Role of Genomic Instability in Immunotherapy with Checkpoint Inhibitors

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Abstract. *Aim: We evaluated whether tumor genome sequencing to detect the number and type of alterations could be used as a valuable biomarker for judging the potential utility of immune checkpoint inhibitors in patients with advanced cancers. Materials and Methods: We identified patients with solid tumors who were treated with checkpoint inibitors and had received commercially available next generation sequencing (NGS). Tumors profiled by Caris Life Sciences, Foundation Medicine and Guardant360 between 2013 and 2015. Patients were divided into 5 quintiles based on mutational load (pathogenic mutations plus variants of undetermined significance). Results: Fifty patients with solid tumors on immunotherapy that had NGS reports available were identified. Top quintile patients had more genomic alterations (median=16.5) than the others (median=2) and had more pathogenic mutations in cell-cycle regulatory genes (100% versus 48%). The overall survival (OS) was significantly superior for patients in the top quintile (722 days) versus the others (432 days). We found no significant difference in progression-free survival (PFS) between the two groups. The objective response rate was numerically higher for the top quintile (50%) vs. others (20%). Programmed cell death protein 1 (PD1) and programmed death-ligand 1 (PDL1) status by immunohistochemistry was not associated with outcomes. Conclusion: The use of immune checkpoint blockade in tumors with higher mutational load was associated with improved OS. Our results suggest that the evaluation of tumor genomes may be predictive of immunotherapy benefit.*

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Genomic instability is one of the hallmarks of cancer (1). Through this acquired instability, malignant cells accumulate non-synonymous coding changes that result in the creation of novel epitopes and proteins unique to the malignant genome. These proteins may serve as potential targets for the host immune system by functioning as neoantigens (2). Enhancing T cell reactivity against these neoantigens may serve as a potent oncolytic therapy.

Checkpoint inhibitors target the regulatory pathways of T cells to enhance anti-tumor activity. Currently available agents target cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) (ipilimumab) and programmed cell death protein 1 (PD-1) (nivolumab, pembrolizumab) and have shown significant clinical activity in various cancers (3). However, it has been difficult to determine biomarkers that predict a response to these agents. Many factors have been considered, such as absolute lymphocyte count (4), tumor infiltrating lymphocytes (5) and expression of PD-1 ligands (6, 7) but a consistent, robust predictive biomarker has remained elusive. We hypothesized that tumors with higher mutation loads, regardless of the functional significance of the individual mutations, including all mutations and variants of unknown significance (VUS), would generate a more robust immune response that results in better survival. To address this hypothesis we conducted a retrospective review to analyze the relationship between mutational burden by next generation sequencing (NGS) and overall survival (OS) in patients with stage IV solid tumors treated with immune checkpoint inhibitors.

Materials and Methods

Patients and samples. Patients who were treated with checkpoint inhibitors and had received commercially available NGS treated at the West Cancer Center (WCC) between August 2013 and October 2015 (n=50) were identified through a retrospective pharmacy database search. Patients' tumor types are included in Table I. The primary end-point was OS. Secondary end-points included progression-free survival (PFS), immune-related response criteria (irRC) objective response rate (complete response + partial

response) and influence of PD-1 and programmed death-ligand 1 (PDL1) expression on OS. This study was reviewed by the University of Tennessee Health Science Center IRB and deemed to be exempt from institutional review board (IRB) review.

Clinical staging and treatment. To be included, patients had to receive at least one dose of ipilibumab, pembrolizumab or nivolumab for any solid tumor with stage IV disease. All patients were also required to have had NGS and imaging studies at baseline. Patients who had incomplete NGS results or did not have measurable disease at baseline or appropriate follow-up imaging were excluded. NGS reports used in this study were generated from tissue biopsy specimens or peripheral blood samples sent to Caris Life Sciences, Foundation Medicine or Guardant360. The genomic alterations were grouped into several categories based on the lab's reporting of suspected pathogenicity: total genomic changes, pathologic/presumed pathologic, benign/presumed benign, VUS and other. Only alterations resulting in coding changes were considered when calculating mutational burden. The functional significance of VUS in DNA damage repair pathways were predicted using PolyPhen (13, 14). PD-1 and PDL1 expression were determined by immunohistochemistry (IHC) by the individual commercial labs.

