## THE UNIVERSITY OF TEXAS MDAnderson Cancer Center



Making Cancer History®

### Spatial multi-omics investigation of high-grade serous ovarian cancer tumor microenvironment provides insight into minimal residual disease and intrinsic chemoresistance

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## High-grade serous ovarian cancer (HGSC)



- ~13,770 deaths in 2021 in the United States (Siegel et al. CA: A Cancer Journal for Clinicians, 2021).
- High-grade serous ovarian cancer (HGSC) is the most common histotype
  - Typically presents as aggressive advanced-stage disease
  - Accounts for over 70% of all ovarian cancer deaths (Coleman et al. Nature Reviews Clinical Oncology, 2013).
  - The current treatment regime of surgery followed by 6+ cycles of chemotherapy, or neoadjuvant chemotherapy followed by interval debulking surgery and adjuvant chemotherapy.
- MD Anderson uses follow up laparoscopy (6-8 weeks) to look for minimum residual disease (MRD)
  - No biomarkers to predict development of MRD or mechanism of chemoresistance



## Spatial multi-omics



- Different techniques that enable detailed molecular analyses directly from tissue
  - Spatial transcriptomics
    - Gene expression data
    - e.g. <u>Visium</u> (10X), CosMx (NanoString)
  - Multiplexed immunofluorescence (mxIF)
    - Cells and cell typing information
    - e.g. CODEX (Akoya), **<u>COMET</u>** (Lunaphore)
  - Mass Spectrometry Imaging (MSI)
    - Molecular information that isn't detectable using other techniques
    - Metabolites, lipids, glycans...
    - e.g. timsTOF fleX (Bruker)

#### **TECHNOLOGY FEATURE** 25 January 2022

#### Seven technologies to watch in 2022

#### **Spatial multi-omics**

The explosion in single-cell 'omics development means researchers can now routinely derive genetic, transcriptomic, epigenetic and proteomic insights from individual cells – sometimes simultaneously (see <u>go.nature.com/3nnhooo</u>). But single-cell techniques also sacrifice crucial information by ripping these cells out of their native environments.

Spatial multi-omics strategy to examine cellular and molecular heterogeneity of tumour immune microenvironment (Ferri-Borgogno S., Burks J., et al., Cancers 2024 - doi.org/10.3390/cancers16050846)

## Materials + Methods - Tissue stack generation



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### Measurement Stack (6 modalities)



**Visium (~55 µm)** - MD Anderson

Aspect Analytics

→ Spatial Transcriptomics

### Immunofluorescence (<1 µm) -**MD** Anderson

20 markers

#### MSI (20 µm) - performed by Erin Seeley (UTA)

- metabolites
- glycans
- peptides
- post -measurement H&E



### Why combine modalities?

Immunofluorescence (IF) provides high resolution images where we can **identify** and spatially segment **individual cells** 

The IF markers allow us to find the **"type"** of the cell using **previous knowledge** 

#### **MSI** provides **novel molecular information** that can only be obtained using the technique





### Multi-modal MSI workflow



(Ferri-Borgogno S., Burks J., et al., Cancers 2024 - doi.org/10.3390/cancers16050846)



### **Tissue measurement stack fusion**





## Tissue measurement stack fusion









### **Tissue measurement stack fusion**





## Weave registration tool for manual, non-rigid registration, MSI $\rightarrow$ post MSI H&E



## Multiplex immunofluorescence cell typing 22 markers $\rightarrow$ 29 cell types



#### Cell phenotyping using Hierarchical Prior Knowledge



Single cell mean





Automatic: Find threshold by fitting **Gaussian Mixture** Model on distribution of IF intensity



