High-Dimensional Analysis of Translational Samples from the NSGO-OV-UMB1 UMBRELLA Clinical Trial of Combined Anti-CD73 and Anti-PD-L1 Immunotherapy in Ovarian Cancer

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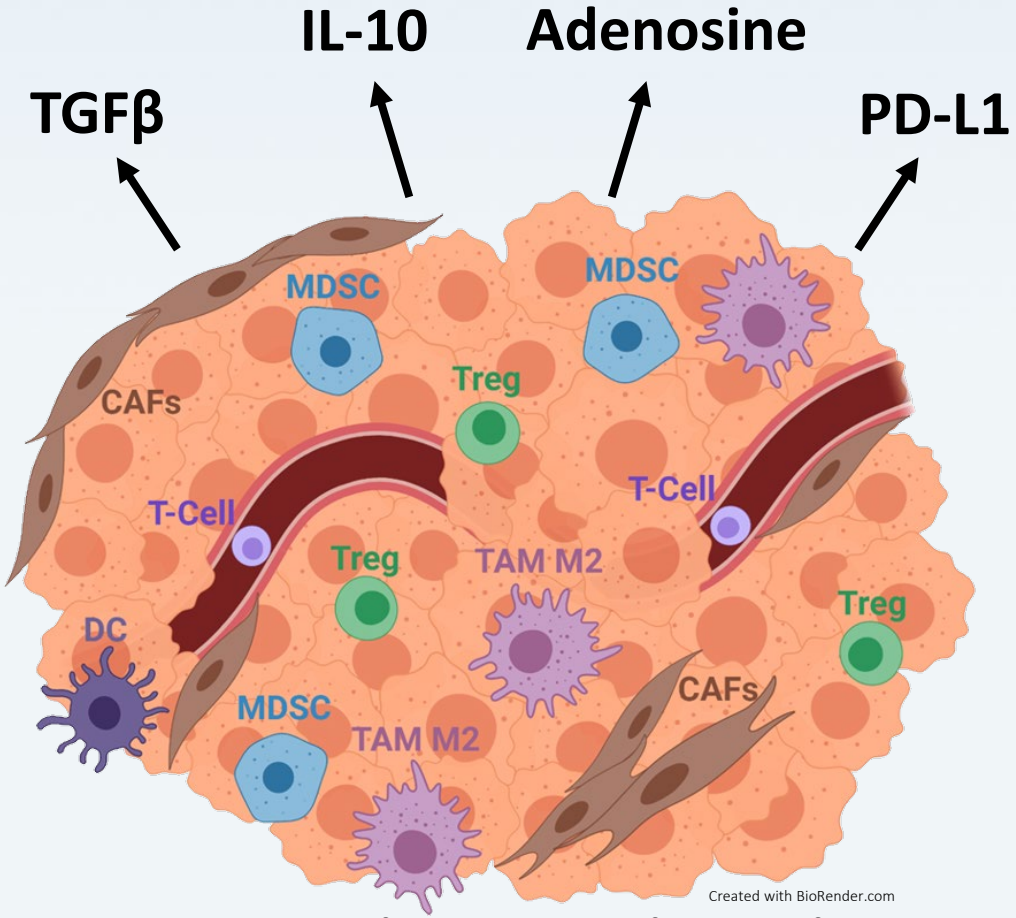
Disclosures:

- AstraZeneca – Research grant

Immunotherapy in OC

- Standardized OC treatment
  - Cytoreductive surgery + carboplatin/paclitaxel
  - 70-80% relapse rate

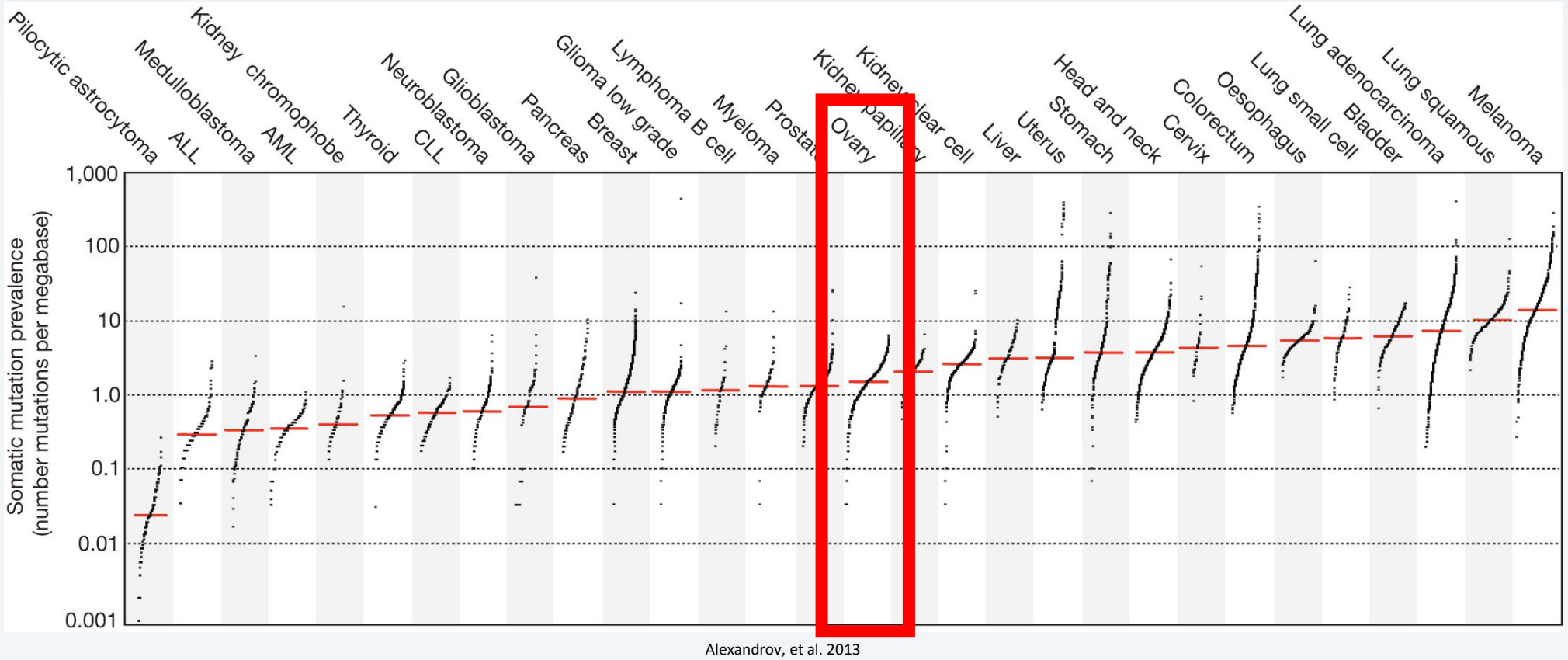
- Immunotherapy in OC
  - Shift to personalized medicine - immunotherapy
  - "Hot" tumor → amenable to immunotherapy
  - Single-agent failure
  - ICI + chemo failure



