



Apports de la Médecine de Précision à la Neurochirurgie : Applications aux Glioblastomes

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THÈSE

Pour obtenir le diplôme de doctorat

Spécialité SCIENCES DE LA VIE ET DE LA SANTE

Préparée au sein de l'Université de Rouen Normandie

Apports de la Médecine de Précision à la Neurochirurgie : Applications aux Glioblastomes

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**Thèse soutenue le 29/11/2021
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**Thèse dirigée par STÉPHANE MARRET et SOUMEYA BEKRI, GENOMICS AND
PERSONALIZED MEDICINE IN CANCER AND NEUROLOGICAL DISORDERS**

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Publications & Communications

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Liste des principales abréviations

ADN	Acide désoxyribonucléique
ARN	Acide ribonucléique
ARNm	ARN messenger
CCS	Collision Cross Section
ESI	ElectroSpray Ionisation
FT-ICR	Fourier Transform-Ion Cyclotronic Resonance
FT-MS	Fourier Transform-Mass Spectrometry
GBM	Glioblastome Multiforme
HDMS	High Definition Mass Spectrometry
HGMD	Human Gene Mutation Database
HMDB	Human Metabolome DataBase
IMS	Ion Mobility Spectrometry
LC	Liquid Chromatography
min	Minute
MS	Mass spectrometry
ms	Millisecond
MS/MS	Tandem Mass spectrometry
m/z	Rapport masse sur charge
NMR	Nuclear Magnetic Resonance
OMIM	Online Mendelian Inheritance in Man
PC	Principal Component
Q	Quadripole
s	Second
TOF	Time of Flight
tR	Temps de rétention
UHPLC	Ultra-High Pression Liquid Chromatography

INTRODUCTION GENERALE

Préambule

Dans la continuité de la médecine de précision, la chirurgie de précision est un concept émergent depuis une dizaine d'année. La chirurgie, qui dans l'histoire a été longtemps une branche à part de la médecine, a mis du temps à s'approprier les énormes progrès technologiques dont nous avons été témoins au cours des trois dernières décennies. En effet, une grande partie de nos pratiques chirurgicales actuelles n'ont pas changées depuis maintenant plusieurs décennies. Bien que nous ayons tenté de rendre les procédures chirurgicales moins morbides en utilisant des voies d'abord mini-invasives ou endoscopiques, lorsque cela était approprié, la technologie accompagnant ces procédures peu invasives reste relativement rudimentaire. Ces avancées ont souvent consisté en une « ouverture » moins large de notre patient, une plus grande prise en compte des contraintes anatomiques naturelles ou le recours à des instruments chirurgicaux plus adaptés et variés. Ce n'est que récemment que les techniques d'ingénierie ont amélioré nos pratiques chirurgicales que ce soit par l'essor des robots chirurgicaux ou des systèmes de navigation per opératoire qui permettent aux chirurgiens d'effectuer des procédures parfois plus précises que la main humaine. Grâce à la collaboration croissante entre l'ingénierie, l'informatique et les chirurgiens, nous commençons à entrevoir un avenir passionnant et peut-être, une prochaine révolution chirurgicale. L'avenir de la chirurgie tournera autour du thème central de la chirurgie guidée par l'image, de la réalité augmentée, de la résolution des images à la navigation et à l'intelligence artificielle, en passant par les résections guidées par des

données de biologie moderne. Néanmoins, un élément central mérite d'être souligné : ces technologies sont des aides à la conception et à la réalisation de l'acte chirurgical et elles ne sauraient se substituer à une connaissance anatomique et gestuelle rigoureuses. Elles viennent faciliter ce geste et son enseignement. Ensuite, la chirurgie de précision n'est pas à confondre avec la précision de l'acte chirurgical même si celui-ci est un élément de ce progrès. Comme la médecine de précision, la chirurgie de précision place le patient comme référentiel de lui-même plus que la procédure elle-même, en y intégrant des données médicales et biologiques individuelles tout en tenant compte de la grande diversité interindividuelle. Les chirurgies oncologiques, mais pas seulement, se sont appropriées ce concept. Traditionnellement, l'évaluation peropératoire de la résection tumorale dépendait largement de l'inspection visuelle, de la palpation des tissus et plus récemment des données d'imagerie ou d'histologie per ou post opératoire. L'apport de la chirurgie de précision a été par exemple de proposer des résections assistées par un spectromètre de masse. Elle repose donc sur de larges collections biologiques issues des sciences omiques (génomique, transcriptomique, protéomique, métabolomique et phénomique) utilisées à l'échelle de l'individu. Son émergence a été rendue possible par l'essor du big data et de l'intelligence artificielle. La neurochirurgie s'est approprié relativement tôt ces avancées technologiques et son développement coïncide avec ces bonds d'ingénierie et de biologie. Elle a été l'une des premières chirurgies à utiliser le microscope opératoire pour la chirurgie hypophysaire puis endocrânienne au sens large ; l'une des premières à utiliser l'imagerie et la navigation peropératoire et les techniques mini-

invasives. Il est donc logique que la neurochirurgie s'enrichisse de ces données nouvelles de biologie moléculaire issues du big data clinico-biologique pour accompagner ce bond technologique vers la chirurgie de précision.

Cette thèse porte sur les apports et applications des sciences omiques aux pathologies neurochirurgicales. Nous développerons les éléments diagnostiques en métabolomiques appliqués au glioblastome, tumeur cérébrale maligne.

Les objectifs de ce travail de thèse sont définis comme suit :

1. Mise en place d'une méthodologie métabolomique non ciblée et ciblée basée sur une stratégie analytique multidimensionnelle comportant la spectrométrie de masse à haute et très haute résolution, la chromatographie liquide ultra-haute performance et la mobilité ionique.
2. Mise en place de la méthodologie de prétraitement, d'analyse et d'exploitation des données
3. Application à une pathologie neurochirurgicale qu'est le glioblastome.

Le manuscrit de cette thèse est structuré en trois parties, Neurochirurgie et chirurgie de précision (**Chapitre I**), le glioblastome (**Chapitre II**) et le travail expérimental (**Chapitre III**).

Le chapitre I comporte une introduction avec une perspective historique de la naissance et pratique de la neurochirurgie ainsi que l'apport des techniques de biologie moléculaire à la neurochirurgie moderne.

Le chapitre II comprend un état des lieux des connaissances actuelles sur l'objet principal du projet de thèse que représente le glioblastome (basés sur une revue publiée) et le chapitre III représente le travail expérimental. Celui-ci consiste en un article (art 3) qui décrit les aspects méthodologiques métabolomiques appliquées au glioblastome.

CHAPITRE I : CHIRURGIE ET MEDECINE DE PRECISION

1.1 Perspectives historiques

1.1.1 La neurochirurgie dans l'histoire

Des techniques et des Hommes

La neurochirurgie est considérée comme la plus ancienne des pratiques chirurgicales. Les premières trépanations ont été réalisées à la période néolithique. En France, des traces de trépanations humaines datant de 7000 ans avant J.C. ont été retrouvées. La civilisation pré Inca usa de techniques de trépanation de façon importante vers 2000 ans avant J.C. et le recours à des plaques en métal était pratiqué pour couvrir le defect osseux. Les indications de trépanation de cette époque étaient les troubles mentaux, l'épilepsie, les céphalées, les maladies organiques et certains traumatismes crâniens. A l'époque, ces pratiques étaient également utilisées pour des raisons magiques ou religieuses. Un outil en bronze utilisé pour « user » la table externe de l'os du crâne a été retrouvé en Amérique du Sud. La cocaïne issue des feuilles de coca était utilisée comme anesthésiant local. Des stigmates de consolidation osseuse attestaient de la survie de certains patients suite à ces procédures ¹⁻³ (Figure 1).



Figure 1. Photographie d'un crâne Inca ayant subi trois trépanations et survécu à la procédure comme en attestent les berges osseuses consolidées ⁴.

Hippocrate, médecin grec au IV^e siècle avant J.C. considéré comme le père de la médecine décrivit l'anatomie fonctionnelle du cerveau. Il enseignait à ses disciples que l'hémisphère cérébral contrôlait l'hémicorps controlatéral et qu'ainsi la trépanation devait se pratiquer du côté opposé aux symptômes. Il usait d'un trépan qui arrêta son œuvre une fois le crâne ouvert en préservant le cerveau sous-jacent. De telles procédures sont toujours couramment utilisées avec des outils chirurgicaux très proches dans leur conception ^{1,5}.

Ambroise Paré, chirurgien-barbier au XVI^e siècle est l'un des fondateurs de la chirurgie moderne. Opérant sur les champs de bataille, il décrit les techniques de cautérisation et de ligature artérielle. Sa description de la trépanation conduit à une diminution des infections et événements hémorragiques. Jean-Louis Petit, chirurgien et anatomiste du XVII^e siècle a décrit l'hématome extradural avec la notion d'intervalle libre. Il décrivit également l'incision de la dure-mère et les deux plans de cette méninge. Ces notions sont toujours enseignées à nos étudiants actuellement. Un exemple classique de convergence entre neurochirurgie et histoire de France est la mort d'Henri II, en 1559 ⁶. Blessé par un fragment de lance lors d'un tournoi, le roi de France meurt d'infection dans les jours suivant cette plaie oculaire. Ambroise Paré, par sa description de l'autopsie du roi, décrivit le phénomène d'empyème cérébral lié à la contamination d'une plaie.

Outre-Manche, le pionnier de la neurochirurgie moderne se nomme Sir Victor Horsley (1857-1924). Il est chirurgien militaire et travaille au Queen Square Hospital à Londres. Appuyé par ses collègues physiologistes, il émet l'hypothèse que l'épilepsie est une maladie du cortex cérébral et que sa résection peut permettre de stopper le développement de l'épilepsie. Il réalise sur des observations cliniques, les premières cortectomies cérébrales chez trois patients. L'un des patients survécut à cette chirurgie avec une diminution de la fréquence des crises. Cette procédure, bien qu'alimentée d'explorations d'imagerie et de physiologie, est couramment pratiquée de nos jours ^{7,8}.

Harvey Cushing (1869-1939), premier neurochirurgien américain à opérer des tumeurs cérébrales, aurait pratiqué plus de 2000 interventions cérébrales. Sa description de ses voies d'abord a permis d'améliorer la survie des patients de neurochirurgie ⁹. En France, les pionniers de la neurochirurgie se nomment Clovis Vincent et Thierry de Martel. Formé à Paris,

Clovis Vincent fonda le service de neurochirurgie de la Pitié-Salpêtrière. Neurologue de formation, il fit progresser son art et forma de nombreux élèves de renom ¹⁰.

Malheureusement, la chirurgie porte encore le poids de son histoire. Du XII^e au XVIII^e siècle, les chirurgiens-barbiers sont distincts des médecins. Les progrès médicaux réalisés en parallèle de la pratique chirurgicale n'enrichissent que peu le savoir-faire chirurgical. Le principal écueil de la neurochirurgie repose sur l'asepsie. La naissance de la neurochirurgie moderne post-guerre a pu se développer grâce aux progrès des conditions per opératoires (asepsie et anesthésie) et à une meilleure appréhension de la pathologie grâce aux progrès de l'imagerie et de la neurophysiologie.

Les explorations en neurochirurgie

Très tôt, les neurochirurgiens se sont définis comme des médecins ayant une pratique chirurgicale. Clovis Vincent disait que « La neurochirurgie, ce n'est pas seulement l'art d'enlever les tumeurs du cerveau, mais c'est le moyen d'apprendre d'une façon précise les fonctions du cerveau humain ». Il fut d'ailleurs le premier médecin-chef d'un service de chirurgie. Au Canada, Wilder Penfield ¹¹, également neurologue et neurochirurgien, décrivit la somatotopie cérébrale. Il contribua, tout comme Clovis Vincent, élève de Babinski, à la description des circuits de la mémoire. S'appuyant sur ses travaux, Harvey Cushing réalisa les premières stimulations peropératoires pour explorer les fonctions des zones cérébrales réséquées. Ces explorations historiques préfigurent le développement de la chirurgie éveillée. L'un des pans de la neurochirurgie moderne la plus liée à ces progrès techniques est certainement la neurochirurgie fonctionnelle. Elle vise à ajuster certaines fonctions du névraxe quand le traitement médicamenteux est insuffisant ou non recommandé. Classiquement d'autres approches y sont associées telles que la stimulation cérébrale profonde, la chirurgie de l'épilepsie, la chirurgie de la douleur et la radiochirurgie. Ses pionniers viennent d'horizons divers. Lars Leksell (1907-1986) est physicien puis neurochirurgien suédois ¹². Nous lui devons l'un des premiers cadres de stéréotaxie (Figure 2) et l'invention de la radiochirurgie. La France est là encore bien représentée : Jean Talaraich (1911-2007) est psychiatre puis neurochirurgien ¹³. Avec Jean Bancaud, neurologue, ils rédigent le premier atlas de stéréotaxie qui reste un ouvrage de référence et proposent un cadre de stéréotaxie qui permet de réaliser implantation

d'électrodes et biopsie cérébrale avec une précision incomparable. Ces techniques chirurgicales ont été développées avant le scanner cérébral (1970's). Les repères anatomiques étaient basés sur des abaques statistiques issus de centaines d'observations cadavériques et de repères indirects. Ces abaques représentent une information stockée, quantifiable, normée et mobilisable pour assister l'acte chirurgical. Conceptuellement, ces abaques sont les bases de données informationnelles précurseurs du big data anatomo-clinico-biologiques actuel. En effet, les repères indirects, avant l'avènement du scanner et de l'IRM, consistaient en une ventriculographie gazeuse (inventée par Dandy) ou l'injection sous-arachnoïdienne de lipiodol tel que décrit par Sicard en 1921 ¹⁴. Il faut imaginer une opération de neurochirurgie avant ces imageries et l'approximation que cela pouvait représenter ainsi que la morbidité associée. La sémiologie clinique nous permettait et permet toujours d'émettre une hypothèse topographique d'une lésion. Une ventriculographie (injection d'air ou de lipiodol) était réalisée puis une radiographie de crâne standard laissait entrevoir la déviation du système ventriculaire et ainsi la localisation supposée de cette lésion. Une « ouverture » du scalp large était réalisée puis des trous de trépan étaient réalisés. Un volet crânien était taillé à la scie Gigli (scie à ruban glissée d'un trou de trépan à un autre). En 1972, Godfrey Hounsfield ¹⁵ se voit décerner le prix Nobel de médecine pour la découverte du premier scanner appelé tomographie. De manière anecdotique, Sir Hounsfield était ingénieur chez EMI, société de musique et d'électronique qui, grâce aux ventes record des Beatles dans les années 1960's finança les travaux de recherche de Hounsfield. Ce fut un bouleversement technologique pour la neurochirurgie. Pour la première fois, nous pouvions voir le cerveau de manière directe sans ouvrir la boîte crânienne. Le scanner a progressivement amélioré sa résolution et l'arrivée de l'IRM au début des années 1990's a permis une technique non irradiante et beaucoup plus fine. En seulement 30 ans, l'IRM est passée de sa naissance à une pratique courante qui paraît évidente à toute une génération.

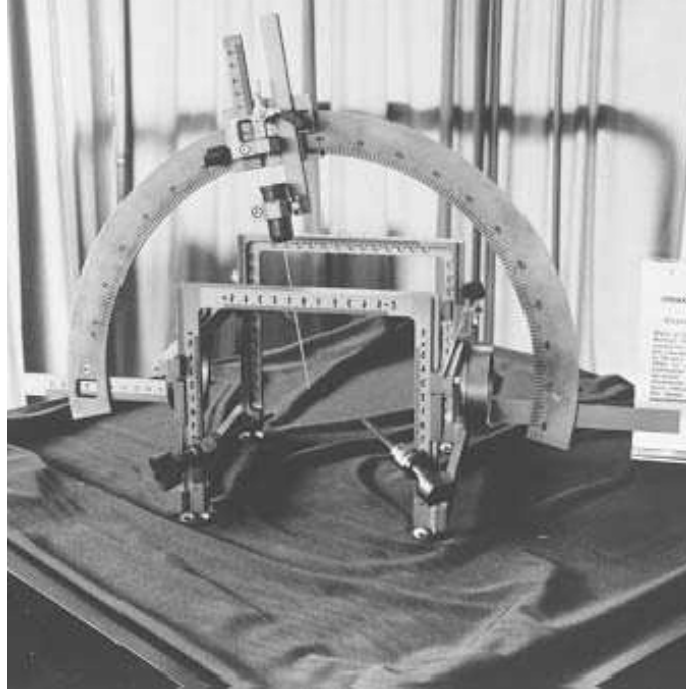


Figure 2. Photographie d'un cadre de stéréotaxie dit de Leksell permettant les biopsies et implantations d'électrodes cérébrales.

En parallèle, les progrès de la magnification optique et des techniques de neuro-endoscopie ont amené à des progrès d'approches opératoires, de gestion de dissection ou d'hémostase voire de nouvelles interventions (ventriculocysternostomie endoscopique par exemple) ¹⁶. Depuis les années 2000, la robotique s'est invitée au bloc opératoire. Des robots chirurgicaux (pour la stéréotaxie cérébrale ou rachidienne pour le moment), des systèmes de navigation voire de réalité augmentée sont utilisés quotidiennement. Plus récemment, cette intégration des progrès humains et techniques vient s'enrichir des progrès de la biologie moléculaire et contribue au développement d'une nouvelle révolution qu'est la chirurgie de précision.

1.1.2. Apports de la biologie moléculaire à la médecine

La naissance de la biologie

Les philosophes grecs, voraces de curiosité, regardent avec intérêt l'éventail des créatures vivantes, de la plante la plus humble à l'homme lui-même. Un nom grec a été inventé par un naturaliste allemand au début du XIXe siècle pour cette étude de tous les aspects physiques de la vie naturelle - la biologie, du bios (vie) et du logos (mot ou discours) ^{17,18}. C'est un sujet qui comporte des subdivisions claires, comme la botanique, la zoologie ou l'anatomie. Mais tous s'intéressent aux organismes vivants. L'un des pères de la biologie est Alcmaeon de Crotone ¹⁹, médecin, astronome et philosophe pythagoricien du Ve siècle avant J.C. Il s'intéresse aux fluides corporels et émet l'hypothèse que les caractéristiques de ces fluides pourraient être des marqueurs de pathologies plus globales : c'est le principe même d'un biomarqueur tel qu'il se conçoit actuellement. Il est suivi un siècle plus tard par Aristote et Théophraste qui développent respectivement la biologie animale et végétale. Dans son ouvrage *Historia animalium*, Aristote ²⁰ recense les caractéristiques biologiques propres à chaque animal, car selon lui, « c'est par l'observation du normal et de ses caractéristiques intrinsèques que l'on peut décrire le pathologique ». Là encore nous verrons tel que développé plus loin dans ce manuscrit, qu'il s'agit d'un principe des sciences Omiques de caractériser sur le plan phénotypique une population et d'établir le pathologique et son biomarqueur par comparaison d'un groupe témoin de sujets indemnes de la pathologie qu'on cherche à identifier. En 158 avant J.C. la ville de Pergame compte un nouveau médecin en chef des gladiateurs. Il est un médecin grec du nom de Galien ²¹. Cette nomination lui donne l'occasion d'étudier des blessures de toutes sortes. Sa connaissance des muscles lui permet d'avertir ses patients du résultat probable de certaines opérations. Mais c'est la pratique de la dissection des singes et des porcs qui donne à Galien des informations détaillées pour ses traités médicaux. Près de 100 de ces ouvrages ont survécu. Ils deviennent la base de la grande réputation de Galien dans la médecine médiévale, incontestée jusqu'aux travaux anatomiques de Vésale. Grâce à ses expériences, Galien est capable de renverser de nombreuses croyances de longue date, comme la théorie selon laquelle les artères contiennent de l'air et non du sang- le transportant vers toutes les parties du corps depuis le cœur et les poumons. Cette croyance est basée à l'origine sur les artères des animaux morts qui semblent vides. Galien démontra

par ses observations des plaies de gladiateurs que les artères vivantes contiennent du sang. Il supposa par contre que le sang va et vient du cœur dans un mouvement de flux et de reflux. Cette théorie a prévalu dans les cercles médicaux jusqu'au XVI^e siècle.

Au cours des siècles profondément chrétiens du Moyen-Âge européen, l'ambiance qui prévaut n'est pas propice à la recherche scientifique. Dieu sait ce qui est le mieux, et il devrait le faire - puisqu'il a tout créé. Lorsque des connaissances pratiques sont nécessaires, il existe des autorités anciennes dont les conclusions sont acceptées sans conteste - Ptolémée dans le domaine de l'astronomie, Galien pour les questions anatomiques. Quelques savants atypiques s'intéressent à la recherche scientifique. Le frère franciscain du XIII^e siècle, Roger Bacon, est l'exemple le plus souvent cité ²²⁻²⁴, mais ses études portent aussi bien sur l'alchimie et l'astrologie que sur l'optique et l'astronomie. Le scepticisme pratique requis pour la science doit attendre la Renaissance.

Des progrès liés à l'imprimerie

En 1454, l'allemand Johannes Gutenberg invente les caractères mobiles. La mécanisation de l'écriture permet une accélération des connaissances biologiques ²⁵. Vers 1489, Léonard de Vinci commence une série de dessins anatomiques ^{26,27}. Pour la précision de l'observation, ils sont bien en avance sur tout ce qui a été tenté auparavant. Au cours des vingt-cinq années suivantes, il dissèque une trentaine de cadavres humains, dont beaucoup dans une morgue à Rome - jusqu'à ce qu'en 1515, le pape Léon X lui ordonne d'arrêter. Ses dessins, au nombre de 750 environ, comprennent des études sur les structures osseuses, les muscles, les organes internes, le cerveau et même la position du fœtus dans l'utérus. Ses études sur le cœur suggèrent qu'il était sur le point de découvrir le concept de la circulation du sang. C'est une coïncidence de grande valeur pour la biologie, dans laquelle l'observation est de première importance, que le regain d'intérêt de la Renaissance pour la science coïncide avec l'invention de l'imprimerie. Dès que les livres peuvent être publiés avec des illustrations gravées sur bois parmi les textes imprimés, les naturalistes ont non seulement un nouveau lectorat important, mais aussi la capacité de montrer ce qu'ils ont observé avec tant de soin. Le premier à utiliser sérieusement cette possibilité est un botaniste, Otto Brunfels, dont les trois volumes *Herbarum vivae eicones* sont publiés à Strasbourg entre 1530 et 1540 ²⁸. Un naturaliste français de cette époque fournit un bon exemple de l'impulsion de la Renaissance à égaler et peut-être même à

surpasser les auteurs classiques. En 1546, Pierre Belon entreprend un voyage de deux ans autour de la Méditerranée orientale dans le but précis de trouver et de représenter les animaux et les plantes décrits par les écrivains anciens. A Bâle, en Suisse, Vésale publie en 1543 son grand ouvrage - *De humani corporis fabrica* (La structure du corps humain) ²⁹. Il s'agit d'un ouvrage en sept volumes comprenant de nombreuses et magnifiques illustrations gravées sur bois. Le livre connaît un succès immédiat, bien qu'il fasse naturellement l'envie des traditionalistes qui suivent Galien. Les théories de Galien ont, après tout, le mérite évident de l'ancienneté. Elles ont maintenant quelque 1400 ans. Ce souci de précision et d'exhaustivité n'est pas sans rappeler les projets actuels que sont par exemple The Human Protein Atlas ou le séquençage du génome humain ^{30,31}. La continuité avec nos ancêtres est que la connaissance biologique des éléments nous constituant nous permet de mieux appréhender et traiter les maladies. Cela nécessite donc de visualiser l'infiniment petit.

La découverte du microscopique

Marcello Malpighi ³², professeur de médecine théorique à l'université de Bologne, a été le pionnier de l'utilisation du microscope en biologie. Un soir de 1661, sur une colline près de Bologne, il utilise le soleil couchant comme source de lumière, l'éclairant dans son objectif à travers une fine section préparée d'un poumon de grenouille. Sur l'image agrandie, il est clair que tout le sang est contenu dans de petits tubes. Malpighi devient ainsi le premier scientifique à observer les capillaires, ces minuscules vaisseaux sanguins dans lesquels le sang circule à travers la chair. Ils sont si fins, et si nombreux, que chacun de nos corps contient plus de 100 000 kilomètres de ces conduits microscopiques. Grâce à leur découverte, le chaînon manquant dans la circulation sanguine de Harvey ³³ a été mis en évidence. Car les capillaires sont littéralement le lien par lequel le sang riche en oxygène des artères fournit d'abord son énergie aux cellules du corps et trouve ensuite son chemin vers les veines pour être renvoyé au cœur. Améliorant le travail de Malpighi avec le microscope, le chercheur néerlandais Anton van Leeuwenhoek ³⁴ atteint un très haut degré de précision et de clarté (certaines offrant un grossissement de 300x). Avec de tels instruments, il est en mesure d'observer des phénomènes jusqu'alors trop petits pour être vus ^{35,36}. En 1674, il est le premier scientifique à donner une description précise des globules rouges. En 1677, il observe et représente des spermatozoïdes

dans le sperme d'un chien. En 1683, il fournit un dessin des animalculae (ou bactéries) présents dans la salive et la plaque dentaire.

Les siècles suivants, l'invention du microscope a permis de perfectionner la connaissance du vivant et notre environnement tout en étayant les classifications pour conduire à la biologie moderne que nous connaissons. Cette histoire est encore incomplète.

1.2. Sciences omiques

Au XXe siècle, la biologie a connu des avancées extraordinaires avec la découverte de l'acide désoxyribonucléique (ADN) par Watson et Cricks en 1953 ³⁷. Le développement de la Polymerase Chain Reaction (PCR) par Mullis en 1988 ³⁸ révolutionne la biologie moléculaire et ouvre la voie aux sciences omiques. Le suffixe -omique fait référence à l'exhaustivité et le haut débit de l'information moléculaire. Les sciences omiques peuvent être définies comme une nouvelle branche de la biologie moléculaire qui repose sur des technologies permettant la caractérisation et la quantification collectives de molécules à partir de différents échantillons biologiques permettant ainsi d'appréhender la structure, fonction et dynamique d'un organismes ³⁹. Le terme « omics » est proposé pour la première fois par Wilkins, généticien de formation en 1994 ⁴⁰. La nécessité de ces sciences nouvelles est apparue criante à l'issue du projet génome humain (HGP) en 2001 ⁴¹. Ce projet a permis une meilleure compréhension de notre génome, mais également mis en exergue les rôles fondamentaux des autres processus de régulation biologiques. Les sciences omiques nécessitent la gestion d'un très grand nombre de données et sont indissociables de la bio-informatique et du big data. La Figure 3 illustre les progrès techniques de biologie moléculaire réalisés au XXe siècle.

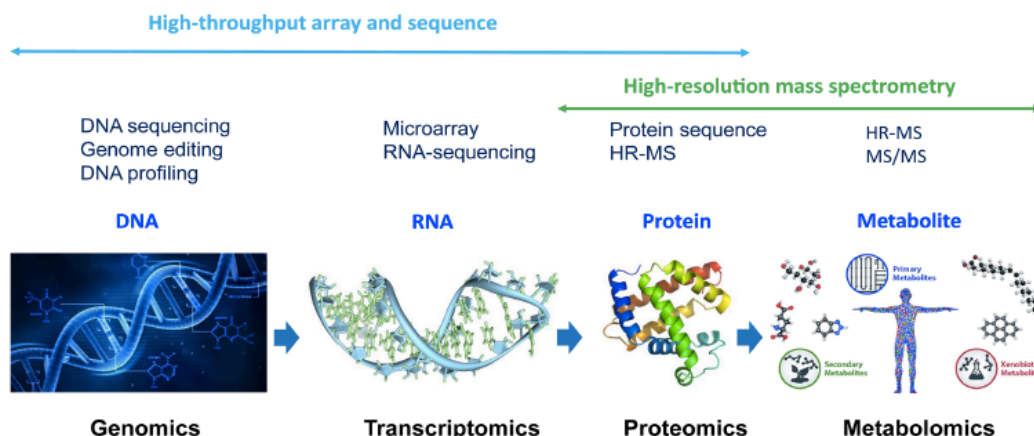


Figure 3. Représentation des techniques de biologie moléculaire des différentes sciences omiques. D'après Khan *et al.*⁴²

Cette technologie a permis de déchiffrer la cellule, le tissu et l'organisme de manière holistique autour d'un dogme central pour la détection des gènes (génomique), de l'ARNm (transcriptomique), des protéines (protéomique), des métabolites (métabolomique) et des profils de méthylation (épigénomique) dans les échantillons. Plus récemment, la phénomique a émergé comme nouvelle branche des sciences omiques qui s'intéresse à l'étude des expressions phénotypiques des organismes. Ces technologies ont été explorées dans diverses branches de la médecine et des sciences de la santé que ce soit au cours du dépistage, du diagnostic ou du pronostic de nouvelles maladies. La recherche de biomarqueurs de ces maladies est aussi l'un des piliers des sciences omiques^{43,44}. La meilleure compréhension de la physiopathologie des maladies offre des perspectives thérapeutiques nouvelles et des approches qui seront prédictives, préventives et personnalisées. Nous allons proposer un aperçu des différentes technologies omiques disponibles ainsi que leurs applications potentielles.

1.2.1. Génomique

La génomique est l'étude complète de l'ensemble des informations héréditaires de chaque organisme vivant qui sont nécessaires à son développement et à son fonctionnement ⁴⁵. Le mot "génome" est une conjonction des mots "gène" et "chromosome". Dans les années 1990, l'ensemble du génome de *Haemophilus influenzae* ⁴⁶, un organisme vivant en liberté, a été séquencé, ce qui représente une contribution importante au domaine de la génomique. L'étude de la génomique caractérise et quantifie collectivement non seulement l'expression, mais aussi son réseau de régulation qui lui est associé. En 1984, l'idée de décoder l'intégrer du génome humain paraît à portée de main du fait des nouvelles techniques de séquençage. Le projet du génome humain (HGP) est lancé aux États-Unis. Le projet a recours aux méthodes de séquençage dites par fusil de chasse (HSS) qui consiste à fragmenter l'ADN en plus petites longueurs pour séquencer séparément chacun de ses fragments. Ensuite, les fragments sont superposés pour recréer le génome ⁴⁷. Deux des premiers objectifs du HGP étaient la préparation de cartes génétiques et physiologiques des génomes de l'homme et de la souris avec des technologies améliorées de séquençage de l'ADN ; le séquençage de génomes plus petits (levures et vers) a été pris comme modèle de test, avec un objectif projeté de transfert de technologie. En 1999, une séquence pour le chromosome 22 humain (Chr 22) a été signalée, la première ébauche du HGP étant détaillée en 2001. Le Chr 22 a été particulièrement choisi en raison de sa petite taille et de son association avec diverses maladies. Trois milliards de lettres du génome de l'*Homo sapiens* ont finalement été partagées dans le projet final du HGP financé par les pouvoirs publics en 2003. Ce projet, bien que relativement complet, comportait quelques lacunes majeures telles que des séquences discontinues. Une version plus actualisée a finalement été complétée en 2006, qui a donné la séquence complète de Chr 1, le plus grand chromosome du génome humain, contenant 8% du matériel génétique. Sept ans plus tard, le génome de l'*Homo neanderthalensis* a été rendu public ^{48,49}.

Le premier catalogue, connu sous le nom de "Index Marker Catalog", a détaillé la cartographie complète avec une résolution de 10-15 cM. Le génome humain est essentiellement constitué de 98,6 % d'ADN de "rebut". Le HGP a permis la découverte de 1800 gènes responsables de maladies, le premier étant associé à la maladie de Parkinson et, étonnamment, 850 sites étant concernés par des maladies courantes. La plupart des points chauds se trouvaient à proximité

des séquences adjacentes plutôt qu'à l'intérieur de celles-ci. Plus de mille tests génétiques et trois cents produits issus de la biotechnologie ont résulté des essais cliniques basés sur les données du HGP ^{50,51}. À ce jour, les génomes de 6070 eucaryotes, 145 357 procaryotes, 17 614 virus, 12 924 plasmides et 11 732 organites ont été séquencés et sont facilement accessibles dans le domaine public dans la base de données sur le génome du National Center for Biotechnology Information (NCBI). Depuis quelques années, la génomique, qui a été la première sciences omique, a permis le développement de thérapeutique innovante en s'inscrivant dans la médecine de précision ^{52,53}.

1.2.2. Transcriptomique

La transcriptomique est la base la plus informative pour commencer un travail de recherche, et avec l'avènement des nouvelles techniques à haut débit, il est devenu très facile et rapide de générer un ensemble de données et d'informations ⁵⁴. Le transcriptome constitue l'ensemble des transcriptions présentes dans une cellule, y compris l'ARNm, les ARN non codants et les petits ARN. La transcriptomique identifie la quantité d'ARN et la structure de la transcription et quantifie les différents niveaux d'expression des transcriptions dans l'espace et dans le temps au cours des différentes étapes du développement et dans des conditions physiologiques variables. Elle fournit des informations sur la diversité, les ARN non codants et la disposition des unités transcriptionnelles dans les régions codantes. L'analyse transcriptomique a commencé par une technique primitive appelée EST, c'est-à-dire des balises de séquences exprimées, suivie d'une autre technique appelée SAGE, c'est-à-dire une analyse en série de l'expression des gènes, basée sur le séquençage de Sanger. Les techniques EST et SAGE étaient laborieuses et permettaient de déterminer un petit ensemble de transcriptions de manière aléatoire, donnant ainsi la moitié des informations sur le transcriptome. Les années 1990 marquent la décennie révolutionnaire de la transcriptomique avec l'introduction d'une innovation technologique de la technique contemporaine appelée "microarray" ^{55,56}. Les microarrays analysent rapidement le transcriptome des grands mammifères et ont été utiles pour le développement de médicaments et la recherche clinique en analysant des milliers de gènes à partir de multiples échantillons. Le principal inconvénient

de cette technique est l'analyse des seules séquences connues et ne permet donc pas de détecter les nouveaux transcrits. La dernière technologie en matière d'analyse du transcriptome est l'ARN-Seq ⁵⁷, basée sur une technologie de séquençage profond. Elle permet d'identifier le gène et l'activité temporelle des gènes dans un génome. L'ARN-Seq in situ est une forme avancée qui donne un aperçu d'une cellule individuelle dans un tissu fixe. De même, l'information des gènes exprimés par une communauté microbienne est appelée métatranscriptomique ⁵⁸. La métatranscriptomique fournit un profil fonctionnel du microbiome dans des conditions physiologiques variables ⁵⁸. Les données générées sont utiles pour l'analyse de l'enrichissement et l'analyse phylogénétique des microbes. Plusieurs pipelines bioinformatiques sont actuellement conçus ou en cours de réalisation pour l'analyse de l'ensemble des données du métatranscriptome ⁵⁹.

1.2.3. Protéomique

L'étude des protéines complètes d'une source et des techniques impliquées pour étudier ces protéines et leurs interactions est le fondement de la protéomique ⁴⁹. La carte tridimensionnelle des protéines et de leurs interactions délimite leur importance et leur fonctionnement dans un organisme. Ces études sont initiées au niveau des protéines en remontant parfois jusqu'à leurs gènes. Le phénomène d'épissage alternatif chez les eucaryotes enrichit sélectivement la diversité du protéome. La protéomique structurale prend en considération la structure tridimensionnelle des protéines, ce qui contribue à la procédure de conception rationnelle des médicaments basée sur la structure. D'autre part, la protéomique fonctionnelle est largement axée sur la compréhension de l'expression des protéines au niveau cellulaire, des modifications des protéines, des interactions entre les protéines, de la signalisation et des mécanismes de la maladie ⁶⁰. Ce domaine a pris de l'ampleur avec l'avènement de la technologie, où différentes techniques telles que les rayons X, la RMN, la spectroscopie de masse, la chromatographie Liquide Haute Performance (CLHP) et la PAGE bidimensionnelle ont permis de générer d'énormes données expérimentales ⁶¹.

Il est difficile de suivre la quantité colossale de données expérimentales générées par les différentes méthodes de détection des protéines. L'analyse effectuée par des procédures de

bio-informatique impliquant des algorithmes, des bases de données et des pipelines pour l'analyse informatique permet une analyse plus rapide et plus précise en quelques jours. Grâce aux bases de données et aux portails de ressources, la gestion, le stockage et le partage des données ont permis aux chercheurs d'obtenir et de rassembler plus facilement les données, ce qui accélère la recherche en protéomique. La protéomique, en combinaison avec d'autres technologies complémentaires comme la génomique et la transcriptomique (une approche au niveau des systèmes), a un énorme potentiel pour répondre à plusieurs questions sans réponse en biologie ⁴⁹.

1.2.4. Métabolomique

1.2.4.1. Définition

La métabolomique est définie comme l'étude des métabolites issus du métabolisme chez un individu ^{49,62}. Les métabolites sont les composés organiques de petite taille participant au métabolisme. Leur étude entre en interaction avec les autres sciences omiques bien que la métabolomique émule directement l'activité biochimique de l'organisme et décrit donc le mieux le phénotype moléculaire. Le métabolome d'un organisme, défini par l'ensemble des métabolites le constituant, est complexe et dynamique, car les métabolites sont continuellement absorbés et dégradés. Les études métabolomiques tentent de fournir un instantané complet de l'état physiologique d'un organisme à un moment donné. De manière générale, l'étude métabolomique peut être réalisée selon deux approches : l'approche ciblée et l'approche non ciblée. Dans le cas de l'approche non ciblée, un certain nombre de métabolites différents sont mesurés sans aucun biais d'échantillon, tandis que dans le cas de l'approche ciblée, des ensembles définis de métabolites sont mesurés avec un objectif du problème à traiter. Cependant, les étapes de ces deux approches sont communes ^{63,64}.

1.2.4.2. Stratégies analytiques

Les études métabolomiques peuvent être divisées en cinq étapes ^{65,66} (Figure 4). La première étape consiste à définir le plan de l'étude en tenant compte d'un certain nombre de facteurs

tels que la taille de l'échantillon, sa nature, la randomisation des analyses. Cette étape permet de s'assurer que tous les facteurs importants sont pris en compte, notamment les métabolites impliqués et leurs interactions potentielles qui pourraient biaiser leur analyse. La deuxième étape est la préparation de l'échantillon, où la collecte, le stockage et la préparation de l'échantillon ont lieu. Une attention particulière sera prise à la méthode d'extraction pour ne pas dégrader les échantillons. De la même manière, la conservation devra s'effectuer à très basse température (-80°C) pour ne pas dégrader les métabolites d'intérêt. Dans la troisième étape, une technique analytique comme la spectroscopie de masse ou la RMN est utilisée pour mesurer et quantifier les métabolites. La quatrième étape consiste à prétraiter les données pour les analyser afin d'en extraire des inférences biologiques. La dernière étape est l'analyse des données. Cette étape comprend l'application d'analyses statistiques aux données générées.

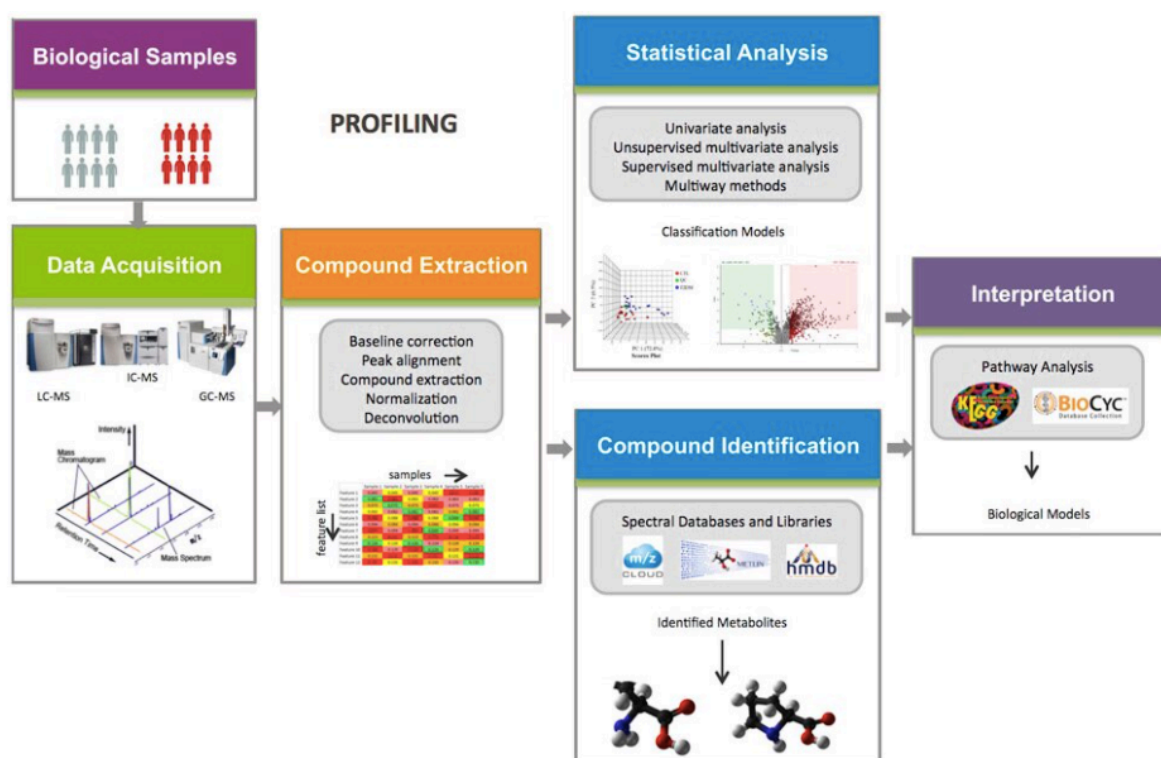


Figure 4. Représentation schématique des différentes étapes d'une étude métabolomique ⁶⁷.

Une fois qu'une étude métabolomique est terminée, elle peut être soumise à diverses applications, car il s'agit d'une approche très proche de la capture du phénotype d'un individu.