Statistical analysis. All statistical analyses were performed on Microsoft Excel version 14.5.7 (www.microsoft.com/en-us/download/details.aspx?id=3) and GraphPad Prism version 6.0 (www.graphpad.com/scientific-software/prism/). Patients were ranked in order of increasing mutational burden and divided into quintiles. Based on the skewed distribution of mutations, patients in the top quintile were compared to the other 4 quintiles combined. This procedure was done before examining mortality to avoid selecting cut-off points that could accentuate the mutation-outcome relationship. Objective response rates were compared with the Fisher's exact test and survival was analyzed using log-rank testing. All *p*-values were two-sided and those less than 0.05 were considered statistically significant. Our report of genomic instability and response to immune checkpoint therapy conforms to the REporting recommendations for tumor MARKer prognostic studies (REMARK) criteria where applicable as defined by McShane *et al*. (15).

Results

One hundred and sixty-one patients treated with immunotherapy were identified. Of these, 54 had NGS reports available. Two patients did not have imaging and two did not have measurable disease at baseline, leaving 50 patients for analysis; most had either melanoma or non-small cell lung cancer (NSCLC) (top quintile 90% *vs*. others 86%, respectively). Median age (66 *vs*. 65) and median number of prior therapies (2 *vs*. 1) were similar. All patients had stage IV disease and were well balanced for other characteristics (Table I). Patients in the top quintile had more genomic alterations (median=16.5) than the others (median=2) ($p<0.001$). Differences were both in median number of pathogenic mutations (3.5 *vs*. 1, $p<0.001$) and VUS (12.5 *vs*. 1, $p<0.001$) (Figure 1).

The Foundation One panel was more commonly used for the NGS in the top quintile, while the NGS panels from Caris was more commonly used in the other group. However, median number of genes analyzed in the top quintile was 343 (range=57-592) *versus* a median of 592 genes (range=33-592) in the others ($p=0.546$). In both groups, nivolumab was the most common checkpoint inhibitor used, followed by ipilibumab and then pembrolizumab.

Top quintile patients were more likely to have pathogenic mutations or VUS predicted to be deleterious by *in silico* analysis in cell cycle checkpoint genes (*TP53, BRCA1/2, ATM*) than the others (100% *vs*. 48%, $p=0.017$) (data in Table II). The presence or absence of mutations in cell cycle checkpoint genes did not independently predict survival ($p=0.256$; data not shown).

OS was significantly different between the top quintile and the others (Figure 2). The median OS was 722 days in the top quintile *versus* 432 in the others (hazard ratio (HR)=5.78; confidence interval (CI)=1.40-15.12; $p=0.029$). There was no significant difference in PFS between the top quintile and the other group with median PFS not reached *versus* 89 days, respectively (HR=2.11; CI=0.80-4.45; $p=0.154$). As measured by irRC, there was no significant difference in objective response rate (ORR) between the top quintile (50%) and others (20%), $p=0.101$.

Neither PD-1 nor PDL1 expression influenced OS (median positive 277 days *versus* negative undefined, HR=1.53; CI=0.38-7.58; $p=0.51$; median positive 277 days *versus* negative undefined, HR=1.76; CI=0.50-6.85; $p=0.39$, respectively) (Figure 2). Though numbers were small, there was no difference in outcome trends based on histology (Figure 3).

Discussion

The use of immune checkpoint blockade in tumors with higher mutational load was associated with improved OS. The OS was significantly different between the top quintile and the others with a median OS of 24 months in the top quintile *versus* 14.5 months in the others. We also found that the top quintile achieved numerically higher ORR as measured by irRC and PFS; however, these did not reach statistical significance.