Immunosuppressive tumor microenvironment

Immunotherapy in OC

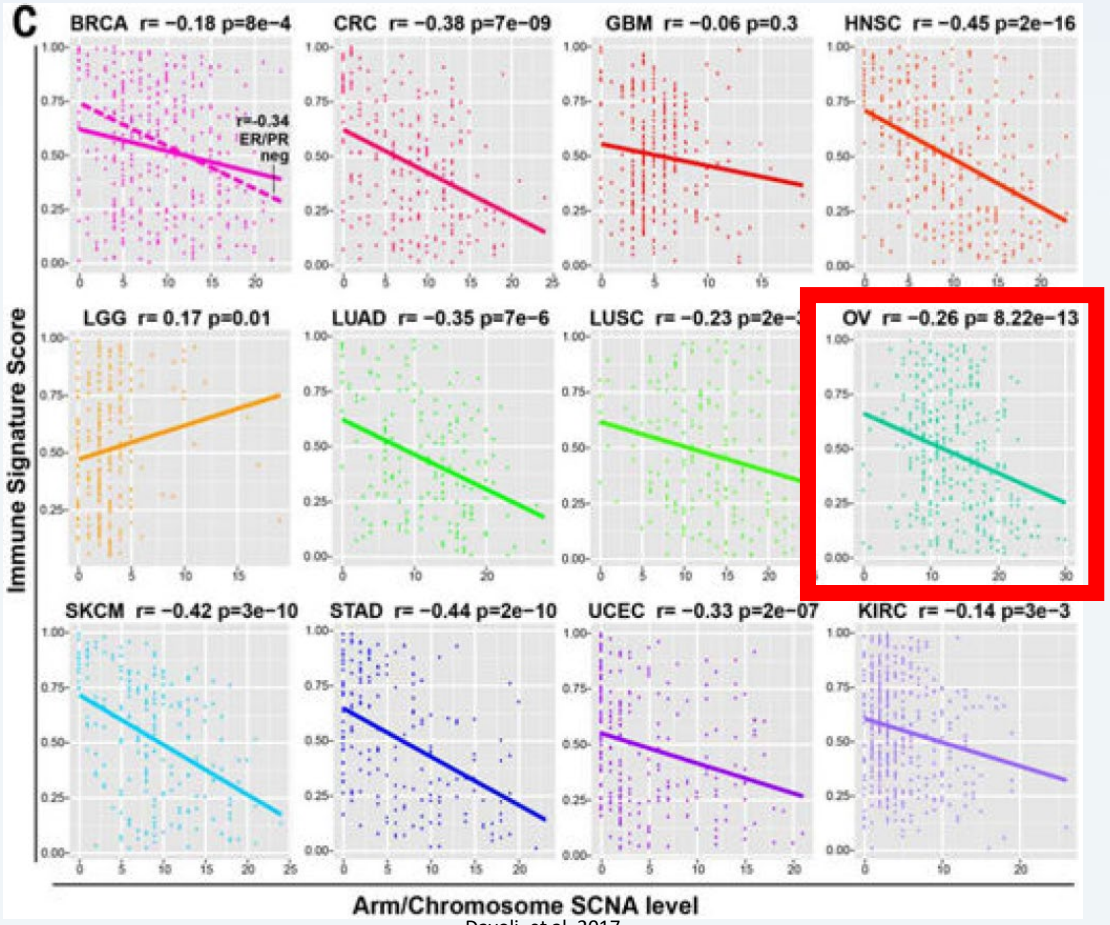
Low-to-intermediate mutational load



Alexandrov, et al. 2013

Immunotherapy in OC

Frequent large-scale SCNAs



Davoli, et al. 2017

NSGO-OV-UMB1/ENGOT-OV30

Relapsed ovarian cancer

COHORT A
n = 25
Oleclumab + Durvalumab
(Anti-CD73 + Anti-PD-L1)

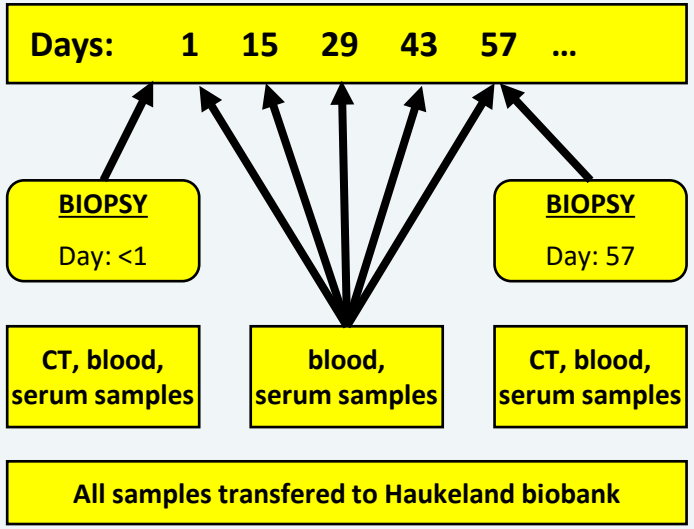
COHORT B
n = 25
Durvalumab + MEDI0562
(Anti-PD-L1 + Anti-OX40)

COHORT C
n = 25
MEDI0562 + Tremelimumab
(Anti-OX40 + Anti-CTLA-4)

Treatment until disease progression

Evaluation of results for each cohort.
Is it feasible to proceed to part 2 for the given cohort?

PART 2: Comparison to standard of care



Patient Criteria

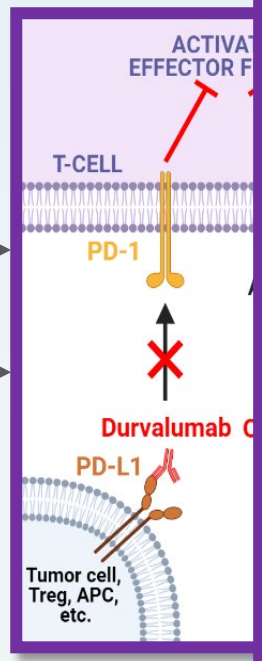
- 25 relapsed HGSOC patients
  - Included between April 2018 and May 2019

