the estimated DFS at both 1 and 3 years was 100% (1-year DFS estimate: 95% CI, 85.2–100) [5].

### 5. Biomarkers for Response

Many studies reported different biomarkers for the prediction of the pCR after neoadjuvant chemotherapy in HER2-positive breast cancer. Nonetheless, no biomarker is clearly validated so far.

**5.1. Role of Hormone Receptors (HR) on the pCR Rate.** Estrogen-receptor- (ER-) negative tumors are usually associated with a higher pCR rate compared to ER-positive tumors [37–39] including HER2-positive tumors [40]. In the retrospective study of Guarneri et al., the rate of pCR was 15% in patients with HR+/HER2+ tumors versus 29%

TABLE 3: Rates of pCR according to HR status.

Study (ref.)	Neoadjuvant regimen	pCR rate HR+	pCR rate HR-	P
NeoSphere [18]	(i) Docetaxel + trastuzumab—(arm A)	20%	36.8%	NR
	(ii) Docetaxel + trastuzumab + pertuzumab (arm B)	26%	63.2%	
	(iii) Trastuzumab + pertuzumab (arm C)	5.9%	27.3%	
	(iv) Docetaxel + pertuzumab (arm D)	17.4%	30%	
Neo-ALTTO [12]	(i) Weekly P + trastuzumab	22.7%	36.5%	NR
	(ii) Weekly P + lapatinib	16.1%	33.7%	
	(iii) Weekly P + trastuzumab + lapatinib	41.6%	61.3%	
CHER-LOB [14, 19]	(i) CT + trastuzumab	25%	26.6%	NR
	(ii) CT + lapatinib	22.7%	35.7%	
	(iii) CT + trastuzumab + lapatinib	35.7%	56.2%	
Buzdar et al. [4]	(i) CT + trastuzumab	61.5%	70%	NR
	(ii) CT alone	27.2%	25%	
NOAH [20]*	(i) CT + trastuzumab	18%	48%	0.51
	(ii) CT alone	17%	22%	
REMAGUS 02 [9]	(i) CT + trastuzumab	20.5%	32%	NR
	(ii) CT alone	20.5%	19%	
NSABP B-41 [15]	(i) CT + trastuzumab	46.7%	65.5%	NR
	(ii) CT + lapatinib	48%	60.6%	
	(iii) CT + trastuzumab + lapatinib	55.6%	73%	

\*ER positive versus ER negative, pCR: pathological complete response; HR: hormone receptors; P: paclitaxel; CT: chemotherapy; NR: not reported; ER: estrogen receptors.

in patients with HR-/HER2+ tumors ( $P < 0.001$ ), after an anthracycline- and taxane-based therapy without trastuzumab [40].

In neoadjuvant trials with trastuzumab or others anti-HER2 therapies, similar results are described. The pCR rates in HR+ and HR- subgroups are summarized in Table 3.

It has been suggested that ER/HER2 crosstalk may be implicated in the escape from HER2-directed therapy. Wang et al. described an increase of ER and its downstream products in 80% of ER+/HER2+ cell lines after a treatment with trastuzumab and lapatinib. Moreover, acquisition of resistance after trastuzumab and lapatinib could require the activation of the ER pathway via Bcl2 family members [41].

**5.2. Activation of PI3K Pathway.** HER2 overexpression leads to an activation of multiple signaling pathways including the PI3K/Akt pathway that result in the modification of cell growth, differentiation, and survival. In a recent study of Dave et al, after neoadjuvant trastuzumab 18.2% (4/22) of patients with low expression of PTEN (IHC) or PI3K mutations achieved pCR whereas 66.7% (6/9) of patients without low PTEN or PI3K mutations achieved pCR ( $P = 0.015$ ) [42]. These findings are consistent with the results published by Berns et al. [43] who used a large-scale unbiased RNA interference screen and identified PTEN as the only modulator of trastuzumab sensitivity, and patients with activation of PI3K pathway had worse clinical outcome. Nagata et al. showed that patients with PTEN-deficient tumors had a poorer response to trastuzumab-containing therapy [44]. With lapatinib, the opposite effect was observed, with 92.3% (12/13) of patients with low PTEN achieving pCR compared with 41.2% (7/17) of patients with normal PTEN [42]. PI3K mutations were not associated with response or resistance

to lapatinib in this study ( $P = 0.007$ ) [42]. Hence, low PTEN expression could select patients with tumors resistant to trastuzumab but sensitive to lapatinib and could be used as a biomarker if the results are confirmed by several large phase 3 studies.

These results are discussed in other studies: in a retrospective study of 129 patients with HER2+ tumors including 26 cases treated with neoadjuvant trastuzumab and 48 metastatic breast cancer receiving a combination of taxane and trastuzumab, any relationship was found between PTEN loss and/or PI3K mutations and response to trastuzumab [45]. A lower clinical benefit rate and a lower overall response rate were observed by Wang et al. on 57 patients treated by lapatinib and capecitabine for metastatic HER2+ breast tumors with PI3K pathway activation (PI3K mutation and/or PTEN expression loss) [46].