Probability that a cell is positive to a marker



Follow phenotype workflow



## Multiplex immunofluorescence cell typing 22 markers $\rightarrow$ 29 cell types



		CD45	Col1A1	aSMA	PanCK	CD31	Ki67	FAP	CD3e	CD8	CD4	CD56	CD11b	CD20	FOXP3	TIGIT	GZMB	CD68	CD66b	CD163	CD86
all	Other Immune cells	pos		]																	
all	Stroma		pos																		
all	CAFs			pos																	
all	Tumor				pos																
all	Endothelial cells			S		pos															
Tumor	Proliferating tumor cells						pos														
Stroma	Col1A1+ CAFs			pos		1								1							
CAFs	FAP+ CAFs							pos													
Col1A1+ CAFs	Col1A1+ FAP+ CAFs							pos													
Stroma	FAP+ Stroma							pos													
Other Immune cells	CD3 T cells								pos												
Other Immune cells	CD8 T cells			S						pos											
Other Immune cells	CD4 T cells										pos										
Other Immune cells	NK cells			1								pos									
Other Immune cells	Myeloid Lineage												pos								
Other Immune cells	B cells								neg					pos							
CD3 T cells	CD3 CD8 T cells									pos											
CD3 T cells	CD3 CD4 T cells										pos										
CD3 T cells	NKT Cells											pos									
CD4 T cells	Regulatory T cells			а С		1									pos						
CD8 T cells	TIGIT+ CD8 T cells															pos					
CD8 T cells	TIGIT- Activated CD8 T cells			1												neg	pos				
NK Cells	TIGIT+ NK Cells															pos					
NK Cells	TIGIT- Activated NK Cells															neg	pos				
Myeloid lineage	Macrophages																	pos			
Myeloid lineage	Neutrophils																		pos		
Myeloid lineage	Myeloid drived suppressor cells (MDSC)			9										1					neg		
CD3 CD8 T cells	TIGIT+ CD3 CD8 T cells															pos					
CD3 CD8 T cells	TIGIT- Activated CD3 CD8 T cells													1		neg	pos				
NKT Cells	TIGIT+ NKT Cells															pos					
NKT Cells	TIGIT- Activated NKT Cells															neg	pos				
Macrophages	M2 Macrophages																			pos	
Macrophages	M1 Macrophages																				pos
TIGIT+ CD3 CD8 T cells	TIGIT+ Activated CD3 CD8 T cells							1									pos				
TIGIT+ NK Cells	TIGIT+ Activated NK Cells																pos				
TIGIT+ NKT Cells	TIGIT+ Activated NKT Cells			1				1		1							DOS				1

A wealth of biological knowledge goes into designing the panel of IF markers

## Multiplex immunofluorescence cell typing 22 markers $\rightarrow$ 29 cell types



		CD45	Col1A1	aSMA	PanCK C	D31 H	Ki67 F.	AP CD	3e CD	08 CD4	CD56	CD11b	CD20	FOXP3	TIGIT	GZMB	CD68	CD66b C	CD163	B CD8	nhenotype	
all	Other Immune cells	pos								-											plicitotype	
all	Stroma		pos																		Ĩ	Proliferating tumor cells
all	CAFs			pos																		Regulatory T cells
all	Tumor				pos																	TIGIT+ CD8 T cells
all	Endothelial cells		-		p	os							-		-							Endothelial cells
Tumor	Proliferating tumor cells					F	oos															CD3 CD4 T cells
Stroma	Col1A1+ CAFs			pos																		FAP+ Stroma
CAFs	FAP+ CAFs					-	p	os												-	and the second	FAP+ CAFs
Col1A1+ CAFs	Col1A1+ FAP+ CAFs					-	p	os			1									-		Myeloid Lineage
Stroma	FAP+ Stroma		-				p	os									1					CD4 T cells
Other Immune cells	CD3 T cells							pos	3													B cells
Other Immune cells	CD8 T cells	-	-			-			pos	s					-			-				TIGIT+ Activated CD3 CD8
Other Immune cells	CD4 T cells		-	-		-	-		-	DOS	-		-		-			S				NK cells
Other Immune cells	NK cells	-	2	-		-			-		DOS						-			-		Stroma
Other Immune cells	Myeloid Lineage		<u>.</u>	-						-	1	DOS										CD3 T cells
Other Immune cells	B cells					-		nec	1	-	-	pee	DOS		-		-			-		Col1A1+ FAP+ CAFs
CD3 T cells	CD3 CD8 T cells		-			-			,	s .	-				-		-					TIGIT+ CD3 CD8 T cells
CD3 T cells	CD3 CD4 T cells	-		2				-		nos					-							Col1A1+ CAFs
CD3 T cells	NKT Cells		-						-	poo	DOS											CAFs
CD4 T cells	Begulatory T cells		2			-			-	-	000			005			-			-		TIGIT+ NKT Cells
D8 T cells		-		8		-					e			poo	005							TIGIT+ Activated NKT Cells
CD8 T cells	TIGIT- Activated CD8, T cells	-	-	-				-		-	-				nea	2005						CD8 I Cells
NK Cells			-	-							-				nos	pos				+		Other Immune cells
IK Celle	TIGIT- Activated NK Cells		1				_	-	-						nea	008						TIGIT- Activated CD3 CD8
Mucleid lineage	Macrophage	-		0							2 3				neg	pos	000					TIGIT- Activated NKT Cells
hyeloid lineage	Neutrophile					-		32		22	10 12						pos	000				NKT Cells
Veloid lineage	Myeloid drived suppressor cells (MDSC)		-			-	_										-	peg		-		
CD3 CD8 T cells	TIGIT+ CD3 CD8 T cells	, 	-			-+			-		-				nos		-					
CD3 CD8 T cells	TIGIT- Activated CD3 CD8 T cells			-							-				nea	DOS						
NKT Cells	TIGIT+ NKT Cells			-				_		_	-				nos	200				+		
NKT Cells	TIGIT- Activated NKT Cells										+				nea	DOS	-					
Macrophages	M2 Macrophages	-									-				. iog	200			nos	+		
Macrophages	M1 Macrophages			2											5 B			P		pos		
TIGIT+ CD3 CD8 T cells	TIGIT+ Activated CD3 CD8 T cells		-						-	0						pos						
TIGIT+ NK Cells	TIGIT+ Activated NK Cells					-										pos						
		-		-			-	-		-	-				-	200	-	-		-		