Cela en fait un outil précieux pour la recherche biomédicale. Des domaines avancés comme la médecine personnalisée utilisent l'étude métabolomique pour le diagnostic et le pronostic médical, utile pour l'identification de la maladie. La métabolomique est utile, car elle a la capacité d'identifier et de caractériser différents métabolites, ce qui nous permet de mieux comprendre les mécanismes de la maladie.

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L'article I présente les grandes avancées dans l'histoire de la neurochirurgie et de la biologie notamment avec l'arrivée des sciences omiques. Il a pour but de montrer l'évolution de la neurochirurgie au XXI^e siècle et comment cette discipline s'est appuyée sur les données de biologie moléculaire pour proposer une chirurgie de précision. Le prisme de la neuro-oncologie a été choisi, car elle illustre au mieux l'interdisciplinarité et l'intégration des données omiques dans la pratique de la médecine personnalisée.

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Precision Neurosurgery: A Path Forward

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Abstract: Since the inception of their profession, neurosurgeons have defined themselves as physicians with a surgical practice. Throughout time, neurosurgery always take advantage of technological advances to provide better and safer care for patients. In the ongoing precision medicine surge that drives the patient-centric healthcare, neurosurgery strives to effectively embrace this data-driven medicine era. Neuro-oncology best illustrates this convergence between surgery and precision medicine with the advent of molecular profiling, imaging and data analytics. This convenient convergence paves the way for new preventive, diagnostic, prognostic and targeted therapeutic perspectives. The prominent advances in healthcare and big data utterly challenge the medical community to deeply rethink current and future medical practice. This work provides a historical perspective on neurosurgery. It also discusses the impact of precision medicine conceptual shifts on neurosurgery through neuro-oncology lenses.

Keywords: neuro-oncology; neurosurgery; precision medicine; personalized medicine; omics; medical imaging

1. Introduction

Neurosurgery is considered as the oldest surgical practice. It has been reported that the first trepanations were performed in the Neolithic period and traces of human trepanations dating back to 7000 years BC have been found with bone consolidation around the drill hole, proof of survival of these first patients [1,2]. Despite its long history, neurosurgery has been able to rely on the great technological advances of its time. In recent years, with the emergence of omics-based biological lenses, precision medicine has emerged as one of the great advances in various fields of medicine [3,4]. Its

contribution can be found in the field of diagnosis with the development of biomarkers, but it also provides prognostic elements and opens the way to new targeted therapies [5]. Neurosurgery is no exception and precision, or personalized medicine is present in our daily surgical practice. Precision medicine (PM) aims to place the patient at the center of the healthcare pathway by integrating individual medical and biological data while taking into account the great diversity between individuals. These new approaches invite us to reinvent ourselves as neurosurgeons and to question our practice and profession. In line with the spirit and training of our mentors, engineering, imaging and molecular biology techniques became more and more familiar components of the operating room. They would not replace the fundamental principles of surgery, which are the core of the operative indication, including a rigorous anatomy knowledge and flawless gestures hardly earned through relentless and long practice in companionship. Nevertheless, these techniques have become very useful in providing high quality and safe care to our patients. One of the fields that best illustrates the contribution of new technologies and interdisciplinarity, in neurosurgery, is undoubtedly neuro-oncology. In the following lines, we aim to give a historical perspective and discuss how neurosurgery is evolving towards its next deep technological shift by fully embracing precision medicine particularly in the field of neuro-oncology.

Neurosurgery: a short historical perspective

Even though the history of neurosurgery seems to begin in the Neolithic period, 5000 years later, it was the pre-Inca civilizations that developed trepanning techniques. The indications of these trepanations were very different from today and had a religious or magical tone [2,6]. Nevertheless, these procedures were also performed in the context of cranial trauma, headaches, epilepsy and behavioral disorders. Later, the Incas have raised to the rank of art their practice of cranial surgery. This is particularly highlighted by the diversity of their metal-based instruments [7,8]. By looking at the survival rate following cranial surgery throughout history, Kushner *et al.* [2] showed that the Incas had a survival rate of 75% compared to 45% during the American Civil War. The main cause of mortality at the time was infection but the Incas applied ointments and metal prosthesis, ancestor of the current cranial vault plasties [7,8]. However, sole technical skills are not enough to provide quality surgery. Understanding the brain physiology is, obviously, important to treat it more effectively. The first Greek physicians helped to better understand these concepts. Hippocrates in the fourth century BC [9,10], who considered as the father of medicine, explained in his *omnia opera Hippocratis*, that the knowledge of physiology allowed for a better understanding of the gesture and that the technique alone was not enough to guarantee high quality surgery. Galen, in the 2nd century A.D., elaborated on Hippocrates' work. In addition to perfecting anatomical knowledge through the observation of a large number of subjects and taking care of injured gladiators [11]. Although ancient Greece practices, such as trepanations, were refined over the centuries, endocranial surgery as such did not appeared until the 16th century. The cruelty of war allowed significant medical leaps in the Middle Ages and the Renaissance. One of the leading figures of modern surgery was undoubtedly Ambroise Paré (1510-1590) [12]. He perfected the trepanation techniques and vessel ligation and introduced the notion of asepsis by using red iron cauterisation. A historical convergence between people's lives and neurosurgery is the death of Henry II of Navarre, who died of an infection following a craniocerebral wound during a tournament. Ambroise Paré, who performed the autopsy, described a cerebral empyema as the cause of death. Across the Channel, the pioneer of modern neurosurgery was Sir Victor Horsley (1857-1924) [13,14]. He was a military surgeon working at the Queen Square Hospital in London. With the support of his neurophysiologist colleagues, he hypothesized that epilepsy was a disease of the cerebral cortex and that its resection could stop the development of epilepsy. Based on clinical observations, he performed the first cerebral cortectomies on three patients. This procedure, although supported by imaging and physiological investigations, is commonly performed today [6,7]. Harvey Cushing (1869-1939) [15] is the first American neurosurgeon to operate on brain tumors. He has performed more than 2000 brain surgeries and the description of his approaches led to improved survival of neurosurgical patients. Unfortunately, surgery still carries the burden of its own history. From the 12th to the 18th century, barber-surgeons were distinct from physicians. The medical progress made in parallel with surgical practice only slightly enriched the surgical know-how. The main pitfall of surgery is asepsis. As so was the case for neurosurgery. The birth of modern post-war neurosurgery was possible through the progress of per operative conditions (asepsis and anesthesia) and to a better understanding of the pathology and the progress in imaging and neurophysiology.

Advances in neurosurgery: first steps towards precision surgery

Very early on, neurosurgeons defined themselves as physicians with a surgical practice. "Neurosurgery is not only the art of removing tumors from the brain, but it is the means to learn in a precise way the functions of the human brain" once said the French neurosurgeon Clovis Vincent (1879-1947) [16]. He was the first physician in charge of a dedicated surgical department. In Canada, Wilder Penfield (1891-1976) [17], also a neurologist and a neurosurgeon, described cerebral somatotopy. He contributed, as did Clovis Vincent, a student of Babinski (1857-1932), to the description of memory circuits. Based on his work, Harvey Cushing performed the first intraoperative stimulations to explore the functions of the resected brain areas. These historic explorations prefigured the development of awake surgery. One of the aspects of modern neurosurgery most related to these technical advances is certainly functional neurosurgery. It aims at adjusting certain functions of the neuraxis when drug treatment is insufficient or not recommended. Classically, it includes deep brain stimulation, epilepsy surgery, pain surgery and radiosurgery. The pioneers come from various backgrounds. Lars Leksell (1907-1986) was a Swedish physicist and neurosurgeon [18]. He is credited with one of the first stereotaxis frameworks and the invention of radiosurgery. In France, Jean Talairach (1911-2007) was a psychiatrist and then a neurosurgeon [19]. With Jean Bancaud (1921-1993), a neurologist, they wrote the first stereotaxis atlas, which remains a reference work. They proposed a stereotaxis framework that allows for the implantation of electrodes and brain biopsy with flawless accuracy. Surgical precision and accuracy are at the core of precision medicine because it relies on the development of engineering techniques. These surgical techniques were developed before the brain scanner (1970's). The anatomical landmarks were based on statistical charts derived from hundreds of cadaveric observations and indirect landmarks. These charts represent a precious stored, quantifiable, standardized and ready-to-use information to assist the surgical act. Conceptually, these charts are the precursor informational databases of the current anatomical, clinical and biological big data. In 1972, Godfrey Hounsfield (1919-2004) ([20] was awarded the Nobel Prize in Medicine for the discovery of the first computerized tomography (CT) scan. It was a technological breakthrough for neurosurgery. For the first time we could, safely and non-invasively, see the brain directly without opening the skull. The scanner resolution progressively improved and the surge of Magnetic Resonance Imaging (MRI) in the early 1990's allowed for a non-irradiating cleaner image. In only three decades, MRI has gone from innovation to a common practice. At the same time, progress in optical magnification and neuro-endoscopy techniques led to advances in operative approaches, dissection management or hemostasis, and even new interventions (i.e., endoscopic ventriculocysternostomy) [21]. At the dawn of this millennium, robotics has entered the operating room. Surgical robots (for cerebral or spinal stereotaxis for the moment), navigation and even augmented reality systems are used on a daily basis. More recently, this integration of human and technical progress has been enriched by advances in molecular biology and is substantially contributing to the development of multimodal techniques paving the way to precision surgery [22].

Precision medicine in daily neuro-oncology

Neurosurgery has always cultivated interdisciplinarity as described above. Some of its prominent members came from different backgrounds. Lars Leksell for example was a physicist by training. So was also Jean Talairach. The different branches of neurosurgery were enriched by their respective backgrounds. With the advent of molecular biology techniques, precision medicine is no longer an outsider of the operating room but became an active component. The most striking example is certainly neurosurgical oncology. This is particularly highlighted through glioblastoma. Indeed, its management has made significant advance [23,24]. Glioblastoma, although the most common primary malignant tumor of the central nervous system, still lacks curative strategies. Its prognosis remains poor, even in case of complete surgical resection with a median survival of 15 months [25]. One of the reasons for treatment resistance in glioblastoma is its heterogeneity. Thus, the integration of molecular biology, genomics and more recently metabolomics and transcriptomics data are of interest and has led to a better understanding of glioblastoma heterogeneity and biological plasticity [26,27]. The digital revolution and the multimodal Big Data surge have made it possible to characterize this tumor in order to better understand its genesis, its clinical heterogeneity, its functional effects and the reasons underlying its resistance to treatment [28,29]. The main goals of precision medicine are to better understand glioblastoma signaling biological networks and to define common and distinct pathways that could explain their

clinical heterogeneity. This will allow for biomarker and prognostic discovery to open the way to targeted and individualized therapies by targeting an alteration in these signaling pathways [30,31].

Historically, the diagnosis of glioblastoma was based on the WHO classification originally derived from histological criteria alone. The latest WHO classification of 2016 has integrated the latest molecular biology data as well as prognostic elements and response to treatment related to their metabolic characteristics [32]. Among the new features of the 2016 WHO classification is the mutation status (mutated or wild type) of the *IDH1* and *IDH2* genes. It is postulated that *IDH*-mutated gliomas inhibit tumor suppressor oncogenes, inducing the development of gliomas. Consequently, *IDH*-mutated status is an important prognostic factor for glioblastomas [33]. The second component of classification of glial lesions is the presence or absence of a 1p/19q codeletion. The 1p/19q co-deletion corresponds to the complete loss of 1p and 19q secondary to a translocation. It is associated with a better prognosis and a better response to chemotherapy for grades II and III gliomas [34]. Another recently described signaling pathway in glioblastoma is the telomerase-related senescence escape pathways through mutation of the *ATRX* (alpha thalassemia/mental retardation syndrome X-linked) gene or *TERT* (telomerase reverse transcriptase) promoter [27]. The *ATRX* gene was initially discovered in patients with X-linked mental retardation. The *ATRX* protein is a chromatin remodeling protein whose main role is to maintain genomic stability. Thus, in case of *ATRX* variation, there is a telomerase lengthening allowing glial cells to escape senescence. *ATRX* variation is a prognosis marker, rarely observed in *IDH*-wild type glioblastoma. Variants in the *TERT* promoter or *ATRX* gene are present in 90% of mutated *IDH* gliomas but are rarely associated. In recent series [35,36], it appears that *TERT* promoter gene mutation is associated with poor outcomes in *IDH* wild type glioblastoma and favorable outcomes in *IDH* mutated glioblastoma. Prognostic value of *TERT* promoter status is discussed due to its high frequency and confounding factors. For example, the association with other molecular alteration such as O(6)-methylguanine-DNA methyltransferase (*MGMT*) methylation is of interest. *MGMT* is an enzyme that repairs O6-methylguanine of DNA to guanine. It protects the tumor cell against cytotoxic damage from alkylating chemotherapies, such as temozolomide [37] [38]. As a consequence, methylation of the *MGMT* promoter leads to a decrease in *MGMT* expression and in the repair of temozolomide-induced lesions. This status is a predictive marker for a better response to alkylating agents and an independent favorable prognostic marker in glioblastoma. Variants in genes encoding histones, that are first identified in childhood glioma, are a promising avenue for a better understanding of glioma signaling pathways [35]. Indeed, these variants are frequently found in various cancers and could contribute to their epigenetic regulation. The heterogeneity of tumors and the large number of molecular biology discoveries yielded through omics highlights the need for a more personalized knowledge through integrative strategies to achieve precision medicine.

The search for a single biomarker for glioblastoma has been the focus of a sustained research for many years [39]. Medical imaging and MRI spectroscopy, in particular, were main stream strategies in clinical practice [40]. It mainly consists in highlighting specific metabolic patterns in the tumor tissue. The development of this technique required a large number of patients. The patient is, in this case, his own control because the healthy brain parenchyma, usually contralateral, is used as a control tissue. Thus, MR spectroscopy in glioblastomas is characterized by a specific increase in choline/N-acetylaspartate and choline/creatinine ratios [41]. In addition, an elevated peak of lactate and lipids as well as a decreased peak of myoinositol are reliable information for the diagnosis of glioblastoma. With the advent of Big Data, radiological diagnosis continues to progress. Recently, researchers at the University of Washington proposed an algorithm based on artificial intelligence and deep learning [42]. Their software has proven effective in diagnosing six categories of brain tumours from conventional MRI sequences. This radiological marker cannot, currently, replace a histological analysis. This highlights the urgent need for a sensitive blood biological signature. The use of large biobanks has allowed for multi omics studies to propose biomarkers defined as a clinical, radiological and biological signature allowing to define sub-groups of glioblastomas [43,44]. This refers to liquid biopsies whose aim is to propose a diagnosis through a simple blood sample. Liquid biopsy has the advantage to prevent from invasive procedure such as brain biopsy and can be repeated easily [29,45,46]. The development of such techniques has its pitfalls even though the concept is still elegant. First, the heterogeneity of glial tumors makes it difficult to find a single biomarker. Secondly, the blood-brain barrier is a powerful obstacle for the discovery of a blood biomarker of an intraparenchymal processes. Finally, an ideal biomarker must allow for monitoring of therapeutic efficacy or relapse. However, therapeutics can modify this biomarker. Thus, currently, de-

spite sustained research, there is no cleared blood biomarker for the diagnosis and follow-up of glioblastoma patients. Nevertheless, recent analyses have highlighted genes or metabolites of interest in the diagnosis of glioblastoma [45,47,48]. Some of them are also prognostic indicators and might pave the way to potential targeted therapies.

Towards a precision surgery

Despite the above-mentioned progress, are we going to see the birth of precision surgery? At the dawn the current century, the main progress in neurosurgery has been based on intraoperative navigation to better locate the tumor from preoperative imaging [49]. Awake surgery has increased the resection rate by decreasing the morbidity of the surgical procedure [50]. Nowadays, imaging is performed during surgery and allows for resection assessment in real time [51]. Augmented reality techniques allow for the smooth integration of these imaging data into the surgeon's microscope to directly visualize the lesion and the peri-tumor area. More recently, intraoperative analysis of resected tissue has been made possible by the miniaturization of omics techniques. Thus, some teams have tested the integration of a mass spectrometer in the cavitronic ultrasound aspirator [52,53]. This allows to limit the resection to the tissue invaded by the tumor. One of the difficulties of this technology is to define the metabolites of interest. These are different depending on whether they concern the fleshy portion of the tumor, a possible cystic portion, the contrast medium or the peri-tumoral zone. Secondly, the contribution of mass spectrometry cannot be free of the functional limits linked to the tumor resection. The surgeon's hand is often stopped by the risk of morbidity of a resection. Nevertheless, *in situ* metabolic phenotyping is a definite contribution and is a complement to navigation and microscopy techniques. The miniaturization of this advanced imaging technologies makes its clinical use possible and opens the door to a new way of understanding the procedure guided by molecular biology data. Conceptually, surgical precision and technological development are only two faces of the same coin: precision surgery. One of its fundamental principles is to put the patient at the center of healthcare to take into account individual and specific biological and clinical attributes [54-56]. To do so, it is necessary to differentiate the normal from the pathological, which relies on the development and analysis of large biobanks integrating the various multimodal data. Worthless to note that in the current technology and regulatory state-of-the-art, these technological shifts cannot yet replace the surgical decision, which still solely guided by the surgeon experience and dexterity.

Conclusion

Neurosurgery is considered as one of the oldest surgical disciplines. Since ancient times, it has been able to embrace major technological advances for the benefit of patients. Its progress has been made possible by the knowledge of anatomy, the development of radiology, microscopy, robotics and, recently, big data. Indeed, the development of the data-driven medicine has considerably modified clinical practice, in neuro-oncology in particular. This has allowed a better pathogenesis understanding and thus opened the way for innovative personalized therapies and disease management. The future of neurosurgery, and medical practice in general, lies in the smooth integration of multidisciplinary perspectives in both translational and clinical neurosurgery to achieve the promise of precision surgery.

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CHAPITRE II : LES GLIOBLASTOMES

1.1 Épidémiologie

Le glioblastome est la tumeur cérébrale maligne primaire la plus fréquente chez l'adulte¹. Au cours des 20 dernières années, une incidence croissante des glioblastomes a été observée en raison de l'augmentation de l'espérance de vie de la population générale et d'un meilleur accès à des outils de diagnostic plus précis tels que l'IRM²⁻⁴. Dans un article récent basé sur un registre prospectif des tumeurs du système nerveux central (SNC) aux États-Unis⁵, les glioblastomes représentent 54% des gliomes du cerveau avec une incidence annuelle de 3,19 pour 100 000. Le pic d'incidence se situe dans la 6^{ème} décennie pour les glioblastomes de type sauvage *IDH* et plus tôt, vers la 4^{ème}-5^{ème} décennie, pour les glioblastomes *IDH* mutés⁶. Malgré de nombreux essais thérapeutiques⁷⁻⁹ et des progrès dans la gestion de ces tumeurs, la survie médiane reste faible, environ 14 à 20 mois, avec un taux de survie à cinq ans de 5% dépendant de l'âge au moment du diagnostic, des caractéristiques moléculaires et de la gestion¹⁰. Nous passons ici en revue les données actuelles concernant la présentation clinique, le diagnostic et la prise en charge des patients présentant un glioblastome.

Les facteurs de risque d'apparition de glioblastomes sont encore inconnus et les études traitant de cette question manquent souvent de puissance. L'exposition aux rayonnements ionisants pour le traitement d'une tumeur maligne pendant la petite enfance (12 ans) est un facteur de risque très rare pour l'apparition d'un gliome. Le risque de développer une tumeur cérébrale après radiothérapie est accru si les radiations se produisent à un âge plus jeune (<5 ans) et semblent liées au volume et à la dose, mais sans seuil clairement établi¹¹. L'incidence accrue des glioblastomes^{12,13} soulève la question des facteurs de risque environnementaux. Une méta-analyse a établi un lien entre l'utilisation d'un téléphone portable et l'apparition de glioblastomes¹⁴. Cependant, ces résultats sont discordants et ont été contestés dans d'autres études¹⁵. Le rôle de l'exposition au tabagisme ou à des agents cancérigènes a été étudié sans

qu'il y ait d'association prouvée avec le glioblastome ^{16,17}. Dans de très rares cas (<1%), il existe une prédisposition génétique au développement de glioblastomes chez les patients atteints des syndromes de Lynch, de Turcot de type 1 ou de Li Fraumeni ¹⁸.

1.2 Présentation clinique

La présentation clinique dépend de la localisation et de la taille de la tumeur au moment du diagnostic. La présentation la plus courante au moment du diagnostic est le mal de tête et/ou la nausée dans le contexte d'une tumeur de grande taille ou d'un œdème important. Les symptômes liés à l'hypertension intracrânienne représentent 30 % des signes cliniques, suivis du déficit moteur (20 %), de la perte de poids et de l'état du corps (17 %), de la confusion (15 %) et du déficit visuel ou de la parole (13 %) ¹⁹. L'épilepsie n'est pas rare (15 à 20 %) et est facilement contrôlée par des thérapies anticonvulsives. L'épilepsie est associée à de meilleurs résultats, probablement en raison de la localisation corticale des glioblastomes présentant une crise ²⁰. Ces symptômes sont souvent associés et conduisent à un diagnostic dans les semaines ou les mois qui suivent leur apparition. Les glioblastomes sont localisés dans l'espace supratentorial dans plus de 85% des cas, ce qui explique les symptômes mentionnés ci-dessus, et très rarement dans le tronc cérébral et la moelle épinière (<5% chacun) ou dans le cervelet (<3%). Jusqu'à 25 % des glioblastomes se produisent dans le lobe frontal ²¹, le plus grand lobe cérébral, et sont donc responsables de troubles de l'humeur et de l'exécution chez 15 % des patients ²².

1.3 Diagnostic radiologique

Les caractéristiques typiques de l'imagerie par résonance magnétique (IRM) des glioblastomes sont bien connues ^{21,23,24}. Elles consistent en des lésions intraparenchymateuses infiltrantes et hétérogènes qui surgissent et se propagent à partir de la substance blanche. L'atteinte du corps calleux est fréquente. Les glioblastomes sont mal circonscrits et présentent

une augmentation du contraste à leur marge en signe de perturbation de la barrière hématoencéphalique (Figure 1A et B). Le centre de la lésion est hypointense sur les images pondérées en T2 en raison de la nécrose et la lésion est entourée d'un œdème cérébral qui apparaît hyperintense sur les images pondérées en T2 et les images FLAIR (fluid-attenuated inversion recovery). L'analyse morphologique présente des limites, car ces lésions peuvent avoir une présentation très hétérogène sur les séquences conventionnelles (Figure 2). Des techniques d'IRM multimodales plus récentes, telles que les séquences de diffusion/perfusion, ont fourni des informations supplémentaires sur les caractéristiques de la lésion elle-même et ont permis un diagnostic plus précis (Figure 3). L'imagerie pondérée par perfusion (PWI) révèle une augmentation du flux sanguin cérébral correspondant à la néoangiogénèse et à la perturbation de la barrière hématoencéphalique. La spectroscopie IRM (Figure 4) dans les glioblastomes est caractérisée par une augmentation spécifique des rapports choline/N-acétylaspartate et choline/créatinine. En outre, un pic élevé de lactate et de lipides ainsi qu'un pic réduit de myoinositol sont des données fiables pour le diagnostic du glioblastome²⁵ et sont utiles pour distinguer les glioblastomes des métastases, des lymphomes et des abcès cérébraux. Comme le suggèrent certains auteurs, ces explorations sont également utiles pour évaluer le degré d'envahissement péri tumoral et peuvent servir de guide pour la biopsie²⁶ et pour suivre l'évolution de la maladie après le traitement.

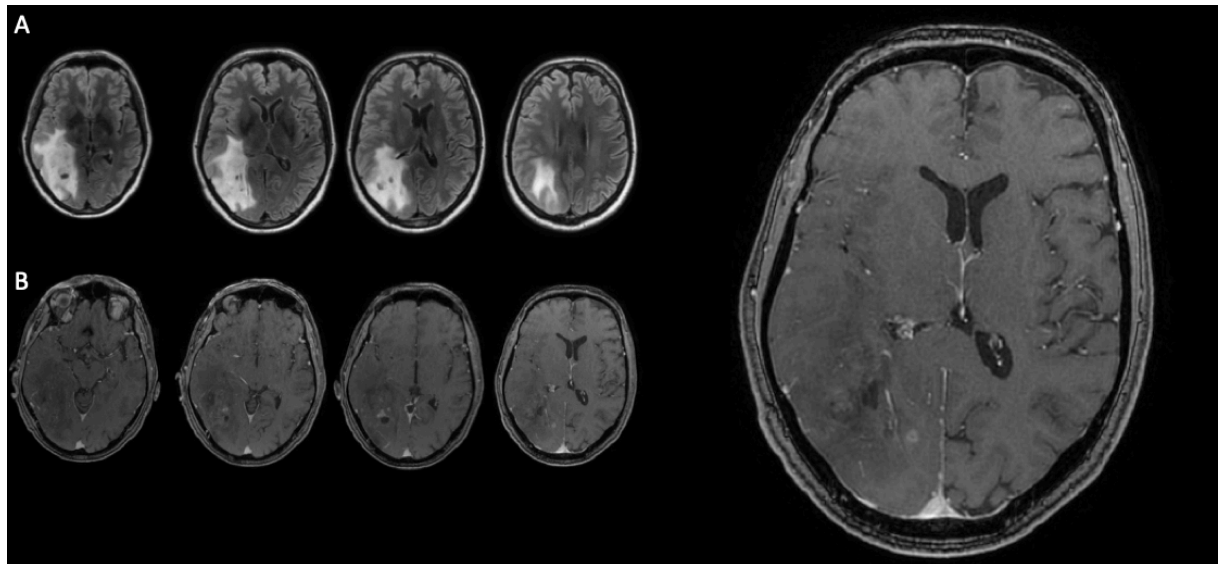


Figure 1. IRM cérébrale en coupes axiales en séquence FLAIR (A) puis en pondération T1 avec injection de gadolinium (B) chez un patient présentant un GBM du carrefour droit.

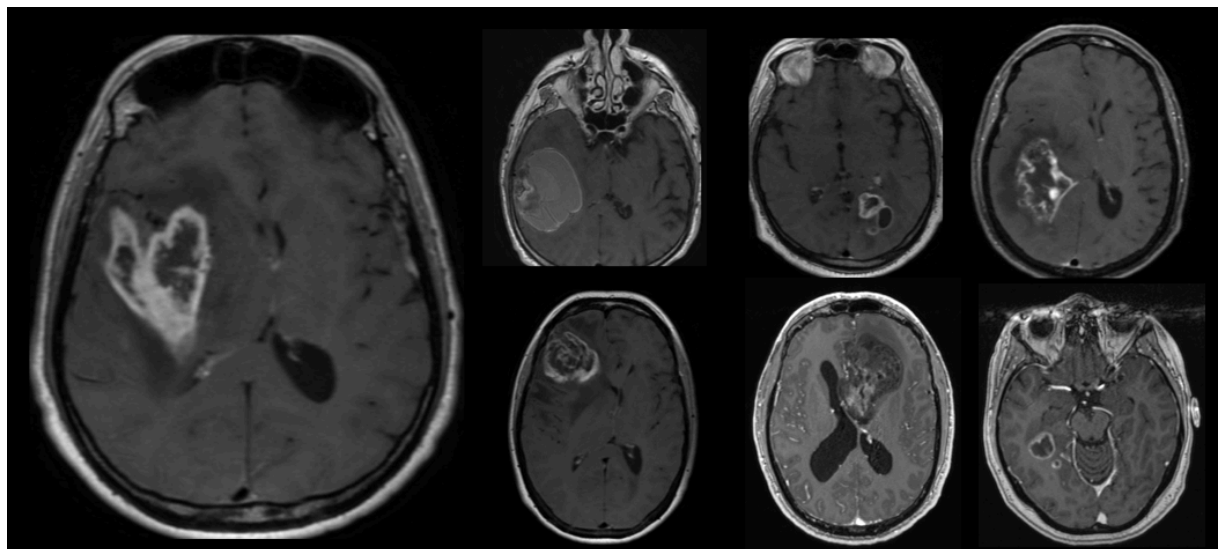


Figure 2. IRM cérébrale en coupes axiales en pondération T1 avec injection de gadolinium chez différents patients présentant un GBM pour illustrer leur hétérogénéité morphologique.

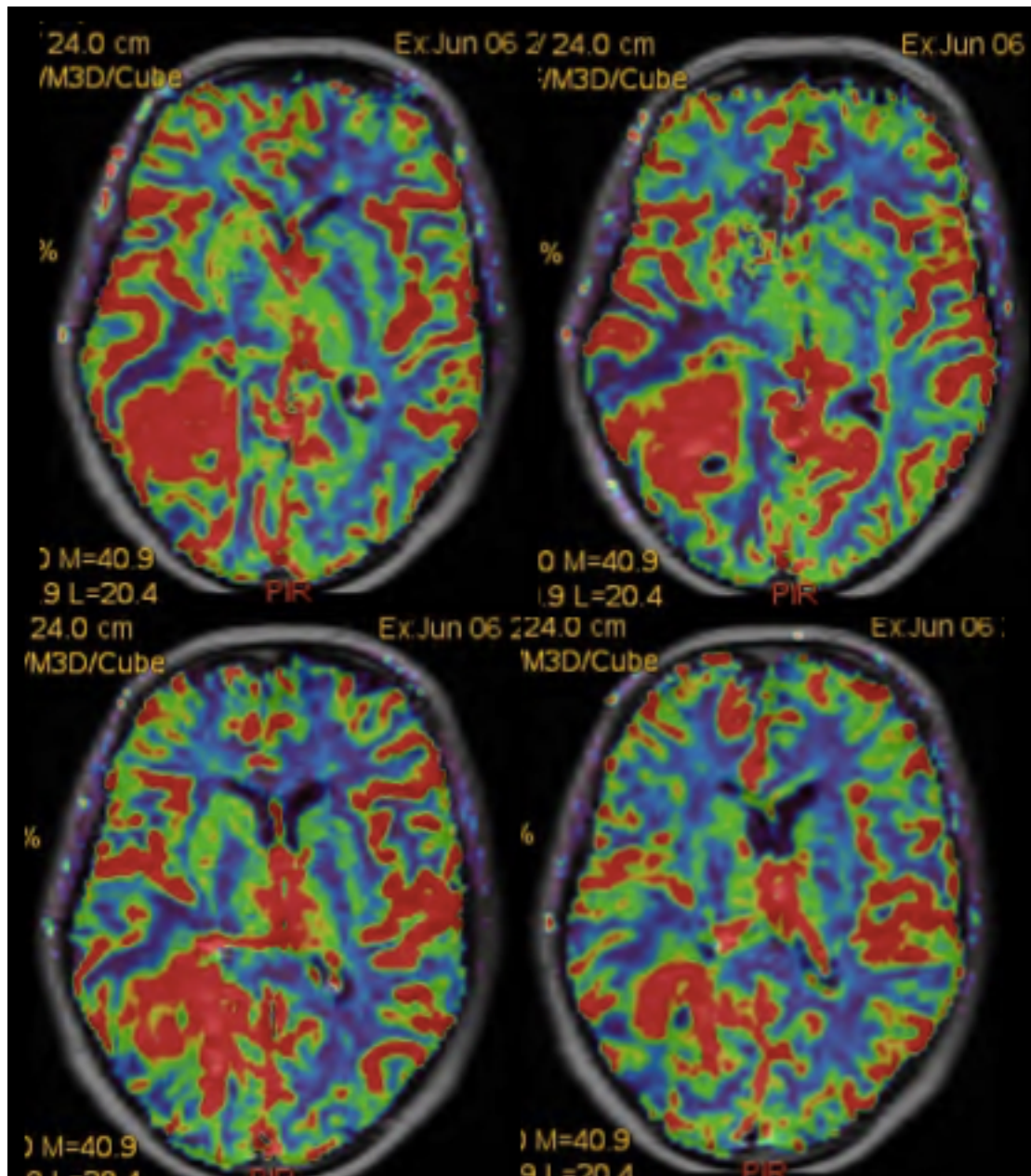


Figure 3. IRM cérébrale en coupes axiales en séquence de perfusion chez un patient présentant un GBM du carrefour droit. A noter l'augmentation du volume sanguin cérébral au sein de la lésion.

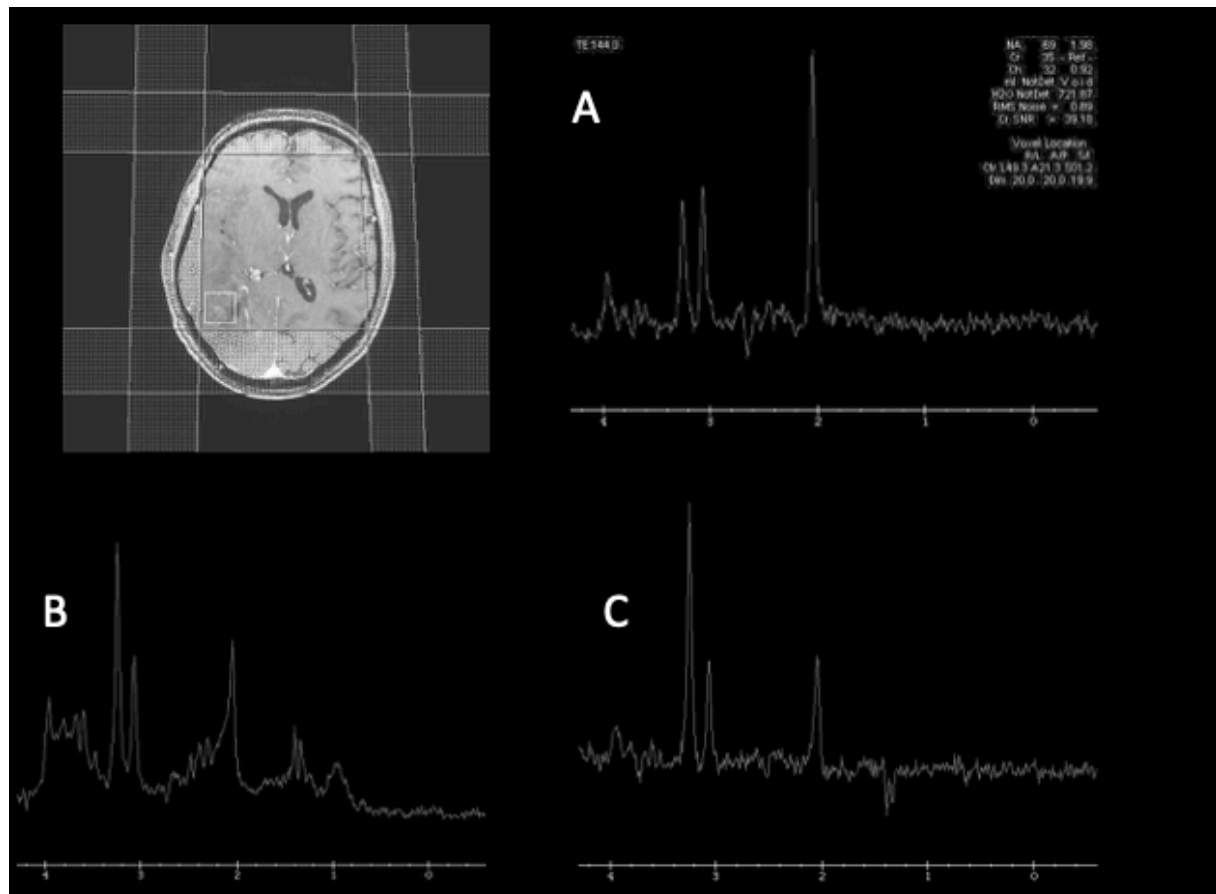


Figure 4 : IRM cérébrale avec séquences de spectrométrie révélant les pics normaux du cerveau sain (4A), la présence de lactates et de lipides au sein de la lésion témoin de la nécrose (4B) ainsi qu'une inversion du rapport choline/NAA (4C), caractéristique d'une lésion gliale de haut grade.

1.4 Histologie et biologie moléculaire

Le diagnostic du glioblastome se fait facilement sur la base de résections chirurgicales ou de prélèvements de biopsie. Les glioblastomes sont des gliomes de haut grade, de grade IV selon la classification de l'Organisation mondiale de la santé (OMS) des tumeurs du système nerveux central ²⁷. Les glioblastomes sont composés de cellules tumorales peu différenciées, souvent pléomorphes, avec une différenciation astrocytaire prédominante ²⁸. Les

caractéristiques histopathologiques comprennent l'atypie nucléaire, le pléomorphisme cellulaire, une forte activité mitotique, la thrombose vasculaire, la prolifération microvasculaire et la nécrose (Figure 5).

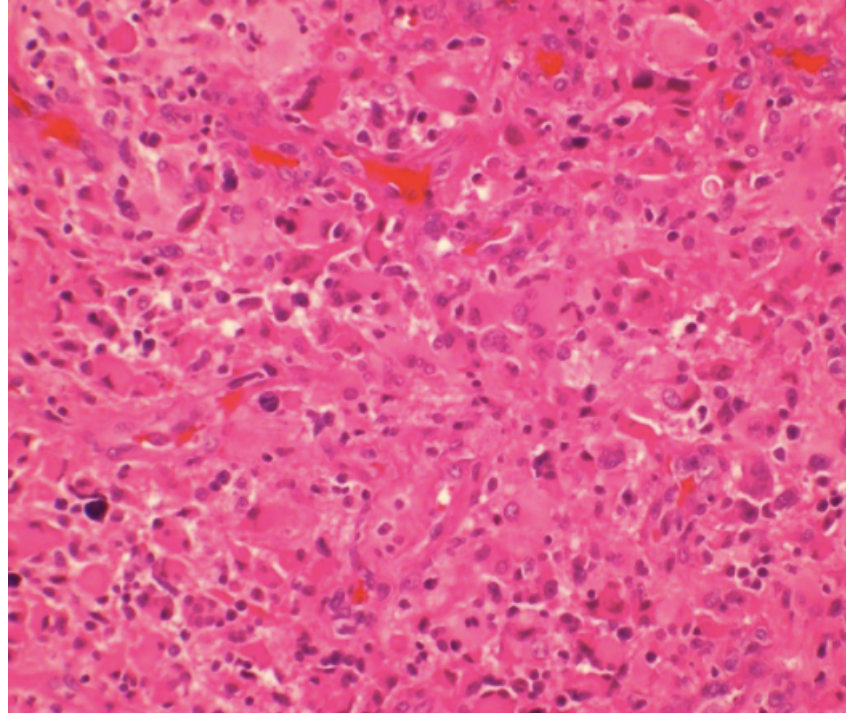


Figure 5. Vue microscopique d'un glioblastome *IDH* muté.

L'immunohistochimie et les techniques moléculaires complémentaires sont désormais couramment utilisées à des fins de diagnostic et de pronostic (Figure 6). Ces études complémentaires consistent en la recherche de l'isocitrate déshydrogénase (*IDH*) et du statut *ATRX* 34, la codéletion du gène 1p/19q ²⁹, l'hyperméthylation du promoteur du gène de la O6-méthylguanine-ADN méthyltransférase (*MGMT*) ³⁰, les mutations du gène *p53* ³¹, les mutations du *TERT* et les altérations du gène du récepteur du facteur de croissance épidermique (*EGFR*)

³².

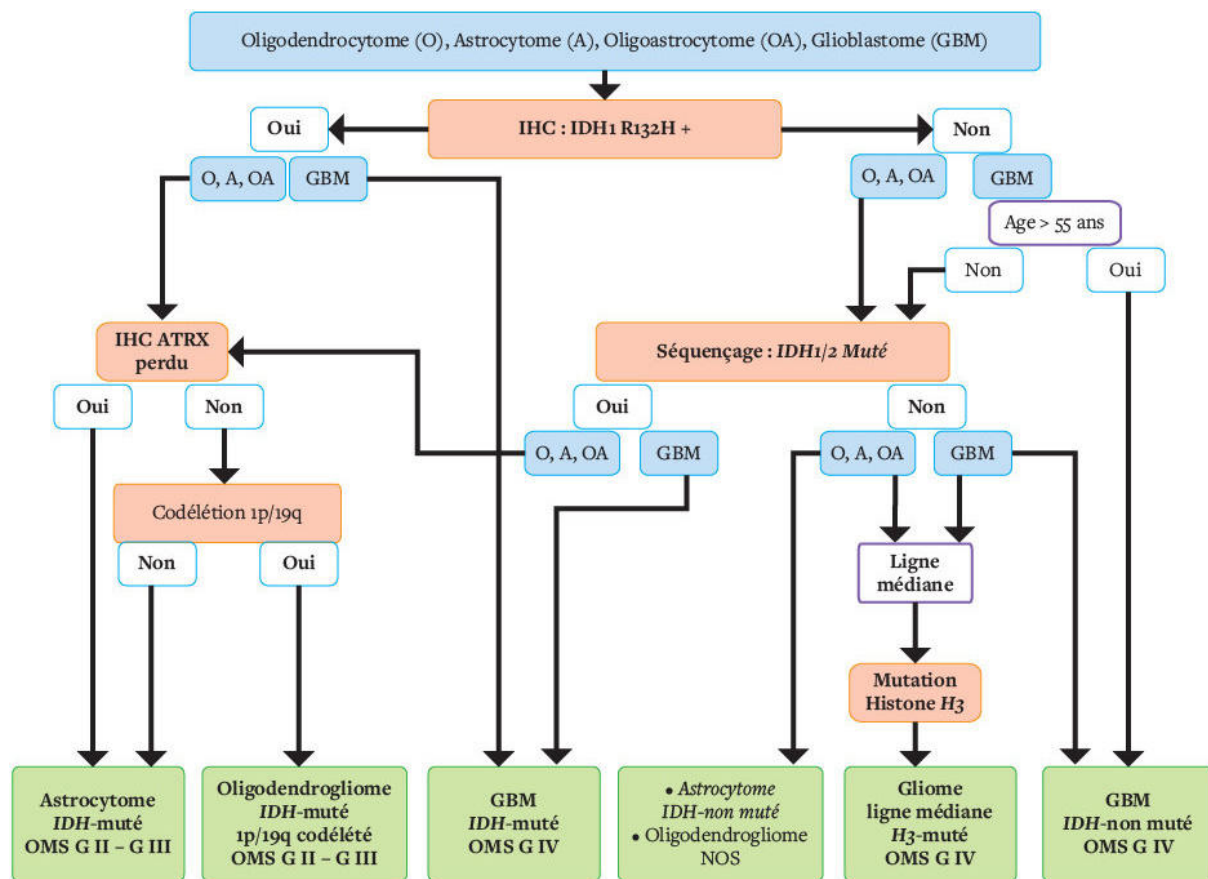


Figure 6. Schéma représentant l'arbre diagnostique de la classification OMS 2016

d'après Louis et al. ²⁷.

