Discordance of PIK3CA and TP53 Mutations Between Breast Cancer Brain Metastases and Matched Primary Tumors

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Research article

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Abstract

Purpose: Extensive data of mutations in breast cancer (BC) metastases has been published in recent years. However, these studies contain very few patients with brain metastasis (BM). Thus, there is limited knowledge of the biology of BCBM. We primarily aimed to compare the mutational and histological pattern of BM with matched primary breast cancer (BC). Secondary aims were to determine mutations in BMs and PT in each BC subgroup (Luminal, HER2+ and TNBC) and survival according to changed or stable mutations between PTs and BMs.

Patients and methods: We investigated 57 BCBMs including 46 cases with the matched primary tumors (PT) by targeted Next Generation Sequencing (NGS) using the Cancer Hotspot Panel v2 (ThermoFisher Scientific) covering 207 targeted regions in 50 cancer related genes. BC subtype according to immunohistochemistry (estrogen- and progesterone receptors, HER2 and Ki67) was obtained by re-evaluation of available tissue from BMs and PT.

Results: NGS results fulfilling sequencing quality criteria were obtained from 52 BM (91.2%) and 41 (89.1%) PT, out of which 37 were matched pairs. Pathogenic mutation was detected in 66% of PTs (27/41), and 62% of BMs (32/52). *TP53* mutations were most frequent; 49% (20/41) of PTs and 48% (25/52) in BMs, followed by *PIK3CA* mutations; 22% (9/42) in PTs and 25% (13/52) in BMs. Mutations in *CDH1, EGFR*, *HRAS, RB1 CDKN2A* and *PTEN* were detected in single pairs or single samples. Mutational pattern was discordant in 24% of matched pairs. Standard BC markers was discordant in 26%, with a loss of the estrogen receptor and a change from Luminal A to other subtypes as the most common. Changes of mutations in BMs compared with PT did not influence survival after diagnose of BM (p=0.4395).

Conclusions: We show a discordance of *PIK3CA* and *TP53* mutations, as well as IHC BC subgroups in 25% of the matched pairs of BM and PT, which confirms the need for re-evaluation of mutations, as well as standard BC markers by immunohistochemistry in the BM. Since there are difficulties in obtaining tissue from BM, analysis of cell-free DNA from cerebrospinal fluid (CSF) may be a way forward.

Introduction

Despite increasingly effective treatment, about 20% of patients with primary breast cancer (BC) suffer from metastatic disease and 15–40% of these patients eventually develop brain metastases (BM) [1]. Due to limited treatment options, and debilitating symptoms that greatly affect quality of life, BM is a dreaded outcome [2]. There is a large variation in survival after diagnosis of BM that ranges from Triple Negative Breast Cancer (TNBC), with a median survival of 4–5 months, to Human Epidermal growth factor Receptor 2 (HER2) positive (HER2+) BC with a median survival following BM diagnosis of 9–16 months [3, 4]. The subtype of BC influences the risk of developing BMs and the BM free interval, with significantly higher incidence and shorter interval from recurrence to diagnosis of BM for patients with TNBC and HER2 + as compared to luminal tumors [5, 6].

Local treatment such as surgery or radiotherapy forms the basis of BM treatment. There is also a clear evidence of systemic therapies having an effect on BM in patients ineligible for local treatment [7, 8]. However, the blood-brain-barrier as well as the blood-tumor-barrier hinders the passage of systemic therapies to the central nervous system, hampering the effect of systemic treatment on BMs [9]. Another clinical problem is the difficulty to access metastatic tissue from the brain. Repeated studies have shown a discordance in the expression of steroid receptor and HER2 between metastatic lesions and the primary BC [10]. Thus, characterization of metastatic lesions is crucial for correct treatment decisions. The introduction of efficient therapies with targets like HER2, CDK4/6, PARP, and PIK3CA are being developed. Hence, the need for analysis of BC tissue in the metastatic setting is becoming apparent.

