Tumor Biology, Pathology, Novel Diagnostics

Tianhong “Tina” Li, MD, PhD

UC Davis Comprehensive Cancer Center, Sacramento, CA, United States

Presented by Tianhong “Tina” Li, UCDCCC, USA, thli@ucdavis.edu

Multi-omics and/or single cell profiling with advanced computational analysis (AI) for new biology and potential biomarkers

Abstract	Title	Focus
OA15.03	Clinicogenomic Landscape of Lymph Node Metastases in TRACERx	1. Tumor cell Clonality in Metastasis + minimal residual disease (MRD)
OA15.04	Multi-Omic Profiling of Paired Non-Small Cell Lung Cancer and Draining Lymph NodesReveals Novel T-Cell Differentiation Patterns	2. Origin and differentiation of Tumor infiltrating lymphocyte (TIL)
OA15.05	Single-Cell Spatial Architectures of Paratumor Zone Determines the Prognosis of Multiple Lung Cancers	3. Heterogeneity of immune cell infiltrates in primary vs. metastatic lung cancer

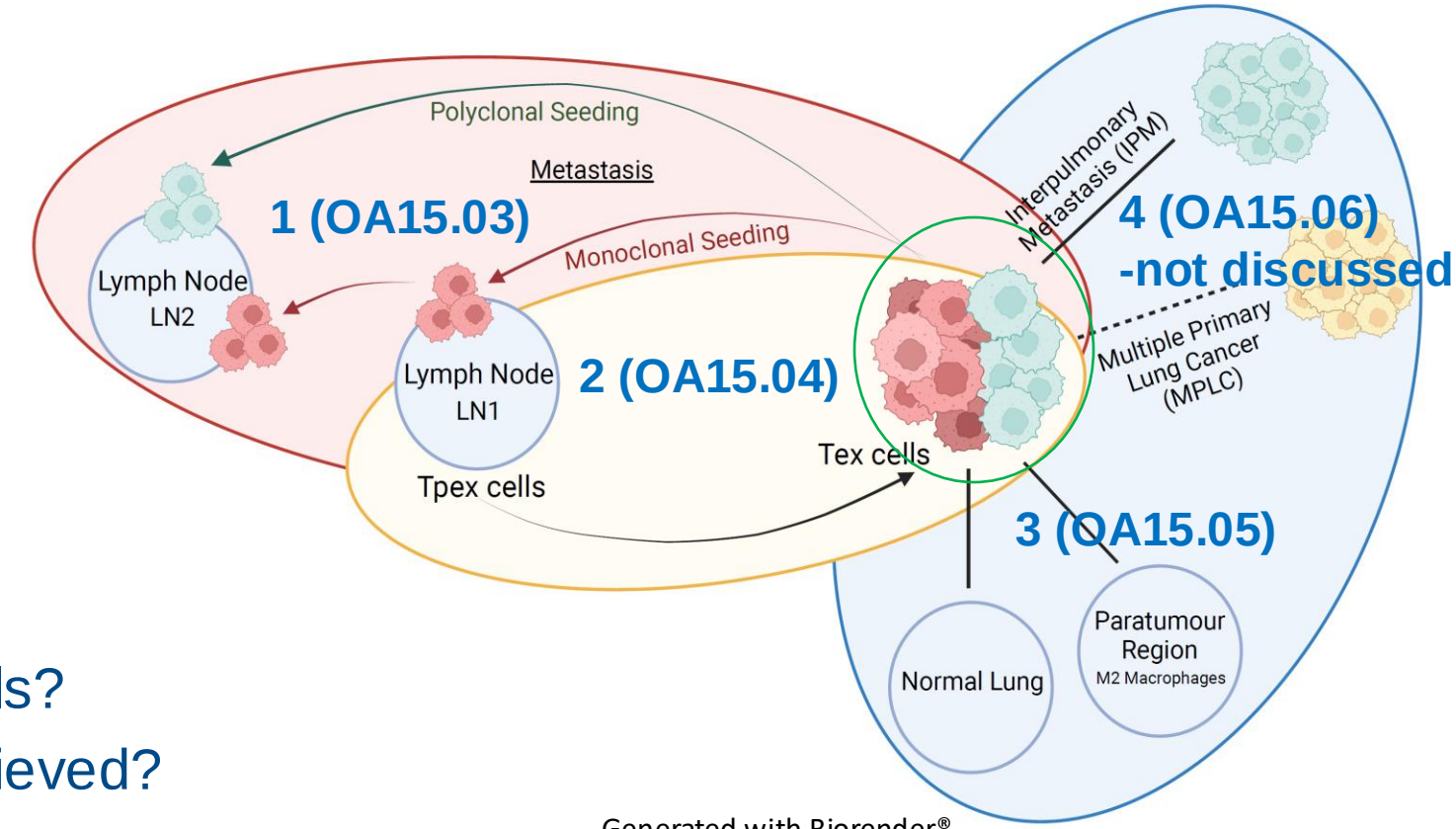
Presented by Tianhong "Tina" Li, UCDCCC, USA, thli@ucdavis.edu

Focus of discussion

- Clinical questions
- Biological questions

Additional issues

- Does the study have hypothesis?
- Does the study have specific goals?
- Have the specific goals been achieved?
- Do the study results have clinical impact?
- Have the results advanced our knowledge in lung cancer biology?



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#1: OA15.03

Clinicogenomic Landscape of Lymph Node Metastases in TRACERx

Takahiro Karasaki[1,2,3],
James Black[1,2], Alexander Frankell[1,2], Christopher Abbosh[1],
Ariana Huebner[1,2], Maise Al Bakir[1,2],
Nicholas McGranahan[2], Mariam Jamal-Hanjani[2], Charles Swanton[1,2]

1. Francis Crick Institute, London, UK
2. UCL Cancer Institute, London, UK
3. Toranomon Hospital, Tokyo, Japan (Current affiliation)

OA15.03: Clinicogenomic Landscape of Lymph Node Metastases in TRACERx

Clinical questions

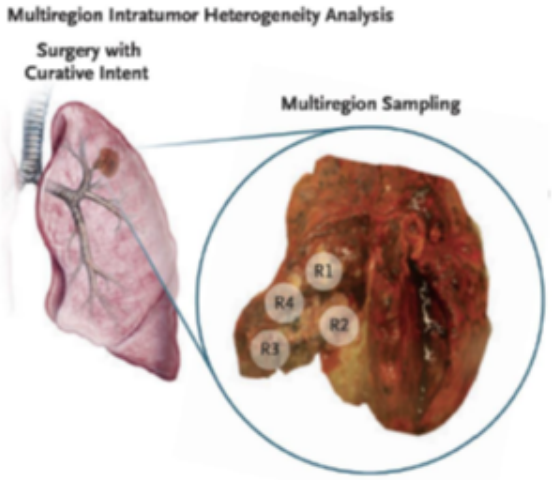
Clonal evolution

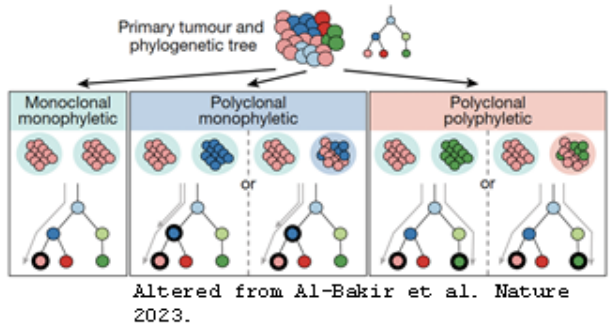
- Is there any difference in dissemination patterns to N1/N2 nodes?
- Are all LN metastases equal when there are multiple LN metastases?

