A sequential treatment strategy for ex vivo profiling of live tumor fragments that mitigates tumor heterogeneity and tissue scarcity from core needle biopsies to characterize response to immunotherapies

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Poster 4

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Introduction

- Immune checkpoint inhibitors (ICIs) are highly effective treatment options for cancer, yet they fail to provide clinical benefit for most patients, primarily due to the low accuracy of approved biomarkers (eg, PD-L1 and Microsatelite Instability (MSI) / Mismatch Repair (MMR))
- Ex vivo cytokine profiling of live tumor samples has shown promise for improved prediction of response to αPD-1 blockade[1]
- This approach has been limited to tumor resections given the need for large amounts of tissue due to challenges associated with intra-tumor heterogeneity
- Here, we present a novel sequential treatment strategy to detect T-cell response to ICI treatment in the limited tissue available from a single core needle biopsy (CNB)

Methods

Specimen processing: Surgical resections or CNBs were cut using specialized cutting instruments to obtain live tumor fragments (LTFs) which were dispensed into assay plates (resections = 200, 300 µm cubed fragments/well; CNBs (12-18 gauge) = ~12 fragments/well) and encapsulated in hydrogel.

Cross-well comparison of human tumor CNBs and surgical resections: LTFs were treated for 24 hours with either IgG or ICI (α PD-1). Conditioned media was then collected and cytokine concentrations were measured using a 30-plex kit from R&D Systems and run on a Luminex instrument.

Validation of the sequential treatment strategy: LTFs were treated with one of three regimens: (1) IgG for 28 hours, (2) αCD3/αCD28 for 28 hours or (3) IgG for 20 hours followed by αCD3/αCD28 for an additional 8 hours. Conditioned media was sampled at 4, 20 and 28 hours for cytokine profiling.

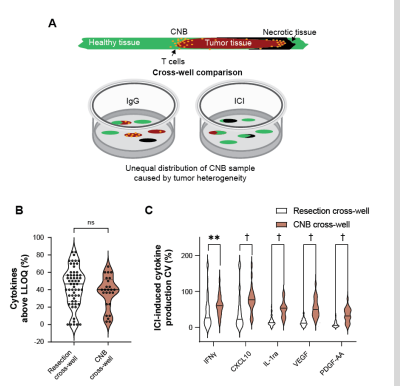
Enrollment of specimens from clinical trials: A total of 167 specimens were received for ex vivo profiling. 65 specimens were excluded due to shipping issues (n=4), sample processing issue (n=4), or having a form factor other than CNB (n=57) (eg, forceps biopsy or fine needle aspirate).

Ex vivo profiling of human tumor CNBs: Human tumor CNBs were fragmented using a specialized cutting instrument to create LTFs which were encapsulated in a proprietary hydrogel for ex vivo culture.[2] LTFs comprising 145 samples from 102 human CNBs were treated for 20 hours with IgG followed by ICI (αPD-1, n=70; αPD-L1, n=6; αPD-1+α CTLA-4, n=26) for an additional 28 hours. Conditioned media was collected at ~4, ~20 and ~48 hours for cytokine profiling.

Derivation of modified Z-scores and hierarchical clustering: A trimmed sample method was used to establish non-response to ICI treatment as a baseline using the population median and median absolute difference (MAD). A trimmed sub-set of CNB samples was identified by including only samples whose fold change in cytokine production rates between ICI treatment and IgG phases lay within the range of median ±2*MAD. This trimmed subset was then used to calculate updated median and MAD values leveraged in the modified Z-score transform for each cytokine. Individual sample cytokine measurements which failed a QC check were replaced by the population median (i.e. 0). Values were also clipped to enforce a modified Z-score range of -20 to 20. Hierarchical clustering was then performed on the resulting sample (columns)and cytokine (rows) values. Ward's linkage method was used to determine cluster formation for both rows and columns.

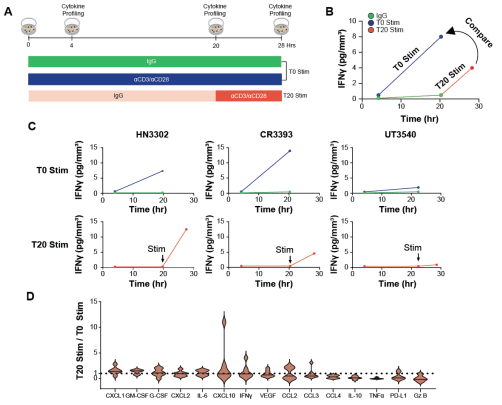
PD-L1 / MMR / MSI status: PD-L1, MMR, and MSI statuses were obtained from patient medical records. Where necessary, PD-L1 and MMR protein expression levels were characterized from treated histological sections collected at the end of the study period. Expression was then quantified by a board certified-pathologist.

1 CNBs are incompatible with cross-well comparisons due to intra-tumor heterogeneity and limited available tissue



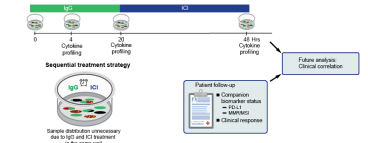
A Schematic showing the distribution of tissue from a CNB in an experiment using cross-well configuration. **B** The percentage of cytokines out of a 30-cytokine panel with concentrations above lower limit of quantitation (LLOQ) for the cross-well configuration for resection LTFs (resection cross-well) and biopsy LTFs (CNB cross-well) following IgG control for 24 hours. There is no significant difference in the ability to detect cytokines between the two form factor protocols (p = 0.175). **C** Five cytokines from resection LTFs and biopsy LTFs plated in the cross-well configuration and treated with ICI for 24 hours. Significantly larger variability in cytokine concentrations between replicate wells of treated groups in biopsy LTFs compared to resection LTFs. ns, not significant, ** p<0.01; † p<0.0001.

