**Title:** Clinicopathologic and Genomic Landscape of Breast Carcinoma Brain Metastases

**Running Header:** Breast Carcinoma Brain Metastases

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**Key Words**: Comprehensive Genomic Profiling; Biomarkers; Breast Carcinoma; Brain Metastases

**Abstract**

**Background:** Among breast carcinoma patients with metastatic disease, 15-30% will eventually develop brain metastases. We examined the genomic landscape of a large cohort of breast carcinoma brain metastases (BCBMs) and compared them to a cohort of primary breast carcinomas (BCs).

**Material and Methods:** We retrospectively analyzed 733 BCBMs tested with comprehensive genomic profiling (CGP) and compared them to 10,772 primary breast carcinomas (not-paired) specimens. For a subset of 16 triple-negative breast carcinoma (TNBC)-brain metastasis (BM) samples, PD-L1 immunohistochemistry was performed concurrently.

**Results:** A total of733 consecutive BCBMs were analyzed. Compared to primary BCs, BCBMs were enriched for genomic alterations in *TP53* (72.0%, 528/733), *ERBB2* (25.6%. 188/733), *RAD21* (14.1%, 103/733)*, NF1* (9.0%, 66/733)*, BRCA1* (7.8%, 57/733), and *ESR1* (6.3%,46/733) (p < 0.05 for all comparisons). Immune checkpoint inhibitor (ICPI) biomarkers such as tumor mutational burden (TMB)-High (16.2%, 119/733), microsatellite instability (MSI)-High (1.9%, 14/733), *CD274* amplification (3.6%, 27/733), and APOBEC mutational signature (5.9%, 43/733) were significantly higher in the BCBM cohort compared to the primary BC cohort (p < 0.05 for all comparisons). When using both CGP and PD-L1 IHC, 37.5% (6/16) of the TNBC brain metastasis patients were eligible for atezolizumab based on PD-L1 IHC, and 18.8% (3/16) were eligible for pembrolizumab based on TMB-High status.

**Conclusion**

We found a high prevalence of clinically relevant genomic alterations in BCBM patients, suggesting that tissue acquisition (surgery) and or cerebrospinal fluid (CSF) for CGP in addition to CGP of the primary tumor may be clinically warranted.

**Implications for Practice**

We found a high prevalence of clinically relevant genomic alterations in BCBM patients, suggesting that tissue acquisition (surgery) and or cerebrospinal fluid (CSF) for CGP in addition to CGP of the primary tumor may be clinically warranted. In addition, we identified higher positive rates for FDA-approved immunotherapy biomarkers detected by CGP in BCBM patients, opening the possibility for new on-label treatments. Last, we noted limited correlation between TMB and PD-L1 IHC which exemplifies the importance to test with both PD-L1 IHC and CGP for ICPI eligibility of TNBC patients with brain metastases.

**Introduction**

Breast cancer remains a leading cause of morbidity and mortality for women globally. Despite therapeutic advances, 30% of women with early disease will relapse with incurable metastatic breast cancer. A growing clinical problem in patients with metastatic disease is the development of brain metastasis, and breast cancer brain metastasis (BCBM) which occurs in 15-30% of patients.[1] In particular, triple negative breast cancer (TNBC) and HER2-positive breast cancer have a propensity for metastasizing to the CNS.[2, 3]

Sequential advances in targeted systemic therapies for metastatic breast cancer have largely been predicated on assessing control of extracranial disease, with predictive biomarkers typically assessed on primary tumor or an extracranial metastasis. Recent advances include the United States Food and Drug Administration (FDA) approval of alpelisib plus fulvestrant for *PIK3CA* mutated disease in estrogen receptor-positive, HER2-negative breast cancer based on the SOLAR-1 clinical trial.[4] While olaparib and talazoparib are now available for patients with metastatic breast cancer (MBC) and germline *BRCA1/2* mutation, the TBCRC-048 trial also demonstrated benefit from olaparib in somatic *BRCA1/2* mutant breast cancer.[5-7]

With regard to immunotherapy, the FDA has recently approved two immune checkpoint inhibitors (ICPIs) for patients with metastatic, triple-negative breast carcinoma (TNBC).[8, 9] First, atezolizumab plus nab-paclitaxel was approved by the FDA in 2019 for TNBC with PD-L1 positivity (immune cell (IC) ≥1) as defined by the Ventana SP142 immunohistochemistry (IHC) assay.[10] The second ICPI, pembrolizumab, was approved for all solid tumor types in patients that are tumor mutational burden (TMB)-High or microsatellite instability-High (MSI-H).[11, 12] In addition, based on the KEYNOTE-355 trial, TNBC patients with PD-L1 positivity utilizing the DAKO 22C3 IHC assay with combined positive score (CPS) ≥10 may benefit from pembrolizumab in combination with chemotherapy.[13] Furthermore, the apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) mutational signature has been associated with ICPI response in tumor types such as non-small cell lung cancer (NSCLC) and breast carcinoma.[14, 15]

