Blood-based tumor mutational burden impacts clinical outcomes of immune checkpoint inhibitor treated breast and prostate cancers

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Abstract

Background Breast and prostate tumors are known to be less responsive to immune checkpoint inhibitors (ICIs). Tissue-based tumor mutation burden (tTMB) has emerged as a predictive biomarker of response to ICIs, including in these "cold tumors". In clinical practice, when tTMB is not available, blood-based TMB score (bTMB) can be used to consider treatment with ICIs.
Methods This retrospective, real-world study included a final cohort of metastatic breast and prostate cancer patients treated with an ICI following a liquid biopsy test. Multiple bTMB-High cut-offs were assessed. Clinical, genomic, and outcomes data were collected. We hypothesized that a cut-off of bTMB ≥10 mut/Mb is not a strong predictor of response to ICIs in this setting. The Guardant Health genomic database (GHGD) was then queried (N = 13,992) for associations of bTMB with genomic alterations.
Results In the clinical cohort (N = 48), ICI treatment is offered after a median of 3 (1–9) lines of treatment. The median bTMB is 16.4 (10–186) mut/Mb. The median time on ICI and PFS is 2.1 (0–1.7) and 3.1 months (95%CI, 1.6–4.6) respectively; no difference by MSI/MMR status (p = 0.152). Response rate among eligible patients (n = 36) is 16.7%; only N = 1/6 in bTMB <16 mut/Mb. High bMSI is associated with higher bTMB (correlation test, r = 0.66, p = 0.000). In the GHGD, patients with bTMB high have significantly more alterations than bTMB low and *TP53, PIK3CA, ATM, ESR1, NF1, BRCA2, ARID1A*, and *APC* were the most frequently altered genes.
Conclusions In this study, the practice of offering an ICIs based on bTMB was uncommon and did not independently predict ICI benefits in patients with refractory, advanced breast and prostate cancers.

Plain Language Summary

The genetic changes seen in cancer tumors can be detected in blood. We aimed to investigate whether these changes detected in the blood could be used to predict the response of people with breast or prostate cancer to a type of treatment called immune checkpoint inhibitors. We found that the genetic changes detected in the blood did not predict response to the treatment. This suggests that additional or alternative information is required about patients to identify those who will benefit from immune checkpoint inhibitor treatment.

Immune checkpoint inhibitors (ICIs) are the standard of care for many cancer types, such as lung, bladder, and melanoma, but some cancers are known to be less responsive to these therapies[1,2]. Breast and prostate cancers are among these classic "cold" tumor types that are less likely to trigger an immune response when exposed to ICIs[3–5]. While generally ineffective, ICIs can be very active among some of these patients with "cold" tumor types thus, identifying impactful predictive biomarkers in this subgroup of patients is crucial[6–9].

High microsatellite instability (MSI-H) and PD-L1 expression have been the primary biomarkers for ICIs and continue to be strong indicators of response in most tumor types[10,11]. In addition, patients with a tumor mutational burden (TMB, a numeric index that expresses the number of mutations per megabase [mut/Mb] harbored by tumor cells) of ≥10 mut/Mb based on tissue-specific data are now able to receive the ICI pembrolizumab as on-label therapy[12]. Tissue-based MSI-H and TMB have been

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shown to be predictors of response in tumors that normally don't respond to ICIs[6,13,14]. New technologies for evaluating TMB have emerged and continue to evolve, including liquid biopsy. Circulating tumor DNA (ctDNA) present in plasma has been shown to predict ICI activity in cases of MSI-H[7].

In clinical practice, providers often perceive a blood-based TMB (bTMB) result as equivalent to tissue-based TMB (tTMB) and can use the same approved cut-off of ≥10 mut/Mb, despite the lack of validation data. Prior studies comparing tTMB and bTMB have suggested different cut-offs for clinical benefit (≥ 10 mut/Mb, ≥16 mut/Mb), which may also be cancer type specific[15–20]. However, it is also important to note that cut-offs may vary between assays.