BM have become increasingly common in BC [6, 11] and consequently, there is need for adequate information regarding the evolution of mutations, and histology in the metastatic process for correct therapy decisions [12–14]. Do targetable mutations in BMs differ compared to that of the previous primary tumor (PT)? There are studies investigating this topic in metastatic lesions [15]. However, due to difficulties to sample tissue, BMs represent very few of investigated metastatic lesions.

We primarily aimed to compare the histology and mutational pattern of BMs with matched primary BC using next generation sequencing (NGS) and immunohistochemistry (IHC). Secondly, we aimed to investigate the mutational patterns in each BC subgroup (Luminal, HER2 + and TNBC), and survival according to changed or stable mutations between PTs and BMs.

Material And Methods

Patients

From hospital records of all diagnostic codes, patients with BMs from BC between 1994 and 2014 were identified. Patients received treatment of the primary BC in any of four hospitals in the western region of Sweden. The diagnoses were confirmed in the patient’s charts and patients with available material from BMs were selected. Clinical characteristics, type of metastasis, progression and survival were extracted from patient charts. PTs and BM tissue were evaluated, as specified below, when sufficient material was available. Data from the original report was utilized if deemed appropriated by the responsible breast pathologist if there was insufficient material available for re-evaluation by IHC. The study was conducted in accordance with the Declaration of Helsinki and the Sahlgrenska University Hospital Ethical Review Board; Gothenburg, Sweden approved the study (460-09, T592-14). Approval for the chart review and bio bank extractions was granted from each head of the participating departments.

Immunohistochemistry (IHC)

The available material was re-evaluated for histological type, nuclear grade, and receptor status. ER/PR, Ki67 and HER2 (HercepTest) immunohistochemistry was performed as per standard procedures using the Dako Autostainer Link and the EnVision™ FLEX detection systems according to the manufacturer's

instructions. HercepTest was followed by SISH when the IHC was judged as 2 + or 3 +. For a more detailed description, see a previous publication. [6].

Next Generation Sequencing

Preparation of sample library and next generation sequencing (NGS)

DNA isolation from FFPE sections containing a minimum of 25% neoplastic cells as assessd by breast pathologists was performed using the QIAamp DNA FFPE tissue kit (Qiagen Gmbh, Hilden, Germany). DNA concentration was determined using NanoDrop™ 3300 with Quant-IT Picogreen dsDNA assay kit (ThermoFisher Scientific, Waltham, MA, USA). Ten ng of DNA was used to prepare barcoded libraries with the Ion AmpliSeq™ Library kit 2.0 (ThermoFisher). The Cancer Hotspot Panel v2 (ThermoFisher Scientific) covering 207 targeted regions in 50 cancer related genes was used (https://tools.lifetechnologies.com/content/sfs/brochures/Ion-AmpliSeq-Cancer-Hotspot-Panel-Flyer.pdf*).* Template preparation and enrichment was performed with the IonChef™ Instrument (ThermoFisher Scientific). Eight barcoded samples were pooled per Ion 318 ^{TM} v2 BC chip and sequenced on the Ion PGM™ System (ThermoFisher Scientific). All steps were performed according to the manufacturer's instruction. Matched germline DNA from the patients was not available in this retrospective investigation.

Data processing

After alignment to the hg19 human reference genome, variant calling by the Torrent Suite Software v4.2.1.0 and filtering of described SNPs was performed. Variants were visually inspected with the Integrative Genomics Viewer (IGV; Broad Institute, Cambridge, MA, USA). Mutations were manually curated as pathogenic, likely pathogenic, variants of unknown significance or as benign. For ten samples, NGS results could not be obtained due to insufficient quality of DNA. For individual variants the minimum accepted read depth was 500 and if the read depth was < 1000, an allele frequency of > 5% was required for positive variant calling.

