Development and Validation of PredicineATLAS, a 600-gene Liquid Biopsy Panel for Simultaneous Detection of TMB and Genomic Alterations in Cancers

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Introduction

Tumor mutation burden (TMB) is a measurement of the number of somatic mutations carried by tumor cells and has been shown to be an effective genomic biomarker for predicting response of anti PD-1/PD-L1 immunotherapy. In the present study, we developed a 600-gene comprehensive liquid biopsy NGS panel, PredicineATLAS, aiming to measure genomic alterations and estimate TMB from human body fluids including blood and urine. PredicineATLAS panel-derived TMB score was shown to strongly correlates with the WES-based TMB using TCGA samples. Analytical validation using the reference materials showed that PredicineATLAS NGS assay is highly robust and sensitive for measurement of TMB scores. The association between TMB scores calculated by the PredicineATLAS panel and clinical outcome in cancer patients was also assessed.

Material and Method

Circulate tumor DNA (ctDNA) was extracted from plasma or urine samples and used to detect genomic alterations and measure MSI and TMB scores.

Fig.1: Workflow for PredicineATLAS TMB measurement

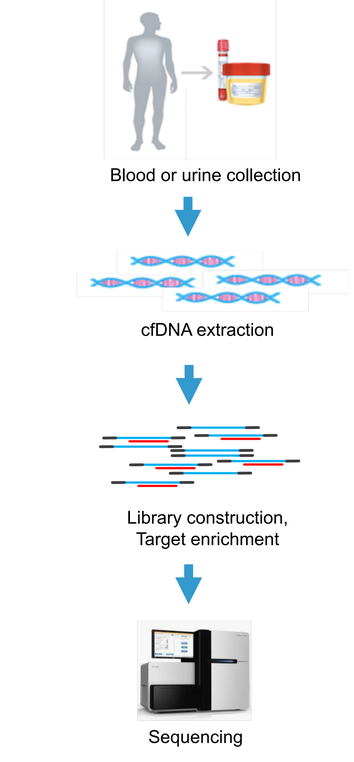

Fig.1a: PredicineATLAS NGS workflow

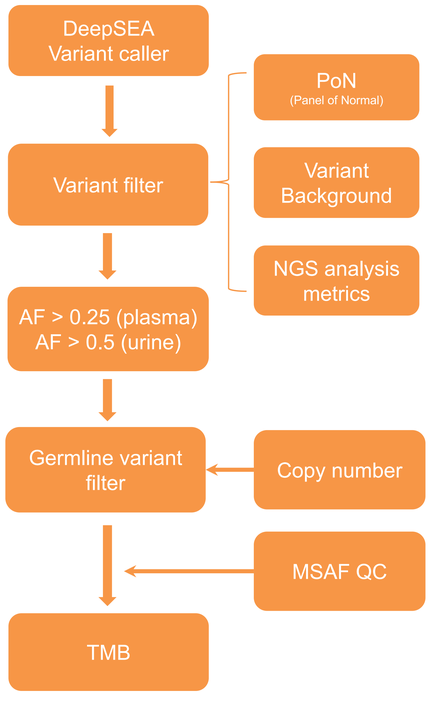

Fig.1b: Mutation call and TMB analysis workflow

Results

Fig.2: PredicineATLAS panel-based TMB scores highly correlates with the WES-based TMB scores

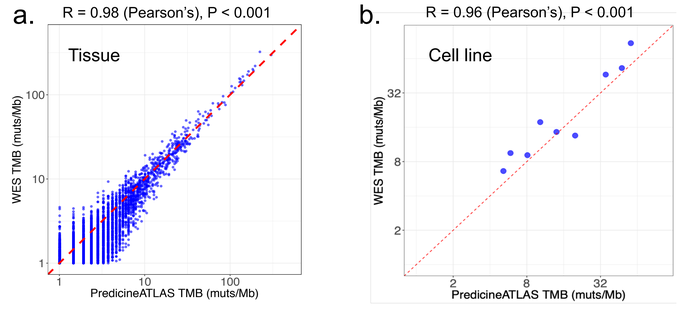

Fig.2: Correlation of the TMB scores from PredicineATLAS panel and WES data. a. *in-silico* assessment in TCGA data. The TMB scores from the TCGA WES datasets were compared to the TMB scores from the PredicineATLAS panel, which contains 600 genes and covers 2.4Mb genomic regions. b. Whole pipeline assessment with the PredicineATLAS panel. Cell lines with Cosmic WES data were used for TMB measurement.

