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INTRODUCTION

Chemoimmunotherapy has improved outcomes in both resectable and unresectable NSCLC, yet primary and acquired resistance remain major challenges. Tertiary lymphoid structures (TLS) and tumor-draining lymph nodes (TDLNs) play critical roles in antitumor immunity, but their spatial immune organization and contributions to treatment resistance remain poorly understood. Using Xenium single-cell spatial transcriptomics, we aimed to characterize spatial immune programs in primary tumors and regional lymph nodes and identify mechanisms and biomarkers associated with T-cell dysfunction, impaired tumor infiltration, and resistance to chemoimmunotherapy in resectable (n=6) and unresectable (n=3) NSCLC.

METHODS

We developed a 480-gene panel to assess tumor- and immune-related gene expression in primary tumors and adjacent lymph nodes from patients with resectable (n=6) and unresectable (n=3) NSCLC following chemoimmunotherapy (chemoICI) or chemotherapy (chemo). Resistance was defined as lack of tumor downstaging after neoadjuvant therapy or disease progression in advanced disease. A pilot Xenium single-cell spatial transcriptomics assay was performed on 16 samples from 10 patients (Table 1) using two tissue microarrays (TMAs), each containing eight 4-mm cores.

Figure 1. Design of the Xenium Panel for NSCLC

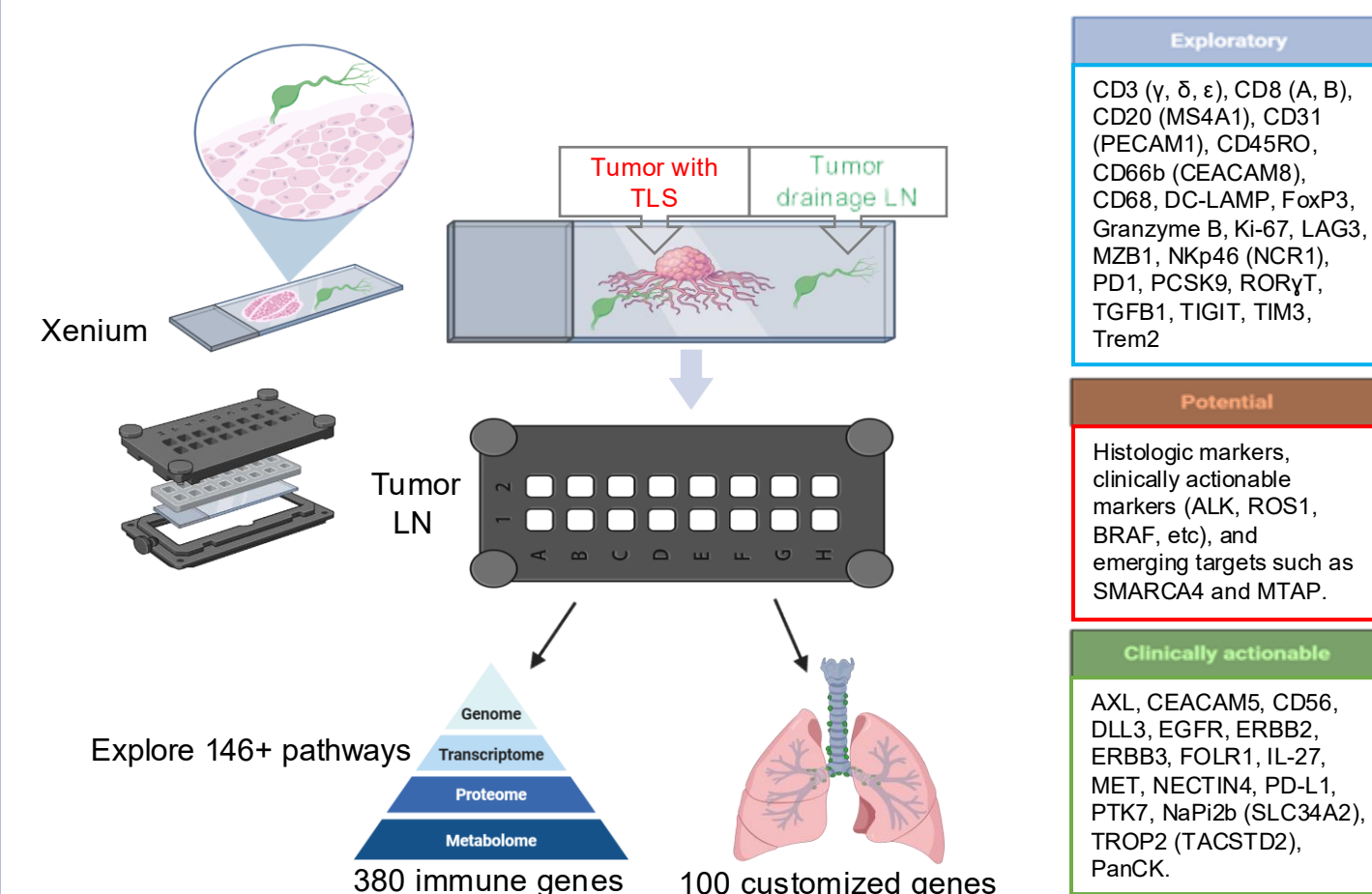
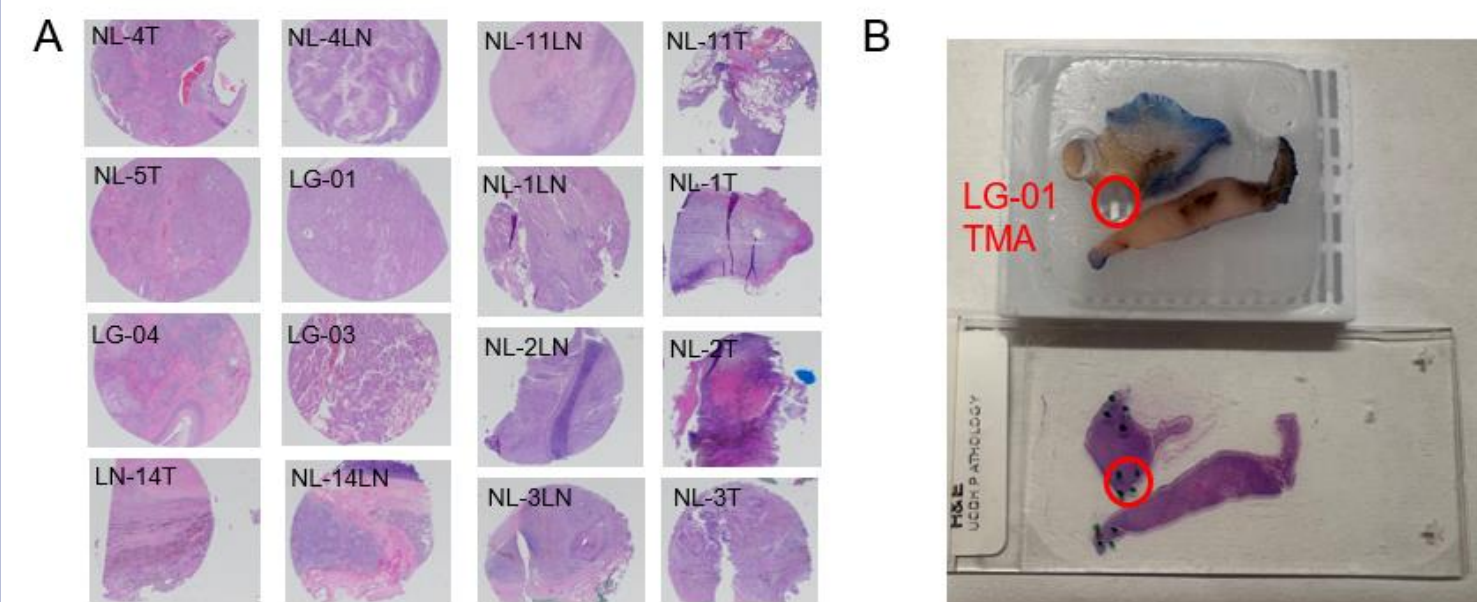


Figure 2. Construction of TMAs



DISCUSSION AND CONCLUSION

We developed a 480-gene spatial transcriptomic panel to characterize mechanisms of resistance to chemoimmunotherapy and chemotherapy in NSCLC. Our findings reveal spatially distinct and potentially targetable tumor-nodal immune programs, supporting the integration of lymph node profiling into biomarker development and compartment-specific therapeutic strategies to overcome treatment resistance.

