CANCER RESEARCH HORIZONS

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T CELL EXTRECT:

USING DNA SEQUENCING TO QUANTIFY T CELL FRACTION FOR THERAPY RESPONSE PREDICTION AND CANCER PROGNOSIS

Sept 2023

OPPORTUNITY OVERVIEW

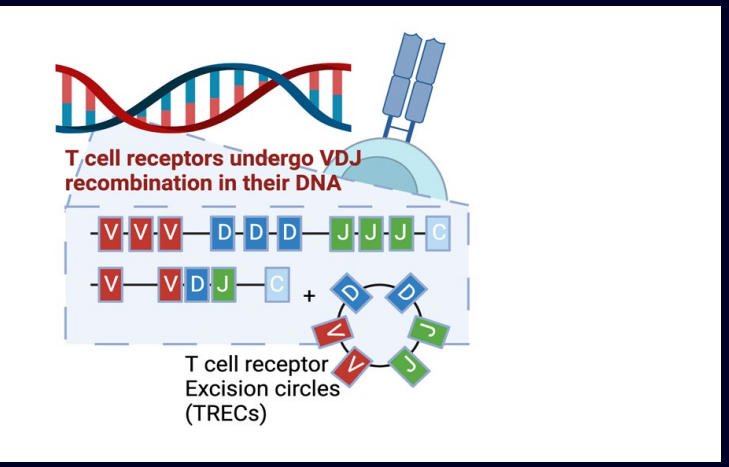
- **Technology:** T Cell ExTRECT uses DNA sequencing data to accurately quantify T cell fraction, with results that correlate positively with other validated methods of immune measurement.
- **Unique value:** This approach is significantly less expensive and less time-consuming than current methods (histopathological analysis, RNA sequencing), especially since DNA sequencing is increasingly performed routinely in the clinic.
- **Findings:** Data from T Cell ExTRECT have been shown to be both predictive of response to checkpoint inhibitor therapy and associated with prognosis in lung adenocarcinoma.
- **The Team:** Robert Bentham, Charlie Swanton, Nicholas McGranaham and Thomas Watkins at UCL and the Francis Crick Institute.
- **Intellectual Property status:** a PCT application was filed in July 2022 with claims covering the T Cell ExTRECT methodology (PCT/EP2022/070694).
- **Publications:** Bentham, Litchfield, Watkins *et al.* Nature 2021
- **Seeking:** We are looking to partner with leaders in genomic sequencing and/or immunotherapy for co-development and/or licensing to translate the technology into a clinical setting.

T CELL EXTRECT OFFERS A POWERFUL TOOL FOR MEASURING T CELL ABUNDANCE IN TUMOURS

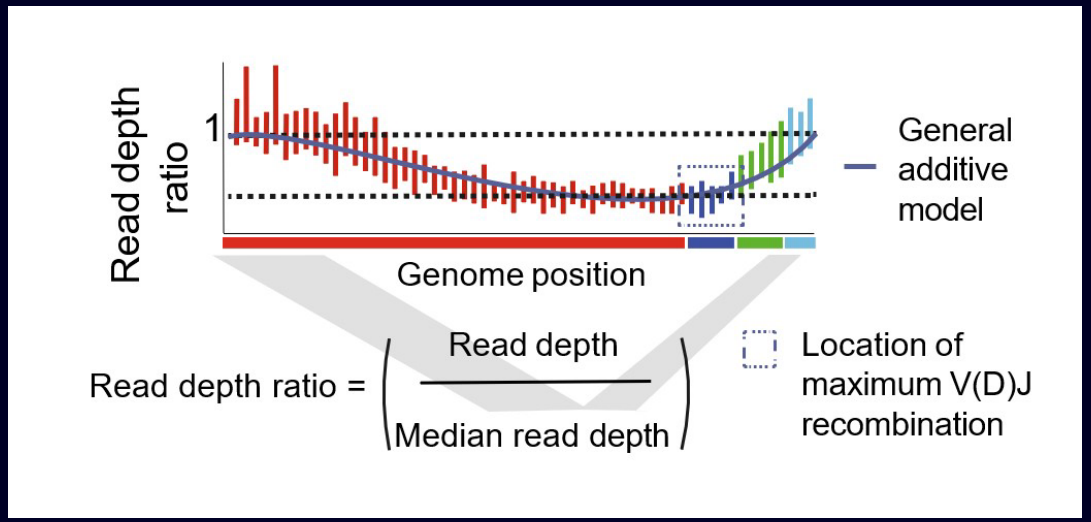
- The immune microenvironment is known to influence tumour evolution and has been shown to predict response to immunotherapy and cancer progression.
- The investigators have established that patients that respond to immunotherapy have significantly higher pre-treatment tumour T cell fraction than non-responders, implicating T cell abundance in response to immunotherapy.
- Current technologies for measuring tumour-infiltrating lymphocytes (TILs) are limited by a shortage of appropriate data, and current methods are time-consuming, not cost-effective and therefore under-utilised in clinical settings.
- T Cell ExTRECT, which is based on DNA sequencing, offers a simple and cost-effective method for estimating T cell fraction in tumour samples, and it is therefore more conducive to widespread clinical use.

