## 900 TBIO BFX 4101: A NEOANTIGEN PRIORITIZATION PIPELINE FOR SELECTED TUMOR-INFILTRATING LYMPHOCYTE THERAPY

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**Background** Tumor-infiltrating lymphocyte (TIL) adoptive cell therapy is an emerging immunotherapy approach for the potential treatment of various types of solid tumors. 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-ACT therapy depends on identifying neoantigens, which are tumor-specific antigens arising from somatic mutations.[1–3] In this study, we present TBio-BFX-4101, a comprehensive bioinformatics pipeline that identifies and ranks neoantigens for the manufacture of a TIL drug product.

**Methods** The TBio-BFX-4101 pipeline begins with pre-processing of high-throughput whole-exome DNA and RNA sequencing data from tumor tissue and normal blood. These data are subjected to quality controls and variant calling to retain only somatic mutations. In addition, TBio-BFX-4101 integrates open source and in-house computational algorithms to determine potential neoantigen peptides based on their likelihood to elicit immune responses. Moreover, machine learning models trained on large-scale datasets with known immunogenicity capture intricate relationships, enabling neoantigen ranking and prioritization.

**Results** A dataset comprising 281 WES and RNAseq files from 145 patients with publicly available evidence of peptide immunogenicity was used to determine peptide prediction accuracy of 100%. It also demonstrated correct ranking of clinically relevant neoantigens with a sensitivity of 95%, providing a valuable resource for personalized cancer immunotherapy. Interestingly, somatic mutation and neoantigen prediction can vary among tumor fragments, indicating technical noise or tumor heterogeneity. Moreover, concordant mutations tend to have higher variant allele frequency, possibly because they originated from the primary tumor. To assess robustness of neoantigen prediction, the same dataset was down-sampled and analyzed with TBio-BFX-4101. When comparing a down-sampled dataset with the corresponding complete, we found that with TBio-BFX-4101, for instance, a drop of 2/3 of the DNA sequence for normal and tumor results in a concordance of 87% and 93% ratio between intersection and union as indicated by a Jaccar score, respectively. We also determined that while diminished RNA expression signal has no impact on identification of immunogenic peptides, decreased RNA reads reduces the power of the peptide ranking score, which varies depending on the tumor mutation burden of the sample.

**Conclusions** We believe TBio-BFX-4101 provides a comprehensive and efficient approach for neoantigen prediction. 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.

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