In contrast to these advances for the control of extracranial disease only tucatinib has been specifically licensed for the treatment of BCBM and only in the context of HER2-positive disease, as patients with active BCBM have been excluded from the vast majority of registration trials in breast cancer.[16] However, limited evidence of potential CNS activity of targeted agents including alpelisib and olaparib is available from case reports.[17, 18] Additionally, in the NALA trial, neratinib in combination with capecitabine has been shown good efficacy in HER2-positive BCBM patients.[19] Studies have previously examined the genomic landscape of BCBM and reported genomically distinct features from primary breast cancer as well as extracranial metastasis.[20] Given the limited systemic options for the treatment of BCBM, identifying the prevalence of biomarkers that may select for patients who may benefit from targeted therapies warrants further study. Here, we examine the genomic landscape of BCBM samples with comprehensive genomic profiling (CGP) in a large cohort of patients to define the potential applicability of recent therapeutic advances.

**Material & Methods**

*Patient Cohort*

This study was approved by the Western Institutional Review Board Protocol No. 20152817. We performed a retrospective analysis of 733 consecutive breast carcinoma samples that metastasized to the brain and were tested with FoundationOne®/FoundationOneCDx® between August 2014 and June 2020 as part of routine clinical care. In addition, 10,772 primary breast carcinoma specimens were used as a comparison group. Age, sex, and site of specimen of patient were extracted from accompanying pathology reports. TNBC status was confirmed in a subset of patients that received PD-L1 IHC testing by reviewing the accompanying pathology report for hormone receptor status and *ERBB2* (HER2) status from CGP.

*Comprehensive Genomic Profiling of Breast Carcinoma Samples*

CGP was performed using FoundationOne®/FoundationOneCDx® in a Clinical Laboratory Improvement Amendments (CLIA)-certified and College of American Pathologists (CAP)-accredited laboratory (Foundation Medicine, Cambridge, MA) using previously described methods.[21]FoundationOne®/FoundationOneCDx® uses a next generation sequencing platform and a hybrid capture methodology that detects base substitutions, insertions/deletions, and copy number alterations in up to 324 genes and select gene rearrangements, as well as tumor mutational burden (TMB) and microsatellite instability (MSI). Hematoxylin and eosin (H&E) stained slides from each sample were reviewed by a board-certified pathologist for presence of adequate tumor (≥20% of nucleated cells are tumor cells) before sequencing. *ERBB2* amplification was determined by CGP and defined as ≥ ploidy + 3 (copy number 5 in a diploid sample) in accordance with the FDA-approved companion diagnostics (CDx) claim for the FoundationOneCDx® assay. TMB was determined on up to 1.14 megabases (Mb) of sequenced DNA and TMB ≥10 mutations/Mb was considered TMB-High per CDx approval.[12, 22] MSI analysis was performed from DNA sequencing across 114 loci and MSI-High was considered positive.[11, 23] APOBEC mutational signatures were called as described by Zehir et al.[24] Genomic ancestry of patients was determined using a principle component analysis of genomic single nucleotide polymorphisms trained on data from the 1000 Genomes Project and each patient was classified as belonging to one of the following super populations: African, Central and South American, East Asian, European, and South Asian.[25, 26] Somatic/germline status for *BRCA1/2* short variant mutations was computationally predicted using previously described methods.[27]

*PD-L1 SP142 CDx Immunohistochemistry Testing*

For a subset of cases, PD-L1 IHC was performed using the Ventana SP142 CDx assay per manufacturer’s instructions in a CLIA-certified and CAP-accredited reference laboratory (Foundation Medicine, Morrisville, NC).[28] PD-L1 IHC slides were interpreted by board-certified pathologists using the tumor-infiltrating immune cell (IC) scoring method where IC = proportion of tumor area that is occupied by PD-L1 staining IC of any intensity per interpretation guide.[29] The CDx cut-off for atezolizumab plus nab-paclitaxel for TNBC is an IC score of ≥1%.[8]

