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ORIGINAL ARTICLE

Breast cancer brain metastases show increased levels of genomic aberration-based homologous recombination deficiency scores relative to their corresponding primary tumors

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Background: Based on its mechanism of action, PARP inhibitor therapy is expected to benefit mainly tumor cases with homologous recombination deficiency (HRD). Therefore, identification of tumor types with increased HRD is important for the optimal use of this class of therapeutic agents. HRD levels can be estimated using various mutational signatures from next generation sequencing data and we used this approach to determine whether breast cancer brain metastases show altered levels of HRD scores relative to their corresponding primary tumor.

Patients and methods: We used a previously published next generation sequencing dataset of 21 matched primary breast cancer/brain metastasis pairs to derive the various mutational signatures/HRD scores strongly associated with HRD. We also carried out the myChoice HRD analysis on an independent cohort of 17 breast cancer patients with matched primary/brain metastasis pairs.

Results: All of the mutational signatures indicative of HRD showed a significant increase in the brain metastases relative to their matched primary tumor in the previously published whole exome sequencing dataset. In the independent validation cohort, the myChoice HRD assay showed an increased level in 87.5% of the brain metastases relative to the primary tumor, with 56% of brain metastases being HRD positive according to the myChoice criteria.

Conclusions: The consistent observation that brain metastases of breast cancer tend to have higher HRD measures may raise the possibility that brain metastases may be more sensitive to PARP inhibitor treatment. This observation warrants further investigation to assess whether this increase is common to other metastatic sites as well, and whether clinical trials should adjust their strategy in the application of HRD measures for the prioritization of patients for PARP inhibitor therapy.

Key words: breast cancer, brain metastasis, cancer therapy

Introduction

The benefit of improved survival in patients with metastatic breast cancer is mitigated by the fact that 15%–20% of such patients will develop brain metastases [1]. In fact, probably due to longer survival, the incidence of brain metastasis, which is usually associated with poor prognosis and severe neurological impairments [2], is rising in breast cancer [1]. Due to its unique location, and perhaps its distinct biology, treatment options have been limited.

Homologous recombination (HR) is an error-free mechanism of double-stranded DNA breaks repair, which is often deficient in breast and ovarian cancer, sensitizing them to PARP inhibitor therapy [3, 4]. Currently three PARP inhibitors (olaparib, niraparib and rucaparib) have been approved by the Food and Drug Administration for the treatment of ovarian cancer, and olaparib has also been approved for the treatment of germline BRCA-mutated HER2– metastatic breast cancer.

These promising clinical results raise the question whether a similar clinical benefit can be achieved with PARP inhibitors in the case of brain metastases as well.

To achieve such therapeutic benefit at least two criteria need to be satisfied. First, crossing the blood–brain barrier, which was shown for both niraparib [5] and veliparib [6], making those potential candidates for the treatment of brain metastases. Secondly, the brain metastases should present HR deficiency to be sensitive to this type of treatment.

Over the past decade, various studies have analyzed human cancer genomes for fingerprints left by homologous recombination deficiency (HRD), and identified predictors to estimate a tumor's response to HRD-specific therapy.

The three earliest predictors: HRD-loss of heterozygosity (HRD-LOH), large-scale state transitions (LST) and the number of telomeric allelic imbalances (ntAI), commonly known as the “genomic scar scores,” are based on the investigated tumors' copy-number profiles and each of them quantifies a different aspect of the increased genomic instability linked to HRD [7–9]. Although all of these scars have the tendency to increase in HRD cases, as of today, none of them has an accepted threshold individually to separate HRD+ from HRD– cases. These methods were later adapted by Myriad Genetics and they are currently part of the myChoice HRD test [10].

Next generation sequencing allowed the identification of the mutational signatures of somatic processes [11]. Analysis of the mutational spectra of nucleotide triplets in tumor specimens found that a signature characterized by a flat mutational spectrum (Signature 3) was connected to HRD; thus, the relative contribution of the corresponding mutational process might be used as an estimator for the deficiency. The relative proportion of deletions caused by microhomology-mediated end joining can also be used as a robust estimator for HRD [12]. All these mutational signatures together with the so-called rearrangement signatures were combined into a robust logistic-regression model, called HRDetect [13].

