Tumor neoantigen prioritization from liquid biopsy whole exome sequencing for selected tumor-infiltrating lymphocyte therapy

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Abstract

Background:

Adoptive cell transfer (ACT) of neoantigen-reactive tumor-infiltrating lymphocytes (TILs) is an emerging therapeutic modality for solid cancers. A growing body of clinical data in the TIL-ACT field supports the potential for the identification, selection, and expansion of tumor-reactive T cells to drive objective response in patients. We hypothesize that improvement in neoantigen identification methods may further increase the breadth and number of tumor-reactive T cells. Tissue biopsy based neoantigen identification can be limiting due to inter- and intra-tumoral heterogeneity and tissue access. Here, we applied whole exome DNA and RNA sequencing on patient liquid biopsy samples to assess the sensitivity of tumor variant detection and prioritization of neoantigen peptides in comparison with tissue data and to potentially improve target yield.

Method:

Matched solid tissue and blood samples were collected from 10 patients (CRC, breast, or NSCLC). For solid tissue, whole exome sequencing (WES) and whole transcriptome sequencing (WTS) libraries were prepared using standard tissue protocols. For blood samples, cell-free DNA (cfDNA) and circulating RNA (cRNA) exome libraries were prepared using Illumina RUO library prep kit reagents. Solid tumor variant calling and neoantigen identification were performed by the Turnstone TBio-4101-BFX platform. Liquid biopsy tumor variants and neoantigens were identified using the DRAGEN™ Bio-IT platform and pVACtools suite[1].

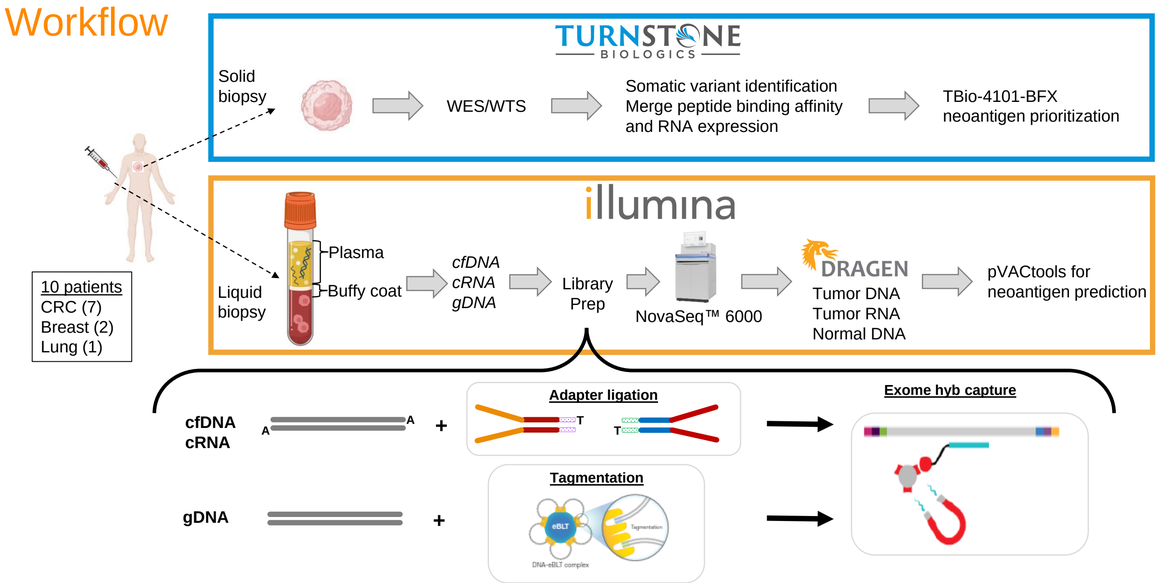
Results:

Ultra-deep WES (>15,000x) of cfDNA resulted in 100% identification of known small variants at 0.5% variant allele frequency (VAF) in control samples, and >80% sensitivity for 0.2% VAF small variant detection in patient samples. Both analytical pipelines achieved 100% sensitivity on a dataset comprising experimentally determined immunogenic peptides. Concordance of somatic variant calls made between the solid and liquid biopsies ranged from 22%-88% concordance in 4 samples, while 6 samples showed no concordance. Further analysis showed a positive correlation between variant concordance and the percent tumor fraction in the liquid biopsy (from 3.6% to 0% tumor fraction in high to low concordance samples, respectively). For those samples with variant level concordance, up to 44% concordance was observed on neoepitope peptide identification between solid and liquid biopsies. In addition, the liquid biopsy data resulted in up to 29x more peptide calls than the solid tissue, suggesting the blood samples may contain unique tumor fragments not detected in solid tissue biopsy.

Conclusions:

Minimally invasive liquid biopsy is viable for detection of somatic variants with the potential to broaden selection of tumor-reactive TILs and improve objective responses.