HOW T CELL EXTRECT WORKS

VDJ Recombination and TRECs



Read depth ratio

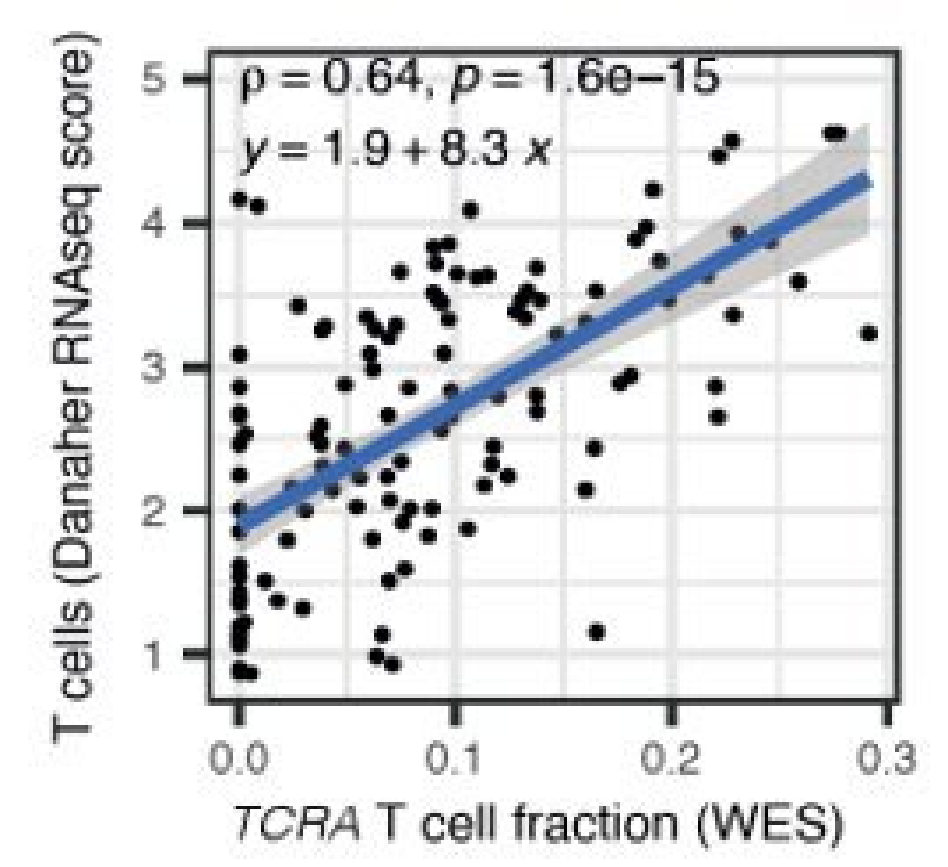


- Recombination in the VDJ region of the T cell receptor gene generates enormous T cell diversity, necessary for the immune system's recognition of foreign antigens.
- This VDJ recombination in the T cell receptor's alpha chain (TCRA) results in the elimination of sequences excised as "excision circles" or "TRECs", hence the name of the technology.
- T Cell ExTRECT measures the ratio ("read depth ratio") of this VDJ sequence in the TCRA gene using DNA sequencing data to directly estimate T cell fraction in WES samples.