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RESULTS

Table 1. Clinicopathologic characteristics, tumor and immune biomarkers, and treatments of the study cohort.

ID	Age	Sex	Histology	Stage Before Treatment	Stage After Surgery	Down-staging history	Smoking history	Treatment	Tumor Res	AGA Biomarkers	Immune Biomarkers
NL-11	78	M	LUSC	IIIA (cT4N0M0)	pCR (ypT0pN0)	Yes	Former	chemoICI	Sensitive	No	PD-L1 TPS 70%
NL-14	76	M	NOS (PSC)	IIIA (cT3N1M0)	IA1 (ypT1aN0M0)	Yes	Former	chemoICI	Sensitive	MET E14sp mutation	PD-L1 TPS 98%, TMB 3.2 muts/Mb
NL-1	74	M	LUSC	IIIA (cT3N1M0)	IA2 (ypT1bN0M0)	Yes	Former	chemoICI	Sensitive	Not available	PD-L1 TPS 60%, CPS 80%
NL-4	67	M	LUSC	IB (cT2aN0M0)	IIIB (ypT1bN1M0)	No	Former	chemoICI	Resistant	No	PD-L1 TPS<1%, TMB 7.4 muts/Mb
NL-5	74	M	LUAD	IIIA (cT4N0M0)	IIIA (ypT4N0M0)	No	Former	chemoICI	Resistant	No	PD-L1 TPS 1%, TMB NA; MSS
LG-01	66	F	LUAD	IIIA (cT4N0M0)	NA	NA	Former	chemoICI	Resistant	MET amp, PIK3CA	PD-L1 TPS 0, CPS 70%, TMB 24
LG-04	76	F	LUAD	IB (cT2N0M0G2)	IIIA (ypT4aN0M0)	No	Former	chemoICI	Resistant	KRAS G12C, STK11	PD-L1 TPS 0%, TMB, MSS
NL-2	77	F	LUAD	IIIA (cT1cN2M0)	IIIB (ypT1bN1M0)	Yes	Former	chemo	Residual tumor	KRAS G12C, MET/HGF amp	PD-L1 TPS 100%
NL-3	71	M	NOS	IIIB (cT3N2M0)	IIIB (ypT2bN1M0)	Yes	Former	chemo	Resistant	EGFR Exon 20 insertion	PD-L1 TPS 3%, TMB 11 muts/Mb
LG-03	70	F	LUAD	IIIA (cT4N0M0)	IVA (TxNxM1b)	NA	Never	chemo	Resistant	KRAS G12C, STK11	PD-L1 TPS 90%, TMB 7 muts/Mb

Figure 3. UMAP visualization of all cells across 16 samples using 10x Xenium data analysis pipeline. Across all sections, an average of 96,794 ± 31,737 cells and 12.5 ± 6.0 million transcripts per section were detected, with 40.1 ± 13.1 genes and 84.5 ± 37.0 transcripts per cell. A total of 29 cell types were identified, with cancer-associated fibroblasts (8.7%), plasma cells (8.1%), and tumor-associated macrophages (8.0%) predominating.