Workflow



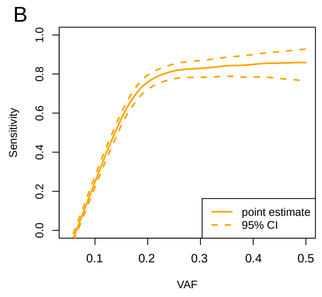
Study workflow: Tissue biopsy samples were processed using standard tissue protocols followed by library preparation using WES and WTS. Data was processed using the TBio-4101-BFX platform to generate variant calls and neoantigen prioritization. Blood samples were processed using standard phase separation protocol to extract cfDNA and cfRNA from plasma and genomic DNA (gDNA) from buffy coat. For cfDNA WES, libraries were prepared using Illumina cfDNA Prep with Enrichment; for cfRNA WTS, libraries were prepared using a modified Illumina Stranded Total RNA Prep; gDNA libraries were prepared using Illumina DNA Prep with Enrichment. All libraries were enriched using the Illumina exome panel. Data was processed using the DRAGEN™ Bio-IT platform for variant detection followed by pVACtools suite for neoantigen prediction. Resulting variant and peptide calls from tissue and liquid biopsy samples were analyzed for concordance.

Deep WES coverage for variant detection for cfDNA

A

Gene	Cosmic	Expected NGS VAF (%)	Observed VAF (%)
NRAS	COSM584	0.8	0.9
KRAS	COSM554	0.5	0.5
KRAS	COSM521	0.6	0.5
KRAS	COSM516	0.7	0.6
AKT1	COSM33765	0.7	0.7
ALK	COSM144250	0.5	0.7
ALK	COSM28055	0.4	0.4
PIK3CA	COSM775	0.5	0.3
KIT	COSM1314	0.6	0.8
EGFR	COSM6240	0.4	0.3
EGFR	COSM6224	0.6	0.8
BRAF	COSM476	0.6	0.4

B



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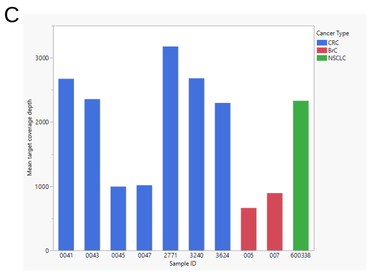

Figure 1: Whole exome cell-free DNA sequencing coverage and variant detection. A) Seracare cfDNA reference material was used to assess the WES SNV recall. B) In silico analysis estimated >80% sensitivity at 0.2% variant allele frequency (SNV). C) Clinical samples were sequenced at >1000x effective coverage (>10,000x raw coverage).

References

1. Hundal et al. *Cancer Immunol Res.* 2020; 8(3): 409-420
2. Select images created with BioRender.com
3. dbGaP Study Accession: phs002735.v1.p1
4. dbGaP Study Accession: phs001003.v1.p1

Concordant immunogenic peptide recall between analysis pipelines

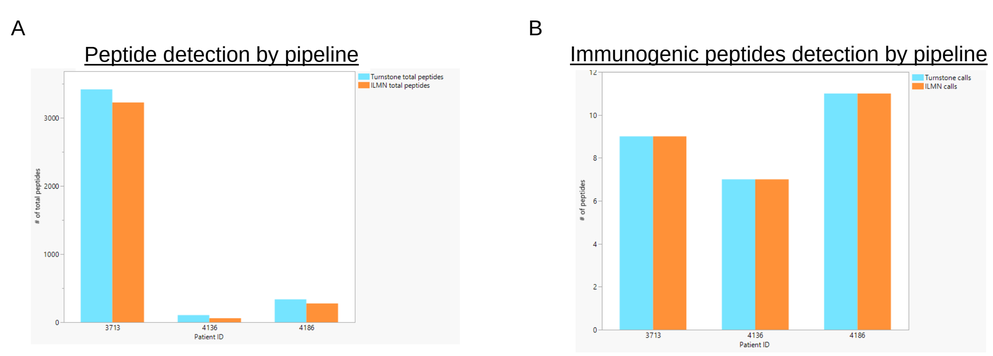

Figure 2: Peptide level detection on validated peptides by analysis pipeline. A) Data from dbGaP studies[3,4] were processed through the Turnstone and Illumina peptide prediction and/or prioritization pipelines to identify the number of total peptides. Samples were from melanoma (3713) and breast cancer (4136 and 4186) patients. B) Both pipelines were able to identify the same validated immunogenic peptides from the study.

