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The genomic landscape of breast cancer brain metastases: a systematic review

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Corresponding Author:	Carlo Palmieri, BSc, MB BS, PhD University of Liverpool Liverpool, UNITED KINGDOM
First Author:	Carlo Palmieri, BSc, MB BS, PhD
Order of Authors:	Carlo Palmieri, BSc, MB BS, PhD
	Athina Giannoudis, PhD
	Alexander Morgan
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Abstract:	Background Breast cancer brain metastases (BCBM) are an increasing clinical problem. Recent studies have shown a distinct genomic landscape in breast cancer brain metastases including mutations absent in the primary tumour. The aim of this study was to produce a systematic review of the genomic landscape of BCBM. Patients and methods We performed a systematic search of Scopus covering dates from inception to 30th April 2020. Mutations data was extracted for mutations, receptor status [immunohistochemistry (IHC) and PAM50] and copy number alterations (CNAs) from the published manuscript and supplementary materials. STRING pathway analysis was performed on the 22 most commonly mutated genes and the drugs-genes interactions database (DGIdb) was used to access their actionability. Results We reviewed 13 studies comprising 164 patients where BCBMs had been sequenced. A list of 268 mutated genes was identified which occurred in at least two of the sequenced BCBMs. Of these, 22 genes were mutated in five or more patients and the most commonly mutated were the TP53 and PIK3CA genes. Pathway enrichment analysis showed that the 22 genes are involved in breast cancer-related signalling pathways, in regulation of gene transcription, cell cycle and DNA repair. Actionability analysis revealed that 68% of these genes are actionable drug targets. In addition, IHC and PAM50 data showed a receptor discordancy between primary breast cancers and paired brain metastases. Conclusions This systematic review provides a conclusive overview of the commonest mutated genes identified in BCBM and their clinical relevance. In addition, the IHC and PAM50 switching between primary BC and BM highlights further the importance of acquiring and assessing BCBMs.

Title

The genomic landscape of breast cancer brain metastases: a systematic review

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Authors list

Alexander J. Morgan[1*], Athina Giannoudis[1*], Carlo Palmieri[1,2]

*AJM and AG contributed equally to the study

Affiliation

1. University of Liverpool, Institute of Systems, Molecular and Integrative Biology, Department of Molecular and Clinical Cancer Medicine, Liverpool, United Kingdom
2. The Clatterbridge Cancer Centre NHS Foundation Trust, Liverpool, UK

Corresponding Author

Professor Carlo Palmieri

University of Liverpool,

Institute of Systems, Molecular and Integrative Biology,

Department of Molecular and Clinical Cancer Medicine,

Liverpool, L69 3BX, United Kingdom,

+441517949813

c.palmieri@liverpool.ac.uk

Abstract

Background

Breast cancer brain metastases (BCBM) are an increasing clinical problem. Recent studies have shown a distinct genomic landscape in breast cancer brain metastases including mutations absent in the primary tumour. The aim of this study was to produce a systematic review of the genomic landscape of BCBM.

Patients and methods

We performed a systematic search of Scopus covering dates from inception to 30th April 2020. Mutations data was extracted for mutations, receptor status [immunohistochemistry (IHC) and PAM50] and copy number alterations (CNAs) from the published manuscript and supplementary materials. STRING pathway analysis was performed on the 22 most commonly mutated genes and the drugs-genes interactions database (DGIdb) was used to access their actionability.

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We reviewed 13 studies comprising 164 patients where BCBMs had been sequenced. A list of 268 mutated genes was identified which occurred in at least two of the sequenced BCBMs. Of these, 22 genes were mutated in five or more patients and the most commonly mutated were the TP53 and PIK3CA genes. Pathway enrichment analysis showed that the 22 genes are involved in breast cancer-related signalling pathways, in regulation of gene transcription, cell cycle and DNA repair. Actionability analysis revealed that 68% of these genes are actionable drug targets. In addition, IHC and PAM50 data showed a receptor discordancy between primary breast cancers and paired brain metastases.

Conclusions

This systematic review provides a conclusive overview of the commonest mutated genes identified in BCBM and their clinical relevance. In addition, the IHC and PAM50 switching between primary BC and BM highlights further the importance of acquiring and assessing BCBMs.

Keywords

Breast Cancer, Brain metastases, Next generation sequencing, genomics, immunohistochemistry, PAM50 subtypes

Research in Context

Evidence before this study:

Searches were performed of PubMed, Embase, and Scopus databases for articles published between the inception of the database and 30th April 2020. Search terms were developed using a combination of MeSH keywords and wildcards. PubMed was searched for breast AND brain AND metast* AND sequenc*. Inclusion criteria were studies of four or more patients, published in English where genomic sequencing of brain metastases had been performed with data presented for single nucleotide variant mutations with at least one specific point mutation identified and presented in the article or supplementary information. We found that studies of BCBM are general much smaller than comparable sequencing studies for extra cranial disease. These studies have often not compared the brain metastasis to the paired primary cancer. These studies that have provide evidence that breast cancer brain metastasis can be genomically distinct from the primaries they arise from. To our knowledge, no publications so far have synthesised the genomic data contained within these studies of breast cancer brain metastasis.

Added value of this study:

Our study provides a comprehensive and detailed overview of sequencing studies related to brain metastasis secondary to breast cancer. By bringing all these brain metastasis studies together we provide a detailed overview and summary of these important data. A direct comparison of the genomic aberrations contained within breast cancer brain metastasis to those reported within large sequencing studies of extra cranial disease demonstrates that while there is some overlap in the genomic aberrations there is a clear divergence between cranial and extra-cranial disease. Undertaking a STRING pathway analysis of these genomic aberrations within breast cancer brain metastasis demonstrates enrichment for breast cancer-related

pathways as well as those involved in the regulation of gene transcription, cell cycle and DNA repair. While an assessment of actionality reveals the majority of these mutations are potentially actionable with clear therapeutic relevance. The data also reveals that PAM50 switching occurs in majority of cases providing further evidence regarding the divergence between the primary and brain metastasis.