Statistical Methods

Tests of differences between subgroups and mutations was performed using Chi-square test or Fischer Exact test, depending on the number of expected values, with 5% significance as the limit to reject the null hypothesis. Brain Metastasis Specific Survival (BMSS) i.e. time from diagnosis of BM to death were estimated by the Kaplan Meier estimator, and compared by the log rank test. Two patients who had not undergone surgery were removed from survival analyses. IBM SPSS Statistics 25 ^{TM} was used for statistical calculations.

Results

A total of 69 patients who had undergone surgery of breast cancer BM were retrieved from chart studies and by diagnostic codes. For 46 of these, archived tissue of the BMs as well as from the PT were

available. For 11 patients, only the BM but not the PT was available. A total of 57 BMs and 46 PTs were thus included in the study. Of 57 patients with BM, 55 had undergone surgery (96%). The median age at time of BM diagnosis was 53 (29–75) years. The 22 patients with extra-cranial disease (ED) prior to BM had a median time from ED to BM of 18.1 months (range 1.4–92 months). For detailed data on clinical characteristics and survival at the time of diagnose of BM, please refer to Tables 1–3.

NGS results fulfilling sequencing quality criteria were obtained for 37 of the 46 matched PT/BM pairs and from 10 of the 11 unmatched BMs. For nine matched pairs, sequencing results were obtained for either only the PT (n = 4) or the BM (n = 5). In total, NGS results from 41 PTs and 52 BMs including the 37 matched PT/BM pairs were obtained (Fig. 1). For IHC subtype

Genetic profile of PT and BM

Twenty-seven PTs (66%) and 32 BMs (62%) exhibited at least one pathogenic mutation (Fig. 2). A pathogenic *JAK2* p.V617F mutation in a patient with previously diagnosed myeloproliferative disease was excluded from further analysis. The most commonly mutated gene in the dataset was *TP53*, with a mutation frequency of 44% (20/42) in PTs and 44% (25/52) in BMs. *PIK3CA* mutations were the second most prevalent, with a mutation frequency of 20% (9/42) in PTs and 23% (13/52) in BMs. Of note, a *CDH1* mutation was present in a PT/BM pair of lobular BC. Moreover, non-recurrent mutations in *EGFR*, *HRAS* and *RB1* were detected in single PT/BM pairs as well as mutations in *CDKN2A* and *PTEN* in single samples of PT or BM.

Mutational findings in matched pairs of BM and PT

In the matched pairs, similarly, the most prevalent mutation was in *TP53* in 43% (16/37) of the matched pairs (Table 5). Mutated *TP53* was found in three Luminal PTs and four paired BMs (27%/36%), eight TNBC PTs and ten paired BMs (47%/59%), and six HER2 positive PTs and five paired BMs (67%/55%). Mutations in *PIK3CA* was the second most prevalent mutation in this material. In matched PT/BM pairs, *PIK3CA* mutations were found in two PTs and four BMs of Luminal B (18%/36%); three PTs and BMs in TNBC (18%/18%), and in one matched case of HER2 positive tumors (11%). The numerical differences of *TP53* and *PIK3CA* mutations in BC subgroups did not reach statistical significance (p = 0.228) (p = 0.552) respectively.

Mutational concordance in matched material

The mutational profile of the genes present in the targeted panel was discordant between the PTs and BMs in nine cases out of 37 (24%). The mutational differences displayed no discernible pattern. There were three cases of mutations present only in the PT, four with only mutations found in the BM, and one case of mutation in the same gene, but at another position BM. Of note, in two PT/BM pairs, a pathogenic *PIK3CA* mutation and five *TP53* mutations were detected only in the BM. An altered mutational status did not affect survival; patients with a mutational change versus stable mutational pattern had a similar brain metastasis specific survival (BMSS) (p = 0.4395).

