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Pan cancer landscape of clonal tumor mutational burden (cTMB)

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ABSTRACT

Background: Response to immune checkpoint inhibitors (ICI) relies on T-cell recognition of neoantigens on the surface of tumor cells. It is hypothesized that the clonality of a neoantigen determines the probability of its presentation to the immune system, with more clonal neoantigens having a higher likelihood of recognition. Using a TMB (tumor mutational burden) metric that is comprised of only clonal variants (cTMB) could refine TMB as a biomarker of ICI response. We sought to describe the pan-cancer landscape of cTMB as compared to TMB.

Methods: Tumor samples were assayed during routine clinical care, by hybrid capture-based comprehensive genomic profiling (Foundation Medicine, MA, USA). TMB was defined as the number of non-driver somatic coding mutations identified on up to 1.1 megabases (Mb) of sequenced DNA. For every sample, TMB variants with a cancer cell fraction < 0.5 were filtered out to calculate the cTMB. Mutational signatures (sig) were assigned based on the COSMIC (Catalogue of somatic mutations in cancer) signatures.

Results: The median TMB and median cTMB of the evaluable cohort (N=233,981) across 46 solid tumor types was 3.7 [1.3-6.7] and 2.7 [1.3-5.3] mutations per Mb (mut/Mb) respectively, with 59.1% of the samples having an identical TMB and cTMB (ITC). Distribution across major tumor types shown here in Figure 3. In tumor types known to be associated with a mutational signature, we compared the ITC between samples associated with the signature and the wildtype samples, and observed that smoking signature in NSCLC (Non-small cell lung cancer; 34.7% vs 50.8%, P=8.9e-105), ultra-violet signature in melanoma (55.1% vs 75.4%, P=1.7e-67), mismatch repair signature in colorectal (26.0% vs 62.7%, P=1.5e-229), alkylating signature in glioma (7.1% vs 73.4%, P=1.4e-55) and POLE signature pan-cancer (17.8% vs 59.7%, P=4.7e-25) had significantly lower ITC compared to their respective wildtypes. Local tumors had a comparable ITC to that of metastatic tumors (59.1% vs 58.9%).

Conclusions: cTMB is diverse across tumor types and it needs to be evaluated as a predictor of ICI response pan-cancer, using tumor-type specific cTMB thresholds.

MATERIALS AND METHODS

- Comprehensive genomic profiling (CGP) of solid tumor clinical cases was performed by Foundation Medicine, Inc., Cambridge, MA, US (Frampton et al., PMID: 24142049), in a Clinical Laboratory Improvement Amendments (CLIA) certified and College of American Pathologists (CAP) accredited laboratory, between August 2014 and December 2021
- All de-identified research consented samples submitted for sequencing featured a minimum of 20% tumor cells and yielded at least 50 ng of extracted DNA. CGP was performed on hybridization-captured, adapter ligation-based libraries (median exon coverage depth >800x), to identify genomic alterations (short variants, copy number alterations, and rearrangements) in all coding exons (FoundationOne® CDx: N = 309 genes; FoundationOne®: N = 395 genes) and select introns of cancer-associated genes (FoundationOne® CDx: N = 36 genes; FoundationOne®: N = 31 genes) and tumor mutational burden (TMB). TMB was calculated as the number of non-driver somatic coding mutations per megabase (Mb) of genome sequenced (Chalmers et al., PMID: 28420421)
- A cancer cell fraction (CCF) defined as the fraction of tumor cells containing the short variant was estimated for every TMB variant in each sample. TMB variants with a CCF < 0.5 were filtered out to calculate the cTMB (Figure 1)
- Mutational signatures (sig) were assigned based on the COSMIC (Catalogue of somatic mutations in cancer) signatures (Alexandrov et al., PMID: 32025018)