## Consolidating and coordinating multimodal data







MSI pixels 1-n... Aggregation to the MSI pixel level....



A consolidated data structure enables making cross modality queries



weave

## Consolidating and coordinating multimodal data













#### Filtering for MSI pixels with purer cell populations

#### Aggregation to the MSI pixel level...

## Consolidating and coordinating multimodal data













#### Filtering for MSI pixels with purer cell populations



## **Correlating molecular readouts**



#### Data Pairs



### **Correlating molecular readouts**

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Mean correlations between Glycan and Peptide in MRD+ samples 0.2 0.0 -0.2 mz: 1809.6416 - Hex5dHex1HexNAc4 mz: 1809,0416 - HeX3dHeX1HeXNAC4 mz: 2100,7378 - HeX3dHeX1HeXNAC4NeuAc1 mz: 1298.4474 - HeX4HexNAc3 mz: 1647.5905 - HeX4dHeX1HeXNAC4 mz: 2174.7175 - HeX6dHeX1HeXNAC5 mz: 1444.5085 - HeX4dHeX1HeXNAC3 mz: 1663.5834 - Hex5HexNAc4 mz: 1743.5859 - Hex8HexNAc2 mz: 1419.4779 - Hex6HexNAc2 m2: 1419.4779 - HexbreixNAc2 m2: 1905.6416 - Hex9HexNAc2 m2: 1905.6416 - Hex9HexNAc2 m2: 1782.4524 - Hex5HexNAc2 m2: 1782.4525 - Hex3Hex1HetMAc3 m2: 1282.4525 - Hex3Hex1HetMAc3 m2: 1285.5373 - Hex3Hex1HexMAc4 m2: 2283.7591 - Hex5HexNAc4NeuAc2 m2: 2396.8306 - Hex5HexNAc4NeuAc2 + 2Na m2: 2306.8306 - Hex5HexNAc4NeuAc2 + 2Na m2: 2306.8316 - Hex3Hex1HexAc7 m2: 1295.4746 - Hex5HexNAc4NeuAc1 m2: 1295.4746 - Hex5HexNAc4NeuAc1 mz: 1954.6746 - HeckSHextMac4NeuAc1 mz: 1460.5004 - HeckSHextMac4NeuAc1 mz: 2025.1125 - HeckSHextMac4 mz: 2539.2012 - HeckSHextMac4 mz: 2539.3039 - HeckTdHextMac4 mz: 245.7702 - HeckSHextMac4NeuAc2 mz: 2455.8669 - HeckGHextHextMac5 mz: 1455.6669 - HeckGHextHextMac5 mz: 1455.6669 - HeckGHextHextMac5 mz: 1606.558 - Hex5dHex1HexNAc3 mz: 1608.6154 - Hex3dHex1HexNAc3 mz: 1257.4229 - Hex3dHex1HexNAc5 mz: 1257.4229 - Hex3dHex1Ac2 mz: 1079.3721 - Hex3dHex1HexNAc2 mz: 1095.367 - Hex4HexNAc2\_ miz: 1093.307 = Nex4HexNAc2 mz: 1704.6058 - Hex4HexNAc5 mz: 1995.7176 - Hex4HexNAc5NeuAc1 + 2Na mz: 2215.8112 - Hex5dHex1HexNAc6 mz: 1403.486 - Hex5dHex1HexNAc2 mz: 1403.460 + Hex3dHex1HexNAc6 mz: 1996.7157 - Hex3dHex1HexNAc5 mz: 2012.7233 - Hex5dHex1HexNAc5 mz: 974.3445 - Hex2HexNAc3 III.: 974.3443 - INEX.ENANG3 mz: 2742.9755 - Hex7dHex1HexNAc7 mz: 2588.9007 - Hex6HexNAc5NeuAc2 mz: 2889.0514 - Hex7dHex2HexNAc7 mz: 2902.0089 - Hex6HexNAc5NeuAc3 + 2Na mz: 2902.0089 - Hex6HexNAcSNeuAc3 + 2Na mz: 3270.1572 - Hex8dHex2HexNAc3 mz: 3196.1212 - Hex8dHex2HexAAc4 Mz: 1398.1212 - Hex8dHex1HexNAc7NeuAc1 + 2Na mz: 1393.477 - Hex3HexNAc4 mz: 2377.8542 - Hex8dHex1HexNAc6 mz: 2506.9162 - Hex3dHex1HexNAc6 mz: 2508.9162 - Hex3dHex1HexNAc6 mz: 2658.9603 - Hex7dHex2HexAAc6 mz: 2658.9603 - Hex7dHex2HexAAc6 mz: 1381.4852 - Hex5dHex1HexNAc2 mz: 2361.8577 - Hex5dHex2HexNAc6 mz: 2320.8268 - Hex6dHex2HexNAc5 mz: 932.0.226 - Hex6HexNAc2 mz: 933.3171 - Hex3HexNAc2 mz: 1866.6564 - Hex5HexNAc5 mz: 1241.4243 - Hex5HexNAc5 mz: 1679.5551 - Hex5HexNAc4 mz: 1679.5551 - Hex5HexNAc4 mz: 771.2636 - Hex2HexNAc2 III: //1.2030 - HEXCHEXNAC2 mz: 2610.9218 - HEX6HEXNAC5NeuAc2 mz: 2319.8175 - HEX6HEXNAC5NeuAc1 mz: 2568.9718 - HEX6HEXNAc6NeuAc1 + 2Na mz: 1971.6953 - Hex6dHex1HexNAc4 mz: 2830.995 - Hex7dHex1HexNAc6NeuAc1 + 2Na 7123 701.9052 -1198. 493.7481 :zu :zu mz:



