**Aim 1: Stratify TCGA melanoma tumors (TCGA-SKCM) by TMB and T cell state phenotype (or TME subtype)**

Rationale: While melanoma is more immunogenic than other tumor types, immune checkpoint blockade (ICB) response is variable. Some high-TMB tumors may fail to respond to ICB. A more nuanced immune stratification is needed to better understand the interaction between tumor antigenicity and immune infiltration and response. This aim addresses that gap by integrating genomic (TMB) and transcriptomic (T cell state or TME subtype) classifications to stratify TCGA-SKCM tumors. This will enable downstream identification of transcriptional and genetic features that drive immune evasion and how they differ between tumors with high and low antigenic potential.

* Classify tumors based on TMB:
  + TMB-low, TMB-medium, TMB-high or quartiles
  + Broad GDAC Firehose “Mutation\_Packager\_Calls”
* Classify tumors based on T cell state:
  + Approach:
    - Lack of harmonized gene signature for inflamed, excluded, exhausted phenotypes
    - Subset TCGA-SKCM expression data for genes included in papers listed below -> custom gene panel
    - Use normalized, PCA-reduced data with Louvain (graph based) or k-means clustering to cluster based on T cell state -or-
    - Gene set scoring -> either clustering or continuous score across each set
  + T cell inflamed
    - Immune system recognizes the tumor and has an ongoing antitumor immune response
    - Gene signature based on Ayers et al. 2017 (18 gene signature) [1] including IFN-γ signaling (e.g., IFNG, CXCL9, IDO1), Antigen presentation (HLA-DRA, TAP1), Immune cell infiltration (CD8A, GZMB, STAT1).
  + T cell excluded
    - At the tumor periphery with physical or functional exclusion
    - Gene signature in Spranger et al. 2016 mostly based on active WNT/β-catenin signaling [2]
    - Active β-catenin -> lack of dendritic cells and cross-presentation of tumor antigens, CCL4 suppression
  + T cell exhausted
    - T cells are present but are functionally impaired
    - Khan et al. (2019) TOX is a transcription factor that regulates CD8+ T cell exhaustion [3]
    - Thommen et al. (2018) High PD-1, CTLA-4, LAG3, TIGIT in PD-1^high subset with upregulation of TOX, NR4A1, BATF, EOMES [4]
    - Miller et al. (2019) TCF7 expression marks progenitor exhaustion versus terminal exhaustion [5]
* Alternatively (try both approaches) use TME subtypes (Bagaev, et al. 2021). Advantage is it was developed from bulk RNA-seq [6]. Can evaluate which approach more robustly stratifies tumors by immune phenotype in TCGA-SKCM.
  + Phenotypes:
    - Immune-inflamed (fibrotic vs. non-fibrotic)
    - Immune-excluded
    - Immune desert (fibrotic vs. depleted)
  + Based on immune genes (CD8A, GZMB, IFNG, CXCL9), stromal/fibrosis genes (COL1A1, MMP11, TGFB1, ACTA2), angiogenesis (VEGFA), and exclusion pathways (WNT/β-catenin)

**Aim 2: Identify transcriptional and genetic signatures associated with exclusion/exhaustion within each TMB group**

Rationale: While TMB has utility as a biomarker of ICB response, some high TMB tumors do not respond to immunotherapy due to immune exclusion or T cell dysfunction. This underscores the need to determine immune evasion mechanisms in a TMB dependent context. This aim will investigate the transcriptional programs and pathways that distinguish inflamed from excluded or exhausted tumors within high-TMB and low-TMB subgroups separately. Biomarkers of resistance in immunologically cold tumors with high mutational load will be identified.

* Question: Within high-TMB tumors, what gene expression programs differentiate T cell–inflamed from T cell–excluded or exhausted tumors? How are these programs different from those in low-TMB tumors?
* Perform differential expression analysis between:
  + T cell inflamed vs excluded/exhausted in high TMB tumors
  + T cell inflamed vs excluded/exhausted in low TMB tumors
* If using quantiles, the approach would use linear modeling but with the same aim
* Identify biomarkers for high and low TMB tumors that are either T-cell exhausted or excluded
* Pathway enrichment (GSEA with Human MSigDB, Reactome) to identify involved pathways—might not have much utility

**Aim 3: Investigate whether specific genomic alterations correlate with exclusion / exhaustion phenotypes in a TMB-cognizant manner**

Rationale: We seek to evaluate whether the genetic basis of immune escape differs between high and low TMB tumors. This may shed light on the evolution of resistance pathways in high TMB tumors and uncover distinct genomic correlates of exclusion or exhaustion across different antigenic contexts.

* Use tumor-normal matched somatic point mutations, indels, and CNV data from TCGA-SKCM
* Identify somatic mutations and CNVs that are enriched in T cell excluded or exhausted tumors separately by TMB
* Assess whether enriched alterations differ by TMB status

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