It is important to emphasize that the phenotypes of the samples (i.e. HR-deficient or HR-proficient) on which all of these estimators relied on were determined from the genotypes of the *BRCA1* and *BRCA2* genes. Hence, determining the HR-status in a *BRCA1/2* wild-type background remains a challenging task.

So far, it has not been determined how often breast cancer brain metastases show HR deficiency. We calculated HR deficiency measures in a published next generation sequencing data of primary breast cancer/brain metastasis pairs (hereinafter referred to as Brastianos et al. [14] cohort), followed by the myChoice HRD test in an independent cohort (validation cohort).

Materials and methods

Brastianos et al. cohort

The whole exome sequencing (WES) data of the Brastianos et al. cohort were downloaded from the database of Genotypes and Phenotypes, under accession number phs000730.v1.p1 [14]. The dataset contains binary alignment files of primary tumors and their corresponding metastatic pairs for 21 breast cancer cases with all primary tumors having at least one matched intracranial metastasis. This breast cancer cohort formed the basis of our initial investigations.

Independent validation cohort

We collected FFPE tissues from 17 cases with primary breast cancer and corresponding brain metastases. Permissions to use the archived tissue have been obtained from the Regional Ethical Committee (No.: 510/2013, 86/2015) and the study was conducted in accordance with the Declaration of Helsinki.

Germline and somatic mutation calling

The samples (Brastianos et al.) were preprocessed, aligned to the hg19 reference genome with the Burrows-Wheeler Aligner mem algorithm, and postprocessed (base-recalibration) by the original authors [14]. We called somatic substitutions along with short insertions and deletions with MuTect2, and germline mutations of the *BRCA1* and *BRCA2* genes with HaplotypeCaller. Both tools constitute a part of the Genome Analysis Toolkit (GATK 3.7) [15]. The majority of the samples were derived from formalin fixed, paraffin embedded (FFPE) specimens, which are well known to contain mutational biases (mostly $C > T/G > A$ transitions) [16]. We used an approach analogous to the OxoG filter [17] to remove mutations that were consistent with this FFPE-bias (supplementary Figures S1–S4 and Tables S5–S7, available at *Annals of Oncology* online).

Genotypic background. As it was reported earlier [14], none of the Brastianos et al. samples had somatic mutations in either *BRCA1* or *BRCA2*; however, four blood-derived normal samples showed deleterious germline mutations being present in at least one of the genes (supplementary Figure S7 and Table S8, available at *Annals of Oncology* online).

Classification of deletions

Deletions were grouped into three classes (supplementary Figure S5 and S6, available at *Annals of Oncology* online): (i) if the entire deleted sequence of a single event was repeated after the breakpoint, it was considered a repeat, (ii) if only the first n nucleotides were repeated after the breakpoint, it was considered a size n microhomology and (iii) if it had no such characteristics, it was treated as a unique deletion. In order to eliminate those microhomologies that have appeared by pure chance instead as the result of the activity of the microhomology-mediated end joining DNA-repair pathway, they had been further divided into $n < 3$ and $n \geq 3$ subgroups. In our prediction models only the $n \geq 3$ subgroup was considered.

Copy number analysis and genomic scar scores

Deriving copy number data from the Brastianos et al. whole exome sequences was executed using the *sequenza* R package [18]. The ranges of ploidy and cellularity were confined into the [1, 5] and [0.1, 1] intervals, respectively, and segmentation data were generated for autosomes only. The three genomic scar scores were calculated using their standard definitions (supplementary Figures S9–S12 and Table S1, available at *Annals of Oncology* online).

