

# Elephas Live™ Method: A novel sequential treatment strategy to address tissue scarcity and mitigate heterogeneity in live tumor fragments generated from core needle biopsies

## Purpose

This white paper demonstrates that Elephas Live™ Method is an effective approach for measuring cytokine response to immune checkpoint inhibition (ICI) from live tumor fragments (LTFs) in a setting where tissue amount is limited, such as in core needle biopsies (CNBs).

## Introduction

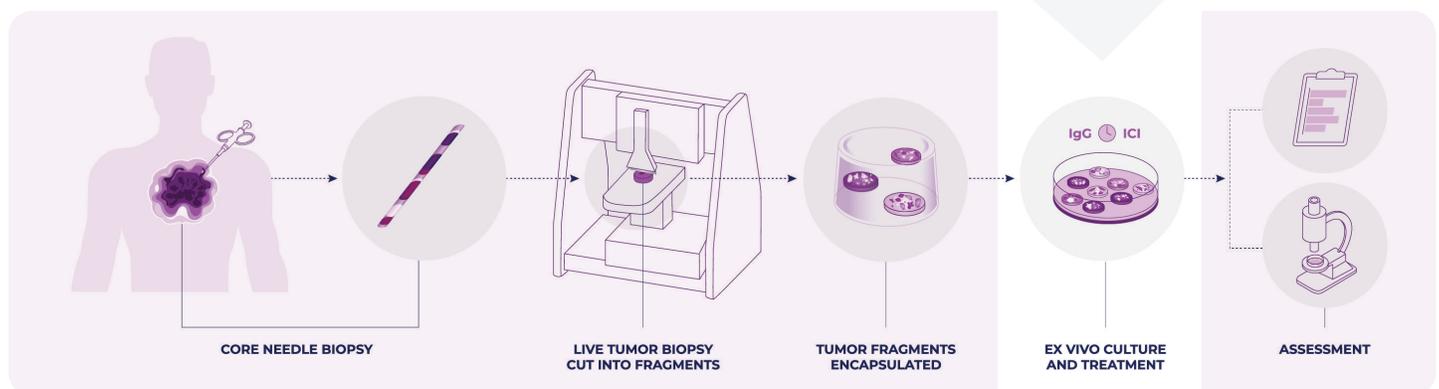
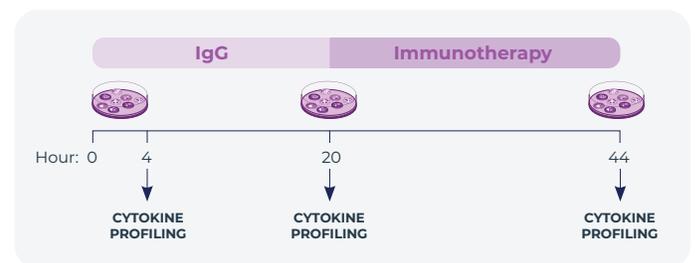
### Problem Statement

Intratumor heterogeneity is a well-known feature of solid tumors that impacts observed cytokine response to immunotherapy in ex vivo profiling experiments<sup>1-5</sup>. CNBs are standard of care for the diagnosis of most solid tumors, but the low tissue yield limits the use of sufficient technical replicates to address the challenge of intratumor heterogeneity in canonical cross-well comparison experiments. Thus, an alternative approach is needed.

using Elephas Live™ Edge, an automated cutting instrument (Figure 1). LTFs are then encapsulated in a proprietary hydrogel (Elephas Live™ Protect) and treated using Elephas Live™ Method, a sequential treatment strategy in which control and treatment conditions are applied to the same LTFs in a single well using a phased approach. Cytokine profiling is then performed at defined time points to assess response to immunotherapy.

### The Elephas Live™ Platform enables ex vivo profiling in settings of limited tissue

The Elephas Live™ Platform enables ex vivo culture of LTFs created from CNBs to assess response to immunotherapy<sup>6</sup>. CNBs are cut into LTFs approximately 300 µm in thickness



**Figure 1.** An overview of the Elephas Live™ Platform, highlighting Elephas Live™ Method. LTFs are created by cutting human CNBs on Elephas Live™ Edge. LTFs are encapsulated in Elephas Live™ Protect and treated using Elephas Live™ Method, an approach that applies IgG control (0-20 hours) and ICI (20-44 hours) sequentially to the same tissue in a single well. Response to ICI is assessed by cytokine profiling at the indicated time points.