*Statistical Analysis*

Clinicopathologic differences between different breast cancer cohorts were analyzed using ANOVA, Chi-Squared Contingency Test, or Fisher’s Exact Test. To examine the differences in the genomic landscape of the different cohorts, we identified the top 25 genes that have genomic alterations (GA) and compared these same genes with a Fisher’s Exact Test. P values were adjusted for multiple comparisons using the Bonferroni method and adjusted p-values of < 0.05 were considered significant.[30]

**Results**

*Breast Cancer Brain Metastasis Patient Cohort*

A total of 733 BCBM samples were included in this study. Median patient age was significantly higher in primary BC compared to BCBM cohort patients (55 and 53, respectively, p < 0.001) (**Table 1**). Predominant genetic ancestry of patients was not significantly different between brain metastasis and primary breast carcinoma samples (p = 0.22) (**Table 1**).

In a subset of samples with PD-L1 IHC testing (n=188), 16 TNBC-BM samples and 172 primary TNBC had CGP performed concurrent with the PD-L1 IHC testing. In this cohort, the age and the predominant genetic ancestry were not significantly different between the TNBC-BM and primary TNBC disease subsets (p = 0.45 and p = 0.81, respectively) (**Table 2**).

*Genomic Landscape of Breast Carcinoma Brain Metastases*

CGP analysis of 733 BCBMs revealed the following top 10 most altered genes: *TP53* (72.0%, 528/733), *PIK3CA* (28.7%, 218/733), *ERBB2* (25.6%, 188/733), *MYC* (25.5%, 187/733), *PTEN* (16.8%, 123/733), *CCND1* (14.9%, 109/733), *RAD21* (14.1%, 103/733), *FGF3* (13.5%, 99/733), *FGF19* (13.5%, 99/733), and *FGF4* (13.4%, 98/733) (**Figure 1A**). BCBMs enriched for GA in *TP53* (72.0% [528/733] vs 59.7% [6405/10772], p < 0.001), *ERBB2* (25.6% [188/733] vs 11.8% [1268/10772], p <0.001), *RAD21* (14.1% [103/733] vs 10.3% [1108/10772], p = 0.046), *NF1* (9.0% [66/733] vs 5.7% [615/10772], p = 0.016), *BRCA1* (7.8% [57/733] vs 4.5% [486/10772], p = 0.005), and *ESR1* (6.5% [46/733] vs 3.7% [397/10772], p = 0.024) compared to the primary BC cohort (**Figure 1B, Supplemental Table 1**). In addition, we identified 2.2% (16/733) of BCBM patients with a fusion, including 3 patients with a *NOTCH1-SEC16A* fusion and 1 patient with a *RFX6-ROS1* fusion (**Supplemental Table 2**).

In this BCBM cohort, 51.7% (379/733) of the patients were positive for at least one CDx biomarker as determined by CGP (**Figure 2**). In terms of specific FDA-approved therapies with an associated biomarker, 24.3% (178/733) had *ERBB2* amplifications, 26.7% (196/733) had *PIK3CA* mutations, 0% (0/733) had *NTRK1/2/3* fusions, 11.9% (87/733) had *BRCA1/2* mutations. Based on the somatic/germline status bioinformatic predictions, 28.6% (22/87) were germline mutations, 27.3% (21/87) were somatic mutations, and the algorithm wasn’t able to make a prediction in 57.1% (44/87) of the mutations. In the BCBM cohort, 26.2% (192/733) had at least one GA in one of the 14 genes involved in the Homologous Recombination Deficiency (HRD) pathway (7.8% (57/733) *BRCA1*, 5.7% (42/733) *BRCA2*, and 3.1% (23/733) *ATM*) (**Supplemental Table 3**). *ESR1* mutations almost always occur following endocrine therapy in estrogen receptor (ER) positive patients and has been shown to be a biomarker of endocrine resistance.[31] In this cohort of BCBM, 4.9% (36/733) of the patients harbored an *ESR1* mutation(s) which could help inform decision making for these BCBM patients as well as highlighting the importance of including such patients in any relevant clinical trials (**Supplemental Table 4**).

In the CGP defined *ERBB2* amplified BCBM cohort (n = 178), there were fewer genomic alterations in *PTEN* (1.7% [3/178] vs 21.6% [120/555], p < 0.001), *RB1* (2.2% [4/178] vs 12.8% [71/555], p < 0.001), and *BRCA1* (1.1% [2/178] vs 9.9% [55/555], p < 0.001) compared to the *ERBB2* non-amplified BCBM cohort (**Supplemental Table 5**). This contrasts with the CGP defined TMB-High BCBM cohort (n = 119), where there was enrichment for alterations in *PIK3CA* (47.4% [55/119] vs 26.5% [163/614], p = 0.001) and *ARID1A* (16.4% [19/119] vs 5.7% [35/614], p = 0.009) compared to the non-TMB-High BCBM cohort (**Supplemental Table 6**).