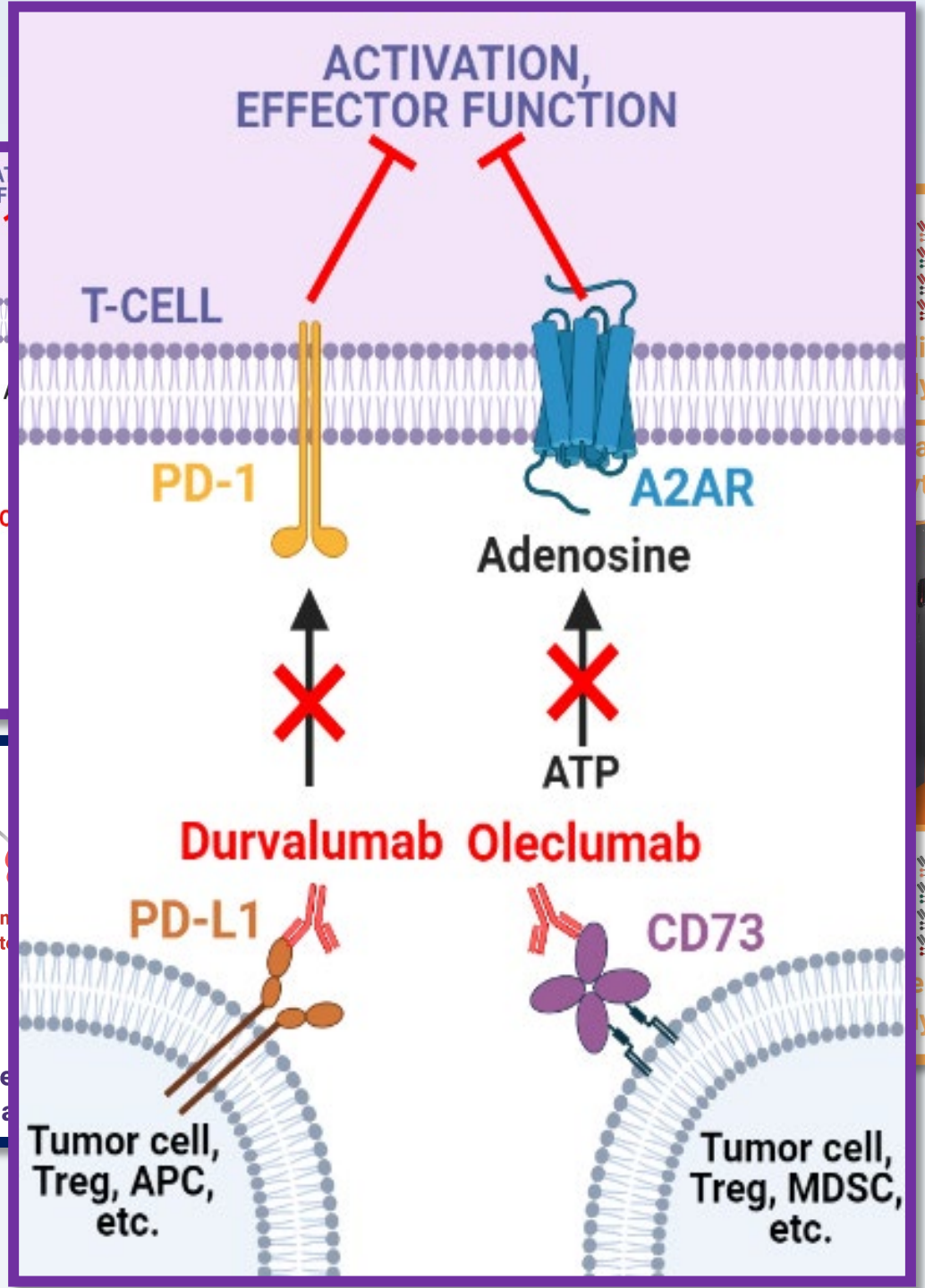
- Inclusion criteria:
  - Histologically confirmed and relapsed ovarian cancer
  - Measurable disease by RECIST 1.1
  - CD73+ archival tumor tissue
    - >10% positive tumor cells OR
    - >50% positive tumor stroma
  - Mandatory tumor biopsy before treatment and at day 56
  - Eastern Cooperative Oncology Group (ECOG) performance status 0-1

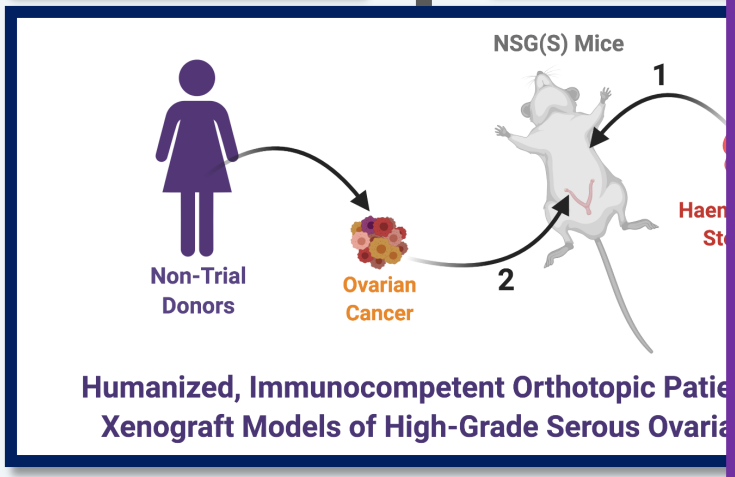
Characteristic (n = 25)	n (%)
Mean age, years (SD)	64.7 (8.2)
Prior chemotherapy	
Only first-line	4 (16)
1 line for relapse	11 (44)
2 lines for relapse	3 (12)
3 lines for relapse	1 (4)
>3 lines for relapse	6 (24)
ECOG Performance Status	
0	10 (40)
1	15 (60)
BRCA mutation	
Yes	3 (12)
No	19 (76)
Missing	3 (12)

Mirza, MR, et al. ESGO 2021

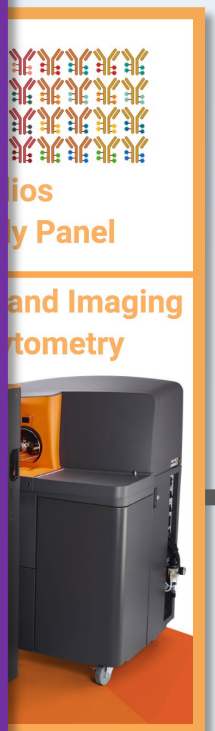
Relapsed High-Grade Serous Ovarian Cancer - 25 Patients

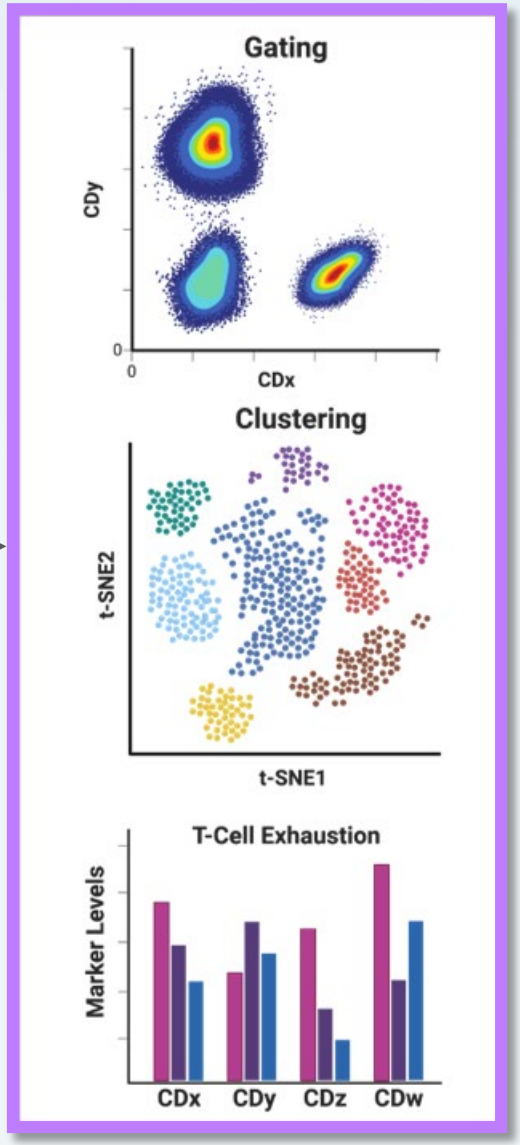






Humanized, Immunocompetent Orthotopic Patie
Xenograft Models of High-Grade Serous Ovaria





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Project Objectives

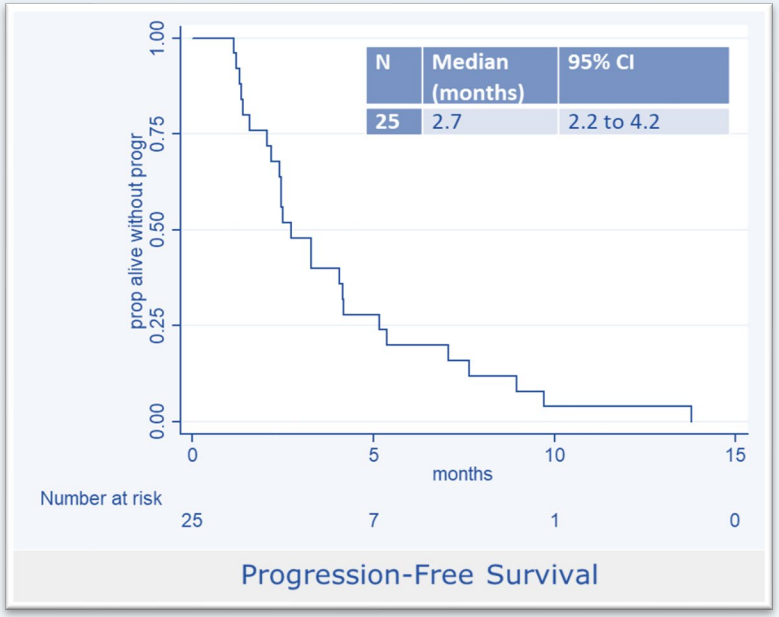
- GENERAL OBJECTIVE: Describe the value of combined CD73 and PD-L1 blockade in patients with relapsed HGSOC.