Prognostic impact

- Why N2 disease is so different from N1 disease in terms of prognosis?
- Why surgical benefit is limited for N2 disease?

Part 1: Clonal evolution and dissemination to the LNs





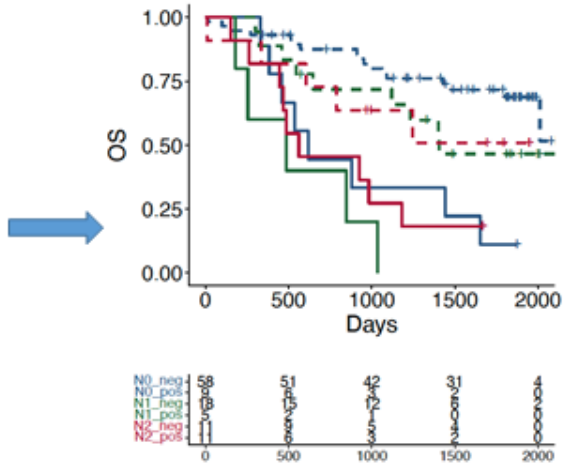
Altered from Al-Bakir et al. Nature 2023.

- Monoclonal? Polyclonal?
- N1? N2?
- Sequential N2? Skip N2?

Part 2: MRD analysis and nodal involvement

Tx421 ctDNA cohort
N=197 patients

Postop landmark analysis
(≥1 plasma samples within 120 POD, before adjuvant treatment or relapse,
known pN status)
N=116 patients



Take home messages

Clonal evolution and dissemination to the LNs

- Most LNs are seeded by a single clone from the primary tumor
- Approximately half of the cases with multi-station LN metastases had distinct clones that metastasized to each LN from the primary tumour.

→ Information from single LN may be insufficient to capture the holistic evolution of tumor clones when multiple LNs were involved.

MRD analysis and nodal involvement

- Landmark positive (MRD+ ≤120 days after surgery) was associated with TNM stage, especially with pN stage.
- Landmark negative patients had better OS than Landmark positive patients, regardless of pN status.

PMID: 37046095 (Al Bakir et al):

- No significant association between clonal dissemination pattern in LN and lung cancer-specific disease-free survival.
- Polyclonal dissemination was enriched for tumours that result in extrathoracic recurrence.

PMID: 37055640 (Abbosh et al):

- Shorter OS in patients with ctDNA exhibiting polyclonal dissemination versus monoclonal dissemination

Details in 2 published manuscripts

Article

The evolution of non-small cell lung cancer metastases in TRACERx

Nature 2023 Apr;616(7957):534-542. (PMID: 37046095)

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Maise Al Bakir[1,2,96], Ariana Huebner[1,2,3,96], Carlos Martínez-Ruiz[1,3,96], Kristiana Grigoriadis[1,2,3,96], Thomas B. K. Watkins[2,96], Oriol Pich[2,96], David A. Moore[1,2,4], Selvaraju Veeriah[1], Sophia Ward[1,2,5], Joanne Laycock[1], Diana Johnson[1], Andrew Rowan[2], Maryam Razaq[1], Mita Akther[1], Cristina Naceur-Lombardelli[1], Paulina Prymas[1], Antonia Toncheva[1], Sonya Hessey[1,6,7], Michelle Dietzen[1,2,3], Emma Colliver[2], Alexander M. Frankell[1,2], Abigail Bunkum[1,6,7], Emilia L. Lim[1,2], Takahiro Karasaki[1,2,6], Christopher Abbosh[1], Crispin T. Hiley[1,2], Mark S. Hill[2], Daniel E. Cook[2], Gareth A. Wilson[2], Roberto Salgado[8,9], Emma Nye[10], Richard Kevin Stone[10], Dean A. Fennell[11,12], Gillian Price[13,14], Keith M. Kerr[14,15], Babu Naidu[16], Gary Middleton[17,18], Yvonne Summers[19], Colin R. Lindsay[19], Fiona H. Blackhall[19], Judith Cave[20], Kevin G. Blyth[21,22,23], Arjun Nair[24,25], Asia Ahmed[24], Magali N. Taylor[24], Alexander James Procter[24], Mary Falzon[4], David Lawrence[26], Neal Navani[27,28], Ricky M. Thakrar[27,28], Sam M. Janes[27], Dionysis Papadatos-Pastos[29], Martin D. Forster[1,29], Siow Ming Lee[1,29], Tanya Ahmad[29], Sergio A. Quezada[1,30], Karl S. Peggs[31,32], Peter Van Loo[33,34,35], Caroline Dive[36,37], Allan Hackshaw[36], Nicolai J. Birkbak[1,2,38,40,41], Simone Zaccaria[1,7], TRACERx Consortium*, Mariam Jamal-Hanjani[1,6,29,97] ✉, Nicholas McGranahan[1,3,97] ✉ & Charles Swanton[1,2,29,97] ✉

Article

Tracking early lung cancer metastatic dissemination in TRACERx using ctDNA

Nature 2023 Apr;616(7957):553-562. (PMID: 37055640)

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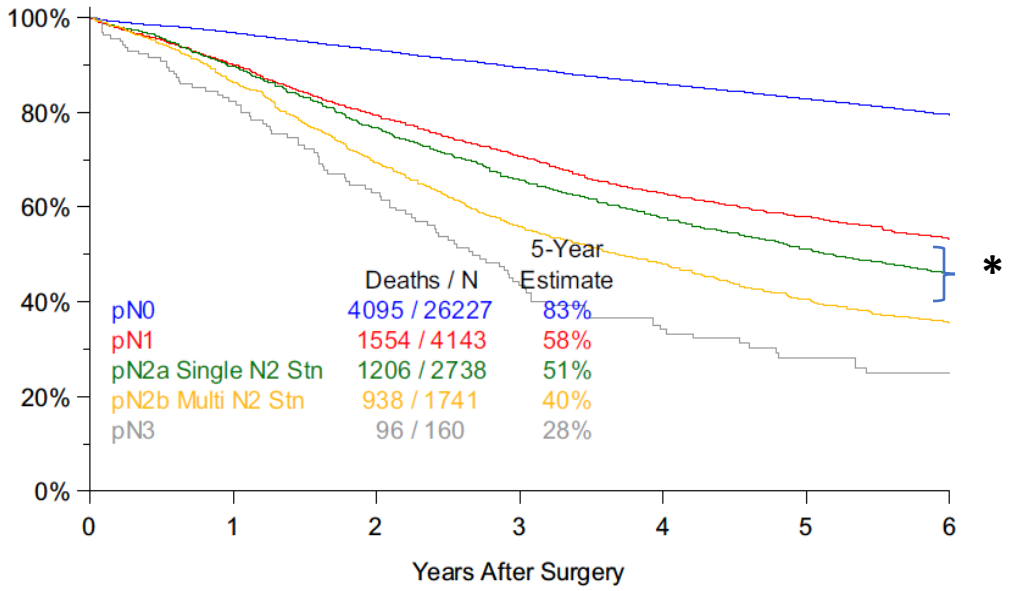
Christopher Abbosh[1,101] ✉, Alexander M. Frankell[1,2,101], Thomas Harrison[3,101], Judit Kisistok[4,5,6,101], Aaron Garnett[3,101], Laura Johnson[3], Selvaraju Veeriah[1], Mike Moreau[3], Adrian Chesh[3], Tafadzwa L. Chaunzwa[7,8], Jakob Weiss[7,8,9], Morgan R. Schroeder[3], Sophia Ward[1,2,10], Kristiana Grigoriadis[1,2,11], Aamir Shahpurwalla[3], Kevin Litchfield[1,12], Clare Puttick[1,2,11], Dhruva Biswas[1,2,13], Takahiro Karasaki[1,2,14], James R. M. Black[1,11], Carlos Martínez-Ruiz[1,11], Maise Al Bakir[1,2], Oriol Pich[2], Thomas B. K. Watkins[2], Emilia L. Lim[1,2], Ariana Huebner[1,2,11], David A. Moore[1,2,15], Nadia Godin-Heymann[16], Anne L'Hernault[16], Hannah Bye[16], Aaron Odell[3], Paula Roberts[3], Fabio Gomes[17], Akshay J. Patel[18], Elizabeth Manzano[1], Crispin T. Hiley[1,2], Nicolas Carey[19], Joan Riley[19], Daniel E. Cook[2], Darren Hodgson[16], Daniel Stetson[20], J. Carl Barrett[20], Roderik M. Kortlever[21], Gerard I. Evan[21], Allan Hackshaw[22], Robert D. Daber[3], Jacqui A. Shaw[18], Hugo J. W. L. Aerts[7,8,23], Abel Licon[3], Josh Stahl[3], Mariam Jamal-Hanjani[1,14,24], TRACERx Consortium*, Nicolai J. Birkbak[1,2,4,5,6,102], Nicholas McGranahan[1,11,102] ✉ & Charles Swanton[1,2,24,102] ✉