Concordant variant detection in samples with high tumor fraction

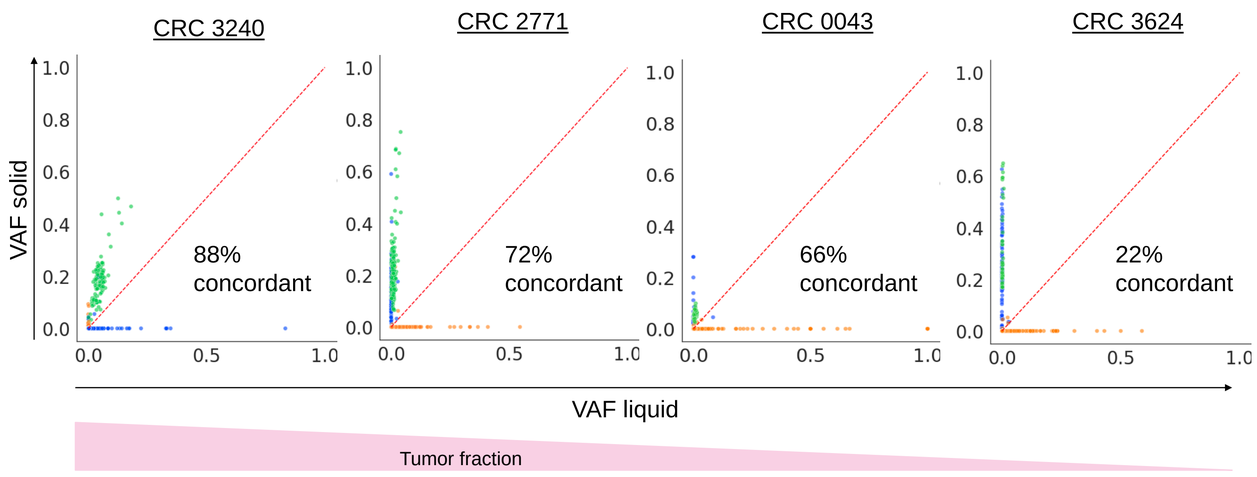

Figure 3: Variant detection comparison between solid and liquid biopsy samples. Tumor variants from WES were assessed from solid tissue and liquid biopsy samples using the Turnstone and Illumina variant detection pipelines, respectively. Concordance between the variant calls were positively correlated with the estimated circulating tumor fraction. Samples with 0% concordance had no detectable tumor fraction (data not shown).

Comparison of neoepitope peptide identification in solid and liquid biopsy samples

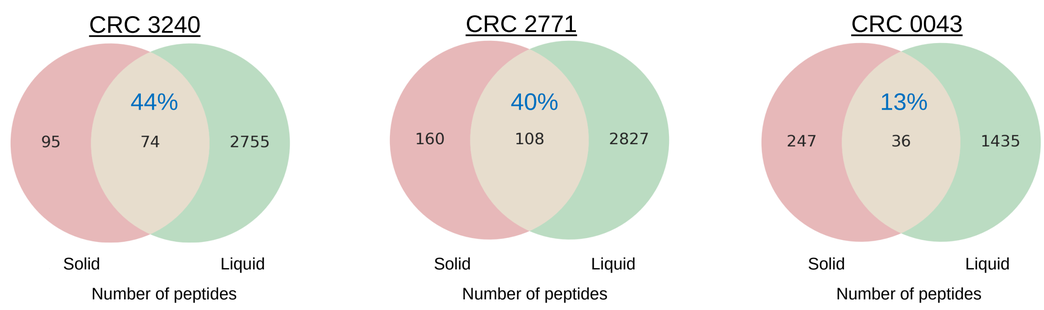

Figure 4: Peptide identification and concordance between solid and liquid biopsy samples. Samples with positive variant level concordance were processed through the peptide prediction pipelines from solid and liquid biopsy samples using Turnstone and Illumina pipelines, respectively. Peptide concordance showed up to 40% concordance (in blue text) between tissue types, with up to 29x more unique peptide calls in the liquid biopsy sample. Sample CRC 3624 did not have sufficient cfRNA data to analyze via the Illumina peptide pipeline.

Conclusions

- **WES from solid and liquid biopsy samples provides an opportunity for variant detection and identification of potentially immunogenic neoantigens.**
- **TBio-4101-BFX and DRAGEN™ Bio-IT platforms can identify the same validated immunogenic peptides.**
- **Variant calling concordance between solid and liquid samples is strongly correlated with liquid tumor-fraction.**
- **Liquid biopsy may represent a minimally-invasive method to comprehensively survey neoepitope profiles associated with a variety of solid tumors.**
- **The potential immunogenicity of neoepitope peptides identified in liquid biopsies will be further investigated using functional bioassays.**

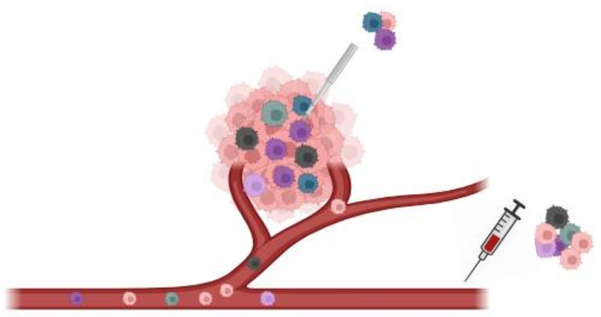

Figure 5: Model for liquid biopsy approach to neoepitope peptide identification. Due to tumor heterogeneity, solid tissue biopsy may result in sub-selection of tumor clones resulting in a smaller pool of tumor antigens. Liquid biopsy is a more accessible sampling method that provides an opportunity to expand neoantigen identification yield.

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