Bentham, Litchfield, Watkins *et al.* Nature 2021

T CELL EXTRECT PROVIDES AN ACCURATE ESTIMATE OF IMMUNE INFILTRATE

Comparison of T Cell ExTRECT and RNA sequencing



- Samples from a cohort of 100 non-small-cell lung cancer (NSCLC) patients within the TRACERx (TRAcking Cancer Evolution through therapy (Rx)) trial were used to calculate T cell fraction using T Cell ExTRECT.
- T Cell ExTRECT scores were compared to scores obtained by existing RNA sequencing methods (Danaher).
- T Cell ExTRECT scores strongly correlated with Danaher RNA sequencing score, thus validating T Cell ExTRECT scores.
- T Cell ExTRECT is significantly less expensive and less time-consuming than methods relying on RNA sequencing, especially since DNA sequencing is increasingly performed routinely in the clinic.

T Cell ExTRECT is an accurate method of estimating immune infiltrate in tumours more efficiently than standard methods.

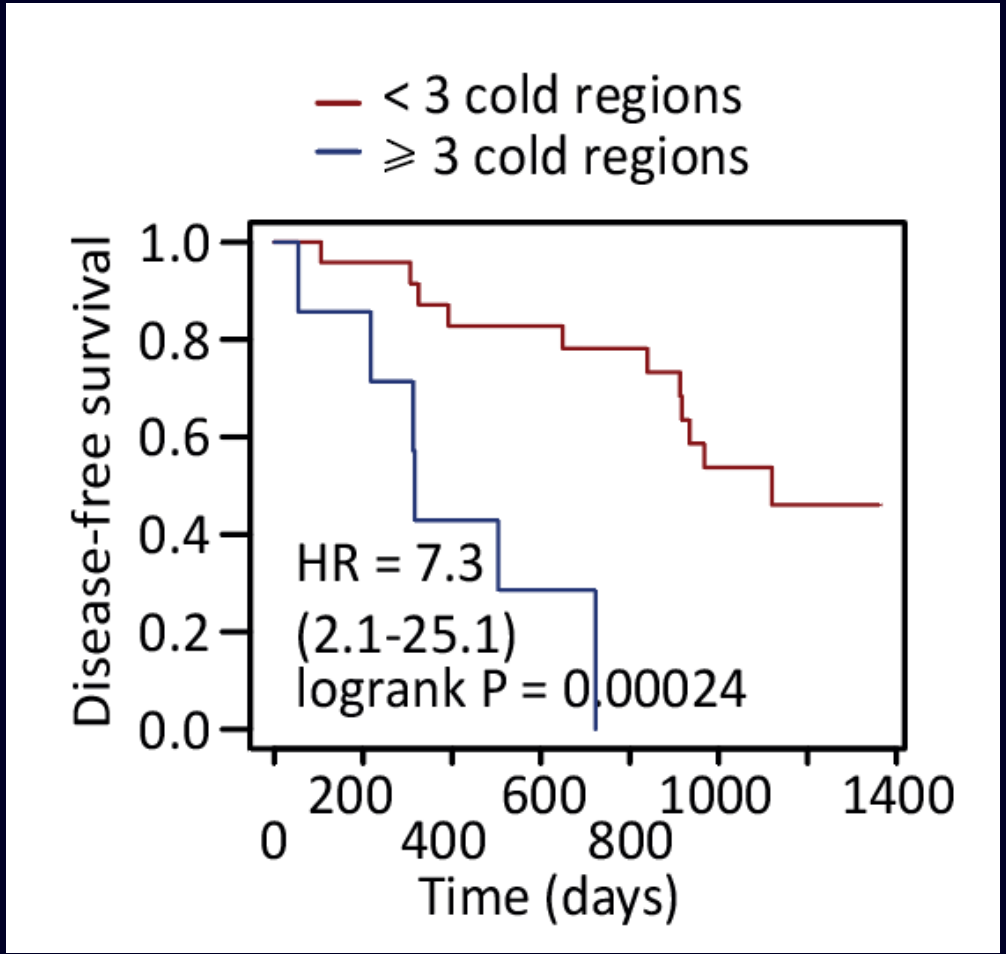
T CELL EXTRECT IS PROGNOSTIC IN LUNG ADENOCARCINOMA

- Tumor samples were characterized as ‘hot’ (red line in figure) or ‘cold’ (blue line in figure) depending on whether the T cell fraction was greater than, or equal to mean in the TRACERx non-small cell lung cancer cohort (ie. ‘hot’ tumors had a higher than average T cell fraction).