**5.3. p95HER2.** Another potential mechanism of resistance to trastuzumab is the accumulation of truncated forms of the HER2 receptor that lack the extracellular trastuzumab-binding domain. Amino terminally truncated carboxyl terminal fragments of HER2, collectively known as p95HER2, are frequently found in HER2+ breast cancer cell lines and tumors [47]. The truncated p95HER2 receptor retains a highly functional HER2 kinase domain where lapatinib can bind. Indeed, lapatinib monotherapy or in combination with capecitabine seems to be equally effective in patients with p95HER2-positive and p95HER2-negative breast tumors [48].

In preclinical models and in 46 metastatic patients treated with trastuzumab, the expression of p95HER2 was correlated with the lack of response to trastuzumab [49]. Loibl et al. reported the pCR (noninvasive residuals in breast and

lymph nodes) and resistance rates in 153 patients included in the GeparQuattro study and then received neoadjuvant trastuzumab according to p95 expression in immunohistochemistry [50]. The pCR rate in the p95-positive tumors (10% cutoff) was 58.2% versus 32.6% in the p95-negative group ( $P = 0.009$ ). The resistance rate in the p95-positive group was 25.8% versus 48.7% in the p95-negative group ( $P = 0.014$ ) [50]. Results were similar when the cutoff was set at 20% and 30% as well as for clinical response. In this translational study, p95 expression indicates response to neoadjuvant trastuzumab but not resistance, in contrast to what was expected [50].

In the CHER-LOB trial the expression of p95HER2 was determined by immunohistochemical assay and was found to be present in 30.7% of cases. However, pCR rates were not different for p95HER2-positive and p95HER2-negative tumors in any treatment group [14].

**5.4. HER2 Serum Level.** The extracellular domain of the HER2 protein can be cleaved from the surface by metalloproteases and detected in the peripheral blood as serum HER2 (sHER2). The remaining cleaved receptor is constitutively activated, suggesting that the presence of sHER2 also reflects a biological process leading to a more aggressive tumor behavior [47]. Trastuzumab inhibits shedding of HER2 [51]. sHER2 can be measured by enzyme-linked immunosorbent assay (ELISA). In the GeparQuattro trial, Witzel et al. found a significant positive association between pCR and elevated sHER2 levels (above 15 ng/mL,  $P = 0.045$ ) and a decrease of sHER2 levels during neoadjuvant therapy ( $P = 0.02$ ) which was also significant in multivariate analysis (OR = 3.29, 95% CI: 1.001–10.89,  $P = 0.049$ ) [52]. In 210 patients of the GeparQuinto trial (52% received trastuzumab and 48% lapatinib), higher prechemotherapy sHER2 levels were associated with higher pCR rates (OR = 1.8, 95% CI: 1.02–3.2,  $P = 0.043$ ) [53]. Moreover, a decline of sHER2 levels (>20%) during neoadjuvant therapy (after 4 cycles) was a predictor of pCR in the lapatinib-treated group only (OR = 11.7, 95% CI 1.03–110,  $P = 0.031$ ) [53].

Nonetheless, the biological relevance of elevated sHER2 is still unknown and other studies have reported a limited predictive utility of baseline sHER2 [54].

**5.5. Other Biomarkers.** Before neoadjuvant chemotherapy with trastuzumab, circulating miR-210 levels were significantly higher in 11 patients who had residual disease than in 18 patients who achieved a pCR ( $P = 0.0359$ ) [55].

Some data suggested that the fragment C  $\gamma$  receptor (Fc $\gamma$ R) polymorphism has an effect on ADCC, which is one of the mechanisms of action of the trastuzumab. Tamura et al. observed in the tumors of 15 patients that the Fc $\gamma$ R2A-131 H/H polymorphism predicts the pathological response to trastuzumab-based neoadjuvant chemotherapy in early-stage breast cancer [56].

Esteve et al. suggested that in 45 patients with HER2+ tumors who received concomitant trastuzumab and paclitaxel followed by FEC, a lower expression of genes involved with CD40 signaling was associated with a greater risk of residual disease [57].

In the NOAH trial, in 171 patients with available biopsies, negative progesterone receptor and c-Myc amplification were associated with higher pCR rates after addition of trastuzumab compared to chemotherapy alone. Overexpression of membranous IGF1R was associated with higher likelihood of residual disease after trastuzumab-based chemotherapy [58].

In the exploratory study presented by Holmes et al. at the 2011 ASCO meeting, 100 patients with stage II/III HER2+ breast cancer were randomized to trastuzumab, lapatinib, or trastuzumab plus lapatinib [16]. Before and after the anti-HER2 therapy all patients had core needle biopsies for tissue microarray, stem cell analysis, and reverse-phase protein microarrays, measuring 60 different phospho-protein/posttranslationally modified protein signaling and gene expression analysis endpoints [16]. Molecular profiles suggested that nonresponders use autophagy and stem-cell-related pathways to evade therapy, while responders have disruption of HER2-HER3 linkages and known downstream regulators of growth and transcription [16].

