Abstract #B032

Characterization of αPD-1, αCTLA-4, and αLAG-3 as mono and combination therapy in live tumor fragments using an ex vivo cytokine profiling platform

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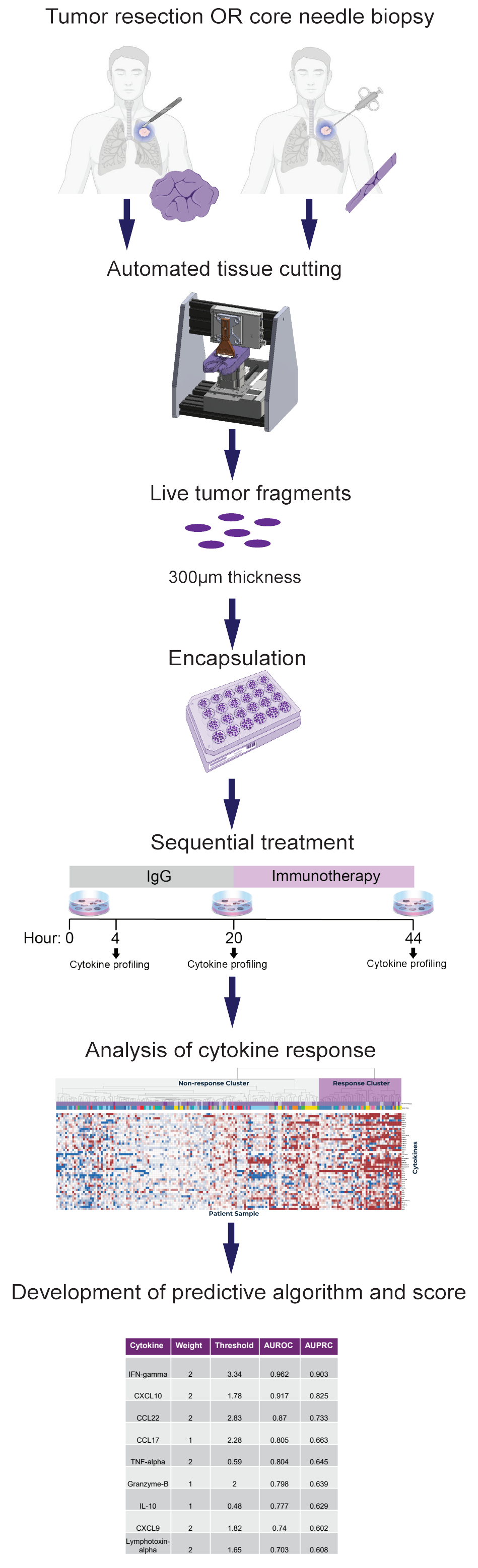
Introduction

- Immune checkpoint inhibitors (ICIs) provide durable clinical benefit in a subset of patients, but current biomarkers to predict response to ICIs lack accuracy
- Ex vivo cytokine profiling has shown promise for improved accuracy but has been mostly limited to ICIs targeting PD-1[1,2], posing the question of whether ICIs beyond αPD-1 - such as CTLA-4 blockade, which has been reported to act within the draining lymph node - would be amenable to similar approaches
- The Elephas platform characterizes response to various mono and combination ICI treatments in live tumor fragments (LTFs) from core needle biopsies (CNBs) and resections using longitudinal cytokine profiling[3]

Methods



Cytokine	Weight	Threshold	AUROC	AUPRC
IFN-gamma	2	3.34	0.962	0.903
CXCL10	2	1.78	0.917	0.825
CCL22	2	2.83	0.87	0.733
CCL17	1	2.28	0.805	0.663
TNF-alpha	2	0.59	0.804	0.645
Granzyme-B	1	2	0.798	0.639
IL-10	1	0.48	0.777	0.629
CXCL9	2	1.82	0.74	0.602
Lymphotoxin-alpha	2	1.65	0.703	0.608

Resections and biopsies (CNBs, 12–22 gauge, ≥3 mm in length) from patients suspected to have cancer and eligible for ICI treatment were collected through 3 ongoing clinical trials (NCT05478538, NCT05520099, and NCT06349642) and a biobank collection study.