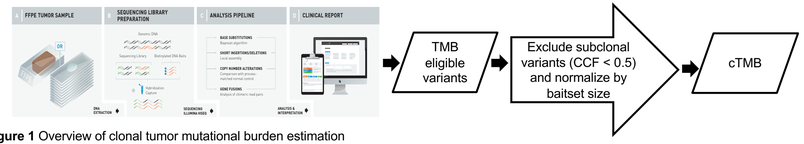

Figure 1 Overview of clonal tumor mutational burden estimation

RESULTS

Characteristic		Study Cohort (N=233, 981)
Median Age (308/233,981 missing age data)		64.0 [55.0-72.0]
Gender	Male	44.2%
	Female	55.7%
	Unknown	0.1%
Site of tissue specimen for CGP	Local	44.0%
	Metastatic	39.6%
	Unknown	16.4%
Median tissue specimen tumor purity		50.6% [37.3%-67.6%]

Table 1 Clinical and demographic characteristics of the study cohort

RESULTS

Tumor Type	N
non-small cell lung cancer (NSCLC)	43256
colorectal cancer	33384
breast	29190
ovary	16314
carcinoma of unknown primary (CUP)	12345
pancreas	11995
prostate	11829
glioma	8498
esophagus	7777
endometrial	7424
melanoma	7147
bladder	5630
cholangiocarcinoma	4687

Tumor Type	N
head and neck	4561
kidney	4051
stomach	3309
cervix	2320
fallopian tube	1666
thyroid	1619
small intestine	1506
uterus	1453
cns non-glioma	1414
liver	1386
gallbladder	1368
anus	1028
salivary gland	1017

Table 2 Distribution of tumor types in the study cohort. Only tumor types with a prevalence greater than or equal to 1000 are shown here.

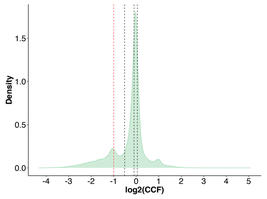

Figure 2 Pan-cancer (N=211,708) CCF distribution of TMB variants. Samples with a TMB = 0 muts/Mb were excluded from this analysis. The red dotted line represents a CCF of 0.5 (CCF threshold for clonality). Observing from left to right, the three black dotted lines each represent the 25th, 50th and 75th CCF percentile. CCF=Cancer cell fraction, TMB=Tumor mutational burden.

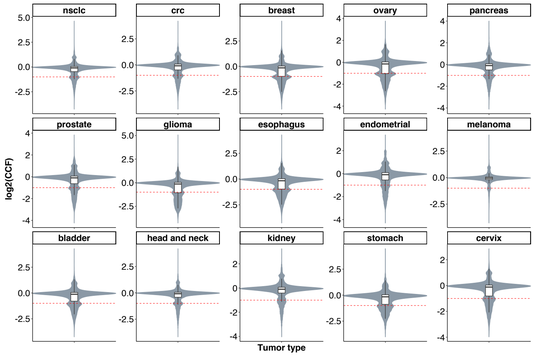

Figure 3 Violin plots describing the CCF distribution of TMB variants per tumor type. Only select tumor types are shown and samples with a TMB = 0 muts/Mb were excluded from this analysis. The red dotted horizontal line in every violin plot represents a CCF value of 0.5 (CCF threshold for clonality). CCF=Cancer cell fraction, NSCLC=Non-small cell lung cancer, CRC=Colorectal cancer

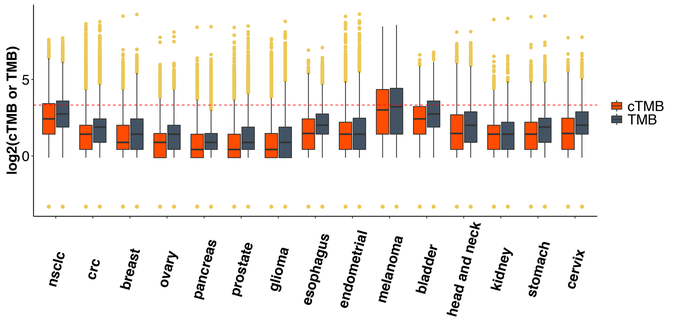

Figure 4 Box plots describing the distribution of cTMB and TMB for select tumor types. The red dotted line represents a TMB threshold of 10 mutations/megabase. cTMB=Clonal tumor mutational burden, TMB=Tumor mutational burden, NSCLC=Non-small cell lung cancer, CRC=Colorectal cancer

