Reduction of Tumor-Infiltrating B Cells Linked to Recurrence of NSCLC Tumors

AACR Annual Meeting 2020
Abstract# 408

Anna Juncker-Jensen • Mate Nagy • Lakshmi Chandramohan • Erinn Parnell • Brigitte Lovell • Lea Moore • Judy Kuo • Eric Leones • Qingyan Au • Josette William
NeoGenomics Laboratories, Aliso Viejo, CA

Background and Results

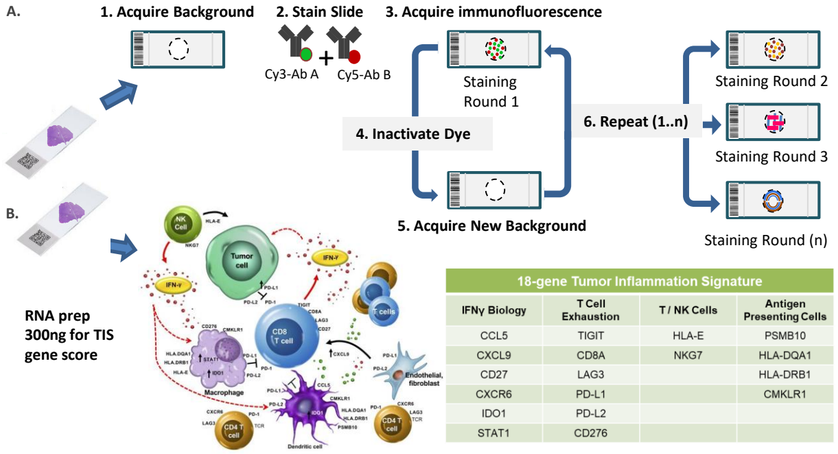
Background: Lung cancer is the leading cause of cancer related mortality worldwide. Non-small cell lung cancer (NSCLC), accounting for approximately 80-85% of all lung cancer cases, is characterized by a poor response to chemotherapy and a low survival rate. Early stage NSCLC patients are typically treated with complete surgical resection of the tumor, but nevertheless 30-50% of these patients end up developing recurrence within 5 years of surgery. While the presence of tumor-infiltrating lymphocytes (TILs) has been shown to be significantly correlated with a positive clinical prognosis in multiple types of cancer the role of tumor-infiltrating B cells (TIBs) remains more controversial.

Methods: To perform comprehensive immunoprofiling of NSCLC FFPE tumor samples we used MultiOmyx™, a proprietary immunofluorescent multiplexing assay. Using a 16-marker panel we have analyzed the proportion of T cell subtypes, B cells, Granulocytes, M1/M2-type tumor-associated macrophages, as well as the expression of PD-1, PD-L1, LAG-3, TIM-3, ICOS, and OX40 in 12 samples from patients with early stage (stage I-II) NSCLC (6 non-recurrent and 6 recurrent). We used an adjacent 10 µm section for a parallel gene analysis of the Tumor Inflammation Signature (TIS) score using the Nanostring PanCancer IO360 Gene expression panel.

Results: Using the MultiOmyx image analysis suite based on NeoGenomics' proprietary deep-learning algorithms, we compared the number and overall density of immune cell populations between non-recurrent and recurrent NSCLC samples and found recurrent samples to have a highly significant decrease in B cells (p-value 0.006). In addition to a decrease in the overall density of B cells, we also observed a decrease in the tumor-infiltration rate, measured as the % of cells found inside the tumor area versus in the stroma of the tumor samples. This demonstrates that not only are there fewer B cells in the recurrent samples, but the B cells present are also less likely to infiltrate into the tumor area.

We did not find the TIS score to be associated with NSCLC recurrence in this study. This correlates with our MultiOmyx T cell protein data in which we did not observe any difference in density between the two groups either.