**5.6. pCR Prediction by Positron Emission Tomography.** In HER2-negative and HER2-positive tumors, the standard uptake value (SUV) decrease, studied with positron emission tomography (PET), is a strong predictor of pCR after only one course of chemotherapy [59]. Other studies are confirmative of these data [60–62]. However PET baseline characteristics and metabolic response to neoadjuvant chemotherapy are highly dependent on the histologic type of breast cancer (i.e., luminal versus HER2-positive versus triple-negative tumors) [63]. By multivariate analysis,  $\Delta$ SUV was found as the only independent predictive factor of pCR in HER2 subtype. A decrease of SUV over 75% ( $\Delta$ SUV < -75%) had a high odds ratio (OR) of 6.31 (95% confidence interval = 1.10–39.16;  $P < 0.03$ ). To identify an optimal threshold for the prediction of the pathological response, receiver operating characteristics (ROC) analysis gave an optimal cut-off value of -70% for  $\Delta$ SUV. At this cut-off value,  $\Delta$ SUV had a sensitivity of 89% while the specificity was 87%. From these results, an SUV decrease greater than 70% allowed for the early identification of the highly responsive tumors, which will be completely eradicated by trastuzumab-based neoadjuvant therapy, with an accuracy of 76% [63]. These data are currently challenged in a prospective study (AVATAXHER Roche no. ML 22229, Eudract no. 2009-013410-26, NCT01142778) aiming at confirming the role of early PET to differentiate the excellent responders to HER2 neoadjuvant trastuzumab-based therapy from the less responders who are then randomized to additional neoadjuvant bevacizumab [64]. This study is the first one to adjust therapeutic choice according to initial PET results. If successful, this type of strategy could be used to lighten or to reinforce future neoadjuvant treatments.

## 6. Conclusion

Trastuzumab (Herceptin) has been a breakthrough in the treatment of HER2-positive breast cancer. Trastuzumab combined with chemotherapy has improved response rates,

pathological complete response, progression free survival and survival in the neoadjuvant setting of these cancers. With the emergence of new anti HER2 therapies like lapatinib (Tyverb), pertuzumab (Tarjeta) and TDM1 (Kacyla), dual HER2 blockade, still associated with chemotherapy, has proven to be superior. In order to better individualize targeted therapies in specific tumor subgroups, additional knowledge is necessary. Different biomarkers have been studied to predict the response to anti-HER2 neoadjuvant therapies but until now, except the HER2 positivity, none has been validated. PET oriented strategy could be used to separate the most responsive tumors from the less responsive ones. Further studies are necessary to define robust, reliable, and reproducible biomarkers.

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## DISCUSSION GENERALE / PERSPECTIVES

Depuis quelques années, les différents traitements systémiques (chimiothérapie, hormonothérapie) ont permis d'améliorer le pronostic des patients pris en charge pour un cancer du sein localisé, avec une diminution du risque de décès par cancer de 20 à 57% à 15 ans [202]. Il est cependant toujours difficile de pouvoir distinguer avec fiabilité les patients qui auront un vrai bénéfice d'un traitement systémique adjuvant, des patients qui n'auront pas d'amélioration de leur pronostic avec un tel traitement, qui sera par ailleurs potentiellement générateur de toxicité et de surcoût pour la société. La recherche de facteurs pronostiques et prédictifs est donc un enjeu majeur dans cette situation.

Ce problème se pose principalement chez les patients ayant une tumeur RE+ / HER2-. En effet, les tumeurs TN (hors sous-type histologique spécial) et les tumeurs HER2+, quel que soit le statut des RH, sont généralement agressives et relèvent d'un traitement adjuvant, et notamment d'une chimiothérapie, à partir du stade pT1b (> 5mm).

Le clinicien a à sa disposition, différents outils, dont « Adjuvant! Online », qui est le plus utilisé. C'est un outil prédictif utilisé dans différents types de tumeurs (sein, colon, poumon). Il s'agit d'un programme accessible en ligne qui prédit le risque de récurrence du cancer du sein à 10 ans, le risque de décès lié au cancer du sein mais également dû aux autres causes et qui calcule le bénéfice attendu des différents traitements systémiques qui peuvent être proposés au patient (chimiothérapie, hormonothérapie) [203]. Ce modèle est basé sur 6 facteurs clinico-pathologiques incluant les comorbidités (âge, statut des RE, taille tumorale, grade SBR, nombre de ganglions positifs). Il a été développé en utilisant de façon rétrospective les données d'un large registre américain (registre SEER = Surveillance, Epidemiology, End-results). Ce registre incluait des femmes âgées de 35 à 69 ans, diagnostiquées entre 1988 et 1992 aux Etats-Unis [203]. Il a ensuite été validé dans d'autres pays, au Canada, en Asie et en Europe. Ce modèle n'est pas adapté chez les patientes âgées de plus de 65 ans [204], de moins de 35 ans ou en cas de tumeur HER2 positive ou d'histologie autre le CCI. La SSR est extrapolée et non mesurée. Certains facteurs pronostiques importants ne sont pas pris en considération tels que le statut du RP, la présence d'embolies vasculaires, le Ki-67 ni le niveau de positivité du RE (facteur prédictif de réponse à l'hormonothérapie).