Immunohistochemical subtype in matched material

IHC subtype was available for 46 matched PT and BM. Receptor status by IHC was discordant in 12 out of 46 (26.1%) of the matched pairs (Fig. 3). The corresponding discordance rate between PT and BM for each BC subtype were as follows; Luminal A 100% (6/6); Luminal B 0% (0), TNBC 9% (2/ 23), HER2+/ ER + 50% (3/6), and HER2+/ ER 20% (1/5). The most common change in BC subtype was from Luminal A to Luminal B.

Survival parameters according subtype

Patients with Luminal and HER2+/ER + BC had the longest BMSS with a median of 28 months (6–64) and 34 months (7–62) respectively, whilst patients with HER2+/ER- and TNBC had BMSS of 11 months (0–46) and eight months (0–46) respectively (p = 0.0851). We found equal BMSS when patients with mutations in *PIK3CA* were compared to patients without *PIK3CA* mutation (p = 0.467). There was no difference in BMSS when patients with a *TP53* mutation were compared with patients without documented *TP53* mutation (p = 0.456).

Discussion

Difficulties in accessing intracranial tissue samples have hampered development of systemic treatments that could have an effect in the treatment of patients with BMs. To our knowledge, this is one of the largest cohorts of genetically characterized BC BM, consisting of 52 analyzed cases, most notably including 37 cases with matched PT, all from one region in Sweden. Despite this, the relatively small number of patients in each BC subgroup is a limitation of the present study, reflecting the difficulty to obtain matched PT/BM tissue. Sequencing results by NGS were successfully obtained in 90% of the BMs. At least one mutation, among 50 cancer driver genes, was present in 62% of the analyzed samples similar to previously published data [16–21]. Concordant with these studies *TP53* and *PIK3CA* are the most commonly mutated driver genes, both in the BMs and PTs. Of these two mutations, *PIK3CA* are considered targetable, whilst *TP53* mutations can be indirectly attacked through restoration of the transcriptional activity resulting in a functional wild-type TP53 protein [22]

The drug alpelisib, is a PI3Kα-specific inhibitor available for patients with recurrent *PIK3CA* mutated Luminal/HER2 negative BC [23]. Previous published data show that approximately 40% of ER + PT harbor a *PIK3CA* mutation [24]. Two studies that compared *PIK3CA* mutations in PTs and BC metastases reported mutations in 33% of PTs and 30% of metastases and 45% of PTs and 53% in metastases respectively [18, 25]. There were few or no patients with BMs in the above mentioned studies. We report lower figures with *PIK3CA* mutations in breast cancer among patients with BMs with a mutation frequency of 20% in PTs and 23% in the BMs. Interestingly, a recently published systematic review investigated 164 BMs with its matched PT in 126 patients extracted from 13 studies, found *PIK3CA* mutations in 22% of the patients with BMs [26]. These similarities raise the hypothesis that *PIK3CA* mutations is lower in BMs compared to other metastatic sites, however, additional studies are required to adequately answer this question. As expected, we found the highest proportion of *PIK3CA* mutations in

Luminal BC. Of note, in our material two out of eight pathogenic *PIK3CA* mutations and five *TP53* mutations were found in the BMs only and not in the PT underlining the need for re-evaluation of metastatic tissue or possibly by analysis of cell free DNA (cfDNA).

We found *PIK3CA* mutations as the second most common mutation after *TP53* in matched pairs of TNBC with 18% *PIK3CA* mutations all concordant in PTs and BMs. This is in line with previous published data in which *PIK3CA* mutations was the second most common mutation after *TP53*, especially in basal-like and luminal androgen receptor subtypes of TNBC [27–29]. The combined treatment of alpelisib and nab-paclitaxel is currently under investigation in pre-treated TNBC with either loss of PTEN expression or a *PIK3CA* mutation (NCT04251533).

The prognostic role of *PIK3CA* mutations in HER2 + BC has been extensively investigated with less effect of HER2 blocking therapy, both in the neo-adjuvant setting, and for recurrent BC in terms of lower pCR rates and shorter survival respectively [30, 31]. We found *PIK3CA* mutations in only 10% of the HER2 + cases. This is lower than previously reported of 20–40%. [24]. The limited number of HER2 + patients in our cohort may be the cause of this discrepancy. The effect of alpelisib in HER2 + breast cancer is under investigation in one ongoing and one completed clinical trial (NCT02038010; NCT04208178).

