

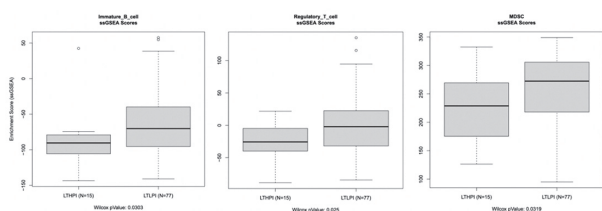
## TUMORS WITH HIGHER HETEROGENEITY WERE ASSOCIATED WITH SUPERIOR SURVIVAL OUTCOME AMONGST STAGE I LUNG CANCER PATIENTS WITH LOW TUMOR MUTATIONAL BURDEN (TMB)

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**Background** Tumor mutational burden (TMB) has been shown to predict response to immune checkpoint inhibitors.<sup>1</sup> Furthermore, the FDA has approved the use of TMB as a biomarker for response to pembrolizumab in solid tumors.<sup>2</sup> Simultaneously, the relationship between tumor heterogeneity and outcome has been studied across a range of cancer indications and has shown predictive value.<sup>3</sup> For Lung Squamous Cell Carcinoma (LUSC) the utility of heterogeneity metrics has not been established. To study this relationship we used both TMB and tumor heterogeneity to stratify patients, compare outcomes, explore differences in immune cell enrichment, and predict driver genes.

**Methods** We obtained Tumor Cancer Genome Atlas (TCGA) LUSC SNP, CNV, and RNASeq data from the GDC Data Portal<sup>4</sup> and clinical data from the PanCancer Atlas dataset through cBioPortal.<sup>5</sup> TMB was calculated by dividing the number of mutations by 38 to yield a mut/Mb value. To estimate tumor heterogeneity we ran PyClone, an algorithm that estimates the number of tumor clones.<sup>6</sup> PyClone uses a random seed and output for the same sample may differ. We ran each sample in triplicate on three separate days yielding 9 runs per sample, yielding an average PyClone clone number. Clones with >2 mutations were counted. Using p-value minimization we chose 5 for the TMB cutoff and 4.6 for the PyClone cutoff. This yielded 4 groups: HTHP, HTLP, LTHP, and LTLPI, where H - high, L- low, T-TMB, and P-PyClone. Immune cell enrichment analysis was accomplished with ssGSEA via the GenePattern platform.<sup>7</sup> Driver gene prediction was performed with OncoDriveClust<sup>8</sup> via the R package maftools.<sup>9</sup>

**Results** A statistically significant difference was found in progression free survival (PFS) between stage I LTHP (LTHPI, N = 15) and stage I LTLPI (LTLPI, N = 77) patients (51.27 months vs. 25.4 months, p-value = 0.0059). Intriguingly, highly heterogeneous tumors revealed superior survival outcomes compared to less heterogeneous tumors in this subgroup. LTLPI patients were enriched for immature B cells, regulatory T cells, and myeloid derived suppressor cells (figure 1). Three driver genes were predicted for the LTLPI cohort (NFE2L2, PIK3CA, and TP53), while none were predicted for the LTHPI cohort.



**Abstract 71 Figure 1** Immune Cell Gene Set Enrichment

**Conclusions** Contrary to previous literature, superior survival outcomes were observed in high tumor heterogeneity, low TMB Stage I LUSC patients. Early stage patients can be

stratified using heterogeneity metrics like PyClone. Given the presence of specific driver genes and an immunosuppressive tumor microenvironment, this population warrants further investigation for therapeutic implications.

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**Trial Registration** N/A

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**Ethics Approval** N/A

**Consent** N/A

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