In the confirmed TNBC-BM cohort, the top 5 genes with GA were *TP53* (87.5%, 14/16), *RAD21* (56.3%, 9/16), *PTEN* (37.5%, 6/16), *MYC* (31.3%, 5/16), and *VEGFA* (18.8%, 3/16)(**Figure 3A**). A significantly higher number of GA were present in the TNBC-BM cohort when compared to the primary TNBC cohort, though significant differences were not found in individual genes (p <0.001, p ≥ 0.05, respectively) (**Table 2, Figure 3B, Supplemental Table 7**).

*Immune Checkpoint Inhibitor (ICPI) Biomarkers*

ICPI biomarkers of TMB-High (16.2% [119/733] vs 5.4% [584/10772], p < 0.001), MSI-H (1.9% [14/733] vs 0.4% [42/10772], p < 0.001), *CD274* (encodes for PD-L1 protein) amplification (3.6% [27/733] vs 1.7% [186/10772], p <0.001), and APOBEC mutational signature (5.9% [43/733] vs 3.6% [384/10772], p = 0.003) were significantly higher in the BCBM cohort when compared to the primary BC cohort (**Table 1**). In addition, we also examined the ICPI biomarker prevalence in *ERBB2* amplification, *PIK3CA* mutations, *BRCA1/2* mutations, and *ESR1* mutation positive and negative disease subsets (**Supplemental Table 8**). In the *PIK3CA* mutation positive cohort, prevalence of TMB-High (26.0% [51/196] vs 12.7% [68/527], p < 0.001) and APOBEC mutational signature (14.8% [29/196], 2.6% [14/537], p < 0.001) was significantly higher when compared to the *PIK3CA* mutation negative cohort. No significant difference was found in the other comparisons.

The frequency of PD-L1 positivity was lower in the TNBC-BM cohort than that in the primary TNBC cohort (37.5% [6/16] vs. 64.0% [110/173], p = 0.057). However, when examining the other ICPI biomarkers (TMB-High: 18.8% [3/16] vs. 2.3% [4/172], p = 0.014; *CD274* amplification: 18.8% [3/16] vs. 2.9% [5/172], p = 0.022), we saw a significantly higher prevalence in the TNBC brain metastatic cohort when compared to the primary TNBC cohort. No MSI-H patients were identified in the TNBC primary and BM cohorts. When using both CGP and PD-L1 IHC, 37.5% (6/16) of the TNBC patients were eligible for atezolizumab based on PD-L1 IHC, and 18.8% (3/16) were eligible for pembrolizumab based on TMB-High status, and 12.5% (2/16) of patients were eligible for both atezolizumab and pembrolizumab based on PD-L1 IHC and TMB-High status (**Supplemental Figure 1**).

*Paired Primary BC and BCBM Samples*

In our cohort, 11 paired primary BC and BCBM samples were identified (**Table 3**). The time between the collection date of the primary BC sample to the BCBM sample ranged from 5.7 months to 8 years. Overall, there were an additional 23 amplifications, 9 mutations, and 1 fusion detected in the paired BCBM samples when compared to the paired primary BC samples. In addition, of the 11 paired cases, 90.9% (10/11) had at least one additional GA discovered in the BCBM sample when compared to the primary BC sample. Also, 45.5% (5/11) of the BCBM samples did not have at least one GA that was found on the primary BC sample. Of importance, case 1 (HR+/HER2-) had gained a *PIK3CA* E545K in the BCBM sample and did not have any *PIK3CA* mutations on the primary BC sample; and case 10 (HR+/HER2-) had gained a *PIK3CA* E726K in the BCBM sample in addition to the *PIK3CA* E545K on the original primary BC sample (**Table 3**). For ICPI biomarkers, case 11 showed a gain of a *CD274* amplification and case 7 had changed to a TMB-High and MSI-H status in their paired BCBM sample; and three cases had lost the TMB-High status in their paired BCBM samples (**Table 3**).