Implications of all the available evidence:

These findings emphasise the importance of accessing and sequencing brain metastasis and for considering brain metastasis as being genomically distinct from extra cranial disease. The number of mutations which are actionable suggest there are a number of possible therapeutic interventions which should be explored in appropriately designed clinical studies.

Introduction

Breast cancer (BC) is the second most common solid malignancy to involve the central nervous system (CNS).[1] BC is a heterogeneous disease which is classified into clinically relevant groups by immunohistochemistry (IHC) and *in situ* hybridisation (ISH) for oestrogen receptor (ER) and progesterone receptor (PR), human epidermal growth factor receptor (HER2) status or lack of receptor expression.[2,3] Alternatively BC can be classified by the PAM50 gene expression profile into luminal A, luminal B , HER2 enriched, basal and normal breast-like subtypes.[2,4] With regard to metastatic behaviour these subtypes display differing predictions for distant organs, including brain metastasis, as identified by Zhang et al.[5] In TNBC, brain metastasis occurs either as the first site of distant spread (26% of patients) or after dissemination to the lungs, whereas in luminal subtype, distant spread to the bones and lungs is more frequent with brain metastasis uncommon.[6] Patients with the luminal subtype of breast cancer tend to have a better prognosis compared to HER2-positive and TNBC. Breast cancer brain metastasis (BCBM) is a particular feature of HER2-positive and triple negative BC, with a 2 to 5-fold increased risk of developing BM compared to luminal cancers.[7,8]

Nearly all breast-cancer deaths are related to metastasis rather than the primary cancer and recent clinical data has shown that BCBM is an increasing clinical problem in metastatic breast cancer (MBC).[9,10] This is likely to be a reflection of women with MBC living longer with improvements in systemic therapy, particularly in the HER2 positive subgroup and increased cross-sectional imaging of the central nervous system.[10,11] A particular clinical challenge is the development of new onset or progressive brain metastases in the setting of extracranial disease that is adequately controlled. BCBM is a cause of significant morbidity and mortality and despite this, patients with BCBM have been disadvantaged by a relative lack of clinical research in this area, in fact such patients are often explicitly excluded from clinical trials.[12] Progress is further hampered by the lack of annotated collection of biological material to

underpin translational research in this area.[13] The current standard of care for newly diagnosed BM includes surgery, stereotactic radiosurgery (SRS), and/or whole brain radiotherapy (WBRT).[6] Systemic therapy is in general the standard of care if there is progressive disease following local therapy.[14] Aside from tucatinib for HER2 positive breast cancer there is a lack of effective systemic treatments making this an area of unmet clinical need and an important avenue of research with the possibility to help a currently underserved sub-population of MBC patients.

The advent of the age of genomic medicine has enabled the characterisation of tumours for diagnosis, progression and the choice of therapeutic options. In addition, it has enabled a deeper understanding of the evolutionary process of breast cancer and the development of metastasis by comparing primaries with secondary cancers.[15] Two recent studies have sequenced a total of 1242 BC metastases , all extracranial,[16,17] however sequencing studies of BCBM have been very limited in the number of patients as it will be further appraised in this review.[18-30] Obtaining samples for genomic work in patients with BCBM is difficult given the inherent risks and complexity of neurosurgery, as well as the fact that the procedure may not be always appropriate due to the occurrence of multiple lesions. The increasing use of SRS has reduced the need for surgical resection but also, the availability of tissue, whereas longitudinal tissue sampling is clinically feasible.[13]

The importance of treating the disease in the brain separately from any prior extracranial disease was demonstrated in a study of 86 paired primary and BM of which 21 were primary BC and their matched BM.[18] This study demonstrated that while the primary and BM shared a common ancestor, both the primary tumour and the metastasis continued to evolve separately, reflected by the presence of distinct mutations so called ‘private mutations’ and each sample continued to develop minor cancer-cell populations. In addition, where the regional lymph nodes, or extracranial metastases were available these were shown not to be reliable surrogates

for the oncogenic alterations found in the BM. Inter-lesional and intra-lesional sampling of the BM demonstrated significant genetic homogeneity; with BM samples having mutations that were not detected in the clinically sampled primary tumour, indicating that the subclones sampled in these lesions were more closely related to one another than to those detected in the primary tumour. In approximately half of cases, clinically informative alterations which were found in the BM were not detected in the matched primary tumour. These data emphasise the need to analyse BMs as a distinct entity rather than relying solely on information from either the primary or the extracranial systemic disease, as well as offering opportunities for innovative treatments with targeted agents based on information derived from the BM.

Methods

Search strategy and selection criteria

This study was designed as a systematic review of the published literature. Searches were made of PubMed, Embase, and Scopus databases for articles published between the inception of the database and 30th April 2020. Search terms were developed using a combination of MeSH keywords and wildcards. PubMed was searched for *breast AND brain AND metast* AND sequenc**. Inclusion criteria were studies of four or more patients, published in English where genomic sequencing of brain metastases had been performed with data presented for single nucleotide variant mutations (SNV) with at least two specific point mutations identified and presented in the article or supplementary information. Data searches and extraction were performed by AM and AG in consultation with CP. There were no disagreements over studies to be included in the review.

Data extraction

This study was performed as a systematic review of the literature in accordance with the PRISMA statement.[31] The flow of information through the different phases of this systematic review are illustrated in Figure 1. As the data used was the published data in the manuscript and the supplementary information and there was observed variation in the genes reported in each study a full meta-analysis was not feasible. Data were extracted from the published manuscript and any supplementary files available from the journal website where it was presented as text, figures and tables. Data were compiled in Microsoft Excel with further analysis and preparation of figures in R3·6.[32] There was no duplication of data between studies. Study information was extracted for the following characteristic; Number of patients and clinicopathological characteristics including immunohistochemistry (IHC) and PAM50 status

where available, whether sequencing was of matched pairs of material or BM alone, sequencing platform, source of material, and sample preservation method. Mutated genes were analysed per-study and per-patient basis. From the study data, a list was generated of the point mutations in the most commonly reported genes. In the cases where genes were reported to be mutated but the point mutations were not presented, the data was excluded. Data on copy number alterations (CNAs) was also included in this review from the studies presenting the highest number of cases.