1.5 Prise en charge chirurgicale

Dans la mesure du possible, la première étape consiste en une résection chirurgicale macroscopique complète. Les données de la littérature suggèrent qu'une résection > 90 % de l'augmentation du contraste de la lésion chez les patients sans comorbidité améliore le résultat du patient au moment du diagnostic et de la récurrence ^{33,34}. La résection chirurgicale est généralement proposée aux patients de moins de 70 ans en bon état (échelle de Karnofsky >70) ³⁵ et ayant une tumeur accessible à l'ablation complète. Sinon, un débulquage chirurgical ou une biopsie stéréotaxique est pratiqué pour confirmer le diagnostic avant le traitement adjuvant ³⁶. En raison de l'importance d'une résection complète sur la survie, des progrès ont été réalisés

dans les techniques chirurgicales ^{37,38} telles que la craniotomie en état de veille ou le neuromonitoring ³⁹ pour améliorer la qualité de la résection et prévenir les déficits ultérieurs. En outre, la chirurgie guidée par fluorescence (Figure 7) a été développée pour guider la résection avec de meilleurs résultats sur la résection et la survie sans progression ^{40,41}. Plus récemment, les dispositifs de résection ont évolué avec l'utilisation de la thérapie thermique interstitielle au laser (LITT) ⁴², qui permet une approche percutanée moins invasive grâce à l'insertion d'une fibre optique. La lésion thermique générée induit une nécrose tumorale ⁴³. Une autre nouveauté dans la résection chirurgicale des tumeurs cérébrales est la surveillance peropératoire des métabolites de la tumeur par spectrométrie de masse. Le contenu cellulaire est analysé, ce qui permet une délimitation moléculaire précise des marges de la tumeur et donc une résection optimale de la tumeur ^{44,45}.

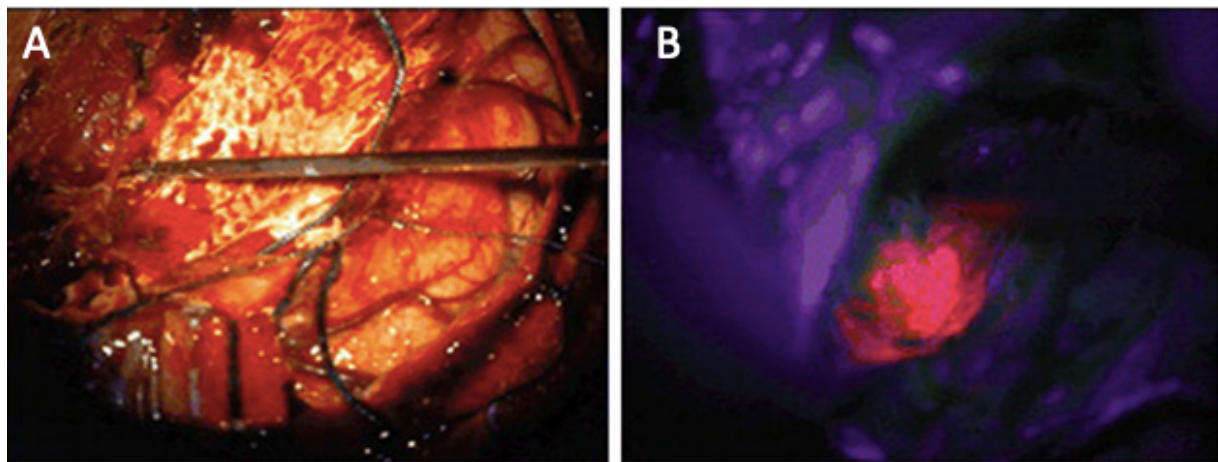


Figure 7. Visualisation peropératoire d'une exérèse chirurgicale ; vue microscopique (7A) et sous fluorescence au 5-ALA (7B).

1.6 Thérapeutiques médicales

La norme de soins pour les patients âgés de moins de 70 ans repose sur la radiothérapie (RT) avec témozolomide concomitant. Il a été prouvé que ce protocole améliore la survie

globale dans un vaste essai randomisé de phase III ⁴⁶. La radiothérapie est administrée pendant une période de six semaines avec une dose totale de 60 grays (Figure 8). Le témozolomide est un agent alkylant administré quotidiennement pendant la RT et ensuite, pendant six cycles de cinq jours consécutifs par mois, un mois après la fin de la RT. L'absence de promoteur MGMT ⁴⁷ hyperméthylé est un facteur de pronostic de l'efficacité du témozolomide. La RT seule peut donc être proposée. Le protocole de traitement proposé par Stupp ⁴⁶ permet d'augmenter le taux de survie moyen de 12,1 mois en utilisant la RT seule à 14,6 mois, et le taux de survie à deux ans de 8 à 26% avec le témozolomide concomitant.

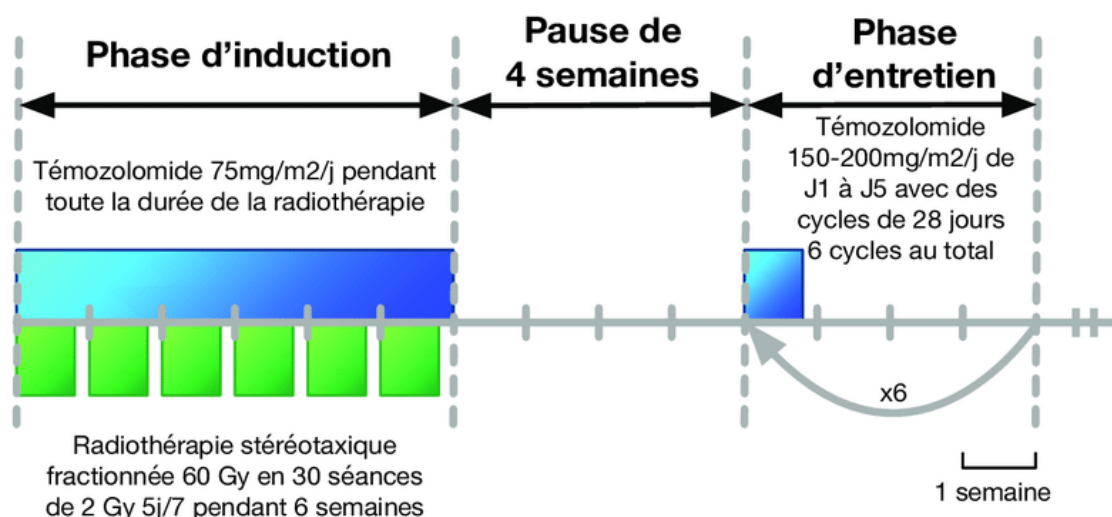


Figure 8. Illustration du traitement d'un patient atteint d'un glioblastome selon le protocole STUPP. D'après Lemée et al ⁴⁸.

Cet essai contrôlé randomisé n'a pas inclus de patients de plus de 70 ans. Dans cette dernière population, il n'existe pas de norme de soins claire et le traitement dépend de l'état général du patient. La RT seule (54 grays) a été proposée avec un impact positif sur la survie (29,1 semaines contre 16,9 semaines chez les patients recevant uniquement des soins de soutien) et sans altération de la qualité de vie ¹⁰. Ces résultats ont été validés chez des patients,

dont le statut de performance Karnofsky ⁴⁹ (KPS) > 60. Plus récemment, certains auteurs ont montré que la RT de courte durée plus le témozolomide était associée à une survie plus longue (9,3 contre 7,6 mois) chez les patients plus âgés (> 65 ans). Chez les patients en mauvaise santé générale, des soins de soutien peuvent être proposés pour préserver la qualité de vie avec une durée d'hospitalisation la plus courte possible ⁴⁹.

L'évolution de la maladie est évaluée par IRM du cerveau tous les 2 à 3 mois selon l'évaluation de la réponse en neuro-oncologie (RANO) selon les critères suivants (Figure 9) ⁵⁰.

REPONSE	CRITERES CLINIQUES/Corticothérapie	CRITERES IRM
Réponse complète (RC)	Amélioration ou stabilité clinique Sevrage complet des corticoïdes	Disparition complète des lésions mesurables et non mesurables prenant le contraste (T1 Gado) pendant >4 semaines Diminution ou stabilité du signal T2/FLAIR Pas de nouvelle lésion
Réponse partielle (RP)	Amélioration ou stabilité clinique Dose stable ou diminuée des corticoïdes	Gd+ $\searrow$ $\geq$ 50 % de la somme des produits des diamètres perpendiculaires des lésions prenant le contraste au T1 gado Diminution ou stabilité du T2/FLAIR Pas de nouvelle lésion
Stabilité	Absence de réponse complète, partielle ou de progression	T2/FLAIR diminution ou stabilité sous une dose stable ou diminuée de corticoïdes.
Progression	Détérioration clinique non attribuable à une autre cause Augmentation de la dose des	Gd+ $\nearrow$ $\geq$ 25 % de la somme des produits des diamètres perpendiculaires des lésions T2/FLAIR $\nearrow$ non lié à une comorbidité

Figure 9. Critères IRM d'évaluation de la réponse en neuro-oncologie. D'après Wen *et al.* ⁵⁰.

Au moment de la récurrence, il n'y a pas de norme de soins. Les principaux déterminants des propositions de traitement sont l'état général du patient et les traitements administrés précédemment. Une deuxième intervention chirurgicale peut être proposée chez les jeunes patients dont le KPS est préservé. Il a été démontré que cette stratégie est associée à une survie plus longue chez certains patients ⁵¹ (14 mois contre 22 mois de survie globale chez les patients ayant subi une seconde chirurgie au moment de la récurrence). Quatre-vingts pour cent des

patients n'ont pas eu besoin de réadaptation après une deuxième chirurgie. Chez des patients sélectionnés, l'utilisation de plaquettes intracavitaires de carmustine (BCNU) a été proposée lors de la première chirurgie ou en cas de récurrence ^{52,53}. L'efficacité et les effets indésirables de cette thérapie restent un sujet de débat ⁵⁴. En l'absence d'une résection possible sûre, une chimiothérapie de deuxième ligne peut être proposée, comprenant des nitrosourées, du témazolomide ou des médicaments antiangiogéniques tels que le bevacizumab, mais sans résultats clairs en termes de bénéfice à ce jour et de mauvais résultats avec un taux de survie global moyen de 6 mois à partir de la récurrence ⁵⁵.

1.7 Nouvelles thérapies et perspectives

En raison de l'issue défavorable chez les patients atteints de glioblastome et de la fréquence élevée de cette maladie, des thérapies innovantes sont testées dans différents essais contrôlés randomisés ⁵⁶⁻⁵⁸. Avec le développement d'une meilleure compréhension des voies moléculaires déclenchant la croissance des glioblastomes, l'approche traditionnelle de la thérapie antitumorale est progressivement complétée par une approche plus personnalisée ^{59,60}. Les calendriers de traitement ont été repensés, ainsi que les médicaments eux-mêmes. Deux inconvénients majeurs consistent en la difficulté pour la plupart des médicaments de passer à travers la barrière hématoencéphalique (BHE) et de cibler les cellules tumorales en raison de la présence de vaisseaux anormaux et de nécrose, ce qui empêche les médicaments d'être délivrés à une concentration appropriée. Certaines techniques émergentes ont été proposées pour améliorer la distribution de la thérapie antitumorale, notamment la conjugaison de médicaments avec des protéines pour faciliter le mouvement à travers la BHE et cibler spécifiquement la tumeur ⁶¹, l'utilisation de la délivrance par convection améliorée consistant en l'administration directe à l'intérieur de la tumeur via un cathéter ⁶² et l'utilisation de nanoparticules. L'augmentation de la perméabilité de la BHE pendant l'administration de la chimiothérapie par

des ultrasons focalisés est également testée ⁶³. Entre-temps, les approches d'immunothérapie sont connues depuis longtemps, avec des résultats prometteurs dans de nombreux cancers tels que le mélanome, mais décevants chez les patients atteints de gliomes ⁶⁴. La vaccination contre le cancer a récemment été proposée en référence à l'activation d'une réponse immunitaire contre les antigènes tumoraux. Ces nouvelles technologies ont été appliquées pour le traitement des glioblastomes avec différents vecteurs ⁶⁴⁻⁶⁶. Deux modalités ont été testées : les vaccins peptidiques ciblant les protéines EGFR, IDH1 ou de choc thermique, et les vaccins à base de cellules consistant en l'injection de cellules modifiées *ex vivo*, principalement des cellules dendritiques. Malgré des résultats encourageants dans les modèles animaux en termes de contrôle de la maladie, la vaccination contre le cancer dans les glioblastomes n'a pas encore prouvé son efficacité sur la survie globale dans les études de phase III. Stupp *et al.* ont récemment mis au point une nouvelle modalité thérapeutique dans le traitement des glioblastomes récurrents, consistant à délivrer localement des champs électriques de faible intensité par l'intermédiaire d'un transducteur non invasif ⁵⁷. Le dispositif (NovoTTF-100A) a été testé dans une étude de phase III et randomisé avec une chimiothérapie active comme bras alternatif. La survie globale a été similaire dans les deux bras (6 mois) avec moins d'effets indésirables dans le groupe NovoTTF-100A et une meilleure qualité de vie.

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L'article II présente un état des lieux des connaissances sur le glioblastome. Les différentes données épidémiologiques et physiopathologiques y sont décrites de même que la présentation clinique et les caractéristiques radiologiques. Une attention particulière a été donnée aux nouvelles avancées thérapeutiques et à l'impact des sciences omiques dans l'appréhension de cette pathologie.

Article II

Gilard V, Tebani A, Dabaj I, Laquerrière A, Fontanilles M, Derrey S, et al. Diagnosis and Management of Glioblastoma: A Comprehensive Perspective. J Pers Med. 2021;11(4):258.

Review

Diagnosis and Management of Glioblastoma: A Comprehensive Perspective

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Abstract: Glioblastoma is the most common malignant brain tumor in adults. The current management relies on surgical resection and adjuvant radiotherapy and chemotherapy. Despite advances in our understanding of glioblastoma onset, we are still faced with an increased incidence, an altered quality of life and a poor prognosis, its relapse and a median overall survival of 15 months. For the past few years, the understanding of glioblastoma physiopathology has experienced an exponential acceleration and yielded significant insights and new treatments perspectives. In this review, through an original R-based literature analysis, we summarize the clinical presentation, current standards of care and outcomes in patients diagnosed with glioblastoma. We also present the recent advances and perspectives regarding pathophysiological bases as well as new therapeutic approaches such as cancer vaccination and personalized treatments.

Keywords: glioblastoma; molecular pathology; omics; pathogenesis; personalized therapies

1. Introduction

Glioblastoma is the most frequent primary malignant brain tumor in adults [1]. Over the last 20 years, a growing incidence of glioblastomas has been observed due to an increase in general population life expectancy and a better access to more accurate diagnostic tools such as MRI [2–4]. In a recent article based on a prospective registry of central nervous system (CNS) tumors in the US [5], glioblastoma accounts for 54% of brain gliomas with an annual incidence of 3.19 per 100,000. The peak of incidence occurs in the 6th decade for glioblastomas isocitrate dehydrogenase (IDH) wild type and earlier, around the 4th–5th decade, for glioblastomas IDH mutant [6]. Despite many therapeutic trials [7–9] and advances in the management of these tumors, the median survival remains poor, approximately 14 to 20 months, with a 5% five-year survival rate depending on age at diagnosis, molecular characteristics and management [10]. Due to the poor outcomes of this pathology, there is a need for a better understanding of the pathogenesis and metabolic pathways of glioblastomas. For a few years, advances in the different areas brought new insights and new therapeutic perspectives. Precision medicine, through the surge of omics technologies, offers new approaches for various oncology diseases [11,12]. Its main goal is to set a personalized perspective of the disease, taking into account individual variability

of the patient along with his environment and predisposition. This approach requires exhaustive collection of glioblastomas data. This work proposed an exhaustive semi-automated review of literature regarding clinical presentation, diagnosis and management of patients presenting with glioblastoma. Moreover, we also emphasize the novelties and perspectives concerning this disease within the precision medicine era.

2. Materials and Methods

2.1. Literature Analysis

In this study, we performed a programmatic literature search for a more efficient and reproducible review process using the Adjutant R package [13]. We searched for articles related to glioblastoma, glioma physiopathology, diagnosis and treatments that were published between January 1990 and February 2020. We used only one query (glioblastoma). The resulting document corpus included articles metadata: PubMed IDs, year of publication, authors, article titles, article abstracts and any associated Medical Subject Heading (MeSH) terms. Titles and abstracts were decomposed into single terms, stemmed and filtered by Adjutant package. The term frequency inversed document frequency metrics for each term and created a sparse Document Term Matrix (DTM) for further analysis. t-Distributed Stochastic Neighbor Embedding (t-SNE) and hdbscan algorithms were used to perform unsupervised clustering using DTM data. The coordinates generated by t-SNE were used in the hdbscan algorithm to derive the topic clusters. Each cluster was then assigned a topic by using the five most frequent terms within the cluster.

2.2. Manual Curation: Inclusion and Exclusion Criteria

Following the topic clustering step, we validated our clusters using external manual curation assessing the correspondence between articles and cluster topics. Each sampled article was examined and either considered acceptable for further analysis or rejected. Inclusion criteria were topic relevance assessed by a neurosurgeon specialized in neuro-oncology; article in English; human data; original research or clinical trial. We further refined the corpus and cluster naming. Supplementary Table S1 contains a list of all the articles along with their corresponding cluster.

2.3. Data Analysis

All the data analysis and visualization have been done using the R software [14].

3. Results

3.1. Literature Mining and Topic Clusters

The first-round analysis generated a document corpus of 2799 articles related to glioblastoma published over the past 30 years (Supplementary Table S1). Using article titles and abstracts, we derived topic clusters in an unsupervised manner, and classified articles according to their clustering status. Articles that never formed part of a cluster were removed from further analysis, leaving 1314 documents that formed 27 clusters. Cluster topics were assigned using the five most frequent terms within the cluster along with a manual curation of the included articles (Figure 1). The full list of articles and related clusters are presented in Supplementary Table S1.

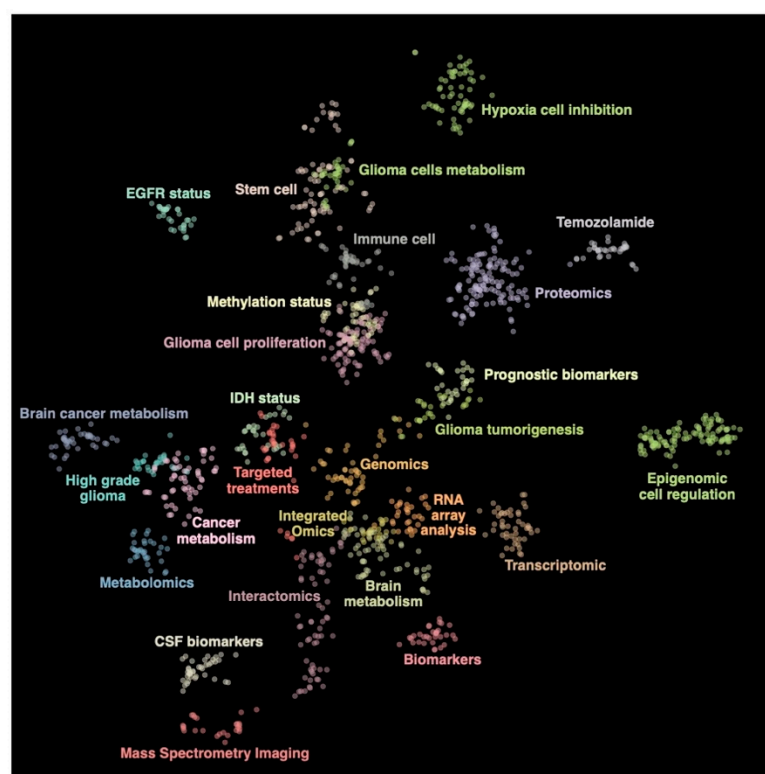


Figure 1. Topic representation of the included literature related to glioblastoma. The figure highlights twenty-seven clusters.

3.2. Pathogenesis of Glioblastoma

3.2.1. Risk Factors for High Grade Gliomas

Risk factors for glioblastoma onset are still unknown and studies dealing with this question frequently lack power. The exposure to ionizing radiation for the treatment of malignancy during infancy [15] is a very rare risk factor for the onset of glioma. The risk of developing a brain tumor after radiotherapy is increased if the radiation occurs at a younger age (<5 years) and seems volume- and dose-related but with no clear reported threshold [16,17]. The increased incidence of glioblastoma [18,19] raises the issue of environmental risk factors. The association between the use of mobile phone and the occurrence of glioblastoma has been reported in a meta-analysis [20]. However, these results are discordant and have been challenged in other studies [21,22]. The role of exposure to smoking or carcinogenic agents has been studied with no proven association to glioblastoma [23,24]. In very few cases (<1%), there is a genetic predisposition to glioblastoma development in patients with Lynch, Turcot type 1 or Li Fraumeni syndromes [25].

3.2.2. Clinical Presentation

Clinical presentation depends on the tumor location and size at diagnosis. The most common presentation at diagnosis is a headache and/or nausea in a context of a large tumor or significant edema. Symptoms related to intracranial hypertension represent 30% of clinical signs followed by motor deficit (20%), loss of body weight and condition (17%), confusion (15%) and visual or speech deficit (13%) [26]. Epilepsy is not uncommon (15–20%) and easily controlled by anticonvulsant therapies. Epilepsy is associated with better outcomes probably due to the cortical location of glioblastomas presenting with seizures [27]. These symptoms are often associated and lead to a diagnosis in the weeks or months following their onset. An overview of clinical presentation at diagnosis is summarized in Figure 2. Glioblastomas are located in the supra-tentorial space in more than 85% of the cases explaining the above-mentioned symptoms and very infrequently in the brainstem and spinal cord (<5% each) or in the cerebellum (<3%). Up to 25% of

glioblastomas occur in the frontal lobe [28], the largest cerebral lobe, and therefore are responsible for mood and executive disabilities in 15% of patients [26].

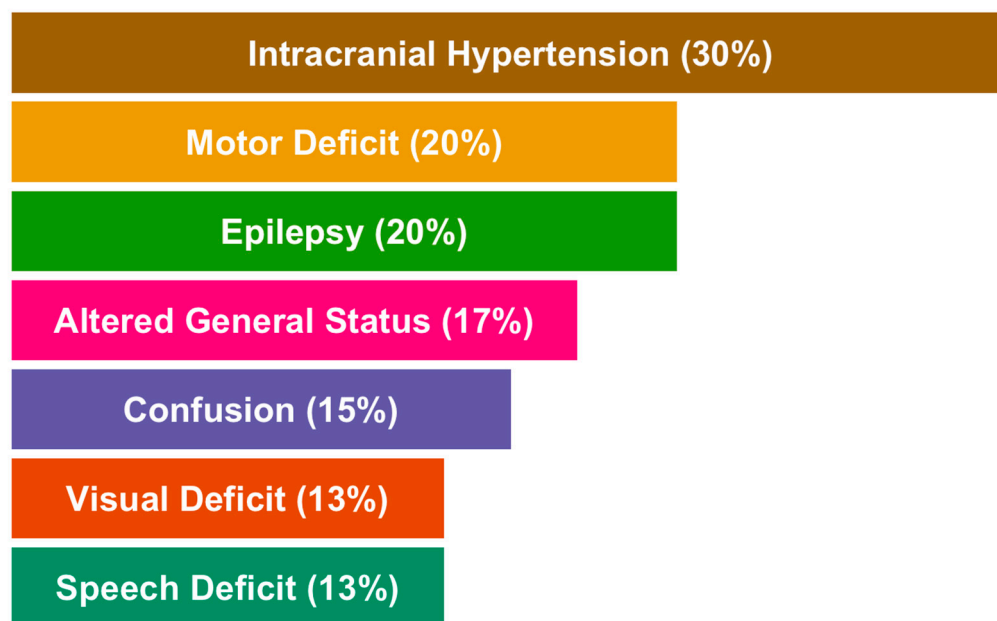


Figure 2. Overview of the main reported clinical features in glioblastomas.

3.2.3. Radiological Characteristics

Typical magnetic resonance imaging (MRI) characteristics of glioblastomas are well known [2,28–31]. They consist in infiltrative, heterogeneous intraparenchymal lesions which arise and spread from the white matter. Corpus callosum involvement is common. Glioblastomas are poorly circumscribed and display contrast enhancement at their margin as a sign of blood–brain barrier disruption. The center of the lesion is hypointense on T1-weighted images due to necrosis and the lesion is surrounded by brain edema which appears hyperintense on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images. Diffusion-weighted images and apparent diffusion coefficients can provide valuable information concerning the suspected degree of malignancy of astrocytic tumors. More recent multimodal MRI techniques such as diffusion/perfusion sequences have provided supplementary information about the characteristics of the lesion itself and have enabled a more accurate diagnosis. Perfusion weighted imaging (PWI) reveals an increase in the cerebral blood flow corresponding to neoangiogenesis and blood–brain barrier disruption. MR spectroscopy in glioblastomas is characterized by an increase in choline/N-acetylaspartate and choline/creatinine ratios. Nevertheless, these characteristics reflect cellular activity and are not, when isolated, specific enough to diagnose glioblastoma. In addition, an elevated peak of lactate and lipids as well as a decreased peak of myoinositol are reliable data for the diagnosis of glioblastoma [30,32] and are helpful to discriminate glioblastomas from metastasis, lymphoma and brain abscess. As suggested by some authors, these explorations are also useful to assess the peritumoral degree of invasion and may be used as a guidance for biopsy [33,34] and to monitor disease evolution after treatment.

3.2.4. Basic and Molecular Pathology

The diagnosis of glioblastoma is easily made on surgical resections or biopsy samples. Glioblastomas are high grade gliomas, grade IV according to the World Health Organization (WHO) classification of the central nervous system tumors [35,36]. Glioblastoma is composed of poorly differentiated, often pleomorphic tumor cells with predominant astrocytic differentiation [37]. Histopathological features include nuclear atypia, cellular pleomorphism, high mitotic activity, vascular thrombosis, microvascular proliferation and necrosis [28]. Since the 2016 WHO classification of the central nervous system tumors [36]

and advances in immunohistochemistry, glioblastomas are now defined by their Isocitrate dehydrogenase (*IDH*) status dividing this entity into glioblastoma *IDH*-mutant or *IDH*-wild type. The latter is, by far, the most frequent accounting for 90% of cases and is predominant in patients over 55 years of age. Glioblastomas *IDH*-mutant (10% of cases) is predominant in younger patients, often results from the transformation of a lower-grade glioma and is associated with longer median survival [38]. Complementary immunohistochemistry and molecular techniques are now routinely used for diagnostics and prognostic purposes. (*IDH*) alterations as well as hypermethylation of the O⁶-methylguanine-DNA methyltransferase (*MGMT*) gene promoter [39] are predictive of longer survival contrary to Telomerase Reverse Transcriptase promoter (*TERT*) variants and chromosome 10 deletion which are poorer prognostic factors. Gain of function variants in the *P53* gene [40] and epidermal growth factor receptor (*EGFR*) gene alterations [41] still are of uncertain prognostic significance. *MGMT* is involved in DNA repair and its expression is associated with drug resistance including temozolamide, the most frequent first-line chemotherapy used in a context of glioblastoma. As a consequence, hypermethylation of the *MGMT* gene promoter is a valuable predictive marker and is associated with longer overall survival and longer progression-free survival [42]. More recently, it has been proven [43] that variants in the tumor suppressor gene Phosphatase and tensin homolog (*PTEN*) or the loss of chromosome 10 is involved in the glioblastoma oncogenesis.

3.3. Current Management

3.3.1. Surgical Procedure

Whenever possible, the first step consists in complete macroscopic surgical resection. Literature data suggest that a resection > 90% of the contrast enhancement of the lesion in patients with no comorbidities improves the patient outcome at the time of diagnosis and recurrence [44–46]. Surgical resection is usually proposed to patients under the age of 70 in good condition (Karnofsky scale > 70) [46] and a tumor accessible to complete removal. Otherwise, surgical debulking or stereotactic biopsy is performed to confirm the diagnosis before adjuvant therapy [47,48]. Due to the importance of a complete resection on survival, advances in surgical techniques have been made [49,50] such as awake craniotomy or neuromonitoring [51] to improve the resection quality and prevent subsequent deficits. Furthermore, fluorescence-guided surgery has been developed to guide the resection with better outcomes on resection and progression-free survival [52–54]. More recently, resection devices have evolved with the use of Laser-Interstitial Thermal Therapy (LITT) [55], which provides a less invasive, percutaneous approach through the insertion of an optical fiber. The generated thermal injury induces tumor necrosis [55–57]. Another novelty in the surgical resection of brain tumors is mass spectrometry-based intraoperative monitoring of tumor metabolites. Cell content is analyzed allowing for an accurate and molecular delineation of tumor margins and thus for an optimal tumor resection [58,59]. A summary of clinical presentations, radiology, biology and treatments together with their interactions is illustrated in Figure 3.

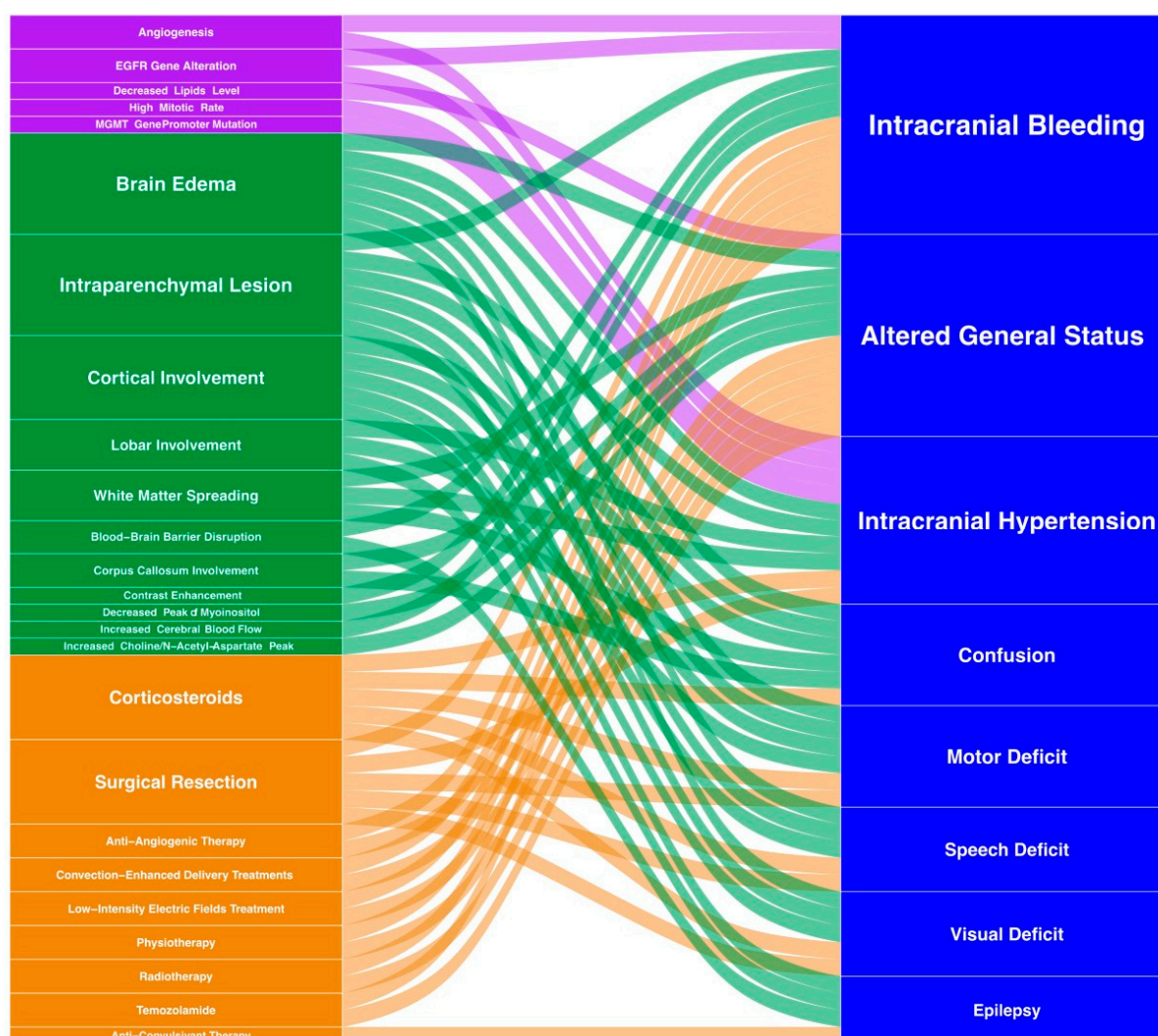


Figure 3. Integrative visualization summary of main clinical symptoms and signs and their interactions with radiology, biology and treatment features. The box and annotation sizes are proportional to the item frequency. Clinical: blue, Biology: purple, Radiology: green, Treatment: orange.

3.3.2. Medical Treatment of Glioblastomas

The standard of care for patients aged less than 70 relies on radiotherapy (RT) and adjuvant temozolomide. This protocol improved the overall survival in a large randomized phase III trial [60]. Radiotherapy is given for a six-week period with a total dose of 60 grays. Temozolomide is an alkylating agent administered daily during the RT and then, for six cycles of five consecutive days per month, one month after the end of the RT. The absence of hypermethylated *MGMT* promoter [61] is a negative prognostic and predictive factor of temozolomide efficiency. The treatment protocol proposed by Stupp allows for increasing the average survival rate from 12.1 months using RT alone to 14.6 months, and the two-year survival rate from 8 to 26% with concomitant temozolomide [60]. This randomized controlled trial did not include patients older than 70. In the latter population, the standard of care is based on hypofractionated radiotherapy and temozolomide [62] whenever feasible, but the treatment depends on the patient's general condition. RT alone (54 grays) has been proposed with a positive impact on survival (29.1 weeks compared to 16.9 weeks in patients with supportive care alone) and with no alteration of the quality of life [63]. These results have been validated in patients with a Karnofsky [64] performance status (KPS) > 60. More recently, some authors have shown that short-course RT plus temozolomide was associated with longer survival (9.3 versus 7.6 months) in older patients

(>65 years). Malmstrom et al. [65] randomized patients aged 60 years and older presenting with a glioblastoma to assess the optimal palliative treatment. The conclusion of the trial was that radiotherapy alone is associated with poor outcomes. On the contrary, both temozolamide and hypofractionated radiotherapy appeared as standards of care especially in patients with methylation of the MGMT gene promoter. In patients with poor general health, supportive care may be proposed to preserve the quality of life with the shortest length of hospital stay if possible [62].

The disease progression is evaluated using brain MRI every 2 to 3 months according to the response assessment in Neuro-oncology (RANO) criteria [66]. At the time of recurrence, there is no standard of care. The main determinants for treatment proposals are the patients' general condition and treatments previously administered [67]. A second surgery can be proposed in young patients with preserved KPS. This strategy has been shown to be associated with longer survival in selected patients [68] (14 months versus 22 months of overall survival in patients with second surgery at recurrence). Eighty percent of patients did not need rehabilitation after a second surgery. In selected patients, the use of intracavitary carmustine wafers (BCNU) has been proposed at initial surgery or in case of recurrence [69]. Efficiency and adverse effects of this therapy remains a matter of debate [70,71]. In the absence of safe possible resection, a second line chemotherapy can be proposed including nitrosoureas, temozolomide or antiangiogenic drugs such as bevacizumab but with no clear results in terms of benefit to date [72] and poor outcomes with an average overall survival rate of 6 months from recurrence [73]. Due to the poor prognosis of glioblastomas, there is an urgent need for new therapies. The REGOMA trial (Regorafenib in Relapsed Glioblastoma) [74] is a phase II randomized trial. The aim was to propose the use regorafenib, an inhibitor of angiogenic and oncogenic receptor of tyrosine kinases at the recurrence of glioblastomas. The results were rather positive on overall survival and with few side effects. A phase III trial is expected.

3.4. Current Research and Perspectives

3.4.1. Omics Approaches

Over the last decade, precision medicine, also through omics approaches (Figure 4), has offered new insights in the diagnosis and management of glioblastomas [75,76]. In the past few years, radiomics brought significant insights in the characterization and predictive models of glioblastomas [77,78]. Radiomics is based on the extraction of a large amount of data from medical images. Radiomics is then enriched with clinical, genomics and proteomics data to establish new diagnosis and prognosis criteria to enhance treatment efficiency. Other promising areas in the fight against glioblastoma are the genomics and transcriptomics approaches. The emergence of big data in the precision medicine offered new therapeutic perspectives. Through ambitious projects such as the human genome project [79], genomics helped us in improving the understanding of glioblastoma. In the continuation of the project, RNA sequencing-based genes proposed genomics signature of life expectancy in patients with glioblastomas [80]. Transcriptomics and other omics technics proposed prognostic tools for the comprehension of the disease [81,82]. Along with these techniques, liquid chromatography and mass spectrometry analyses from different samples such as CSF (Cerebrospinal fluid), urine, blood or glioblastoma cell lines [83] have provided a comprehensive view of the altered metabolic pathways in patients with glioblastoma [84,85]. More recently, special attention has been given to alterations of lipid metabolism in glioblastomas. Based on omics human studies, Guo et al. have described a decrease of 90% of lipid levels in tumor tissue except for phosphatidylcholine and cholesterol ester levels which appeared high in glioma tissue while they are absent in normal brain tissue [86]. These data may emphasize the key role of certain lipids in the glioblastoma metabolism to facilitate tumor growth. Moreover, the metabolic signature of brain tumors in the plasma is of interest for the grading and prognosis of these diseases. It has been shown that the plasma level of metabolites of interest can help to define the grading of brain glioma and to provide prognostic information in patients with a similar

glioma grade [87]. Nevertheless, data are still rare for these emerging approaches; however, the promise of precision medicine and the surge of multimodal data-driven strategies can provide valuable tools for the development of biomarkers and innovative therapies in glioblastomas [88].

3.4.2. Novel Therapies for Glioblastomas

Due to the adverse outcome in patients with glioblastoma and the high frequency of this disease, innovative therapies are being tested in different randomized controlled trials [73,89,90]. With the development of a better understanding of molecular pathways triggering glioblastoma growth [27–29], the traditional approach of antitumor therapy is being progressively complemented by a more personalized approach [91,92]. Treatment schedules have been rethought as well as the drugs themselves. Two major drawbacks consist in the difficulty for most of the drugs is to pass through the blood–brain barrier (BBB) and to target tumor cells due to the presence of abnormal vessels and necrosis, which hampers drugs being delivered at a suitable concentration. Some emerging techniques have been proposed to improve the distribution of antitumor therapy, notably the conjugation of drugs with protein to facilitate the movement across the BBB and specifically target the tumor [93], the use of convection-enhanced delivery consisting in the direct administration inside the tumor via a catheter [94] and the use of nanoparticles. The increase in BBB permeability during chemotherapy administration via focused ultrasounds is being also tested [95]. Meanwhile, immunotherapy approaches are known for a long time, with promising results in many cancers such as melanoma but deceiving results in patients with gliomas [96]. Cancer vaccination has recently been proposed referring to the activation of an immune response against tumor antigens. These new technologies have been applied for glioblastoma treatment with different vectors [97–99]. Two modalities have been tested: peptide vaccines targeting EGFR, IDH1 or heat shock proteins, and cell-based vaccines consisting in the injection of ex vivo modified cells, mainly dendritic cells [100]. Despite encouraging results in animal models in terms of disease control [101], cancer vaccination in glioblastomas has not yet proven its efficacy on overall survival in phase III studies [9]. Recently, the role of tumor-associated macrophages (TAM) has been highlighted in the genesis and resistance to treatment of glioblastoma cells [102–104]. Landry et al. [102] showed that the TAM located in the core have different characteristics and metabolic pathways compared to those located in the periphery of the glioblastoma. For these reasons, they reaffirm the need for a multi-targeted approach through a modulation of the TAM. Furthermore, tumor-associated neutrophils (TAN) are found to be involved in necrosis onset in glioblastoma patients [105,106]. In this context, the mechanism of necrosis could be a neutrophil-mediated ferroptosis. The latter could have a pro-tumorigenic role [105]. Thus, targeted therapies are potential novel therapies for glioblastomas to prevent TAN recruitment.

Stupp et al. recently developed a new therapeutic modality in the treatment of recurrent glioblastomas consisting in the local delivery of low-intensity electric fields via a non-invasive transducer [73]. The device (NovoTTF-100A) was tested in a phase III study and randomized with active chemotherapy as an alternative arm. The overall survival was similar in both arms (6 months) with fewer adverse events in the NovoTTF-100A group and a better quality of life. For this reason, the tumor-treating field is considered as a standard of care in some guidelines [107]. Moreover, in the last years, the association of molecularly targeted drugs such as tyrosine kinase administration or others combined with X-rays to decrease radioresistance due to hypoxia showed encouraging results [108–110].