We next compared the genomic profiles of the 11 primary breast cancer cases that eventually metastasized to the brain with the overall primary BC cohort. Here, we saw higher prevalence of GA in *TP53* (90.9% [10/11] vs 59.4% [6395/10761], p = 0.860), *PIK3CA* (36.4% [4/11] vs 31.4% [3375/10761], p = 1), *ERBB2* (27.3% [3/11] vs 11.8% [1265/10761], p = 1), and *MYC* (36.4% [4/11] vs 21.1% [2272/10761], p = 1), in the primary BC that eventually metastasized to the brain, though no significance was found due to the limited number of these samples (**Supplemental Table 9**).

**Discussion**

This retrospective cohort study of 733 BCBM and 10,772 primary BC specimens revealed that brain metastases were more likely to exhibit *TP53, ERBB2, RAD21, NF1, BRCA1*, and *ESR1* GAs. These genes have important clinical implications. For example, *TP53* mutations has been shown to have distinct prognostic relevance; *ERBB2* amplifications are an indication for the use for HER2 inhibitors; *RAD21* expression confers resistance to chemotherapy; *NF1* GA are associated contralateral breast cancer and poor survival, *BRCA1/2* mutations are an indication for the use for PARP inhibitors; and *ESR1* mutations are resistance biomarkers for aromatase inhibitor therapy.[5, 6, 31-35] In the confirmed TNBC cohort, the number of GA per sample was also increased in the brain metastases cohort and in many cases it was different from the primary BCs. Due to these differences, for patients with breast carcinoma that metastasized to the brain, the metastatic tissue in the brain or CSF specimen when safely available, should be considered as specimens for CGP testing.[36, 37]

In the eleven-paired primary BC and BCBM samples we found that there was at least one additional GA in 90.9% (10/11) BCBM (post treatment) sample when compared to the primary BC sample and 45.5% (5/11) of the BCBM lost a least one GA when compared to the primary BC sample. One case had gained a *PIK3CA* mutation on the BCBM sample with no *PIK3CA* mutation detected in the primary BC sample, thus making the BM targetable with *PIK3CA* inhibitors. Another BM case gained an additional *PIK3CA* mutation on top of the original *PIK3CA* mutation, which likely portends a higher sensitivity to *PIK3CA* inhibitors when compared to a single *PIK3CA* mutation.[38, 39] Last, one case gained a *CD274* amplification in the BCBM samples not previously detected in the primary BC sample which confers sensitivity to ICPI.[40] Previously, it was shown that HER2+ and TNBC breast carcinoma have an increased risk of developing brain metastasis.[20] In our 11 primary BC that eventually developed BM, we discovered a higher prevalence of GA in *TP53*, *PIK3CA*, *ERBB2*, and *MYC* which suggests that GA alterations in these genes could play a role in the metastasis of BC to the brain. It is important to note that our cohort is small, and so conclusions cannot be made on this data alone, but these findings do highlight important trends that should be expanded upon in a larger dataset.

In the overall breast carcinoma cohort, the ICPI biomarkers of TMB-H, MSI-H, *CD274* amplification, and APOBEC mutational signature, were all enriched in the BCBM samples when compared to the primary BC samples. Importantly, several have documented efficacy for immune checkpoint therapy in brain metastases (mostly from lung, melanoma and renal cell cancers).[41] These results further suggest that a subset of BCBM are positive for ICPI biomarkers and could be considered for treatment with ICPI. We detected enrichment of TMB-High and *CD274* amplification in the TNBC-BM samples; however, we also found a lower PD-L1 positivity rate among TNBC-BMs compared to the TNBC primary cohort similar to what was previously described.[42] One possibility for this observed difference in PD-L1 positivity rate could be due to the small sample size. However, it is more likely pre-analytic factors in processing brain specimens or biologic reasons caused a lower PD-L1 positivity rate in TNBC-BM specimens when compared to primary TNBC specimens, and this difference could be considered when choosing a sample for PD-L1 IHC testing for treatment purposes or enrollment into a clinical trial.

Last, in the TNBC-BM confirmed cohort with PD-L1 testing (n=16), we saw that 37.5% (6/16) of the TNBC patients were eligible for atezolizumab based on PD-L1 IHC, 18.8% (3/16) were eligible for pembrolizumab based on TMB-High status, and 12.5% (2/16) patients were eligible for both atezolizumab and pembrolizumab based on PD-L1 IHC and TMB-High status. While there is a subset of patients eligible for both atezolizumab and pembrolizumab based on TMB and PD-L1 IHC, there is a distinct subset of patients only eligible for atezolizumab or pembrolizumab, exemplifying the importance of testing with both CGP and PD-L1 IHC in these patients. Given the recent approval of Dako 22C3 PD-L1 IHC in TNBC for pembrolizumab, it would be important to determine if the positivity for this assay overlaps with TMB and Ventana SP142 PD-L1 IHC.