In this study, we aimed to investigate the role of bTMB in predicting response to ICIs in classic cold tumors. We hypothesized that a bTMB score is being frequently used in clinical practice to offer ICI to patients with advanced cold tumors in the cases where tTMB is not available. We further hypothesized that bTMB ≥10 mut/Mb is not a strong predictor of response to ICI in these "cold tumors". We assessed data from a commercial liquid biopsy assay (Guardant360) to evaluate molecular associations with bTMB in the clinical cohort of patients with advanced cold tumors treated with ICIs. We then evaluated the Guardant Health genomic database for associations of bTMB with relevant genomic alterations.

In this evaluation, the practice of offering an ICIs based on bTMB is uncommon and does not independently predict ICI benefits in patients with refractory, advanced breast and prostate cancers.

Methods
Clinical Cohort and genomic database
This retrospective, real-world study was IRB-approved at University Hospitals (STUDY20230043) and collaborating institutions through data transfer agreements at all eleven participating sites (Duke Cancer Institute, Emory University, Massachusetts General Hospital Cancer Center, Mayo Clinic Jacksonville, Mayo Clinic Phoenix, Mayo Clinic Rochester, Miami Cancer Institute Baptist Health, Moffitt Cancer Institute, Rutgers University, Tulane Cancer Center, UT Southwestern University). Informed consent was waived by IRB at University Hospitals (STUDY20230043) given the retrospective nature of this project. Per protocol, patients with metastatic breast (excluded triple negative) ovarian, pancreatic, and prostate cancer who received treatment with an ICI following an NGS result with Guardant360 that included a bTMB score, were eligible to be included in this study. However, given the very low number of patients with ovarian and pancreatic cancers identified among the participating institutions (n = 1 pancreatic cancer; n = 0 ovarian cancer), only breast and prostate cancer was included in the final analysis. Different bTMB cut-offs were assessed, including ≥10 mut/Mb and ≥16 mut/Mb, to determine if implementing a higher bTMB-H benchmark would improve survival outcomes. Clinical and outcomes data were collected, and the first liquid biopsy drawn was considered for each patient.

The Guardant Health genomic database was then queried (2020–2023) for patients with breast or prostate cancer who had ctDNA NGS, which included evaluation for a TMB score (Total n = 13,992; Guardant360, Redwood City, CA), performed as part of clinical care. Mutation profiles were evaluated for patients within the Guardant database, utilizing two different bTMB-H benchmarks: ≥10mut/Mb (n = 5327; 38.1%) and ≥16mut/Mb (n = 2039; 14.6%). These groups were compared to the associated bTMB low group (bTMB-L), which were <10 mut/Mb (n = 8665; 61.9%) and <16 mut/Mb (n = 11953; 85.4%), respectively. Blood MSI-H (bMSI-H) detection with liquid biopsy has been previously described and was assessed for these patients as well[21]. Synonymous alterations, fusions, and copy number variants were excluded unless otherwise noted.

Statistical analysis
Descriptive statistics were used to summarize baseline characteristics. Blood TMB benchmarks ≥10 mut/Mb or ≥16 mut/Mb defined bTMB-H and is specified in each analysis. The median follow-up of the clinical cohort was

calculated by the inverse Kaplan-Meier method. Overall survival (OS) was calculated from the time of initiation of ICI treatment to death from any cause or censored at the time of last follow-up. Progression-free survival (PFS) was calculated from the time of ICI initiation to radiologic or clinical progressive disease (PD), death from any cause, or censored at the date of the last follow-up. Kaplan-Meier method was used to estimate PFS and OS. Kaplan-Meier methods and log rank tests were performed to compare them between categorical subgroups. Overall response rate (ORR) was assessed by investigator review according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1. Statistical analyses were performed using SPSS V.23 and all statistical tests used for comparison were 2-sided. For genomic analyses, Fisher's Exact test was utilized to statistically compare groups. Correlation between MSI and TMB was measured using the Pearson correlation test. Figures were generated using cBioPortal and Graph Pad Prism 9 software (*p-value < 0.05, **p-value ≤ 0.01, ***p-value ≤ 0.001, and ****p-value ≤ 0.0001; exact p values included in Tables S2–4).

Reporting summary
Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Results
Clinical cohort IO results
Between 10/2020 and 12/2022, a total of 596 patients with advanced solid tumors [breast, ovarian, pancreatic, and prostate cancers] among the participating collaborators were found to have bTMB ≥10 mut/Mb (Fig. 1). Patients who received an ICI prior to G360 (n = 21), had an unavailable bTMB or a bTMB of <10 mut/Mb prior to ICI initiation (n = 6), were excluded. Our final analysis included 48 bTMB high patients treated with ICI therapy (n = 36 prostate, n = 12 breast).