9th Edition TNM staging findings: Metastasis to multiple N2 station or multiple extra thoracic organs have poorer prognosis than single N2 or organ metastasis

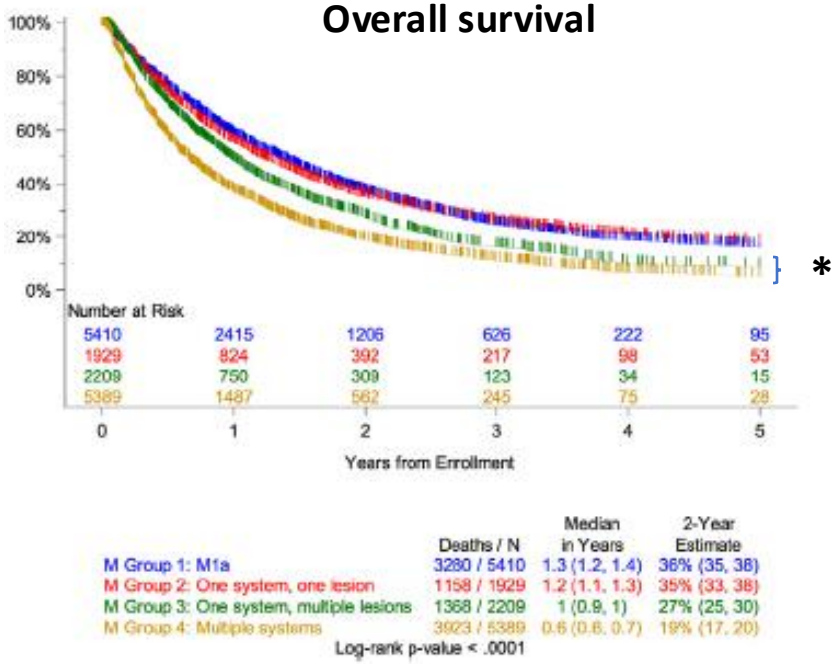
Pathologic stage



N2		Metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s)
	N2a	Single N2 station involvement
	N2b	Multiple N2 station involvement

Huang J., et al. *J Thorac Oncol 2024 May;19(5):766-785 (PMID: 37866624)*

Overall survival



M1c		Multiple extrathoracic metastases
	M1c1	Multiple extrathoracic metastases in a single organ system[c]
	M1c2	Multiple extrathoracic metastasis in multiple organ systems

Fong K., et al. *J Thorac Oncol 2024 May;:786-802. (PMID: 38320664)*

OA15.03: Clinicogenomic Landscape of Lymph Node Metastases in TRACERx

Clinical:

- Do the findings apply to all types (histology and driver status) of lung cancers?
- Is pattern of spread (polyclonal vs. monoclonal) prognostic?
- Can clonal dissemination patterns explain the survival difference between N2a (single) vs N2b (multiple), or M1c1 (single organ) vs. M1c2 (multiple organ) metastatic patients?
- Does clonality determination in ctDNA have impact on treatment strategy and outcome?

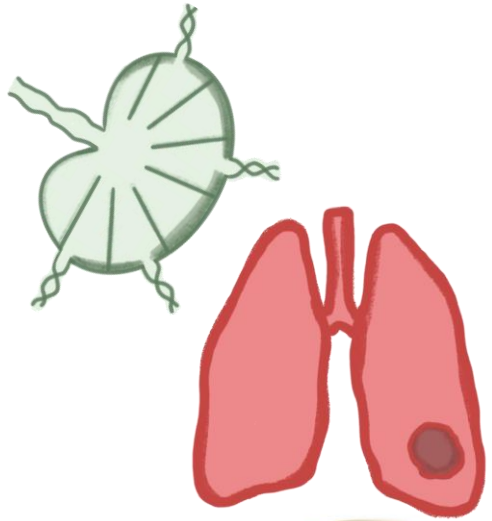
Biological

- What are the mechanisms that drive mono vs. poly clonal metastatic patterns?

Hypothesis	YES (inferred)
Study goals	YES
Hypo./Goals answered?	YES
Clinical impact	Somewhat
Advancing knowledge	Substantial (p.1) Supportive (p.2)
Caveats or further needs: - Sample size - Mechanistic explanation	

OA15.04

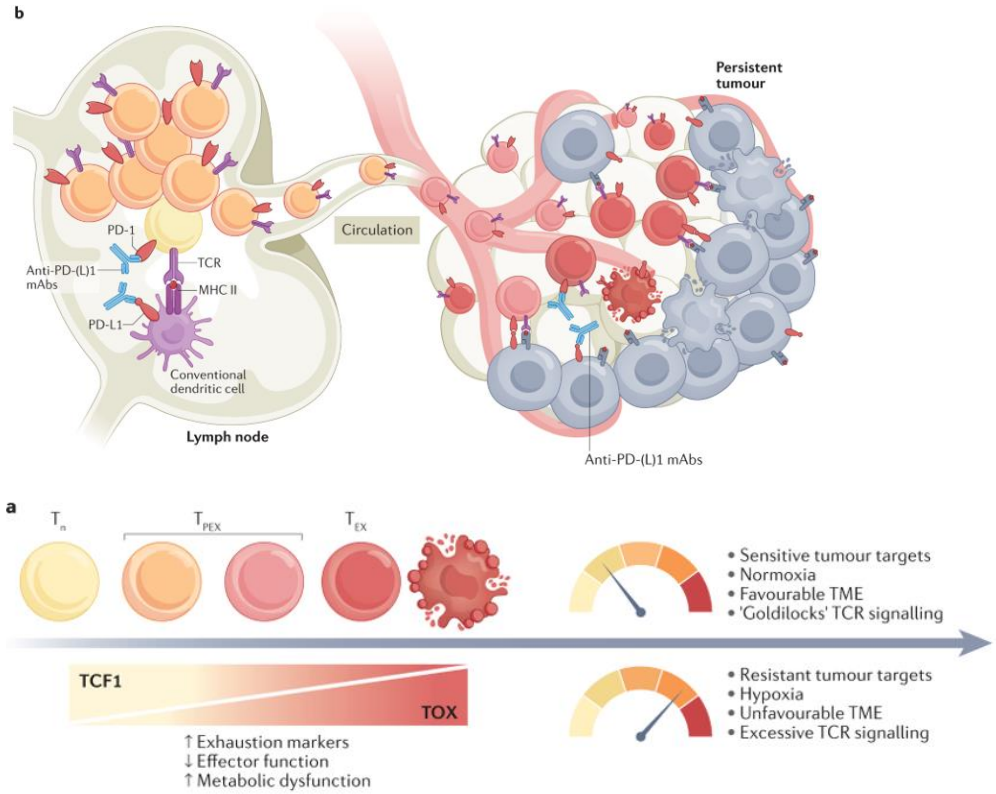
Multi-omic profiling of paired NSCLC tumor and draining lymph node reveals novel T cell differentiation patterns