The WES-HRDetect model

The original HRDetect model [13] was trained on mutational features derived from 560 breast cancer whole genome sequences, and although the authors have created an artificial whole exome variant (limiting WGS to typical WES genomic coverage area), the exact details of this computational pipeline were not published. Therefore, in order to use this predictor for our WES samples, we had to retrain the LASSO logistic regression model on the 560 artificial WES dataset, with the HRD-LOH scar scores included [13] (supplementary Figures S18–S21 and Tables S4 and S11, available at *Annals of Oncology* online).

myChoice HRD analysis

DNA was extracted from FFPE tissue sections and analysis carried out as previously described by Myriad Genetics [10]. Briefly, genome-wide SNP data and sequence spanning the coding regions of 111 genes were generated using a custom hybridization panel [10]. Allelic imbalance profiles were generated to determine the scores for the three genomic scars (ntAI, HRD-LOH and LST). The combined HRD score was derived from these three independent genomic scars. A myChoice HRD threshold of 42 has previously been developed to identify HR-deficient tumors using this test [10]. Tumors are considered HRD+ if they have a high myChoice HRD score (≥ 42) or a tumor BRCA1/2 mutation and HRD– if they have a low myChoice HRD score (< 42) and wild-type BRCA1/2.

Results

Genomic scars–based HRD measures

We determined the three genomic scar scores (HRD-LOH, LST and ntAI) in the Brastianos et al. dataset. All three WES-based scores, and their sum, had significantly increased in brain metastases ($P \ll 0.01$ for all the three measures and for their sum; Figure 1, supplementary Figures S13 and S14 and Tables S9 and S10, available at *Annals of Oncology* online), regardless of the mutational backgrounds of the samples. We also compared the scar scores of patients who received brain radiation treatment before the resection of their brain metastasis (5 of 21) to the scores of those patients who did not and found no significant difference between the two groups (supplementary Figures S15 and S16, available at *Annals of Oncology* online), suggesting that brain irradiation did not introduce bias into our analysis.

To independently validate these observations, we have assembled a cohort of primary breast cancer brain metastasis pairs, which were processed and assessed for the myChoice HRD score as described in [10]. Two of 17 of these patients (patients 1 and 12; Figure 2A and supplementary Tables S2 and S3, available at *Annals of Oncology* online) had biallelic BRCA1 mutations (a deleterious somatic deletion and LOH in both cases), and two showed signs of monoallelic BRCA2 mutations (4 and 5); however, the latter two mutations' clinical significance could not be

determined. In one case (patient 7), the HRD score of the primary tumor could not be determined.

Figure 2B shows that in 14 of 16 cases the brain metastasis had a higher myChoice HRD score than the primary tumor. In four cases, the brain metastasis “switched myChoice HRD status” relative to the primary tumor from a low myChoice HRD score (HRD–) to a high myChoice HRD score (HRD+), based on the currently accepted threshold value of 42 [10]. One of these donors' (patient 3) metastasis, however, was likely to be derived from a HRD+ recurrent tumor that was diagnosed 4 years after the appearance of the initial tumor. The remaining three patients had estrogen receptor positive (ER+) primary tumors and metastases, which means that 38% of the ER+ patients' metastases in our validation cohort had switched HRD status. In a single case (patient 6), the metastatic sample turned out to be HRD–, while its primary counterpart was HRD+.

As expected, the myChoice HRD scores of the two BRCA1 mutants were larger than 42, i.e. both of these samples were HRD+. The statistical comparison of the myChoice HRD scores yielded a significant difference between the primary and metastatic groups (Figure 2B), with a P -value of $5.4E-6$, supporting our findings on the Brastianos et al. dataset.

We also found a significant correlation between the changes in the scar scores and the time elapsing between the detection of the primary tumor and the brain metastasis ($r_{\text{Brastianos}} = 0.52$, $r_{\text{control}} = 0.54$) in the case of both cohorts (supplementary Figures S17 and S23, available at *Annals of Oncology* online).

