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TBio BFX 4101: A Neoantigen Prioritization Pipeline for Selected Tumor-infiltrating Lymphocyte Therapy

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INTRODUCTION

Tumor-infiltrating lymphocyte (TIL) adoptive cell therapy is an emerging immunotherapy approach for the potential treatment of various types of solid tumors [1-4]. The selective expansion of tumor-reactive TILs may enable the balance of the immune system to shift and focus the T-cell more specifically toward the malignant tumor cells. However, the success of selected TIL therapy depends on identifying neoantigens, which are tumor-specific antigens arising from somatic mutations. In this study, we present TBio BFX 4101, a bioinformatics pipeline that identifies and ranks neoantigens for the manufacture of a selected TIL drug product.

METHODS

TBio BFX 4101 Workflow:

Acquire sequencing data

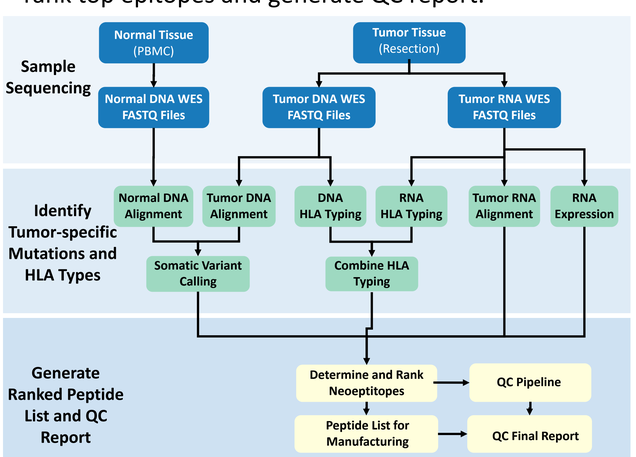
- The TBio BFX 4101 pipeline begins with read quality trimming of high-throughput whole-exome (WES) DNA and RNA sequencing data from tumor tissue and normal blood, followed by alignment and removal of duplicates.

Identify tumor-specific mutations and HLA types

- A comparison of WES data from normal and tumor tissue identifies somatic variant calls.
- Tumor RNA-seq reveals expression patterns of mutated alleles.
- WES and RNA-seq data provide genotyping of HLA-A, HLA-B and HLA-C alleles.

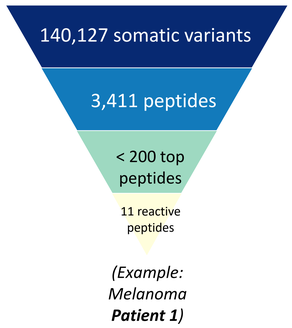
Generate ranked peptide list and QC report process

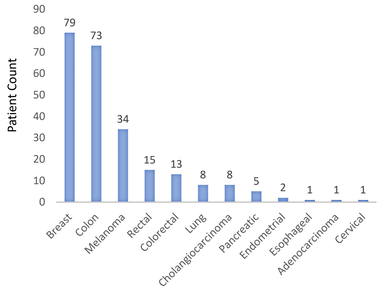
- From normal and tumor DNA, determine peptide sequences for associated somatic variants.
- Calculate binding affinity for all potential variant peptides, as well as RNA expression from tumor.
- Use binding affinity and RNA expression to score and rank top epitopes and generate QC report.

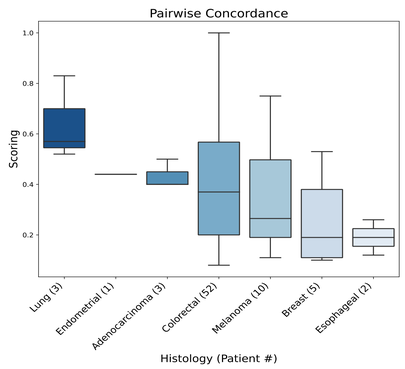


RESULTS

TBio BFX 4101 pipeline achieves a sensitivity of **95%** in neoantigen detection for solid tumors

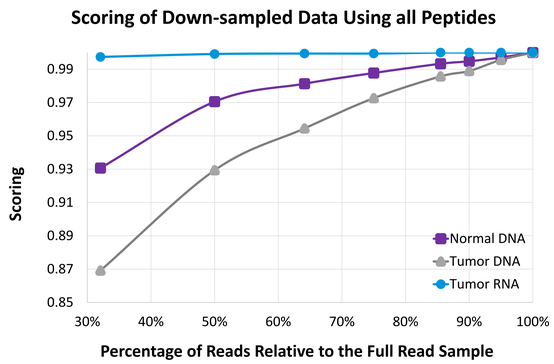


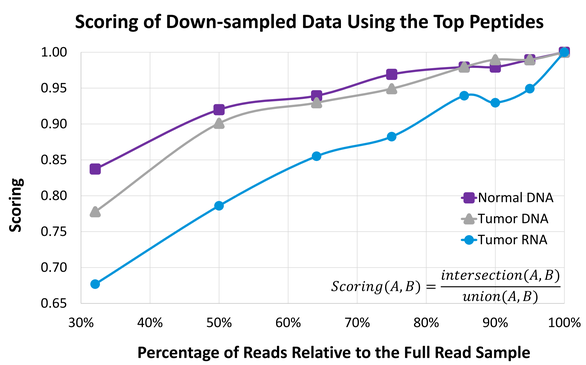




- A dataset containing 145 samples, 240 fragments with 567 reactive peptides from 145 patients representing a variety of solid tumor types was collected from two dbGaP datasets [5].
- TBio BFX 4101 correctly identified 540 of 567 validated neoantigen peptides.