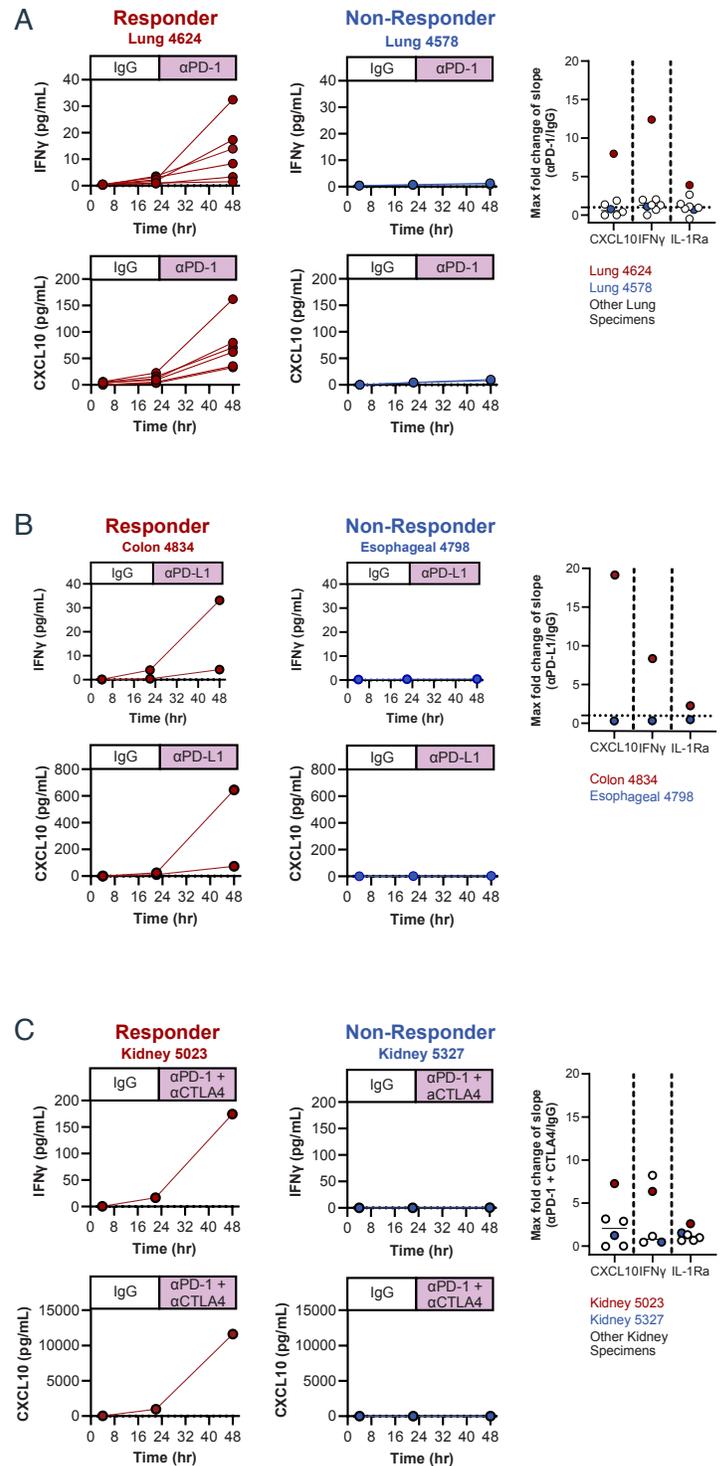
## Elephas Live™ Method is a sequential treatment strategy to mitigate intratumor heterogeneity in settings of tissue scarcity

Elephas Live™ Method is comprised of a control phase and treatment phase applied to the same LTFs in a single well during ex vivo culture. During the control phase, LTFs are treated with IgG for 20 hours, and an aliquot of supernatant is taken at 4 hours and 20 hours for cytokine profiling. After the end of the control phase, the treatment phase begins with the addition of immune checkpoint inhibitor (ICI) or an investigational therapeutic. After 24 hours of treatment, an additional aliquot of supernatant is taken for cytokine profiling at 44 hours. A multiplex Luminex assay is used to measure cytokine concentrations for over 60 cytokines at the beginning and end of each phase. From these data, cytokine production rates for each phase are calculated. Delta and/or fold changes of cytokine production rates during the treatment phase compared to the control phase for each analyte are used to determine a cytokine response profile for each sample, and a sample is classified as a cytokine responder or a cytokine non-responder. To view data showing Elephas Live™ Method is comparable to canonical cross-well experiments, [view our poster](#) from AACR 2025.

