Tumor microenvironment heterogeneity is not identified across multiple histologically similar tumors from the same patient

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Background

Tumor heterogeneity has been well documented for mutational analysis in virtually all types of tumors and is accepted as a true finding. Heterogeneity of the tumor microenvironment (TME) in the context of response to checkpoint inhibitors has not been well studied, but the belief is that variation will be identified across multiple tumors from the same subject. The expectation is that multiple tumors from a single subject would demonstrate extensive TME heterogeneity driven by the neoplastic component.

Methods

We validated and utilized a targeted RNA-seq immune panel of >350 genes to interrogate the TME of 35 different tumors from 16 unique subjects. These samples for one subject typically represented primary and metastatic tumors that were often separated by multiple years in time. Prior to this study we built a reference database of RNA-seq immune results for this panel for 167 samples. An in-depth analysis of genes associated with checkpoint blockade and tumor infiltrating lymphocytes (TILs) were the focus of the comparative analysis. Unsupervised analysis and gene score by RNA-seq were the primary modes of comparison.

Formalin-fixed paraffin embedded (FFPE) cancer samples were evaluated by RNA-Seq with the OncomineTM Immune Response Research Assay and DNA seq with AmpliSeq capture of 409 cancer related genes with Comprehensive Cancer PanelTM using the Ion ChefTM and S5XLTM. RNA-Seq analysis was performed with the TorrentSuiteTM v5.2.0, followed by data normalization.

• FPPE specimens
• DupSpec-35 Cohort
• 10ng RNA & 30ng DNA
• OncomineTM Immune Response Research Assay (ThermoFisher)
• Comprehensive Cancer panelTM (ThermoFisher)
• Ion Chef and S5XL (ThermoFisher)
• Torrent Suite and immuneResponseRNA plugin (ThermoFisher)

Immune Response NGS Workflow

Figure 1: Immune Response NGS workflow (NYS CLEP approval pending)

Immune response gene expression panel was used to measure normalized reads per million (nRPM) for all immune response genes. nRPM values were then ranked from 0 to 100 based on a reference population of 167 patients to derive genes expression score.

Concordance

Table 1: DupSpec-35 cohort showing multiple comparisons within subjects for various diseases.

Vehicle Response Mutational Burden

Figure 3: Interpretation plot of Mutational Burden (MuB) for multiple specimens for a single subject in the DupSpec-35 cohort.

Conclusions

For approximately one-half of cases for TILs and TCRS for different tumors from a single subject often separated by multiple years in time showed minimal heterogeneity. For the other one-half of cases heterogeneity was present with the more recent specimen showing immune suppression. Additional analysis did not show an association with prior chemotherapy or other treatment. Minimal to no heterogeneity was identified for MuB, which is distinctly different from individual gene analysis for mutations. This result would support some baseline genomic instability that is driven by the host and not the tumor with a consideration that this process is immune related. Our results support a paradigm shift in the influence of the host on TME heterogeneity with evidence that the host and not the neoplastic cells are the primary determining factor, at least for a subset of cases. This study did not evaluate multiple primary tumors from the same subject, but is an additional study we have planned.