Somatic point-mutation signatures in the Brastianos et al. cohort

As a result of our signature extraction process, 3 of 21 primary samples (149, 244 and 296) and 1 of 27 metastatic samples (296) failed to pass the reconstruction step, hence their somatic signature composition could not be determined (supplementary Figure S8, available at *Annals of Oncology* online). The rest of the signature compositions are summarized in Figure 3A.

We found that the primary breast tumor mutational profiles were dominated by signature 1 (aging-related signature), with lesser contributions from signature 2 (APOBEC signature), signature 3 (BRCA mutation associated), signatures 5 and 6. Strikingly, signature 3, the BRCA mutation–associated signature increased in 13 of 18 patients' brain metastases. Interestingly, in about 20%–25% of the brain metastasis cases, signature 5 (another aging-related signature) has become similarly dominant as signature 1. Finally, in a single case (302), signature 17 (unknown relevance and biological origin) became the predominant mutational signature in the metastasis.

Analysis of deletions

Since the Brastianos dataset was whole-exome sequenced, the number of high fidelity deletions in the samples was low (7.33 ± 1.44 in primary tumors and 9.36 ± 1.49 in metastases), and although the contribution of microhomology-mediated deletions increased in the metastases (supplementary material subsection 2.3.3, available at *Annals of Oncology* online), the difference between the two groups was not significant ($P = 0.13$).

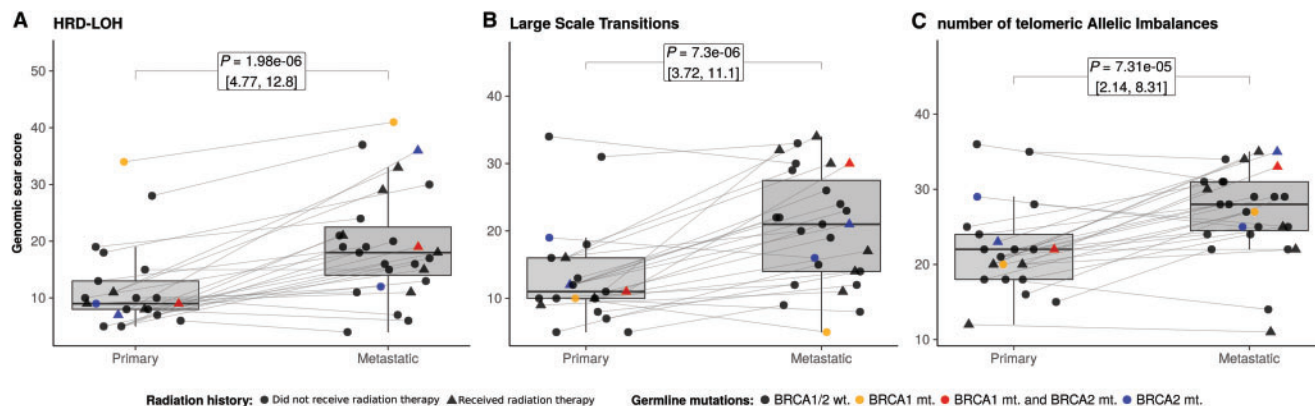


Figure 1. Genomic scar scores of the Brastianos et al. breast cancer brain metastasis samples. Distributions of the homologous recombination deficiency–loss of heterozygosity (HRD-LOH) (A), large-scale state transitions (LST) (B) and number of telomeric allelic imbalances (ntAI) (C) scores. The corresponding primary-metastatic pairs are connected by thin lines. Scores for each of these measures were increased in metastases compared with primary tumors with P -values of the paired t -tests: $pLOH = 1.98 \times 10^{-6}$, $pLST = 7.3 \times 10^{-6}$ and $pntAI = 7.31 \times 10^{-5}$, respectively. The brain radiation history and the germline status of the *BRCA1/2* genes are encoded by different shapes and colors, respectively.

WES-HRDetect scores

The original HRDetect model, developed using WGS data, is not directly applicable to WES data. Since the Brastianos et al. dataset contained only WES data, we extracted the features of HRDetect that could be calculated based on the available sequences (HRD-LOH, substitution signatures, and deletion classes) and retrained the LASSO regularized logistic regression model on them. Using the same principles as the original publication [13], we developed a similar complex measure of HR deficiency using WES data. (For details, see [supplementary material](#) section 4, available at *Annals of Oncology* online.)