L'index NPI (Nottingham Prognostic Index) permet de classer les patientes en 6 catégories pronostiques mais ne repose que sur 3 facteurs histologiques (pT, pN, grade) [205]. Le caractère discriminant n'est donc certainement pas optimal.

L'Institut National du Cancer (INCA) ne recommande pas l'utilisation seule de ces outils pour décider d'un traitement adjuvant [206].

Nous avons vu au cours de ce travail, que la seule histologie lobulaire ne permettait pas de se passer d'une chimiothérapie (néoadjuvante ou adjuvante), et que cette décision devait être basée sur les critères histopathologiques habituels : taille tumorale, grade SBR, emboles vasculaires, statut ganglionnaire, RH, HER2 ainsi que sur l'âge du patient et ses comorbidités.

Nous avons également montré que la classification moléculaire des cancers du sein basée sur PAM50 ou sur le Ki-67 avec un cut-off à 14% n'était pas suffisamment fiable pour aider à la décision thérapeutique. Dans le sous-groupe des tumeurs RH+ / HER2-, l'une des questions fondamentales est : quels sont les patients guéris par une hormonothérapie seule, et qui, à l'inverse a besoin d'une chimiothérapie ? En effet, certaines tumeurs RE+ / HER2- sont de bas grade, prolifèrent peu et sont de bon pronostic après un traitement loco-régional +/- une hormonothérapie. A l'inverse, d'autres ont une prolifération plus importante, ce qui majore le risque de récurrence à distance et les rend plus sensibles aux cytotoxiques qui doivent donc être envisagés. L'hésitation à prescrire un tel traitement s'observe surtout en cas de tumeur de grade SBR II et / ou qui expriment peu ou pas le RP (hormonosensibilité intermédiaire selon St Gallen). Si la mesure de la prolifération par le Ki-67 apporte des informations relativement robustes sur le pronostic et la chimiosensibilité lorsqu'il est élevé (> 30%), son utilisation dans l'aide à la décision est très controversée, notamment du fait du manque de reproductibilité et de standardisation [207]. Ainsi, des tests génomiques ou signatures moléculaires ont été développés, tels que Oncotype DX<sup>TM</sup>, MAMMAPRINT<sup>®</sup>, Prosigna<sup>TM</sup> (RORscore), EndoPredict et Breast Cancer Index (BCI). Oncotype DX<sup>TM</sup> quantifie l'expression de 21 gènes, dont 16 gènes cibles, par qRT-PCR sur du tissu tumoral fixé et inclus en paraffine. Les 16 gènes incluent principalement des gènes associés à la prolifération, à l'expression de RE, RP et HER2. Le test donne une valeur de Recurrence Score (RS) qui correspond à un niveau de risque de rechute pour chaque tumeur : risque bas, intermédiaire ou haut. Chez les patientes pN0, RE+ sous tamoxifène, ce test a une valeur pronostique en termes de risque de métastases à distance à 10 ans (niveau

de preuve intermédiaire selon Simon, rapport INCA) [134]. Cette conclusion repose sur 3 études rétrospectives - prospectives réalisés à partir de 3 essais cliniques (NSABP-B14, NSABP-B20, ATAC) [208-210]. Dans l'étude initiale de Paik *et al*, on observait une relation statistiquement significative entre le RS et le risque de métastase à distance : RS faible (6,8% [4,0 - 9,6%]), RS intermédiaire (14,3% [8,3 - 20,3%]), RS élevé (30,5% [23,6 - 37,4%]) ; HR = 3,21 ;  $p < 0,001$  [208]. Ce test a une valeur pronostique intermédiaire selon le dernier rapport de l'INCA chez les patientes pN0 ou pN+ sous tamoxifène ou anastrozole [134]. Chez les patientes pN0, RE+, sous tamoxifène, ce test a une valeur prédictive de réponse à la chimiothérapie adjuvante par CMF (niveau de preuve intermédiaire) [134]. Cette conclusion repose sur une étude rétrospective de 651 patientes traitées ou non par CMF [211]. En cas de RS élevé, si la chimiothérapie est ajoutée au tamoxifène, la survie sans métastases à distance passe de 60% à 88% (RR = 0,26 [0,13 - 0,53]). Ce bénéfice est moins net dans le groupe à RS intermédiaire (89,1% *versus* 90,9% ; RR = 0,61 [0,24 - 1,59]), et dans le groupe à RS faible (95,6% *versus* 96,8% ; RR = 1,31 [0,46 - 3,78]). Ces résultats sont similaires pour la SSM et la SG. Le degré d'interaction entre chimiothérapie et RS est plus élevé qu'entre chimiothérapie et les autres variables classiques (âge, taille, RH, grade) [211]. Chez les patientes pN+, RE+, sous tamoxifène, ce test a une valeur prédictive de réponse à la chimiothérapie adjuvante à base d'anthracyclines pour les patientes à RS élevé (niveau de preuve intermédiaire) [134, 212]. Les 1<sup>ers</sup> résultats de l'étude TAILORx viennent juste d'être publiés : dans cette étude prospective incluant 10 253 femmes ayant une tumeur RE+, HER2-, pN0, en cas de RS bas (0 à 10, contrairement au seuil de 18 initialement retenu [211]), les patientes ne recevaient pas de chimiothérapie adjuvante mais uniquement une hormonothérapie [213]. Pour ces 1 626 (15,9%) femmes le taux de SSM à 5 ans est de 93,8% (IC 95%, 92,4 - 94,9) et le taux de SG de 98% (IC 95%, 97,1 - 98,6). Trente pourcent de ces femmes n'étaient pas ménopausées, la très grande majorité avait une tumeur de taille < 30 mm (92%) voire 20 mm (69%), 34% avaient une tumeur de grade 1 et 59% une tumeur de grade 2 (donc seulement 7% de grade 3) et 98% une tumeur RP+ [213]. Cette étude confirme en prospectif la validité clinique du test pour sa capacité à reconnaître les patientes à très bas risque de rechute à distance, pour lesquelles une chimiothérapie en sus d'une hormonothérapie n'est pas nécessaire. Cependant il ne confirme pas l'utilité clinique de ce test : en effet nous ne connaissons pas la valeur ajoutée des résultats de ce test en deçà des caractéristiques clinico-pathologiques classiques (intensité d'expression du