- Lung adenocarcinoma patients harboring an elevated number of ‘immune-cold’ tumor samples were associated with significantly inferior survival.

A low T Cell ExTRECT score is indicative of a worse outcome in lung adenocarcinoma.

Higher T cell fraction correlates with higher survival rate in lung adenocarcinoma patients

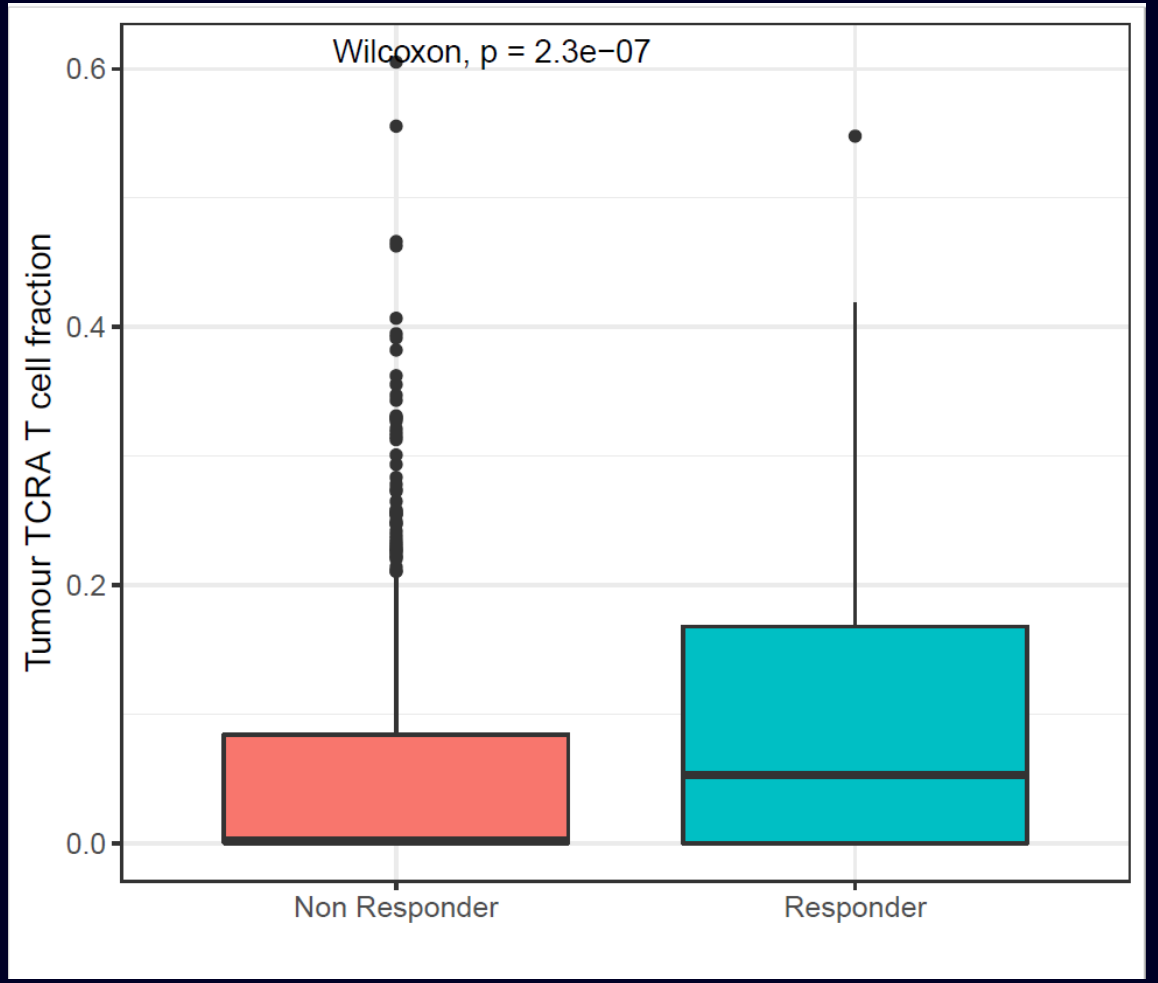


Bentham, Litchfield, Watkins *et al.* Nature 2021

T CELL EXTRECT IS PREDICTIVE OF IMMUNOTHERAPY RESPONSE

- The clinical utility of T Cell ExTRECT was validated using a pan-cancer cohort of 1,070 checkpoint inhibitor therapy (CPI)-treated tumors across 8 main cancer types.