Almost 50% of the PTs in the present study population with a high proportion of TNBC harbored a *TP53* mutation, in contrast to the 20–35% mutation prevalence reported in unselected primary BC [32]. The Cancer Genome Atlas reveals an enrichment of *TP53* mutations in basal-like and HER2 enriched BC [24]. Interestingly, previous data reveal that a high proportion of patients with a *TP53* mutation in the primary BC developed BM [33, 34]. We lack data on the molecular subtypes in our material, but find an increased number of *TP53* mutations in TNBC and HER2/ER- BC. Enrichment of *TP53* mutations in BMs was not seen in the HER2 amplified subgroup, a finding that must be interpreted with caution due to the very small sample size of the HER2 group.

Roughly 25% of the matched pairs changed IHC based BC subtype in the BMs. The most common change was from Luminal A in PT to other subtypes, in most cases Luminal B in BMs. This is in accordance with previous results in which 219 patients showed a 36% overall discordance with the most common change in form of loss of PgR [35]. In a review pooling a total of 3384 matched pairs of BC and metastases from all organs, BM showed a discordant median rate of 22% compared to 45% in liver metastases and 16% in lymph node metastasis [36]. TNBC was the most stable group with less than 10% showing a gain in ER (one case) and HER2 (one case). Other detected mutations in our series, *CDH1*, *EGFR*, *HRAS, RB1, CDKN2A* and *PTEN* were rare, in general found in single or both samples from one patient.

A limitation of our current study is that genetic profiling was performed using a relatively small NGS panel of 50 genes. Even though the panel gives broad coverage of important cancer driver genes, more comprehensive sequencing might have provided further details in the landscape of mutation discordancy between PTs and BMs. Still, we believe that the relatively large number of matched PT and BM may

increase knowledge about the biology of BMs, how the metastatic process affects actionable genes, ultimately helping patients suffering from BM.

Conclusions

In conclusion, we confirm mutations in *TP53* and *PIK3CA* to be common in both primary breast tumors and BMs but the proportion varied depending on the subgroup. Mutation pattern, as well as IHC based subtypes were discordant in approximately 25% of the patients underlining the need for re-biopsy at disease progression. In this context, analysis of mutations in liquid biopsies by use of cell-free DNA (cfDNA) from cerebrospinal fluid has shown promising results [37–39].

Declarations

Ethical Approval: The study has been conducted in accordance with the Declaration of Helsinki and the Sahlgrenska University Hospital Ethical Review Board, Gothenburg, Sweden approved the study (460-09, T592-14). The need for informed consent was waived under the approval of the Ethical Review Board due to the retrospective design.

Consent for publication: Not applicable

Availability of data and materials: All pathological and uncertain mutations are added as a supplemental table. Clinical data is not included as this would allow for possibly identifying individuals.

Competing interests: The authors declare no conflicts of interest.

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Author Contributions: BL, AT, and FE conceived of the study. CA, SDL, AK, ER, FE, and BL developed the methodology for the study. CA, ER, SHD, CC, AS, AK, FE and AT participated in data acquisition. AT, FE, HF, and BL participated in data analysis and interpretation. AT, BL, and HF wrote and/ or edited the manuscript. All authors read and approved the final manuscript.

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Tables

Due to technical limitations, Table 1 to 5 are only available as a download in the Supplemental Files section.