Table 1 summarizes the baseline characteristics of the study cohort (bTMB ≥10 mut/Mb and a subgroup of patients with bTMB ≥16 mut/Mb). In this cohort, liquid biopsy was performed after a median of 3 (range: 1–9) lines of treatment and ICI was administered after a median of 3 (range: 1–9) lines of treatment. Most patients were previously exposed to chemotherapy (52%). All patients except one were ICI-naïve prior to bTMB results. The median time between G360 test and initiation of ICI was 35 (0–514 days). Less than one third of patients (n = 15) received any systemic therapy between G360 test and ICI and included chemotherapy [taxanes n = 9, platinum n = 2, capecitabine n = 2), novel hormonal therapies [enzalutamide n = 5, abiraterone acetate n = 1, exemestane n = 1], mTOR inhibitor (n = 1) and sipuleucel-T (n = 1). Most patients were treated with ICI monotherapy including a PD-1 inhibitor while only three patients were offered a combination regimen of two different immunotherapies (Table 1).

The median bTMB was 16.4 (range: 10–186) mut/Mb; no significant difference between the median bTMB in prostate cancer [15.8 (range: 10–147.9)] and breast cancer [24.3 (range: 11.5–174.5)] was observed (Figure S1). bMSI-H was detected in eight (16%) bTMB-H (≥ 10 mut/Mb) patients treated with ICI (prostate n = 6; breast n = 2). Only four patients had MMR status known (Table 1): one patient had both bMSI-H and MMR defect, one patient had bMSI-H with preserved MMR and one patient had MMR defect without bMSI-H. The median bTMB among patients with bMSI-H/MMR defect was 91.2 (range: 19.3–186.0) as compared to the median bTMB among patients without bMSI-H/ MMR defect, at 16.3 (range: 10.0–147.9). bMSI-H was associated with higher bTMB (correlation test, r = 0.66, p = 0.000). Median bTMB was 91.2 (range: 19.3–186.0) mut/Mb in bMSI-H vs 16.3 (range: 10.0–147.9) mut/Mb in bMSI-Not Detected tumors. Patients with bMSI-H had a mPFS of 10.3 months (CI 95% 0–15.4) compared to a mPFS of 2.8 months (CI 95% 2.1–4.0) in those patients with bMSI-Not Detected (Fig. 2A). With a median follow up of 10.9 (range: 0–34.3) months, the median time on ICI was 2.1 (range: 0–1.7) months (no significant difference by bMSI/MMR status, p = 0.214). Most patients (26) discontinued ICI due to disease progression and/or death, irAES (5 pts: 1 – hepatitis; 1 – ARF, 2 – dermatitis, 1 - colitis), or unknown (4 pts). The
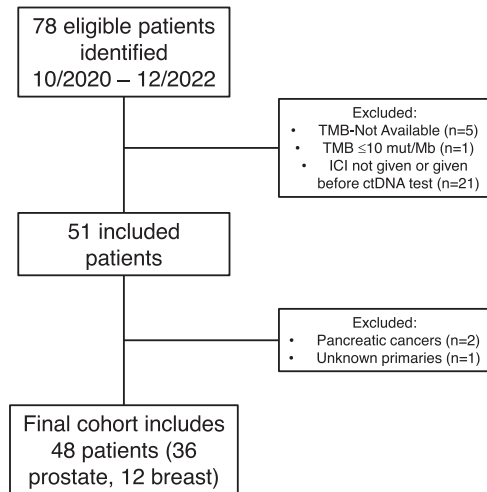
Fig. 1 | Clinical study cohort diagram. From 78 eligible patients (10/2020-12/2022), 30 patietns were excluded; final analysis include 48 patients (*n* = 36 prostate, *n* = 12 breast).

median progression free survival was 4.0 months (95% CI, 2.4–5.6) and 37 patients (77%) progressed on therapy. There was no significant association between median bTMB score and mPFS (HR 0.99 CI 0.99–1.0; *p* = 0.56). Similarly, there was no significant difference in mPFS between bMSI-H and bMSI-Not Detected patients on ICI therapy at different bTMB-H cut-offs of ≥10, ≥16, ≥20 (Fig. 2A–C) and ≥50 mut/Mb (not shown).