Pathway and actionability analysis

Functional pathway enrichment analysis (KEGG and REACTOME) of the 16 commonly mutated genes was performed using the STRING (Functional protein association networks) database v11 (string-db.org). Clustering was also performed using the K-means algorithm that aims to group a given dataset into clusters. The genes in the same cluster are considered more similar than other ones in different clusters.

The actionability of the 16 commonly mutated genes was assessed by the Drug-Gene interactions database (DGIdb) v3.1 (http://www.dgidb.org/).[33] DGIdb includes 39 potentially druggable categories with at least 35 interaction types as defined by several source datasets; DrugBank, Therapeutic Target Database (DTT), PharmGKB, OncoKB, Cancer Genome Interpreter (CGI), Memorial Sloan Kettering IMPACT (MskIMPACT), Clinical Interpretetions of Variants in Cancer (CIViC), FDA Pharmacogenomic biomarkers, Database of Curated Mutations (DoCM), The Jackson Laboratory Clinical Knowledgebase (CKB), NCI Cancer Gene Index, the ChEMBL Bioactivity Database and ClinicalTrials.gov.

Results

Study details

Of the 430 articles returned by the search, 13 were included in the final review after the application of our inclusion criteria. These 13 studies comprised 167 patients of which 164 were sequenced, all female, with the patients' characteristics summarised in Table 1 whereas additional information regarding age and median times for diagnosis of BM and survival are presented in supplementary table 1. Sequencing of matched pairs of primary breast tumour and brain metastases was conducted in 129 of these patients. Of the 13 studies, 4 (30·8%) were of BCBMs only, 5 (38·5%) studies reported BCBM as part of sequencing various MBC sites and four (30·8%) reported BCBM as part of sequencing from different primaries. Eight studies (61·5%) used material from resection with 3 (23·1%) from post-mortem, 1 from both (7·7%) and 1 (7·7%) did not report where the material derived from (Table 2). Three studies (23·1%) used snap-frozen samples, 6 (46·2%) used formalin-fixed paraffin-embedded tissue and 4 (30·1%) used both preservation methods. This information of the different sequencing platforms, the clinical material and their receptor status by IHC or PAM50 including the source of the extracted data is summarised in Table 2.

BCBM Mutations and pathway analysis

Overall, 268 genes were reported as mutated in two or more of the 164 BCBMs (Supplementary file 1: Mutated genes). Of these, 22 genes (8·2%) (Supplementary file 1: Mutated genes, highlighted in blue) were reported to be mutated in five or more brain metastases and they were selected for further review. These genes in order of decreasing frequency were (n= number of BCBMs with mutations in brackets and percentages); TP53 (n=85, 51·8%), PIK3CA (n=36, 22·0%), KTMT2C (n=10, 6·1%), RB1 (n=8, 4·9%), ZFHX3 (n=8, 4·9%), BRCA2 (n=7,

4·3%), ERBB2 (n=7, 4·3%), KMT2D (n=7, 4·3%), MLH1 (n=7, 4·3%), PTEN (n=7, 4·3%), ATR (n=6, 3·7%), BRCA1 (n=6, 3·7%), CDH1 (n=6, 3·7%), COL6A3 (n=6, 3·7%), FAT1 (n=6, 3·7%), FLT3 (n=6, 3·7%), IGFN1 (n=6, 3·7%), ARID1A (n=5, 3·0%), ATM (n=5, 3·0%), CHEK2 (n=5, 3·0%), MAP3K1 (n=5, 3·0%), MET (n=5, 3·0%) (Figure 2A). We compared our list of the 22 most frequently mutated genes to the data of two large-scale extracranial sequencing studies of BC metastasis (Supplementary table 2).[16,17] Seven out of the 22 (31·8%) genes reported in five and more patients in our aggregation were also present in the top 21 reported mutated genes in the study by Angus et al.[17] with five found in the top 10 (50·0%) reported by Bertucci et al.[16] The remaining 13 (61·9%) genes (ATM, ATR, BRCA1, BRCA2, CHEK2, COL6A3, FAT1, FLT3, IGFN1, KMT2D, MET, MLH1, ZFHX3) were not present anywhere on the list of 21 cancer-driving genes presented in either of the extracranial sequencing studies.[16,17] It is of note that the 2 most commonly reported genes in both the extracranial studies and in this review were TP53 and PIK3CA.

The 22 frequently mutated genes were further subjected to functional pathways analysis. The functional protein-protein interaction network as generated by STRING is illustrated in Figure 2B. KEGG pathway enrichment analysis indicated that the 22 highly mutated genes are present in pathways in cancer, breast cancer, drug resistance, cellular senescence and PI3K-AKT, TP53, MAPK, FOXO signalling pathways among other cancer related pathways with false discovery rate (FDR) less than 0.001. The breast cancer related pathways including glioma are illustrated in Figure 2C. REACTOME functional enrichment showed that mutated genes are involved in the regulation of gene transcription, cell cycle and DNA repair whereas k-means clustering (Figure 2D) grouped the genes into 3 clusters mainly involved in cell cycle, growth arrest, DNA repair and signal transduction/transcription. (red), signalling pathways (yellow) and transcriptional activation and cell migration (green). Two genes, the COL6A3 (cyan) and IGFN1 (blue) remained individually. COL6A3 as a cell binding protein is involved in

extracellular matrix and adhesion but the functional role of IGFN1 (Immunoglobulin-like and fibronectin type III domain containing 1) is not established. All the pathway analysis data is available in Supplementary file 1: KEGG, REACTOME and kmeans clustering.