RESULTS

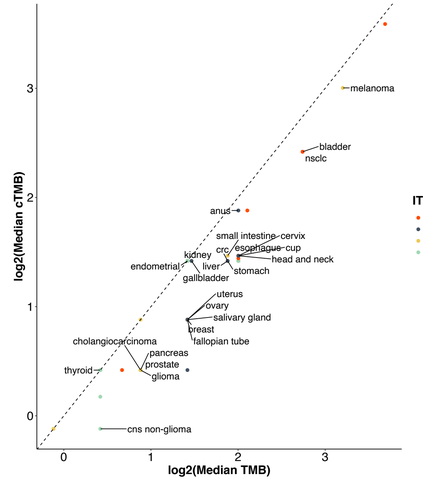

Figure 5 Scatterplot of the log2 median TMB vs. log2 median cTMB for all 46 tumor types studied. ITC (% of samples having an identical TMB and cTMB) values across the 46 tumor types have been binned into quartiles. First=[38.5%-58.0%), Second= [58.0%-62.0%), Third=[62.0%-70.9%), Fourth=[70.9%-90.9%]. Only tumor types with a prevalence greater than or equal to 1000 are labelled. The black dotted line represents y=x. cTMB=Clonal tumor mutational burden, TMB=Tumor mutational burden, NSCLC=Non-small cell lung cancer, CNS=Central nervous system, CRC=Colorectal cancer, CUP=Carcinoma of unknown primary.

	Signature	N	Median TMB	Median cTMB	ITC (%)
NSCLC	Smoking	5220	14.7[10.1-21.4]	12.9[8.3-18.7]	34.7
	Smoking wt	33127	5.5[2.7-10.1]	4.6[2.7-8.3]	50.8
Colorectal	dMMR	2021	10.7[6.4-41.4]	8.3[5.3-36.1]	26.0
	dMMR wt	28648	2.8[1.8-5.3]	2.7[1.3-4.0]	62.7
Glioma	Alkylation	127	65.5[39.8-106.9]	40.1[17.4-62.6]	7.1
	Alkylation wt	7595	1.8[0.9-2.8]	1.3[0.9-2.8]	73.4
Melanoma	UV	3394	21.2[12.0-38.8]	20.1[12.0-36.8]	55.1
	UV wt	3184	2.7[1.3-5.3]	2.7[0.9-4.0]	75.4
Breast	APOBEC	1862	12.0[8.3-18.7]	9.2[5.5-13.8]	23.6
	APOBEC wt	23215	2.7[1.3-4.0]	1.8[0.9-2.7]	61.8
Bladder	Smoking	25	9.4[6.7-17.4]	6.4[5.3-13.4]	28.0
	Smoking wt	5170	6.7[3.7-2.0]	5.3[2.7-9.4]	44.4
Pan-Cancer	POLE	146	69.5[9.2-159.1]	50.6[7.4-133.4]	17.8
	POLE wt	208416	2.8[1.3-6.7]	2.7[1.3-5.3]	59.7

Table 3 Distribution of TMB and cTMB for select tumor types and subgroups based on relevant COSMIC mutational signatures. Note that not all samples were eligible for COSMIC mutational signature estimation. TMB=Tumor mutational burden, cTMB=Clonal tumor mutational burden, COSMIC=Catalogue of somatic mutations in cancer, wt=wildtype, NSCLC=Non-small cell lung cancer, dMMR=Mismatch repair deficiency, UV=Ultra-violet, APOBEC=Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide, POLE=DNA polymerase epsilon

CONCLUSIONS

- cTMB can be estimated from a single bulk-tumor tissue biopsy specimen taken during routine clinical care
- cTMB is diverse across tumor types and the neoantigenic potential of cTMB variants needs to evaluated
- Further clinical studies are needed to understand the predictive value of cTMB in stratifying response to immune checkpoint inhibitor therapy, using tumor type specific cTMB thresholds

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