The major strength of this study is the large number of BCBM samples all undergoing centralized CGP using a single assay. However, a primary limitation of this study is the limited clinical information available with the patient samples. It is likely that some primary BC samples in our study could also have concurrent BCBM; however, in the absence of clinical histories, we do not know the extent of their disease. However, in general, most samples received at our institution are from patients with advanced disease at time of testing. Also, the rates of BCBM patients who undergo surgery and obtain a surgical specimen are low (Sperduto et al [21.1%, 521/2473] and (Lin et al [15.8%, 46/291]) and this study only represents the patients who had a tissue specimen for CGP testing.[43, 44] In addition, while we have the HER2 status of the patients in this study based on CGP testing, we do not have the ER/PR status of most of the patients to further stratify the patients based on ER/PR status. Another limitation is that the only FDA approved therapy specifically for BCBM is tucatinib. While the actionability of the biomarkers described in this study have been associated with breast carcinoma patients, they have not been FDA approved for BCBM patients, and so further clinical studies are needed to formally assess the actionability of these biomarkers in BCBM patients.

**Conclusion**

We found a higher prevalence of clinically relevant GA in patients with BCBM which suggests that metastatic tissue to the brain or CSF specimen should be considered as specimens for CGP testing.[36] In addition, we saw a higher frequency of immunotherapy biomarker positivity in the BCBM cohort suggesting that patients with breast carcinoma metastasized to the brain should be assessed for ICPI biomarkers and advanced to such treatment modalities when clinically appropriate. Last, we found only a weak relationship between TMB and PD-L1 IHC which exemplifies the importance to test with both PD-L1 IHC and CGP for ICPI eligibility in TNBC patients with brain metastases.

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**Figure Legend**

**Figure 1** Genomic landscape of breast carcinoma brain metastases (BCBM). A) Co-mutation plot of the 733 BCBM samples. The top 5 genes with genomic alterations (GA) were *TP53*, *PIK3CA*, *ERBB2*, *MYC*, and *PTEN*. B) Longtail plot of genes with GA and comparison between the BCBM and primary BC cohort. The genomics of the breast carcinoma brain metastases was different from primary breast carcinoma samples. Enrichment of GA in *TP53*, *ERBB2,* *RAD21, NF1, BRCA1*, and *ESR1* was present in the BCBM cohort when compared to the primary BC cohort (p < 0.05).

**Figure 2** BCBM patient eligibility for therapy based on CGP CDx dataset. In this BCBM cohort, more than half of the patients (51.7%, 379/733) were positive for at least one companion diagnostic biomarker as determined by CGP.

**Figure 3** Genomic landscape of triple negative breast carcinoma brain metastases (TNBC-BM). A) Co-mutation plot of the TNBC-BM cohort. In the confirmed TNBC-BM cohort, the top 5 genes with genomic alterations (GA) were *TP53*, *RAD21*, *PTEN*, *MYC*, and *VEGFA.* B)Longtail plot of genes with GA and comparison between the TNBC-BM and primary TNBC cohort. A significantly higher number of GA were present in the TNBC-BM when compared to the primary TNBC cohort, though significant differences were not found in individual genes with GA (p < 0.001, p ≥ 0.05).

**Table 1** Patient Characteristics of Primary Breast Carcinoma and Breast Carcinoma Brain Metastases

|  |  |  |  |
| --- | --- | --- | --- |
| **Patient Characteristics** | **Primary Breast Carcinoma (n=10772)** | **Breast Carcinoma Brain Metastases (n=733)** | **ap-value** |
| **bAge (years old)** |  |  | <0.001 |
| Median | 55 | 53 |  |
| Mean | 55 | 53 |  |
| **cPredominant Ancestry** |  |  | 0.22 |
| African | 14.8% (1564) | 13.0% (94) |  |
| Central and South American | 11.9% (1250) | 14.3% (103) |  |
| East Asian  | 4.2% (442) | 3.7% (27) |  |
| European | 67.3% (7094) | 66.7% (481) |  |
| South Asian | 1.8% (192) | 2.2% (16) |  |
| **Immunotherapy Biomarkers** |  |  |  |
| TMB-High(≥10 mutations/Mb) | 5.4% (586) | 16.2% (119) | <0.001 |
| MSI-High | 0.4% (42) | 1.9% (14) | <0.001 |
| *CD274* amplification | 1.7% (186) | 3.6% (27) | <0.001 |
| APOBEC mutational signature | 3.6% (384) | 5.9% (43) | 0.003 |

aAll p-values from Fisher’s exact test except for age which is from ANOVA and predominant ancestry which is from Chi-squared contingency test; bAge was not available for 3 breast carcinoma brain metastases and 28 primary breast carcinoma samples; cPredominant genetic ancestry could not be determined for a few cases (n=721 for total breast carcinoma brain metastases and n= 10542 for total primary breast carcinoma)