The median OS was 12.8 months (95% CI, 6.1–19.4) and no difference was observed by bMSI/MMR status (16.5 months [CI 95%, 14–19.1] bMSI-H/ MMR defect vs 10.3 months [CI 95%, 0–27.2], *p* = 0.906) in bMSI-Not detected/ MMR preserved. For prostate cancer, the median OS was 12.1 months (CI 95%, 6.1–18.1), and for breast cancer the median OS was 16.6 months (CI 95%, 0–39.7). The best overall response rate among eligible patients (*n* = 36) was 16.7% (complete response *n* = 1; partial response *n* = 5); all responses, except one, were observed in patients with bTMB ≥16 mut/Mb. All partial responses were observed in bMSI-H and the single complete response was observed in a patient with bMSI-Not Detected.

Genomic analysis
The Guardant Health genomic database (GHGD) was queried for patients with breast (BC; *n* = 7899; 56.5%) or prostate (PR; *n* = 6093; 43.5%) cancers who had a bTMB score identified by ctDNA NGS (Total *n* = 13,992; Guardant360, Redwood City, CA) for associations of bTMB with genomic alterations. Mutation profiles were evaluated for patients within the Guardant database, utilizing two different bTMB-H benchmarks: ≥10mut/Mb (*n* = 5327; 38.1%) and ≥16mut/Mb (*n* = 2039; 14.6%). These groups were compared to the associated bTMB low group (bTMB-L), which were <10 mut/Mb (*n* = 8665; 61.9%) and <16 mut/Mb (*n* = 11953; 85.4%), respectively. These cut-offs were chosen to align with the tTMB approval of ≥10 mut/Mb and with previous studies suggesting that a bTMB-H benchmark of ≥16 mut/Mb would best correlate with the tTMB-H cut-off of ≥10 mut/Mb[15,16].

Genomic Database –bTMB ≥10 mut/Mb. More than a quarter of patients (28.4%, 5327/13992) had a bTMB ≥10 mut/Mb (bTMB-10). Over 5% (290/5327) of bTMB-10 patients and only 0.01% (1/8665) of patients with bTMB <10 mut/Mb were bMSI-H. Most bTMB-10 patients (94.56%; 5037/5327) were bMSI-Not Detected.

Genomic Database –bTMB ≥16 mut/Mb. Approximately 15% (2039/13992) of patients had a bTMB ≥16 mut/Mb (bTMB-16). Almost 14% (285/2039) of bTMB-16 patients and 0.05% (6/11953) of patients with bTMB <16 mut/Mb also were bMSI-H, respectively. Most bTMB-16

Table 1 | Baseline Characteristics of Clinical Cohort (*N* = 48)

Baseline Characteristics	bTMB ≥10, (*N* = 48)	bTMB ≥16, (*N* = 27)
Median Age, *N* (range)	72 (37–87)	72 (37–86)
Race, *N* (%)		
NH White	37 (77.1)	21 (77.8)
NH Black	4 (8.3)	1 (3.7)
Other (Hispanic, American Indian)	5 (10.4)	5 (18.5)
Unknown (%)	2 (4.2)	0 (0)
Primary tumor, *N* (%)		
Prostate	36 (75.0)	18 (66.7)
Breast	12 (25.0)	9 (33.3)
MSI Status, *N* (%)		
bMSI-H	8 (16.7)	8 (29.6)
bMSI-Not Detected	32 (66.7)	17 (63.0)
Unknown	8 (16.7)	2 (7.4)
MMR deficient, *N* (%)	3 (6.3)	3 (11.1)
Histology, *N* (%)		
Adenocarcinoma	47 (97.9)	27 (100)
Small cell	1 (2.1)	0 (0)
Location of metastases, *N* (%)		
Bones	36 (75.0)	21 (77.8)
Lymph nodes	24 (50.0)	14 (51.9)
Visceral		
Liver	11 (22.9)	7 (25.9)
Lung	7 (14.6)	4 (14.8)
Other (bladder, adrenals, pleura, brain	8 (16.7)	7 (25.9)
soft tissue)	4 (8.3)	2 (7.4)
Prior systemic therapies prior to IO, *N* (range)	3 (1–9)	3 (1–9)
Prior systemic therapies, *N* (%)		
ARPI	31 (64.6)	14 (51.9)
Aromatase inhibitors	7 (14.6)	5 (18.5)
Chemotherapy	25 (52.1)	14 (51.9)
Sipuleucel-T	9 (18.8)	2 (7.4)
Targeted therapies	13 (27.1)	7 (25.9)
Investigational agents	1 (2.1)	0 (0)
PD(L)−1 agents	1 (2.1)	1 (3.7)
ICI received, N (%)		
Pembrolizumab	42 (87.5)	26 (96.3)
Nivolumab	1 (2.1)	0
Atezolizumab	1 (2.1)	0
Investigational anti-PD-1	1 (2.1)	0
Ipilimumab/nivolumab	1 (2.1)	1 (3.7)
Anti-PD-1/CTLA4 bispecific	1 (2.1)	0
Atezolizumab/BiTE	1 (2.1)	0