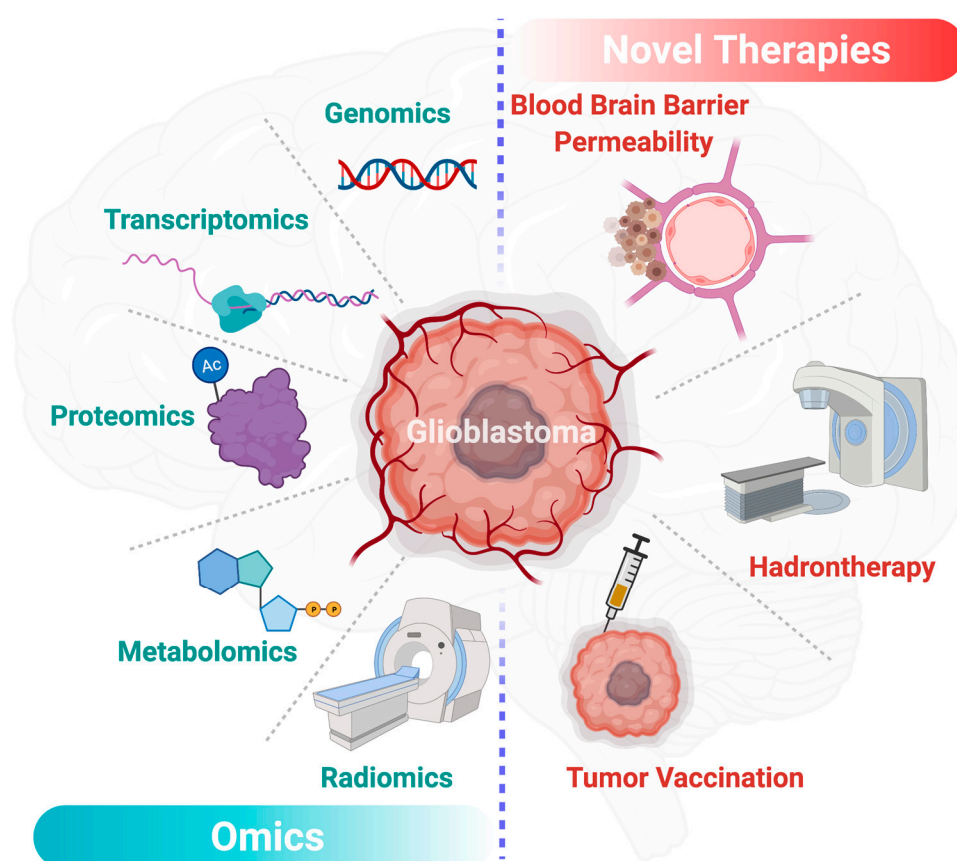


Figure 4. Overview of the main driving omics technologies and therapeutic perspectives for glioblastoma in the precision medicine era. This figure has been created with [BioRender.com](https://www.biorender.com) (accessed on 9 March 2021).

4. Conclusions

The authors are aware of the limits of using such automatic topic literature search tools. This limits might be related to recency or coverage scope of such tools that might lead to some key literature outputs being missing. This highlights the importance of manual curation combined with the use of multiple tools to consistently cover the area of interest.

In conclusion, despite a better understanding of the molecular pathways leading to glioblastoma development and growth, outcomes remain poor in terms of survival. Many trials are currently in progress with new therapeutic approaches to go from a global approach to a more personalized approach. The main objective of these approaches is to deliver the chosen drug inside the tumor and to adapt its concentration to the tumor characteristics. Then, the modality of drug delivery has become as challenging as the drug itself.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/jpm11040258/s1>, Table S1: Articles and clusters related to glioblastoma published in the past 30 years.

Author Contributions: Conceptualization, V.G.; software, A.T.; formal analysis, A.T.; data curation, V.G., S.B. and S.M.; writing—original draft preparation, V.G. and S.M.; writing—review and editing, A.T., I.D., A.L., M.F., S.D. and S.B.; visualization, A.T.; supervision, S.B. and S.M. All authors have read and agreed to the published version of the manuscript.

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CHAPITRE III : TRAVAIL EXPERIMENTAL

1. Définition du problème

Le travail réalisé dans les deux premiers chapitres de cette thèse met en exergue plusieurs problématiques. Dans le Chapitre I, nous avons montré par une perspective historique, comment la neurochirurgie, qui est une discipline ancienne, a su s'approprier les grandes avancées technologiques de chaque époque pour progresser. Ensuite, nous avons rappelé le rôle émergent de la médecine de précision dans de nombreux domaines médicaux en insistant sur la neuro-oncologie qui est aujourd'hui à l'orée d'une révolution grâce à l'interdisciplinarité et les outils du big data qui permettent d'intégrer et exploiter les données des sciences omiques. L'exploitation de ces informations va permettre de servir la neuro-oncologie et d'en faire une médecine de précision centrée sur le patient. Dans de nombreux essais cliniques récents, les thérapies innovantes sont fréquemment adaptées à chaque patient, se basant sur les caractéristiques intrinsèques de la tumeur ce qui est l'essence même de la médecine personnalisée.

En parallèle de ce bond technologique, nous avons développé dans le Chapitre II un résumé des connaissances actuelles du glioblastome. Cette pathologie est extrêmement complexe, infiltre la substance blanche et s'accompagne ainsi d'une symptomatologie insidieuse. Historiquement, les avancées sur cette pathologie se sont faites par des analyses de la sémiologie, de la radiologie et de l'histologie rendues possibles par les progrès des techniques d'imagerie en coupes et de la microscopie optique puis électronique. Depuis quelques années, la biologie moléculaire a modifié les outils diagnostiques et bousculé les classifications établies. Néanmoins, le diagnostic, et la thérapeutique du glioblastome passent par un geste chirurgical, invasif par définition et à risque de morbidité. Sur le plan thérapeutique, la chirurgie doit s'accompagner d'une chimiothérapie et d'une radiothérapie. Malgré ces traitements lourds, la médiane de survie est inférieure à 2 ans. Il y a donc une nécessité d'une nouvelle approche pour améliorer le diagnostic de cette maladie et son pronostic.

Le concept de biopsie liquide a émergé depuis quelques années. Son concept est de pouvoir établir un diagnostic de manière non invasive pour le patient en évitant cette étape de chirurgie. Les sciences omiques, et notamment la métabolomique, pourraient permettre de proposer ces nouveaux outils diagnostiques. La petite taille des métabolites leur permet de franchir la barrière hématoencéphalique et ainsi d'exprimer dans le plasma des patients, un profil métabolomique des tumeurs intracérébrales telles que le glioblastome. L'objectif de notre travail expérimental était ainsi de définir le profil métabolomique du glioblastome dans le plasma en le corrélant au tissu tumoral.

2 Mise en place du design expérimental

2.1 Sélection des patients contrôles

Notre étude reposant sur une étude métabolomique ciblée et non ciblée, la sélection d'un groupe contrôle est une étape prépondérante dans la mise en place d'un tel type d'étude. Pour le choix du tissu contrôle, une analyse de la littérature retrouvait comme principaux groupes contrôles du cortex cérébral issu de cortectomies ou les méningiomes. Nous avons fait des études préliminaires avec ces deux tissus et retenu le cortex cérébral. La principale raison est que même si plus rare, le tissu est macroscopiquement similaire et le méningiome correspond à une lésion extraparenchymateuse ce qui est moins proche du gliome, lésion par définition intraparenchymateuse. Concernant le plasma, nous avons opté pour du plasma de sujets dits sains issu du commerce. En effet, il est facilement accessible, relativement peu onéreux et le caractère hautement symptomatique du glioblastome fait qu'il peut être supposé aisément les patients comme indemnes d'un gliome.

2.2 Type d'étude métabolomique

Les études métabolomiques peuvent être ciblées ou non ciblées. La différence est le postulat des métabolites d'intérêt. Ainsi, une étude métabolomique ciblée permettra de chercher au sein de classes de métabolites (lipides par exemple), ceux qui diffèrent entre le groupe d'intérêt et le groupe contrôle. Une étude non ciblée explorera le maximum de métabolites d'intérêt sans préjugés de classes ou de tailles des métabolites. Ces études ont des avantages et inconvénients qui leur sont propres. Une étude ciblée sera plus précise dans le screening, mais pourra écarter à tort des métabolites hautement discriminants si le postulat de base est faux. A l'inverse une étude non ciblée donnera une analyse moins fine des métabolites, mais en exclura moins. Nous avons opté pour deux types d'analyses ciblées et non ciblées pour profiter de leurs avantages respectifs. Il s'agit également d'une validité externe de notre étude de vérifier si les métabolites d'intérêt en analyse non ciblée sont discriminants dans l'analyse ciblée.

Enfin, notre schéma d'étude s'appuie sur l'imagerie métabolomique à l'aide de la technologie FTICR (spectrométrie de masse à résonance cyclotronique ionique). La technique FTICR est une technique à très haute résolution qui permet de déterminer les masses moléculaires avec une grande précision. De nombreuses applications de la FTICR-MS utilisent cette précision des masses pour aider à déterminer la composition des molécules sur la base de la masse exacte. Il s'agit d'une technologie de pointe, encore peu répandue dans la recherche médicale, mais qui dispose d'un

potentiel significatif. L'intérêt est de permettre une analyse de coupes histologiques avec leurs métabolites d'intérêt. De plus, après traitement des données, il est possible d'obtenir une analyse spatiale des métabolites avec leur répartition au sein de la coupe étudiée. Ceci est particulièrement intéressant pour l'analyse de phénomènes dynamiques, dans notre cas, la différence entre le cerveau sain, le centre tumoral et la zone péri lésionnelle.

3 Application : Article III

Ce chapitre est présenté sous forme d'article accepté dans le journal *Cancers*.

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Article

Integrative Metabolomics Reveals Deep Tissue and Systemic Metabolic Remodeling in Glioblastoma

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Simple Summary: This study aims to explore metabolic remodeling in plasma and tissue samples in patients with glioblastoma through an integrated targeted and untargeted metabolomics-based strategy. We report phospholipids, sphingomyelins, acylcarnitines, and acylglycerols as key impaired metabolic classes in glioblastoma.

Abstract: (1) Background: Glioblastoma is the most common malignant brain tumor in adults. Its etiology remains unknown in most cases. Glioblastoma pathogenesis consists of a progressive infiltration of the white matter by tumoral cells leading to progressive neurological deficit, epilepsy, and/or intracranial hypertension. The mean survival is between 15 to 17 months. Given this aggressive prognosis, there is an urgent need for a better understanding of the underlying mechanisms of glioblastoma to unveil new diagnostic strategies and therapeutic targets through a deeper understanding of its biology. (2) Methods: To systematically address this issue, we performed targeted and untargeted metabolomics-based investigations on both tissue and plasma samples from patients with glioblastoma. (3) Results: This study revealed 176 differentially expressed lipids and metabolites, 148 in plasma and 28 in tissue samples. Main biochemical classes include phospholipids, acylcarnitines, sphingomyelins, and triacylglycerols. Functional analyses revealed deep metabolic remodeling in glioblastoma lipids and energy substrates, which unveils the major role of lipids in tumor progression by modulating its own environment. (4) Conclusions: Overall, our study demonstrates in situ and systemic metabolic rewiring in glioblastoma that could shed light on its underlying biological plasticity and progression to inform diagnosis and/or therapeutic strategies.

Keywords: glioblastoma; high-grade glioma; brain tumor; neuro-oncology; metabolomics; omics; mass spectrometry; machine learning

1. Introduction

Glioblastoma (GBM) is the most common malignant brain tumor in adults [1]. Its incidence, 2 to 5 per 100,000 [1,2], is still increasing [3,4], and its etiology remains unknown in most cases. Glioblastoma pathogenesis consists of a progressive infiltration of the white matter by tumoral cells leading to progressive neurological deficit, epilepsy, and/or intracranial hypertension [5,6]. Multimodal magnetic resonance imaging (MRI) is the first-line investigation to diagnose glioblastoma and guides treatment strategies [7]. The current standard of care in the management of glioblastoma is based on surgical resection or biopsy followed by adjuvant chemotherapy and radiotherapy [8–10]. Despite many therapeutic trials [11–13], the prognosis of glioblastoma is poor, with a mean survival between 15 to 17 months [14]. Given this poor prognosis, there is an urgent need for a better understanding of the underlying mechanisms of glioblastoma to unveil new diagnosis strategies and therapeutic targets through a deeper understanding of its biology. During the last few years, Precision Medicine (PM) has been a new paradigm that has opened a new era in personalized diagnosis and therapeutic approaches in oncology [15–18]. It is a tailored approach in disease management driven by the patient's attributes to deliver personalized healthcare. The concept of PM is penetrating steadily into the clinical setting with increasing evidence in different areas, including oncology [15], autoimmunity [19], or inborn errors of metabolism [20]. The PM surge is shaped by omics-based technologies and the data-driven medicine revolution [21–23]. Omics are technologies that allow for a multilayer molecular deciphering of the cell, tissue, and organism in a holistic fashion. These technologies have been explored in various avenues of medicine and health sciences, such as screening, diagnosis, and prognosis [24]. Given their capacity to interrogate biology at an unprecedented depth and breadth, omics are also reshaping the biomarker discovery landscape [25]. Metabolomics, one of these technologies [26], directly emulates the biochemical activity of a biological system with high time sensitivity and spatial resolution. Metabolomics is the closest biological information layer to the phenotype, given its position in the downstream information flow. Thus, interrogating the metabolome, which is a set of metabolites in a given biological system, is an appealing opportunity to parse the biological process. Two main technologies are used in metabolomics: mass spectrometry (MS) and nucleic magnetic resonance (NMR) [27,28]. The latter is nondestructive and robust; however, the former exhibits higher sensitivity and broader metabolome coverage. High-resolution mass spectrometry (HRMS) coupled to other analytical modalities, such as liquid chromatography (LC-MS) or MALDI-MS, offers a great opportunity to achieve higher metabolic coverage, thus, biological scope. Furthermore, ultra-high resolution mass spectrometry instruments based on Fourier-transform ion cyclotron resonance (FTICR) exhibits unique resolution, sensitivity, and specificity [29–31]. Thus, this technology offers unprecedented exhaustive metabolome coverage and non-ambiguous molecular formula assignments [31]. Thus, disease-related metabolic phenotype could be described by retrieving metabolomic profiles through biospecimens, such as blood, urine, tissue samples, or cerebrospinal fluid [32–34]. Previous human-based studies have described glioblastoma-related metabolites [35–43], and to the best of our knowledge, no study has reported results based on both plasma and tissue analysis from the same glioblastoma patients. The aim of this study is to explore the glioblastoma biological landscape through an integrated metabolomics analysis of plasma and tissue samples of glioblastoma patients. This strategy presents a great opportunity to explore both the in-situ and systemic metabolic phenotypes and unveil predictive metabolomic patterns that could help early non-invasive glioblastoma diagnosis at presentation or recurrence. It might also provide new insights regarding glioblastoma biology and ultimately guide targeted treatment strategies.

2. Materials and Methods

2.1. Patients and Blood Samples

From January 2020 to November 2020, twenty-nine patients with histologically documented glioblastoma were selected for the study. All patients were referred to our neurosurgery department, and resection or biopsies of the tumor were performed in all cases. Plasma and tissue samples were collected at surgery before initiating any treatment. Medical charts were reviewed, and the following data were collected: gender, age at diagnosis, past medical history, revealing symptoms, radiological characteristics, type of surgery performed, and histological and biological characteristics of the tumor. All samples used in this study belong to a declared collection located in the Pathology Department (Prof. Annie Laquerrière) of Rouen University Hospital in accordance with the relevant guidelines and regulations and with the permission of the local authorities. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

2.2. Targeted Metabolomics

2.2.1. Reagents

All reagents, internal and calibration standards, quality controls, test mix, and a patented 96-well filter plate required for the AbsoluteIDQ[®]p180 analysis are included in the kit or provided by Biocrates Life Science AG (Innsbruck, Austria).

2.2.2. Sample Preparation

Sample preparation was carried out according to the manufacturer's protocol [44,45]. Briefly, 10 µL of plasma were transferred to the upper 96-well plate and dried under a nitrogen stream. Thereafter, 50 µL of a 5% PITC solution was added to derivatize amino acids and biogenic amines. After incubation, the spots were dried again before the metabolites were extracted using 5 mM ammonium acetate in methanol (300 µL) into the lower 96-well plate for analysis after further dilution using the MS running solvent A. Quantification was carried out using internal standards and a calibration curve [44,45]. The full list of 188 measured metabolites is presented in Table S1.

2.2.3. Liquid Chromatography and Tandem Mass Spectrometry

The AbsoluteIDQ[®] p180 kit is a fully automated assay based on phenyl isothiocyanate (PITC) derivatization of the target analytes in bodily fluids using internal standards for quantitation. Amino acids and biogenic amines are determined in LC-MS mode, acylcarnitines, phospholipids, sphingomyelins, and the sum of hexoses are analyzed in flow injection analysis (FIA). The analyses were performed following the instructions of the kit manufacturer using the liquid chromatography instrument prominence Shimadzu UFLC System (Shimadzu, Prominence, Kyoto, Japan) coupled to the 4000 Qtrap mass spectrometer (Sciex, Framingham, MA, USA) with an electrospray ion source. A system suitability test was conducted before each batch of the samples (analysis of a standard mixture) to warm up the LC-MS/MS system and check the inter-day performance of the system. Data acquisition and processing were performed using the Analyst 1.5 software (Sciex, Framingham, MA, USA). Twenty microliters of the sample extract were used in the flow injection analysis (FIA) in positive mode to capture acylcarnitines, glycerophospholipids, and sphingolipids, while hexoses were monitored in a subsequent negative mode run. All FIA injections were carried out using the Biocrates MS Running Solvent. All metabolites were identified and quantified using isotopically labeled internal standards and multiple reaction monitoring (MRM) as optimized and provided by Biocrates Life Sciences AG (Innsbruck, Austria).

2.3. Untargeted Metabolic Phenotyping

2.3.1. Reagents

Water, acetonitrile, isopropanol, and formic acid were optima LCMS grade and purchased from Fisher Chemicals. Ammonium formate was LCMS grade (Sigma–Aldrich, Saint-Quentin Fallavier, France). Leucine Enkephalin (Sigma–Aldrich, Saint-Quentin Fallavier, France) at a concentration of 2 mg/mL (in isopropanol/water, 50/50) was used as a reference for mass measurements. Poly-DL-alanine was prepared in 50:50 (*v/v*) water/acetonitrile at 10 mg/L and used for ion mobility cell calibration.

2.3.2. Sample Preparation

Human plasmas were prepared using a liquid biphasic approach. Lipids were extracted from 100 µL of plasma by adding 280 µL chloroform, 140 µL methanol, and 84 µL water. The mixtures were vortex-mixed, cooled in ice for 15 minutes, and centrifuged 10 min at 12,000 rpm. The bottom phase was removed, transferred to glass UPLC vials, and N₂ evaporated. The resulting phase was re-suspended in 200 µL ACN/H₂O/IPrOH (1:1:1). A pooled quality control (QC) was prepared by mixing 5 µL of each plasma sample. Four QC dilutions at 1:2, 1:4, 1:8, and 1:16 were prepared from the QC sample. Samples were stored at −20°C before use.

2.3.3. Chromatographic Conditions

The liquid chromatography separation was performed with a UHPLC system (Dionex Ultimate 3000 UPLC+, Thermo Scientific, San Jose, CA, USA). Separation was carried out at 50 °C using a 1.0 × 100 mm Acquity UPLC HSS T3 column (Waters), with a particle size of 1.8 µm, equipped with a 0.2 µm prefilter. Mobile phase A consisted of ACN/H₂O (40/60) containing 10 mM ammonium formate; mobile phase B consisted of IPrOH/ACN (90/10). Both solvents contained 0.1% formic acid. The flow rate was maintained at 100 µL min^{−1}. A mobile phase gradient separation was performed over 20 min according to the following steps: 10% B at 0 min, 56% B at 2 min, 75% B at 10 min, 99% B at 12–15 min, 10% B at 16–20 min. Sample analysis order was randomized to avoid the potential for confounding critical variables with analytical run order effects.

2.3.4. Ion Mobility and Mass Spectrometry

The U-HPLC system was coupled to a hybrid quadrupole orthogonal time-of-flight (TOF) mass spectrometer (SYNAPT G2 HDMS, Waters MS Technologies, Manchester, UK). The mass spectrometer was operated in both positive and negative electrospray ionization mode. A mass range of *m/z* 50–1200 was used. The source and IMS parameters are summarized in Table S2. Leucine enkephalin was used as the lock mass [*M* + *H*]⁺ at *m/z* 556.2771 and [*M*−*H*][−] at *m/z* 554.26202. Sodium formate solution was used for external instrument calibration. The Synapt G2 HDMS was equipped with a traveling wave “Triwave™” geometry, including an ion mobility cell (IMS T-wave). The TOF analyzer was operated in the V resolution mode with an average mass resolution of *m*/Δ*m* 20,000 (full-width at half-maximum definition). Data acquisition of an ion mobility experiment consisted of 200 bins. The cross-collision section (CCS) values, obtained in nitrogen, were experimentally determined using singly charged Poly-DL-alanine oligomers as the TWIM calibrant species for ESI+. The CCS values were derived according to previously reported procedures [46] with the following general equation $\ln(\text{CCS}') = x \ln(\text{dt}') + \ln(A)$. Calibration values and curves are shown in the Supplementary Materials (Table S3). The ion mobility resolution was ~40 Ω/ΔΩ (fwhm). The N₂ CCS values reported were determined at the apex of the ion-mobility peak.

2.3.5. Raw Data Processing

All LC-IM-MS raw data files data processing, peak detection, and peak matching across samples using retention time (*t_R*) correction and chromatographic alignment along with drift time were performed using Progenesis QI (Waters MS Technologies, Manchester,

UK) to yield a data matrix containing retention times, accurate masses, drift time, and peak intensities. Experimental CCS were determined, as stated above, to include CCS instead of drift time values in the data matrix. CCS errors between experimental and theoretical values (%) were then calculated from the LipidsCCS Web Server (Zhu Lab) [47]. The preprocessing step resulted in an X-matrix where t_R , CCS, and m/z values were concatenated into " t_R_m/z_CCS " features (in columns) present in each sample (in rows) with corresponding peak areas.

2.3.6. Quality Control

Aliquoted 10 μ L extracts were mixed to generate pooled quality control samples (QCs). The QCs and solvent blank samples (mobile phase) were injected sequentially in-between the plasma samples. In addition, a dilution series of QC samples (1:2, 1:4, 1:8, and 1:16 original concentration) were used to assess the quality of the extracted features. In this study, we used a filter strategy in which the features' intensity must be correlated to the matrix concentrations in a series of diluted QC samples in order to be included in further analysis. Feature groups with a correlation coefficient of less than 0.70 were removed from the dataset. Furthermore, datasets were refined by the removal of feature groups that did not meet the threshold of peak area measurement precision prior to data analysis. This approach used RSD values derived from repeated measurements of a pooled QC sample. The threshold was set to $RSD < 25\%$ to enhance the biological interpretation of metabolomics data.

2.4. Tissue Metabolic Phenotyping

2.4.1. Reagents

MALDI matrices, including 2,5-dihydroxybenzoic acid (DHB) and 9-aminoacridine hydrochloride monohydrate (9-AA), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium trifluoroacetate (NaTFA, Sigma-Aldrich) at 0.1 mg mL^{-1} (ACN/ H_2O 50:50 [v/v]) was used for external calibration before each analysis.

2.4.2. Sample Preparation

Fresh frozen 10- μ m sections of human brain tissue were mounted on conductive ITO-coated glass slides $75 \times 25 \text{ mm}$ (Bruker, Bremen, Germany) and stored at -80°C until analysis. Matrix solutions were applied with an automatic micro sprayer HTX TM-Sprayer (HTX Imaging, Chapel Hill, NC, USA) as previously described [31]. In the positive-ion mode, the DHB matrix (30 mg mL^{-1} in $\text{MeOH}/\text{H}_2\text{O}$ 50:50 [v/v]) was deposited with the following parameters: nozzle temperature 80°C , nozzle velocity 1200 mm min^{-1} , N_2 pressure 10 psi, N_2 flow rate 2 L min^{-1} , number of passes 12, flow rate $100 \mu\text{L min}^{-1}$, and track spacing 3 mm. In negative-ion mode, 9-AA matrix (10 mg mL^{-1} , $\text{EtOH}/\text{H}_2\text{O}$ 70:30 [v/v]) was sprayed using the following parameters: nozzle temperature 90°C , nozzle velocity 1200 mm min^{-1} , N_2 pressure 10 psi, N_2 flow rate 3 L min^{-1} , number of passes 2, flow rate $120 \mu\text{L min}^{-1}$, track spacing 3 mm, and drying time 30 s between passes. Each slide was vacuum-dried before analysis.

2.4.3. Data Acquisition and Data Processing

Data were acquired on a FTICR instrument (Solarix XR, Bruker, Bremen, Germany) equipped with a 12-Tesla superconducting magnet and a dynamically harmonized ICR cell. This instrument was also equipped with both a laser desorption ionization source (Smartbeam II, Nd:YAG $\times 3$ laser at 355 nm, Bruker, Bremen, Germany) and an electrospray (ESI) source. Each MALDI spectrum for each position was the result of 1 scan and 500 consecutive laser shots. Spectra were acquired over a $100 \mu\text{m}$ raster. Before imaging analyses, the instrument was externally calibrated in the required mode by NaTFA infusion via an ESI source, then internally calibrated by assigning known metabolites from m/z 150–1000 via MALDI source. Thus, the instrument was auto calibrated during image acquisition. In positive-ionization mode, calibration was performed by assigning $\text{C}_7\text{H}_6\text{O}_4$

(m/z 155.033885 $[M+H]^+$, matrix peak), $C_7H_{15}NO_3$ (m/z 162.112470 $[M+H]^+$, carnitine), $C_7H_6O_4$ (m/z 177.015829, $[M+Na]^+$, matrix peak), $C_5H_{14}NO_4P$ (m/z 184.073321, $[M+H]^+$, phosphocholine), $C_9H_{17}NO_4$ (m/z 204.123034, $[M+H]^+$, acetyl carnitine), $C_{14}H_8O_6$ (m/z 273.039364, $[M+H]^+$, matrix peak), $C_{21}H_{12}O_9$ (m/z 409.055408, $[M+H]^+$, matrix peak), $C_{40}H_{80}NO_8P$ (m/z 734.569432, $[M+H]^+$, lipid), and $C_{42}H_{82}NO_8P$ (m/z 798.54096, $[M+K]^+$, lipid). In the negative-ion mode, the assigned peaks were $C_{13}H_{10}N_2$ (m/z 193.077122, $[M-H]^-$, matrix peak), $C_{10}H_{15}N_5O_{10}P_2$ (m/z 426.022139, $[M-H]^-$, adenosine diphosphate), $C_{10}H_{16}N_5O_{13}P_3$ (m/z 505.988470, $[M-H]^-$, adenosine triphosphate), $C_{41}H_{80}NO_8P$ (m/z 744.554879, $[M-H]^-$), and $C_{47}H_{83}O_{13}P$ (m/z 885.549853, $[M-H]^-$). Data size was set at 2 million points for a transient length of 0.87 s, and spectra were acquired with a 97% data file reduction. A single MSI measurement was performed for each specimen. Images were captured using FTMS control and FlexImaging (v 5.0, Bruker) software. Images were processed with SCiLS Lab Pro software (Bruker Daltonics, Bremen, Germany). The total ion current method was used for normalization, and m/z intervals were automatically set at ± 1 ppm. Images were viewed using both FlexImaging and SCiLS Lab software (Bruker Daltonics, Bremen, Germany).

2.5. Metabolite Annotation and Identification

Preliminary assignments based on accurate mass measurements were performed using the mass spectrometry databases, METLIN [48] and HMDB [49], using a threshold of ± 2 ppm. For some metabolites, the precise raw formula led to one hit. For CCS-based annotations, the AllCCS database was used [50]. Only positive ion mode highlighted discriminative ions. Other ions were identified via “on-tissue” tandem mass spectrometry experiments using MALDI tandem MS/MS. Ions of interest were first isolated using a window of ± 1 Da, then fragmented by collision-induced dissociation with energy levels between 10 and 40 eV. For each MS/MS analysis, 50 scans were accumulated for better sensitivity. Spectra were reprocessed using Data Analysis 4.4 software (Bruker Daltonics, Bremen, Germany) and recalibrated with the single-point calibration option. The full list of annotated features is presented in Tables S4 and S5.

2.6. Circulating Cell-Free DNA Acquisition

Circulating cell-free DNA was extracted from blood samples right before surgery and as previously described [51]. Briefly, six milliliters of whole blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA). Within two hours after blood collection, the tubes were centrifuged; the plasma was then extracted and stored at -80°C until use. cfDNA was extracted from 1 mL to 5 mL of plasma using the QIAamp Circulating Nucleic Acid kit[®] (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The sample was eluted in a final volume of 30 μL and stored at -20°C . Double-stranded DNA quantification was performed by a fluorometric method using a Quantit[™] PicoGreen[®] dsDNA Assay kit (Invitrogen, Carlsbad, CA, USA) and a Twinkle LB970 microplate fluorimeter (Berthold, Bad Wildbad, Germany). For each sample, cfDNA quantification was performed in duplicate from 2 μL of eluate, and normalization was performed using a standard calibration curve of known concentrations of standard dsDNA (from 0 to 10 ng). Overall, cfDNA concentration reflects both cell-free genomic DNA as well as circulating tumor DNA (ctDNA).

2.7. Data Analysis

Ion intensities or absolute concentrations were log-transformed and Pareto-scaled [52]. Missing values were imputed using the nearest neighbor averaging algorithm using the `impute.knn` function in the `impute` R package. The full data matrices are presented in Tables S6–S8. Univariate analyses were performed using *t*-tests or Mann–Whitney U depending on the normal distribution of the data. The `Limma` package [53] was used for differential analysis using sex, and age was taken into account by adding it as a covariate. Spearman correlation analysis was performed using R software. Euclidean distance was

used as a similarity measure in the clustering analysis. Principal Component Analysis was used as a dimension reduction technique using log-transformed and Pareto-scaled datasets. False discovery rates were corrected using the Benjamini–Hochberg–Yekutieli method [54], and $p < 0.05$ was considered statistically significant. For network analysis, the first step was to compute several glasso-based networks (GLN) [55]. Three kinds of GLNs were calculated; control + disease samples, control samples only, and all the combinations of samples, including control + “disease-minus-one-patient” to get patient-specific networks. Networks were then constructed from each GLNs data matrix and pruned with each other to get specific networks. The idea of network pruning is to remove edges in a general network that are also found in a more specific network. So, we pruned the “disease + control” network with the “control” network in order to keep only the edges that were disease-specific. Thus, this step resulted in a “disease-specific” network. This step was done using the CTD R package [56]. Using the same strategy, networks of controls + “disease-minus-one” samples were pruned with the controls samples network to obtain a “disease-specific-minus-one-patient” network. This network was then pruned with the “disease-specific” network calculated above in order to extract “patient-specific” metabolic signatures. A summary overview of the network strategy is presented in Figure S1. The metabolites present in more than 50% of these “patient-specific” networks were selected to build a Consensus-Network, enabling the visualization of key metabolic signatures for the disease. To test the discriminatory power of this signature, Random Forest models were tuned for every possible combination of metabolites from the Consensus-Network. Random Forest models were built using the ranger package [57] and the caret package in R [58]. The models were tuned over ~50 repeats to obtain robust classification probabilities. Performances of the models were assessed with the MLeval package in R. The main metric for predictive performance assessment was the area under the curve (AUC) for the resulting receiver operating characteristic (ROC) curve. All analyses were done using R software [59]. All data generated or analyzed during this study are included in this published article and its Supplementary Materials.

3. Results

3.1. Cohort Description

The mean glioblastoma showed a male predominance of 82%. The mean age at diagnosis was 66 ± 11.4 years for females and 64 ± 11.1 years for males. Regarding the performed surgery, it consisted of a biopsy in 56.7% and resection in the other cases. The mean controls age was 39 ± 12.5 years for females and 37 ± 10.9 years for males. A cohort overview is presented in Figure 1, and a summary is presented in Table 1. The detailed clinical and biological data are listed in Table S9. Three different metabolomics methods were used to explore metabolic profile differences between glioblastoma and control samples. They included untargeted mass spectrometry imaging on the tissue samples and both targeted and untargeted analysis on plasma samples. The full list of analyzed metabolites is presented in Tables S4 and S5, and the full data matrices are presented in Tables S6–S8.

Table 1. Main cohort summary.

Characteristic	Control (Brain), $n = 6$ ¹	Control (Plasma), $n = 60$ ¹	Glioblastoma, $n = 22$ ¹	p -Value ²
Sex				0.023
Female	2 (33%)	30 (50%)	4 (18%)	
Male	4 (67%)	30 (50%)	18 (82%)	
Age (Years)	39 (25, 46)	34 (27, 42)	62 (54, 66)	<0.001
ATRX_mutation				
Absent			1 (6.2%)	
Present			15 (94%)	

Table 1. Cont.

Characteristic	Control (Brain), <i>n</i> = 6 ¹	Control (Plasma), <i>n</i> = 60 ¹	Glioblastoma, <i>n</i> = 22 ¹	<i>p</i> -Value ²
Unknown			6	
IDH_mutation				
Absent			15 (88%)	
Present			2 (12%)	
Unknown			5	

¹ *n* (%); Median (IQR), IQR: Interquantile range; ² Fisher's exact test; Kruskal–Wallis rank-sum test.

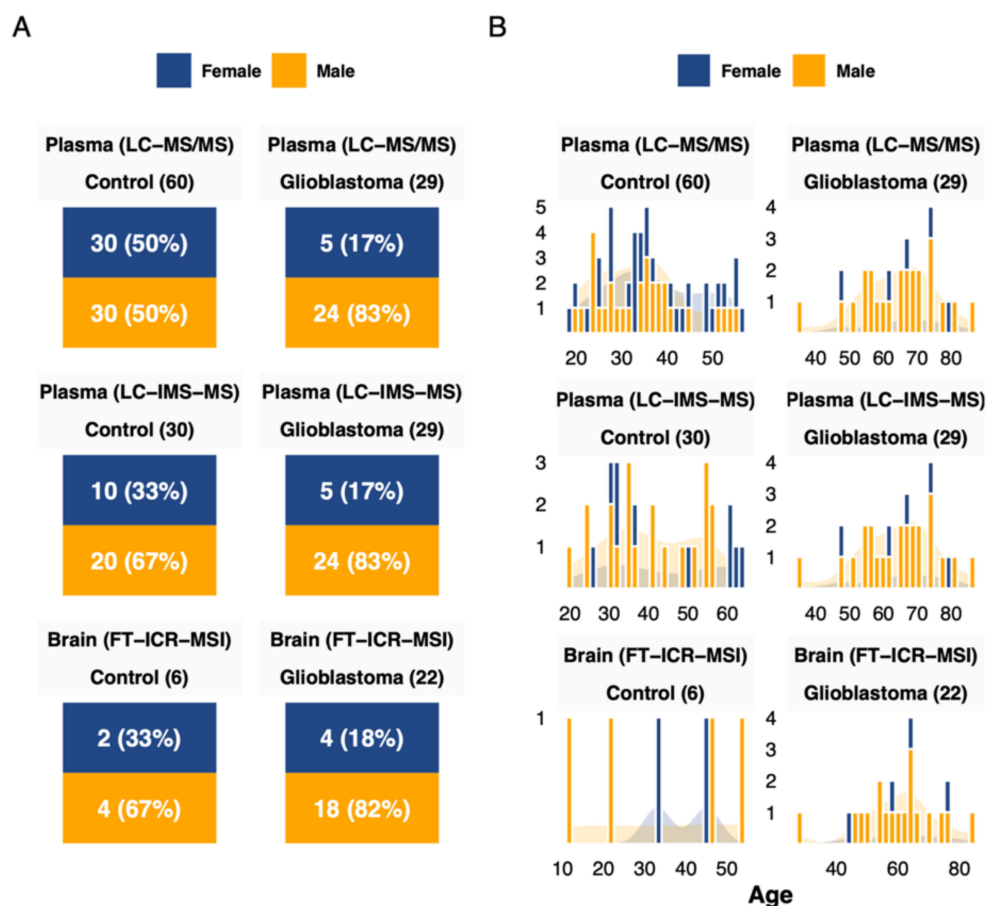


Figure 1. Cohort overview. (A) Cohort description and sex distribution related to each dataset. (B) Age distribution by sex and dataset.

3.2. Unsupervised Exploratory Analysis

To analyze the data, an exploratory approach was first used. This unsupervised analysis aimed to track samples' clustering trends based on their underlying metabolic profiles. We unveiled these grouping trends using Spearman correlations and hierarchical clustering of the samples. The results showed a clear separation of two distinct groups related mainly to control and GBM samples Figure 2A–C. This was observed in all three datasets (MSI, untargeted, or targeted plasma metabolomics) as shown in the heatmaps in Figure 2A–C. Higher-resolution heatmaps are presented in Figure S2, and full correlation matrices are presented in Tables S10–S12. The same clustering trends were also observed using the PCA scores plots, Figure 2D, that showed two clear groups related to GBM and control samples. This separation was mainly observed on the PC1 dimension. The PCA scores matrices are presented in Table S13.

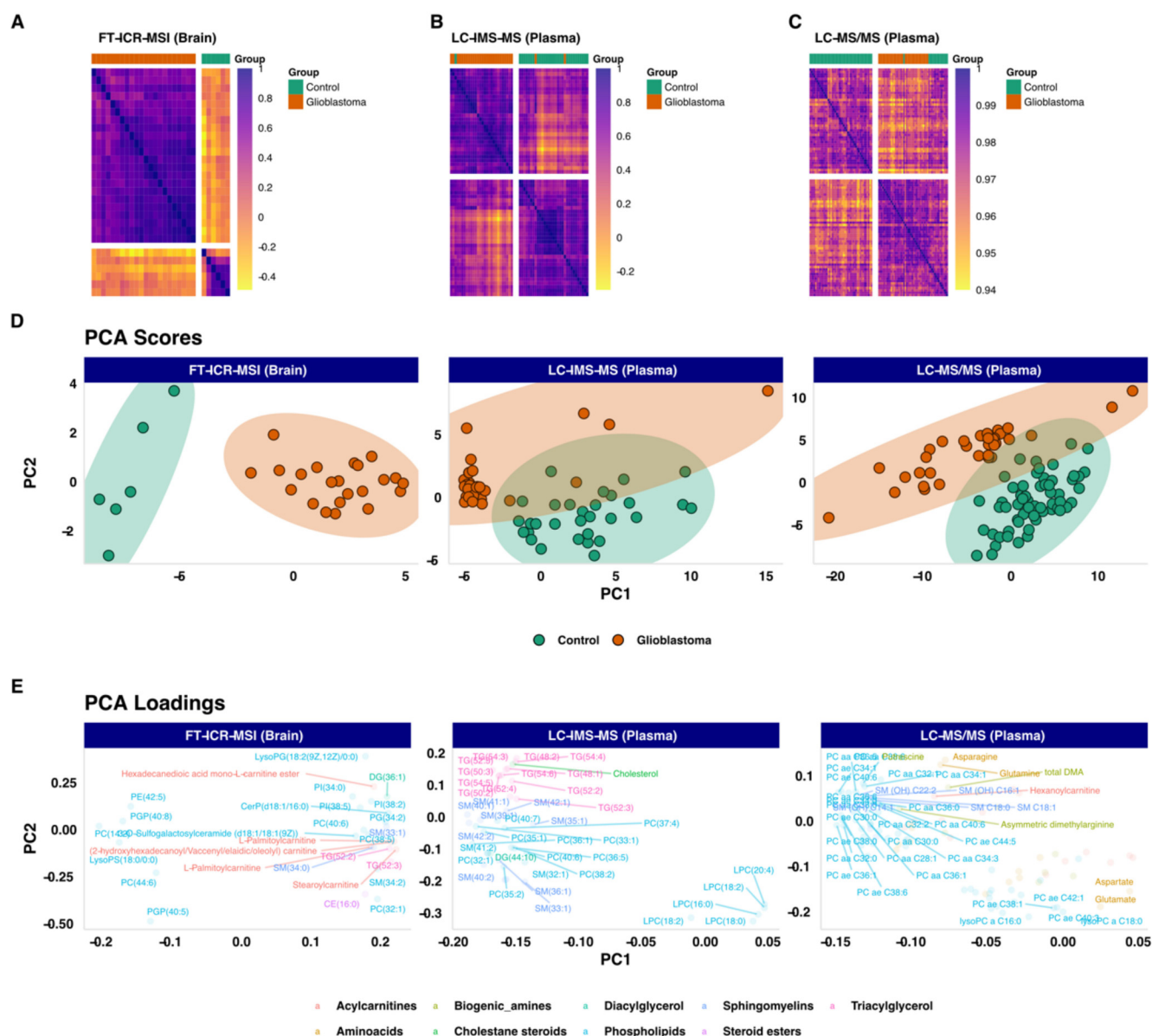


Figure 2. Unsupervised exploratory analysis overview. (A) Heatmap of the sample's spearman correlation analysis based on untargeted FT-ICR-MS data. (B) Heatmap of the sample's spearman correlation analysis based on untargeted LC-IMS-MS data. (C) Heatmap of the sample's spearman correlation analysis based on targeted LC-MS/MS data. (D) Principal Component Analysis scores plot based on untargeted FT-ICR-MS (Explained variance: PC1 = 65%, PC2 = 7%), untargeted LC-IMS-MS (Explained variance: PC1 = 55%, PC2 = 15%) and targeted LC-MS/MS (Explained variance: PC1 = 32%, PC2 = 16%) datasets, respectively. (E) Principal Component Analysis loadings plot based on untargeted FT-ICR-MS (Explained variance: PC1 = 65%, PC2 = 7%), untargeted LC-IMS-MS (Explained variance: PC1 = 55%, PC2 = 15%) and targeted LC-MS/MS (Explained variance: PC1 = 32%, PC2 = 16%) datasets, respectively.