Resections or CNBs were cut into LTFs with a thickness of ~300μm using an automated cutting device. LTFs were seeded in a 24-well plate and encapsulated in a proprietary hydrogel.

Following encapsulation, LTFs were treated using a sequential treatment strategy in which IgG control followed by ICI (mono or combination treatment) was added to the same tissue in a single well. Concentrations of 46 cytokines were measured longitudinally at indicated time points using Luminex multiplex assays. An increase in the slope of cytokine production from the treatment compared to the control phase indicated response.

A cytokine-based classifier trained on data from 130 specimens (treated mainly with αPD-1 or αPD-1+CTLA-4) was used to generate scores measuring LTF cytokine response, with platform scores >30 indicating response to ICI and scores ≤30 indicating no response to ICI. Quality control metrics were developed to assess specimen and assay quality.

Results

1 Ex vivo cytokine responses to ICIs beyond αPD-1 are observed on the platform

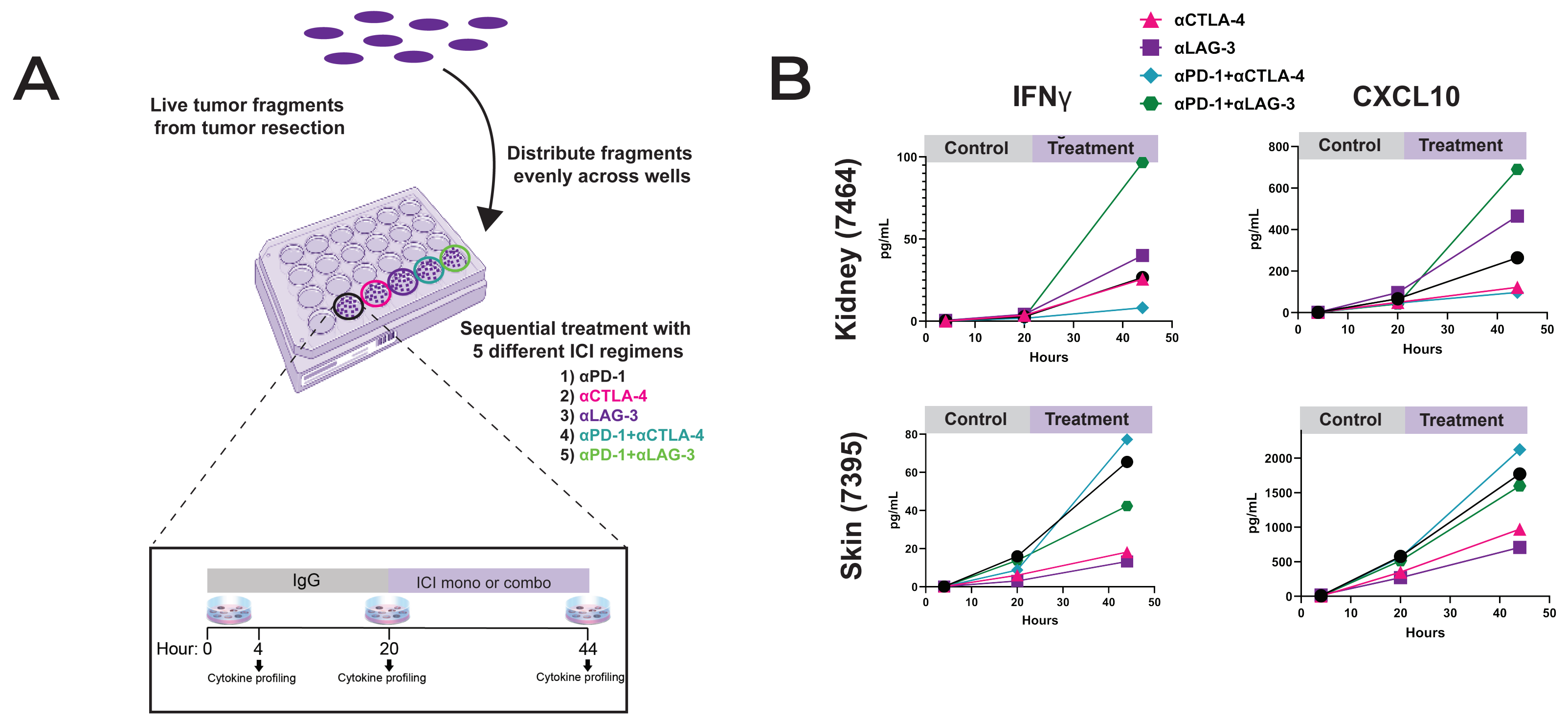


Figure 1. A) LTFs from individual tumor resections were divided across 5 wells and treated with 5 different ICI regimens using a sequential treatment strategy. Changes in cytokine production rate in the treatment phase compared to the IgG control phase were used to determine response status and score. If a speicmen provided enough tissue for more than 5 wells, replicate wells were included. **B)** Cytokine responses in 2 specimens that were platform responders show varying upregulation in IFNγ and CXCL10 (2 cytokines known for their role in T-cell activation) across treatment conditions.