Vivian Gerretsen MD
Supervisors: Floris Dammeijer, Joachim Aerts

Erasmus MC | IRC-VIB
Rotterdam, The Netherlands | Ghent, Belgium

OA15.04 T cell development in tumor and beyond – origin of TILs



Chow et al. *Nat Rev Clin Oncol* 2022

Improving our **understanding of T cell dynamics** in tumor will aid in new immunotherapy and biomarker development

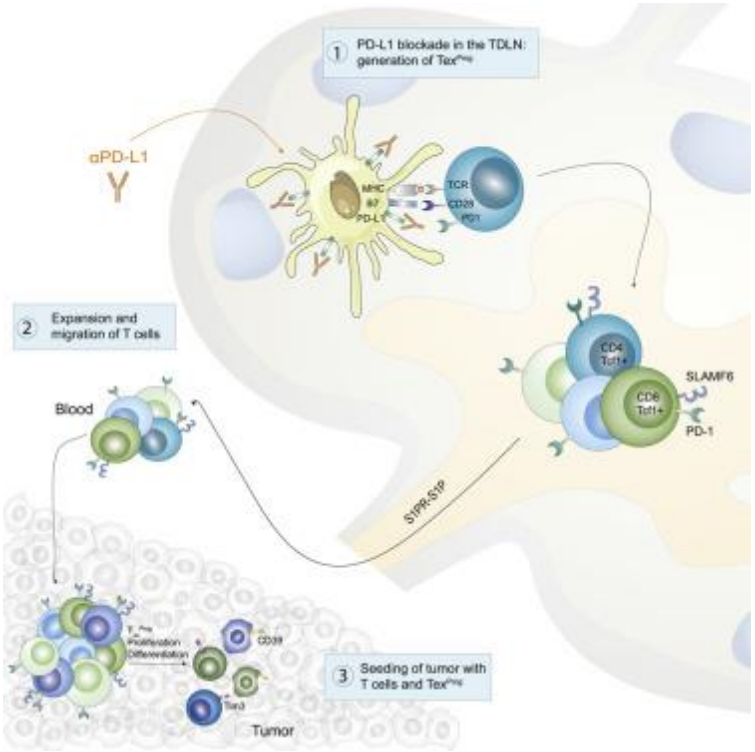
Tumor infiltrating lymphocytes (TILs) become progressively **exhausted**

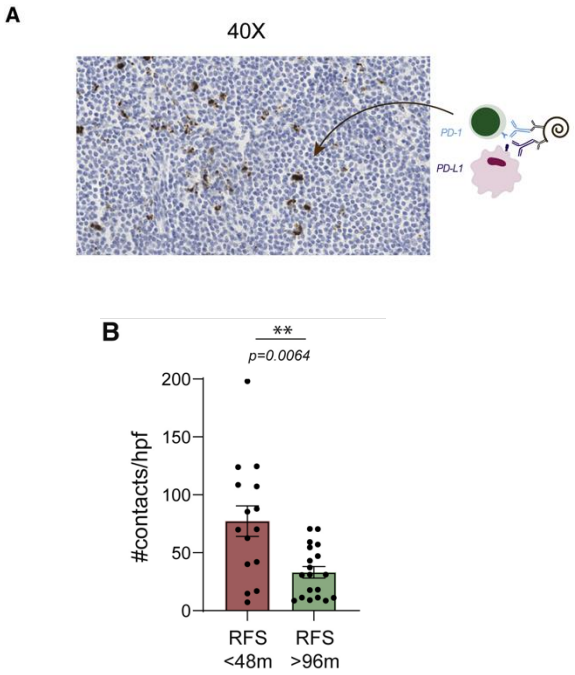
A linear **developmental trajectory** (Tpex → Tex) has been proposed

How and whether this **cross-tissue** trajectory occurs in human remains unknown

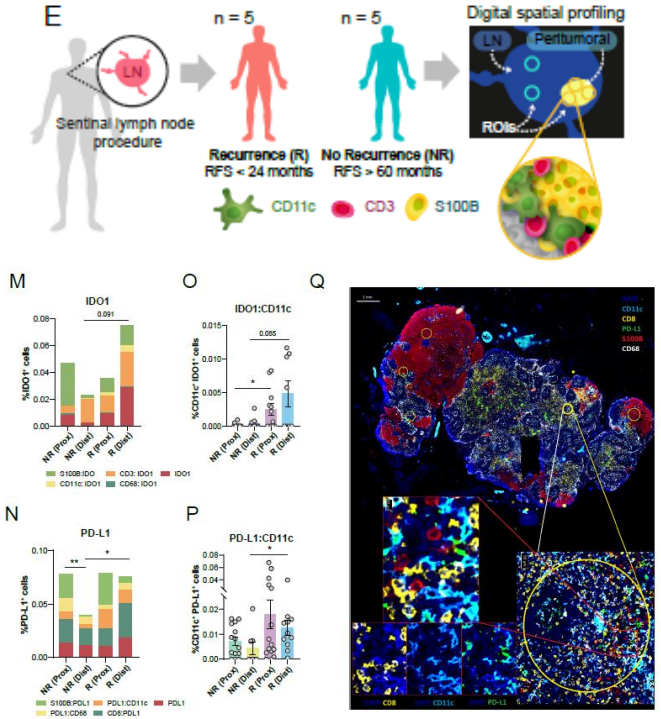
OA15.04

Tumor-draining lymph nodes (TDLNs) are essential to mount an anti-tumor T cell response