Our findings provide support to the hypothesis that tumors with greater genomic instability generate a more robust immune response and this can result in better survival. This resonates with findings from other studies (8, 9), Topalian *et al*. reported that two of the tumor types that were most responsive to PD-1 blockade, lung cancers and melanomas, had high numbers of somatic mutations (10). Further, in colon cancer, it has been reported that mismatch-repair status predicted clinical benefit of immune checkpoint blockade

Table I. *Demographics and patients' characteristics*.

	Top Quintile	Others
Patients	10	40
Median age (range)	65 (42-80)	66 (42-81)
Gender	30% female	45% female
Ethnicity	70% white	83% white
Malignancy	p=0.7774	
Melanoma	50%	33%
Lung Adenocarcinoma	30%	33%
Lung Squamous Cell	10%	18%
Head and Neck Squamous Cell	0%	5%
Various*	10%	11%
Next generation platform (%)		
Caris	40	90
Precipio	0	2.5
Foundation One	60	2.5
Guardant 360	0	5
Median number of genes analyzed (range)	343 (57-592)	592 (33-592)
Median coding changes (range)	16.5 (9-60)	2 (0-8)
Median number of prior therapies (range)	2 (0-7)	1 (0-4)
Immunotherapy received (%)		
Nivolumab	60	65
Pembroluzumab	0	10
Ipilumumab	40	25
PD1 IHC (%)		
Positive	40	50
Negative	0	28
Unknown	60	22
PDL1 IHC (%)		
Positive	20	28
Negative	20	55
Unknown	60	17

*Included thymoma, squamous cell carcinoma of the thymus, small cell lung cancer, adenoid cystic carcinoma of the nasopharynx; IHC, immunohistochemistry.

with pembrolizumab suggesting that higher mutational loads may be more responsive to immunotherapy (11).

While different platforms were used for genomic analyses, the number of genes analyzed in the top quintile and the others was well-balanced and the majority of the coding changes were VUS. Moreover, in both groups, nivolumab was the most common immune checkpoint inhibitor therapy. We also found that PD-1, PD-L1 expression had no significant effect on OS.

This study is limited by its sample size and retrospective nature. Further, while increased mutations may predict for neo-epitopes, there are more sophisticated epitope prediction methods that were not used here (12). Additionally, a multivariate analysis was not performed due to small numbers in each group, thus limiting the power of subgroup comparisons. Future studies looking at genomic instability