NH non-Hispanic, *ADT* androgen deprivation therapy, *ARPI* androgen receptor signaling inhibitor, *ICI* immune checkpoint inhibitor, *BiTE* bispecific T-cell engager.

patients (86.02%; 1754/2039) were MSI-Not Detected. Altogether, bTMB-16 had a higher percentage of bMSI-H than patients with bTMB <16 mut/Mb (13.98% vs 5.4%).

The top 20 most frequently altered genes were altered at a significantly elevated rate in both bTMB-10 and bTMB-16 groups compared to patients
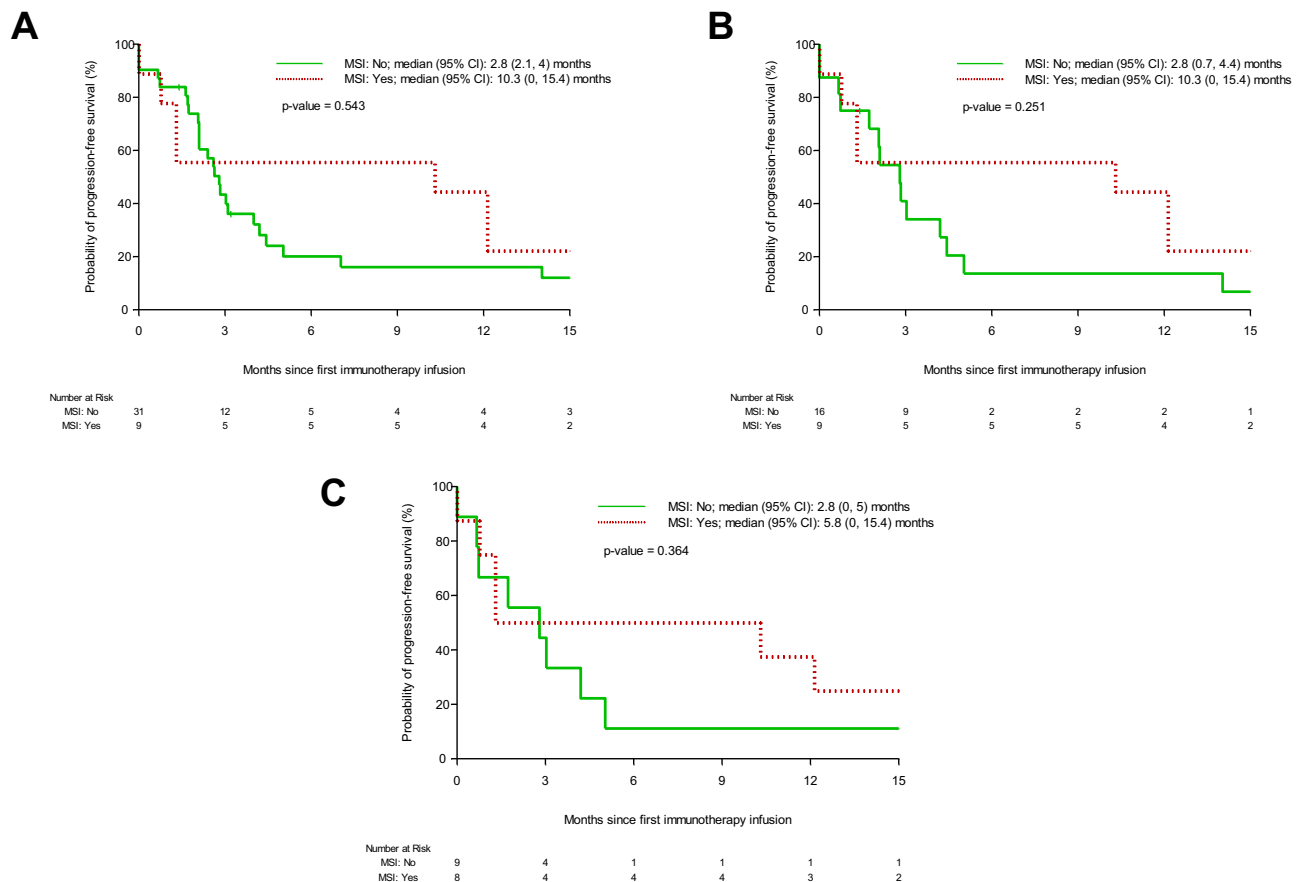
Fig. 2 | Kaplan-Meier plot of PFS by MSI status. A Kaplan-Meier plot of PFS by MSI status for all patients with TMB ≥ 10 mut/Mb. **B** Kaplan-Meier plot of PFS by MSI status for all patients with TMB ≥ 16 mut/Mb. **C** Kaplan-Meier plot of PFS by MSI status for all patients with TMB ≥ 20 mut/Mb

