elive™ characterizes immunotherapy response in live tumor fragments from patient biopsies

Purpose

This white paper demonstrates how elive™ combines an automated cutting device, proprietary hydrogel, and novel sequential treatment strategy to characterize cytokine responses to immunotherapy in live tumor fragments (LTFs) generated from clinically relevant biopsies.

Introduction

Problem Statement

Immunotherapies have revolutionized cancer care, but current biomarkers lack predictive accuracy[1,2]. Only about 55% of patients are eligible for immune checkpoint inhibitors (ICIs) based on existing approved biomarkers, and of those, only about 20% respond. Conventional biomarkers such as programmed death-ligand 1 (PD-L1), high microsatellite instability (MSI)/mismatch repair deficiency (dMMR), and tumor mutational burden (TMB), are typically measured from fixed tissue, such as formalin-fixed paraffin embedded (FFPE) slides, and at a single time point from a small sample of the biopsy. Strategies exploring ex vivo functional profiling of immune responses have shown that maintenance of the native tumor microenvironment (TME) is essential for enabling the measurement of treatment response[3-9]. Ex vivo functional profiling approaches to predict response that maintain the native TME offer the potential to improve predictive accuracy, but these strategies often require large amounts of tumor tissue (ie, from resections). elive™ maintains the native TME in LTFs from core needle biopsies (CNBs) to characterize cytokine response to ICI over 72 hours, providing dynamic functional profiling of LTFs that mirrors how living tumor tissue responds to immunotherapy.

elive™ enables cytokine profiling of live tumor tissue using multiple innovative approaches

elive™ comprises elive™ Edge, an automated cutting instrument that creates uniform LTFs from CNBs, and elive™ Gel, a proprietary hydrogel that preserves features of the native TME. Additionally, the platform incorporates elive™ Method, a novel sequential treatment strategy that addresses the challenge of intraspecimen heterogeneity in a setting of tissue scarcity (Figure 1).[10] Unlike conventional biomarker approaches that rely on FFPE tissue, elive™ profiles live tumor tissue from CNBs within 24 hours of collection, allowing a rapid assessment of treatment response. The platform preserves large contiguous areas of TME to provide a functionally relevant model for predicting response to immunotherapy. Cytokine profiling is performed longitudinally to assess treatment response. Together, these components enable a novel form of immunotherapy response assessment—live tumor profiling that preserves the native TME and captures functional cytokine responses to treatment.

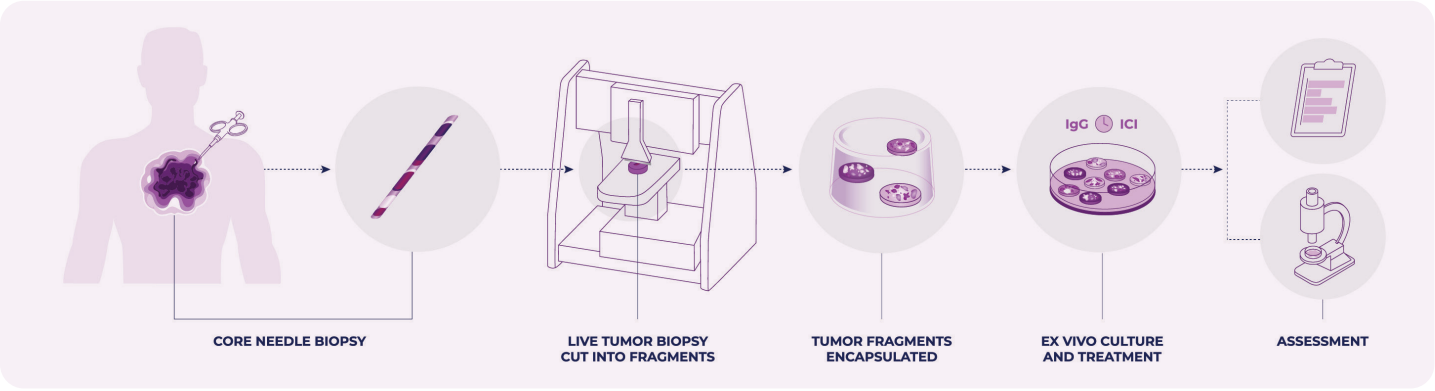

Figure 1. An overview of elive™. LTFs from human CNBs are cut with elive™ Edge, encapsulated in elive™ Gel, sequentially treated with IgG control then ICI (elive™ Method), and assessed for response.

