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Abstract

Osteosarcoma is a rare malignancy—affecting 3.4 patients per 1 million people per year worldwide. The survival rate at five years post-diagnosis is less than fifty percent. While other malignancies have seen outcomes improve with immunotherapies, the immune phenotype of osteosarcoma tumors remains poorly understood.

With the goal of understanding the immune phenotype of osteosarcoma primary and metastatic tumors before and during therapy, samples from 39 patients were sequenced and analyzed. To assess for expression changes in response to neoepitope status, bulk RNA sequencing data was evaluated. (Figure 1,2).

Tumor expression for predicted binding of f neoepitopes to HLA was evaluated (Figure 3), primary samples were ranked based on neoepitope burden, and differential gene expression between low versus high-burden primary samples was evaluated (Figure 4).

Methods

- Whole genome DNA sequencing was performed on tumor samples and matched germline samples and somatic variants were called using Mutect2. Variants were filtered for coding mutations.
- HLA Haplotypes were calculated from pre-mapped WGS DNA sequencing data using a method currently in development.
- Using a filtered list of coding variants (above), Variant peptide—HLA binding prediction was assessed by MHCFlurry.
- Tumor bulk WGS RNA sequencing was performed and counts were generated using STAR.Align. Differential gene expression was calculated using DESeq2 and gene ontology calculation was calculated using clusterProfiler.

Conclusions

- The number of predicted HLA-bound neoepitopes increases with therapy and with metastatic site (Fig. 3)
- Clonally related neoepitopes can be found longitudinally across samples and tumor sites (Fig 3)
- As assessed by variant allele fraction, neoepitopes predicted to have higher binding scores and epitopes seen across time points do not demonstrate selection effects (Fig 3)
- Among primary tumors, bulk tumor gene expression varies significantly with neoepitope burden in gene ontology domains related to epigenetic factors (such as nucleosome assembly) and megakaryocyte differentiation (Fig 4,5)