This WES-HRDetect model also distinguished well between HR-deficient and HR-proficient primary breast cancers, as determined by e.g. *BRCA* mutation status ([supplementary material](#) section 4, available at *Annals of Oncology* online). Therefore, we calculated the WES-HRDetect scores for the primary breast cancer brain metastasis pairs as well. Compared with the results obtained by the genomic scar scores, this new measure showed an even more substantial increase of HR-deficiency in brain metastases relative to the primary tumors (Figure 3B,C and [supplementary Figure S22](#), available at *Annals of Oncology* online). The distribution of these predictor scores was not affected by either the *BRCA1/2* germline genotypes of the samples, or the brain radiation history of the patients.

The distribution of the breast cancer brain metastatic WES-HRDetect scores was compared with the distribution of the same model ran on the original 560 breast cancer exomes. We found a significant difference between the two cohorts (Figure 3D) suggesting that there were significantly more tumors in the Brastianos et al. brain metastasis cohort with high ($\gg 0$) WES-HRDetect scores than in the pure primary cohort of breast cancers. By using the score ≥ 0.7 threshold that was determined for the original model, we concluded that four patients' metastases changed HRD status from HRD- to HRD+, among which two had germline mutations in at least one of the *BRCA1/2* genes. One of the *BRCA1* germline patient's (222), however, went through an opposite change; while the primary sample was HRD+, the metastasis was HRD-.

Discussion

This work is the first demonstrating that the various DNA aberration-based HRD measures are significantly higher in brain metastases relative to their primary breast tumor counterparts. The possibility of such an increase was previously suggested in a publication showing that a *BRCA1* deficient-like gene expression signature is higher in breast cancer brain metastases, but that study did not use patient-matched primary metastasis sample pairs [19].

This discrepancy has several clinical consequences. Ideally, only patients sensitive to a given therapy should receive that particular treatment. In the case of PARP inhibitors, it is likely that mainly patients with HR deficiency benefit from this form of therapy. Therefore, correlating the clinical benefit of PARP inhibitor therapy with HR status of the tumors has been intensely investigated [3]. However, HR status is often determined in biopsies derived from the primary tumor but clinical benefit is determined at a more advanced, often metastatic stage of the disease. If the HR deficiency status is significantly different in the metastases relative to the primary tumors then the observed correlations will be corrupted leading to contradictory clinical results. For example, in the case of breast cancer repeated observations demonstrated a correlation between the ntAI score and response to platinum-based therapy [7]. The TNT trial [20], however, studied the efficacy of platinum-based therapy in locally advanced or metastatic triple-negative breast cancer and failed to find a correlation between the myChoice HRD score and response to carboplatin. It should be noted, however, that the myChoice HRD score was determined on the primary tumors and not on the metastases. The discrepancy between the myChoice HRD score of the primary and metastatic sites presented in this study may be partially responsible for this lack of correlation.

There are several ongoing clinical trials evaluating the potential clinical benefit of PARP inhibitor treatment in metastatic breast cancer, including NCT02723864 (<https://clinicaltrials.gov/ct2/show/NCT02723864>). This trial, however, excludes patients with active brain metastasis. Based on our results, it seems likely that breast cancer brain metastases might be a particularly sensitive population.