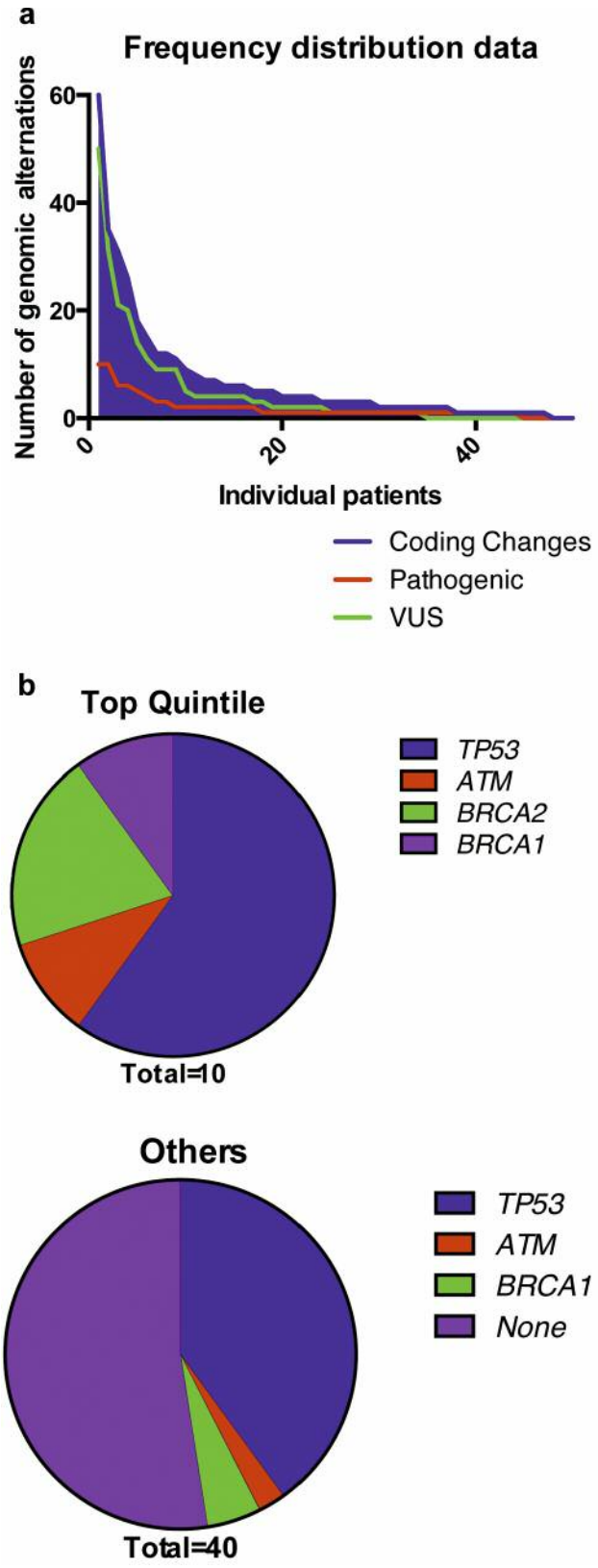

Figure 1. *Distribution of genetic alternations (a) and pathogenic cell-cycle checkpoint mutation profile (b).*