2 A sequential treatment strategy that compares cytokine production between treated and control conditions within the same tissue addresses tumor heterogeneity



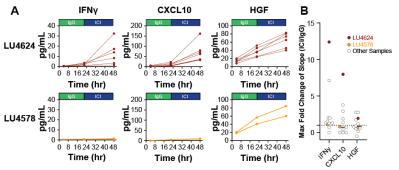
A Protocol to validate the sequential treatment strategy. Resection LTFs were used due to the availability of sufficient tissue to address inter-well heterogeneity and enable meaningful cross-well comparisons. LTFs encapsulated in hydrogel were treated with control IgG antibody for 28 hours (green), αCD3/αCD28 for 28 hours to stimulate T cells (blue), or IgG for the first 20 hours and α CD3/αCD28 for the final 8 hours of culture (red). **B** Cytokine production rates (slope) following stimulation at T0 and T20 were compared. **C** IFNγ induction in 3 human tumors (head and neck [HN3302], colorectal [CR3393], uterine [UT3540]) was observed following αCD3/αCD28 stimulation (Stim) at T0 (blue) and T20 (red). **D** Fold change in slope from Stim at T20 relative to slope from Stim at T0 shows a similar rate of induction for multiple cytokines (ratio of 1 indicates equivalent induction rates) for 7 human tumors.

3 A sequential treatment strategy enables characterization of ICI response in CNBs where tissue is limited



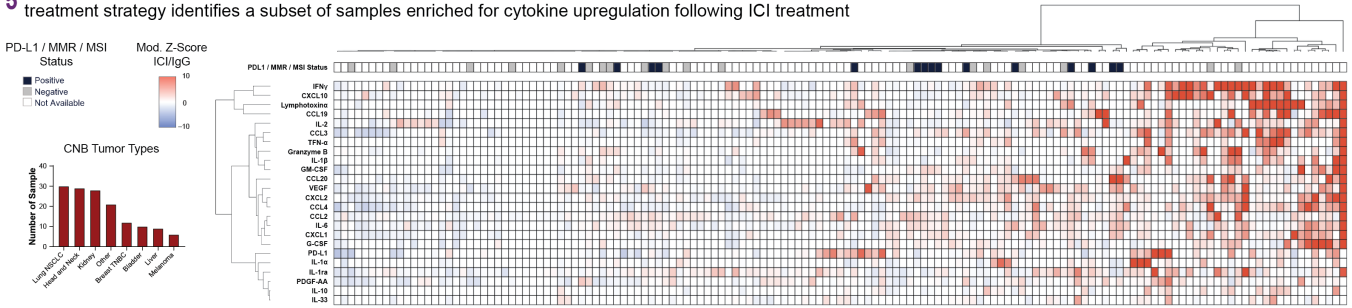
Encapsulated LTFs from CNBs are sequentially treated with IgG control followed by ICI. Conditioned media is collected at the 4-, 20- and 48-hours to determine cytokine production rate during the control (4-20 hours) and ICI (20-48 hours) treatment phases. Fold change in the rate of cytokine production (slope of ICI treatment phase / slope of control treatment phase) is used to assess ex vivo treatment response.

4 Differential cytokine response observed across 12 non-small cell lung cancer (NSCLC) CNB specimens



A LU4624 is a NSCLC CNB exhibiting an increased rate of IFNγ and CXCL10 production following ICI treatment suggesting a potential cytokine response (multiple CNBs were obtained from this patient enabling profiling of 6 replicate wells). LU4578 is a NSCLC CNB exhibiting a similar rate of IFNγ and CXCL10 production between the ICI and IgG treatment phases suggesting cytokine response is unlikely (multiple CNBs were obtained from this patient enabling profiling of 2 replicate wells). **B** Fold change in slopes of ICI and IgG treatment phases for IFNγ and CXCL10 across 12 NSCLC specimens. Data points report maximum fold change for those specimens where multiple CNBs/replicate wells were profiled. HGF is a cytokine for which little change in cytokine production rate was observed between the IgG and ICI treatment phases for all specimens.

5 Unsupervised hierarchical clustering of cytokine profiling data from 145 CNB samples collected with the sequential treatment strategy identifies a subset of samples enriched for cytokine upregulation following ICI treatment



Unsupervised hierarchical clustering of cytokine profiling data from 145 CNBs (from 102 human tumors) using modified Z-scores of fold change in cytokine secretion rates comparing ICI treatment phase to the control IgG phase (ICI/IgG). Z-scores reflecting increased secretion during the ICI treatment phase relative to the population baseline are depicted in red while decreases are depicted in blue. Samples with no difference in cytokine production rate are depicted in white. Biomarker status (PD-L1 / MMR / MSI) is reported in the top track where biomarker positive samples (PD-L1+, dMMR or MSI-H) are depicted in black, biomarker negative samples (PD-L1-, pMMR or MSI-L) are depicted in gray and samples for which biomarker status was not available are depicted in white.

Conclusions

- A sequential treatment strategy for the assessment of response to immunotherapy is an effective approach to capture treatment-induced changes in cytokine response
- Sequential treatment of CNBs circumvents issues of tumor heterogeneity inherent to cross-well comparison in settings of limited tissue availability
- Unsupervised hierarchical clustering of cytokine profiling data collected with the sequential treatment strategy identifies a subset of specimens enriched for cytokine response following ICI treatment
- This platform provides a scalable approach with the potential to change clinical practice for cancer patients being considered for treatment with immunotherapy

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- Some figures were created in Biorender.com.

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