Figures

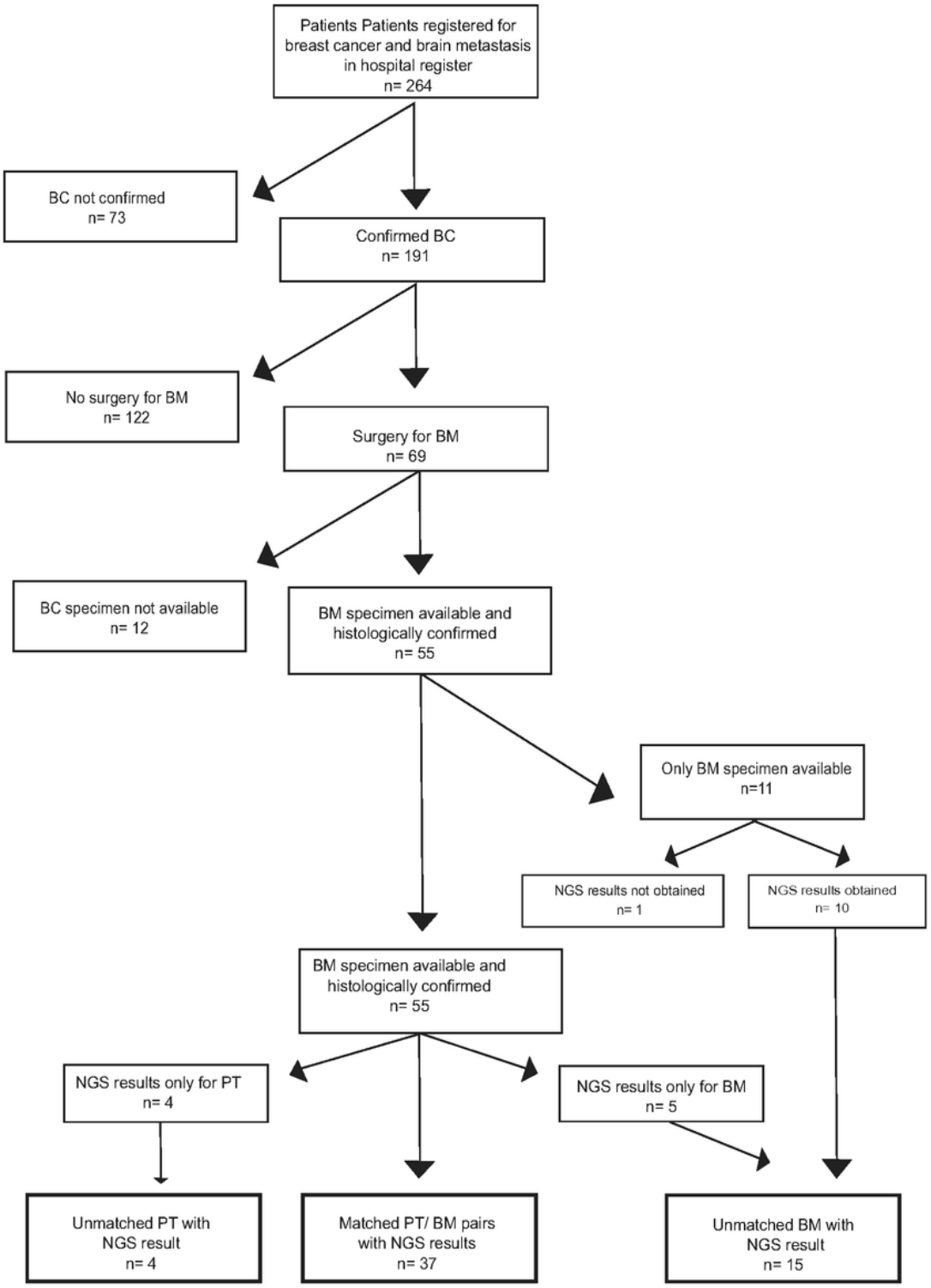

Figure 1

Consolidated Standards of Reporting Trials (CONSORT) diagram listing inclusions and exclusions and final numbers of primary tumors, brain metastases and matched primary and brain metastases.