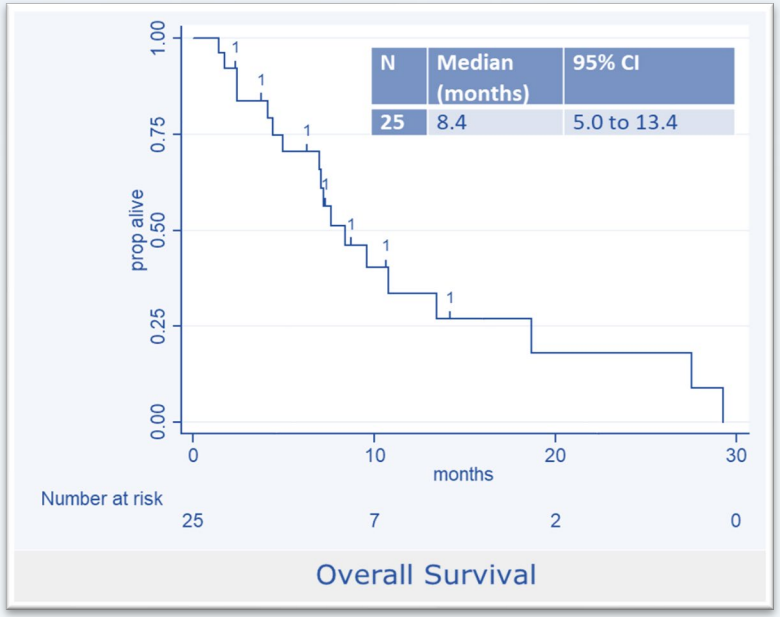
- TRANSLATIONAL OBJECTIVES:
  - Identify biomarkers associated with clinical outcome parameters:
    - **Primary endpoint:** DCR at 16 weeks
    - **Secondary endpoints:** PFS at 6 and 12 mos.; OS; ORR; DoR; Safety and tolerability
  - Establish criteria for improved patient selection for studied treatment.
  - Test combinatorial immunotherapy in preclinical ovarian cancer mouse models.
  - Portray the *in vivo* activity of the study drugs (oleclumab & durvalumab).

Study Endpoint Results

- Efficacy (week 16):
  - Disease control – 6/22 (27%)
  - Response – 1/22 (5%)
  - Progressed – 16/22 (73%)

  - Median PFS: 2,7 mos.
  - Median OS: 8,4 mos.
- Manageable toxicity
- Phase III recommendation

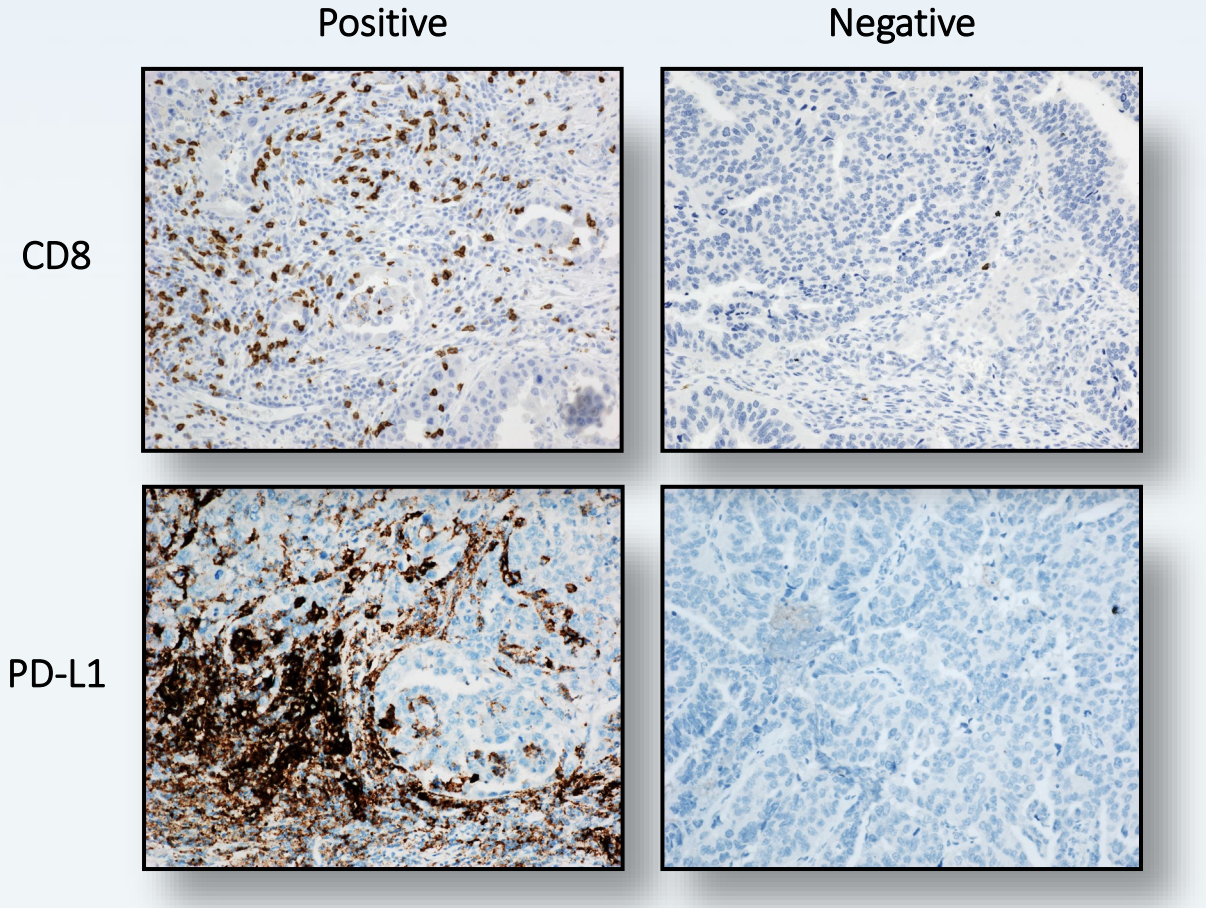




Mirza, MR, et al. ESGO 2021

Evaluation of Primary Tumor Biomarkers

- Correlation of CD8 / PD-L1 expression to response (DCR at week 16).
- Primary tumors (archival)
  - 3 slides from each patient (CD8, PD-L1, ctrl.)
- **74%** $CD8^+$, **42%** $PD\text{-}L1^+$, **37%** double-positive
- Spearman's rank correlation coefficient = 0,1707; p = 0,5124
  - Not significant.