3.3. Differential Expression Analysis

To go further in the analysis, we performed a differential analysis between the two groups GBM versus Control, using each of the three datasets. The analysis identified 176 annotated metabolites that were differentially expressed between GBM and control samples. The plasma-based comparison yielded 148 metabolites, including 75 phospholipids (69 glycerophosphocholines, 4 phosphatidylcholines, and 2 phosphosphingolipids), 20 sphingomyelins, 15 acylcarnitines, 14 amino acids, 12 triacylglycerids, 10 biogenic amines, 1 cholestan steroid, and 1 diacylglycerol. The tissue-based comparison unveiled 28 metabolites, including 17 phospholipids (4 glycerophosphocholines, 3 phosphoinositols, 1 phosphatidylcholine, 2 phosphosphingolipids, 2 glycerophosphoglycerophosphate, 2 glycerophosphoglycerid, 1 glycosphingolipid, 1 glycerosphingosine, and 1 glycerophosphoethanolamine), 5 acylcarnitines, 1 diacylglycerol, 2 sphingomyelins, 2 triacylglycerols, and 1 steroid ester. The summary results are presented in Figure 3A. The list of metabolites and their statistics-related metrics is presented in Table S14. Furthermore, we compared the retrieved metabolites with previously reported human GBM metabolomics-based literature [38–41,60]. The summary results are presented in Figure 3B, and the full results are presented in Table S15. The overlap with previously reported metabolites includes 4 biogenic amines (Putrescine, cis-4-Hydroxyproline, trans-4-Hydroxyproline, Spermine), 8 amino acids (Glutamine, Asparagine, Ornithine, Lysine, Tryptophan, Citrulline, Threonine, Valine), 1 steroid ester (CE(22:6)) and Carnitine. The related boxplots and correlations are presented in Figures S3 and S4, respectively. In contrast, the metabolites that were not reported in the above-mentioned literature include mainly Acylcarnitines, Biogenic amines, Cholesterol, Phosphatidylcholines, and Triacylglycerols. Based on adjusted *p*-values, the top metabolites are presented in Figure 4, and their Spearman correlations are presented in Figure S5.

3.4. Correlation Analysis

Given the opportunity to have in-tissue metabolomics with matched-plasma samples, we compared the plasma-based and tissue-based metabolic profiles and found ten lipids that overlap between the two lists. These include, phosphatidylcholines LPC(18:0), PC(36:5), PC(40:6), PC(32:1), sphingomyelins SM(33:1), SM(36:2) and triacylglycerides TG(56:7), TG(52:2), TG(52:4), TG(52:3). The boxplots of the top five lipids are presented in Figure 5. They include PC(40:6), PC(32:1), SM(33:1), TG(52:2), and TG(52:3). We also evaluated these lipid intercorrelations between tissue samples and their matched plasma samples. The figure did not show a high correlation between plasma and brain levels. However, the heatmap showed a high intra-matrix correlation. The top metabolites ($|\log \text{fold change}| > 1.5$) and adjusted *p*-value < 0.01 are presented in Figure 5. Furthermore, we explored the overall intra-GBM group variability using the coefficient of variation as a proxy in both tissue and plasma samples. The results showed high inter-tissue variability (CV median = 127 %) compared to plasma (CV median = 36%) (Figure S6A,B). This highlights an inter-tumor metabolic heterogeneity. We also explored the variability of the above-mentioned overlap lipids that clearly showed the same observation (Figure S6C). The full metabolite correlation matrix is presented in Table S16.

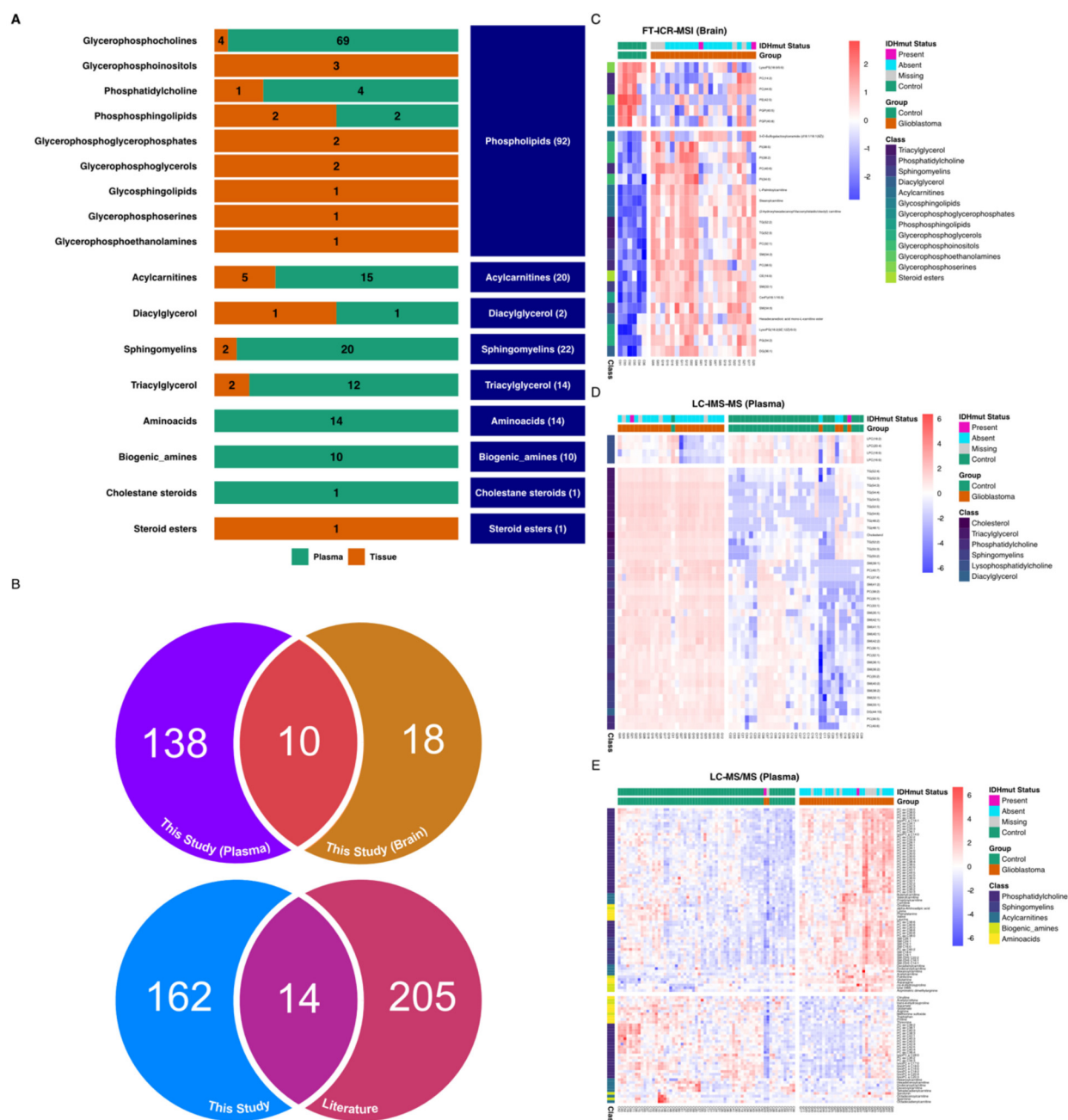


Figure 3. Differential analysis overview. (A) Differential analysis results between control and glioblastoma samples highlighting metabolic classes. One hundred and seventy-six metabolites were identified based on an adjusted p -value of 0.05. One hundred and forty-eight plasma-based metabolites and 28 Tissue-based metabolites. (B) Upper: Ven diagram representation of the overlap between the identified differentially expressed metabolites in Plasma versus Brain. Lower: Ven diagram representation of the overlap between the identified differentially expressed metabolites in this study versus reported human metabolomics-based GBM reported literature. (C) Heatmap showing the relative levels of statistically differential metabolites in different patients' tissues using FT-ICR-MS data. (D) Heatmap showing the relative levels of statistically differential metabolites in different patients' plasma using LC-IMS/MS data. (E) Heatmap showing the relative levels of statistically differential metabolites in different patients' plasma using LC-MS/MS data. Detailed results are presented in Table S13.

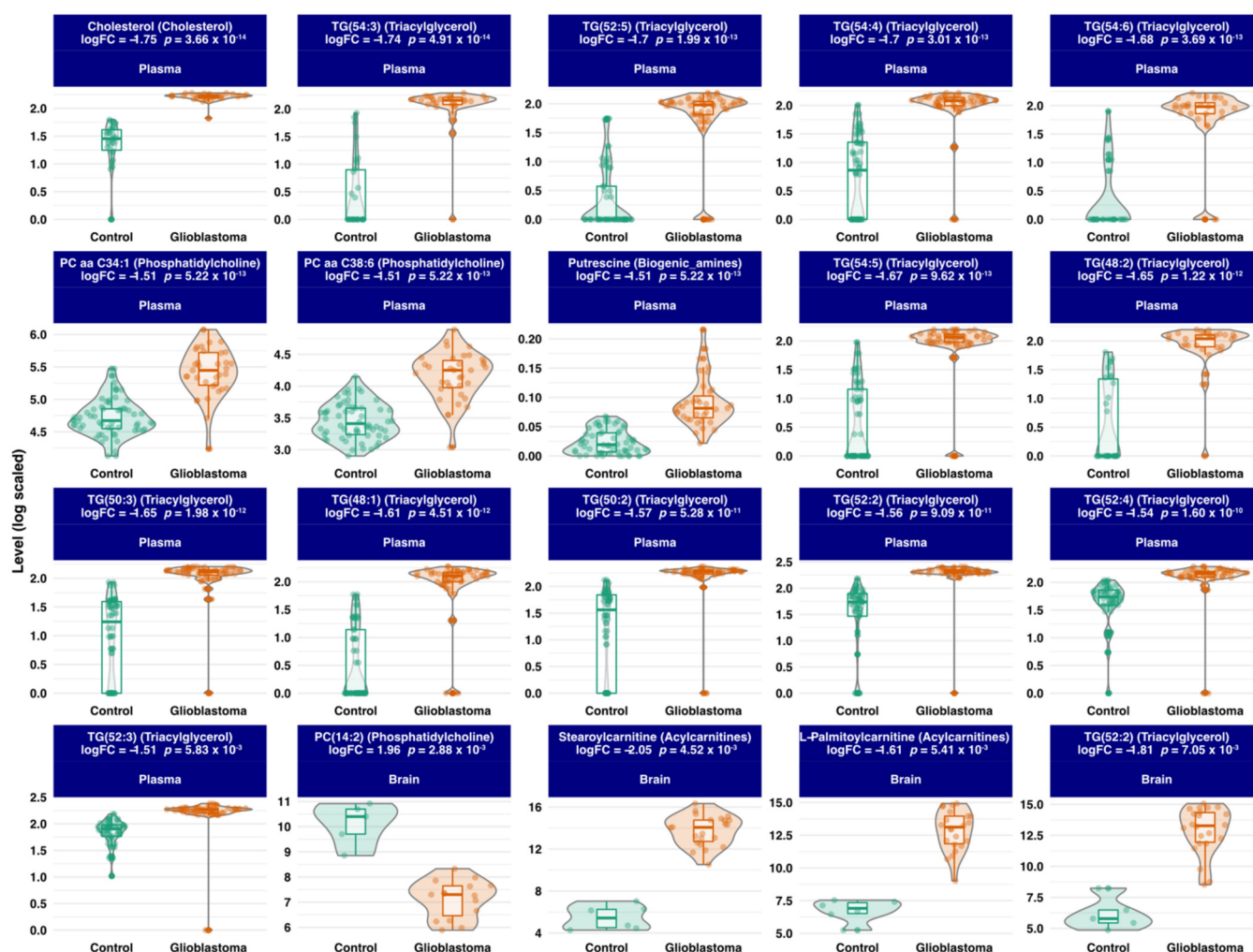


Figure 4. Boxplots of the top (based on adjusted p -values) twenty newly reported metabolites and related biological matrix.

Given the rising interest in using circulating free DNA (cfDNA) in oncology [51], we also assessed the associations between the retrieved metabolic profile and cfDNA using Spearman correlations. The analysis yielded 13 positive correlations and 2 negative correlations with a cut-off set at adjusted p -value < 0.05 and $\text{abs}(\text{Spearman } \rho) > 0.25$. The positively correlated metabolites included Ornithine (Spearman $\rho = 0.52$), Lysine (Spearman $\rho = 0.49$), Phenylalanine (Spearman $\rho = 0.46$), Propionylcarnitine (Spearman $\rho = 0.45$), Carnitine (Spearman $\rho = 0.45$), Hexadecenoylcarnitine (Spearman $\rho = 0.42$), Tetradecenoylcarnitine (Spearman $\rho = 0.4$), Hexenoylcarnitine (Spearman $\rho = 0.35$), Spermine (Spearman $\rho = 0.3$), SM C18:1 (Spearman $\rho = 0.28$), Butenylcarnitine (Spearman $\rho = 0.29$), SM C16:1 (Spearman $\rho = 0.29$), and Asymmetric dimethylarginine (Spearman $\rho = 0.27$). The negatively correlated metabolites included two biogenic amines Serotonin (Spearman $\rho = -0.68$) and Methionine sulfoxide (Spearman $\rho = -0.29$). A network visualization is presented in Figure 6, and full results are presented in Table S17. The full heatmap correlation matrix is presented in Figure S7.



Figure 5. Overlap analysis between tissue-based and plasma-based metabolomics results. **(A)** Brain tissue section distribution of selected overlap lipids. Ion images were generated using SCiLs Lab software. **(B)** Brain tissue sections boxplots of the selected top five differentially expressed metabolites among the overlap lipids with related adjusted p -values. The y -axis shows the log-scaled average intensity. **(C)** Plasma boxplots of the selected top five differentially expressed lipids among the overlap metabolites with related adjusted p -values. The y -axis shows the log-scaled intensity. **(D)** Spearman correlation analysis between the selected top five lipids that overlap between tissue-based and plasma-based metabolomics analysis.

3.5. Network and Predictive Machine Learning Analysis

Using the targeted plasma metabolomic dataset, different correlation networks were built using control, disease, or both samples. Using these correlation networks, 29 patient-specific metabolic signatures were extracted (Figures S8–S36, Tables S18–S20). These patient-specific metabolic networks highlighted the metabolic individuality of each patient. Based on this, we explored the similarity of these signatures between patients. The results revealed 69 unique metabolites spanning phosphatidylcholines (57%), amino acids (14%), acylcarnitines (12%), sphingomyelins (12%), and biogenic amines (6%). Eleven metabolites were found in only one signature and included aspartate, proline, valine, hexanoyl-carnitine, SM C16:0, lysoPC a C16:0, PC aa C42:1, PC aa C42:4, PC aa C42:5, PC ae C32:2, PC ae C34:0, PC ae C38:6. Two patients with mutated IDH status, PGB_04 and PGB_24, both males at 35 and 45 years old, respectively, yielded the following signatures.

For PGB_04 the signature included 11 metabolites; 6 phosphatidylcholines, 3 acylcarnitines, and 2 sphingomyelins (Figure S11) and for PGB_024 male at 35 years old, the signature included 18 metabolites; 14 phosphatidylcholines, 1 acylcarnitine, 1 biogenic amine, and 2 sphingomyelins (Figure S31). Then, we identified the most redundant metabolites found in more than 50% of the patients' signatures which included eight metabolites, seven phosphatidylcholines (PC aa C28:1, PC aa C32:2, PC aa C34:1, PC aa C36:0, PC aa C36:5, PC aa C36:6), and two sphingomyelins (SM C18:0, SM C24:1) (Figure 7A,B, Table S21). The related boxplots are presented in Figure 7C. Based on this signature, we explored the predictive performance of each lipid and all its combinations using predictive machine learning. We built Random Forest predictive models based on each lipid alone as well all possible combinations of the eight lipids. Area under curve and ROC curves were used as performance metrics (Table S22). The eight univariate models and their combinations are shown in Figure 7C. These models included PC aa C36:6 (AUC = 0.949: 0.891–1.000), PC aa C34:1 (AUC = 0.907: 0.83–0.983), PC aa C36:5 (AUC = 0.864: 0.773–0.955), SM C18:0 (AUC = 0.789: 0.68–0.897), PC aa C28:1 (AUC = 0.757: 0.643–0.871), PC aa C36:0 (AUC = 0.72: 0.601–0.839), PC aa C32:2 (AUC = 0.712: 0.591–0.832), SM C24:1 (AUC = 0.686: 0.563–0.809). All included lipids models showed an AUC = 0.936: 0.871–1.000. It is worth mentioning that the most predictive model was the PC aa C36:6 (AUC = 0.949: 0.891–1.000). The full list of investigated models is presented in Table S23.

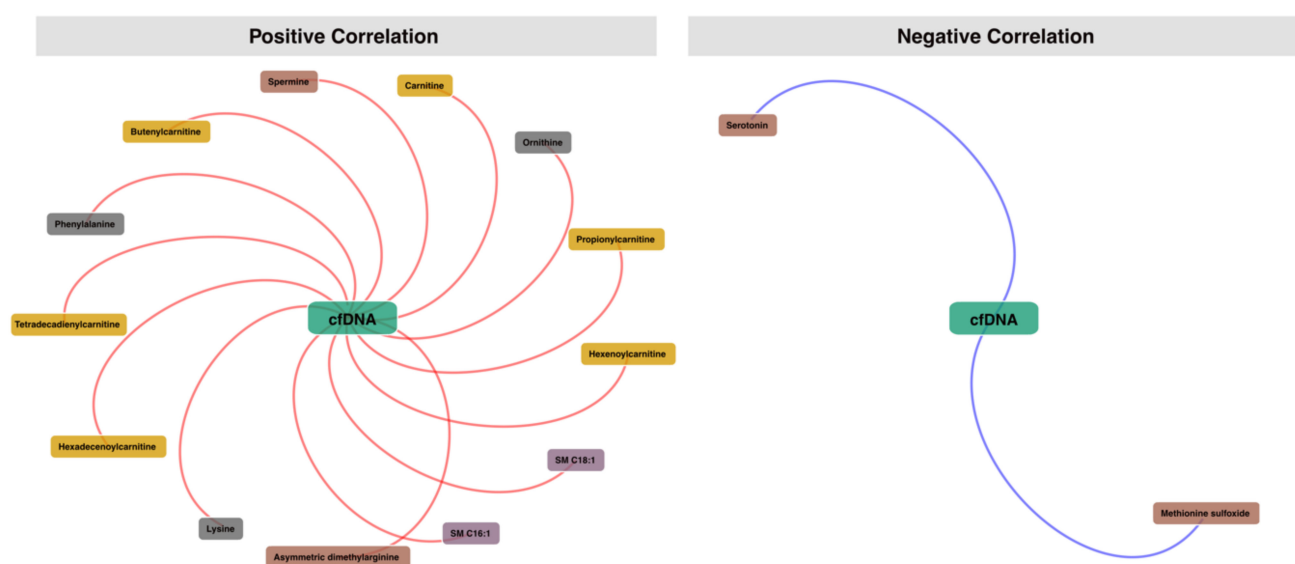


Figure 6. Spearman correlation analysis between metabolic profiles and free circulating DNA. Cut-off was set at adjusted p -value > 0.05 and $\text{abs}(\text{Spearman } \rho) > 0.25$. Multiple test correction was applied for p -values using Benjamini and Hochberg method is used.

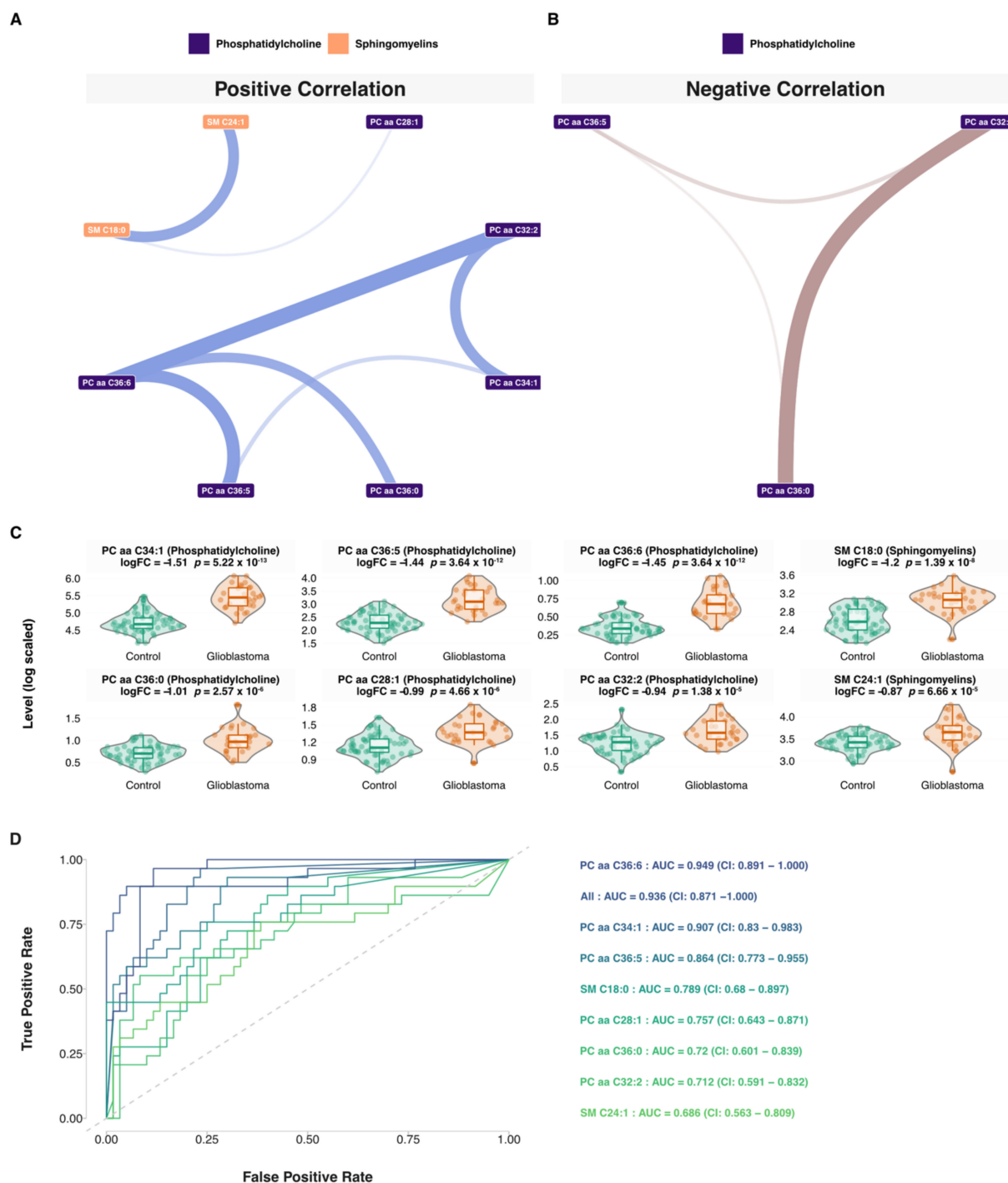


Figure 7. Network and machine learning analysis. (A) Negative correlation network visualization of the glioblastoma consensus metabolic signature. (B) Positive correlation network visualization of the glioblastoma consensus metabolic signature. Line width is proportional to the Spearman correlation. (C) Boxplots of the consensus plasma metabolic signature. (D) ROC curves of the Random Forest predictive models, including seven phosphatidylcholines (PC aa C36:6, PC aa C32:2, PC aa C36:0, PC aa C36:5, PC aa C34:1, and PC aa C28:1) and two sphingomyelins (SM C18:0 and SM C24:1). AUC and 95% confidence intervals (CI) were calculated using balanced subsampling with 50 repeats.

4. Discussion

Despite a sustained research effort in the fight against glioblastoma, the prognosis of this condition remains devastating, and its metabolic signaling pathways are barely understood. To tackle this challenge, omics-based technologies could shed light on this aggressive disease. Metabolic phenotyping through metabolomics is one of the promising tools to interrogate functional metabolic readouts.

In this study, we identified four metabolites' classes as proxies of deep metabolic remodeling in plasma and tissue samples from glioblastoma patients, which included phospholipids, acylcarnitines, sphingomyelins, and Triacylglycerols. Some of the highlighted metabolites in our study have been previously reported. This is the case for a number of amino acids, such as asparagine, glutamine, lysine, citrulline, or valine, for example (Figure 3) [38,39,41]. Indeed, amino acid metabolism is an area of interest in the race for new therapies for glioblastoma. The discovery of the Isocitrate Dehydrogenase (IDH) metabolic pathway has revived this antimetabolic approach, which aims at reducing the synthesis of these molecules, which play an essential role in tumor development and progression [61]. Recently differences in the levels of certain amino acids in the blood of patients with glioblastoma compared to healthy subjects have been demonstrated [62]. These data empower the central role of amino acids in the genesis of glioblastoma. In parallel to amino acid metabolism, our study also highlighted the role of lipids in this disease. Glycerophosphocholines (GPC) were one key class highlighted in this study. Most prominent GPC were PC aa C34:1, PC aa C38:6, PC (14:2), PC aa C36:5, lysoPC a C18:0, PC aa C28:1, PC aa C32:2, PC aa C36:0, and PC aa C36:6 (Figures 3A, 4, 5 and 7). GPC are precursors lipids of acetylcholine [63]. Cholines are important membrane components and known as markers of tumor progression [64]. A choline peak visualized by MRI spectroscopy is a diagnostic criterion for glioblastoma that has been used in clinical practice for several years [38]. Similarly, it has been shown that choline blood levels in glioblastoma patients are correlated with tumor progression [65]. More recently, following in-vivo brain micro dialysis studies [66], it appears that choline levels in the peri-tumor tissue also correlate with its degree of invasion. One of the pathophysiological hypotheses is that glioblastoma modifies its environment to make it favorable to its development and proliferation [67]. Furthermore, one of the key elements for glioblastoma progression is energy substrate availability. The cerebral high lipid enrichment helps deliver the energy necessary for tumor growth [68]. Unlike normal cells, which deliver energy from mitochondrial phosphorylation, tumoral glial cells get their energy from glycolysis and have intense lipidogenesis. Taïb et al. [69] showed that oleic acid, a monounsaturated fatty acid, increases triacylglycerol production in malignant glial cells but not in normal glial cells, arguing for a metabolic reprogramming of the malignant glial cell environment to allow them to spread [70]. Sphingolipids are also involved in this phenomenon and were highlighted in our analysis through SM (OH) C22:2, SM (OH) C16:1, SM C18:0, and SM C18:1 and SM C24:1 (Figures 3A, 4, 5 and 7). These lipids are abundant in the brain and are essential membrane constituents highly expressed in glial cells. Alterations of the sphingolipid pathway resulting in lower levels of ceramides which have pro-apoptotic properties, are thought to play a role in malignant cell dissemination, and malignant glial cells have also been reported to evade apoptosis by converting ceramides to sphingosine-1-phosphate, thus preventing apoptosis [71]. Thus, the action of oncological treatments of glioblastoma, which generate sphingomyelinases to lyse sphingomyelins into ceramides, escape this cell death phenomenon [67]. The exact drivers of this process are not yet well understood. Steroylcarnitine and L-palmitoylcarnitine, which belong to the Acylcarnitines (AC) class, are also modulated during glioma genesis (Figures 3A and 5). AC primary function is to allow fatty acids transport to feed mitochondrial β -oxidation and thus ATP production. Based on a recent study on patient-derived xenograft models of GBM, it appears that AC are abundantly found within and at the edge of these tumors, and this may provide a fertile ground for glioblastoma spread [72]. These advances on how glioblastoma modulates its biological environment to promote its spread, notably through lipid metabolism, opens,

obviously, promising ways to new targeted therapeutic strategies and biomarker discovery. Interestingly, this study showed that circulating free DNA level was positively correlated with sphingomyelins (SM C16:1 and SM C18:1) and Acylcarnitines (Figure 6), which are major substrates of tumor progression by providing the glioblastoma with the energy. Lipid droplets (LDs) are organelles that are commonly found in fatty tissues storing lipids, triglycerol, and cholesterol esters. Recently, the presence of LDs has been demonstrated in glial and glioblastoma cell cultures *in vivo* [73]. These LDs are not only a simple reservoir of fatty acids but have an impact on important cell processes such as cell cycles, cell migration of glial cells, and their resistance to apoptosis and conventional treatments [69]. LDs seem to be promising biomarkers to probe tumor progression [74]. It has been established for a long time [75] that GBM diverts brain lipid metabolism to its advantage to sustain the energy stores needed for their own expansion. By focusing on these LDs, it has been shown that they play a central role in this lipid metabolism reprogramming [73]. Furthermore, it has been reported that autophagy-mediated hydrolysis of these LD maintains energy homeostasis [76]. Thus, limiting the access of glioblastoma to this lipid stock represents a potentially innovative treatment avenue. Despite intensive chemoradiotherapy, the median survival of patients with GBM remains at around 15 months. In recent years, lipidomics has opened the way to new perspectives [77]. The goal is to prevent glial cells from spreading by modulating their environment. Sphingolipids are one of these targets by promoting the formation of apoptosis-inducing ceramides. The action of temozolomide, the first-line chemotherapy for glioblastoma, on the biological environment of glial cells is being actively studied. Temozolomide modifies the extracellular vesicles both in size and content [78]. These extracellular vesicles release fatty acids and proteins that modulate the action of tumor-associated macrophages [78]. Chemotherapy may thus have a modulating effect on the environment of glial cells via these vesicles. The macrophages associated with tumors are major components of tumor spread. Thus, targeting these macrophages could counteract the chemo- and radio resistance of glial cells. As lipids are ubiquitous in the brain, they have been used as vectors for targeted therapies using CRISPR-Cas9 technology [79]. Based on the seminal paper by Garofano et al. [80], a classification of glioblastomas based on cell signaling pathways has revealed a therapeutic sensitivity in the mitochondrial glioblastoma subgroup. These preliminary results, although encouraging, need to be validated and replicated in humans. Some limitations have to be noted regarding our study. The sample size was limited for the tissue-based metabolomics which is mainly related to practical constraints and patients' recruitment. Larger cohorts are needed to confirm the present results, ideally, including other bulk and/or single-cell panomics to have a broader view of the impaired biological pathways and their clinical effect.

5. Conclusions

In summary, our study demonstrated the potential of systems-based metabolomics strategies to holistically interrogate biological plasticity in glioblastoma and parse the role of lipids in tumor progression by the modulation of its own environment. The next step of our work would be the study of therapeutic modulations of these metabolic signatures in order to probe treatment effectiveness. Applying these plasma metabolic patterns could also inform clinical practice to adapt treatment before MRI or clinical modification. A better understanding of metabolic impairments underlying glioblastoma spread would also drive the development of targeted therapies to prevent glioblastoma from promoting its own extension to the surrounding tissues. Such systems-based strategies highlight the importance of multi-omics and multimodal investigations to understand glial lesions to pave the way to more personalized therapies and, ultimately, achieve the promise of precision medicine.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/cancers13205157/s1>, Figure S1: Network analysis overview. Networks are built using Partial Correlation, Figure S2: Spearman correlations between samples, Figure S3: Boxplots of previously reported metabolites, Figure S4: Heatmap of Spearman correlations of previously reported metabolites, Figure S5: Heatmap of Spearman correlations of newly reported metabolites, Figure S6: Intra-group (GBM) variation, Figure S7: Heatmap of Spearman correlations between all significant metabolites, Figure S8: Network-based patient specific plasma metabolic signature (Patient PGB_01), Figure S9: Network-based patient specific plasma metabolic signature (Patient PGB_02), Figure S10: Network-based patient specific plasma metabolic signature (Patient PGB_03), Figure S11: Network-based patient specific plasma metabolic signature (Patient PGB_04), Figure S12: Network-based patient specific plasma metabolic signature (Patient PGB_05), Figure S13: Network-based patient specific plasma metabolic signature (Patient PGB_06), Figure S14: Network-based patient specific plasma metabolic signature (Patient PGB_07), Figure S15: Network-based patient specific plasma metabolic signature (Patient PGB_08), Figure S16: Network-based patient specific plasma metabolic signature (Patient PGB_09), Figure S17: Network-based patient specific plasma metabolic signature (Patient PGB_10), Figure S18: Network-based patient specific plasma metabolic signature (Patient PGB_11), Figure S19: Network-based patient specific plasma metabolic signature (Patient PGB_12), Figure S20: Network-based patient specific plasma metabolic signature (Patient PGB_13), Figure S21: Network-based patient specific plasma metabolic signature (Patient PGB_14), Figure S22: Network-based patient specific plasma metabolic signature (Patient PGB_15), Figure S23: Network-based patient specific plasma metabolic signature (Patient PGB_16), Figure S24: Network-based patient specific plasma metabolic signature (Patient PGB_17), Figure S25: Network-based patient specific plasma metabolic signature (Patient PGB_18), Figure S26: Network-based patient specific plasma metabolic signature (Patient PGB_19), Figure S27: Network-based patient specific plasma metabolic signature (Patient PGB_20), Figure S28: Network-based patient specific plasma metabolic signature (Patient PGB_21), Figure S29: Network-based patient specific plasma metabolic signature (Patient PGB_22), Figure S30: Network-based patient specific plasma metabolic signature (Patient PGB_23), Figure S31: Network-based patient specific plasma metabolic signature (Patient PGB_24), Figure S32: Network-based patient specific plasma metabolic signature (Patient PGB_25), Figure S33: Network-based patient specific plasma metabolic signature (Patient PGB_26), Figure S34: Network-based patient specific plasma metabolic signature (Patient PGB_27), Figure S35: Network-based patient specific plasma metabolic signature (Patient PGB_28), Figure S36: Network-based patient specific plasma metabolic signature (Patient PGB_29), Table S1: Metabolites List used in Targeted Metabolomics, Table S2: Sources and IMS parameters for Ion Mobility Mass Spectrometry analyses, Table S3: Cross Collision Section Calculation Curve, Table S4: Variable metadata (Untargeted LC-IMS-MS Metabolomics), Table S5: Variable metadata (Untargeted FT-ICR-MS Metabolomics), Table S6: Data matrix (Targeted LC-MS/MS Metabolomics), Table S7: Data matrix (Untargeted LC-IMS-MS Metabolomics), Table S8: Data matrix (Untargeted FT-ICR-MS Metabolomics), Table S9: Cohorte Overview, Table S10: Sample Spearman correlation (Targeted LC-MS/MS Metabolomics), Table S11: Sample Spearman correlation (Untargeted LC-IMS-MS Metabolomics), Table S12: Sample Spearman correlation (Untargeted FT-ICR-MS Metabolomics), Table S13: Principal Component Analysis Scores, Table S14: Differential Analysis Results, Table S15: Analysis of overlap with literature, Table S16: Spearman Metabolite Correlation Matrix, Table S17: Spearman Correlations with circulating free DNA, Table S18: Partial Correlation Matrix including all samples, Table S19: Pruned network using all samples network, Table S20: Partial Correlation Matrix including only control samples, Table S21: Patient specific pruned networks, Table S22: Consensus network, Table S23: All Random Forest Models.

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Supplementary material: Integrative Metabolomics Reveals Deep Tissue and Systemic Metabolic Remodeling in Glioblastoma

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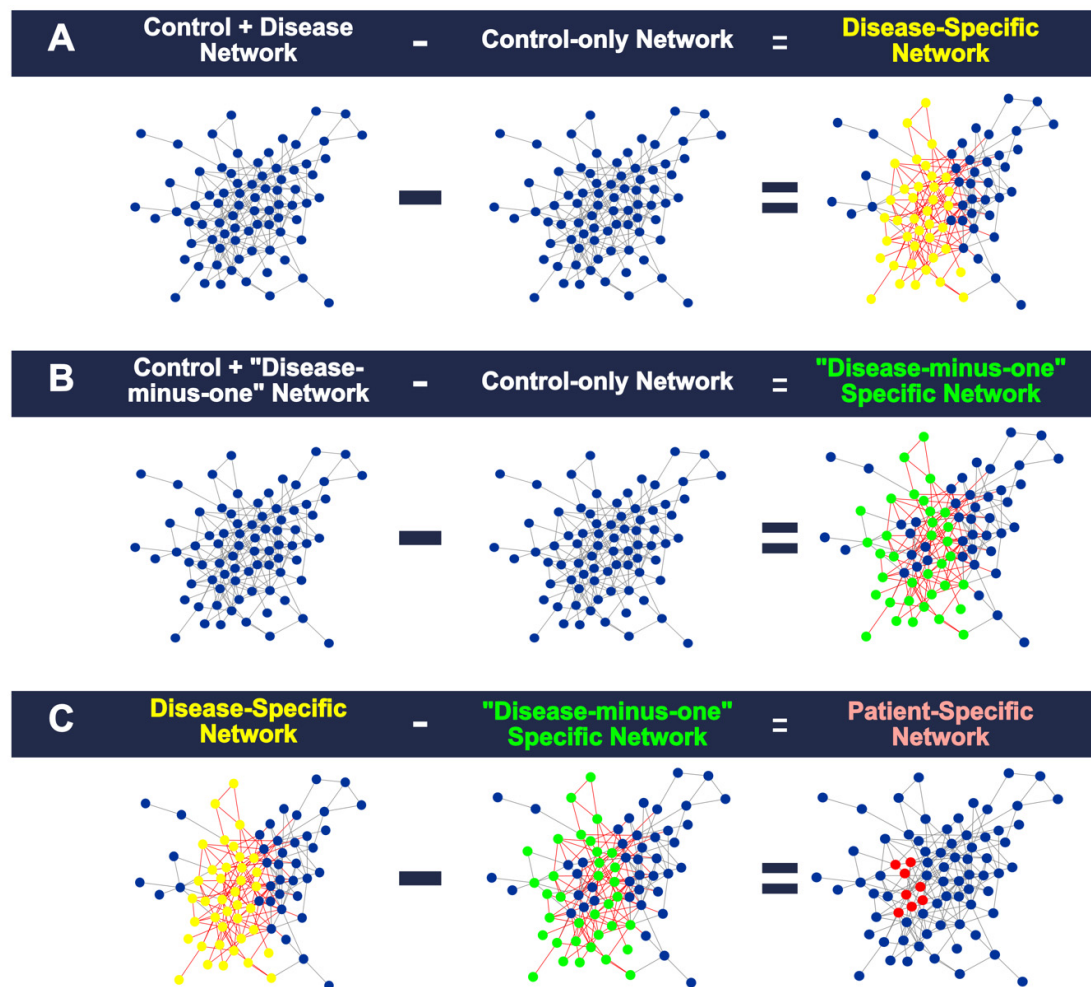


Figure S1. Network analysis overview. Networks are built using Partial Correlation. The first network ("disease-control" network) is learned from disease profiles and control profiles. A second network composed of only control ("control-only" network). A third network is composed of control profiles and disease excluding one patient at a time ("Disease-minus-one" network). This third network is built iteratively to cover all the patients. A final pruning stage subtracts edges from the disease-control network that are also found either in A) the control-only network, outputting the disease-specific network B) "Disease-minus-one" specific network or C) Patient-specific network.

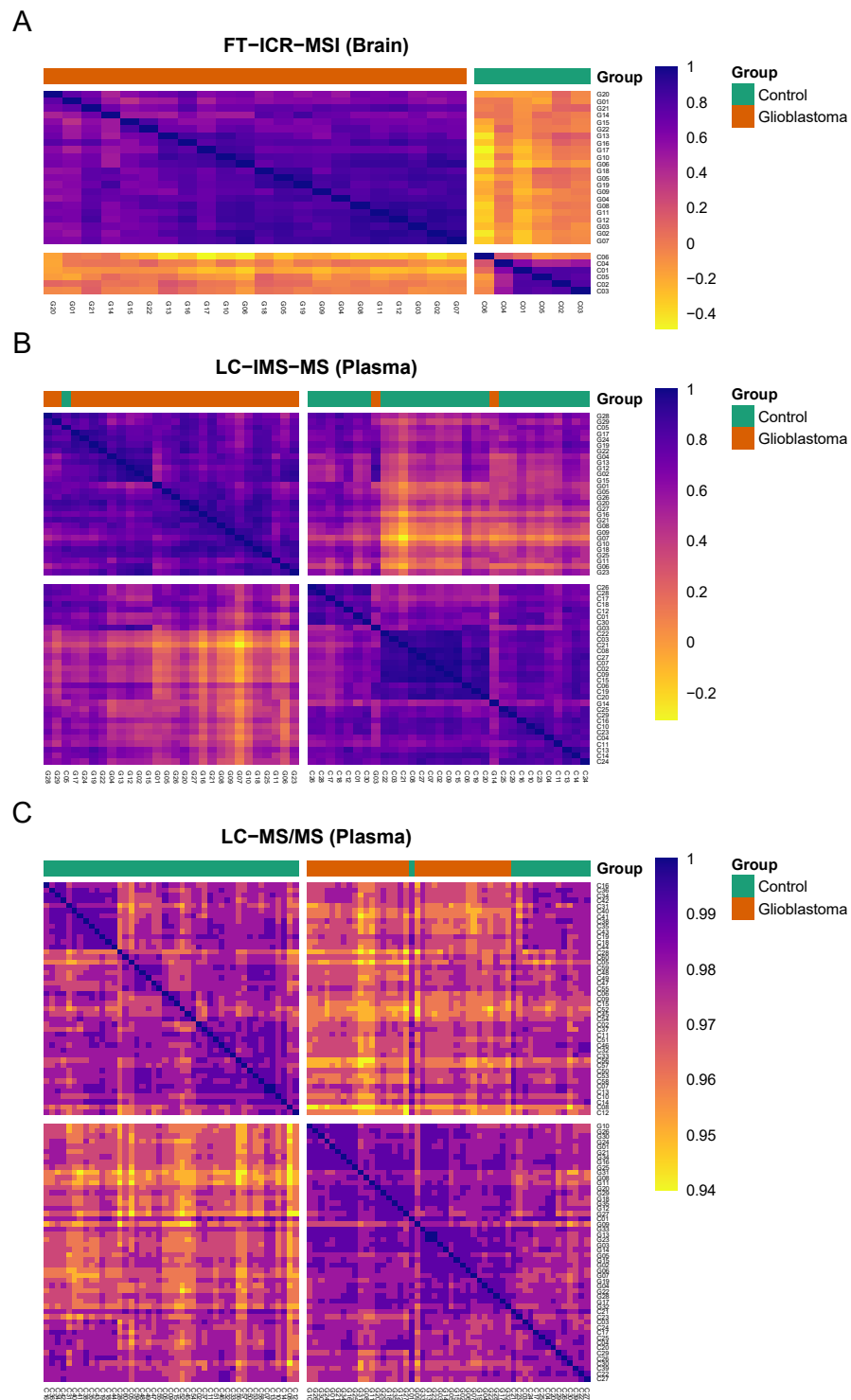


Figure S2. Spearman correlations between samples.