**Table 2** Patient Characteristics of Primary TNBC and TNBC Brain Metastases

|  |  |  |  |
| --- | --- | --- | --- |
| **Patient Characteristics** | **Primary TNBC (n=172)** | **TNBC Brain Metastases (n=16)** | **p-value** |
| **Age (years old)** |  |  | \*0.454 |
| Median | 54 | 58.5 |  |
| Mean | 54.1 | 56.9 |  |
| **Predominant Ancestry** |  |  | \*\*0.805 |
| African | 25.0% (43) | 18.8% (3) |  |
| Central and South American | 12.2% (21) | 18.8% (3) |  |
| East Asian  | 2.9% (5) | 0% (0) |  |
| European | 57.6% (99) | 62.5% (10) |  |
| South Asian | 2.3% (4) | 0% (0) |  |
| **PD-L1 Positivity Rate (IC ≥ 1)** | 64.0% (110) | 37.5% (6) | \*\*\*0.057 |
| **# of GA / sample** |  |  | \*<0.001 |
| Median | 5 | 8 |  |
| Mean | 5.7 | 8.3 |  |
| **Immunotherapy Biomarkers** |  |  |  |
| TMB-High(≥10 mutations/Mb) |  2.3% (4) | 18.8% (3) | \*\*\*0.014 |
| MSI-High | 0% (0) | 0% (0) | \*\*\*1 |
| *CD274* amplification | 2.9% (5) | 18.8% (3) | \*\*\*0.022 |