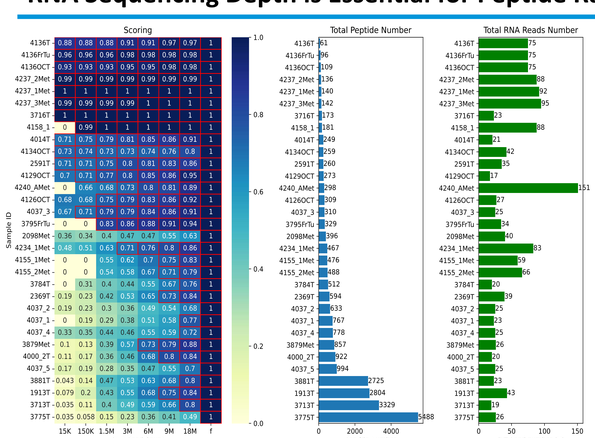
DNA and RNA Sequencing Data Depth has Distinct Effects on Neoantigen Prioritization

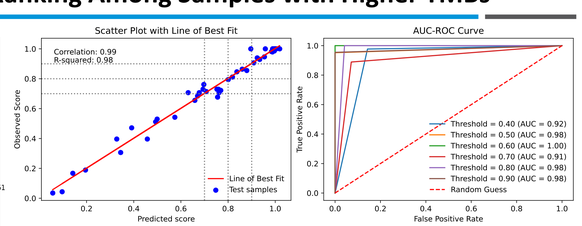




- To assess robustness of the neoantigen pipeline, the same dataset was down-sampled and analyzed with TBio BFX 4101.
- Peptide calls using as little as 33% of the total WES data from normal or tumor tissue contained 87% and 93% of the immunogenic peptides, respectively.
- However, while a diminished RNA expression signal has no impact on identification of immunogenic peptides, decreased number of RNA reads reduces the power of the peptide ranking score, which varies depending on the tumor mutation burden of the sample.

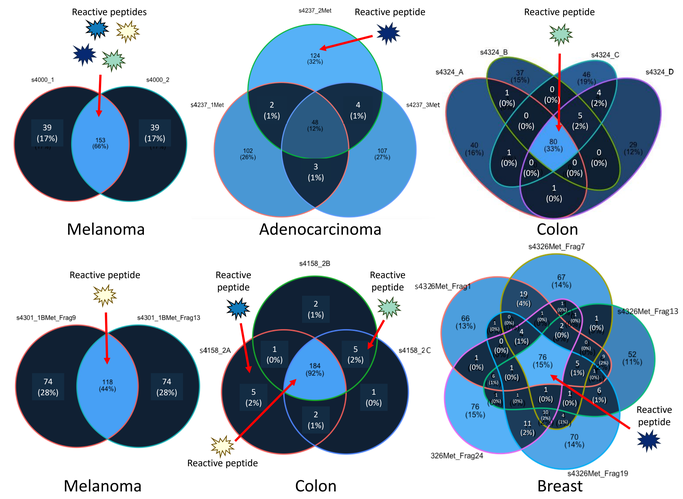
RNA Sequencing Depth is Essential for Peptide Ranking Among Samples with Higher TMBs



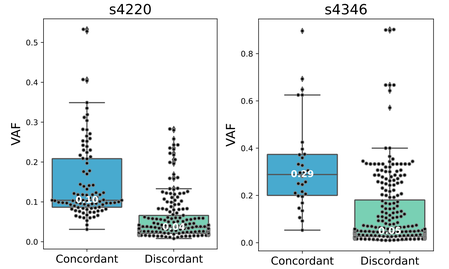


- A concordance test performed among 31 samples using different RNA read counts as input revealed a positive correlation to the number of peptides and thus TMB.
- It is possible to predict the concordance with high accuracy (R^2=0.98) given the number of reads and number of peptides.

Neoantigen Peptides can Vary Among Tumor Fragments



- Venn diagrams of top peptide lists from six patients exemplifies the degree of overlapping among fragments.
- Interestingly, somatic mutation and neoantigens can vary among tumor fragments, indicating technical noise or tumor heterogeneity.



Also, concordant mutations tend to have higher variant allele frequency, possibly because they originated from the primary tumor.

CONCLUSIONS

- TBio BFX 4101 provides a comprehensive and efficient approach for neoantigen prioritization.
- A decrease in RNA depth can impact peptide prioritization, especially for samples with higher TMBs
- Tumors can be highly heterogeneous, with different mutations among fragments of the same tumor.
- The identification and prioritization of neoantigens have the potential to meaningfully advance the field of cancer immunotherapy and facilitate the development of personalized treatment strategies.

REFERENCES AND ACKNOWLEDGEMENTS

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5. Patient dataset used on this work was obtained from dbGaP (phs002748.v1.p1 and phs002735.v1.p1).