with bTMB <10 and <16 mut/Mb, respectively (Fig. 3A, C; p-values in Table S2). This is not unexpected, as an increased TMB score confers an increase in genomic alterations. Among bTMB-10 and bTMB-16 cohorts, patients with bMSI-H also harbored significantly more frequent alterations in the top assessed genes, with one notable exception (Fig. 3B, D and S2, 3; p-values in Table S3). In the bTMB-16 group, alterations in *PIK3CA* were not significantly different in those with bMSI-H vs. MSI-Not Detected (Fig. 3D).

In breast cancer, most of the genomic alterations were significantly more frequent in bTMB-H patients (bMSI-H were removed from this cohort) among both the bTMB-10 and bTMB-16 groups (Figure S3A, B). Some genes of note here are *PIK3CA, ESR1, CDH1, ARID1A, BRCA2, BRCA1, ERBB2, MTOR, EGFR, CHEK2*, and *PTEN* genes. In bTMB-10, *GATA3* was significantly more frequently altered than in patients with a bTMB <10 mut/Mb, but there was no significance observed in the bTMB-16 group compared to the bTMB <16 mut/Mb cohort.

Results in prostate cancer were similar to that of the breast cancer cohort, with most alterations being significantly more frequent in bTMB-H patients in both the bTMB-10 and bTMB-16 groups (bMSI-H were removed from this cohort as well). Some of these altered genes included *TP53, ATM, BRCA2, CHEK2, EGFR, PIK3CA, CDK12, ARID1A, PTEN, MTOR*, and *BRCA1* genes (Figure S3C, D). In the bTMB-10 group, *AR* was significantly more frequently altered than in patients with a bTMB <10, but there was no significance observed in the bTMB-16 group compared to patients with a bTMB <16 mut/Mb. *CTNNB1* showed no significant difference in both bTMB-10 and bTMB-16 compared to their respective bTMB-L groups.

Interestingly, in prostate cancer, the bTMB-16 group has a significantly higher frequency of alterations in the majority of HRR genes compared to the bTMB-10 group (Table S4), including *ATM* (32.53% vs. 26.26%;

$p = 0.0018$), *BRCA2* (31.02% vs. 17.46%; $p = 3.09E-13$), *BRCA1* (15.36% vs. 8.25%; $p = 3.54E-07$), *CDK12* (13.25%vs. 10.09%; $p = 0.0267$), *PALB2* (11.14% vs. 5.02%; $p = 1.12E-07$), and *RAD51D* (3.31% vs 1.38%; $p = 0.0025$). *CHEK2* (16.27% vs. 14.42%; $p = 0.2616$) was the only HRR gene without a significant difference between groups. Similarly, when MSI status was assessed, alterations in HRR genes were significantly more frequent in bMSI-H vs bMSI-Not Detected, with the exception of the *CHEK2* gene which was not significant between groups.