## Results

### Elephas Live™ Method is a scalable approach that enables distinction of cytokine responders from non-responders

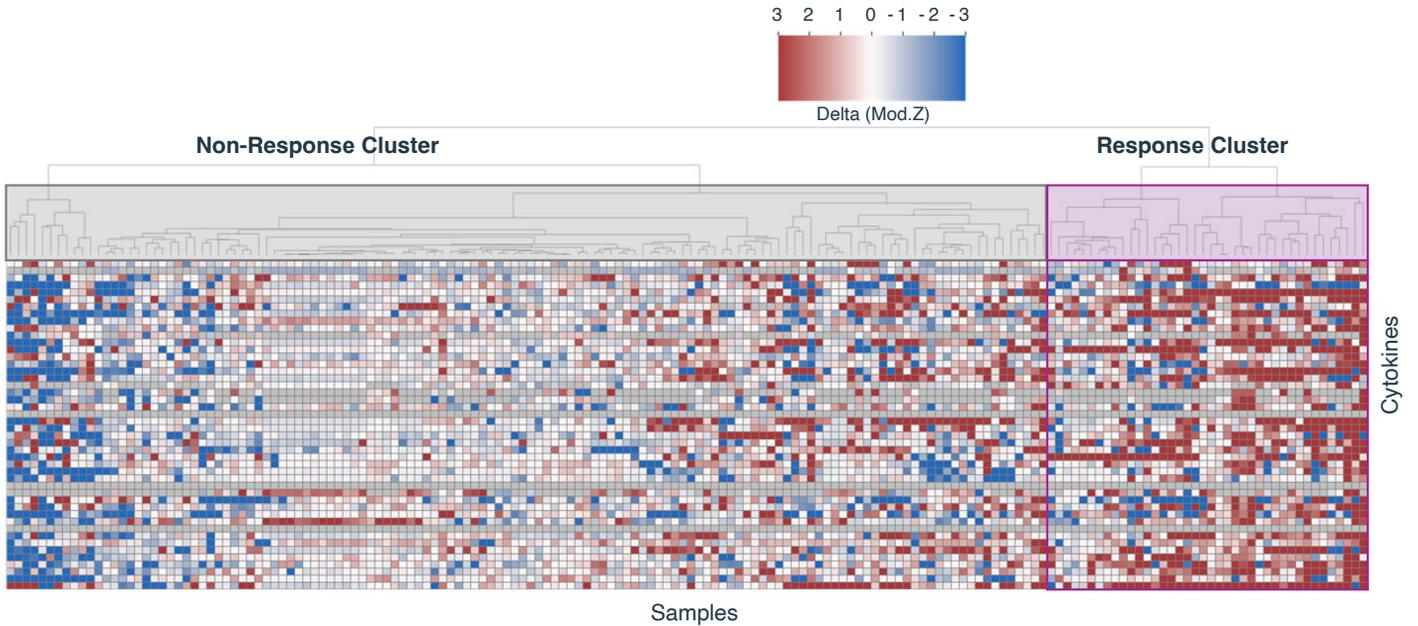
LTFs from a variety of human tumors were treated with Elephas Live™ Method and cytokine profiling was performed at indicated time points. The concentrations of IFN $\gamma$  and CXCL10, two cytokines known to increase in concentration following T-cell activation by immune checkpoint inhibition, were observed. An increase in the slope (cytokine production rate) of the treatment phase compared to the IgG control phase indicates a cytokine response. Elephas Live™ Method differentiated cytokine responders (Figure 2A-C: Lung 4624, Colon 4834, Kidney 5023) from non-responders (Figure 2A-C: Lung 4578, Esophageal 4798, Kidney 5327). ICI treatment varied depending on the tumor type ( $\alpha$ PD-1 for lung specimens,  $\alpha$ PD-L1 for gastrointestinal [GI] specimens, or  $\alpha$ PD-1 +  $\alpha$ CTLA-4 for kidney specimens) (Figure 2A-C), demonstrating that Elephas Live™ Method can be used to show cytokine response to various ICIs across diverse tumor types.