Glioblastoma – Previously Reported Metabolites

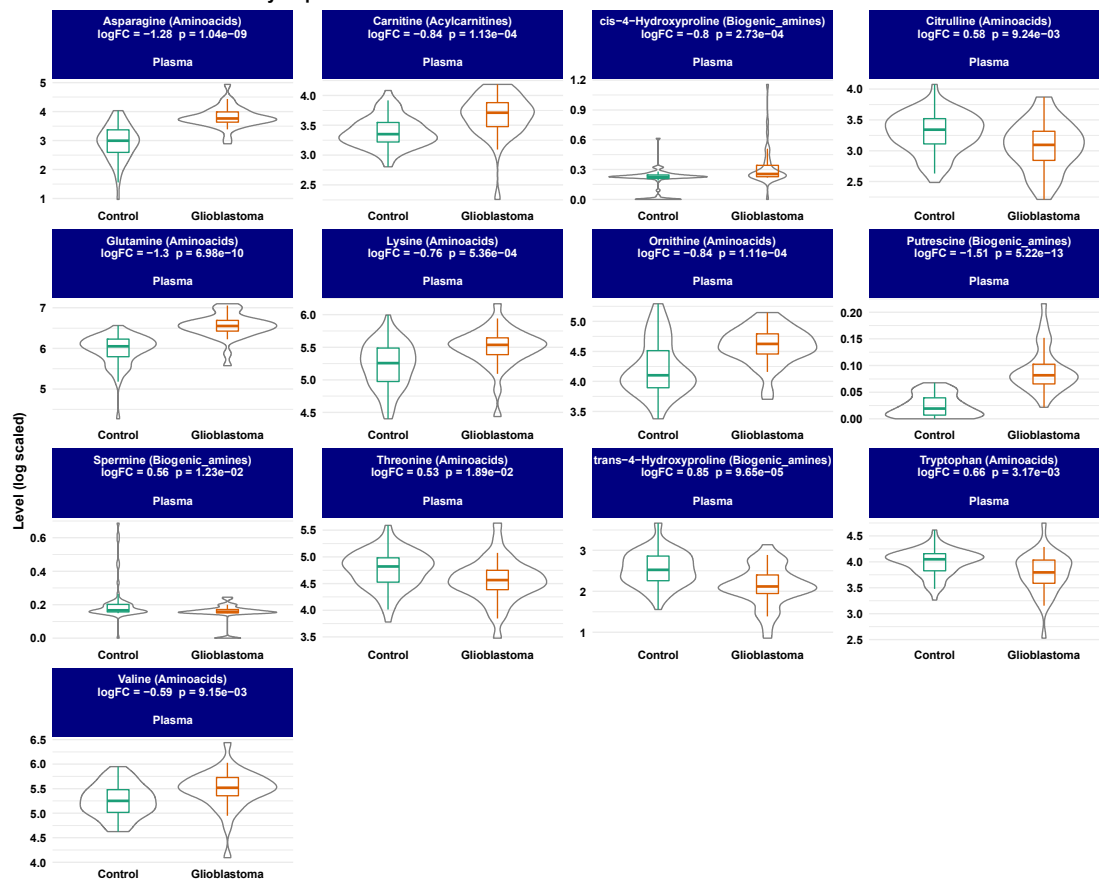


Figure S3. Boxplots of previously reported metabolites.

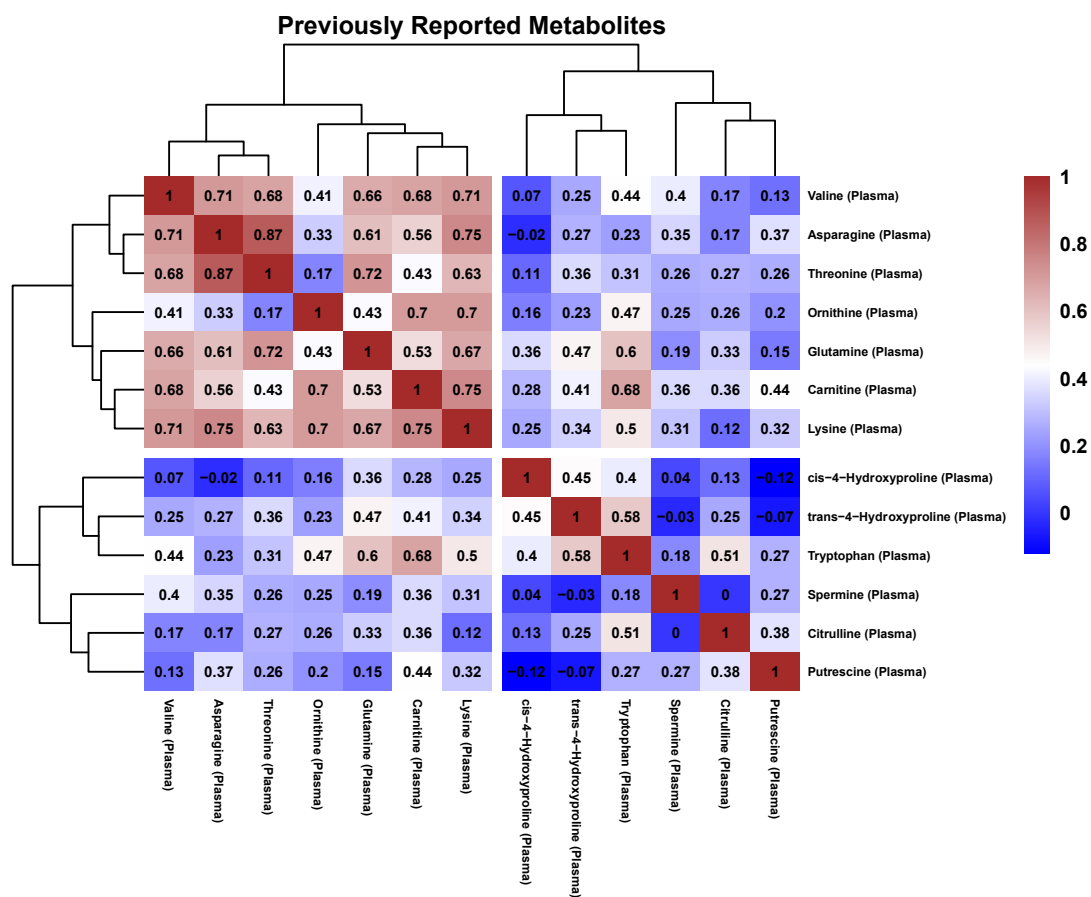


Figure S4. Heatmap of Spearman correlations of previously reported metabolites.

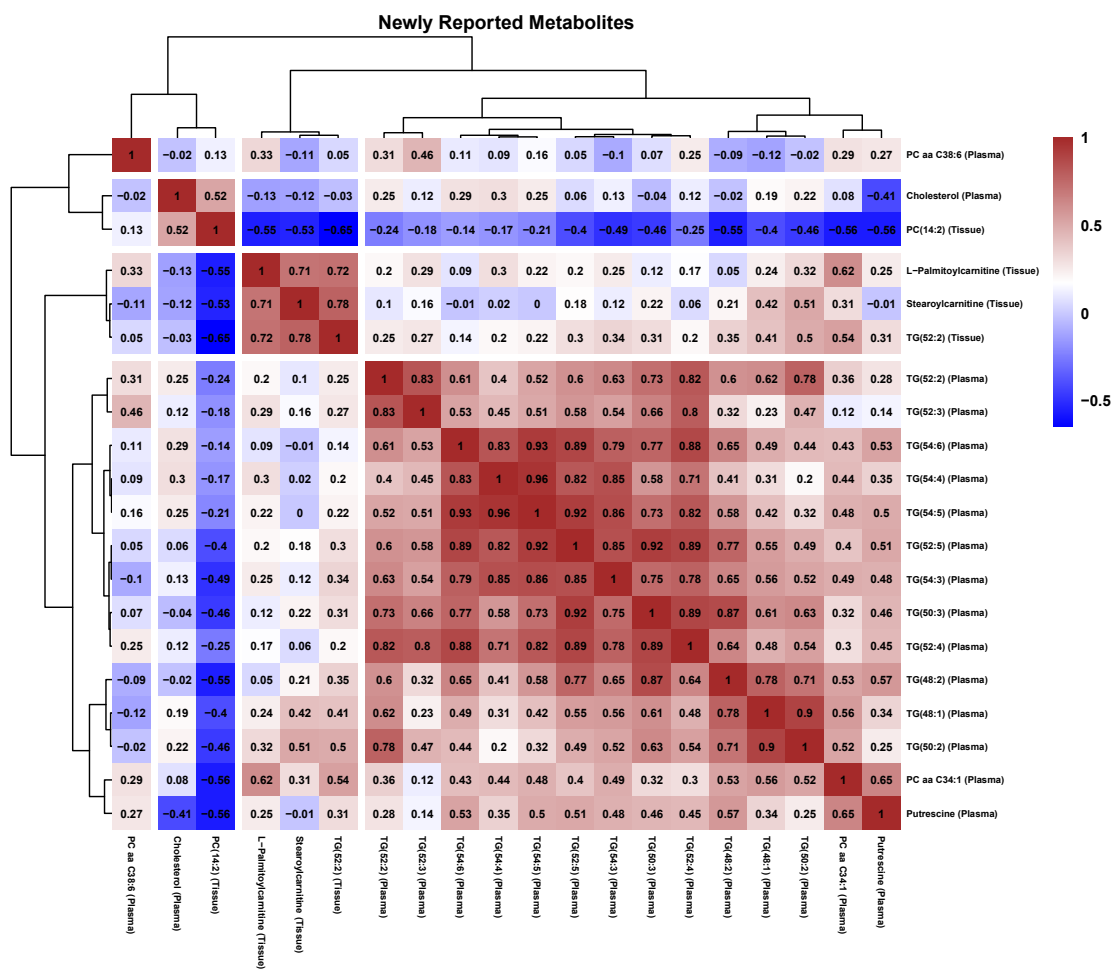


Figure S5. Heatmap of Spearman correlations of newly reported metabolites.

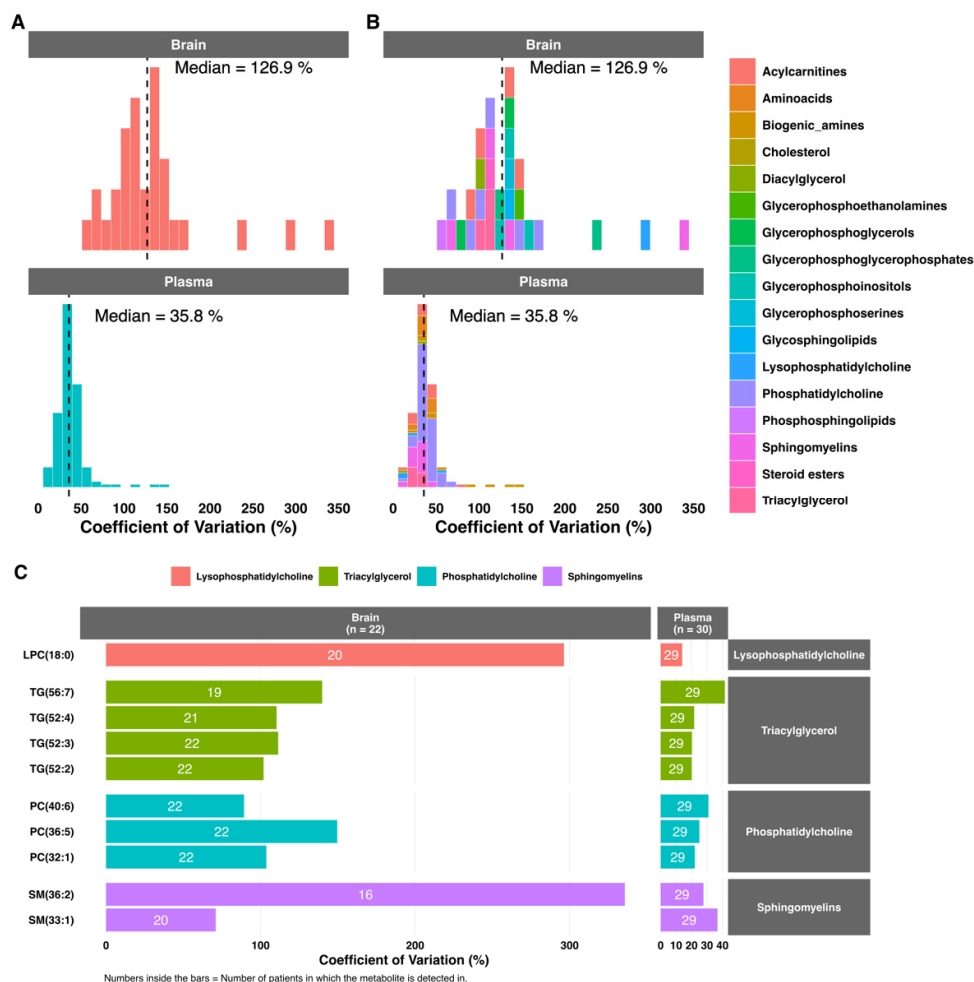


Figure S6. Intra-group (GBM) variation. A) Histogram distribution of coefficient of variation in brain and plasma (Colored according to matrice). B) Histogram distribution of coefficient of variation in brain and plasma (Colored according to biochemical classes). C) Coefficient of variation of the 10 lipids correlated between plasma and brain samples.

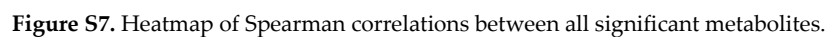


Figure S7. Heatmap of Spearman correlations between all significant metabolites.

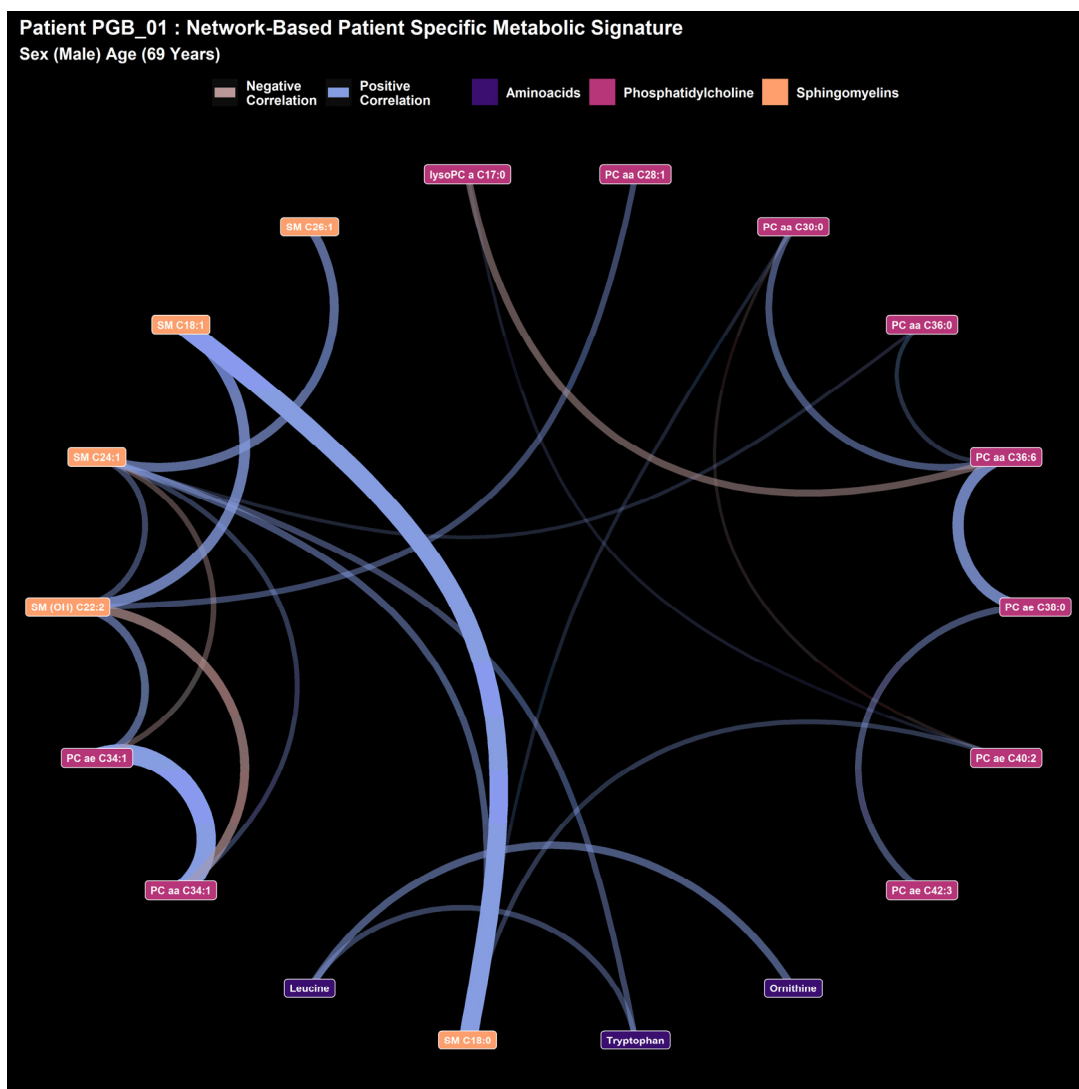


Figure S8. Network-based patient specific plasma metabolic signature (Patient PGB_01). The signature includes 18 metabolites; 10 phosphatidylcholines, 5 sphingomyelin and 3 aminoacids.

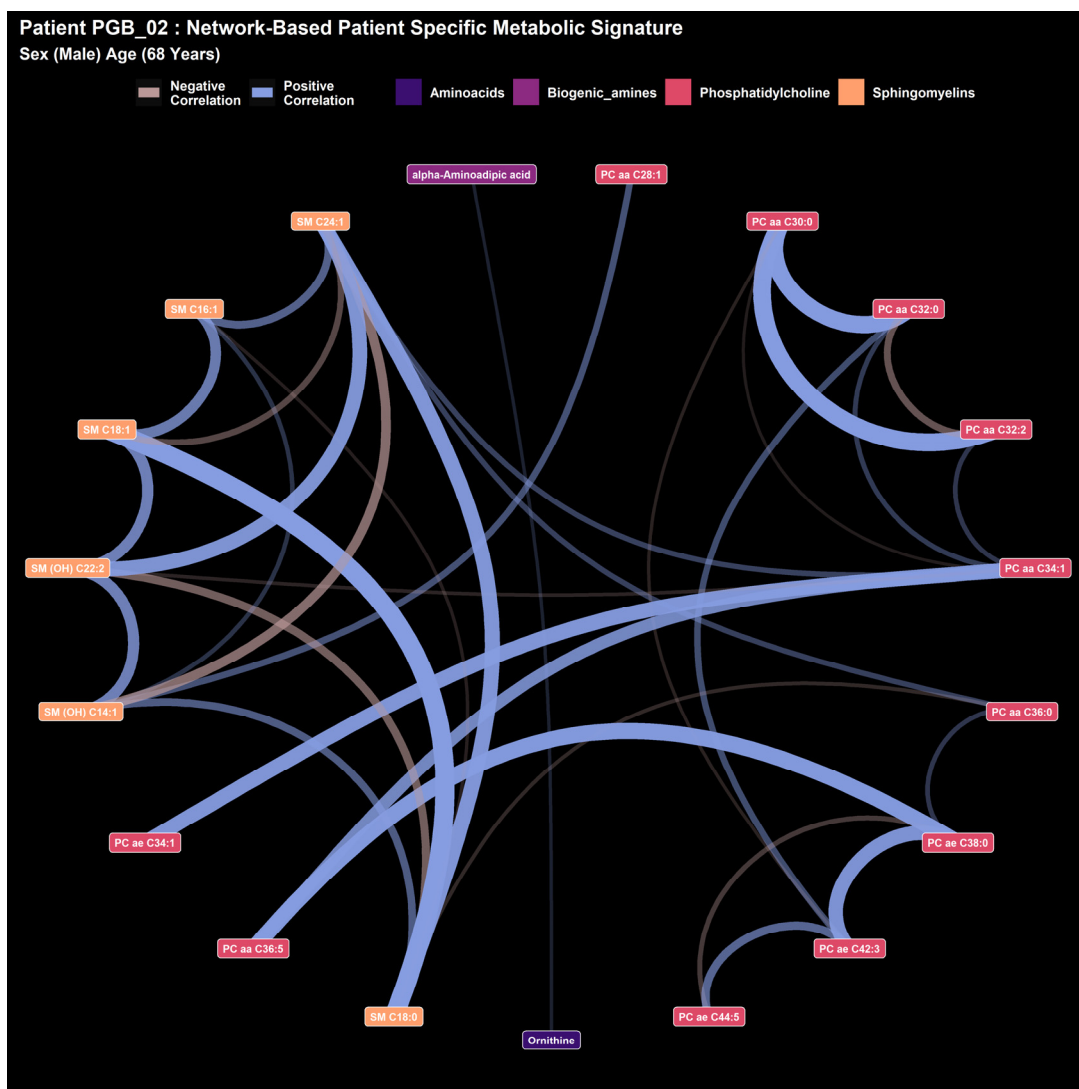


Figure S9. Network-based patient specific plasma metabolic signature (Patient PGB_02). The signature includes 19 metabolites; 11 phosphatidylcholines, 6 sphingomyelin, 1 aminoacid and 1 biogenic amine.

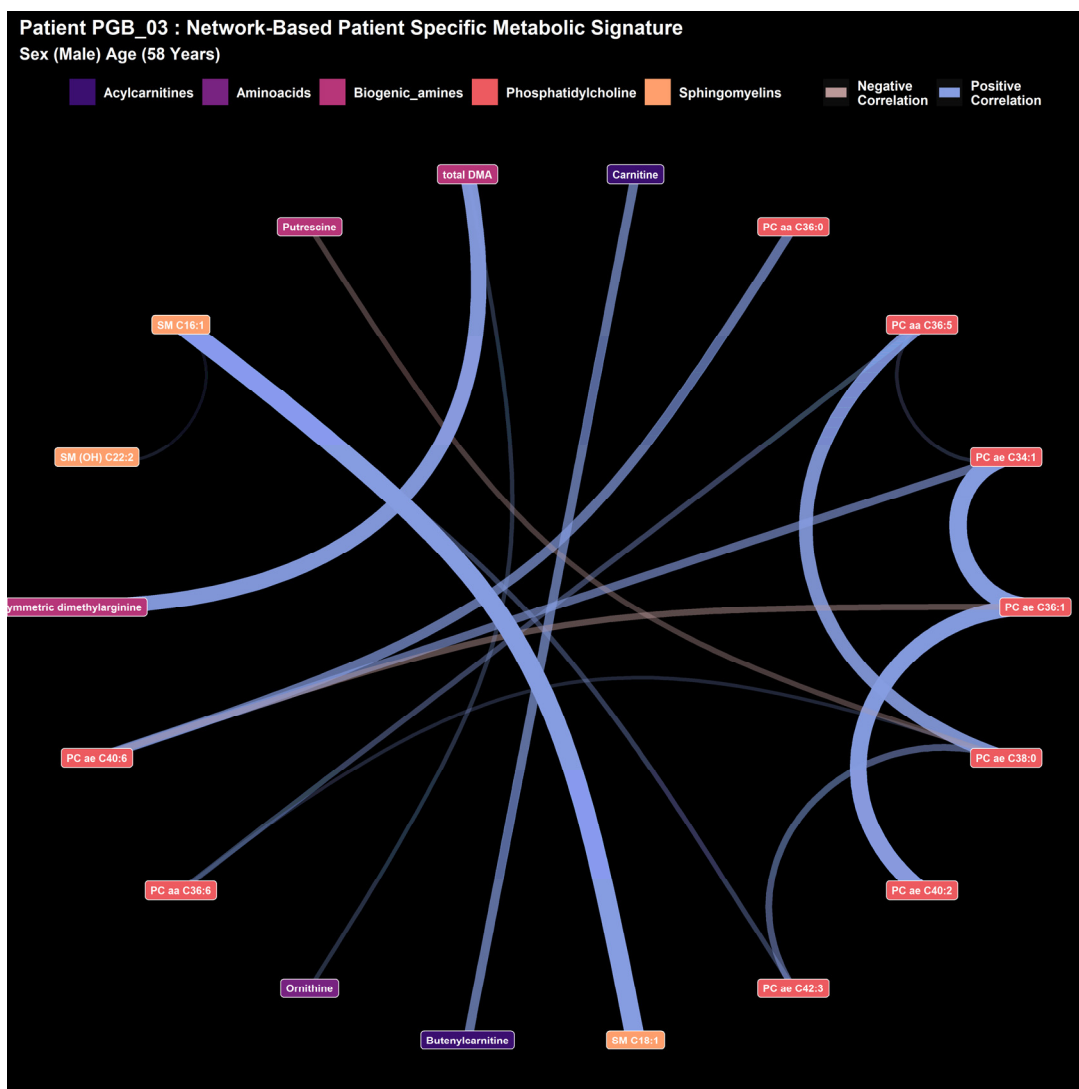


Figure S10. Network-based patient specific plasma metabolic signature (Patient PGB_03). The signature includes 18 metabolites; 9 phosphatidylcholines, 3 sphingomyelin, 1 aminoacid and 3 biogenic amines.

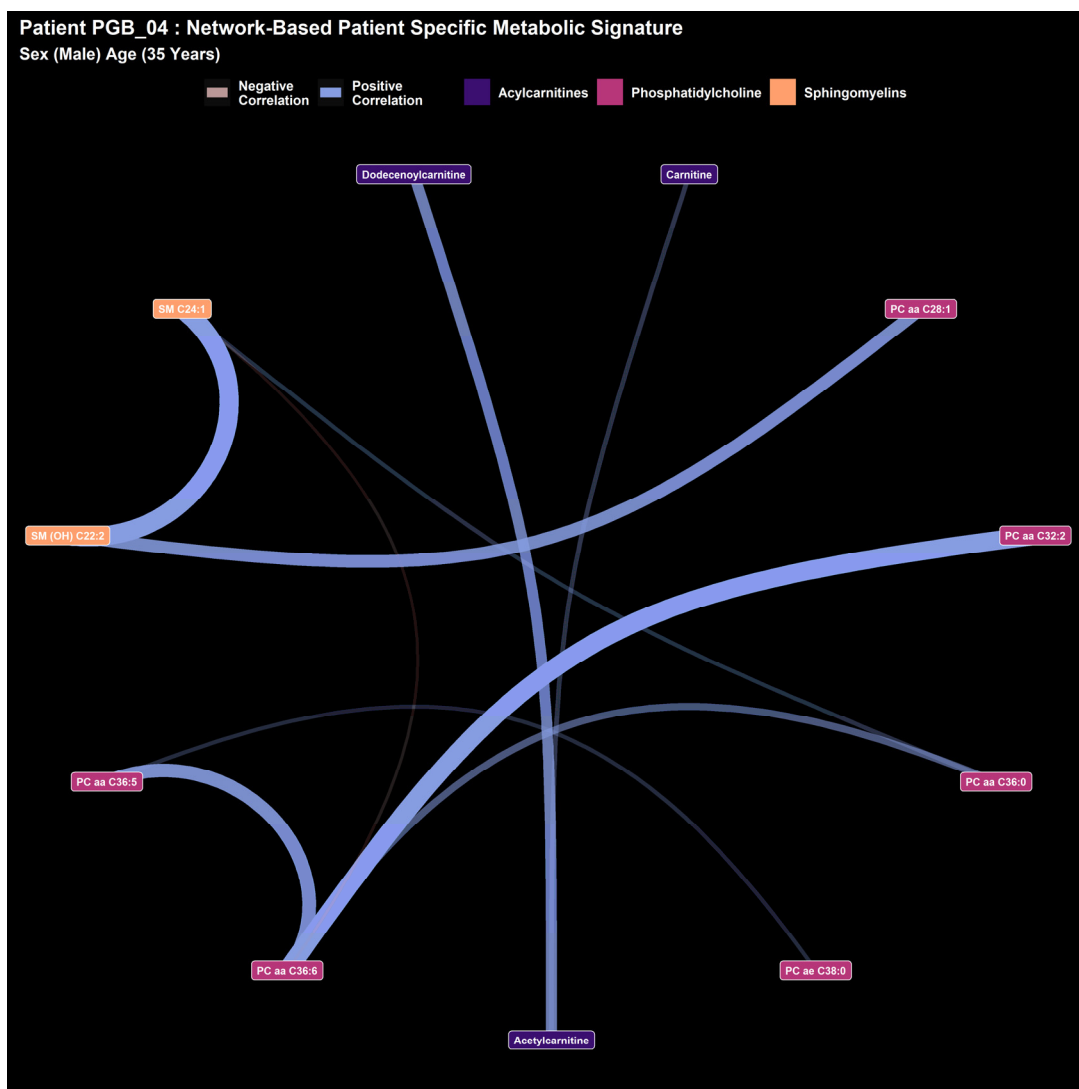


Figure S11. Network-based patient specific plasma metabolic signature (Patient PGB_04). The signature includes 11 metabolites; 6 phosphatidylcholines, 3 acylcarnitines and 2 sphingomyelins.

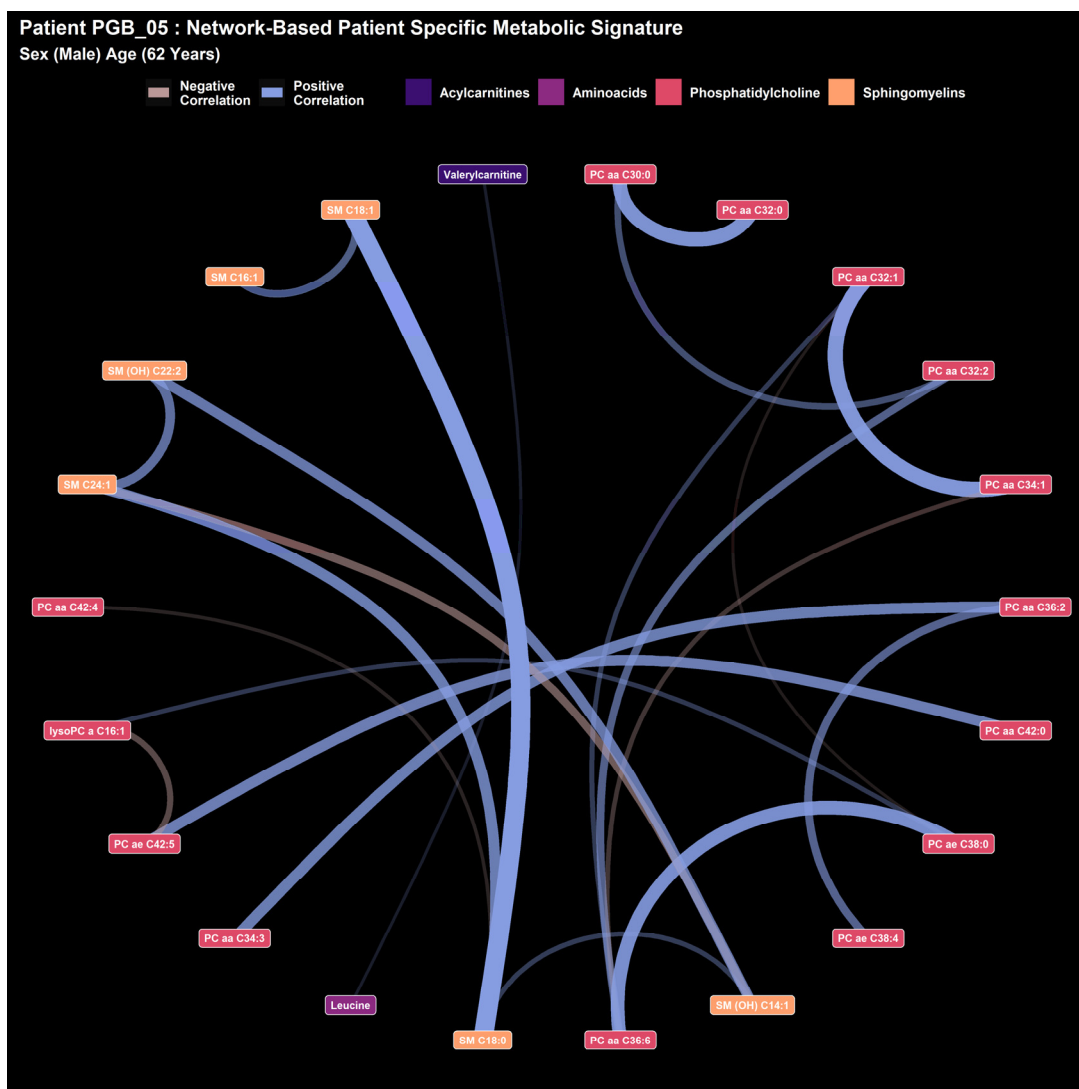


Figure S12. Network-based patient specific plasma metabolic signature (Patient PGB_05). The signature includes 22 metabolites; 14 phosphatidylcholines, 6 sphingomyelin, 1 aminoacid and 1 acylcarnitine.

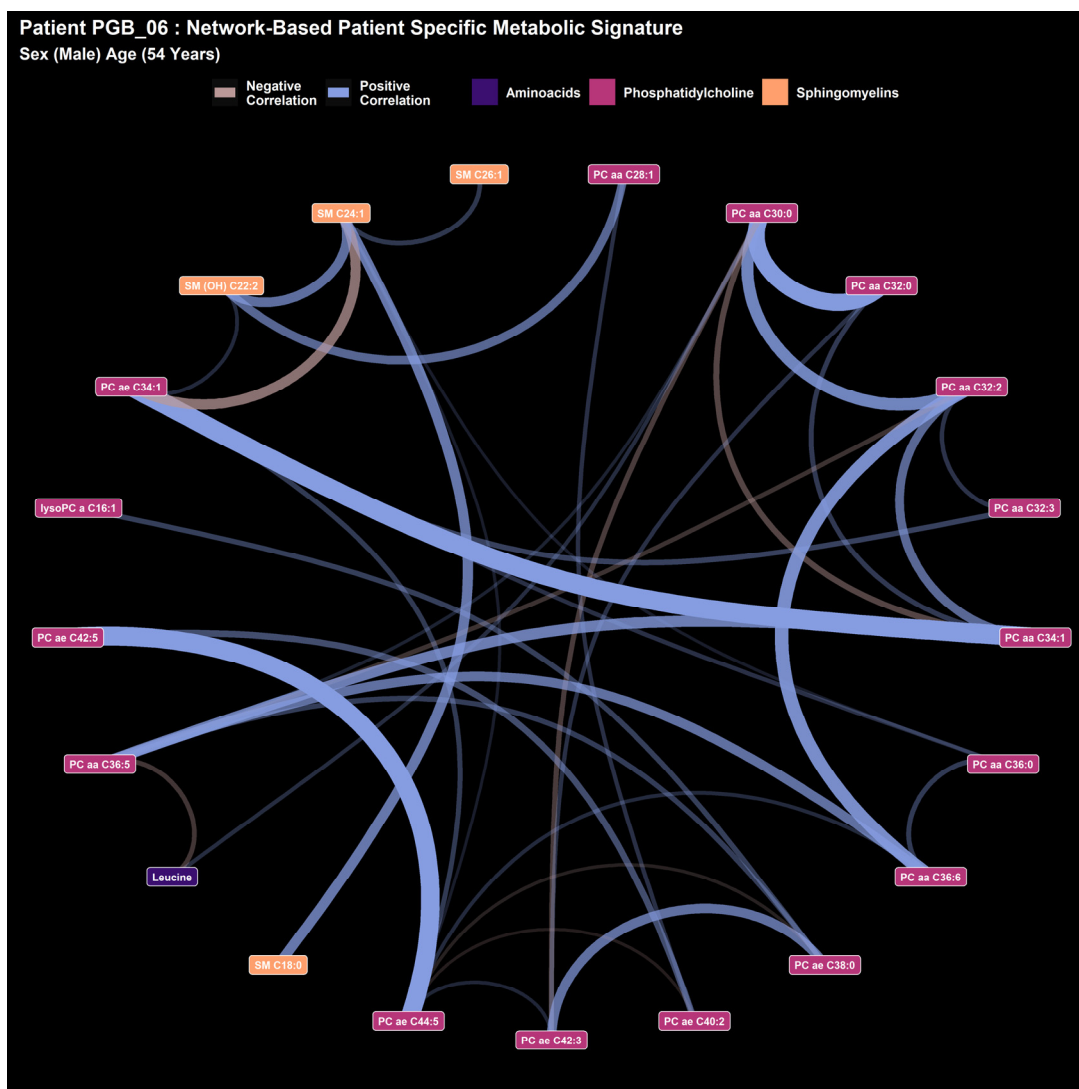


Figure S13. Network-based patient specific plasma metabolic signature (Patient PGB_06). The signature includes 21 metabolites; 16 phosphatidylcholines, 4 sphingomyelins and 1 aminoacid.

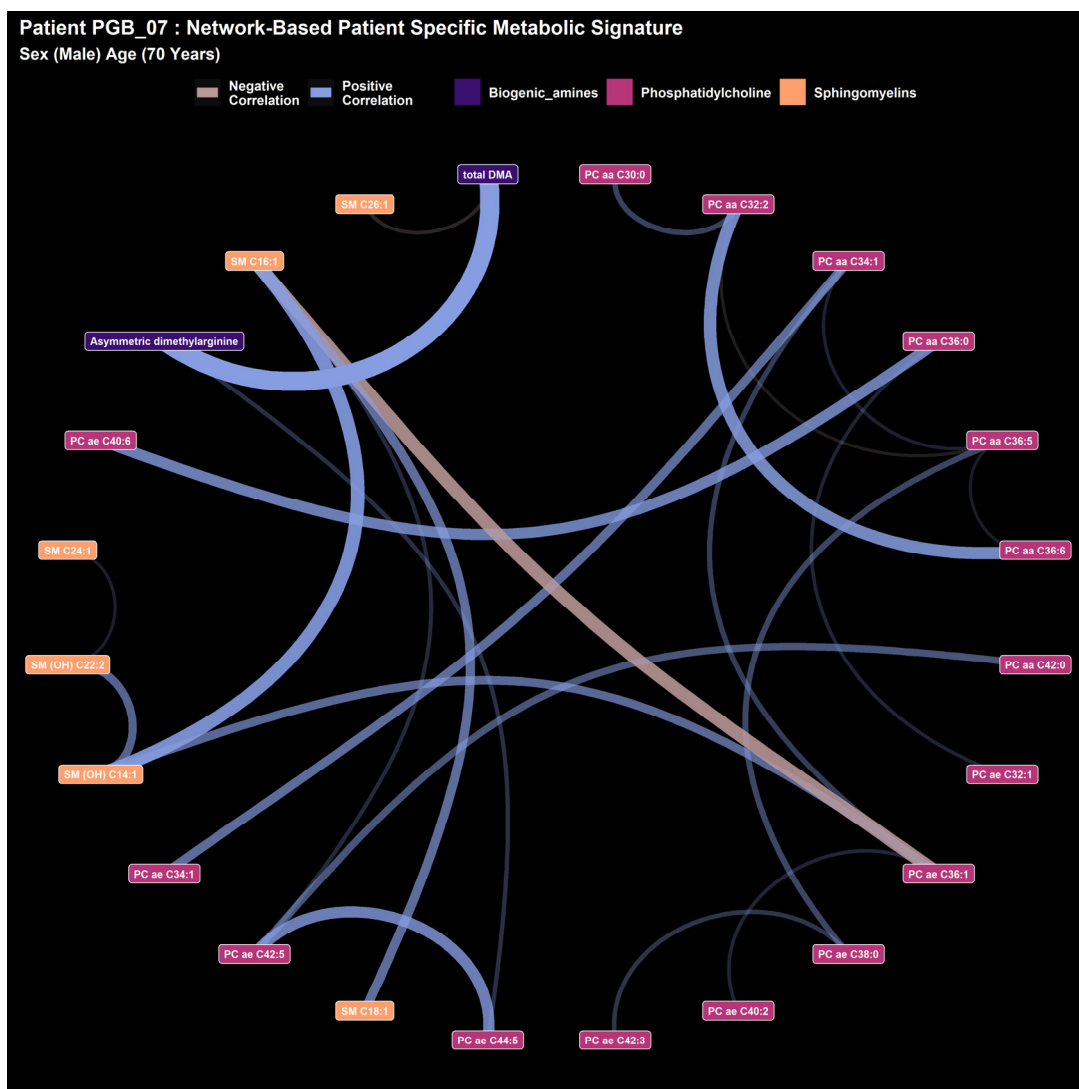


Figure S14. Network-based patient specific plasma metabolic signature (Patient PGB_07). The signature includes 24 metabolites; 16 phosphatidylcholines, 6 sphingomyelins and 2 biogenic amines.