2 Responders are observed across all treatments, with specimen response scores varying across treatments

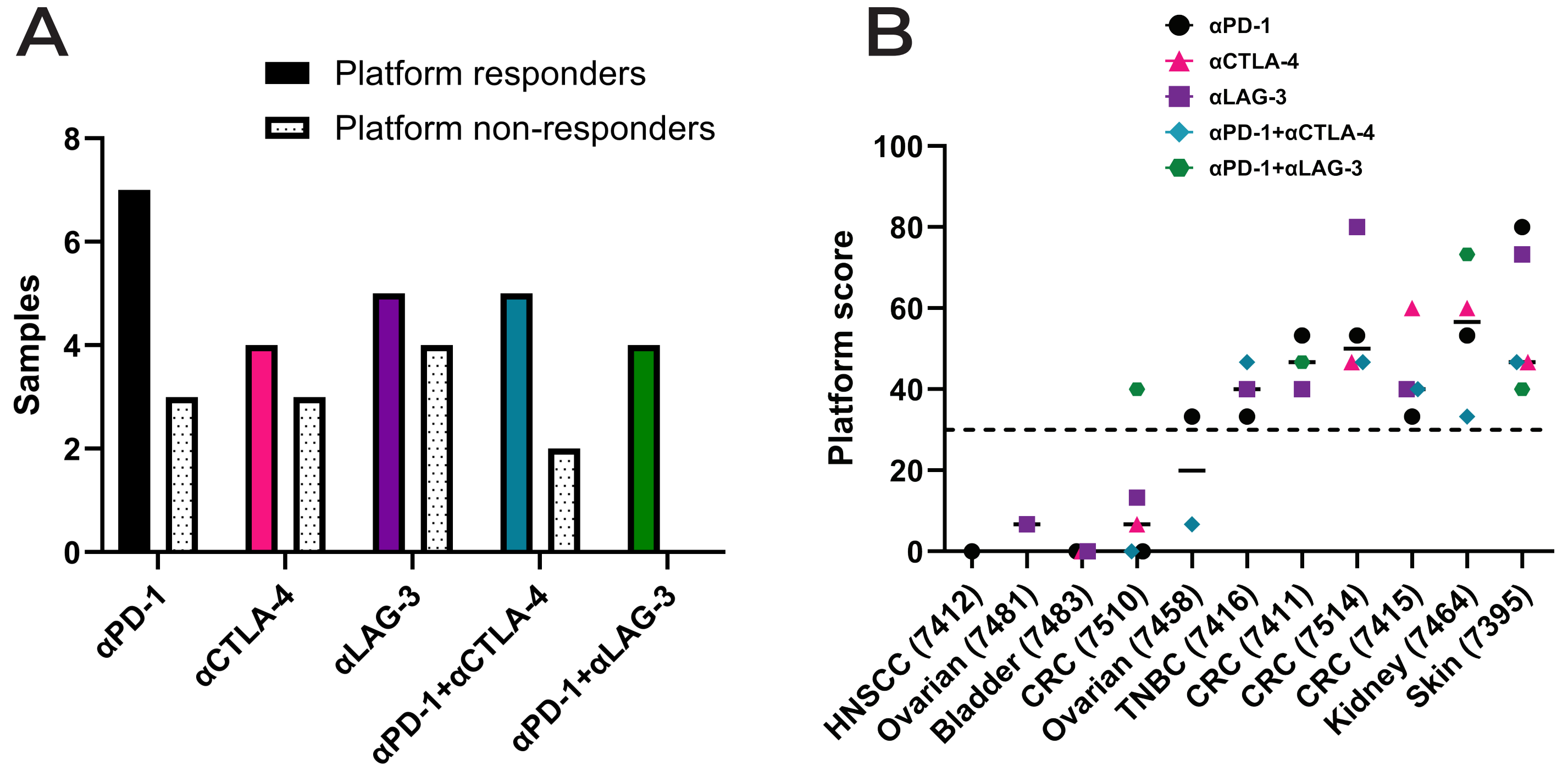


Figure 2. A) Distribution of platform responses (score >30 = responder) for all samples for each specimen (N=13). **B)** Platform score variation for each specimen. Some specimens respond to all ICIs (right) and others respond to few or no ICIs (left). For specimens with replicate samples for a given treatment condition, the highest score was used; dashed line indicates platform responder threshold of 30.