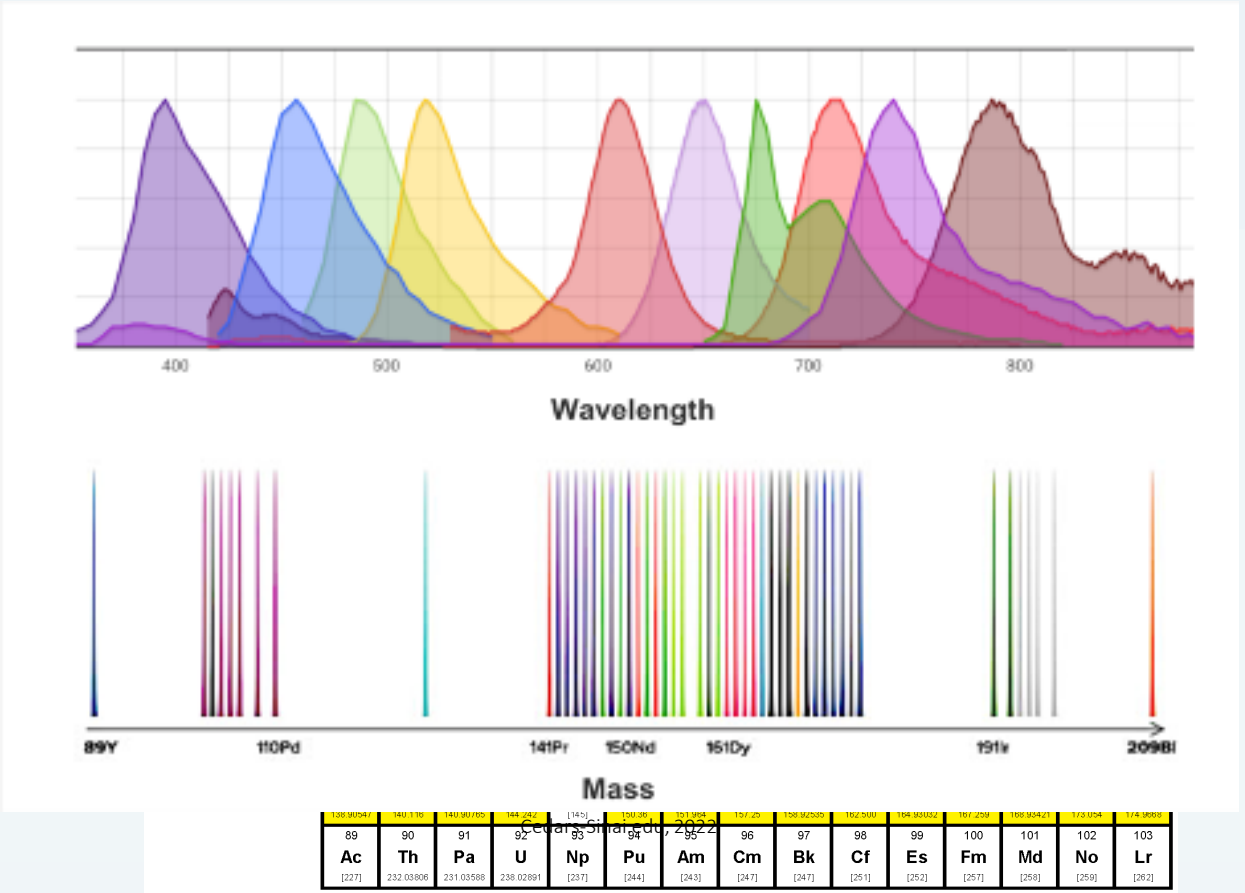
Positivity thresholds: PD-L1: ≥5% positive cell frequency; CD8: ≥5% area occupied by CD8+ cells

Mirza, MR, et al. ESGO 2021

Blood Biomarker Discovery – Suspension Mass Cytometry

CyTOF®XT™ mass cytometer

Standard BioTools Inc., 2021

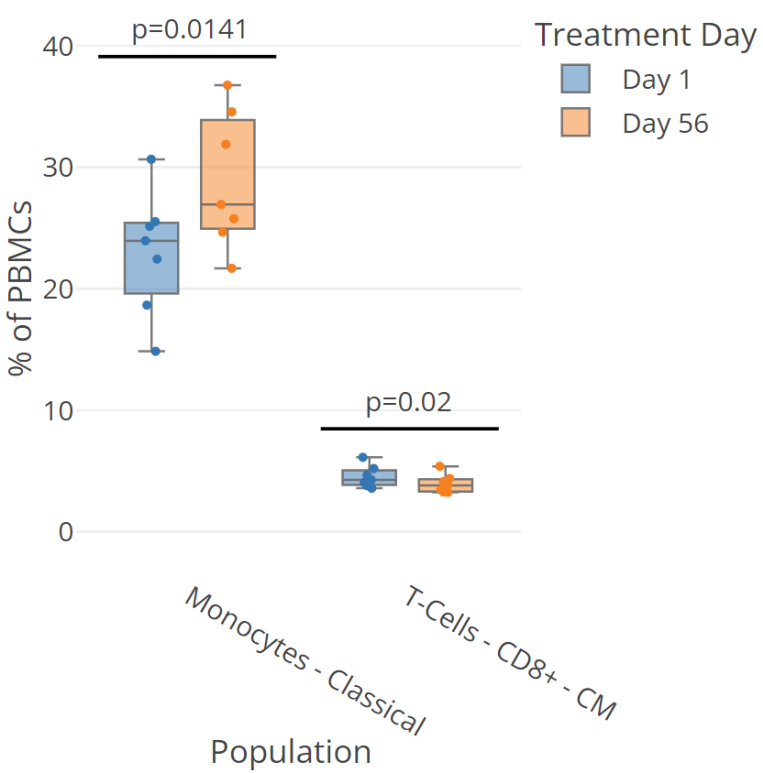


Blood Biomarker Discovery – 40-Marker Panel

CD1c	CD3	CD4	CD8	CD11b
CD11c	CD14	CD16	CD19	CD20
CD25	CD27	CD33	CD34	CD38
CD39	CD45	CD45RA	CD45R0	CD56
CD57	CD66b	CD73	CD86	CD123
CD141	CTLA-4	FoxP3	GranB	HLA-DR
IDO1	LAG-3	OX40	PD-1	PD-L1
p-CREB	p-S6	TCRgd	TIGIT	TIM-3

- T-Lymphocytes
- B-Lymphocytes
- Myeloid Lineage
- Other Leukocytes
- Exhaustion / Activation