Features of elive™

The sequence of events in elive™ can be divided into 3 steps:

1

Prepare

Fully automated, proprietary cutting method for LTFs, followed by ex vivo culture and maintenance of native TME features

2

Assess

Sequential treatment strategy to determine changes in cytokine production rates in response to immunotherapy

3

Result

Secure, cloud-based web portal providing access to large datasets to help inform clinical trial participation and assess treatment response

Prepare

Automated cutting: Fresh CNBs are cut into LTFs using elive™ Edge, which slices CNBs at a 20° angle to generate LTFs with large contiguous areas of native TME (Figure 2). This angled cut yields 3.5 times more contiguous TME area than conventional 90° slicing, enabling more biologically relevant ex vivo profiling from limited biopsy material. Automated cutting streamlines the workflow, allowing higher-throughput sample processing with consistent precision and minimal manual intervention.

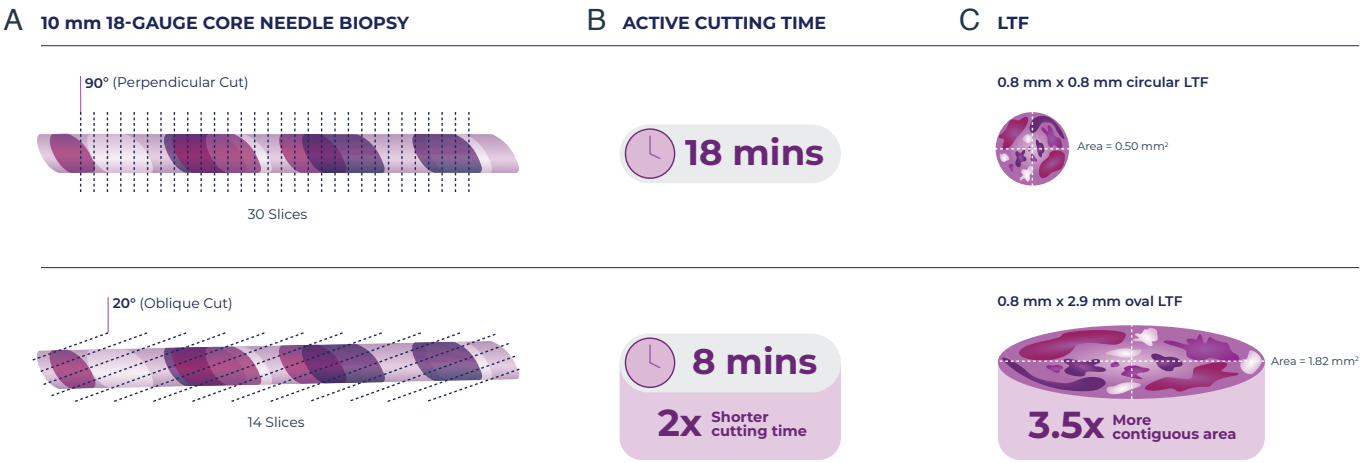

Figure 2. Illustrations of CNBs depicting cutting angles of 90° and 20°. A) The difference in the number of LTFs created from a 90° cut and a 20° cut on a 10-mm biopsy. B) CNBs cut at 90° take 2 times longer than CNBs cut at 20°. C) At 20°, 3.5 times more contiguous area is present in an individual LTF compared with 90°.

Prepare (cont'd)

Preservation of TME: LTFs are then encapsulated in elive™ Gel, a proprietary hydrogel that supports cell viability and preserves features of the native TME. A comparison of unencapsulated and elive™ Gel encapsulated LTFs showed significantly more T cells (CD3+ cells) are retained in encapsulated LTFs after 48 hours of ex vivo culture (Figure 3A). Further, histological features and various immune cell types are maintained (Figure 3B-C).