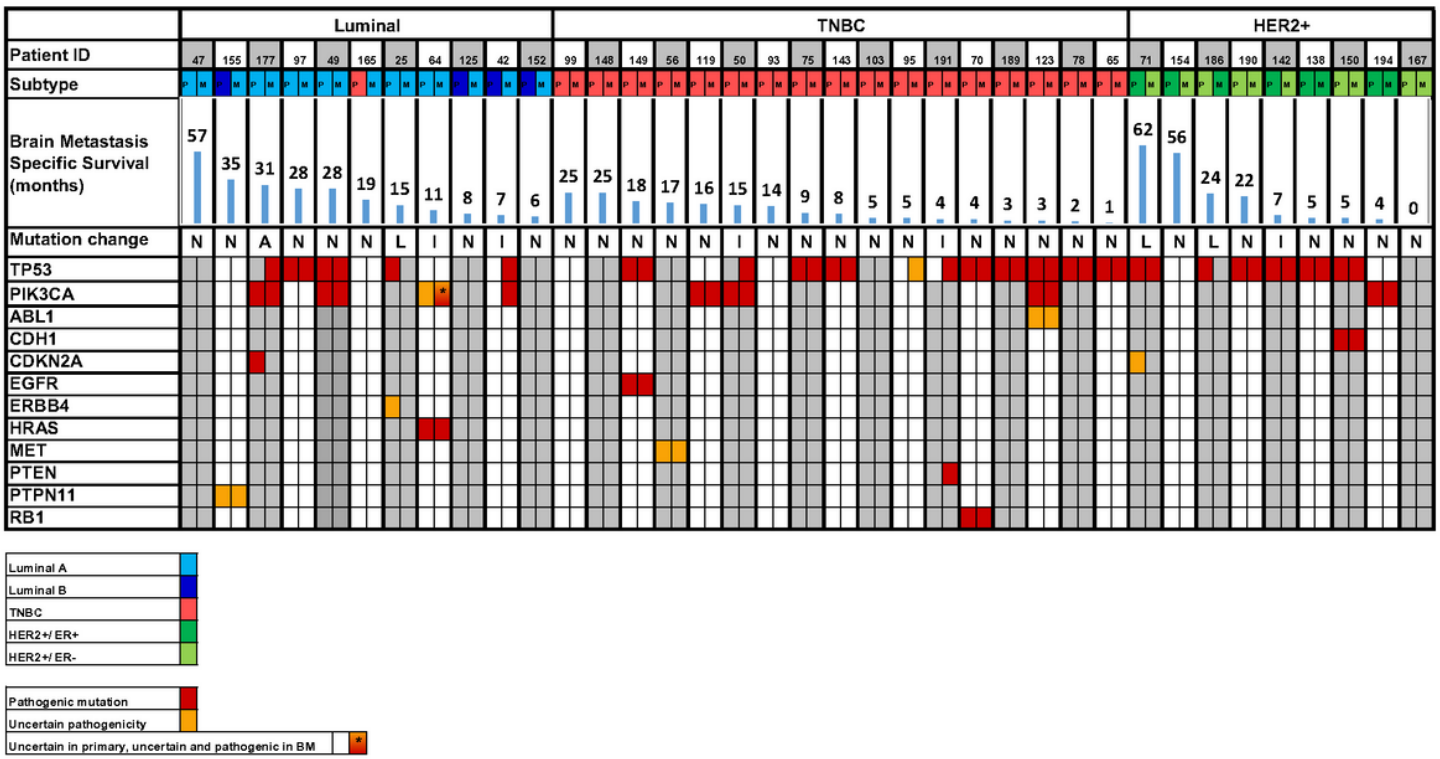

Figure 2

All the matched pairs of primary and brain metastatic tumors color coded in to sub groups with pathological and uncertain mutations shown as well as brain metastasis specific survival.