Blood Biomarker Discovery – Preliminary Results

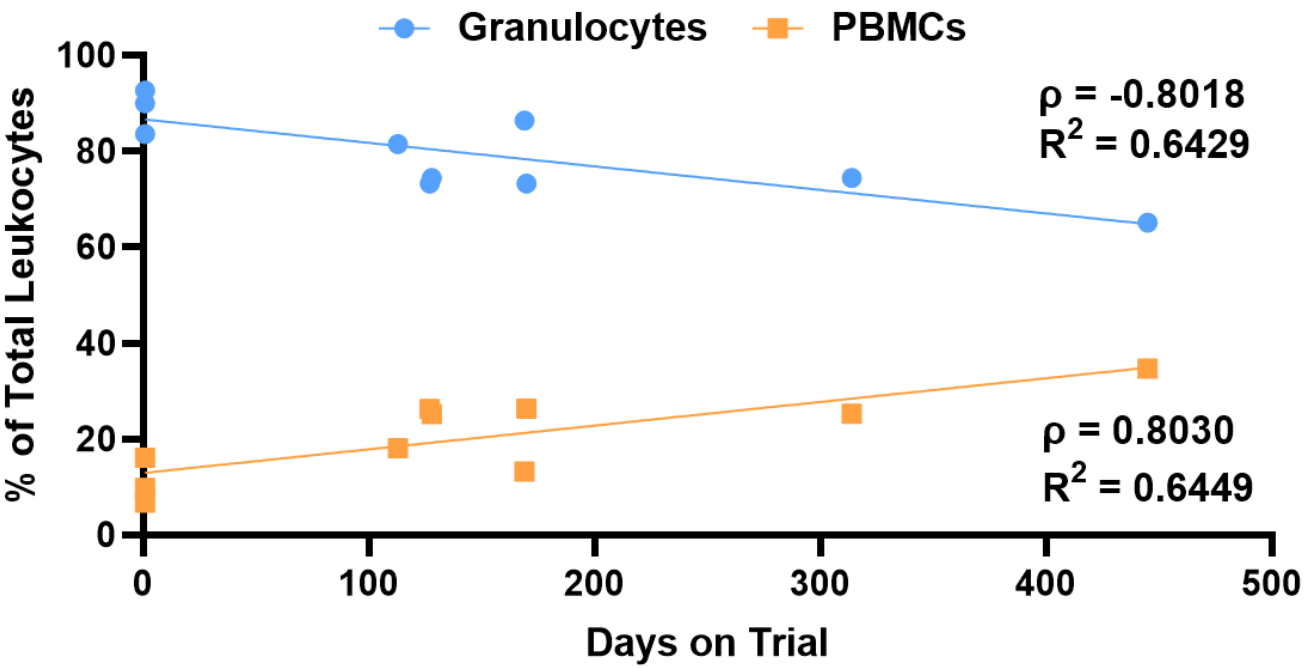


Baseline (Day 1) vs. Day 56 of treatment

- Significantly higher proportions of:
  - Classical monocytes
  - Central memory CD8+ T-cells

- Immunological response to immunotherapy

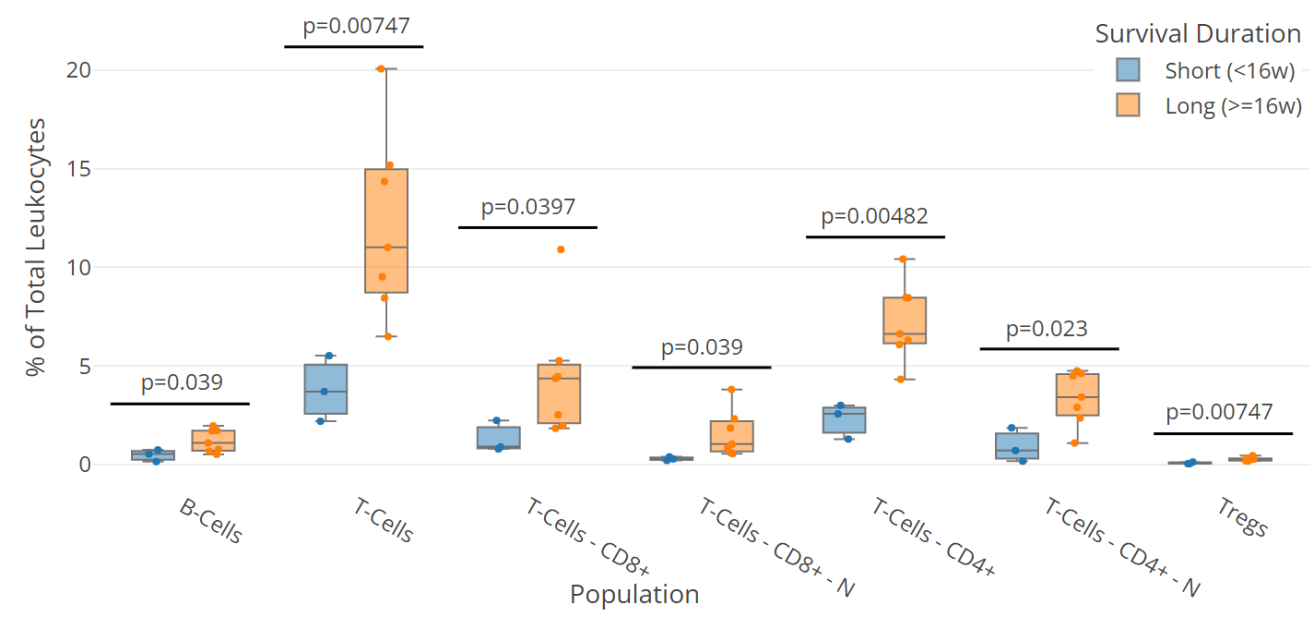
Blood Biomarker Discovery – Preliminary Results



Strong correlation (+ 0,8) between treatment length and proportion of PBMCs at baseline (Day 1)

Potential predictive biomarker

Blood Biomarker Discovery – Preliminary Results



Baseline (Day 1) PBMCs vs. Survival

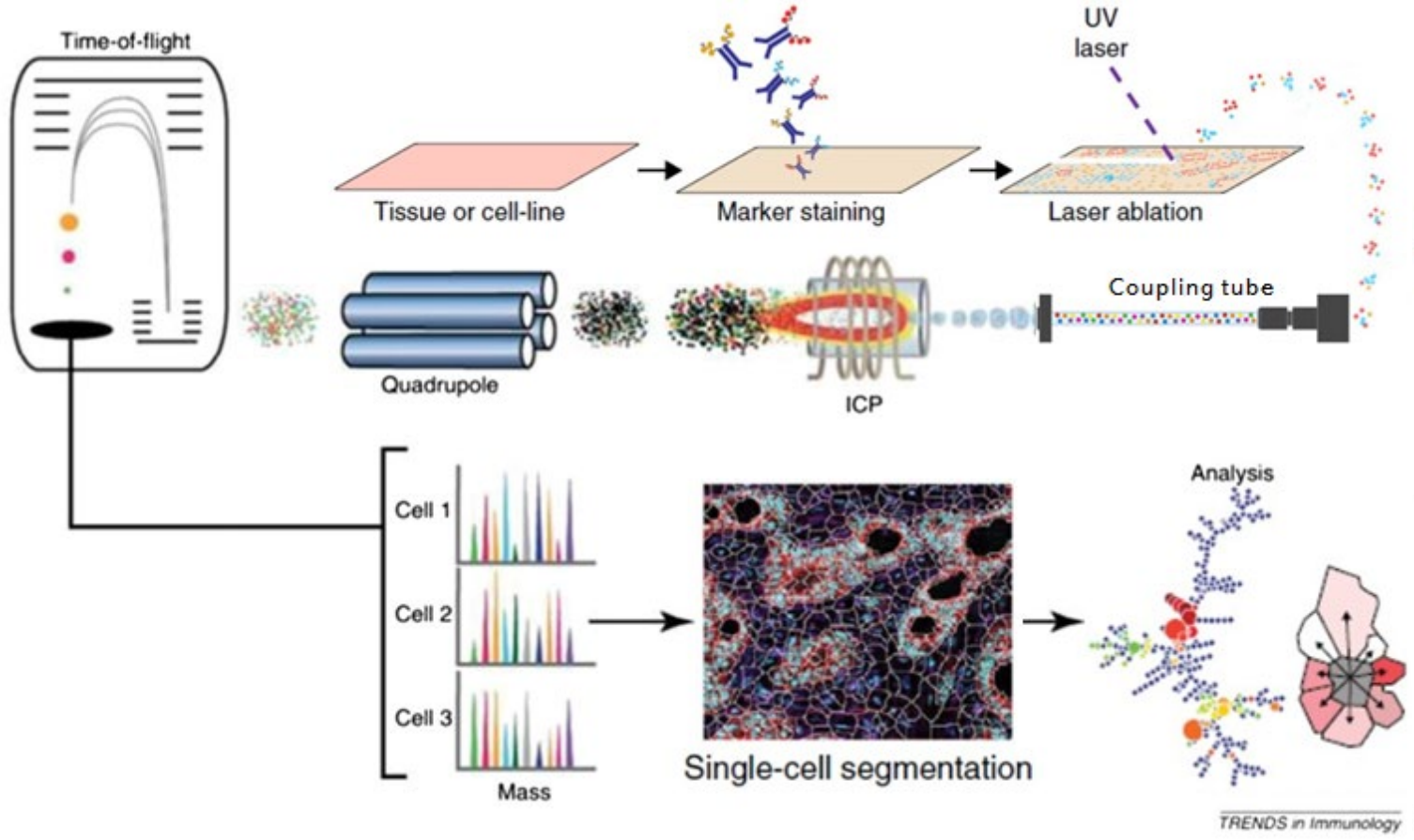
- Long-term survivors (>= 16wks) show higher proportions of:
  - B-cells
  - Total T-cells
  - Total and Naïve CD4+ & CD8+ T-cells
  - Tregs