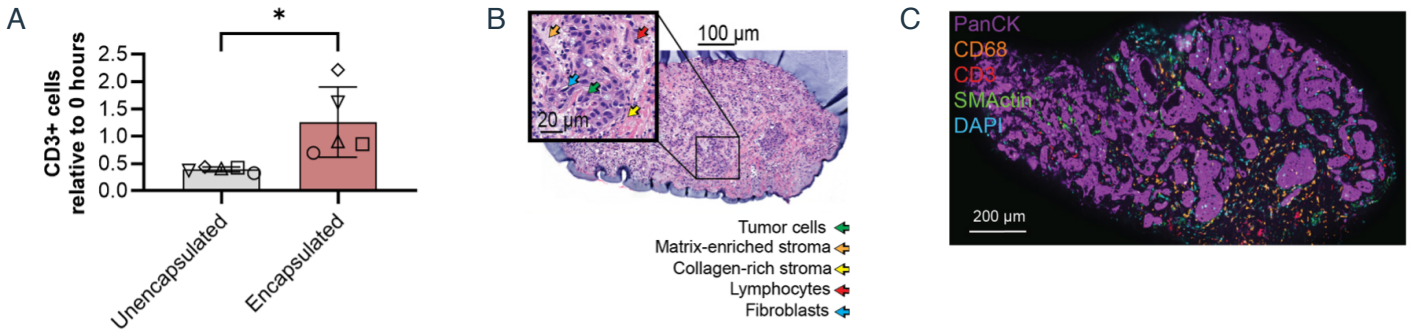

Figure 3. elive™ Gel preserves features of the native TME. A) Quantification of CD3+ T cells in unencapsulated LTFs and LTFs encapsulated in elive™ Gel after 48 hours of ex vivo culture, where each symbol represents one tumor (N=5 tumors per group, * indicates $P < 0.05$). B) H&E-stained section of encapsulated LTF after 48 hours of culture. C) Multiplex immunofluorescence of an encapsulated LTF after 48 hours of culture shows the presence of tumor cells (PanCK – purple), macrophages (CD68 – orange), T cells (CD3 – red), and fibroblasts (SMActin – green).

Assess

elive™ Method is a sequential treatment strategy that applies control and treatment conditions to the same tissue in a single well. This method mitigates the challenge of heterogeneity in a setting of tissue scarcity, as is the case with CNBs, because the tumor sample response to immunotherapy is compared to itself at baseline. LTFs are treated with IgG control followed by ICI for 24 hours. Aliquots of supernatant are taken at indicated time points (Figure 4A) and cytokine concentrations are measured using a multiplex Luminex assay to determine changes in cytokine production rates (slope of lines in Figure 4B). An increase in slope during the treatment phase reflects an increase in the cytokine production rate and a response to immunotherapy. A comparison of the cytokine production rates (slopes) between the control phase and the treatment phase using elive™ Method distinguishes platform responders (red lines, Lung 4624) from non-responders (blue lines, Lung 4578) (Figure 4B).

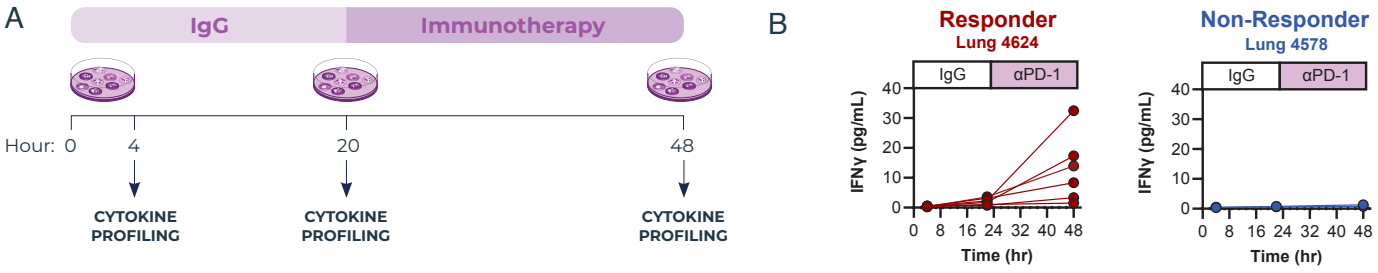

Figure 4. elive™ Method distinguishes platform responders from non-responders in human CNB LTFs. A) Schematic of elive™ Method treatment timeline. LTFs are treated with IgG control followed by ICI, and aliquots of supernatant are taken at hours 4, 20, and 48 to assess cytokine response. B) Human lung LTFs treated with αPD-1 and assessed for cytokine response show a cytokine responder (Lung 4624) and a cytokine non-responder (Lung 4578). Each line indicates a replicate well.

Result

Cytokine profiling data from more than 150 patients reveal different cytokine response profiles, with a subset of samples enriched for response (Figure 5, purple box). This result highlights that elive™ Method is a scalable approach that can be used for patient stratification in clinical trials.