## Differential correlations



### MRD+



MRD-

## Differential MSI signals by cell type aggregation (tumor vs CAFs)



Tumor

#### m/z 1742.5924 -Hex5HexNAc4NeuGc1\_mouse

m/z 1325.757 - histone H4

#### m/z 128.0354 - Pyroglutamic acid



# Differential MSI signals by cell type aggregation (CAFs vs Tumor)



Cancer Associated Fibroblasts





m/z 2369.8306 -Hex5dHex1HexNAc4NeuAc2 + 2Na





m/z 89.0246 - L-Lactic acid





## Differential MSI signals by cell type aggregation (proliferating tumor vs tumor)





Proliferating tumor (PanCK+/Ki67+)

Tumor (PanCK+)

m/z 2028.7172 - Hex6HexNAc5



m/z 1742.5924 -Hex5HexNAc4NeuGc1



## Spatial distance based exploration of Tumor-Stroma interface



Area No	ormaliz	ed Cell C	ounts per	Relative	Interface	Distance 199	Distance Bins × 2	29 Cell Typ	<b>\$</b> -
- Distance Bin Set	B cells CAFs	CD3 CD4 T cells CD3 CD8 T cells CD3 T cells	CD4 T cells CD8 T cells CD8 T cells Col1A1+ CAE	Col1A1+ FAP+ CA Endothelial cells FAP+ CAFs	FAP+ Stama Myeloid Bineage NK cells <u>e</u>	NKT Cel <b>ig</b> Other Immune celi, Proliferating tumor Regulatory T	Strom <sub>a</sub> TIGIT <sub>+</sub> Activated C TIGIT <sub>+</sub> Activated C TIGIT <sub>+</sub> CD3 CD8 : TIGIT <sub>+</sub> CD3	TIGIT + NKT Cell. TIGIT + NKT Cell. TIGIT - Action 1 C TIGIT - Action 1 C	Unknown
istance Bins									
Ö									



## Spatial distance based exploration of Tumor-Stroma interface





## Conclusion



- We demonstrate a multimodal data acquisition workflow for combined spatial multi-omics
- We describe tooling and a cross modality data structure for correlative, differential, and spatial multimodal data analysis
- Using cell phenotype information, we found interesting preliminary MS markers
- We preliminary describe the tumor microenvironment with spatial analysis of tumor interface using both cell type and MSI data



## Thank you for your attention!

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Making Cancer History®

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Erin Seeley



#### Aspect Analytics team



Want to know more and see this data live?

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