Actionability of mutated genes

The actionability of the 22 commonly mutated genes was investigated by the DGIdb (http://www.dgidb.org/).[33] Eighteen of the 22 genes (81·8%) were present on the generated list of drugs and genes interactions (Supplementary file 1: DGIdb) and 15 (68·2%) of these are or can potentially be useful drug targets. Eight genes (36·4%), PIK3CA, MET, ERBB2, FLT3, ATR, ATM, TP53 and MAP3K1 were targetable. TP53 cannot be directly targeted but re-activated by several strategies that include targeting of molecules that modulate its posttranslational modifications, localization, synthesis and its degradation.[34,35] Seven genes (31·8%), BRAC1, BRAC2, RB1, PTEN, CDH1, CHEK2 and MLH1 were actionable providing biallelic inactivation was present. MLH1 can also act as a biomarker for selecting immunotherapy efficacy.[36] Three of the genes (13·6%) ARID1A, KMT2D and COL6A3 were present on our DGIdb list but the information was poor to justify any clinical relevance.[33] No interactions were identified for four genes (18·2%) FAT1, ZFHX3, KMT2C and IGFN1.

Copy Number Alterations

Whilst copy number alterations (CNA) were not a primary outcome of this review we have presented the summarised data below. Five of 13 studies (38·5%)[18,21,26,27,29] comprising 67 patients in total (40·1%) reported CNA in 2 or more patients in a total of 50 genes (Supplementary file 1: CNAs). Of these, 19 genes (22·6%) had CNA in five or more BCBM patients presented here as n= number of patients with reported CNA and percentage of patients

with reported CNA from these five studies, ERBB2 (n= 27, 40·3%), MYC (n=18, 26·9%), CCND1 n=10, 14·9%), RB1 (n=8, 11·9%), ADAM28 n=7, 10·4%), CDK12 (n=7, 10·4%), NRG1 (n=7, 10·4%), AURKA (n=6, 9·0%), DPYSL2 (n=6, 9·0%), FGF3 (n=6, 9·0%), MDM4 (n=6, 9·0%), ROBO3 (n=6, 9·0%), TP53 (n=5, 7·5%), BRCA2 (n=5, 7·5%), EGFR (n=5, 7·5%), FGF4 (n=5, 7·5%), FGFR1 (n=5, 7·5%), SLIT1 (n=5, 7·5%), UNC5D (n=5, 7·5%).

Immunohistochemistry

Immunohistochemistry (IHC) status was reported in all studies, in a total of 136 primary and 121 metastatic samples (Supplementary file 2: Primary IHC, Metastasis IHC). The studies reporting IHC status of the primary BC and of the BM are presented in Table 3A and 3B respectively. It was possible to determine the status of all 3 receptors in 77 (56·6%) primary and 66 (55%) metastatic cases. In the primary BC, 34 of 136 samples (25·0%) were reported as TN, 47 (34·6%) as ER+/HER2± and 59 (43·4%) as HER2+/ER±. In the metastatic samples, 34 (28%) were reported as TN, 44 (26·3%) as ER+/HER2± and 60 (35·9%) as HER2+/ER±. Of the ER- cases where PgR status was available, 4 out of 50 (8%) primary BCs and 3 out of the 37 (8%) BMs were PgR-positive.

Paired samples for 67 patients were available from the studies by Da Silva, Lee, Saunus, Diossy, and Tyran[19-21,28,29] (Supplementary file 2: Pair cases IHC). After the removal of incomplete data and patients with multiple primary or secondary samples, data was available for 51 patients with an overall discordancy of 22% between primary and metastasis. ER status was discordant in 4 cases (8%), PgR in 4 cases (8%), and HER2 was discordant in 5 cases (10%). As can be seen in figure 4A there is a trend towards TNBC and HER2-positive subtypes from primary to metastasis (39% to 45%) and (22 to 24%) respectively and away from the ER+HER2+ subtype (14% to 10%).

PAM50

PAM50 status was reported in 3 of the 12 studies, 2 reported both primary and metastatic status and 1 study reported the PAM50 status of the primary tumour alone as summarised in Table 4. These two studies were Lee et al.[20] and Siegel et al.[25] comprising 21 patients in total (13%). Of these, 20 patients had complete data available with 1 patient with unknown status in the metastasis. The summary of those with complete data showed discordancy in 11 of 20 (55%) of cases between primary and metastasis. Figure 3B illustrates the switching of PAM50 profiles between primary and metastases. There was significant discordancy between PAM50 subtypes in primary and metastatic tissue with a trend away from basal-like (45% to 30% primary to secondary) in the primary tissue to normal-like in the metastatic tissue (10% to 30%). Two cases switched from luminal-A to luminal-B subtypes

Discussion

In this systematic review we have compiled the mutation data and CNAs presented in twelve BCBM sequencing studies and generated a comprehensive overview of the genomic landscape of BCBM. We also extracted the data for IHC and PAM50 status from these studies and elaborated further on the discrepancies between primary BC and their matched brain metastases.

Of the twelve BCBM studies included in this review, nine utilised a variety of Illumina NGS platforms, two utilising the ThermoFisher Ion-PGM platform whereas Da Silva et al.[19] used the OncoCarta™ v1.0 kit on the MassARRAY system (Agena Bioscience, San Diego, USA), a mass spectrometry based on matrix assisted laser desorption/ionisation-time of flight (MALDI-TOF). Despite having a different methodology to the others, the study was included due to the large number of matched BC and BM patients (12 matched cases). In addition, the main difference of the MassARRAY in comparison to NGS on the identification of mutations is the use of predefined mutations across different oncogenes; it cannot therefore detect unknown mutations and CNAs.[37]

Comparison between these comprehensive BCBM dataset and those from two datasets of sequenced extracranial metastases identified several differences with genes mutated in BCBM but not in extracranial metastases and *vice versa*.[16,17] Moreover, the coefficients of correlation between this review and the extracranial studies highlighted further the divergences between BCBM and other metastatic sites secondary to BC, endorsing the investigation of BCBM as distinct entities to extracranial metastases. The most frequently mutated genes in our current study, as in Bertucci[16], and Angus[17] were TP53 followed by PIK3CA. Similarly, in a recent study by our group utilising the UltraSEEK™ breast cancer panel (Agena Bioscience, San Diego, USA) on the MassARRAY system, the common mutated genes within a cohort of 32 paired BCs and BCBMs were TP53, PIK3CA, AKT1 and ESR1.[38] The presence of ESR1 and

AKT1 mutations within our cohort is a reflexion of the large number of ER positive cases in comparison to ER negative whereas in this systematic review only the 30% of the cases were ER-positive.