- Potential predictive biomarkers

Tissue Biomarker Discovery – Imaging Mass Cytometry

- Tissue section
- Laser ablation
- Spatial data

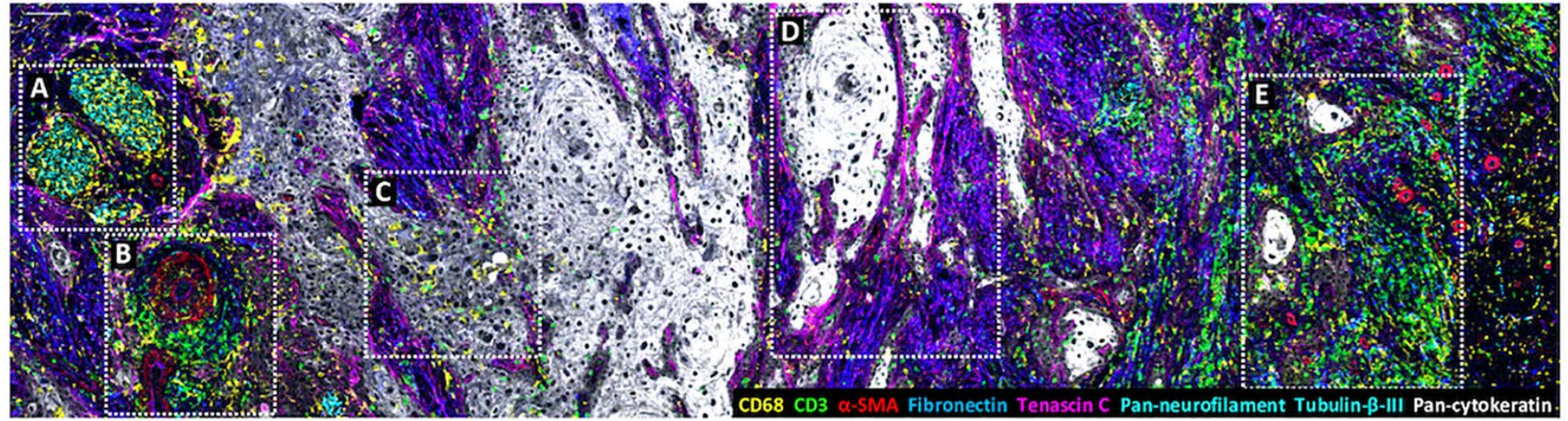
Hyperion™ mass cytometer

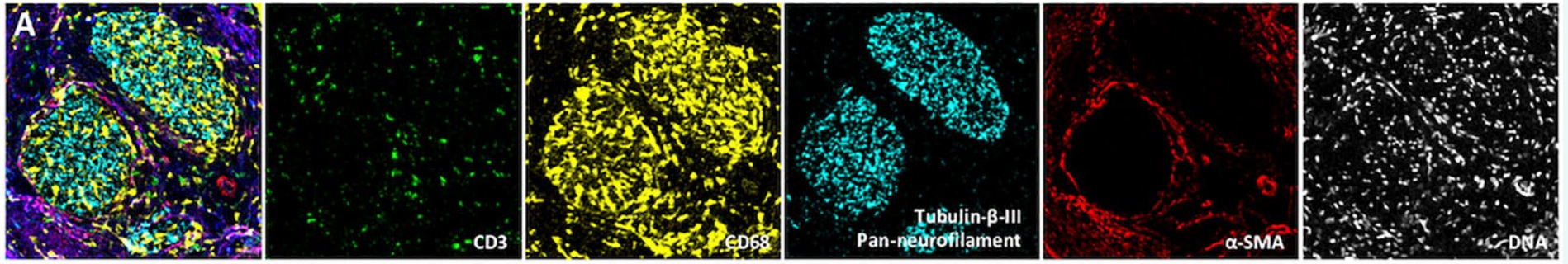


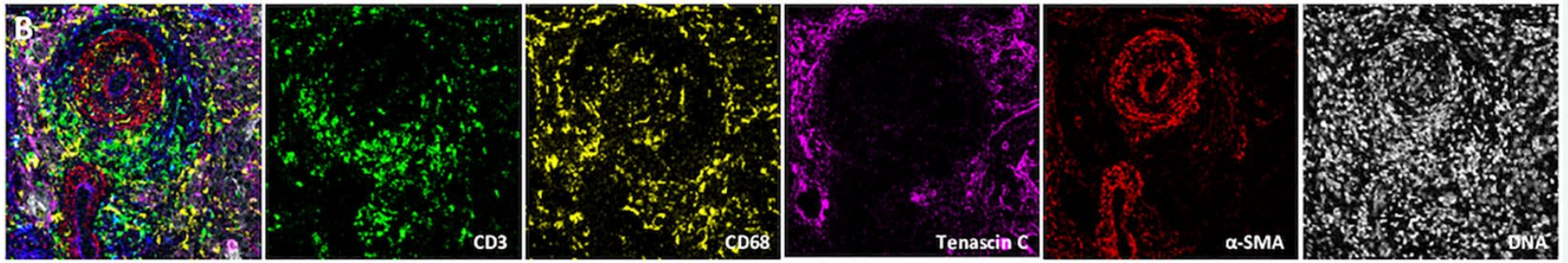
Adapted from Kondala et al., 2015

CyTOF®XT™ mass cytometer

Standard BioTools Inc., 2021







Elaldi et al., 2021

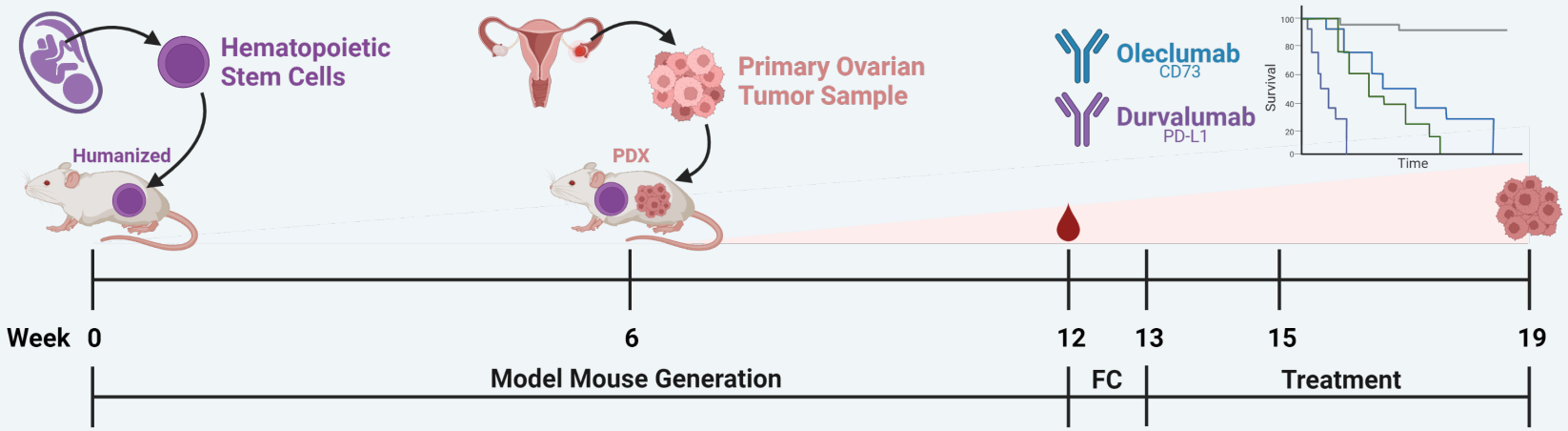
Tissue Biomarker Discovery – 38-Marker Panel