RE et du RP, KI-67 ou index mitotique, présence ou pas d'embolies vasculaires, type histologique). Les résultats sur la population avec un RS intermédiaire sont en attente ainsi que les résultats de 2 autres études prospectives chez des patientes pN+ (RxPONDER et OPTIMA ). Le test Oncotype DX<sup>TM</sup> est réalisé au niveau d'une plateforme unique (laboratoire Genomic Health, US), sa validité pré-analytique n'est pas démontrée et la phase analytique n'est pas suivie de contrôles de qualité externes.

MammaPrint<sup>®</sup> (signature génomique d'Amsterdam) permet l'analyse simultanée de l'expression de 70 gènes par puces à ADN à partir de tissu tumoral congelé. L'analyse est centralisée au niveau d'un seul laboratoire en Europe (Agendia, Amsterdam). Chez les patientes pN0, de moins de 61 ans, avec une tumeur de moins de 5 cm, ce test présente une valeur pronostique en termes de risque de métastases à distance à 5 ans (niveau de preuve bas) [134]. Cette conclusion repose principalement sur des études rétrospectives [214-216]. Peu de données évaluent la valeur prédictive du test (niveau de preuve bas) [134].

Prosigna<sup>TM</sup> a l'avantage de pouvoir être réalisé sur plateforme locale. Les données disponibles sont moins robustes que pour Oncotype DX<sup>TM</sup>. Ce test aurait surtout l'avantage de pouvoir distinguer les patientes à risque de récurrence tardive après hormonothérapie adjuvante [217], et ainsi pouvoir offrir à ces patientes une prolongation de l'hormonothérapie par tamoxifène ou IA au-delà des 5 années habituelles.

EndoPredict est une signature incluant 11 gènes, développée chez les patientes RE+ / HER2- [218, 219]. Il pourrait distinguer les patientes à risque de récurrence tardive [220, 221]. Ce test est décentralisé sur plateforme locale.

Breast Cancer Index (BCI) a été jusqu'à présent moins développé. Certaines données suggèrent que cette signature pourrait avoir une valeur pronostique [222], pourrait prédire la pCR après chimiothérapie néoadjuvante [223] ou le risque de récurrence tardive [224].

Les populations à partir desquelles ces tests ont été développés ne sont pas identiques, par conséquent les informations apportées ne sont pas les mêmes [225].

L'utilisation en routine de ces tests reste controversée. Aux Etats-Unis, selon les recommandations NCCN 2015, le test Oncotype DX<sup>TM</sup> peut être utilisé pour les patientes ayant une tumeur RE+, HER2- et pN- de taille > 5 mm. En Europe, selon la conférence de consensus de St Gallen 2015, la position n'est pas si tranchée mais ces tests sont reconnus comme ayant une valeur pronostique et Oncotype DX<sup>TM</sup> apporte des informations prédictives utiles pour ajouter une chimiothérapie à une hormonothérapie [122, 226]. En France, les résultats des études prospectives (TAILORx et MINDACT) sont attendus avant de se prononcer. Malgré ces signatures génomiques, il est probable qu'il persiste des zones « grises » intermédiaires pour certaines patientes où il sera toujours difficile d'apprécier le risque individuel de récurrence et la prise en charge thérapeutique optimale (chimiothérapie ?, prolongation de l'hormonothérapie ?).