**Figure 2.** Elephas Live™ Method distinguishes cytokine responders from cytokine non-responders in human CNB LTFs. A-C) Examples of cytokine responders (Lung 4624, Colon 4834, and Kidney 5023) and non-responders (Lung 4578, Esophageal 4798, Kidney 5327). Scatter plots show fold change in slope of ICI/control phase for 7 lung, 2 GI, and 6 kidney specimens.



Analysis of cytokine profiles from a population of samples allowed for distinguishing a subset of samples enriched for cytokine response that are potentially treatment responders. Unsupervised hierarchical clustering of cytokine response data collected using Elephas Live™ Method showed that samples fall into two major clusters: responders and non-responders (Figure 3). Data shown is from 170 samples, demonstrating that Elephas Live™ Method is a scalable approach that offers the opportunity to stratify patients for clinical trials.

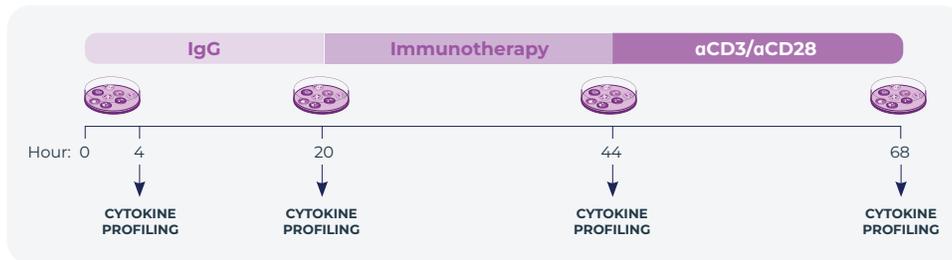


**Figure 3.** Changes in cytokine production rates (ICI-IgG), displayed as delta modified z-scores, for 46 cytokines in CNB LTFs from a variety of human tumors. Unsupervised hierarchical clustering reveals a subset of samples enriched for cytokine response (purple box, right).

### A positive control T-cell stimulation phase of Elephas Live™ Method supports quality control and provides insight into T-cell activation and cytokine response

After the end of the treatment phase at 44 hours of ex vivo culture, αCD3/αCD28 is added as a third and final phase of Elephas Live™ Method to serve as a positive control for

T-cell activation (Figure 4). At the end of 68 hours of ex vivo culture, an aliquot of supernatant is taken for a final measurement of cytokine production. Comparison of the cytokine production rate in this third, positive control phase to the cytokine production rate of the treatment phase yields potential insights into the extent of T-cell activation from the test therapeutic.

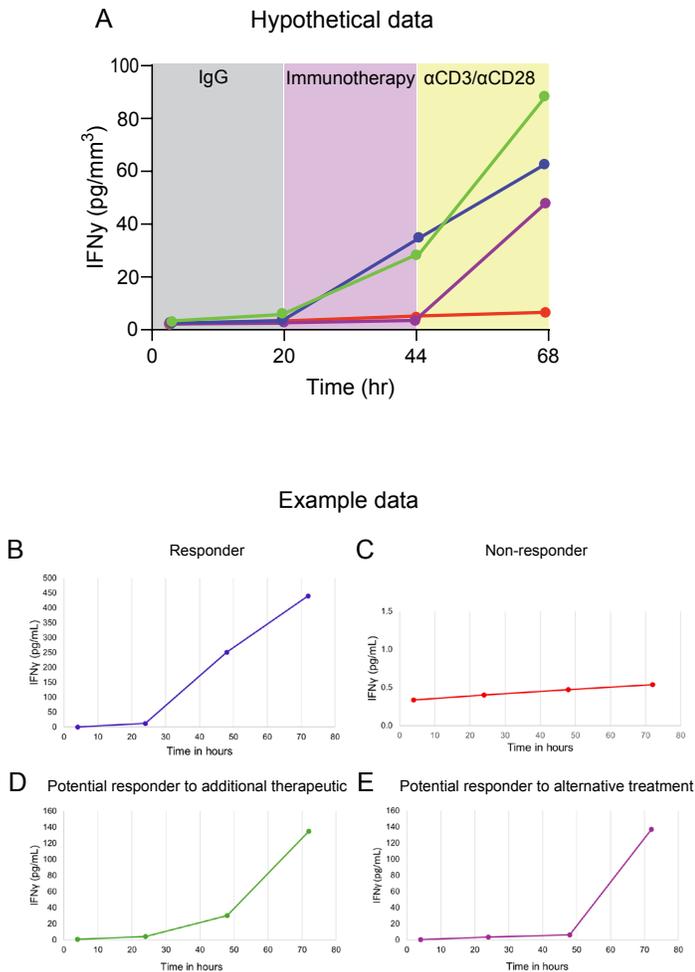


**Figure 4.** A schematic of the three phases of Elephas Live™ Method through 68 hours of ex vivo culture.



## Interpretation of four potential cytokine response profiles

A comparison of the cytokine production rates (slopes) from the treatment phase to the T-cell stimulation phase can indicate whether T cells have been maximally stimulated in response to treatment. The following four scenarios may further inform results obtained using Elephas Live™ Method (Figure 5A).