Fig 1. Graphical abstract

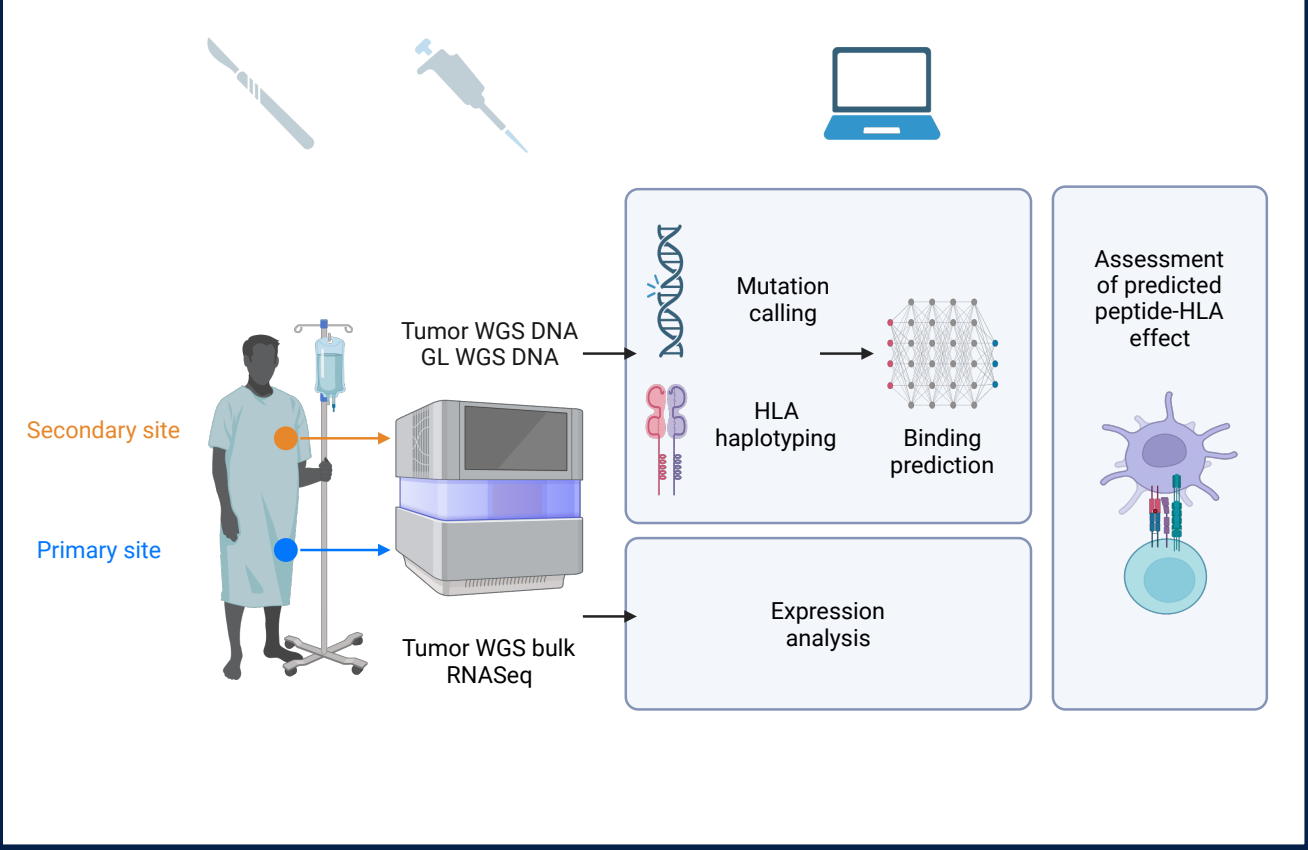


Fig 2. Cohort summary

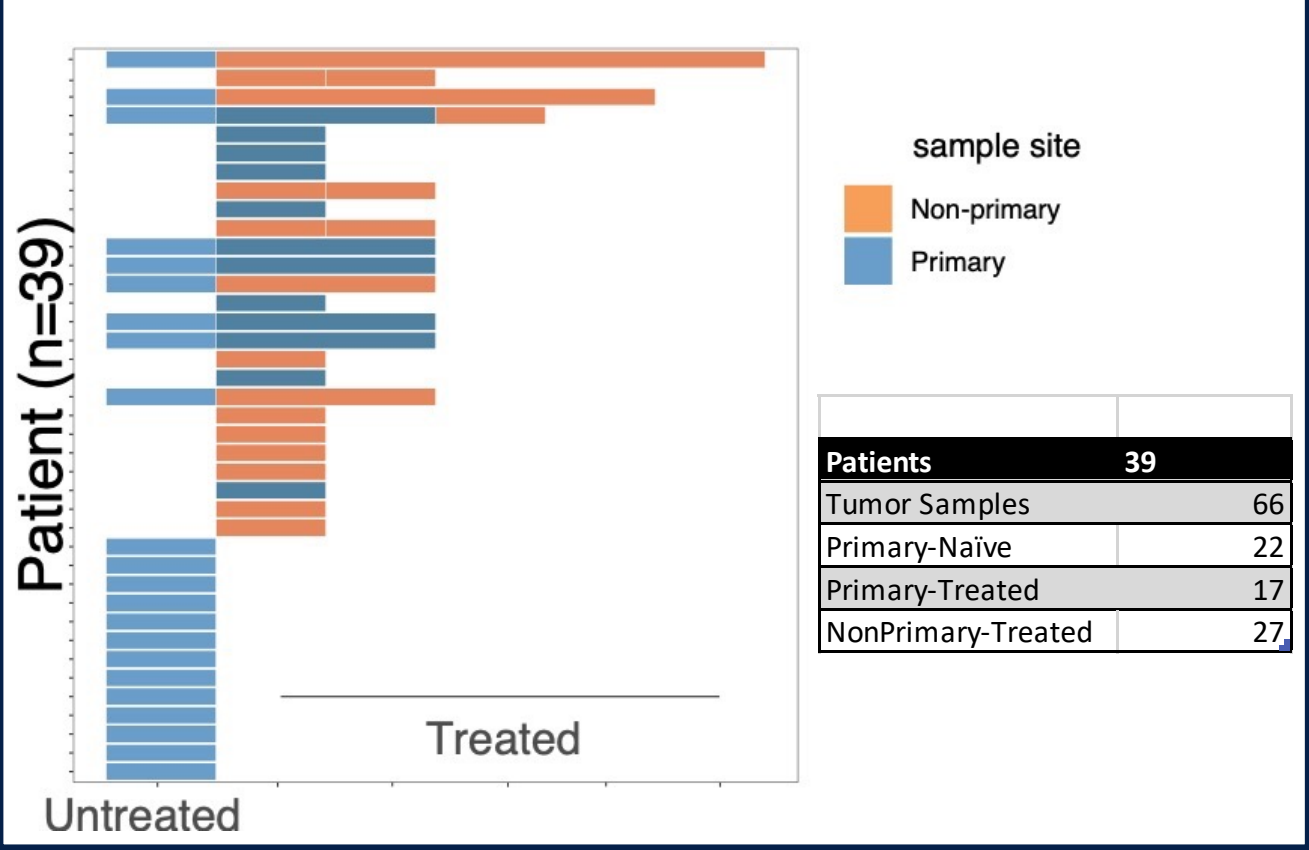


Figure 5. Difference in gene expression based on predicted neoepitope burden in primary tumors