Discussion

In this study including advanced, refractory breast and prostate cancer patients, bTMB was a weak predictor of response to ICI, irrespective of the cut off used. While very limited activity was observed in the group of patients with bTMB/MSI-Not Detected, clinical benefit was seen in patients with MSI-H/MMR defects. Despite no statistical difference in outcomes, most responses were observed in patients with MSI-H/MMR defects who also had a numerically longer median survival of over 6 months compared to patients with MSI-Not detected/MMR preserved.

In routine clinical practice, providers might perceive a bTMB result as equivalent to tissue-based TMB (tTMB) and therefore may use a bTMB cut-off of ≥10mut/Mb to treat patients with ICI, yet data supporting this approach is lacking. In fact, in our clinical cohort from eleven academic institutions that routinely used G360, only a small number of patients with "cold" tumors prostate and breast cancers were offered ICI based on a bTMB cut-off of ≥10mut/Mb and it was rare in other tumors, such as ovarian or pancreatic cancer. Thus, the practice of offering an ICI in these tumor types, solely based on bTMB, appears infrequent in academic practices.

Further, ICIs were used late in the metastatic setting and achieved limited activity in most cases. Most patients discontinued ICI due to disease
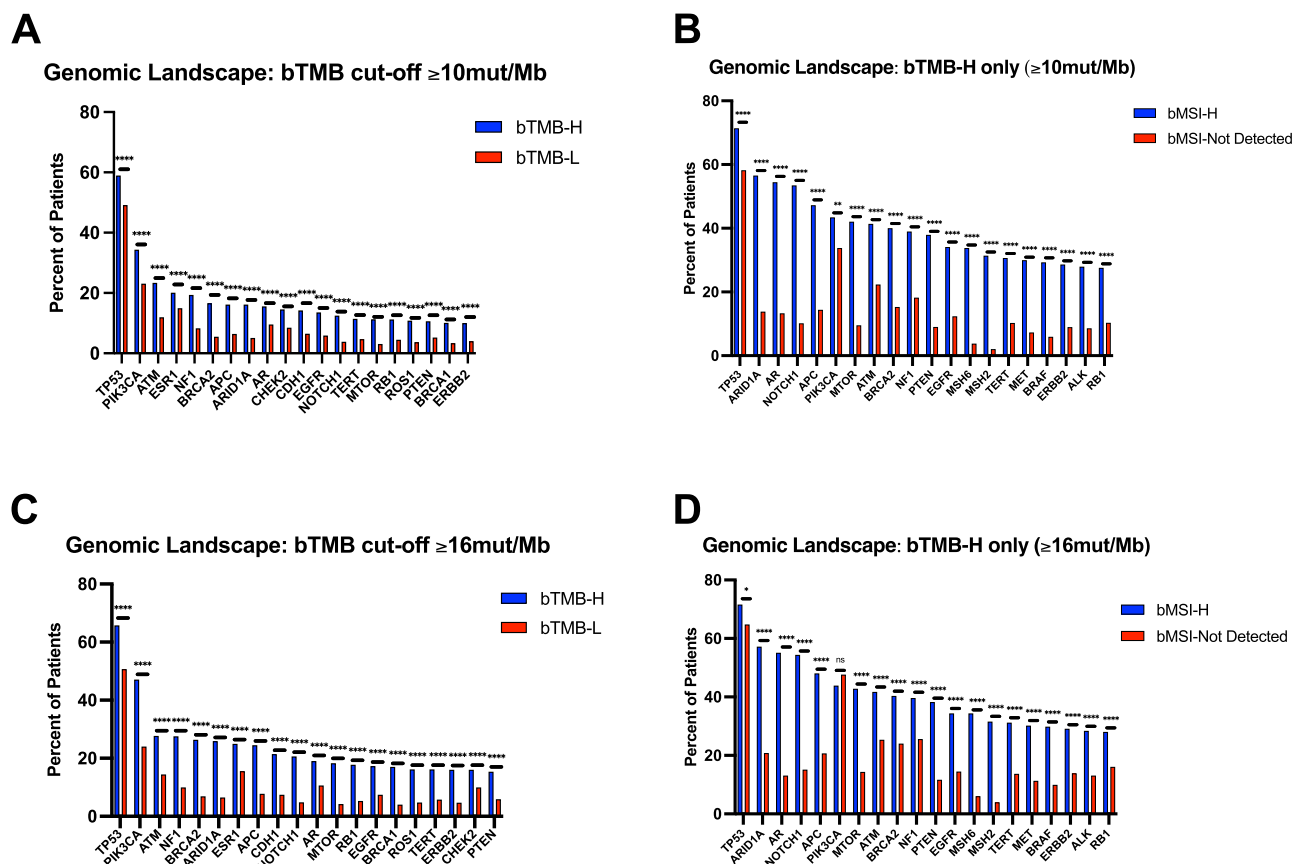
Fig. 3 | Genomic Landscape. Genomic Landscape in cohort of tumors with bTMB ≥10 (TMB-H) and bTMB ≤10 (TMB-L) (**A**) and bTMB-H (≥ 10 mut/Mb) only patients stratified by MSI (**B**), followed by tumors with bTMB ≥16 (bTMB-H) and bTMB ≤16 (bTMB-L) (**C**) and then bTMB-H (≥ 16 mut/Mb) only patients stratified by MSI (**D**).