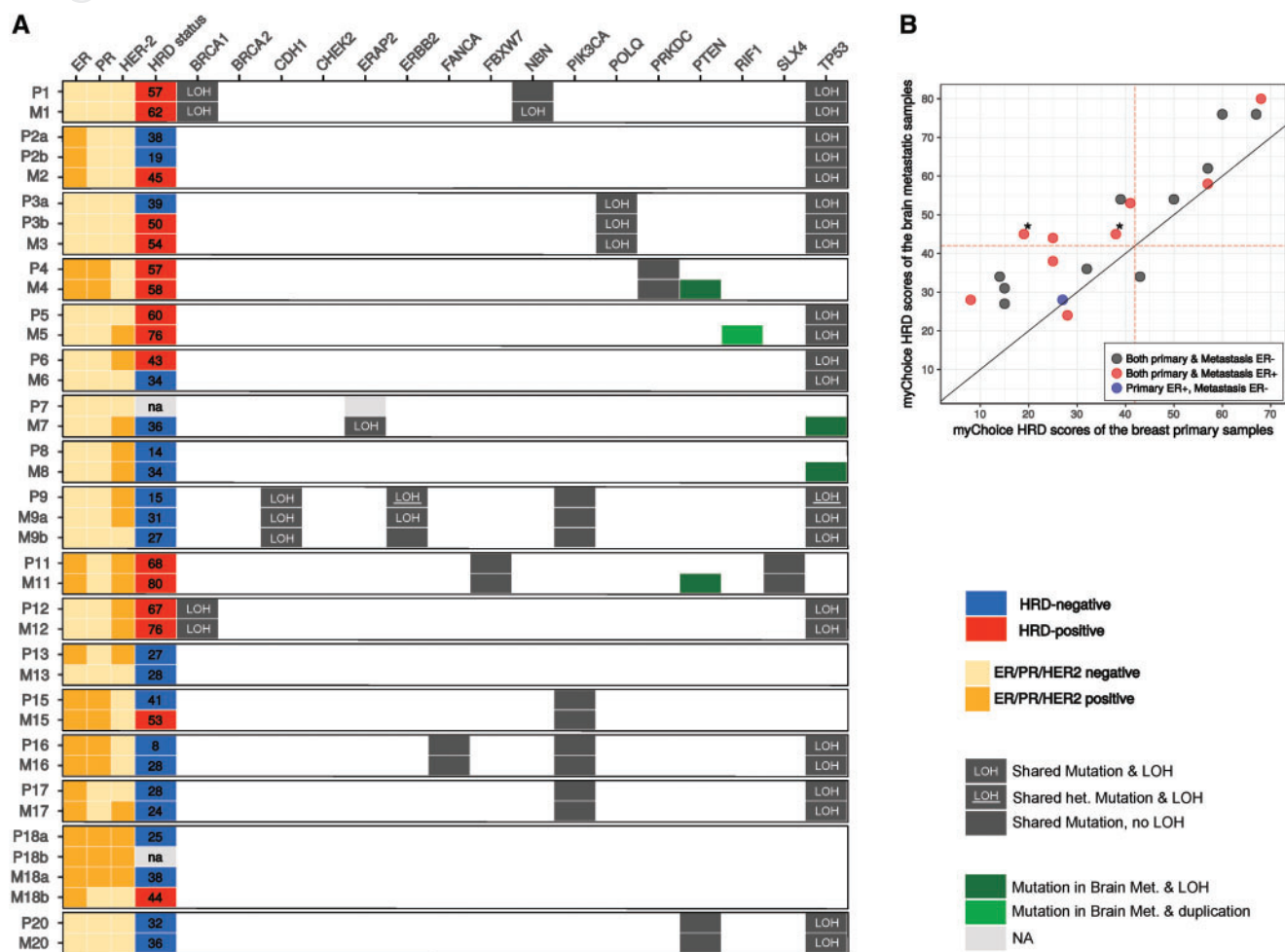


Figure 2. Summary of the analysis carried out on the validation cohort by Myriad Genetics. (A) Hormone receptor and HER-2 status along with the mutational profile and HRD status of the control samples determined by Myriad Genetics. In the sample, names P stands for primary and M stands for metastasis. In the majority of cases (14 of 16), the HRD score was higher in the metastasis compared with the primary tumor. In four cases, three of which were ER positive, the HRD status of the metastasis switched to positive from an HRD negative primary. Patients 4 and 5 had uncertainly significant BRCA2 monoallelic mutations. Mutations of either *PTEN*, *RIF1* or *TP53* were gained in five of the brain metastases. LOH was more common in the brain metastases. (B) Genomic scar scores determined by Myriad Genetics summed together into a single HRD score. HRD scores were increased in metastases compared with primary tumors ($P = 5.4E-6$). The figure is divided into four quadrants by the HRD score = 42 lines, among which the top left quadrant contains those four cases, whose HRD status changed from HRD- to HRD+. Two of these samples (marked with asterisks) belong to the same patient (patient 2).