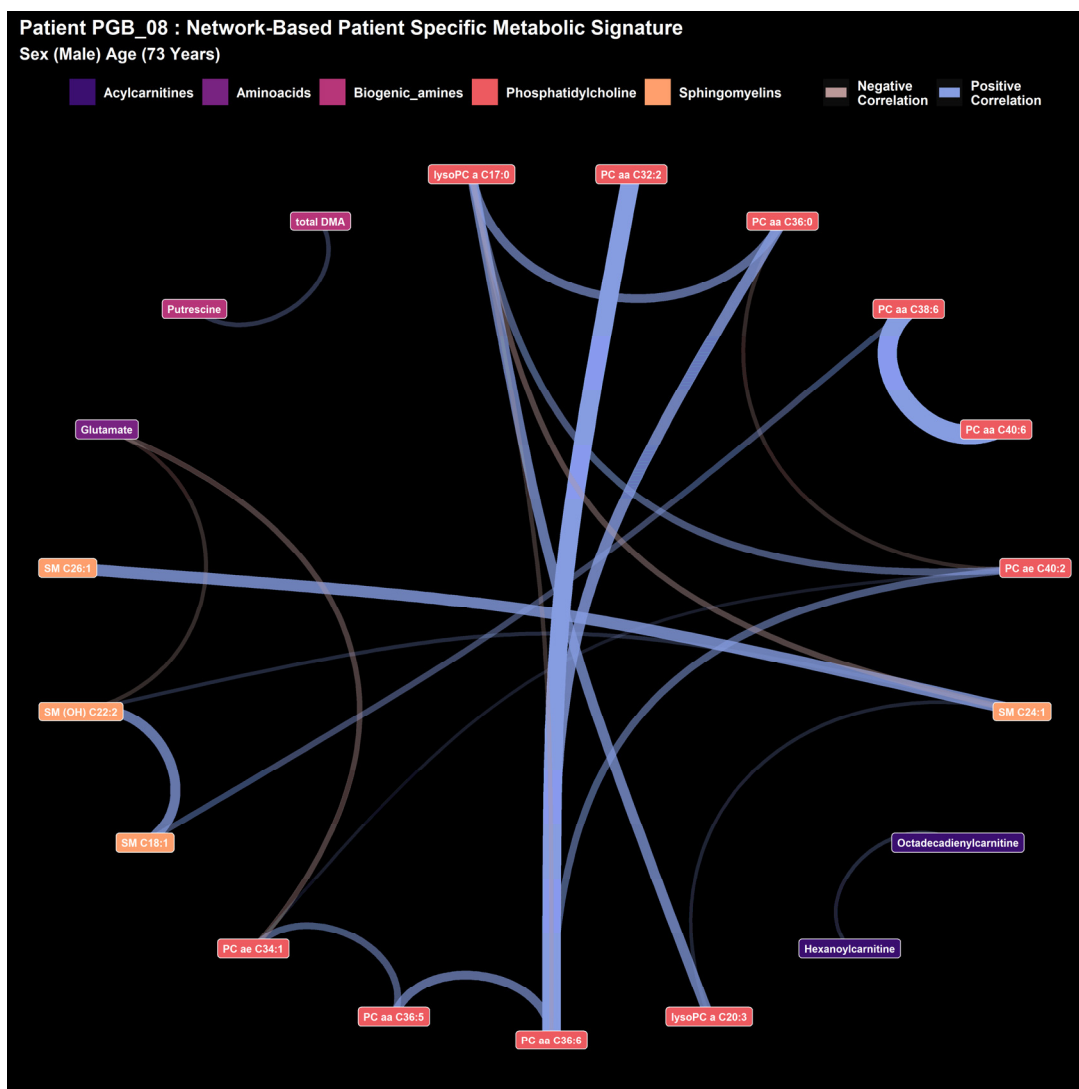


Figure S15. Network-based patient specific plasma metabolic signature (Patient PGB_08). The signature includes 19 metabolites; 10 phosphatidylcholines, 4 sphingomyelins, 2 acylcarnitines, 2 biogenic amines and 1 aminoacid.

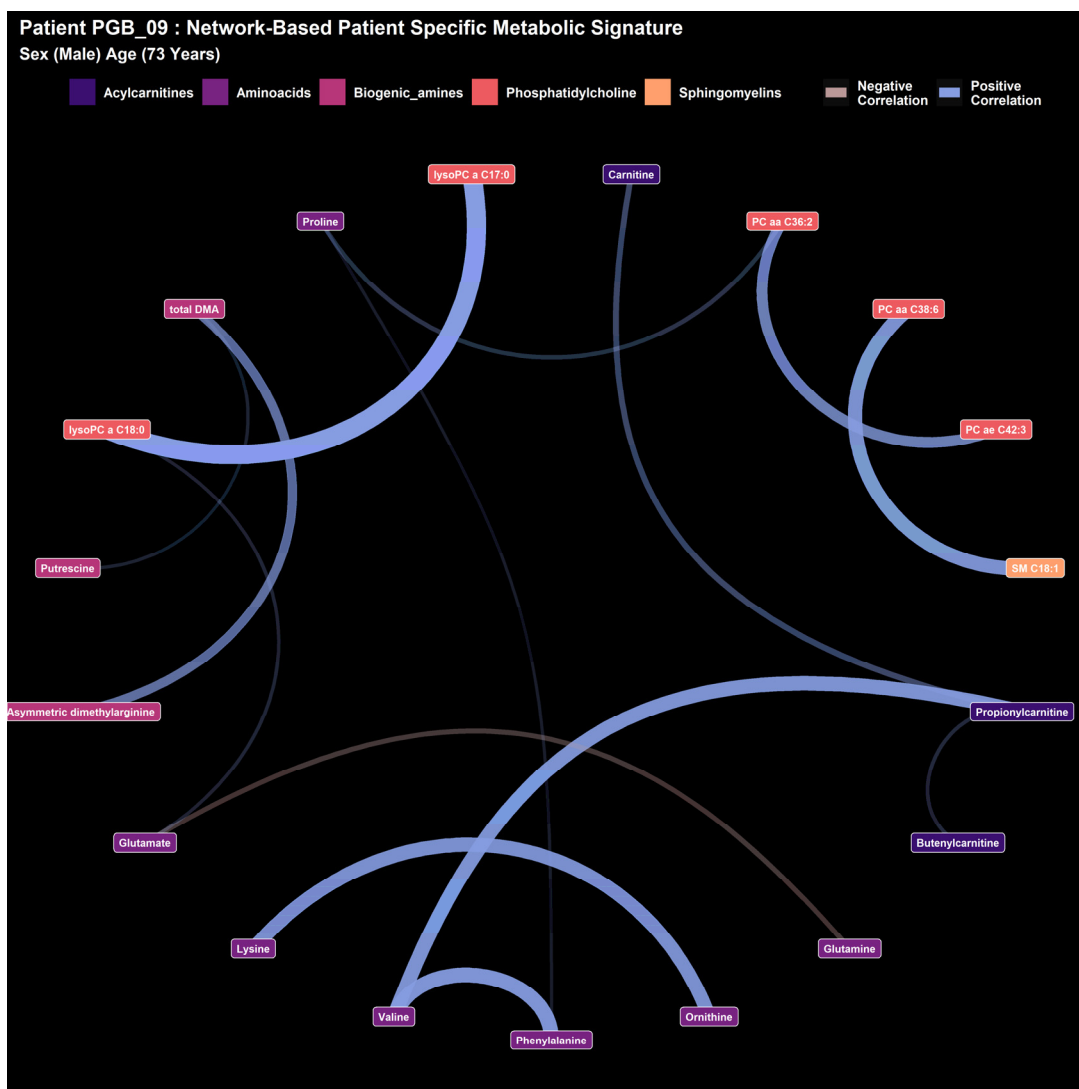


Figure S16. Network-based patient specific plasma metabolic signature (Patient PGB_09). The signature includes 19 metabolites; 5 phosphatidylcholines, 7 aminoacids, 3 acylcarnitines, 3 biogenic amines and 1 sphingomyelin.

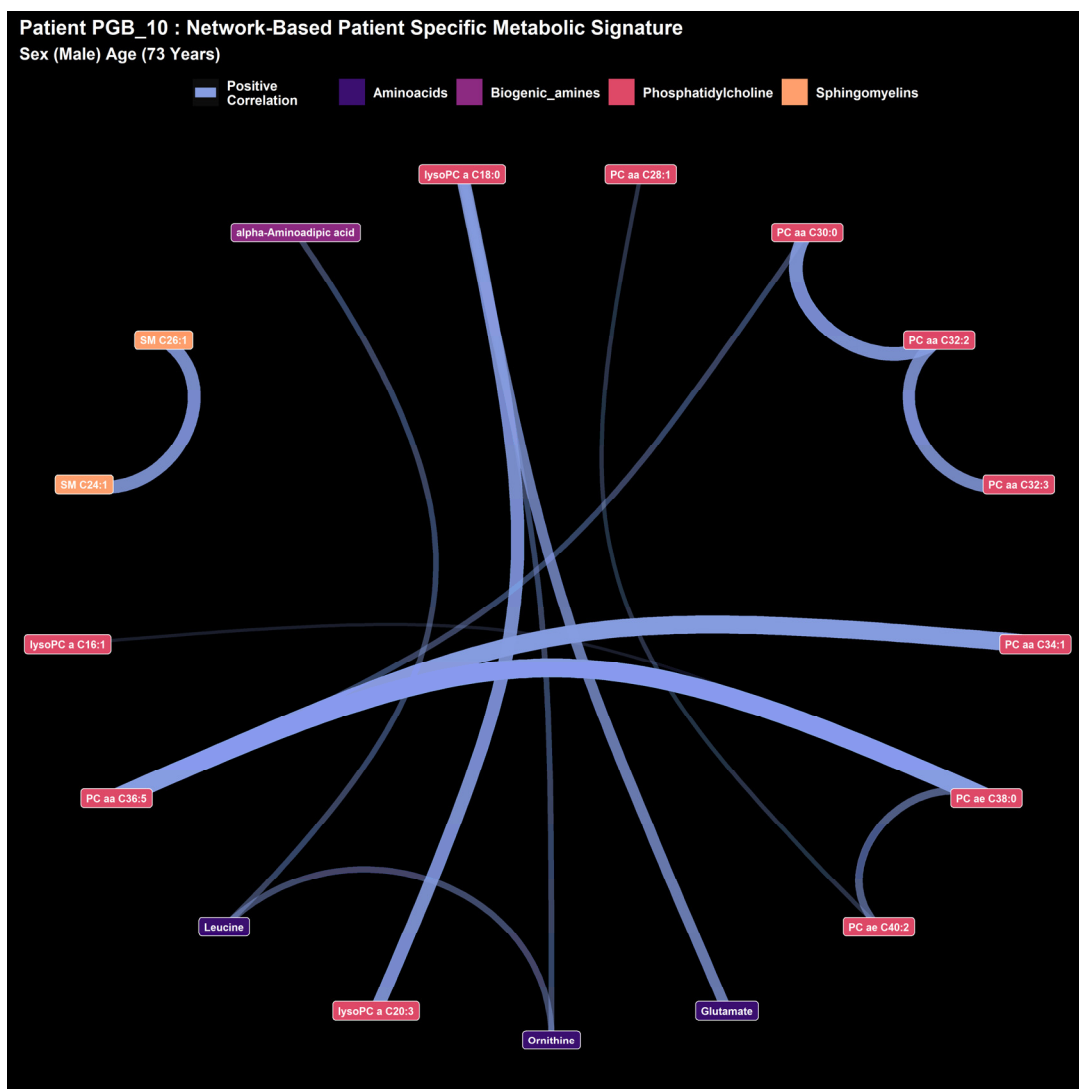


Figure S17. Network-based patient specific plasma metabolic signature (Patient PGB_10). The signature includes 17 metabolites; 11 phosphatidylcholines, 3 aminoacids, 1 biogenic amine and 2 sphingomyelins.

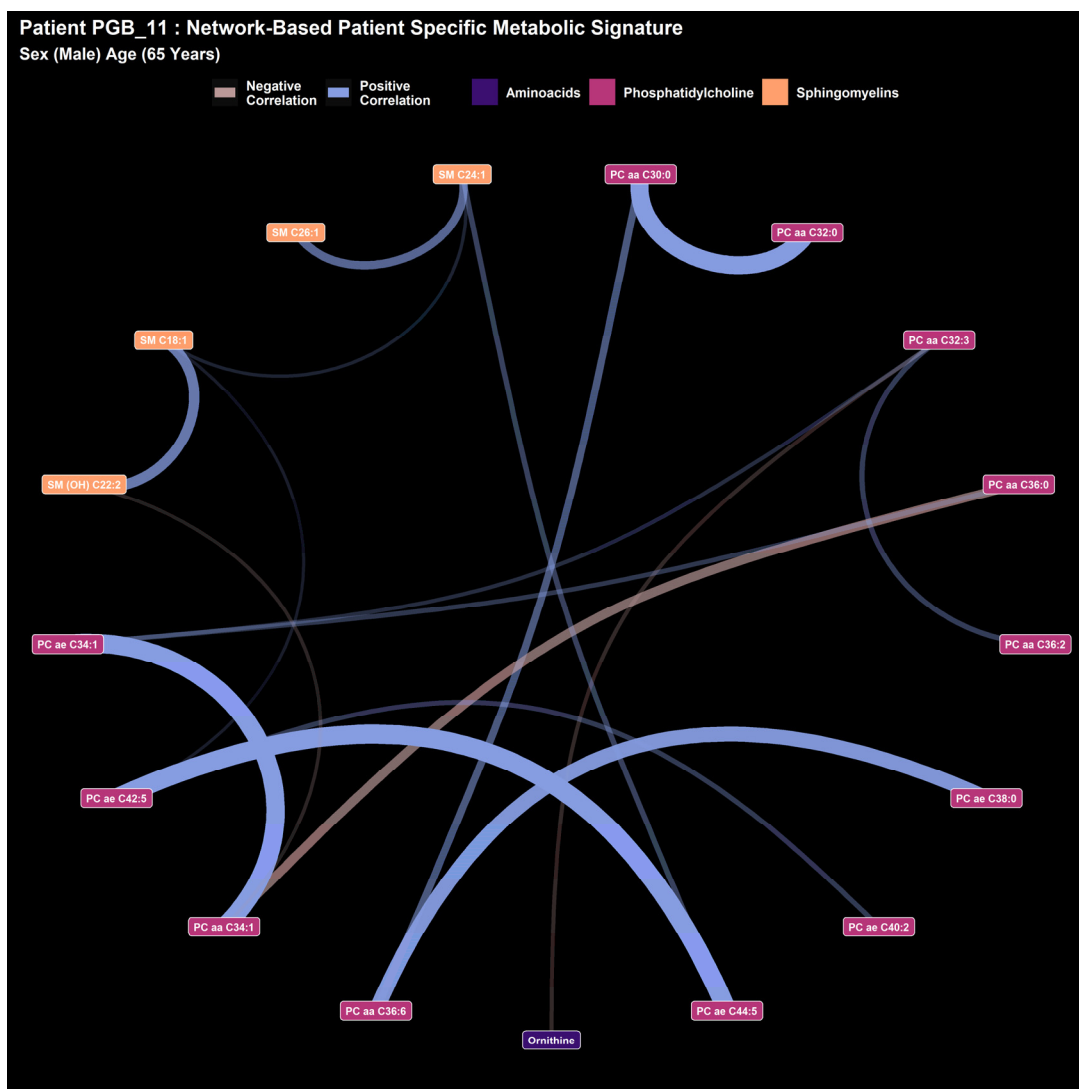


Figure S18. Network-based patient specific plasma metabolic signature (Patient PGB_11). The signature includes 17 metabolites; 12 phosphatidylcholines, 1 aminoacid, and 4 sphingomyelins.

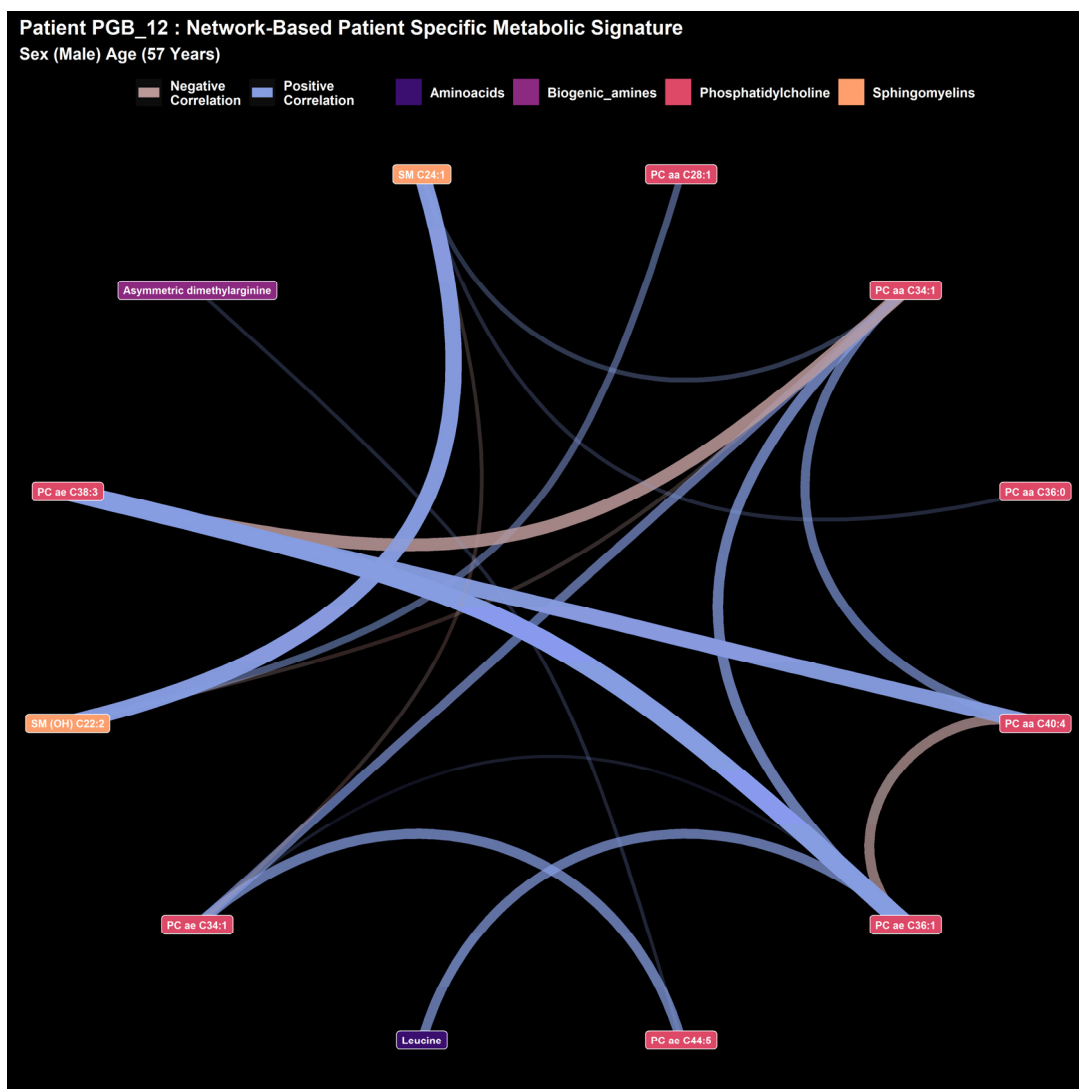


Figure S19. Network-based patient specific plasma metabolic signature (Patient PGB_12). The signature includes 12 metabolites; 8 phosphatidylcholines, 2 aminoacids, 1 biogenic amine and 2 sphingomyelins.

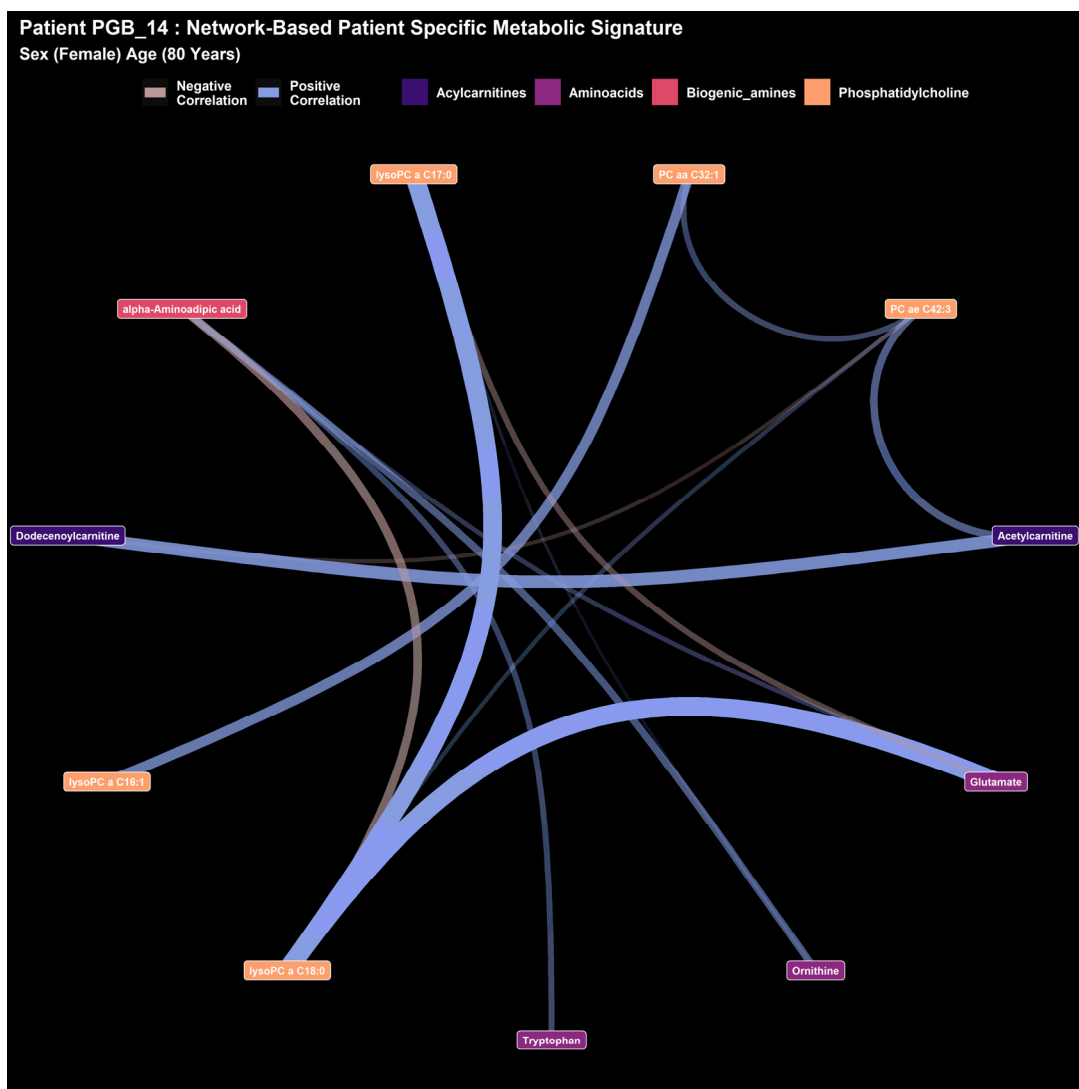


Figure S21. Network-based patient specific plasma metabolic signature (Patient PGB_14). The signature includes 11 metabolites; 5 phosphatidylcholines, 3 aminoacids, 2 acylcarnitines and 1 biogenic amine.

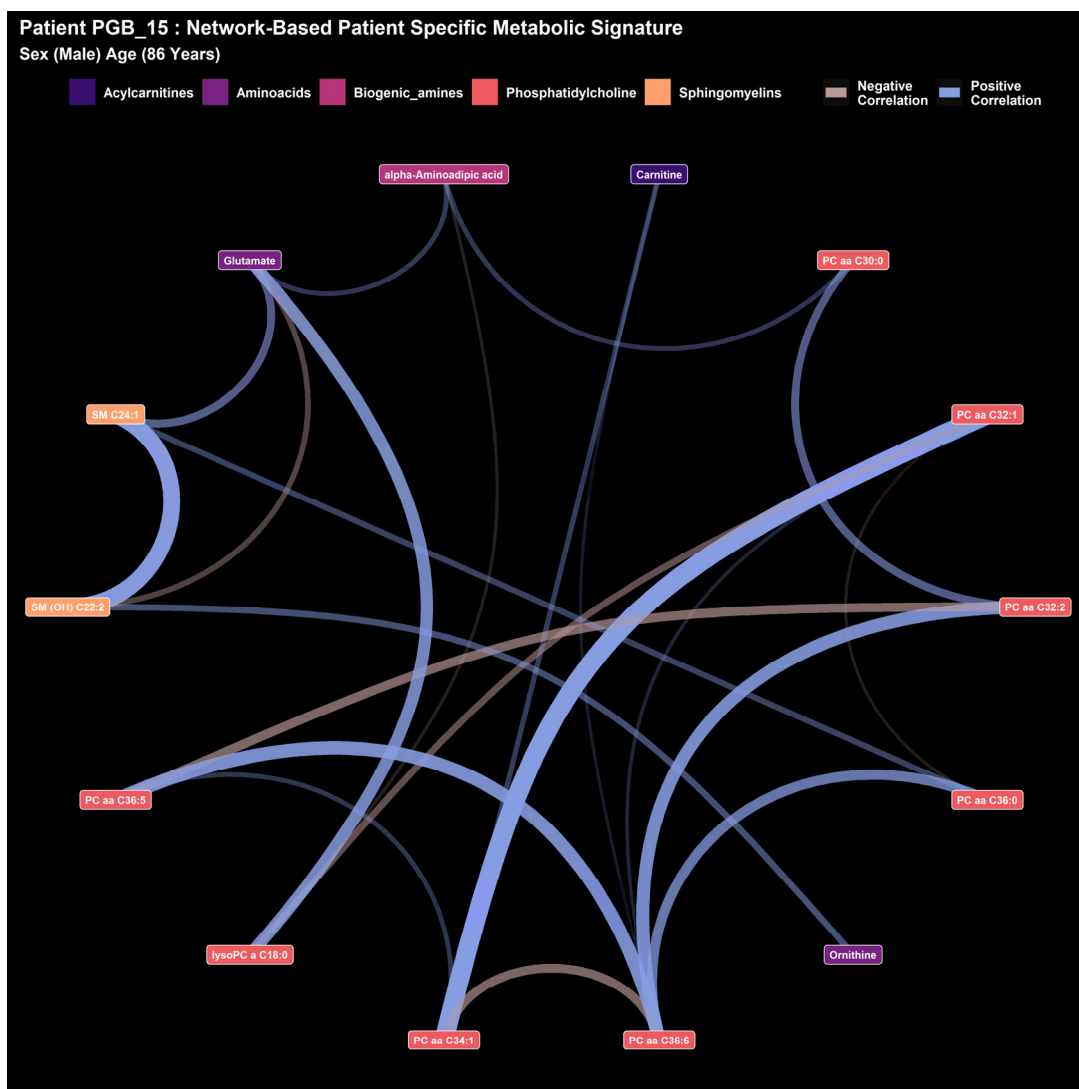


Figure S22. Network-based patient specific plasma metabolic signature (Patient PGB_15). The signature includes 14 metabolites; 8 phosphatidylcholines, 2 aminoacids, 1 acylcarnitine, 1 biogenic amine and 2 sphingomyelins.

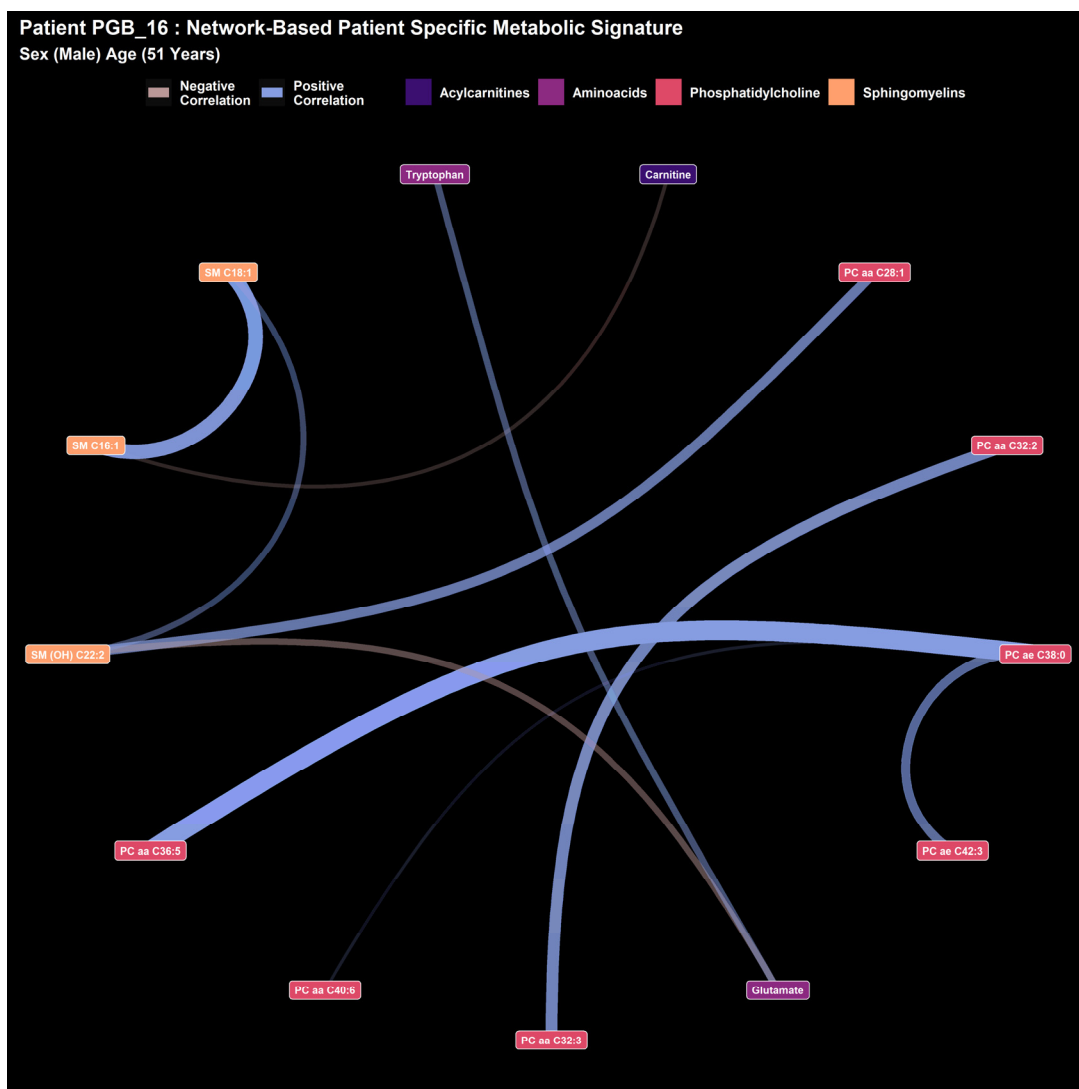


Figure S23. Network-based patient specific plasma metabolic signature (Patient PGB_16). The signature includes 13 metabolites; 7 phosphatidylcholines, 2 aminoacids, 1 acylcarnitine and 3 sphingomyelins.

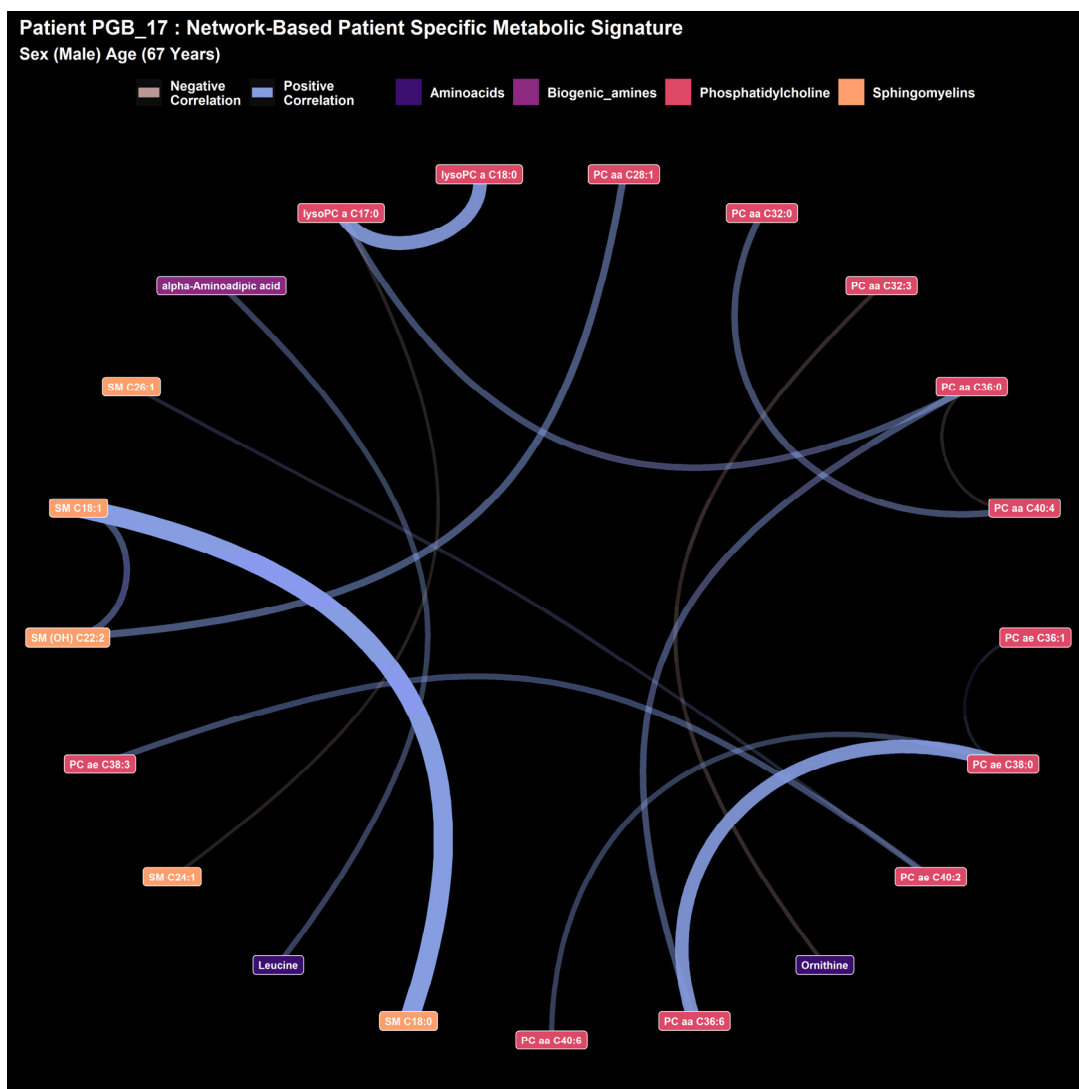


Figure S24. Network-based patient specific plasma metabolic signature (Patient PGB_17). The signature includes 21 metabolites; 13 phosphatidylcholines, 2 aminoacids, 1 biogenic amine and 5 sphingomyelins.

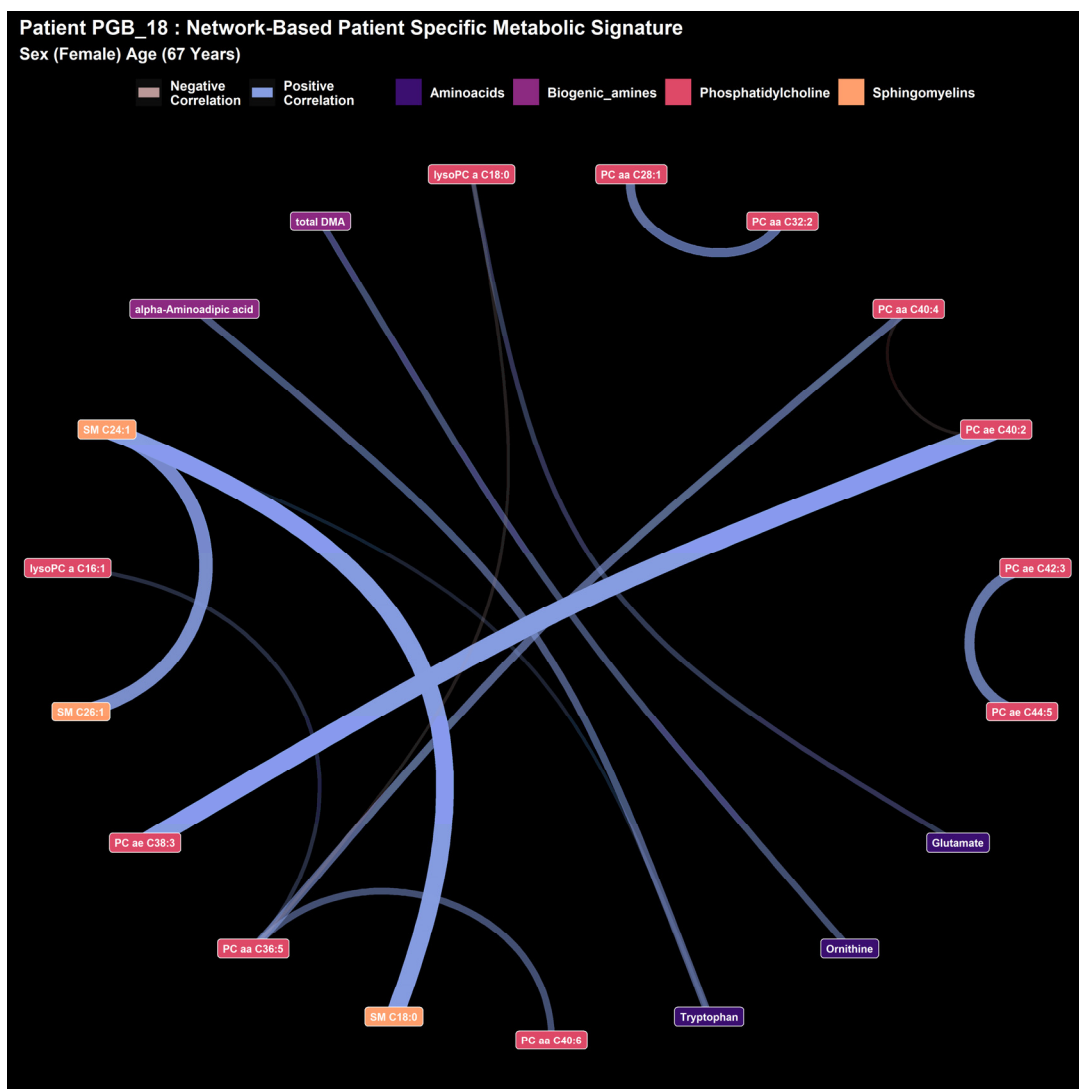


Figure S25. Network-based patient specific plasma metabolic signature (Patient PGB_18). The signature includes 19 metabolites; 11 phosphatidylcholines, 3 aminoacids, 2 biogenic amines and 3 sphingomyelins.

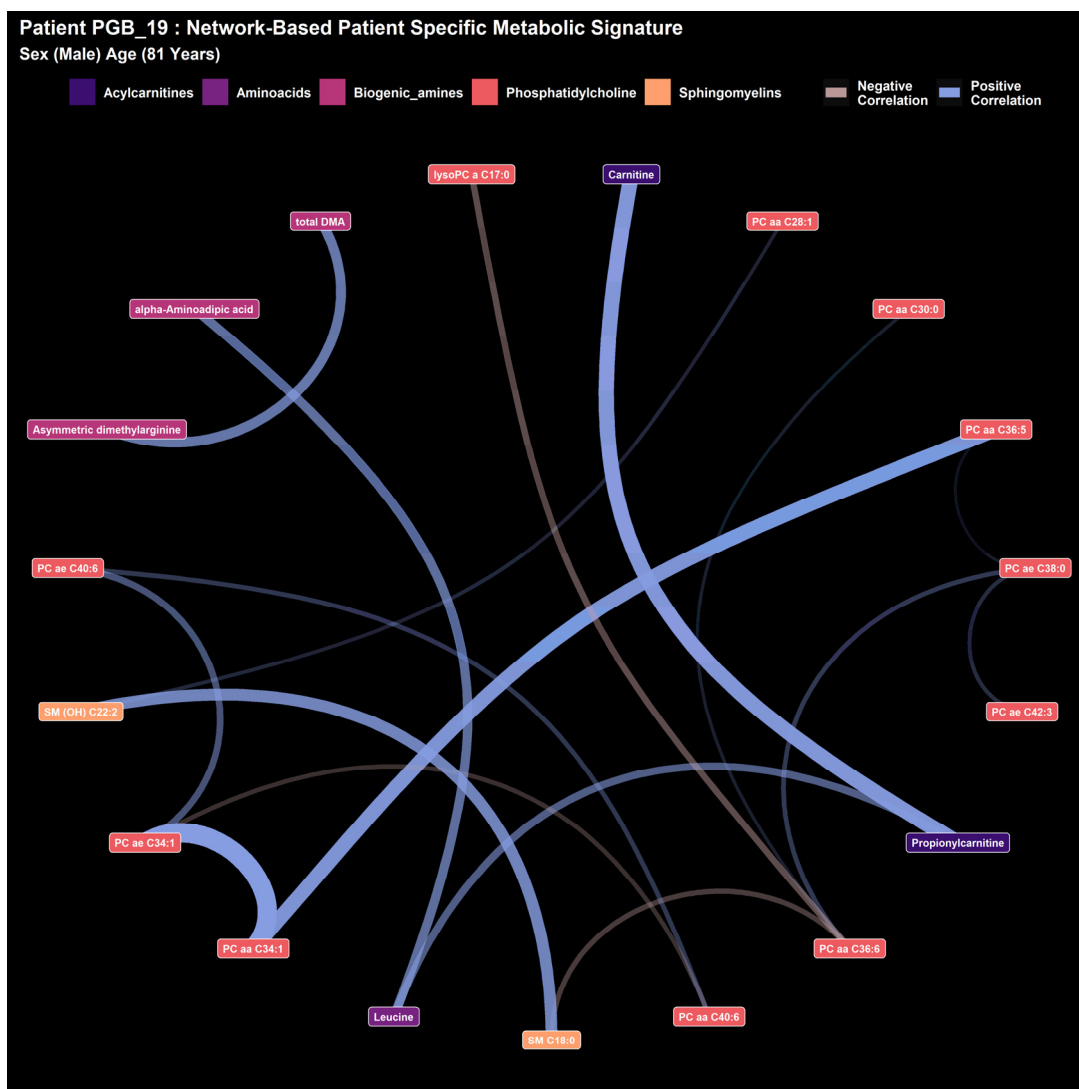


Figure S26. Network-based patient specific plasma metabolic signature (Patient PGB_19). The signature includes 19 metabolites; 11 phosphatidylcholines, 1 aminoacid, 2 acylcarnitines, 3 biogenic amines and 2 sphingomyelins.