- Significantly higher pre-treatment T Cell ExTRECT scores were observed in the blood of responders to CPI compared to non-responders.
- Furthermore, in an additional univariate analysis, T Cell ExTRECT score was found to be comparable to RNA-Seq measures (such as clonal tumour mutation burden and CD8 expression) for predicting response to immunotherapy.

T cell fraction in responders and non responders to immunotherapy in a pan-cancer cohort



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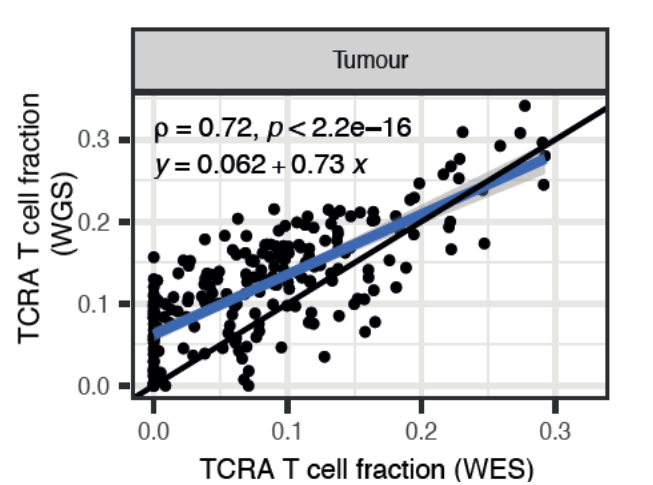
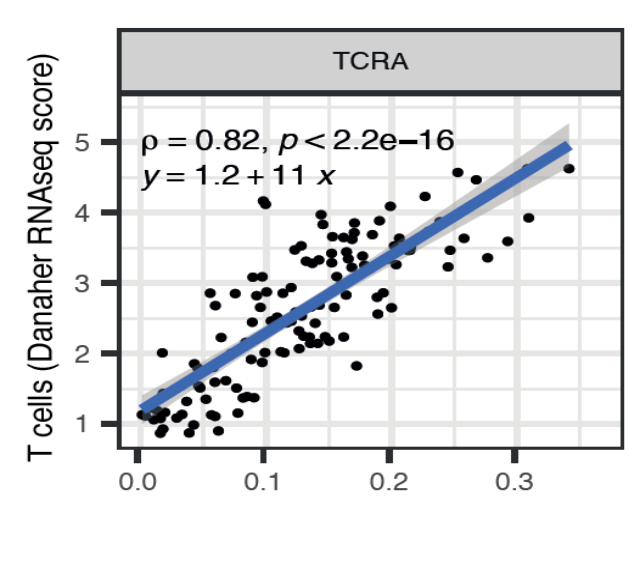
T CELL EXTRECT CAN ALSO BE APPLIED TO WHOLE GENOME SEQUENCING DATA