The SWOG trial S1416 is a Phase II Randomized Placebo-Controlled Trial of Cisplatin with or without ABT-888 (Veliparib) in Metastatic Triple-Negative Breast Cancer (TNBC) and/or BRCA Mutation-Associated Breast Cancer. This trial includes patients with brain metastasis but excludes patients with estrogen receptor positive breast cancer. As we showed in our validation cohort, the majority of estrogen receptor positive breast cancer brain metastasis cases showed high myChoice HRD scores. It might be worth establishing the proportion of HRD+ ER+ breast cancer cases on a larger cohort as those patients might be responding to PARP inhibitors as well.

The significant increase in HR deficiency scores in brain metastases may arise due to at least two distinct mechanisms. (i) Clonal selection of tumor cells with higher HR deficiency score. Tumors cells with higher levels of genomic instability, higher levels of HR deficiency may be able to adjust more readily to a distinct environment such as the brain. (ii) It is also possible that the growing brain

metastases become gradually more genomically unstable due to some unidentified mechanism. Both mechanisms are consistent with the fact that the time elapsing between primary tumors and brain metastases often takes up to 5–10 years in breast cancer, and the length of this time showed a strong correlation with the increase in the various HRD measures in brain metastases.

Brain metastases of breast cancer patients are surgically removed with palliative intent in a significant number of cases. According to the guidelines, surgical resection is recommended in patients with a limited number (1–3) of newly diagnosed brain metastases, especially with controlled systemic disease and good performance status [21]. In such cases, when the histological material is already available, it might be worth considering determining the HR deficiency score in order to determine the likely sensitivity of the tumor to PARP inhibitors versus other second-/third-line therapeutic options.