Multiplexing Setup – MultiOmyx™ (protein) & NanoString TIS (mRNA)

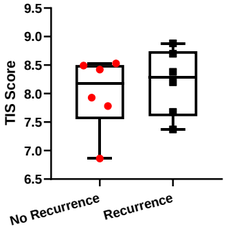


18-gene Tumor Inflammation Signature			
IFNγ Biology	T Cell Exhaustion	T / NK Cells	Antigen Presenting Cells
CCL5	TIGIT	HLA-E	PSMB10
CXCL9	CD8A	NKG7	HLA-DQA1
CD27	LAG3		HLA-DRB1
CXCR6	PD-L1		CMKLR1
IDO1	PD-L2		
STAT1	CD276		

Figure 1. Assay Workflow. Two adjacent sections were cut from each FFPE tumor sample. **A. MultiOmyx multiplexing IF staining protocol**. For each round of staining, conjugated fluorescent antibodies were applied to a 4 µm section, followed by image acquisition of stained slides. The dye was erased, enabling a subsequent round of staining with another pair of fluorescent antibodies. Proprietary cell segmentation algorithms generate unique IDs for every cell allowing them to be tracked through multiple rounds of staining. **B. NanoString nCounter assay**. RNA was extracted from an adjacent FFPE section for analysis of a Tumor Inflammation Signature (TIS) containing 18 genes (**Table 1**) that measure a peripherally suppressed immune response and distinguishes tumors as immune hot and cold (Ayers et al, JCI 2017).

NanoString TIS

TIS was not significantly associated with NSCLC recurrence in this study.



Patient Cohort Characteristics

CHARACTERISTICS OF NSCLC PATIENT POPULATION								
Diagnosis	Age	Sex	Stage	TNM	PD-L1 TC%	Therapy	Disease Status	TIS
Adeno	57	F	IA	T1aN0MX	100	Surgery only	No recurrence	8.42
Adeno	62	F	IB	T2aN0MX	90	Surgery only	No recurrence	7.78
SCC	74	M	IB	T2aNXMX	60	Surgery only	No recurrence	8.49
SCC	68	M	IIA	T1bN1MX	60	Chemotherapy	No recurrence	8.53
Adeno	0	M	IA	T1bN0MX	0	Chemotherapy Radiation	No recurrence	6.86
SCC	58	M	IIIA	T1bN2MX	50	Chemotherapy Radiation	No recurrence	7.93
Adeno	76	M	IIA	T2bN0MX	95	Radiation	Recurrent	7.37
Adeno	80	M	IB	T2aN0MX	90	Surgery only	Recurrent	8.38
Adeno	59	F	IIA	T1aN1MX	95	Chemotherapy Radiation Nivolumab	Recurrent	8.7
Adeno	70	F	IB	T2aN0MX	40	Chemotherapy Radiosurgery	Recurrent	8.88
SCC	59	M	IB	T2aN0MX	25	Chemotherapy	Recurrent	8.2
Adeno	72	M	IIA	T1bN1MX	0	Chemotherapy	Recurrent	7.68

Figure 2. 300ng of RNA was extracted from FFPE sections adjacent to the FFPE section used for MultiOmyx analysis, and analyzed according to NanoString nCounter protocol. **Table 2. Study Cohort Information.**

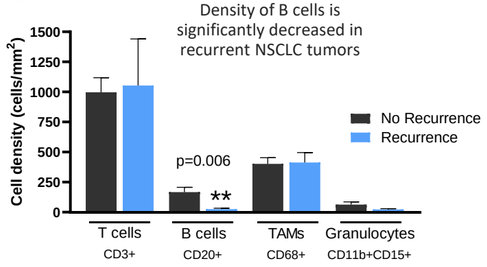
MultiOmyx Panel

A.

16-Marker Panel		
#	Cy3	Cy5
1	CD15	PD-L1
2	PanCK	ICOS
3	CD11b	LAG-3
4	CD3	OX40
5	CD8	TIM-3
6	CD4	FoxP3
7	CD20	CD163
8	CD68	PD-1

NSCLC Immuno Phenotypes

B.



B CELL CHARACTERISTICS				Granulocytes	
	Mean Density (#cells/mm²)				
NSCLC Diagnosis	CD20+ cells	CD20+PD1+ cells	CD20+TIM3+ cells	CD15+ cells	CD11b+CD15+
No recurrence	166.8	3.9	4.7	109.7	62.9
Recurrent	26.2 **	0.7*	1.2	76.1	23.6

Figure 3. A, MultiOmyx Protein Panel Compositon. B, Bargraph displaying densities of T cells, B cells, tumor-associated macrophages (TAMs), and granulocytes in non-recurrent versus recurrent NSCLC samples. **Table 3,** Densities of B cells co-expressed with PD-1 or TIM-3, and granulocytes.