Dammeijer et al. *Cancer Cell* 2020



Van Krimpen et al. *Cancer Cell* 2022

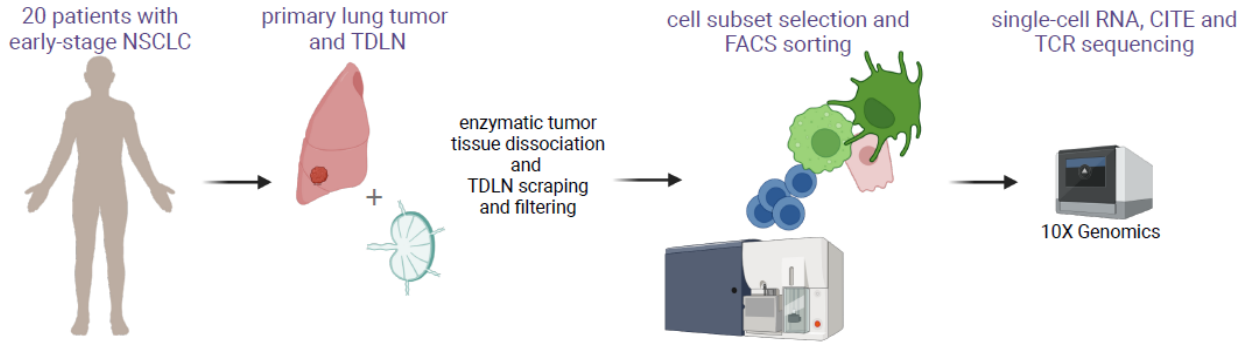
OA15.04

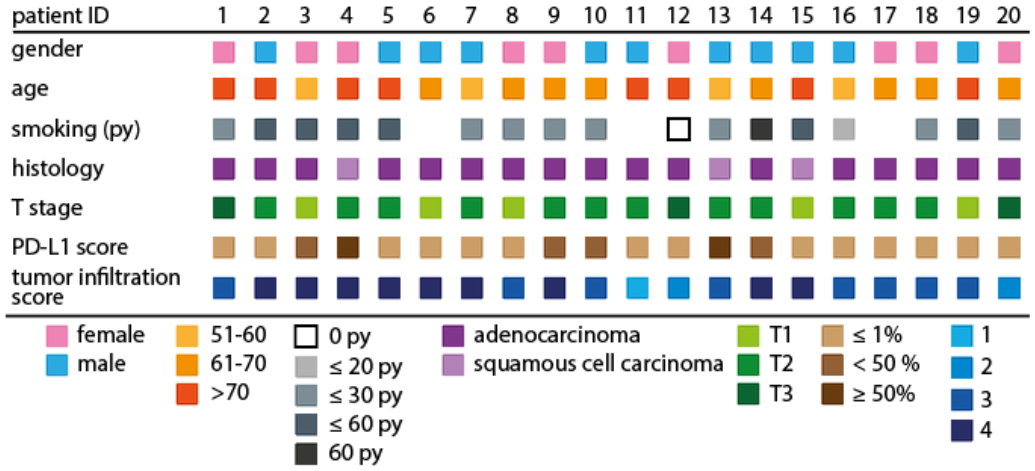
Hypothesis:
Improving understanding of T-cell dynamics in tumor will aid in new immunotherapy and biomarker development

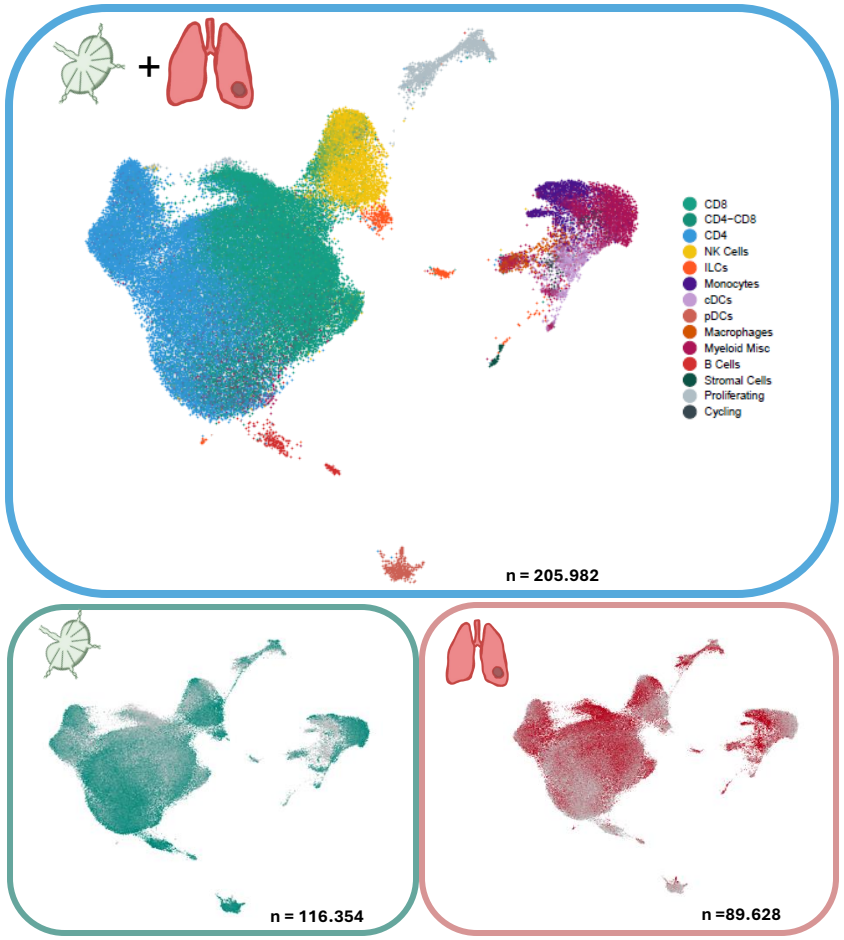
Objectives:
1) Better understand the T cell dynamics in early-stage NSCLC
2) Investigate the clonal relatedness of TDLN and tumor T cells

OA15.04

Multi-omic characterization of paired tumor and TDLN in patients with early-stage non-small cell lung cancer (NSCLC)

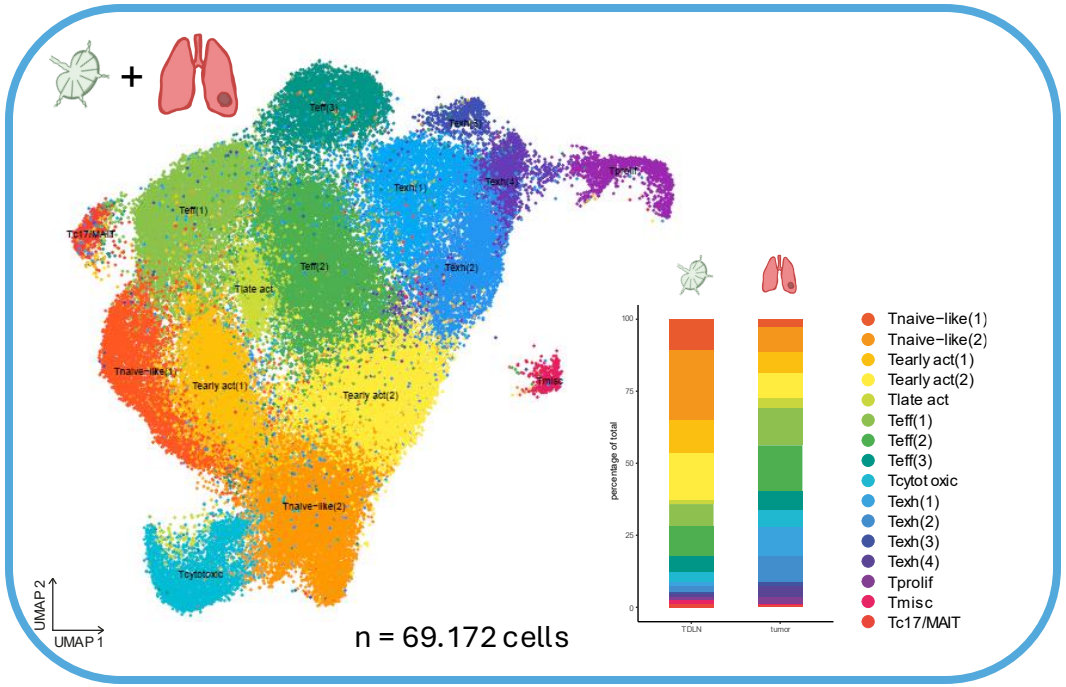




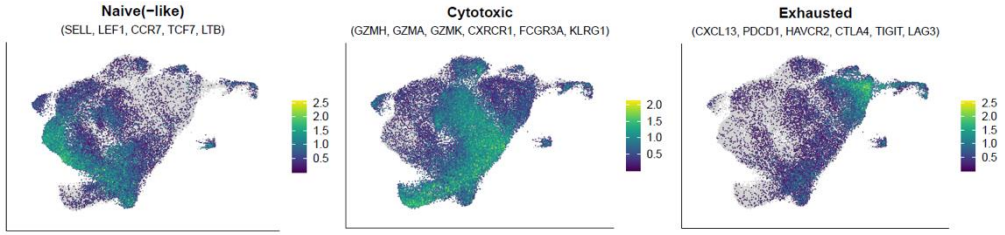


TDLNs are enriched for earlier CD8⁺ T cell differentiation subsets, while tumor is enriched for exhausted CD8⁺ T cells