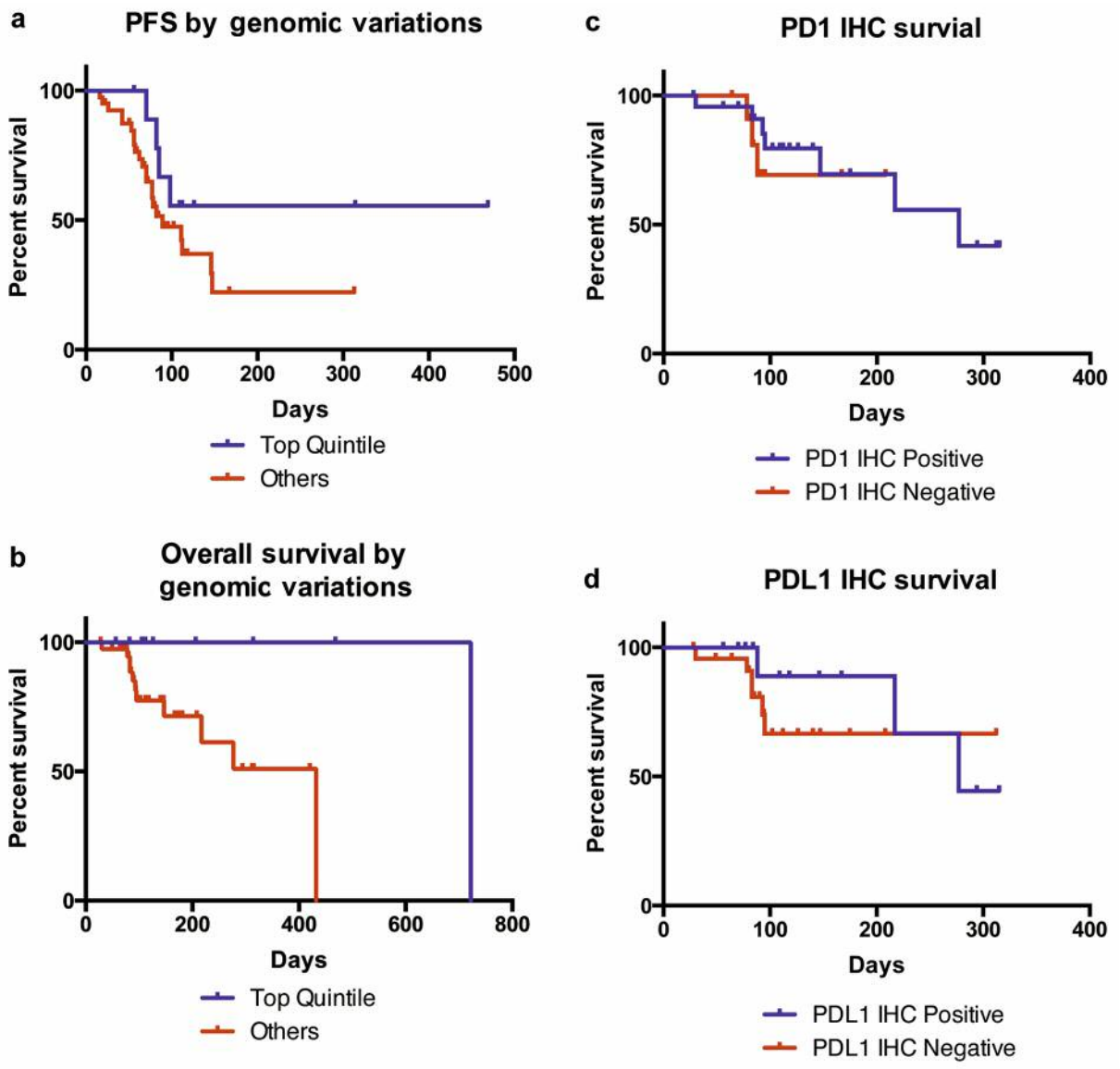

Figure 2. *Survival outcomes by genomic alternations and PD-1 and PDL-1 status. PFS (a) and OS (a) by mutational burden and OS by PD-1 (c) and PDL-1 (d) status.*

and immunotherapy prospectively and in a larger population are warranted.

Translational Relevance

Acquired genomic instability generates neoantigens on cancer cells, which enhances T cell reactivity against these neoantigens, and may serve as a potent oncolytic therapy. Currently available checkpoint inhibitors targeting the inhibitory molecules CTLA4 (ipilimumab) and PD-1 or PDL-1 ligands (nivolumab, pembrolizumab) have shown significant clinical acitvity against a variety of cancers. However, biomarkers that predict responses to these agents have remained elusive. We hypothesized that tumors with greater genomic instability generate a more robust immune response and result in better survival. This article demonstrates that the use of immune checkpoint blockade in tumors with higher mutational load was associated with improved overall survival. Furthermore, it suggests that the evaluation of tumor genomes may be predictive of immunotherapy benefit, whereas the number and type of alterations may prove to be valuable for judging the potential utility of immune checkpoint inhibitors.