Même si nous avons montré dans le sous-groupe des tumeurs HER2+ que nous manquons de biomarqueurs prédictifs d'efficacité des différentes thérapies anti-HER2, nous avons vu que c'est dans ce sous-groupe qu'il y a eu au cours de ces dernières années le plus d'avancées thérapeutiques (au-delà du trastuzumab, l'arrivée du pertuzumab et du T-DM1). C'est finalement dans le sous-groupe des tumeurs TN qu'il y a eu le moins de progrès. Nous avons vu que le terme « triple négatif » inclue des tumeurs très différentes, et qu'il existe une grande hétérogénéité moléculaire au sein de ce sous-groupe. Dans l'étude de Prat *et al*, on constate que parmi 412 tumeurs TN en IHC, après analyse par PAM50, seulement 78,6% des tumeurs sont classées dans le sous-groupe « basal-like » et qu'une partie des tumeurs classées « HER2-enriched » ou « luminal » expriment les gènes du RA et de FOXA1 [169]. Après évaluation indépendante de tumeurs TN du TCGA (The Cancer Genome Atlas), on constate qu'aucune des tumeurs classées dans les sous-groupes « HER2-enriched » ou « luminal » ne présente de mutation somatique ou germinale de *BRCA1* [169]. Nous avons vu dans notre étude que les cancers du sein TN en IHC qui co-expriment RA et FOXA1 se comportent comme des tumeurs de type luminal [227] et que les lignées cellulaires et les tumeurs exprimant les RA répondent moins à la chimiothérapie néoadjuvante [172] mais sont répondeuses à des thérapies anti-androgènes comme le bicalutamide [171, 179]. De façon récente, certaines données suggèrent que l'immunothérapie pourrait avoir un rôle dans la prise en charge des patients avec un cancer du sein TN. En effet, les patientes avec des TILs (tumor-infiltrating lymphocytes) ont un meilleur pronostic [228] et les tumeurs TN

ont un taux plus élevé d'infiltrat de lymphocytes T (LcT) CD8+, qui est un facteur pronostique favorable indépendant [229]. PD-1 (Programmed cell death protein 1) est un récepteur exprimé à la surface des cellules immunitaires dont les LcT et activé par sa liaison avec le ligand PD-L1 exprimé par les cellules présentatrices d'antigènes mais également de façon aberrante à la surface de certaines cellules tumorales (rein, mélanome, poumon non à petites cellules) [230]. L'activation de PD-1 (après fixation du ligand) entraîne une inhibition de la prolifération et de l'activation des LcT, conduisant à un arrêt de la réponse immunitaire anti-tumorale, en faisant ainsi une cible thérapeutique intéressante [230]. PD-L1 est exprimé dans 1,7 à 60% des cancers du sein, chiffres extrêmement variables du fait de populations d'étude et de techniques de dosage différentes [231-237]. L'expression de PD-L1 est associée à des critères de mauvais pronostic (taille tumorale importante, grade élevé, RE-, RP-, HER2+, Ki-67 élevé) [231-233, 237], à la présence de TILs [232, 235, 237] et est plus fréquente dans les tumeurs basales que luminales [231, 234, 236]. Dans les tumeurs TN, l'expression de PD-L1 est décrite dans 19 à 59% des cas selon les séries [231, 236, 238, 239]. C'est un facteur pronostique favorable indépendant dans 2 études [231, 235] et péjoratif dans une autre [233]. Il est associé à un meilleur taux de pCR après chimiothérapie néoadjuvante [231, 237]. Deux études de phase 1 (une avec le pembrolizumab, anti-PD-1 et une avec le MPDL3280A, anti-PD-L1) ont montré des résultats prometteurs avec des taux de réponse de 19%, des réponses durables et une survie sans progression de 27% à 6 mois [240, 241], résultats non habituels chez des patientes avec un cancer TN déjà lourdement prétraitées. Une présentation au dernier congrès de l'ASCO 2015 rapporte une expression 3,8 fois plus fréquente du PD-L1 dans la population des cancers du sein TN RA+ que dans les autres TN [239]. Cependant, pour l'instant, l'impact pronostique de cette co-expression est inconnu.

Les cancers basal-like présentent fréquemment des mutations ou des pertes de l'expression de PTEN (35%), corrélées à une activation de la voie PI3K [242]. Dans les tumeurs TN, les mutations de PI3K sont plus fréquentes en cas de positivité du RA (36% *versus* 9%) [175, 177]. Dans l'étude de Millis *et al*, les cas avec perte de l'expression de PTEN en IHC (56,5%) ou de mutation de PTEN (12,9%) représentent au total 57,6% des tumeurs TN RA+ [177]. Mittendorf *et al* suggèrent que PTEN intervient dans la régulation transcriptionnelle de l'expression de PD-L1 dans les cancers TN et que la perte de PTEN peut être responsable d'une augmentation de l'expression de PD-L1 par les cellules tumorales, limitant la réponse