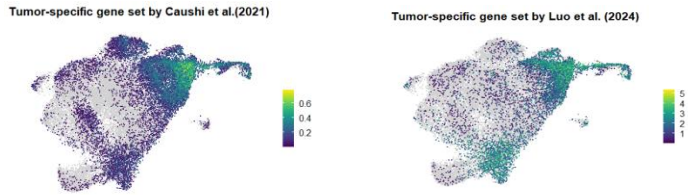
TDLN and tumor CD8⁺ T cells



Gradient of T cell differentiation states present in TDLN and tumor



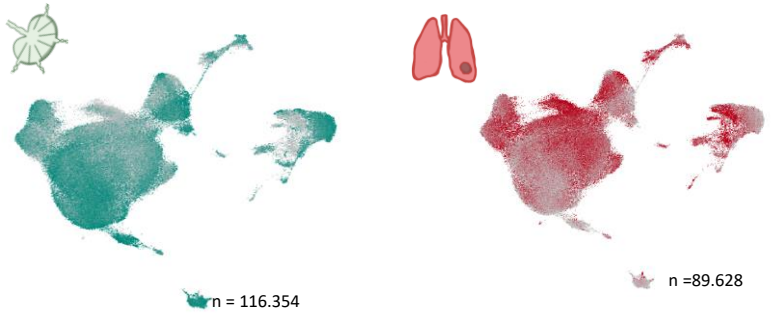
Tumor-specific genes enriched mainly but not solely in Texh cells

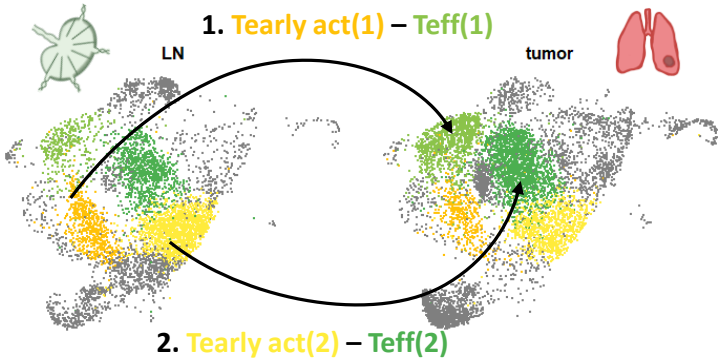


Can we trace back where Texh cells originate from?

OA15.04: Multi-Omic Profiling of Paired Non-Small Cell Lung Cancer and Draining Lymph Nodes Reveals Novel T-Cell Differentiation Patterns

Single cell analyses





CONCLUSIONS

Clonal relatedness between TDLN CD8⁺ T cells and TILs in **all** included patients

Distinct clonal patterns of T cell subsets, related to commonly known (exhausted) TILs

Different 'routes' to exhaustion may have **consequences for therapeutic targeting**

Currently ongoing:
Validating existing scRNAseq databases including those incorporating **neo-antigen specificity**

Ex vivo tumor slice cultures examining T-cell subset **functionality** and **response to immune checkpoint blockade**

OA15.04: Multi-Omic Profiling of Paired Non-Small Cell Lung Cancer and Draining Lymph Nodes Reveals Novel T-Cell Differentiation Patterns

Questions/Comments

Clinical:

- How can knowledge on “routes” of exhaustion improve current treatment strategy in early-stage NSCLC?
- Would the route of exhaustion be affected by neoadjuvant therapies, including immuno, chemo or radiation therapies?

Biological:

- Would the route and phenotype of T-cell exhaustion identified in this study be applicable to advanced stage patients?

Hypothesis	YES
Study goals	YES
Hypo./Goals answered?	YES
Clinical impact	Currently Unclear
Advancing knowledge	Substantial
Caveats or further needs: - Additional validation	

OA15.05

Single-Cell Spatial Architectures of Paratumor Zone Determines the Prognosis of Multiple Lung Cancers

Yuan Xiaoqiu
Department of Thoracic Surgery & Thoracic Oncology Institute
Peking University People's Hospital, Beijing, China

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北京大学 PEKING UNIVERSITY
北京大学人民医院 PEKING UNIVERSITY PEOPLE'S HOSPITAL
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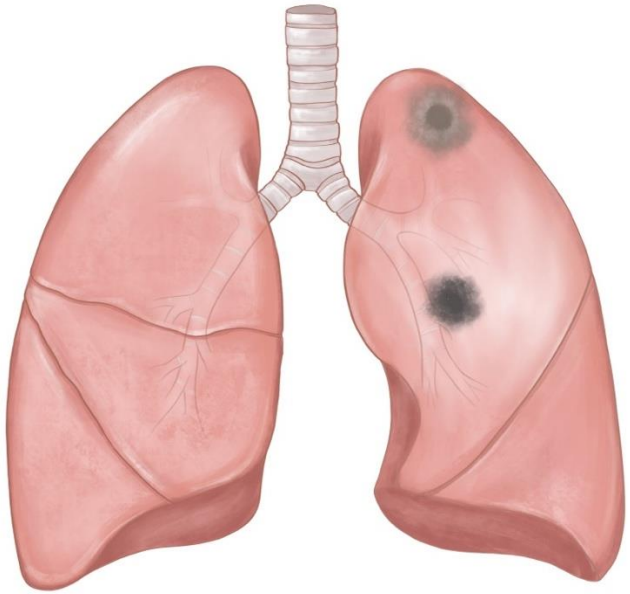
OA15.05

Background



Multiple lung cancers (MLCs)

~2.6-8.4% lung cancer

Multiple primary lung cancers (MPLC)