As 85 (51·8%) and 36 (22·0%) patients had reported mutations in TP53 and PIK3CA respectively, these 2 genes represent the most obvious targets for intervention. Most recently, PI3Kα-specific inhibitor alpelisib, has shown activity in PIK3CA mutant breast cancer.[39] While the brain penetrant inhibitor paxalisib (GDC-0084) has demonstrated activity in pre-clinical models of breast cancer brain metastasis[40] as well as preliminary clinical activity in glioblastoma.[41] In addition, a number of ongoing clinical trials are using p53-reactivating compounds in combination with different chemotherapeutic drugs but there are still certain limitations such as the development of systemic toxicities to overcome.[33-35]

While TP53 and PIK3CA are commonly mutated in brain and extracranial metastases, our aggregated BCBM data also shows that only 31·8% of the frequently mutated genes overlapped with those frequently mutated in the extracranial metastases.[16,17] This supports the previous observations showing divergence between extracranial and CNS metastases. The genes that divert from the extracranial mutated genes are mainly involved in cell cycle and DNA repair pathways and MET signalling. Of these genes, MLH1, ATR, CHEK2, BRCA1, BRCA2, FLT3 and MET have been identified as druggable targets by our DGIdb analysis highlighting their importance in BCBM and the need for further investigation.

In addition, four of the genes identified with CNAs (TP53, ERBB2, RB1, BRAC2) in 5 or more BCBM patients were also frequently mutated in this study, highlighting further the significance of genomic alterations in these genes and their relevant pathways.

A pathway analysis was conducted on the 22 commonly mutated genes identified in this systematic review and we observed that they are mainly involved in the regulation of gene

transcription, cell cycle, DNA repair and signalling. As expected, since most of them are well known oncogenes or tumour suppressors, they were identified in KEGG pathways in cancer, breast cancer, drug resistance, senescence and PI3K-AKT, TP53, MAPK signalling. This data highlights further the importance of these mutated genes and the need for targeted therapies as alterations in cell cycle, DNA repair, gene transcription and signalling comprise some of the hallmarks of cancer.[42]

A comprehensive study of receptor conversion in breast cancer reported that ER discordance between primary and CNS metastases was 20.8% [95% confidence interval (CI) 15-28%] whereas the HER2 CNS discordance was 12.5% (95% CI: 7.8-13.6%).[43] In another study within our group, analysing 40 cases of primary BC and their matched BM, we observed that the distribution of the subtypes changed in the BM setting with the majority of ER-positive primaries losing their ER status (47.6% ER-positive) whereas no change was observed between the HER2-positive and the TNBCs.[44] In this systematic review, we identified an 8% discordancy in ER status and a 10% discordancy in HER2 status between primary BC and BM. We also observed a total discordancy of 55% in the PAM50 status between primary BC and BM. This variation justifies further the need to assess the receptor status of the metastases. The heterogeneous findings between the studies can be due to the primary tumour characteristics and the time interval between primary BC and brain metastasis. Indeed, ER positive BCs have a better prognosis and a slow rate of relapse and distant metastasis whereas HER2 positive and triple negative BC can relapse quicker and sometimes, brain is their first site of metastasis. Moreover, receptor conversion is the result of both clonal selection and selective pressure of therapy.[18,26,43] Although clonal selection can be observed due to the different mutations between primary BCs and BMs,[18,26] we did not assess the therapeutic regimes as it was not the primary scope of this review. Nevertheless, this data confirms the heterogeneity of BC and

supports further the importance of accessing the receptor status and/or intrinsic subtype of BCBM.

The challenges however to obtain BM samples for sequencing or receptor screening are reflected in the total number of patients included in the studies analysed in this systematic review. Some of these challenges include the inherent risks of neurosurgery, as samples can only be taken when surgical resection is clinically indicated, making longitudinal studies of the changes in the BM genome unethical. In addition, for multiple metastases resection is often not indicated. Instead, the usage of SRS for the treatment of BM has been increasing. The benefits of this method to patients are obvious, however it makes it impossible to collect surgical samples and therefore, samples can only be collected post-mortem.

There have been several investigations into the use of circulating cell-free DNA (cfDNA) in the CSF as a 'liquid biopsy' of BCBMs. Despite promising initial results, these studies have not been conclusive.[18,22] Should cfDNA prove a reliable surrogate for biopsy of BMs it is likely that in the future patients will be able to have tailored treatments based on the results of sequencing cfDNA from CSF rather than relying on the genomics of the primary lesion.

It is important to note that the lack of a report of a mutation in this review is not confirmation of absence of the mutation. For this reason, it was not appropriate to calculate mutation prevalence over the entire study population as in a meta-analysis. In addition, it was beyond the scope of this review to attempt to discriminate between pathogenic variants, passenger variants, and variants of unknown significance.

Conclusion

This is the first systematic review into the genomic landscape of BCBM that combined multiple smaller studies to produce more convincing and powerful findings. Several of the genes found

to be mutated present potential therapeutic targets whereas the receptor status switching between primary BC and BCBM suggests that the divergence of the genome of BCBM from that of the primary tumour may present new avenues of treatment in addition to the current challenges.

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Disclosure

The authors have declared no conflicts of interest.

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Figure Legends

Figure 1. Flow of information through the different phases of the systematic review. The diagram illustrates the number of records identified throughout the literature, the number of included and excluded records, and the reasons for exclusions according to the PRISMA statement.