**Table 3** Genomics of 11 Paired Primary Breast Carcinoma and Breast Carcinoma Brain Metastasis Samples

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Case** | **Days b/w Specimen Collection** | **Pt Age at pBC** |  **Ancestry** | **Histologic Subtype** | **HR/HER2 Status**  | **\*Primary Breast Carcinoma** | **\*BCBM** | **Addition of Genomic Findings**  | **Loss of Genomic Findings** |
| 1 | 720 | 56 | European |  invasive ductal carcinoma  | HR+/HER2- | *MYC* amp, *MDM2* amp, *ZNF703* amp, *ESR1* D538G, TMB-High | *MYC* amp, *MDM2* amp, *ZNF703* amp, *FRS2* amp, *ARFRP1* amp, *PIK3CA* E545K, *ESR1* D538G | *PIK3CA* E545K*, FRS2* amp, *ARFRP1* amp | TMB-High |
| 2 | 2937 | 58 | European |  invasive ductal carcinoma  | HR-/HER2- (TNBC) | *EGFR* amp, *TP53* R342fs\*3, *PIK3CA* V105\_R108del | *EGFR* amp, *EMSY* amp, *FGF19* amp, *CCND1* amp, *FGF3* amp, *FGF4* amp, *TP53* R342fs\*3, *IKZF1* G141\*, *PIK3CA* V105\_R108del | *EMSY* amp, *FGF19* amp, *CCND1* amp, *FGF3* amp, *FGF4* amp, *IKZF1* G141\* | N/A |
| 3 | 343 | 33 | East Asian  |  invasive ductal carcinoma  | HR(unk)/ HER2- | *FGFR2* amp, *MYC* amp, *TP53* A159P, *PIK3R1* N673fs\*19, *STK11* V337fs\*22 | *FGFR2* amp, *TP53* A159P, *CDH1* duplication, *STK11* V337fs\*22 | *CDH1* duplication | *MYC* amp, *PIK3R1* N673fs\*19 |
| 4 | 608 | 41 | European |  invasive ductal carcinoma  | HR+/HER2- | *CCND1* amp, *FGFR2* amp, *PIK3CA* H1047R, *TP53* R280K | *FGFR2* amp, *CCND1* amp*, EMSY* amp*, PIK3CA* H1047R*, TP53* R280K | *EMSY* amp | N/A |
| 5 | 172 | 31 | European |  invasive ductal carcinoma  | HR-/HER2- (TNBC) | *REL* amp, *PDGFRA* amp, *KIT* amp, *KDR* amp, *TP53* M237I | *REL* amp, *KIT* amp, *KDR* amp, *TP53* M237I | N/A | *PDGFRA* amp |
| 6 | 639 | 27 | European |  invasive ductal carcinoma  | HR-/HER2+ | *ERBB2* amp, *MYC* amp, *TP53* R175H | *ERBB2* amp, *MYC* amp, *TP53* R175H, *RB1* splice site 1390-16\_1421+29del77 | *RB1* splice site 1390-16\_1421+29del77 | N/A |
| 7 | 610 | 65 | European |  invasive lobular carcinoma  | HR(unk)/ HER2+ | *ERBB2* amp, *MLH1* del, *TP53* R267G, *ERBB2* V777L, *CDK12* truncation, *BCORL1* P1681fs\*20, *CDH1* N315fs\*6, *CHD4* Q1596\*, *NOTCH3* N1961fs\*5 | *ERBB2* amp, *MLH1* del, *ERBB2* V777L, *TP53* R267G, *ABL1* P310fs\*9, *CDK12* truncation, *NOTCH3* N1961fs\*5, *MSH3* K383fs\*32, *BCORL1* P1681fs\*20, *CDH1* N315fs\*6, *SMO* P694fs\*82, TMB-High, MSI-H | *ABL1* P310fs\*9, *MSH3* K383fs\*32, *SMO* P694fs\*82, TMB-High, MSI-H | *CHD4* Q1596\*, |
| 8 | 1008 | 61 | African |  invasive ductal carcinoma  | HR-/HER2+ | *TOP2A* amp, *ERBB2* amp, *SMAD2* del, *PIK3CA* G1049R, *TBX3* E275fs\*7, *TP53* D281fs\*24 | *ERBB2* amp, *SMAD2* del, *ZNF703* amp, *FGFR1* amp, *TOP2A* amp, *PIK3CA* G1049R, *TBX3* E275fs\*7, *TP53* D281fs\*24 | *ZNF703* amp, *FGFR1* amp | N/A |
| 9 | 618 | 39 | African |  invasive ductal carcinoma  | HR-/HER2- (TNBC) | *MYC* amp, *LYN* amp, *MYST3* amp, *BCL2L2* amp, *TP53* E204\*, *EP300* truncation | *LYN* amp, *MYST3* amp, *MYC* amp, *BCL2L2* amp, *NCOR1* G150R, *TP53* E204\* | *NCOR1* G150R | *EP300* truncation |
| 10 | 784 | 51 | European | breast carcinoma (nos) | HR+/HER2- | *ZNF217* amp, *GNAS* amp, *AURKA* amp, *TP53* R196Q, *XRCC2* R91Q, *PIK3CA* E545K, *ARAF* E568\*, *NF1* splice site 4577+1G>A, *IRF2* E30\*, *GATA3* S408\*, APOBEC mutational signature, TMB-High | *AURKA* amp, *ZNF217* amp, *GNAS* amp, *PIK3CA* E726K, *PIK3CA* E545K, *XRCC2* R91Q, *NF1* S1954\*, *CDKN1B* S138\*, APOBEC mutational signature | *PIK3CA* E726K, *NF1* S1954\*, *CDKN1B* S138\* | *TP53* R196Q, *ARAF* E568\*, *NF1* splice site 4577+1G>A, *IRF2* E30\*, *GATA3* S408\*, TMB-High |
| 11 | 473 | 41 | Central and South American | breast carcinoma (nos) | HR-/HER2- (TNBC) | *EGFR* amp, *PRKCI* amp, *TERC* amp, *FGF12* amp, *TP53* P300fs\*6,TMB-High | *RAD21* amp, *SOX2* amp, *MYC* amp, *CCNE1* amp, *MYCL1* amp, *RPTOR* amp, *CD274* amp, *PRKCI* amp, *EGFR* amp, *VEGFA* amp, *PIK3CA* amp, *TERC* amp, *PDCD1LG2* amp, *FGF12* amp, *NOTCH3* amp, *JAK2* amp, *CCND3* amp, *MYB*-*AHI1* rearrangement, *TP53* P300fs\*6 | *RAD21* amp, *SOX2* amp, *MYC* amp, *CCNE1* amp, *MYCL1* amp, *RPTOR* amp, *CD274* amp, *VEGFA* amp, *PIK3CA* amp, *PDCD1LG2* amp, *NOTCH3* amp, *JAK2* amp, *CCND3* amp, *MYB*-*AHI1* rearrangement | TMB-High |

\*Immune checkpoint inhibitor biomarkers of TMB-High, MSI-H, *CD274* amplification, and APOBEC mutational signature are also included when present.