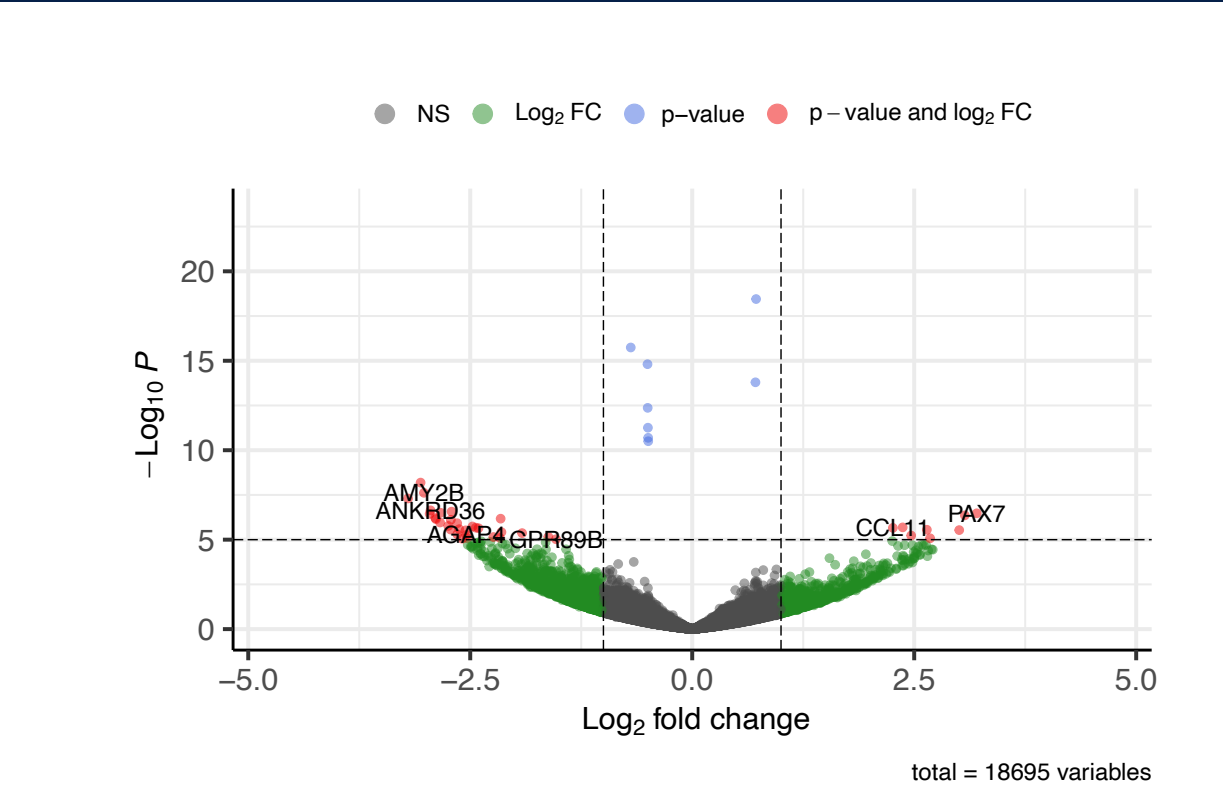


Figure 3. Burden of predicted-bound neoepitopes increases with therapy but epitope variant allele fractions are independent of binding score and exposure to therapy