3 Clustering of cytokine profiling data shows unique cytokine signatures across specimens

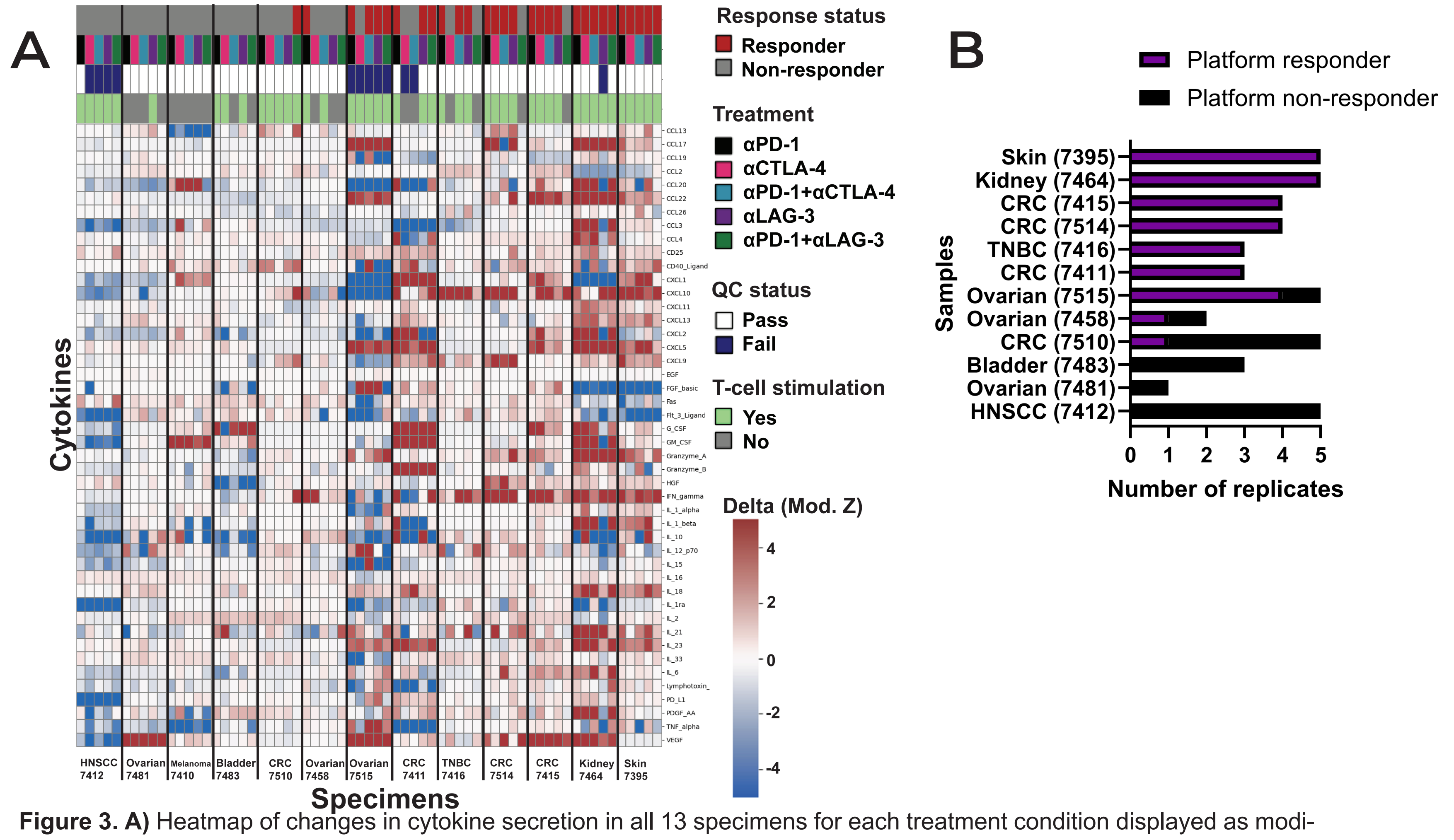
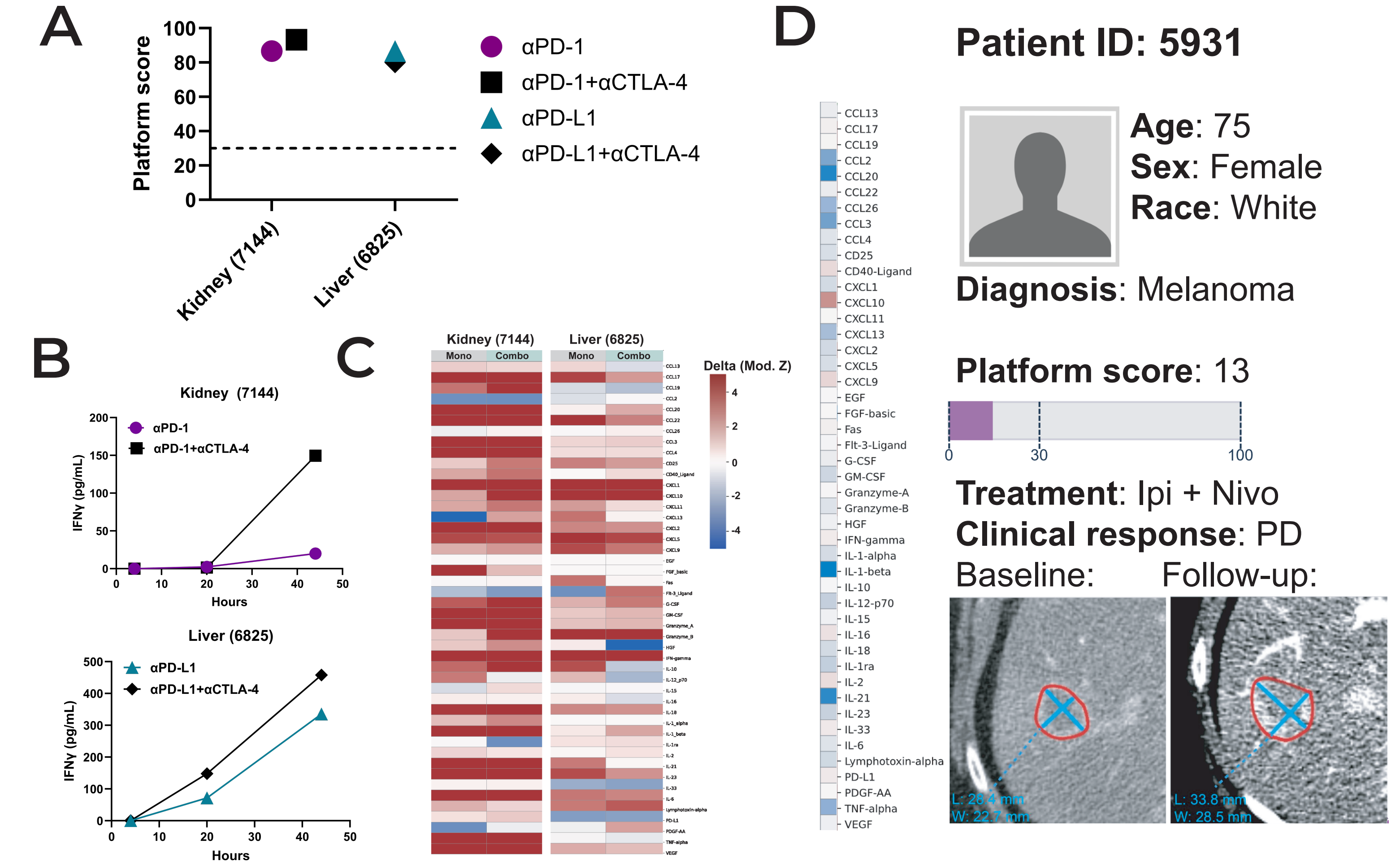