**Figure 5.** A third phase of Elephas Live™ Method provides insight into T-cell function. A) Schematic of hypothetical cytokine profiling data showing four potential cytokine response scenarios during each phase of sequential treatment. B-E) Cytokine responses from human CNB LTFs matching each of the potential cytokine response profiles: potential responder to test therapeutic (B), potential non-responder to test therapeutic (C), potential responder to additional therapeutic (D), and potential responder to a therapy with an alternative mechanism of action (E).

### Potential responder to test therapeutic (Figure 5B)

An increase in slope from the treatment phase to the control phase is observed, but there is no further increase in slope from the treatment phase to T-cell stimulation phase. This suggests that T cells achieved maximal stimulation during application of the treatment, and the sample is responsive to the test therapeutic.

### Potential non-responder to test therapeutic (Figure 5C)

No increase in slope is observed during the treatment or T-cell stimulation phases. Given the sample has been shown to contain viable tumor tissue, this response suggests that the TME is highly immunosuppressive or lacks suitable T cells.

### Potential responder to test therapeutic and combination therapy (Figure 5D)

A further increase in slope is observed in the T-cell stimulation phase compared to the treatment phase. This response suggests that the sample is responsive to the test therapeutic, but that the treatment alone does not maximally stimulate T cells. This profile may reflect a potential responder to the test therapeutic with potential for further T-cell stimulation with a combination therapy.

### Potential non-responder to test therapeutic, but potential responder to alternative therapy with a different mechanism of action (Figure 5E)

An increase in slope is observed in the T-cell stimulation phase, but not the treatment phase, when compared to the control phase. This response suggests that, while the sample is a non-responder to the test therapeutic, T-cell stimulation can be achieved. Thus, the sample may be a potential responder to an alternative therapy with a different mechanism of action.



## Conclusion

Elephas Live™ Method mitigates the challenge of intratumor heterogeneity in settings of tissue scarcity by enabling the measurement of cytokine response from a single well of tissue after sequential control and treatment phases. Elephas Live™ Method offers a scalable approach to test response to immunotherapy and provides a mechanism for patient stratification in clinical trials. The T-cell stimulation positive control phase of Elephas Live™ Method may generate additional insights into observed cytokine responses.

Learn more about how the Elephas Live™ Platform enables prediction of response to immunotherapy at [our website](#) and view previous publications highlighting Elephas Live™ Method here:



**Watch a short overview of our 2025 AACR poster:**  
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



**2025 AACR Poster:** 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



**BioRxiv Preprint Publication:** A live tumor fragment platform to assess immunotherapy response in core needle biopsies while addressing challenges of tumor heterogeneity

## Methods

### Sample preparation

Human CNBs from 12- to 20-gauge needles were cut on Elephas Live™ Edge at a 20° angle into slices with a thickness of 300 µm and plated in a 24-well culture plate. Elephas Live™ Protect hydrogel (300 µl) was added to each well. After hydrogel polymerization, culture media containing the indicated treatment was added to each well.

### Treatments

ImmunoCult™ Human CD3/CD28 T-Cell Activator (STEMCELL Technologies Inc, 10971) was used at a final concentration of 25 µL/mL, except in Figure 4 where 100 µL/mL was used. IgG isotype controls (RecombiMAb and BioXCell) and ICI antibodies (BioXCell) were used at a concentration of 50 µg/mL.

### Cytokine Profiling

Conditioned media collected from individual culture wells at defined time points were assessed using the Human XL

Cytokine Luminex Performance Assay (R&D Systems, FCSTM18B). The cytokine concentrations from samples were interpolated from a standard curve generated for each analyte. If the concentration was above the limit of quantitation, the value was set to the upper limit of quantitation; values below the limit of quantitation were unchanged and removed from analysis.

## References

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