immunitaire anti-tumorale [238]. Des associations de thérapeutiques ciblant la voie PI3K et la liaison PD-1 / PD-L1 pourraient augmenter ainsi la réponse immunitaire adaptative anti-tumorale. Nous avons pour projet d'évaluer l'expression du RA, de FOXA1 et de PD-L1 en IHC dans une série de 400 cancers du sein TN non métastatiques lors du diagnostic initial, pour lesquels nous réaliserons également les analyses suivantes : expression en IHC des marqueurs « basal-like » (cytokératines 5/6, EGFR), recherche de mutations *PIK3CA*, recherche de délétions / insertions de *PTEN*, expression de *BRCA1*. La recherche d'une hyperméthylation du promoteur de *BRCA1* sera également réalisée. En effet, l'absence ou la diminution d'expression de la protéine *BRCA1*, en l'absence de mutation *BRCA1*, semble liée à l'hyperméthylation du promoteur de *BRCA1* [243]. Par ailleurs, Gong *et al* suggèrent que *BRCA1* maintient l'expression de la protéine FOXA1 en supprimant la méthylation du gène *FOXA1* [244]. Dans cette série de cancers du sein TN, nous disposons des principales caractéristiques anatomo-cliniques et nous disposons des données de survie avec un recul d'au minimum 3 ans. Etant donné le risque de récurrence à court terme de ce type de tumeurs, ceci nous permettra d'évaluer l'association de ces différentes anomalies d'intérêt avec les caractéristiques anatomo-cliniques et les paramètres de survie dans une large population homogène de cancers du sein TN. L'ensemble des données résultant de cette étude devrait nous permettre : i) de mieux caractériser les associations de ces différentes anomalies d'intérêt au sein des cancers du sein TN ; ii) d'en évaluer l'impact pronostique ; iii) identifier une sous-population de tumeurs TN accessible à des essais cliniques ultérieurs utilisant des agents ciblant ces différentes anomalies d'intérêt.

Un traitement néoadjuvant est une option thérapeutique dans le cancer du sein [189, 190]. Bien que la proportion de patients présentant une pCR ait augmenté ces dernières années grâce à l'introduction des taxanes et des thérapies anti-HER2, 40 à 80% des patients ont un résidu tumoral viable après traitement. Nous avons montré que la pCR est un bon critère substitutif de la survie chez les patientes ayant une tumeur RH- (TN ou HER2+) [245]. Ceci a également été retrouvé dans de larges séries regroupant des patientes traitées par chimiothérapie néoadjuvante [168, 192, 199, 246]). Malgré cela, on constate parfois des rechutes chez les patients en réponse complète et fréquemment une absence de rechute chez des patients n'ayant pas obtenu une réponse complète. Il semble important d'identifier précocement les patients susceptibles de moins répondre au traitement, afin d'intensifier,



voire de changer de stratégie de traitement. Ceci a été étudié par une équipe allemande. Chez 1 390 patients ayant une réponse en imagerie après 2 cycles de TAC (docetaxel, adriamycine, cyclophosphamide) néoadjuvant, la moitié ont reçu 4 cycles complémentaires de TAC et l'autre moitié 6 cycles supplémentaires [247]. Il n'y a pas eu de différence statistiquement significative en termes de pCR entre les 2 groupes mais une toxicité plus importante en cas de prolongation du traitement [247]. Chez les non-répondeuses après 2 cycles de TAC, les mêmes auteurs ont randomisé les patientes entre 4 cycles supplémentaires de TAC et 4 cycles d'un schéma sans résistance croisée (vinorelbine et capecitabine) [248]. Il n'y a pas eu de différence en termes de pCR (aux alentours de 5% dans les 2 bras) mais une meilleure tolérance en cas de traitement par vinorelbine et capecitabine [248]. Une analyse exploratoire de ces 2 études a permis de mettre en évidence une amélioration de la SSM (HR = 0,71, IC 95% 0,60 - 0,85,  $p < 0,003$ ) et de la SG (HR = 0,79, IC 95% 0,63- 0,99,  $p = 0,048$ ) en cas d'adaptation du traitement néoadjuvant selon la réponse initiale même en l'absence d'augmentation du taux de pCR [249]. Ceci était constaté uniquement en cas de tumeur RH+ (HER2- et HER2+) mais pas chez les patientes ayant une tumeur RH- (TN ou HER2+) [249]. Il faut souligner que dans cette étude, les schémas utilisés comportaient des molécules de chimiothérapie standard, sans thérapie ciblée.

Au-delà de la biologie tumorale (moins bonne réponse mais meilleure survie en cas de tumeur RH+ [192]), des facteurs cliniques et des biomarqueurs ont été identifiés comme marqueurs prédictifs de réponse à un traitement néoadjuvant. Parmi eux, l'âge (plus de pCR chez les patients jeunes), l'IMC (moins de pCR en cas d'IMC élevé), une réponse précoce après 2 à 4 cycles de chimiothérapie (22% de pCR *versus* 5% dans l'étude de Von Minckwitz *et al* décrite plus haut), l'expression du RA (moins de pCR si RA+), la prolifération (grade, Ki-67, grade génomique), la présence de TILs (augmentation de la pCR si présence), la présence d'une mutation *BRCA1*, une mutation de *PI3KCA* en cas de tumeur HER2+ (moins de pCR si présence d'une mutation) [250].

