Introduction

Neoantigens are increasingly critical in immuno-oncology as a therapeutic target for neoantigen-based personalized cancer vaccines and as a potential biomarker for immunotherapy response. An important step in identifying neoantigens is comprehensive exome and transcriptome sequencing of a tumor biopsy, which can identify both tumor derived neoantigens and (2) track neoantigens in the cfDNA related systematic mutations, we constructed a panel of normal from 20 healthy plasma donors (PON20). This PON20 data was used to "polish" or correct the pileup level evidence seen in the CRC plasma samples. This "polishing" has been shown to greatly decrease false positive calls.

Methods

Seracare ctDNA reference standards

We first wanted to estimate ACE ctDNA exome assay's ability to detect clinically relevant SNVs. To do this we used Seracare CTDNA Standards that harbor 25 SNVs across 21 cancer relevant genes (Table 1) at 0.5%-100ng starting DNA amounts (Figure 1). At 0.5% AF we see a considerable loss in sensitivity for gold SNVs, using our somatic variant calling pipeline (Figure 1 green bars). By interrogating the pileups we found ~5200 heterozygous variants that are private mutations in D1 compared to D2. We designated these as "Gold" variants. Our somatic variant calling assay had a sensitivity of 95.5% identifying gold variants at AF 1.25% and 82.5% at AF 0.625% (Figure 3L). To assess specificity, we used a PON20 polished realistic tumor sample and the 25 somatic variants called in the mixed. Figure 3L shows precision recall cutoffs at the 1.25% mse. Each point (and adjacent number) on the plot refers to the minimum number of polished reads required to confirm a tumor variant. For a variant to be called tumor, it was required that the variant was at least 2 polished reads supporting a variant in ctDNA, we observed between 41% and 90% of tumor variants in matched plasma (Figure 4, grey bars indicate variants unique to tumor). We also called somatic variants directly in plasma and set a cutoff of 5 polished reads of evidence. Even with such a stringent cutoff we observed variants that are unique to plasma (dashed circle Figure 5).

CRC tumor vs ctDNA SNV comparison

We analyzed late stage CRC tumor samples and identified between ~120-900 somatic variants with AF >10%. We then queried the matched plasma from these same patients for the same variants. We found ~4000 SNVs across 21 genes in 8 late stage CRC plasma samples. This "polishing" has been shown to greatly decrease false positive calls.

Conclusion

ACE ctDNA Exome has a sensitivity of > 95% to monitor and PPV of >97% to detect SNVs in plasma, down to an AF of 1.25%. The assay's high sensitivity is important in cases when monitoring of tumor variants in plasma is desired. The very high PPV is useful when identifying variants derived solely from plasma without a tumor biopsy. We believe the assay can be used a complement to the results from sequencing of the tumor biopsy alone.