Figure 2. Frequently mutated genes and pathway enrichment analysis by STRING.
(A) The 22 genes mutated in five or more brain metastases presented in order of decreasing frequency. (B) Protein–protein interaction network of the 22 mutated genes as visualized by STRING. The colour saturation of the edges represents the confidence score of a functional association; higher the confidence score, the darker the grey lines. (C) KEGG pathway enrichment analysis plot of the main breast cancer-related pathways with FDR<0.001. The orange line represents the log10 ratio of observed/background gene count. (D) k-means clustering grouped the genes into 3 main clusters: cell cycle, growth arrest, DNA repair and signal transduction/transcription. (red), signalling pathways (yellow) and transcriptional activation and cell migration (green). The COL6A3 (cyan) and IGFN1 (blue) did not clustered with any other genes. Inter-cluster edges are represented by the dash-lines.

Figure 3. The receptor switch between primaries breast cancer and brain metastases.
(A) Represents the receptor switch between primaries BC and BCBM as recorded by immunohistochemistry (IHC) whereas in (B) is the PAM50 receptor switch between primary breast cancer and breast cancer brain metastasis.

Tables

	Total Number of breast cancer patients with brain metastasis.		Median Age (Range)/years	Median time between diagnosis and BM (Range) / years	Median Survival time (Range)/years
	Total	Matched primary / metastatic pairs			
Da Silva (2010) [19]	12	12	48·5	3·5	NR
Lee (2015) [20]	45	15	44·6 (22·4-64·1)	2·5 (0-17·7)	5.1 (0·8-20·0)
Saunus (2015) [21]	11	11	51·0 (35·8-74·9)	2·3 (0·7-25·9)	0·5 (0·3-0·9)
Brastianos (2015) [18]	21	21	46.2	3·7	NR
De Mattos-Arruda (2015) [22]	4	4	36·5 (33·0-56·0)	7·9	NR
Muller (2016) [23]	4	0	47·5 (64·0-75·0)	NR	NR
Richichi (2017) [24]	12*	12	46·0 (32·0-52·0)	NR	NR
Siegel (2018) [25]	6*	6	51·0	1·6	3·1
De Mattos-Arruda (2018) [26]	6	6	40·0	1·9	NR
Schrijver (2018) [27]	9	9	NR	NR	NR
Diossy (2018) [28]	17	17	55·90 (39·0-69·0)	2·3 (0·03-5·4)	NR
Tyran (2018) [29]	14	14	51·0 (29-66)	4·3 (0-20·8)	1·3 (0·2-5·6)
De Mattos-Arruda (2019) [30]	6	2	NR	NR	NR
Totals	167*	129			

Table 1. Summary of patient characteristics in the 12 included studies. The table summarises the total number of patients of each study, the number of matched primary breast cancers and their brain metastases (BM), age, time between diagnosis and BM and survival time. All are median values including (range)/years. Please refer to supplementary table 1 for per-paper definitions of characteristics * Richichi et al.[24] sequenced 10 out of 12 patients *Siegel et al.[25] sequenced 5 out of 6 patients taking the total number of breast cancer brain metastasis patient with sequencing data to 164.

	Sequencing platform	Source of BM material	Sample preservation method	IHC reported	PAM50 reported	Sequencing data format
Da Silva (2010) [19]	OncoCarta V1*	Resection	FFPE	b	NR	table
Lee (2015) [20]	Ion-PGM	Resection	FFPE	b	b	table
Saunus (2015) [21]	Illumina HiSeq 2000	Resection	SF	b	NR	table
Brastianos (2015) [18]	Illumina HiSeq	Resection	SF/FFPE	b*	NR	Diagrams
De Mattos-Arruda (2015) [22]	Illumina HiSeq 2000	PM	FFPE	p	NR	xls
Muller (2016) [23]	Ion-PGM	Resection	SF/FFPE** 1 case SF	m	NR	table
Richichi (2017) [24]	Illumina HiSeq 2000	Resection	FFPE	p	p	table
Siegel (2018) [25]	Illumina HiSeq 2500	PM	SF/FFPE	p	b	xls
De Mattos-Arruda (2018) [26]	Illumina HiSeq 2000	PM	SF/FFPE	p	NR	table
Schrijver (2018) [27]	Illumina HiSeq 2500	Resection	FFPE	p	NR	xls
Diossy (2018) [28]	Illumina HiSeq**	NR	FFPE	b	NR	Diagram
Tyran (2018) [29]	Illumina NextSeq500	Resection	SF	b	NR	table
De Mattos-Arruda (2019) [30]	Illumina HiSeq 2500	PM/Resection	SF	p	NR	xls

* Validated

** MyChoice HRD performed on Illumina

Table 2. Summary of sequencing methodologies and clinical material in the 13 included studies. The table summarises the different sequencing platforms used within the 13 studies, the clinical material and their receptor status by immunohistochemistry (IHC) and PAM50. NR: not recorded, PM: post-mortem, FFPE: formalin-fixed paraffin-embedded, SF: snap frozen, p: primary tissue only, m: metastasis (brain) only and b: both primary and metastasis.

