The mutational landscape of circulating ctDNA in patients pre- and post-intervention was investigated. The most commonly identified differences are Somatic SNVs in 3 patients at both treatment timepoints. MSI, which is monitored in ctDNA status, was initially found in one subject at baseline, and was not observed post-intervention. In two additional patients who post-intervention, appearance of these mutations in the ctDNA pre-intervention could be due to an increase in tumor burden or shedding in response to therapy.

Dynamic VAF in response to intervention

Large shifts in variant allele frequency (VAF) were detected in all patients, despite the relatively short treatment time. For patients 8 and 12, who present with predominantly increasing tumor neoantigen burden and dynamic VAF in response to intervention, we observed a marked increase in Th1 transcription which expanded from "pre-intervention to post-intervention." The timecourse analysis of the peak identified SNVs, and remained consistent through TREAT. Across both treatments, we observed a marked increase in Th1 transcription which expanded from "pre-intervention to post-intervention." Concordant somatic events were detected between plasma and tumor at pre- and post-treatment timepoints. Neoantigens predicted to arise from these somatic events were reduced in solid tumor post-treatment, but increased in ctDNA, when compared to pre-treatment timepoints. This suggests that ctDNA is a better indicator of disease burden in patients who are progressing on treatment.

Neoadaptogen detection in solid and liquid biopsies

Composite neoantigen presentation score (nICOS), which accounts for impairment to neoantigen presentation and other established resistance markers, was calculated for both solid and liquid biopsies. In patients 2 (non-responder, blue) and 3 (progressor, orange), we observed decrease in neoantigen burden post-treatment. Conversely, in patient 1 (progressor, green), we observed an increase in both solid and liquid neoantigen burden. We hypothesize that the dominant treatment response is neoantigen exhaustion due to increased cytolytic activity, as captured by the cytolytic activity score (CYT).

We investigated tumor infiltration as it is a favorable prognostic factor in a number of cancer types. TILs are associated with increased levels of tumor neoantigen antigen, which may influence response to therapy and survival benefit. To evaluate this, we compared neoantigen burden from plasma vs. solid biopsies from all patients. Using ssGSEA, we found consistent enrichment of specific neoantigen signatures, suggesting that both plasma and solid biopsies are valuable in identifying potential therapeutic targets. Using scRNAseq data from both plasma and solid biopsies, we also detected a trend of increased cytolytic activity, as captured by the cytolytic activity score (CYT).

Leveraging the TILs data, we investigated the CD8+/Treg cell ratio, and found that it trended higher in the immune-high population compared with the immune-low population, with notable increases at the subject level, suggesting that despite higher overall immune infiltration in the immune-high population, immune-high tumors experience reduced infiltration. When stratifying MSI cases for treatment timepoint, we observed small subject-level variances. Prior to treatment, tumors display generally increased cytolytic activity, as captured by the cytolytic activity score (CYT). We then used unsupervised clustering on findings from both plasma and solid biopsies, and used these data to identify a distinct treatment response signature.

We used unsupervised clustering to identify distinct response signatures from both plasma and solid biopsies. These signatures were then used to create a comprehensive immune cell specific reference gene signature library. Using this library, gene expression profiles were generated from plasma and solid biopsies for each patient, enabling quantification of cellular abundances. Using ssGSEA, we found consistent enrichment of specific gene signatures, which were then used to classify subjects into different cell lineages. These profiles were then used to create immune cell specific signature libraries, allowing for the identification of potential therapeutic targets. Using these libraries, we identified a number of subjects, likely resulting in reduced neoepitope presentation in those cases. Immune cell infiltration increased in the tumor microenvironment following treatment, with no changes to the CD8+/Treg cell ratio, suggesting consistent immunotherapy.

Conclusions

Concordant somatic events were detected between plasma and tumor at pre- and post-treatment timepoints. Neoantigens predicted to arise from these somatic events were reduced in solid tumor post-treatment, but increased in ctDNA, when compared to pre-treatment timepoints. This suggests that ctDNA is a better indicator of disease burden in patients who are progressing on treatment.

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