MultiOmyx Images – Immune cells in NSCLC tumors

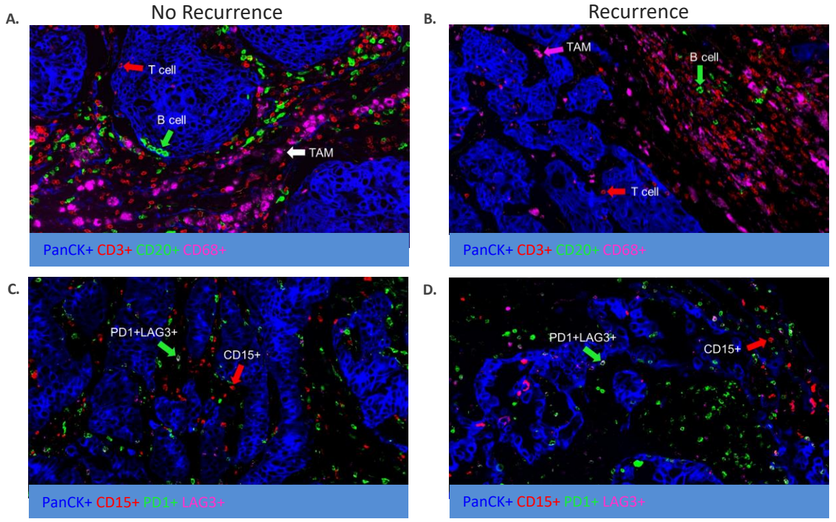


Figure 4. Multiplexed overlay images of NSCLC tumors. In **A+B**, T cells (red), B cells (green), and TAMs (magenta) can be seen in the stromal compartment and infiltrating into the tumor area. **In C+D**, cells positive for CD15 (granulocytes) can be seen in red, while PD-1 positive cells are green. In both C + D cells co-expressing PD-1 and LAG-3 are indicated by green arrows.

MultiOmyx Spatial Analytics – Tumor Infiltration

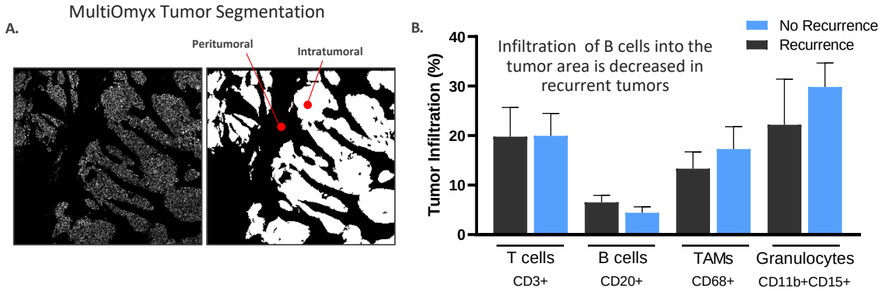


Figure 5. MultiOmyx Analytics Workflow. A, Proprietary cell segmentation algorithms generate unique IDs for every cell allowing them to be tracked through multiple rounds of staining. Deep learning based cell classification algorithms identify positive cells for each and a tumor marker such as pan-cytokeratin is used to generate a tumor mask in order to classify all cells as intra- or peri-tumoral. **. B,** Tumor infiltration is calculated as the % of cells found inside the tumor mask, out of the total number of cells.

Key Findings

- Evaluating B-cell density in primary NSCLC tumors is a good biomarker that can predict prognosis and recurrence in this patient population.
- The spatial location of B-cells (intratumoral versus stromal) should also be considered as a tool for the prediction of NSCLC prognosis.
- Further studies to sub-classify the infiltrating B-cells and analyze potential interactions with T cells via the CD40/CD40L pathway will be performed to evaluate the effect of B-cells on NSCLC recurrence.

anna.juncker-jensen@neogenomics.com