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Poster 529

Comparison of the clinical biomarkers dMMR, MSI-H and PD-L1 with cytokines secreted from αPD-1 treated human live tumor fragments on an ex vivo platform

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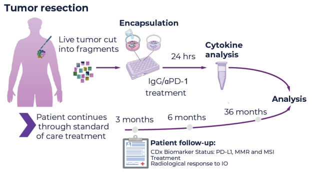
elephas

Introduction

- There is an unmet need for a diagnostic platform to guide immunotherapy treatment for cancer patients
- A significant challenge associated with developing such a platform is obtaining human tumor specimens from known clinical responders and non-responders to immunotherapy
- Tumors positive for immunotherapy companion diagnostic (CDx) biomarkers (PD-L1, dMMR or MSI-H) exhibit an enrichment for response to αPD-1 treatment, providing a setting to test a diagnostic platform in the absence of corresponding clinical response data
- Patients positive for CDx biomarkers do not always respond to immunotherapy and some patients negative for these biomarkers would respond but are excluded from treatment, highlighting the need for improved response prediction
- Here, we leveraged tumor specimens with known CDx biomarkers status to determine the time course of αPD-1-induced changes in secreted cytokines and compared the levels of Response cytokines to specimen biomarker status
- The Elephas platform uses Live Tumor Fragments (LTFs™), which preserve the tumor microenvironment, to assess ex vivo responses to immunotherapy treatment using molecular assays and advanced imaging

Methods


Tumor resection
Encapsulation
Live tumor cut into fragments
24 hrs
IgG/αPD-1 treatment
Cytokine analysis
Analysis
Patient continues through standard of care treatment
3 months
6 months
36 months
Patient follow-up:
CDx Biomarker Status: PD-L1, MMR and MSI
Treatment
Radiological response to IO

Enrollment: We enrolled specimens from patients with the following tumor types which have FDA-approved indications for αPD-1 treatment based on CDx biomarkers: colorectal[1], uterine[2], head and neck[3], lung[4], and triple-negative breast[5].

Ex vivo cytokine analysis: Tumor resections were fragmented on the Elephas platform into 300 µm × 300 µm × 300 µm LTFs, encapsulated, and treated for 24 hours with either IgG isotype control (50 µg/mL) or αPD-1 (50 µg/mL, pembrolizumab biosimilar). Conditioned media from culture plates were collected for multiplex cytokine analysis. A set of 8 "Response cytokines" (CCL2, CXCL1, CXCL10, CXCL2, IFNγ, Granzyme B, IL-10, and IL-6) was selected for further analysis. These cytokines exhibited significant upregulation in at least two tumor samples following immune checkpoint inhibition and were documented in the scientific literature as playing a role in T-cell-activation or whose expression was correlated with response to immune checkpoint inhibition[6,7,8,9,10,11,12,13].

CDx biomarker status: PD-L1, MMR and MSI status were obtained from medical records. Where necessary, PD-L1 (n=14) or MMR (n=4) expression was characterized from untreated histological sections immunostained with biomarker specific antibodies. Expression was then quantified by a board-certified pathologist. PD-L1 immunostaining was confirmed using an external clinical biomarker assessment and MMR staining was confirmed using human tonsil as a positive control.

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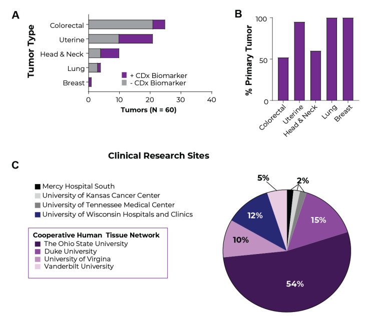
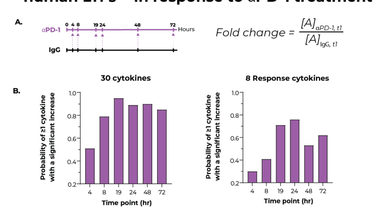
1 Breakdown of patient tumor characteristics


A
Tumor Type
Colorectal
Uterine
Head & Neck
Lung
Breast
+ CDx Biomarker
- CDx Biomarker
Tumors (N = 60)
B
% Primary Tumor
C
Clinical Research Sites
Mercy Hospital South
University of Kansas Cancer Center
University of Tennessee Medical Center
University of Wisconsin Hospitals and Clinics
Cooperative Human Tissue Network
The Ohio State University
Duke University
University of Virgina
Vanderbilt University
5%
2%
12%
15%
10%
54%

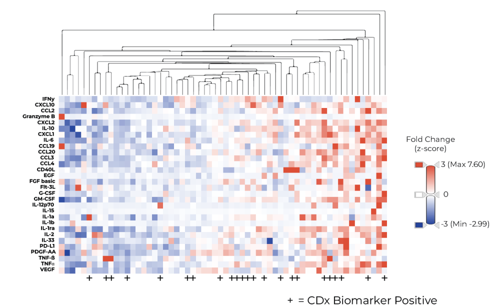
(A) Bar graph reports the number of patient tumors by type and CDx biomarker status. Tumors included in this study were primary or metastatic. The percentage of primary tumors for each tumor type are presented in (B). (C) The percentage of tumors received from each clinical site.