(A) BC	N	HER2+							HER2-						HER2 NK / ER+ / PgR NK	TNBC
		ER+			ER-			ER NK / PgR NK	ER+			ER-		ER NK / PgR NK		
		PgR+	PgR-	PgR NK	PgR+	PgR-	PgR NK		PgR+	PgR-	PgR NK	PgR+	PgR NK			
Da Silva (2010) [19]	12					4						3				5
Lee (2015) [20]	18						6				5					7
Saunus (2015) [21]	10			1			4				2		2		1	
Brastianos (2015)[18]	21							12						6		3
De Mattos-Arruda (2015) [22]	4			1			1				1				1	
Muller (2016) [23]	0															
Richichi (2017) [24]	12	1				4			5	1						1
Siegel (2018) [25]	6								1							5
De Mattos-Arruda (2018) [26]	6			3			3									
Schrijver (2018) [27]	9						5									4
Diossy (2018) [28]	19	2	2			4			3	3						5
Tyran (2019) [29]	14	2				1			5	3						3
De Mattos-Arruda (2019) [30]	6			2			1				2					1
(B) BM	**N**	**HER2+**							**HER2-**						**HER2 NK / ER+ / PgR NK**	**TNBC**
		ER+			**ER-**			**ER NK / PgR NK**	**ER+**			**ER-**		**ER NK / PgR NK**		
		PgR+	**PgR-**	**PgR NK**	**PgR+**	**PgR-**	**PgR NK**		**PgR+**	**PgR-**	**PgR NK**	**PgR+**	**PgR NK**			
Da Silva (2010) [19]	12				3	5										4
Lee (2015) [20]	42			5			8				11					18
Saunus (2015) [21]	11			2			3				3					3
Brastianos (2015)[18]	19							11						8		
Muller (2016) [23]	4		2						1							1
Diossy (2018) [28]	19	1	2			5			3	2						6
Tyran (2019) [29]	14	2			1				3	2				1		5

NK: Not Known

Table 3. A summary of studies by the IHC status of the (A) primary tumour and (B) brain metastasis. The studies presenting immunohistochemistry status of (A) primary breast cancer and (B) brain metastasis are presented with the number of patients (N) included in each study.

	N	LumA		LumB		HER2		Basal-like		Normal-like		Not known	
		P	M	P	M	P	M	P	M	P	M	P	M
Lee (2015) [20]	15	5	2	0	2	4	4	5	6	1	1	0	0
Richichi (2017) [24]	12	1	NR	5	NR	5	NR	1	NR	0	NR	0	NR
Siegel (2018)[25]	6	0	0	0	0	0	0	4	0	2	5	0	1

Table 4. Reported PAM50 status of paired primary and brain metastasis samples.

The 3 studies presenting the PAM50 status of primary breast cancer (P) and brain metastasis (M) are presented with the number of patients (N) included in each study.

Figure 1.

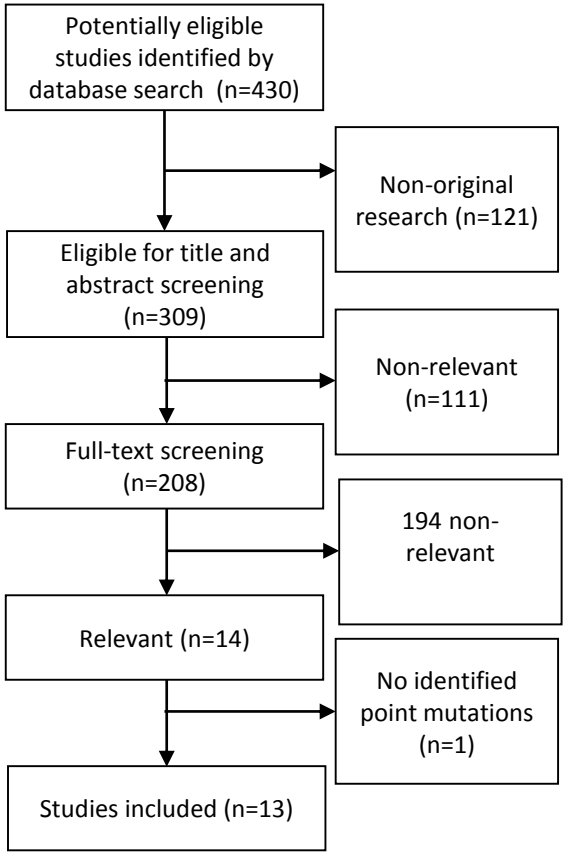

Potentially eligible studies identified by database search (n=430)
Non-original research (n=121)
Eligible for title and abstract screening (n=309)
Non-relevant (n=111)
Full-text screening (n=208)
194 non-relevant
Relevant (n=14)
No identified point mutations (n=1)
Studies included (n=13)

Figure 2

Figure 2.