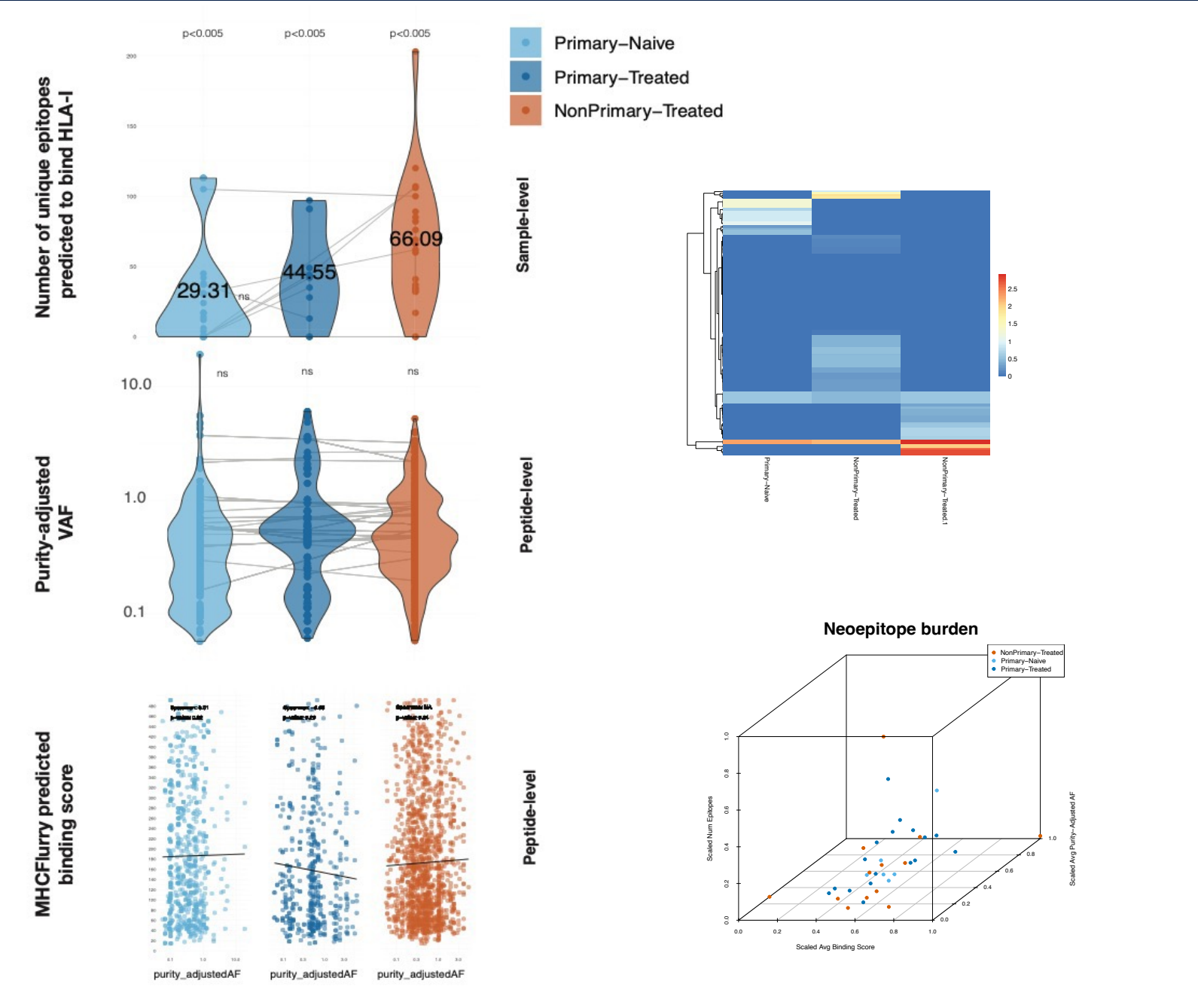
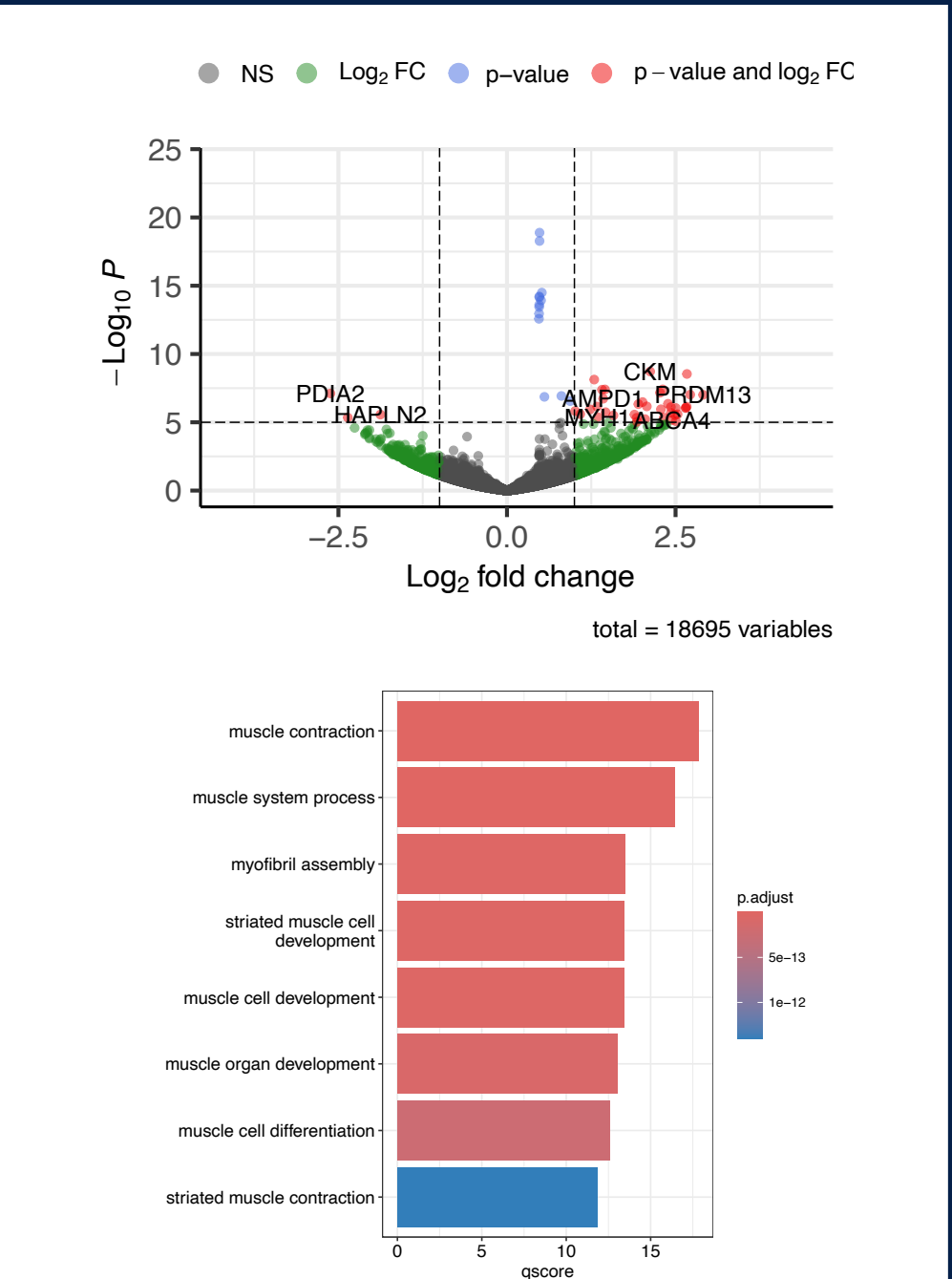


Figure 4. Difference in gene expression based on predicted neoepitope burden in primary, treatment-naïve tumors



Ongoing Directions and Acknowledgements

- Characterization of immune components of RNA gene expression using CiberSortx
- Further exploration of RNA gene expression patterns as a predictor of tumor neoepitope burden
- Integration of neoepitope-immune interaction analysis with clinical outcomes
- The authors would like to express thanks to the members of the Sweet-Cordero Laboratory, the lab's collaborators, and the patients and families involved in the study.