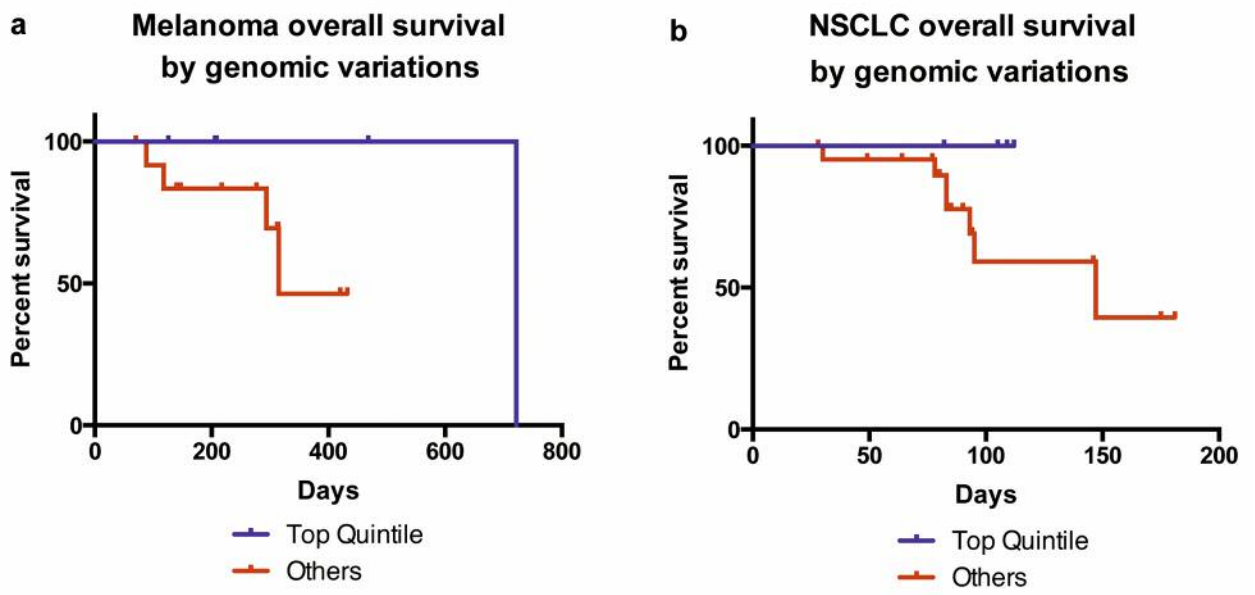

Figure 3. *Histology-specific overall survival (OS) outcomes for melanoma (a) (p=0.1729, HR=3.290 (0.7535 to 25.53)) and non-small cell lung cancer (NSCLC) (b) (p=0.2050, HR=undefined (0.5262 to 25.70)).*

Table II. *In silico analysis of variants of unknown significance (VUS) in cell-cycle checkpoint genes in patients without known pathogenic mutations in cell cycle checkpoint genes.*

	Gene with coding change	UniProtKB identification	PolyPhen score (sensitivity; specificity)	Prediction
Top quintile (patient number)				
6	*BRCA2* (S2326F)	P51587	0.933 (0.80; 0.94)	Possibly damaging
8	*ATM* (P1759L)	Q13315	0.998 (0.27; 0.99)	Probably damaging
8	*FANCE* (E138K)	Q9HB96	0.000 (1.00; 0.00)	Benign
8	*FANCG* (A265V)	O15287	1.00 (0.00; 1.00)	Probably damaging
9	*BRCA2* (G1976)	P51587	1.00 (0.00; 1.00)	Probably damaging
11	*BRCA1* (M1783T)	P38398	0.992 (0.70; 0.97)	Probably damaging
11	*ATM* (N1650S)	Q13315	0.00 (1.00; 0.00)	Benign
11	*FANCA* (E633D)	Q0VAP4	0.028 (0.95; 0.81)	Benign
Others (patient number)				
22	*BRCA1* (D232N)	P38398	0.981 (0.75; 0.96)	Probably damaging
28	*ATM* (L2332P)	Q13315	0.005 (0.97; 0.74)	Benign
31	*BRCA2* (A2727P)	P51587	0.449 (0.89; 0.90)	Benign
35	*ATM* (R2719C)	Q13315	1.00 (0.00; 1.00)	Probably damaging
42	*BRCA1* (A102V)	P38398	1.00 (0.00; 1.00)	Probably damaging

Conclusion

Our results suggest that the evaluation of tumor genomes may predict benefit from checkpoint inhibitors and should be studied further. They support the view that the total number and type of genomic alterations may prove to be valuable for judging the potential utility of immune checkpoint inhibitors.

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