Figure 3. A) Heatmap of changes in cytokine secretion in all 13 specimens for each treatment condition displayed as modified Z scores reveals specimen-specific cytokine signatures. T-cell stimulation was assessed via a third, terminal phase of sequential treatment in which fragments were treated with αCD3/αCD28 to stimulate T cells following the ICI treatment phase. The replicate with the highest platform score was selected for each treatment condition. **B)** Distribution of response status for samples that were positive for T-cell stimulation for each specimen (12).

4 Cytokine responses to mono and combination ICI therapy in LTFs from core needle biopsies



Patient ID: 5931

Age: 75
Sex: Female
Race: White

Diagnosis: Melanoma

Platform score: 13

Treatment: Ipi + Nivo
Clinical response: PD
Baseline: Follow-up:

Figure 4. A) LTFs from 2 CNBs treated ex vivo with αPD-1 (kidney) or αPD-L1 (liver) mono or combination with αCTLA-4 show similar platform scores for both treatments; dashed line indicates platform responder threshold of 30. **B)** Increase in IFNγ secretion is observed in the treatment phase for both treatment conditions in each specimen. **C)** Heatmaps showing changes in cytokine secretion for mono and combination treatment. **D)** LTFs from patient 5931 treated ex vivo with αPD-1+αCTLA-4 show limited cytokine upregulation (left heatmap), with a platform score of 13 (platform non-responder). The patient, treated with ipilimumab and nivolumab, displayed progressive disease (PD) at 3 month follow-up (scans, bottom).

Scan to learn more

Platform score development:
(SITC 2025)

PDF copy of poster:

Conclusions

- The Elephas platform identifies cytokine responders and non-responders to ICI mono and combination therapies beyond αPD-1
- While no clear differences in cytokine response signatures were observed across treatments, specimens displayed unique cytokine signatures regardless of treatment condition
- Ex vivo cytokine profiling is feasible in resections and clinically relevant CNBs, and preliminary data suggests responses on the platform correlate with clinical response

Acknowledgments

- We thank the patients, clinicians, research staff, and Elephas employees for their contributions
- Tissue samples were provided by the Cooperative Human Tissue Network which is funded by the National Cancer Institute

References

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