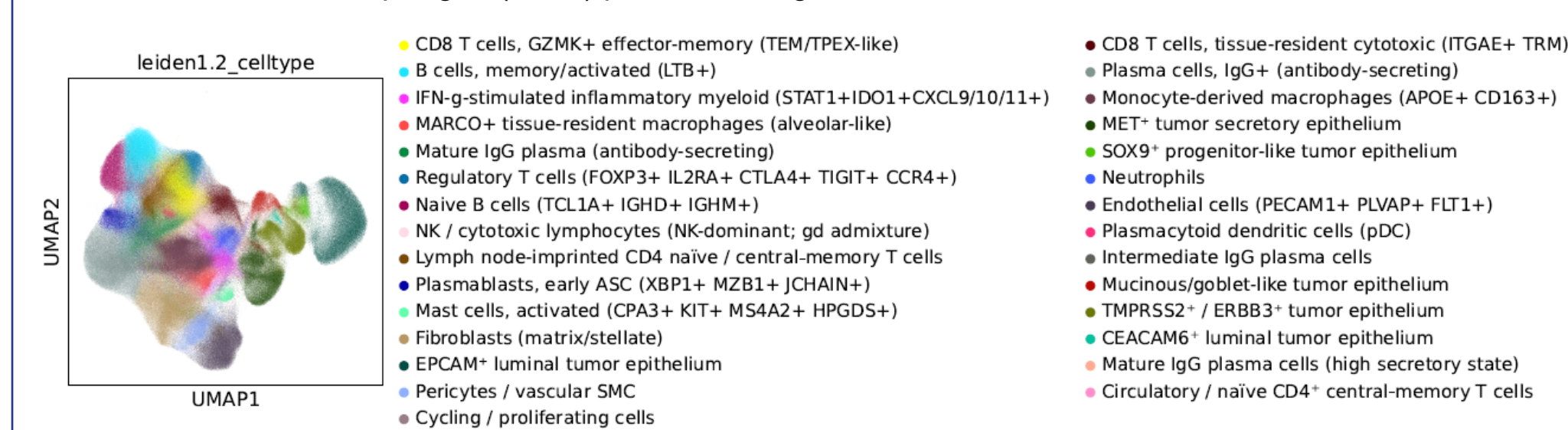


Figure 4. Differential gene expression between six paired primary tumors and regional lymph nodes after neoadjuvant chemoICI.