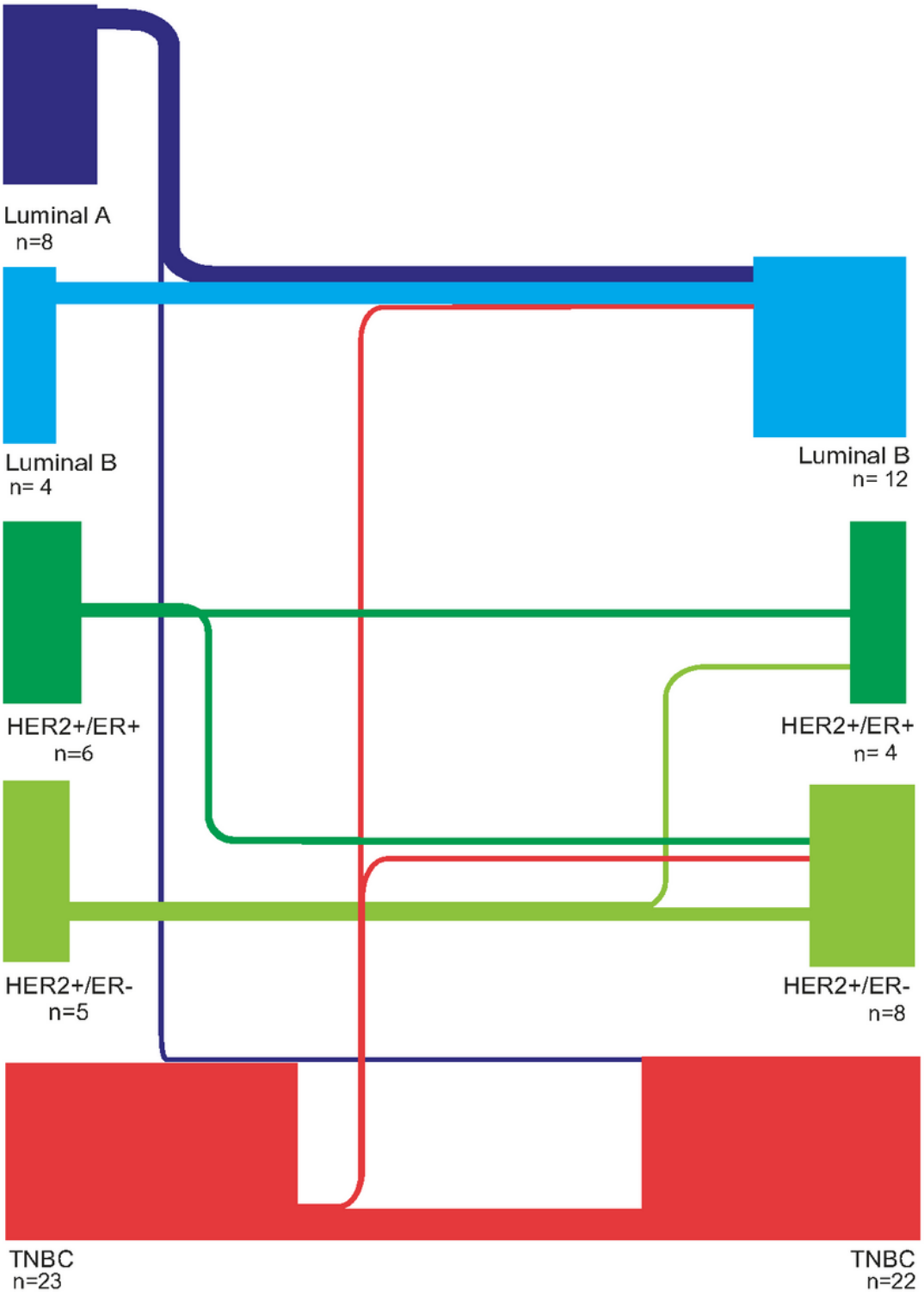

Figure 3

Histological expression in primary tumors and brain metastases, divided in to groups to depict the number of tumors that had significantly changed receptors so that the subgroup was altered. The thickness of the line represents the number of altered in each group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalData.xlsx
- Tables.docx