progression in less than 3 months, suggesting lack of benefit except for cases with concurrent bTMB-H and bMSI-high status. This data suggests bTMB may simply be a marker of genomic instability. Despite the small numbers of bMSI-H in our clinical cohort, the differences in clinical outcomes favored bMSI-H as a predictor of response to ICI, concordant to other reports in these and other tumors[7,13].

Differences between tTMB and bTMB have been observed in various patient cohorts[22–24]. Notably, prior research has shown a weak association between tTMB of ≥10 mut/Mb and a bTMB of ≥10 mut/Mb[15]. A pan-tumor bTMB-H benchmark of ≥16 mut/Mb has been suggested as a better correlative to the tTMB ≥10 mut/Mb cutoff[16] in certain tumors such as lung and colorectal cancers, but not in the present cohort including breast or prostate tumors. Additionally, the timing of blood collection and exposure to different therapies could have affected the overall bTMB score, and it has been reported[25].

As anticipated, genomic alterations were found more frequently in patients with bTMB-H and some of the genes altered are known to be targetable. However, in most cases, they were *bystander* events and also likely monoallelic, including some HRR genes which have been shown to not always be predictive of response to Poly (ADP-ribose) polymerase (PARP) inhibitors[26]. Although MSI-H and mutations in the HRR gene family tend to be mutually exclusive, they may co-occur as shown here and other prostate cancer studies[26–28].

Further research is needed to better understand the role of bTMB in these traditional "immune-cold" tumors. It is possible that in certain cancer types, bTMB and bMSI status may not be sufficient to identify which patients could receive clinical benefit from ICI therapy. Development of an immuno-oncology signature that encompasses TMB and MSI, as well as other markers such as specific genomic alterations and methylation patterns, should be

considered in future studies and may help match patients with ICIs more effectively. As TMB may also vary by genomic ancestry, further research in racially and ancestrally diverse populations is also recommended[29].

Limitations of our study include the retrospective design and the inclusion of a heavily pretreated population which might have impacted bTMB results by inducing additional mutations. The timing of blood collection can also be a limitation: if drawn too soon after a patient receives therapy, this can suppress ctDNA shed and thus impact bTMB results. Similarly, if drawn during disease progression, bTMB is more likely to be observed at higher levels.

In conclusion, the findings of this multi-institutional study suggest that bTMB alone is a weak predictor of response to ICI in patients with advanced, refractory breast and prostate cancers. Higher bTMB was associated with more genomic alterations and with potentially additional treatment implications. As high tTMB is an approved biomarker of response to ICI in solid tumors, further research in liquid biopsy-based markers is needed to determine equivalency as well as to identify and validate novel biomarkers of response to ICI in these tumors.

Data availability

The source data for Figs. 2, 3 is in Supplementary Table S1–S3, respectively. Restrictions apply to the availability of ctDNA sequences in the national cohort which was obtained from Guardant Health due to data privacy regulations and restrictions for use in the patient consent form. Requests are to be made to Pedro Barata describing the nature of the proposed research and the extent of data requirements. Data recipients may require a collaborative research agreement, which describes the conditions for data release and requirements for data transfer, storage, archiving, publication, and intellectual property.

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