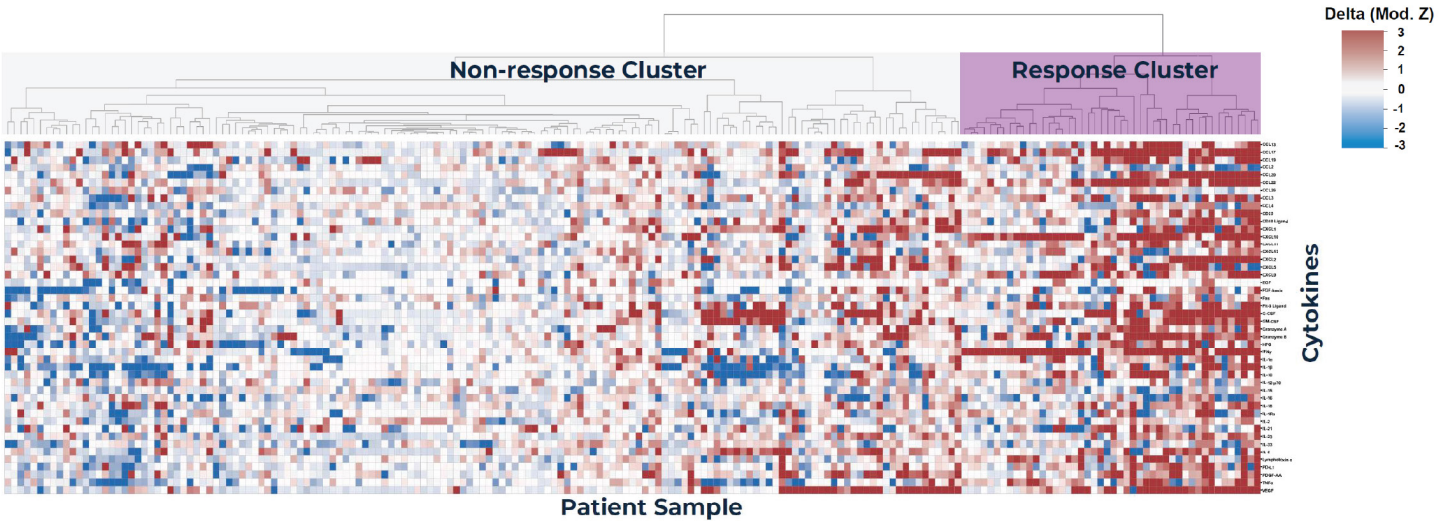

Figure 5. Cytokine responses measured on elive™. Changes in cytokine production rates displayed as delta modified z-scores (ICI-IgG) for 46 cytokines from CNB LTFs across a variety of human tumors. Unsupervised hierarchical clustering of the data reveals responder and non-responder clusters.

elive™ generates large datasets with corresponding clinical data that can be leveraged to accelerate the development of therapeutics and investigate immune response to treatment. Raw cytokine profiling data are shared in the form of a report, which can be used for additional analyses (i.e. pathway analysis).

Conclusion

elive™ enables ex vivo cytokine profiling of live tumor fragments from patient biopsies to predict potential response to immunotherapy. Cytokine profiling is performed from limited tissue in a single CNB over 72 hours, enabling quick and efficient characterization of cytokine response. elive™ is a scalable approach that is amenable to patient stratification for clinical trials, potentially minimizing the likelihood of trial failure, leading to lower overall cost and accelerated development of candidate therapeutics.

Learn more about how elive™ enables prediction of immunotherapy response and read our publications on **our website**:

Methods

LTF Generation

CNBs of 12- to 20-gauge sourced from 3 observational clinical trials (ELEPHAS-01 [NCT05478538], ELEPHAS-02 [NCT05520099], and ELEPHAS-04 [NCT06349642]) and a biopsy collection study were cut into LTFs using elive™ Edge, an automated, proprietary cutting instrument. The LTF slices were cut at a 20° angle, with a thickness of 300 µm. LTFs were counted manually and equally distributed into the wells of a 24-well plate.

Encapsulation

After LTFs were plated, 300 µL of elive™ Gel was added to each well. LTFs were exposed to a 395-nm UV light for 90 seconds to polymerize the hydrogel and washed 3 times with Dulbecco's phosphate-buffered saline. After polymerization, 500 µL of culture media was added to each well for downstream analysis. Specimens were maintained at 37 °C and 5% CO_2 throughout culture.

Cytokine Profiling

Conditioned media collected from individual culture wells at defined time points were assessed using the Human XL Cytokine Luminex Performance Assay Premixed Kit per manufacturer's instructions (R&D Systems, FCSTM18B-30). If the concentration was above the limit of quantitation, the value was set to the upper limit of quantitation.

References

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