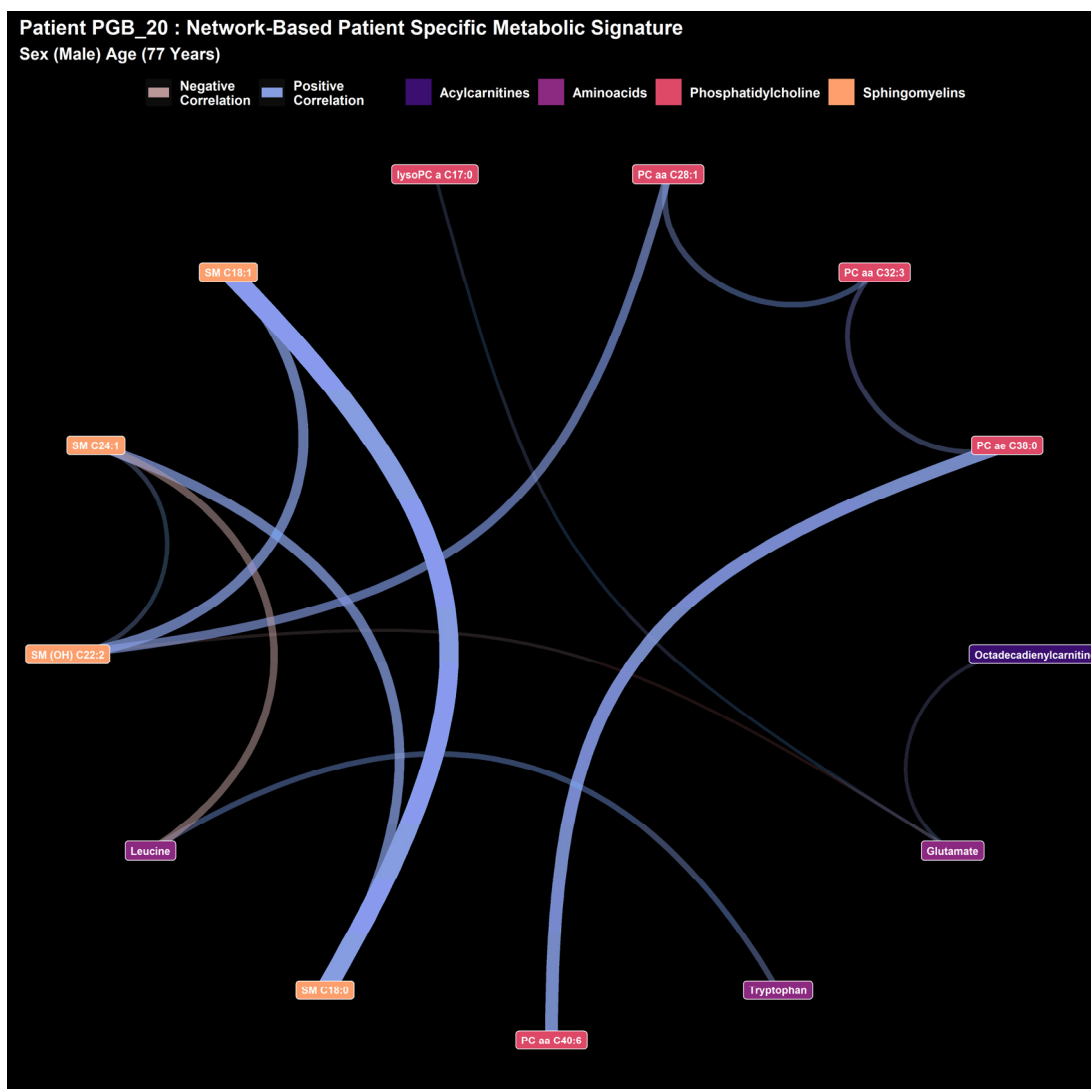


Figure S27. Network-based patient specific plasma metabolic signature (Patient PGB_20). The signature includes 13 metabolites; 5 phosphatidylcholines, 3 aminoacids, 1 acylcarnitine, and 4 sphingomyelins.

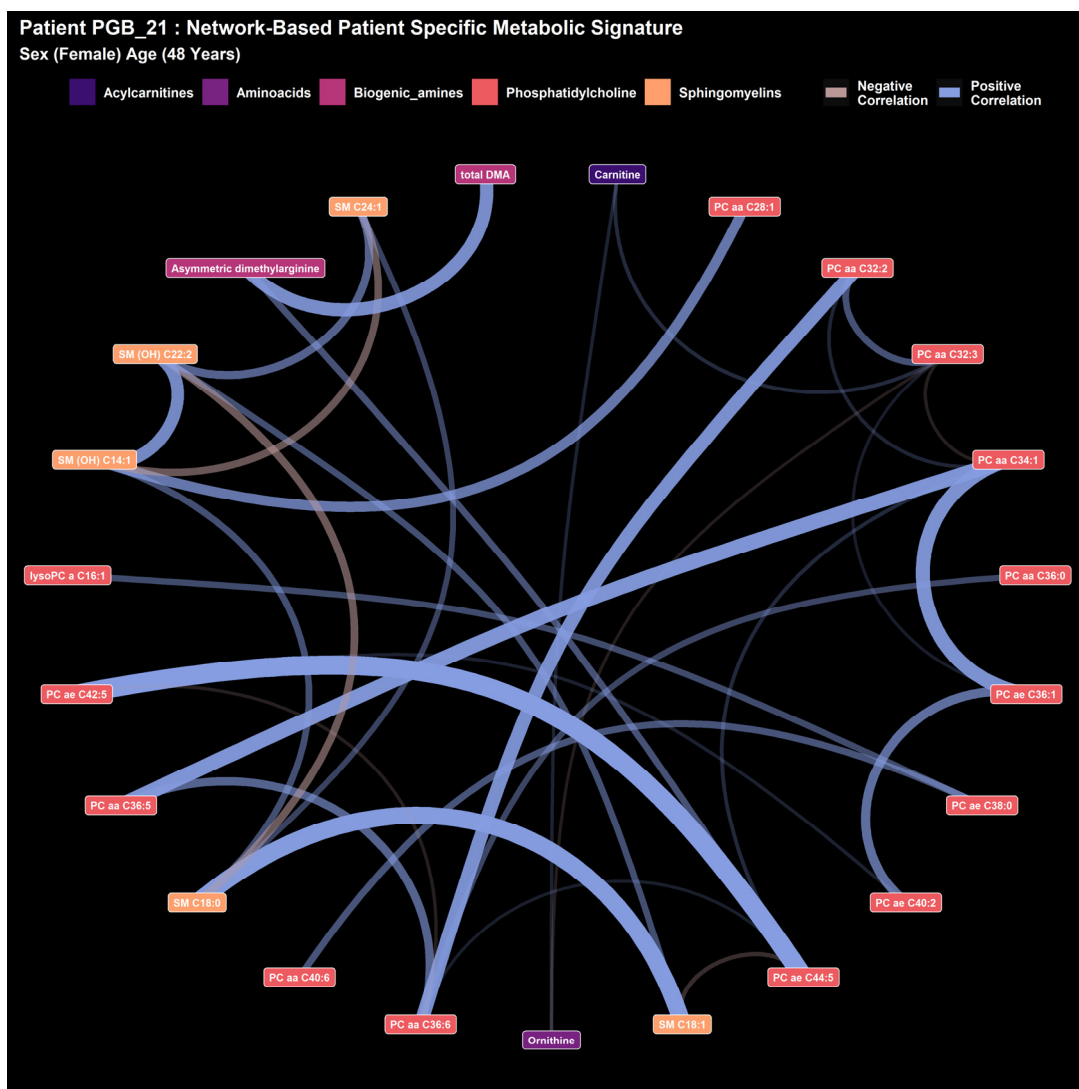


Figure S28. Network-based patient specific plasma metabolic signature (Patient PGB_21). The signature includes 23 metabolites; 14 phosphatidylcholines, 1 aminoacid, 1 acylcarnitine, 2 biogenic amines and 5 sphingomyelins.

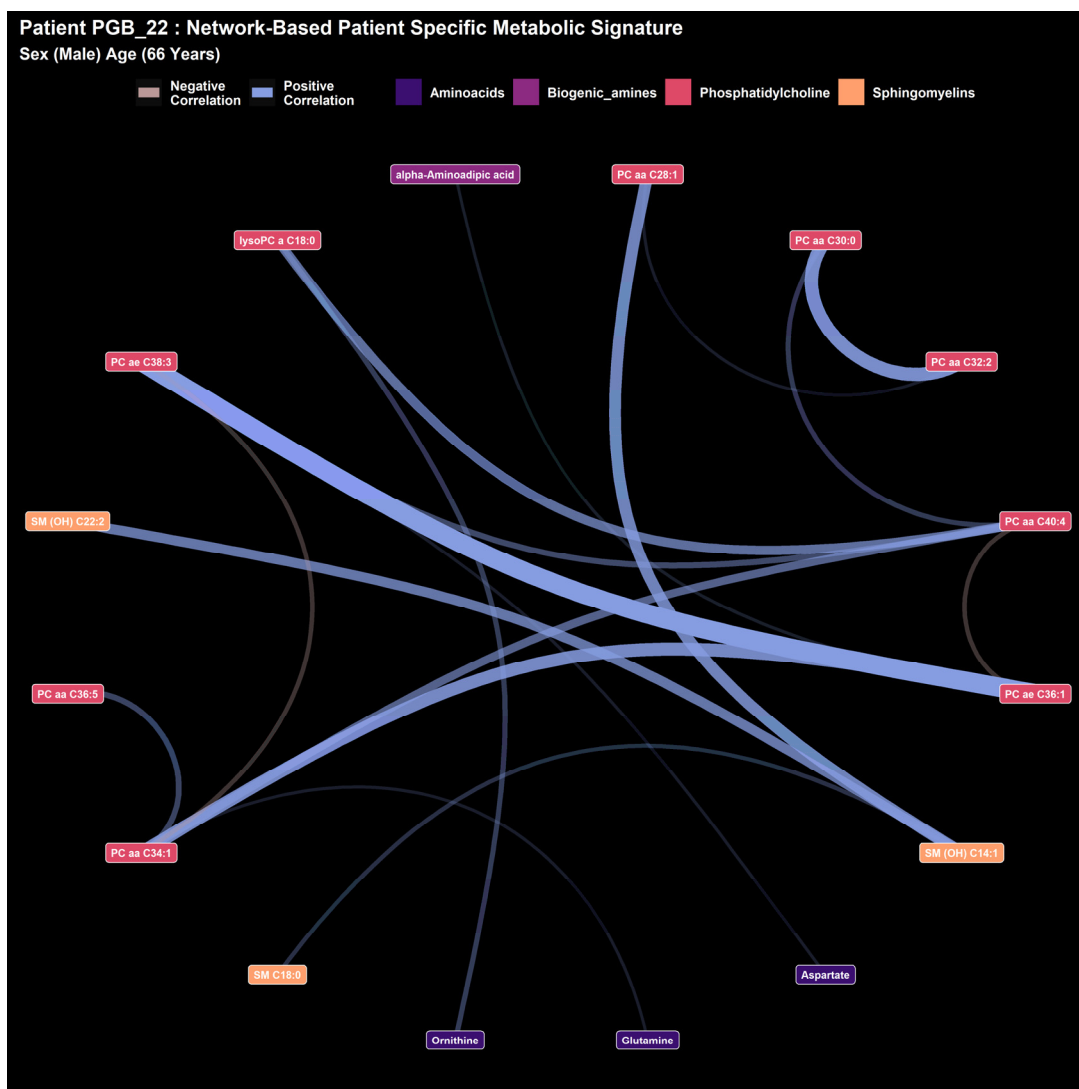


Figure S29. Network-based patient specific plasma metabolic signature (Patient PGB_22). The signature includes 22 metabolites; 9 phosphatidylcholines, 3 aminoacids, 1 biogenic amine and 3 sphingomyelins.

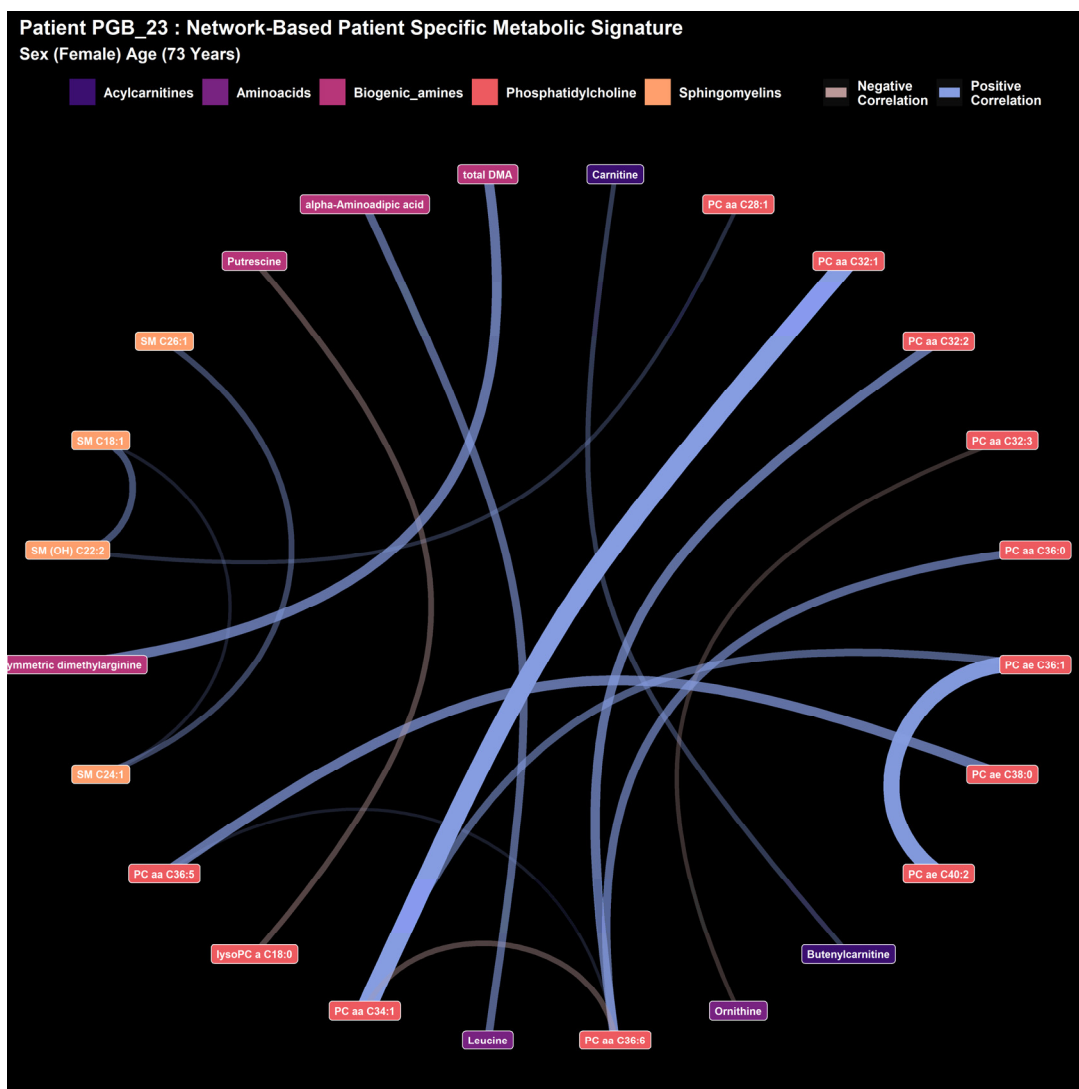


Figure S30. Network-based patient specific plasma metabolic signature (Patient PGB_23). The signature includes 24 metabolites; 12 phosphatidylcholines, 2 aminoacids, 2 acylcarnitines, 4 biogenic amines and 4 sphingomyelins.

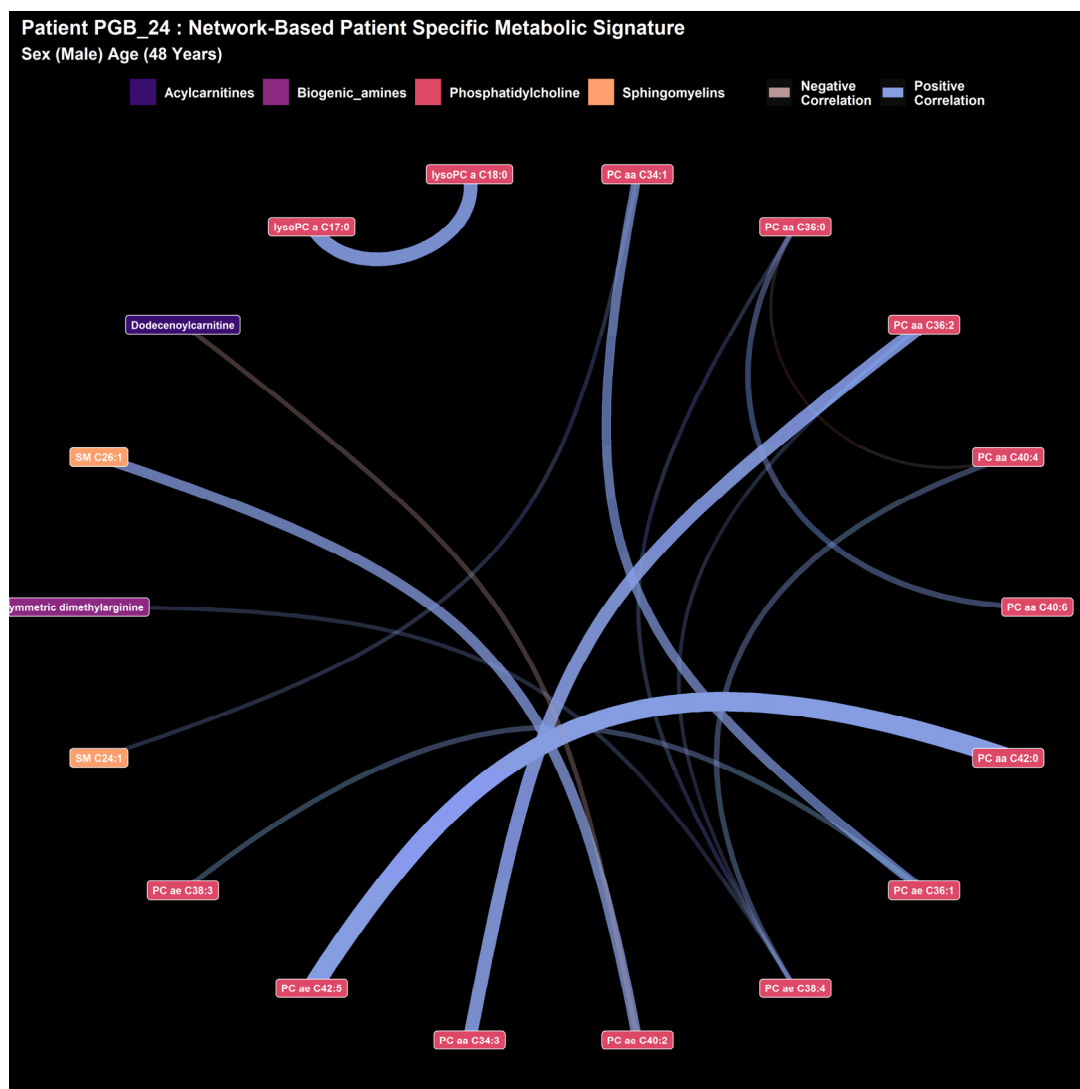


Figure S31. Network-based patient specific plasma metabolic signature (Patient PGB_24). The signature includes 18 metabolites; 14 phosphatidylcholines, 1 acylcarnitine, 1 biogenic amine and 2 sphingomyelins.

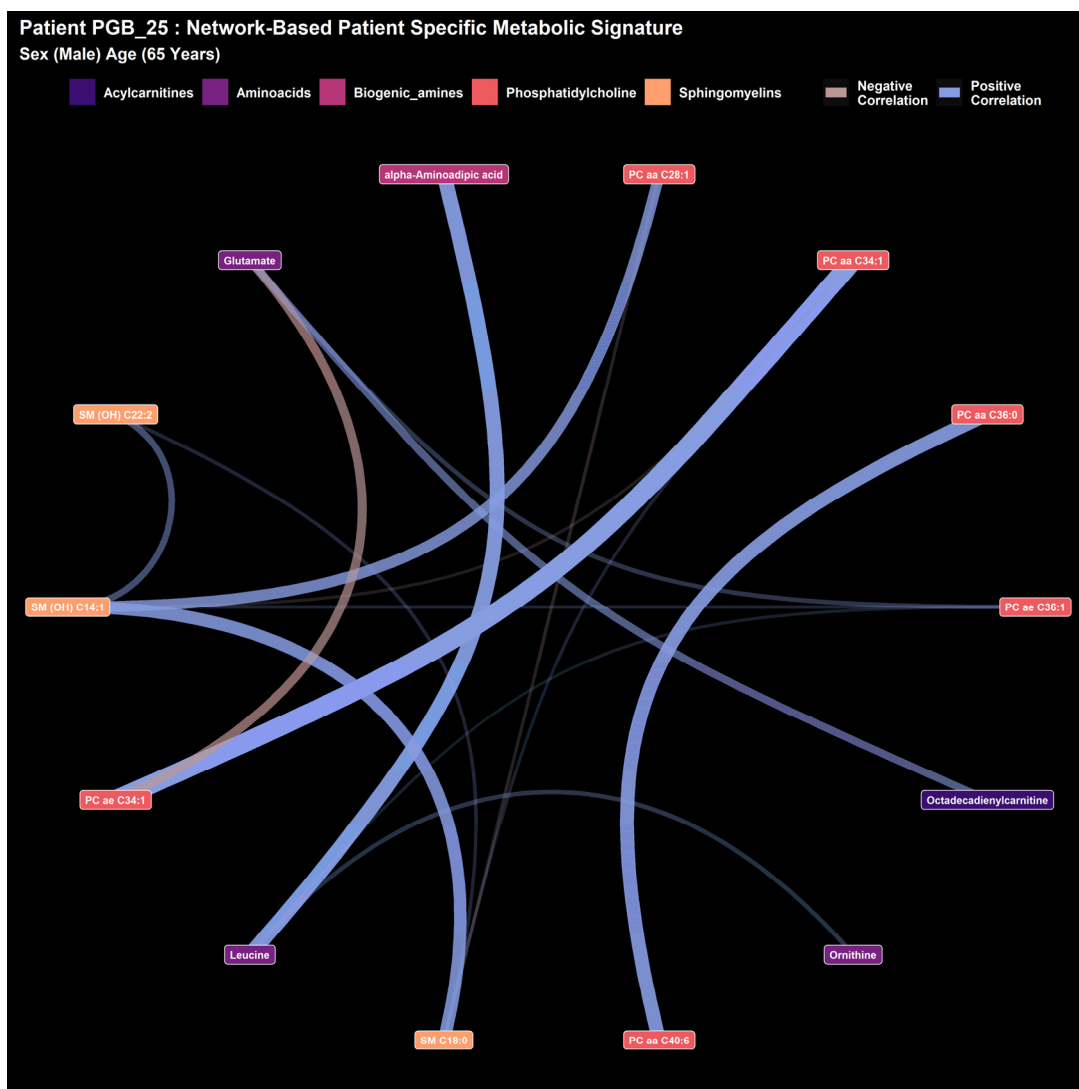


Figure S32. Network-based patient specific plasma metabolic signature (Patient PGB_25). The signature includes 14 metabolites; 6 phosphatidylcholines, 3 aminoacids, 1 acylcarnitine, 1 biogenic amine and 3 sphingomyelins.

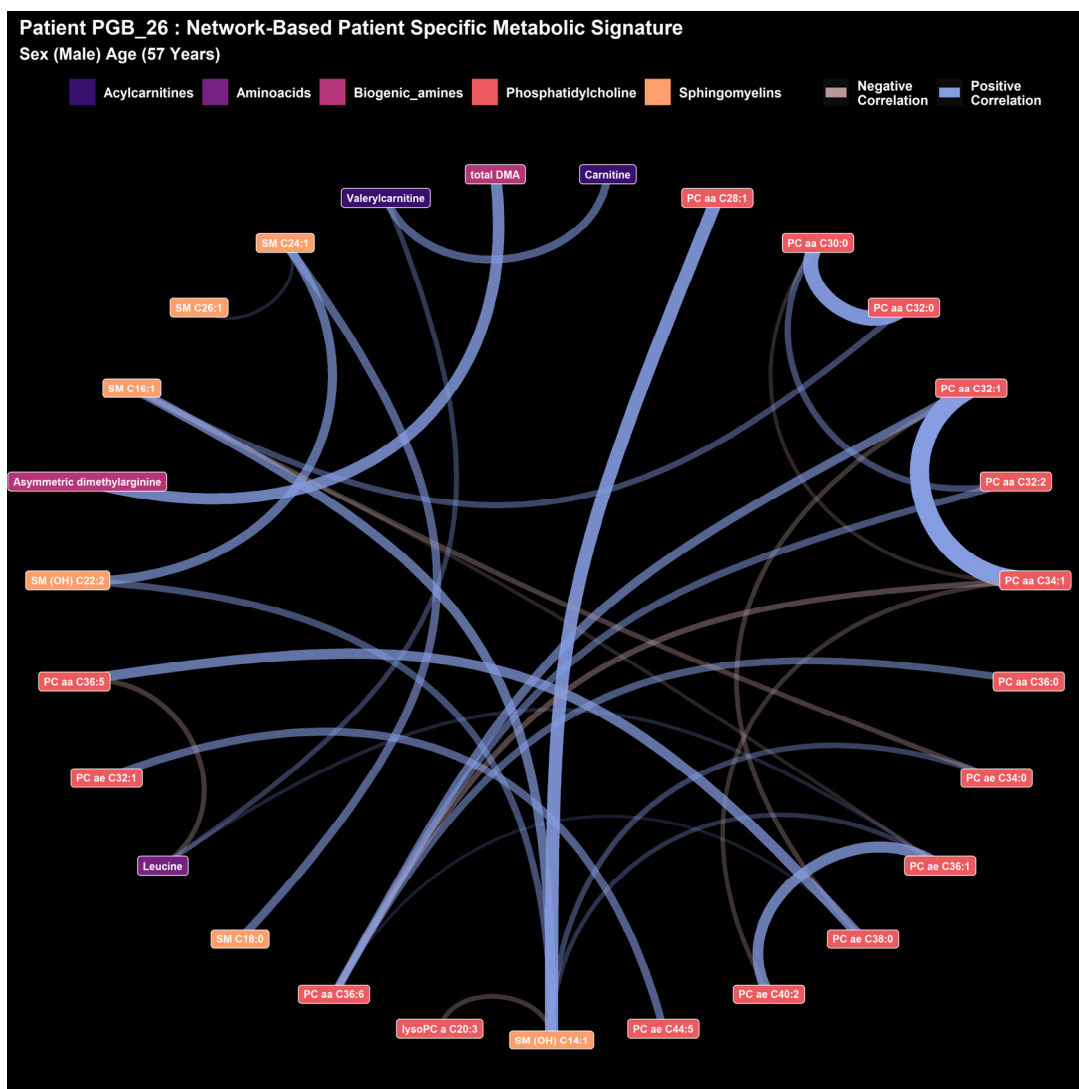


Figure S33. Network-based patient specific plasma metabolic signature (Patient PGB_26). The signature includes 27 metabolites; 16 phosphatidylcholine, 1 aminoacid, 2 acylcarnitines, 2 biogenic amines and 6 sphingomyelins.

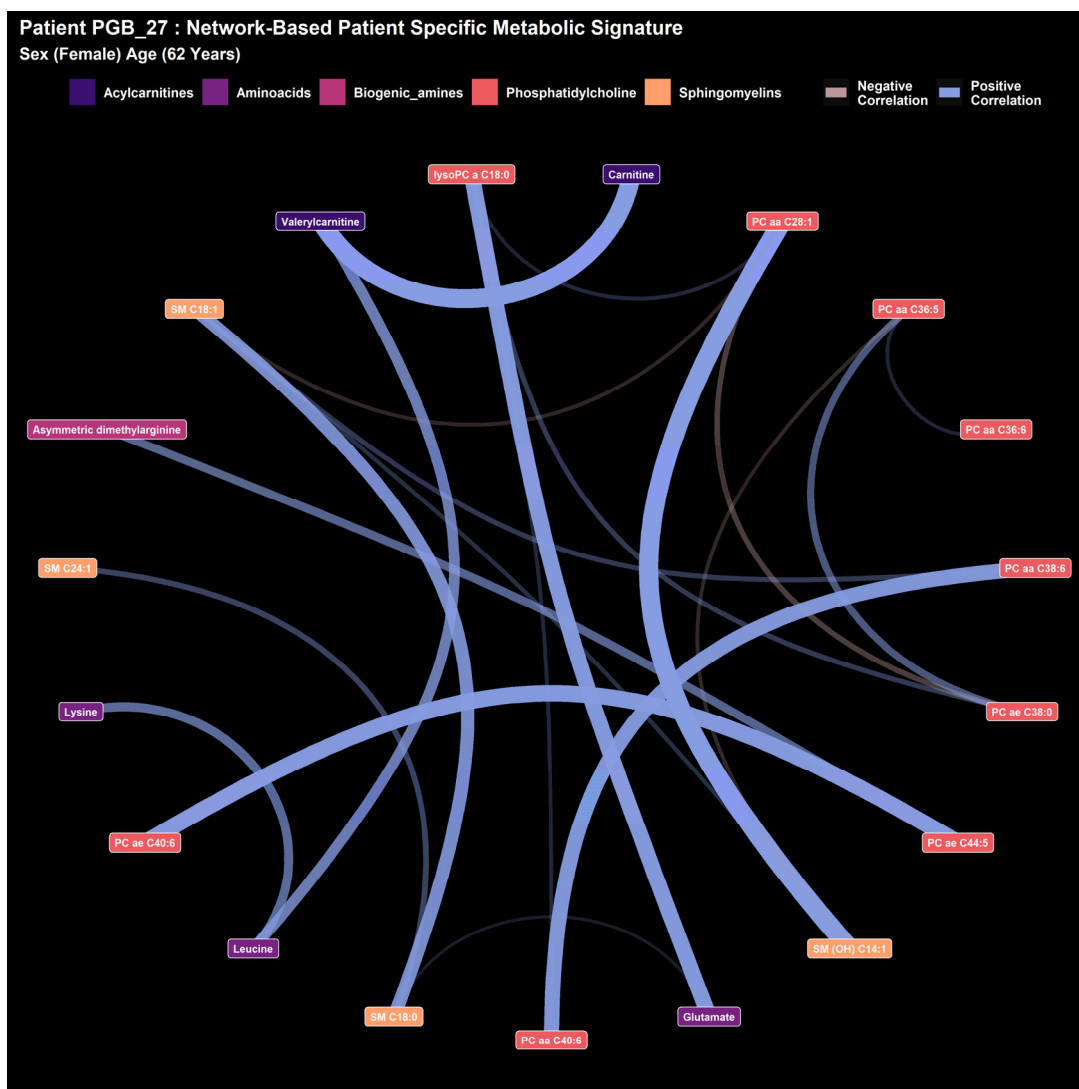


Figure S34. Network-based patient specific plasma metabolic signature (Patient PGB_27). The signature includes 19 metabolites; 9 phosphatidylcholines, 3 aminoacides, 3 acylcarnitine, 1 biogenic amine and 3 sphingomyelins.

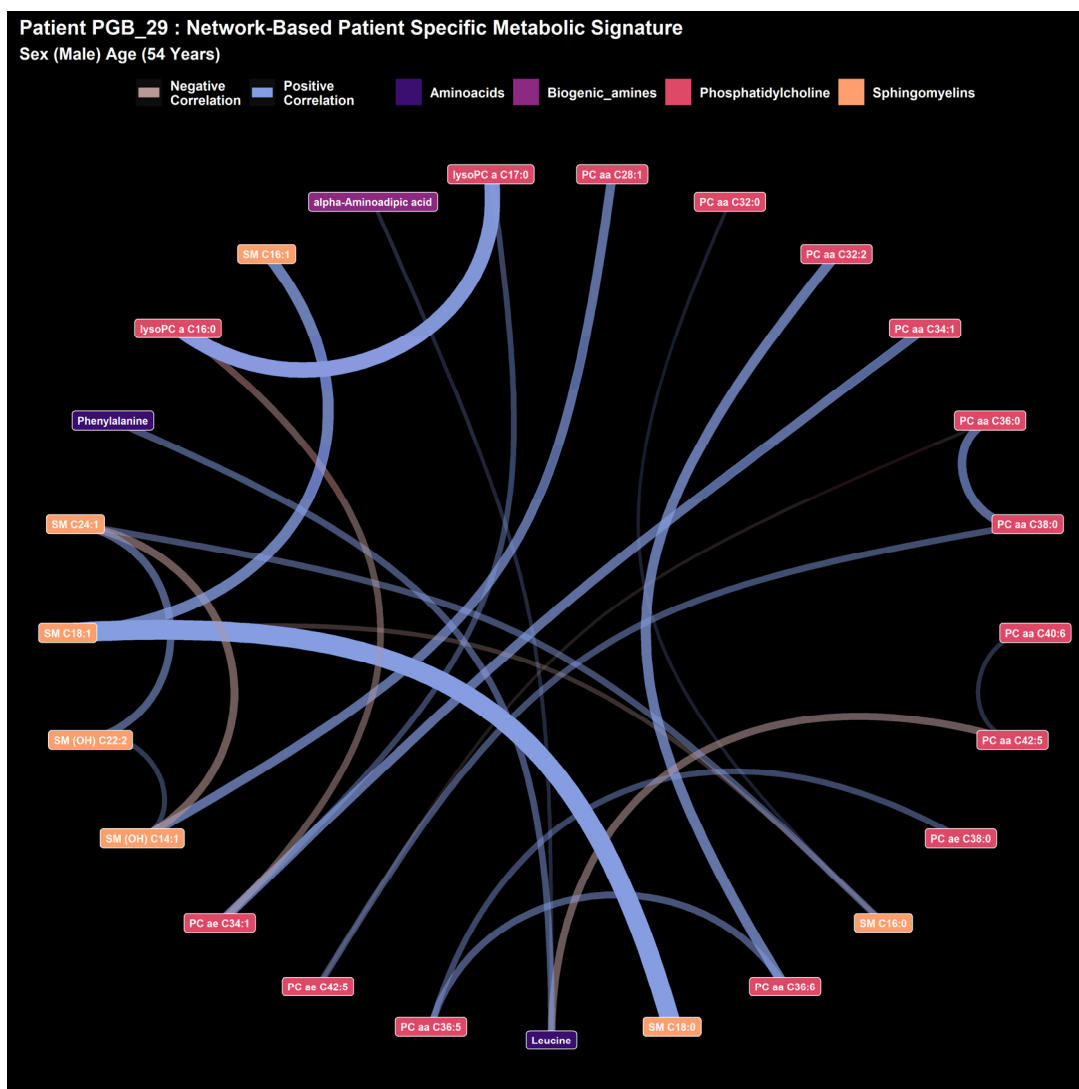


Figure S36. Network-based patient specific plasma metabolic signature (Patient PGB_29). The signature includes 25 metabolites; 15 phosphatidylcholines, 2 aminoacides, 1 biogenic amine and 7 sphingomyelins.

CONCLUSIONS ET PERSPECTIVES

La neurochirurgie s'est enrichie de tout temps grâce aux progrès techniques de son époque que ce soit par les avancées en radiologie, magnification optique ou de biologie. Un domaine qui illustre au mieux cette interdisciplinarité est certainement la neuro-oncologie. Les glioblastomes, qui sont les tumeurs cérébrales primitives les plus fréquentes s'accompagnent toujours aujourd'hui d'un pronostic sombre, et ce malgré les travaux de recherche qui leur sont consacrés. C'est pourquoi il y a une nécessité de repenser l'approche que l'on peut avoir de cette pathologie en terme diagnostique et thérapeutique.

La médecine de précision propose une médecine centrée sur le patient avec des approches personnalisées. Elle a contribué à des avancées dans de nombreux domaines de la médecine. L'essor du Big Data permet d'intégrer aux données classiques médicales, les différentes sciences Omiques. La métabolomique nous paraît particulièrement intéressante, car cette approche permet l'étude du produit final du métabolisme.

La compréhension des mécanismes métaboliques mis en jeu dans le cadre du Glioblastome permettra à terme de proposer des stratégies diagnostiques et thérapeutiques. Le travail que nous présentons dans cette thèse montre quels outils la biologie, et les sciences Omiques en particulier, peuvent apporter à la neurochirurgie.

Les revues présentées sont une réflexion sur le changement de paradigme que connaît le neurochirurgien actuellement en s'appropriant une chirurgie de précision et en dressant un état des connaissances de la lutte contre le Glioblastome. Notre travail expérimental, qui est une étude multi-omique des patients présentant un Glioblastome a permis d'émettre des hypothèses quant à la propagation des lésions gliales intracérébrales. L'un des déterminants mis en évidence est la modulation par le Glioblastome de son environnement, notamment énergétique, lui permettant de croître. Nous avons déterminé des perturbations dans le métabolisme lipidique et relevé des métabolites d'intérêt dans le sérum et le tissu chez des patients malades. Ces données pourraient permettre de proposer des marqueurs diagnostiques après validation dans de plus grandes cohortes. La caractérisation plus précise des lésions gliales est un prérequis indispensable pour proposer des biomarqueurs efficaces et ouvrir la voie à des thérapies ciblées, permettant ainsi de s'inscrire pleinement dans la médecine de précision.

Résumé : La médecine de précision vise à placer le patient au centre du parcours de soins en intégrant les données médicales et biologiques individuelles tout en tenant compte de la grande diversité entre les individus. Ces nouvelles approches nous invitent à nous réinventer en tant que neurochirurgiens et à remettre en question notre pratique et notre métier notamment en neuro-oncologie. Le glioblastome est la tumeur cérébrale maligne la plus fréquente chez l'adulte et consiste en une infiltration progressive de la substance blanche par des cellules tumorales. La survie moyenne est de 15 à 17 mois. Compte tenu de ce pronostic agressif, il est urgent de mieux comprendre les mécanismes sous-jacents du glioblastome afin de dévoiler de nouvelles stratégies diagnostiques et des cibles thérapeutiques grâce à une compréhension plus approfondie de sa biologie. Pour ces raisons, dans ce travail, nous avons réalisé des études métabolomiques ciblées et non ciblées sur des échantillons de tissus et de plasma provenant de patients atteints de glioblastome. Cette étude a révélé 176 lipides et métabolites exprimés de manière différentielle, 148 dans le plasma et 28 dans les échantillons de tissus. Les principales classes biochimiques comprennent les phospholipides, les acylcarnitines, les sphingomyélines et les triacylglycérols. Les analyses fonctionnelles ont révélé un remodelage métabolique profond des lipides et des substrats énergétiques des glioblastomes, ce qui dévoile le rôle majeur des lipides dans la progression tumorale en modulant son propre environnement. Dans l'ensemble, notre étude démontre un remodelage métabolique *in situ* et systémique dans le glioblastome qui nous éclaire sur sa plasticité biologique sous-jacente et sa progression afin de faire évoluer le diagnostic et/ou les stratégies thérapeutiques.

Mots clés : Glioblastome ; gliome de haut grade ; tumeur cérébrale ; neuro-oncologie ; métabolomique ; omique ; spectrométrie de masse ; apprentissage automatique.

Abstract: Precision medicine aims to bring the patient at the center of the healthcare ecosystem by integrating individual medical and biological data while taking into account the great diversity between individuals. These new approaches invite us to reinvent ourselves as neurosurgeons and to question our practice and profession especially in neuro-oncology. Glioblastoma is the most common malignant brain tumor in adults and consists in a progressive infiltration of the white matter by tumoral cells. The mean survival is between 15 to 17 months. Given this aggressive prognosis, there is an urgent need for a better understanding of the underlying mechanisms of glioblastoma to unveil new diagnostic strategies and therapeutic targets through deeper understanding of its biology. For these reasons, in this work, we performed targeted and untargeted metabolomics-based investigations on both tissue and plasma samples from patients with glioblastoma. This study revealed 176 differentially expressed lipids and metabolites, 148 in plasma and 28 in tissue samples. Main biochemical classes include phospholipids, acylcarnitines, sphingomyelins and triacylglycerols. Functional analyses revealed deep metabolic remodeling in glioblastoma lipids and energy substrates, which unveils the major role of lipids in tumor progression by modulating its own environment. Overall, our study demonstrates *in situ* and systemic metabolic rewiring in glioblastoma that could shed light on its underlying biological plasticity and progression to inform diagnosis and/or therapeutic strategies.

Keywords: Glioblastoma; high grade glioma; brain tumor; neuro oncology; metabolomics; omics; mass spectrometry; machine learning.