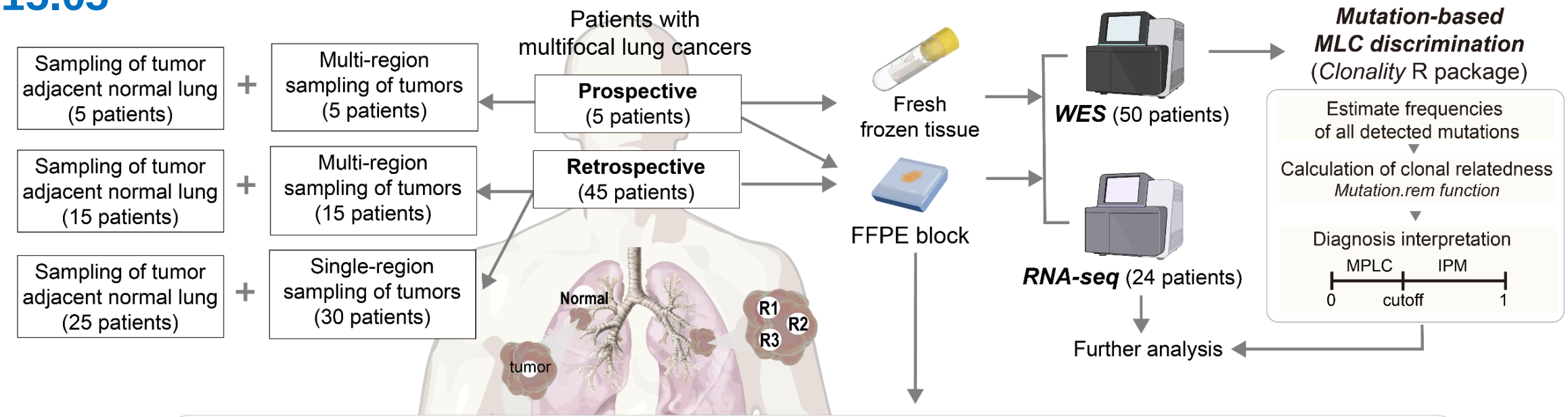
- Independent clonal origins
- Usually good prognosis

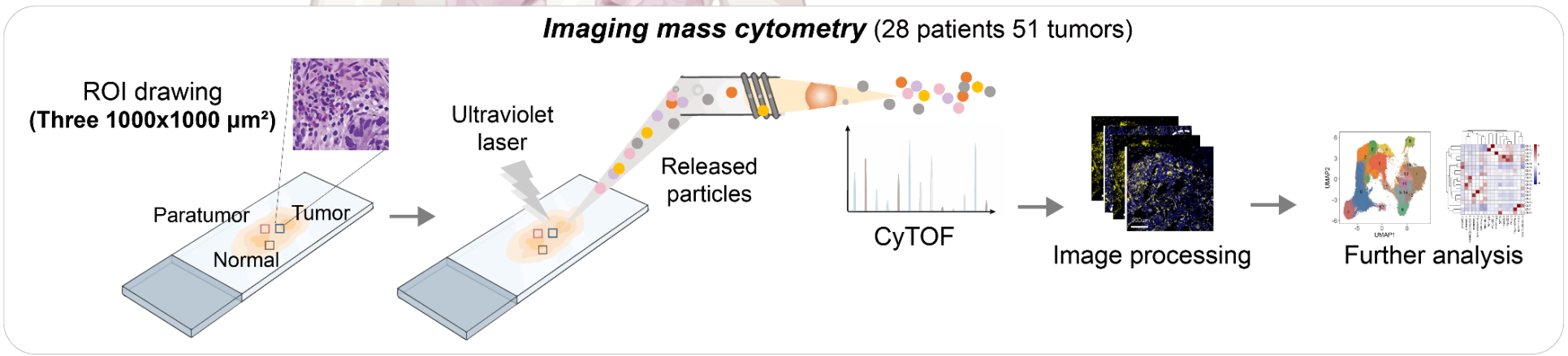
Intrapulmonary metastasis (IPM)

- Shared clonal origins
- Worse prognosis

Methods

OA15.05



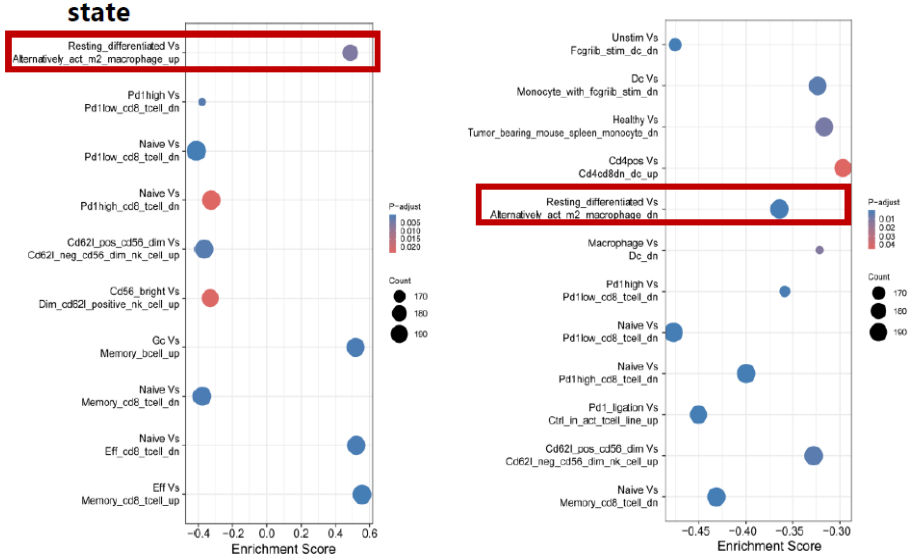


OA15.05

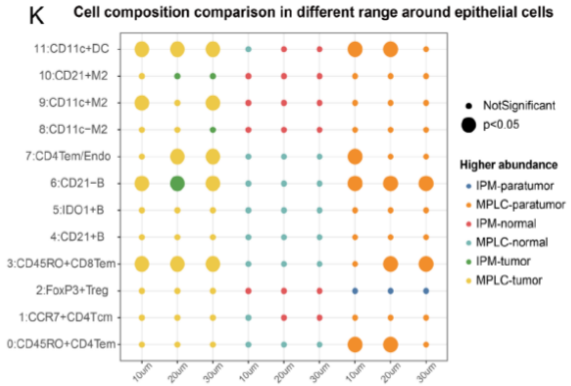
Understanding immunology is quite challenging for non-immunologists

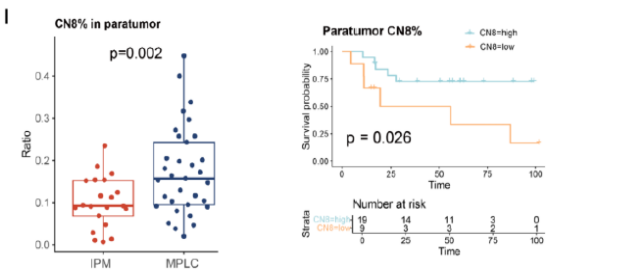
M2 different functional states

- Compared with the adjacent normal tissues of MPLC, MPLC tumor macrophages are more in the resting differentiated state
- IPM tumor macrophages significantly activated M2 compared to adjacent normal tissues

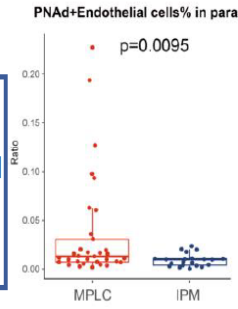


Activation of anti-tumor immunity in MPLC paratumor zone against immune escape in IPM





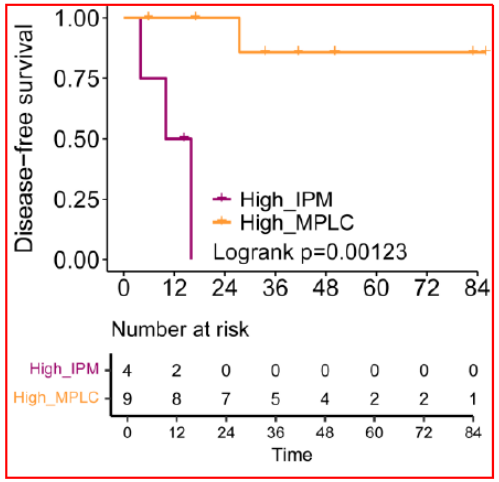
- CN8 (characterized by M2) was enriched in MPLC and was positively correlated with survival
- CD45RO+CD4/8+ Tem cell、CD21- Bcell accumulate around epithelial cells in the paratumor area of MPLC
- Stronger anti-tumor immunity in the MPLC paratumor region
- PNAd+ endothelial cells (tertiary lymphoid structures) are enriched in the paracancerous region of MPLC