1. Comparing T Cell ExTRECT scores from WES and WGS data



2. Comparing T Cell ExTRECT scores from WGS data and RNA-seq derived T cell score

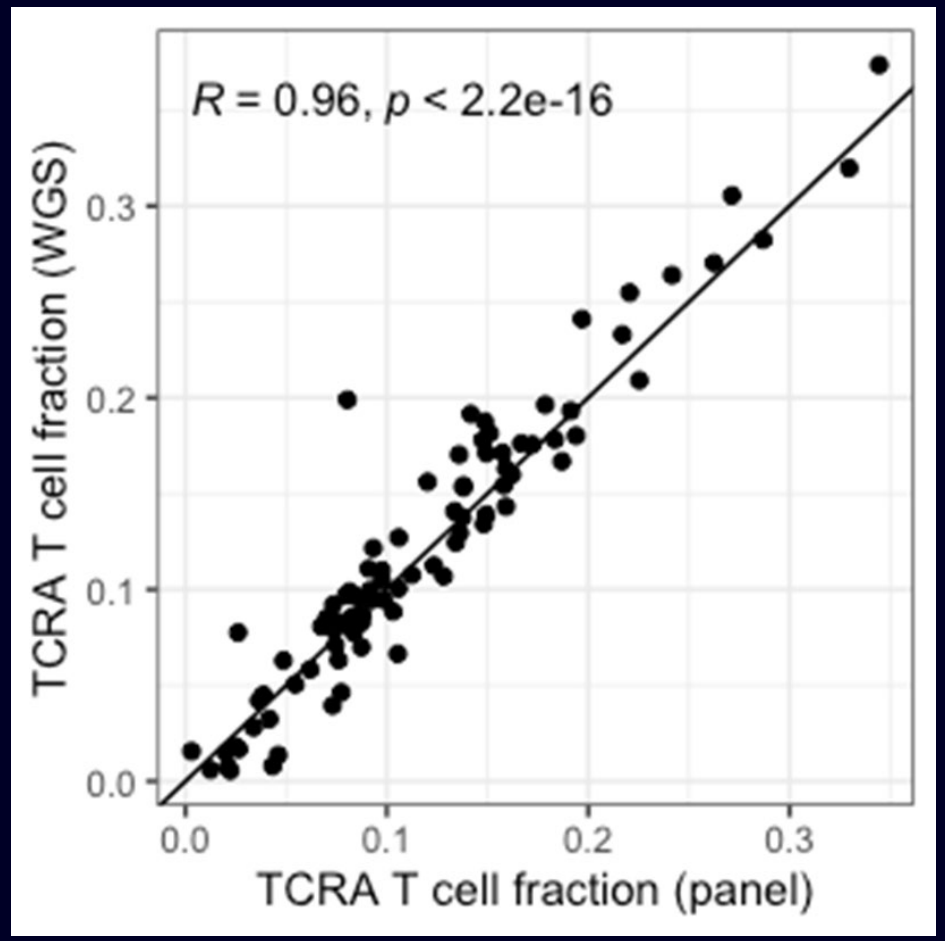


- The researchers have adapted the T Cell ExTRECT algorithm to whole genome sequencing data.
- Samples from a cohort of 100 NSCLC patients within the TRACERx trial were used to calculate T cell fraction using T Cell ExTRECT on whole genome sequencing data.
- Comparison of T Cell ExTRECT scores from whole exome sequencing (WES) data to whole genome sequencing (WGS) showed a significant positive correlation.
- T Cell ExTRECT scores obtained from WGS data were compared to scores obtained by RNA sequencing (Danaher).

T Cell ExTRECT scores from WGS data strongly correlated with immune infiltrate scores obtained from RNA sequencing, validating the use of the methodology on WGS data as an accurate measure of immune infiltrate in tumours.

T CELL EXTRECT CAN BE ADDED TO SEQUENCING PANELS AT LOW COST

Panel and WGS comparison



- T Cell ExTRECT was incorporated into a sequencing panel to verify whether the T cell fraction could be measured in a more cost-effective way and more accurately than WGS/WES derived T cell fraction.
- Samples from a cohort of 96 samples (both germline blood and tumour) within the TRACERx trial were used to calculate T cell fraction using the T Cell ExTRECT methodology in a gene panel.
- The researchers found a high correlation between the T Cell ExTRECT score calculated from a sequencing panel and the T Cell ExTRECT score derived from WGS data.
- T cell ExTRECT can therefore be incorporated in a sequencing panel to determine T cell fraction with equivalent or greater accuracy to WGS-derived T Cell ExTRECT scores, but at much reduced cost.

In this way, T Cell ExTRECT can easily be used to measure tumour immune cell content in a clinical setting.

FURTHER DEVELOPMENT PLANS

- The T Cell ExTRECT methodology is now being adapted for B cell fraction estimation.

- The team are incorporating the methodology into mutation panels that are already used in the clinic.

We are seeking an industry partner for co-development and/or licensing, with the goal of validating the utility of T Cell ExTRECT in a clinical setting.

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