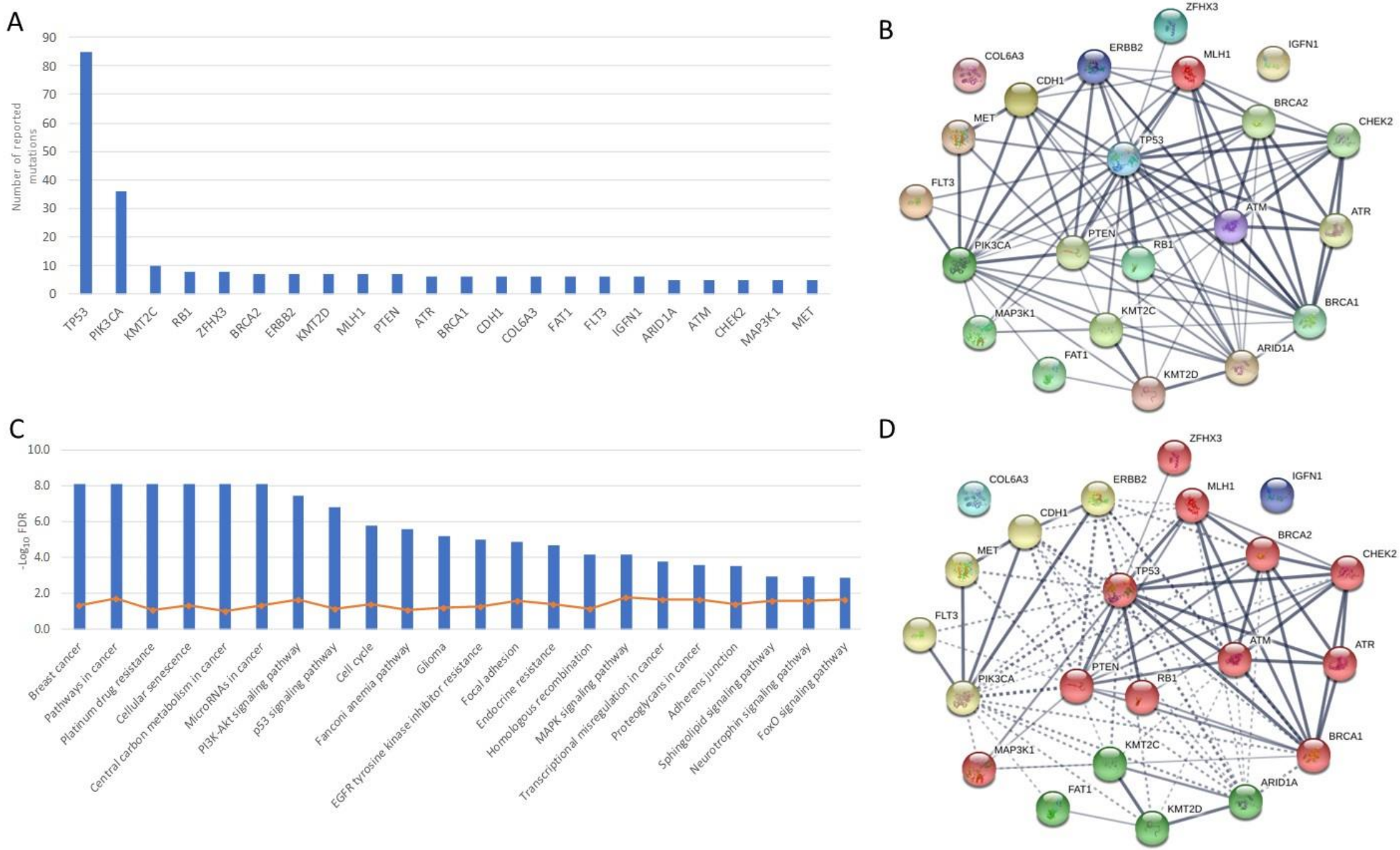
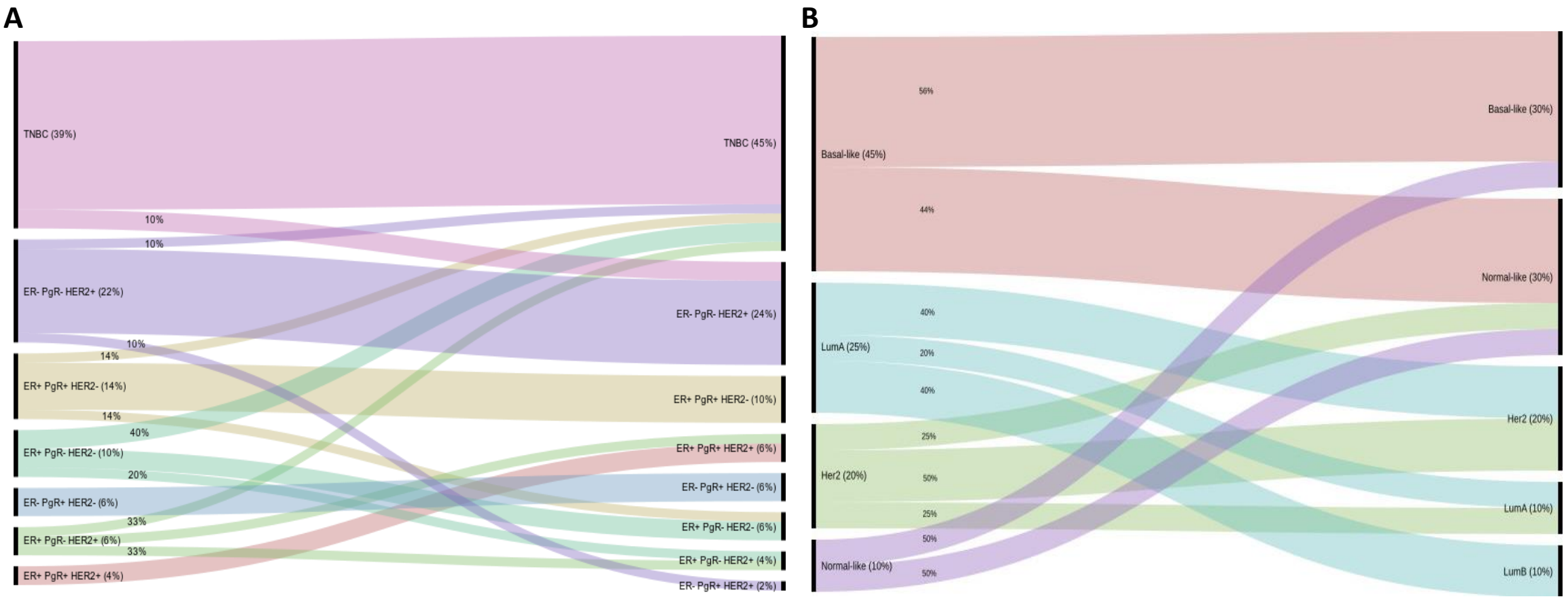


Figure 3.


A
TNBC (39%)
ER- PgR- HER2+ (22%)
ER+ PgR+ HER2- (14%)
ER+ PgR- HER2- (10%)
ER- PgR+ HER2- (6%)
ER+ PgR- HER2+ (6%)
ER+ PgR+ HER2+ (4%)
10%
10%
10%
14%
14%
40%
20%
33%
33%
TNBC (45%)
ER- PgR- HER2+ (24%)
ER+ PgR+ HER2- (10%)
ER+ PgR+ HER2+ (6%)
ER- PgR+ HER2- (6%)
ER+ PgR- HER2- (6%)
ER+ PgR- HER2+ (4%)
ER- PgR+ HER2+ (2%)
B
Basal-like (45%)
LumA (25%)
Her2 (20%)
Normal-like (10%)
56%
44%
40%
20%
40%
25%
50%
25%
50%
50%
Basal-like (30%)
Normal-like (30%)
Her2 (20%)
LumA (10%)
LumB (10%)

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