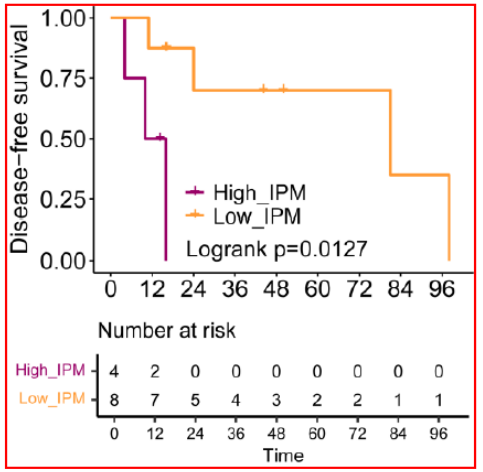


OA15.05: Single-Cell Spatial Architectures of Paratumor Zone Determines the Prognosis of Multiple Lung Cancers

CONCLUSIONS

- Different anti-tumor immunity in paratumor region between MPLC and IPM
- M2 macrophages may play a key role
- The microenvironment score incorporating paratumor features can identify high-risk phenotypes of multiple lung cancers



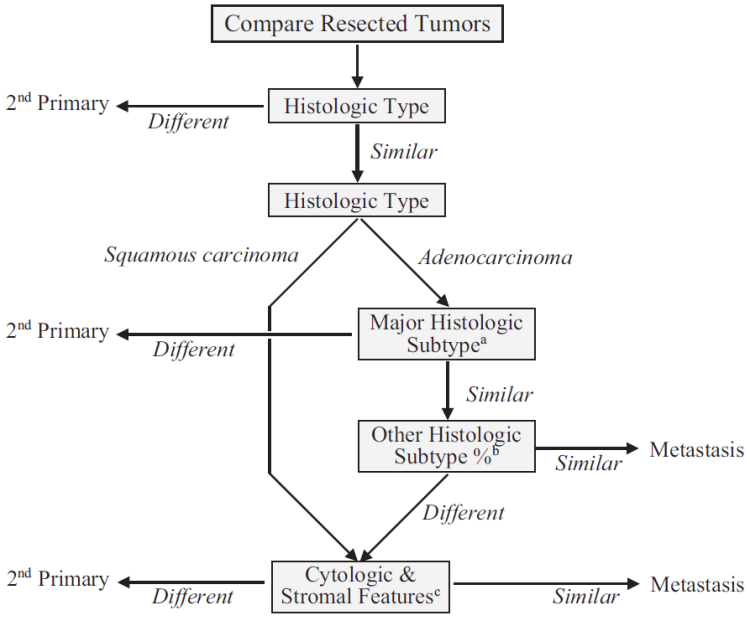


- **Most of the prognostic microenvironmental features come from the adjacent area**
- **The characteristics of the paratumor microenvironment, patients with higher risk of IPM can be screened out → more actively intervened**

Current approach (by pathologists) to distinguish separate primary vs. intrapulmonary metastatic lung cancers?

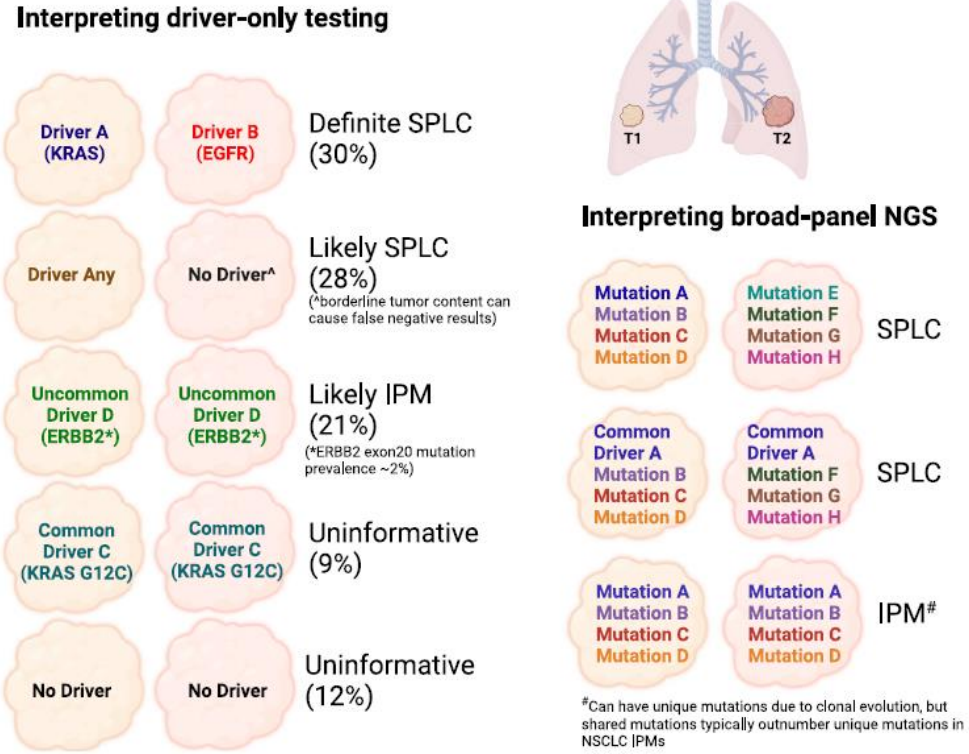
Combination of histology and molecular profiles:

- Different histologies -> metastasis
- Different LUAD subtypes -> metastasis
- Similar LUAD subtypes -> molecular profiling



Detterbeck FC, et al. J Thorac Oncol 2016;11(5):651-665 (PMID: 26944304)

Molecular profiling



Chang J and Reckthman N, et al. Mod Pathol 37 (2024) 100453 (PMID: 38387831)

Current approach (by pathologists) to distinguish separate primary vs. intrapulmonary metastatic lung cancers?

Questions/Comments

Clinical:

- Unclear what is the clinical utility and impact of the results

Biological:

- Each ROI is 1x1 mm^2. How were they chosen and how representative are they for the entire tumors.
- Biological meaning of the results is unclear.

Hypothesis	Missing
Study goals	Unclear
Hypo./Goals answered?	Not sure
Clinical impact	Unclear
Advancing knowledge	Possible
Caveats or further needs: - Validation with multiplex IHC/IF - Input from pathologists in project development	

Take home message

- Advanced omics profiling and computational technologies enable broad and deep molecular analysis at single cell levels
- These data provide unprecedented opportunities to explore new frontiers and gain new insights in cancer biology, with potential transformative clinical impacts
- However, results from computational analysis are hypothesis generating and require robust biological and clinical validation

My Takeaway Messages

1. In 9th Edition TNM staging, metastasis to multiple N2 station or multiple extra thoracic organs have poorer prognosis than single N2 or organ metastasis.
2. Polyclonally determination in ctDNA might have prognostic impact on risk of cancer recurrence.
3. Tumor-draining lymph nodes (TDLNs) are essential to mount an anti-tumor T cell response, and might have complex interaction with primary tumor.
4. M2 macrophage in paratumor microenviorment might be imporant in determing anti-tumor response.

Presented by Tianhong "Tina" Li, UCDCCC, USA, thli@ucdavis.edu