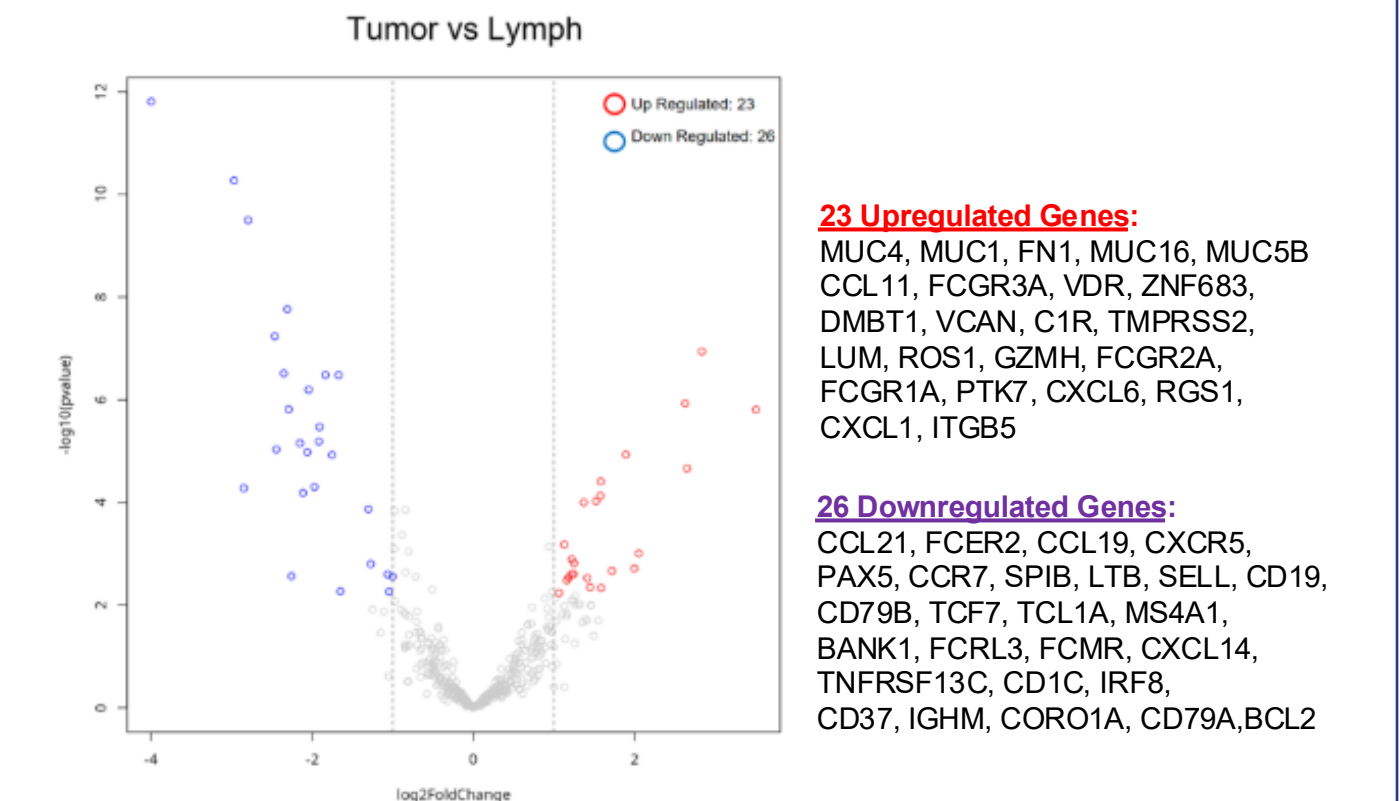
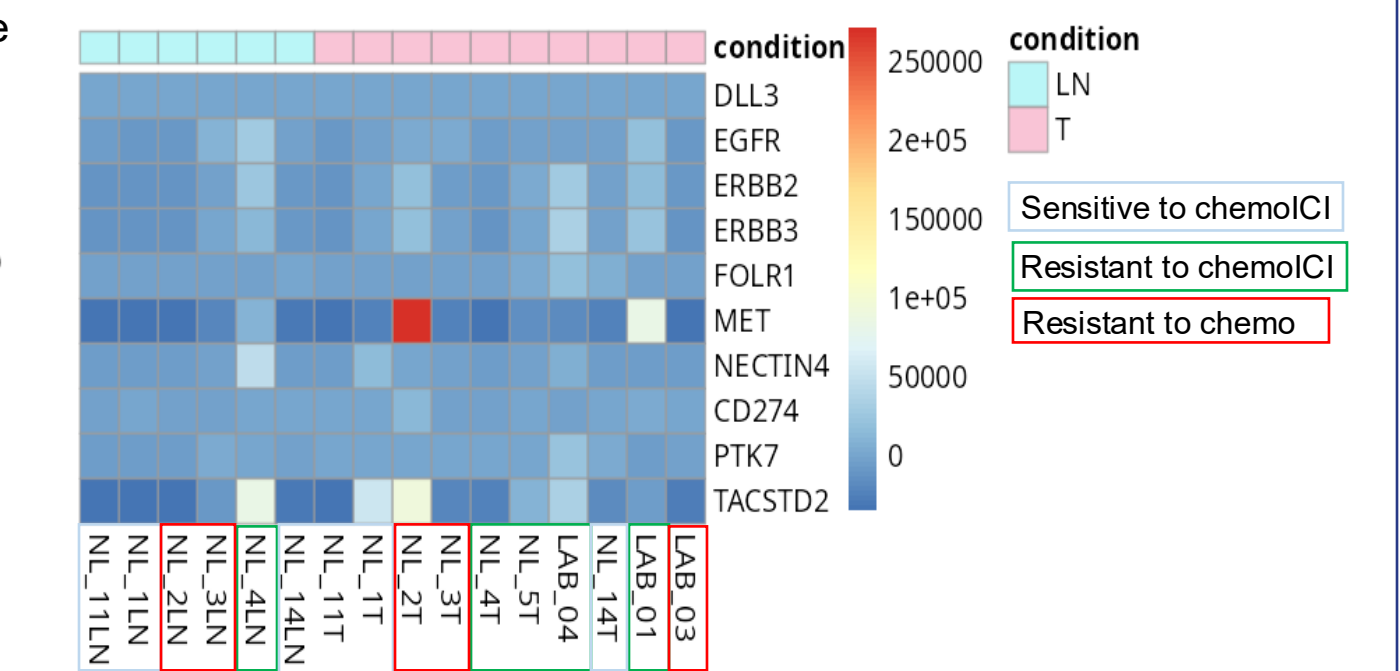


Figure 5. Potential actionable targets in NSCLC tumors refractory to chemoimmunotherapy or chemotherapy.



Spatial analyses revealed discordant expression patterns between tumors and lymph nodes.

Figure 6. Representative Xenium analysis of paired tumor and lymph node specimens from a patient with an EGFR exon 20 inserti on after neoadjuvant chemotherapy.