CD3	CD4	CD8	CD11b	CD11c
CD14	CD15	CD16	CD19	CD25
CD27	CD28	CD31	CD39	CD45
CD45R0	CD56	CD68	CD73	CD103
CD123	CD206	CD298	CTLA-4	EpCAM
FAPa	Fibronect.	FoxP3	GranB	HLA-DR
LAG3	PAX8	PD-1	PD-L1	aSMA
TIGIT	TIM-3	Vimentin		

- T-Lymphocytes
- B-Lymphocytes
- Myeloid Lineage / Macrophages
- Other Leukocytes
- Exhaustion / Activation
- Tumor
- Stroma / Fibroblasts
- Vasculature

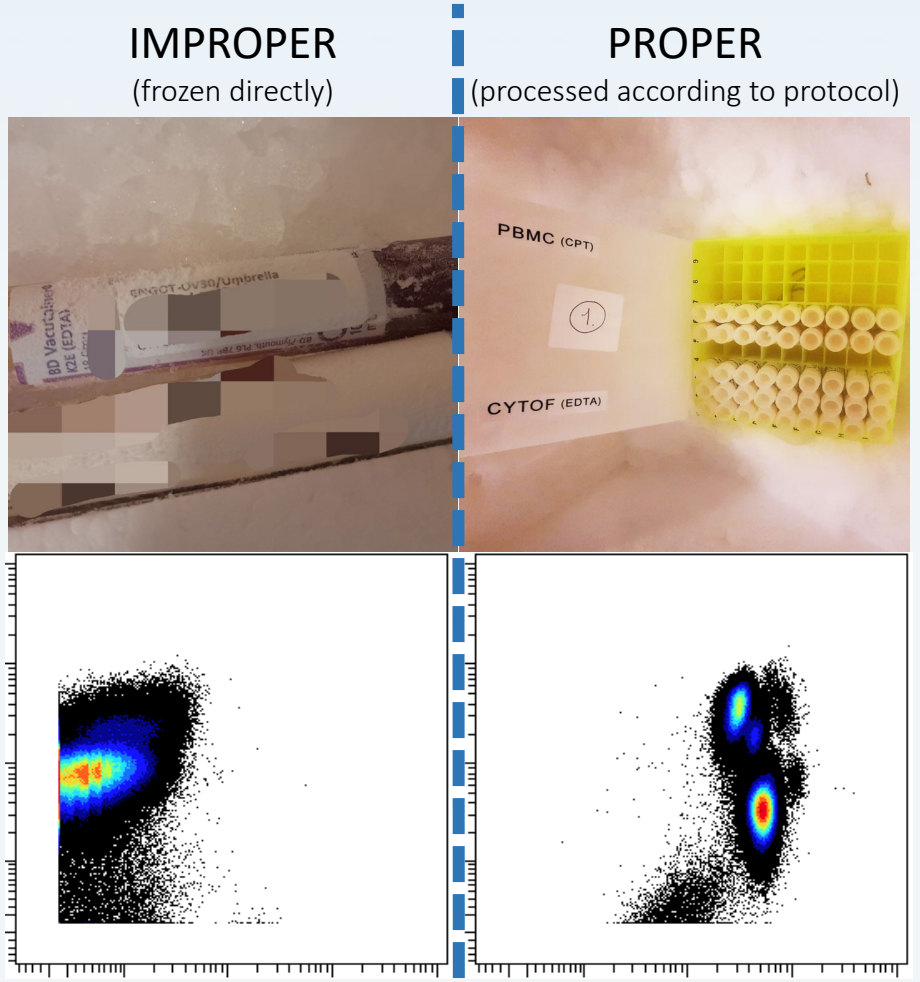
Humanized Mouse Models

- Two orthotopic PDX models
  - Previously established, ready for xenotransplantation
  - Non-trial, therapy-naïve HGSOC
  - Known CD73 and PD-L1 status
- Humanized mice (18 mice per PDX model)
  - NSGS strain (highly immunodeficient)
  - HSC from umbilical cord blood
- Oleclumab + durvalumab treatment
  - Combinatorial (n=5), single (n=5 x 2), saline control (n=3)
  - 20 mg/kg, IP
- Tumor sampling at endpoint
- Analysis with imaging mass cytometry panel with added anti-mouse antibody.



Sample Inconsistency

- Same instructions for all sites – different results.
- Sampling issues
  - 50% (40/77) improperly processed or not taken at all.
  - 50% (37/77) in pristine condition.
  - 10 / 25 patients.
- Significant loss of statistical power.



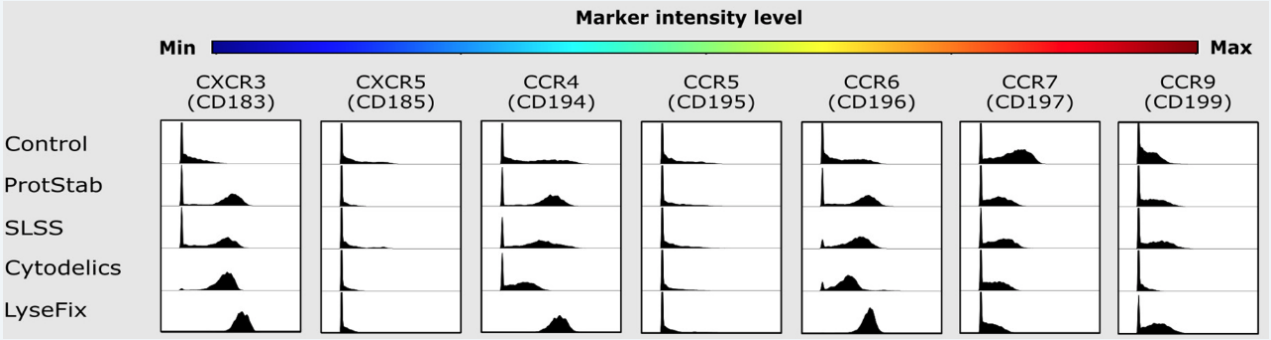
Large clots in improperly processed samples

Sample Inconsistency - Prevention

- Protocol writing
  - Prioritize clarity
  - Remove presumptions of previous knowledge
  - Translation into native language
  - Demonstration meetings
  - Tutorial videos
  - Time / quality ratio
- Feasible study nurse scheduling

Choice of Sample Preservation Method

- Evaluate preservation method beforehand
  - Loss of information vs. protocol complexity
- UMBRELLA trial: Lyse/Fix
  - Simple protocol, but harsh fix
    - (4% PFA, 1,5% MeOH, diH_2O)
  - Epitope destruction
- SmartTube ProtStab
  - Add to blood → freeze.



Sakkestad et al., 2019

Thank you for your attention.

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