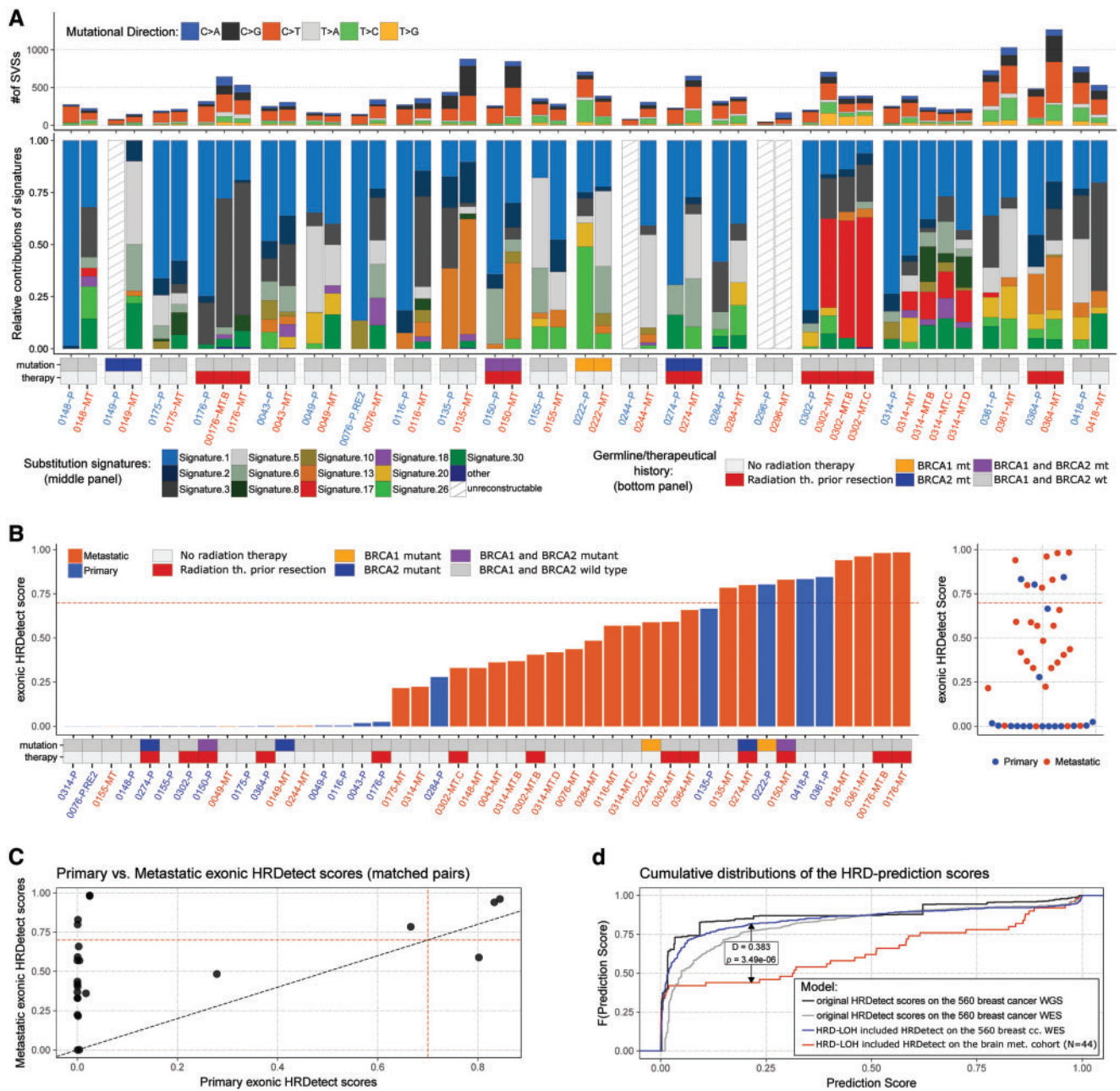


Figure 3. Somatic signature composition and WES-HRDetect scores of the Brastianos et al. samples. (A) Summary of somatic point mutations. The figure can be divided into three panels. The top panel contains the total number of point mutations in the samples, colored by the relative abundance of the six mutational directions. The middle panel shows the somatic point-mutation signature compositions of the triplet mutational spectra. The reconstruction errors (supplementary material section 3, available at *Annals of Oncology* online) of specimens 0149-P, 0244-P, 0296-P and 0296-MT did not pass the cosine similarity threshold, hence those samples have no colors on their bars in the plot. The bottom panel of the figure indicates the germline genotype of the *BRCA1/2* genes in the normal sample, and the brain radiation history of the patient. (B) WES-HRDetect scores of the primary and metastatic samples. The germline genotypes of the *BRCA1/2* genes and brain radiation history of the patient is also presented below the bars. The red dashed line indicates the score ≥ 0.7 threshold, determined by the original creators of the HRDetect model. (C) Primary WES-HRDetect scores versus metastatic WES-HRDetect scores. Noticeably, there is only a single case (PB0222) for which the primary sample's score is significantly higher than its metastatic pairs. The difference between the two groups was tested with a paired Wilcoxon signed rank test: $P = 1.64E-6$. (D) Black and gray curves; cumulative distributions of the original HRDetect model's scores ran on the 560 WGS and WES. Red and blue curves: cumulative distributions of the WES-HRDetect model's scores ran on the 560 breast cancer whole exomes and on the Brastianos et al. breast cancer dataset. The latter two distributions were compared with each other by a Kolmogorov–Smirnov test, the D statistic and P -value of which is indicated on the plot.

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Disclosure

ZS and ACE are inventors on a patent used in the myChoice HRD assay. KMT, CN, CS and DP are employees of Myriad Genetics. All remaining authors have declared no conflicts of interest.

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