Fig.3: PredicineATLAS panel-based TMB scores correlates with clinical outcome

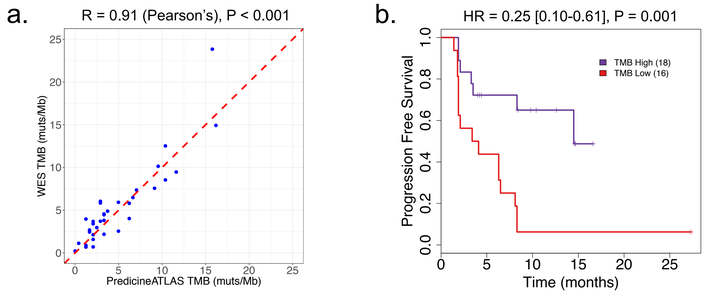

Fig.3: *In-silico* simulation of public data[1] for TMB scores derived from PredicineATLAS panel showed correlation with clinical outcome of PD-1 immunotherapy. a. Correlation of TMB scores from PredicineATLAS panel and WES. b. Samples with high TMB defined by PredicineATLAS panel associate favorable PFS. Patients were split into TMB-High and TMB-Low by the median TMB score.

PredicineATLAS Panel Information

Table 1. Panel Analysis Metrics / Sample Requirement	
Regions Analyzed	600 genes
Panel Size	2.4Mb genomic regions (1.4Mb CDS regions)
Sequencing and Bioinformatics	Illumina NGS / in-house pipeline
Report Range	SNV/Indels/CNV, MSI and TMB scores
Sequencing Depth	>20,000X for biofluid, >2,000X for tissue
Sample Requirement	Blood or plasma, urine, FFPE tissue

Fig.4: PredicineATLAS panel-based TMB score measurement for samples with tumor content greater than 1%

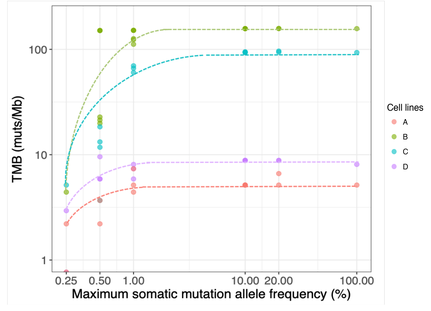

Fig. 4: Titration study of the tumor content for evaluating PredicineATLAS-based TMB scores. To determine the minimal tumor content for the TMB measurement, cell lines having different TMB scores were titrated into five dilution levels with different MSAFs (maximum somatic allele frequency). All the cell line gDNA fragments mimic the cfDNA size distribution profile. TMB scores across all the dilution levels are compared and plotted.

Fig.5: PredicineATLAS panel-based MSI measurement for samples with tumor content greater than 1%

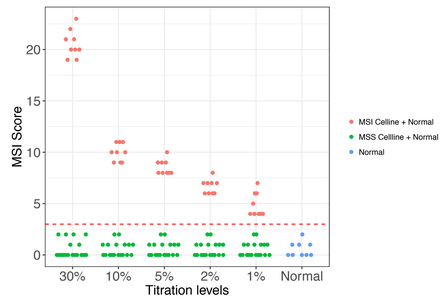

Fig. 5: Titration study of the tumor content for evaluating PredicineATLAS-based MSI scores. MSI and MSS cell lines were mixed with normal plasma samples with five titration levels *in silico*. MSI status can be accurately determined for samples with tumor contents greater than 1%.

Conclusions

PredicineATLAS liquid biopsy NGS assay was analytically validated for detection of genomic alterations, MSI and TMB scores with as little as 1% tumor content. Further development and improvement towards CLIA-certified assay remains actively ongoing.

1. *Rizvi et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348(6230):124–128.*