La TEP (tomographie par émission de positons) au  $^{18}\text{F}$ -fluorodéoxyglucose (18-FDG) est un outil non invasif prometteur pour identifier précocement les patients susceptibles de ne pas obtenir une réponse complète à la chimiothérapie néoadjuvante. L'imagerie conventionnelle peut être mise en défaut pour distinguer des cellules tumorales actives d'une fibrose réactionnelle au traitement [251]. En étudiant la variation du métabolisme glucidique tumoral, la TEP peut permettre de distinguer ces 2 aspects. C'est notamment la variation de

SUV (standard uptake value) avant et après 1 ou 2 cycles de chimiothérapie qui est étudiée et qui pourrait prédire la pCR [252]. Ceci est particulièrement vrai en cas de tumeur HER2+ [252]. Une étude de phase II randomisée (AVATAXHER) a d'ailleurs évalué le bénéfice de l'addition d'un traitement par bevacizumab à un schéma de docetaxel / trastuzumab en cas de mauvaise réponse métabolique ( $\Delta\text{SUV} < 70\%$ ) après 2 cycles de docetaxel / trastuzumab [253]. Ce changement de stratégie thérapeutique a permis d'augmenter le taux de pCR (24% *versus* 43,8%) [253]. La valeur pronostique de la réponse métabolique dans ce sous-type biologique n'a pas encore été évaluée. Des études suggèrent également un rôle prédictif de la réponse métabolique chez les patients ayant une tumeur TN [252]. En cas de tumeur luminaire (RH+, HER2-), ceci n'est pas aussi clairement montré et dans ce sous-groupe, la valeur de SUV à baseline et le changement après un cycle de chimiothérapie sont plus des critères substitutifs précoces de survie à 5 ans, indépendamment de la pCR. Dans l'étude de Humbert *et al*, 2 groupes ont été identifiés avec des pronostics différents : haute valeur de SUV initialement et faible réponse métabolique à 1 cycle (taux de SSR à 5 ans de 33%), et faible SUV initiale ou haute valeur de SUV initiale avec une bonne réponse métabolique après 1 cycle (taux de SSR à 5 ans de 93% et 88%, respectivement) [254].

En conclusion, bien que d'énormes progrès aient été réalisés dans la compréhension de la biologie du cancer du sein et de son hétérogénéité, il reste encore beaucoup à faire pour pouvoir distinguer les patients à risque de récurrence élevé nécessitant un traitement plus agressif, et notamment une chimiothérapie, des patients guéris par un traitement loco-régional adéquat et éventuellement une hormonothérapie adjuvante. En attendant, la décision doit reposer sur les critères histopronostiques classiques que nous avons abordés, en tenant compte non seulement des comorbidités du patient mais également de sa préférence notamment en cas de zone « grise », considérée à risque intermédiaire.

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## **Annexe. Classification pTNM (7<sup>ème</sup> édition AJCC / UICC) [79, 80]**

### **T : Tumeur primitive**

pTis : carcinome in situ

pT0 : pas de signe de tumeur primitive

pT1 : tumeur de 2 cm ou moins dans sa plus grande dimension

pT1a : ≤ 0,5 cm

pT1b : > 0,5 cm et ≤ 1 cm

pT1c : > 1 cm et ≤ 2 cm

pT2 : tumeur dont le plus grand diamètre est compris entre 2 et 5 cm

pT3 : tumeur de plus de 5 cm dans sa plus grande dimension

pT4 : tumeur de toute taille avec extension directe à la paroi thoracique ou à la peau

pT4a : avec fixation à la paroi thoracique.

pT4b : avec œdème, infiltration ou ulcération de la peau, présence de nodules cutanés satellites limités au même sein

pT4c : T4a + T4b.

pT4d : carcinome inflammatoire

### **N : Adénopathies régionales**

pN0 : absence d'atteinte ganglionnaire

pN1 : micrométastases ou métastases dans 1 à 3 ganglions axillaires homolatéraux et/ ou mammaires internes

pN1mi : micrométastases (> 0,2mm et < 2mm)

pN1a : atteinte de 1 à 3 ganglions axillaires (dont au moins 1 > 2mm)

pN1b : atteinte ganglions mammaires internes détectés par biopsie du GS

pN1c : pN1a + pN1b

pN2 :

pN2a : atteinte de 4 à 9 ganglions axillaires (dont au moins 1 > 2mm)

pN2b : métastases ganglions mammaires internes détectées en imagerie en l'absence d'atteinte ganglionnaire axillaire

pN3 :

pN3a : atteinte de 10 ou plus ganglions axillaires (dont au moins 1 > 2mm) ou atteinte ganglions sous-claviculaires

pN3b : métastases ganglions mammaires internes détectées en imagerie et atteinte ganglionnaire axillaire ou atteinte de plus de 3 ganglions axillaires et de ganglions mammaires internes avec micro ou macrométastases détectés par la biopsie du GS

pN3c : métastase ganglion sus-claviculaire homolatéral

### **M : Métastases à distance**

pMx : détermination impossible de l'extension métastatique

pM0 : absence de métastases à distance

pM1 : présence de métastases à distance