2 Temporal dynamics in cytokine levels in human LTFs™ in response to αPD-1 treatment



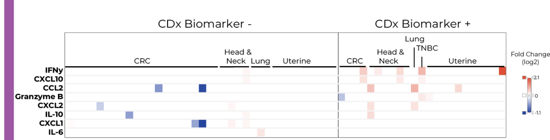
(A) Experimental timeline and calculation for fold-change comparison in cytokine levels from 10 unique patient specimens treated with αPD-1 versus IgG at each time point. [A] = cytokine concentration. Cytokine concentrations were adjusted to account for earlier supernatant collections. Any concentration outside the limits of quantification of the assay was adjusted to the limit of quantification, either lower or upper limit. All concentrations were then normalized to total tissue volume within individual treatment wells. (B) The probability of at least 1 cytokine showing a significant increase in the αPD-1-treated group over the IgG-treated group at each supernatant collection time point. Two-tailed, unpaired Student's T-test was used to determine the statistical significance between the two treatment regimens for each specimen. *P*-value cutoff was 0.05. The left bar graph shows the cumulative probability considering all 30 cytokines assayed. The right bar graph shows the cumulative probability limited to Response cytokines. The time point when Response cytokines were most likely to show a significant change was at 24 hrs of treatment.

3 Hierarchical clustering groups CDx biomarker-positive samples amongst those with greatest cytokine upregulation following αPD-1 treatment



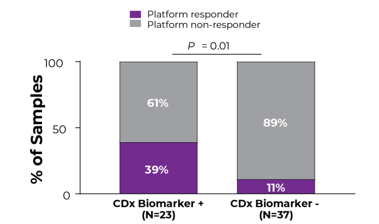
Hierarchical clustering analysis of patient tumor samples using z-scores of fold change (αPD-1/IgG) in cytokine concentrations following 24 hrs of treatment (normalized for tissue volume). αPD-1 mediated increases in cytokine concentration are depicted in red and decreases are depicted in blue. Samples with no change in cytokine concentration are depicted in white. N=60 specimens.

4 CDx biomarker-positive samples exhibit a clear enrichment in αPD-1 mediated increase in Response cytokines



Heatmap reports significantly up- or down-regulated Response cytokines following 24 hrs of treatment with αPD-1. The legend reports colors based on log2 fold change (αPD-1/IgG) in cytokine concentrations (normalized for tissue volume and adjusted to be within the limits of quantification of the assay). All non-significant changes are reported in white. N=60 total patient tumor samples, composed of 23 CDx biomarker-positive and 37 CDx biomarker-negative samples.

5 CDx Biomarker-positive samples were 3.5x enriched for platform responders



Platform responder (purple) and non-responder (gray) frequencies broken down by biomarker status. Platform responders were defined as those tumor samples exhibiting ≥1 significantly elevated response cytokine following treatment with αPD-1 (n=9 for CDx biomarker positive, n=4 for CDx biomarker negative). Platform non-responders were defined as those tumor samples exhibiting no significantly elevated Response cytokines following treatment with αPD-1 (n=14 for CDx biomarker positive, n=33 CDx biomarker negative). The calculated Chi Square p-value was 0.01 for the null hypothesis of non-enrichment of CDx biomarker positive samples amongst platform responders.

6 Overview of ongoing observational clinical trials at Elephas

	ELEPHAS-01:	ELEPHAS-02:	ELEPHAS-04:
Setting	Metastatic & recurrent	Metastatic & recurrent, Advanced	Metastatic & recurrent, Advanced, Neoadjuvant, Adjuvant
Study Endpoints	• Sensitivity and specificity of Elephas platform for predicting clinical response to immune checkpoint inhibitors • Compare to existing FDA approved biomarkers (PD-L1, MSI, MMR and TMB) • Compare clinical benefit of predicted responders and non-responders (ORR, DCR, PFS, OS) • Measured per RECIST v1.1 and iRECIST		

Elephas is conducting three observational clinical trials. The primary objective of these studies is to determine the ex-vivo predictive accuracy of the Elephas platform across a variety of solid tumors types.

Conclusions

- The Elephas platform is capable of detecting changes in cytokine response to αPD-1 treatment in human LTFs within 24 hours of treatment
- Consistent with clinical studies and evidence from real-world clinical use, we observed an enrichment of platform responders among CDx biomarker-positive specimens
- The Elephas platform demonstrates the potential to identify responders in CDx biomarker-negative specimens

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Some figures were created in Biorender.com.

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