Biomarkers for perioperative therapy

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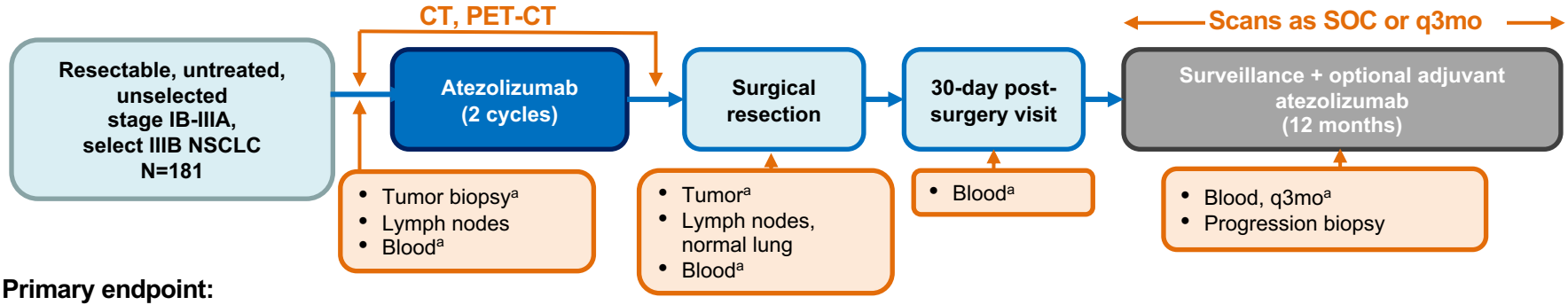
We are regressing to one-size-fits-all for IO

- We made dramatic and clinically significant progress by matching targeted therapies to specific features of tumors
- Most non-driver mutant NSCLC in the USA get a combination of chemotherapy and pembrolizumab, regardless of PD-L1
  - 30-60% response rate, 5 yr. survival ~20%
- PD-L1 is good but has intrinsic variability and is continuous
- Immunotherapy is much more complicated than single-gene correlations, and involves host as well as tumor factors
- The neoadjuvant setting is ideal for collection of relevant pre- and post-intervention tissue samples for biomarker studies!

LCMC3: designed to discover and test biomarkers

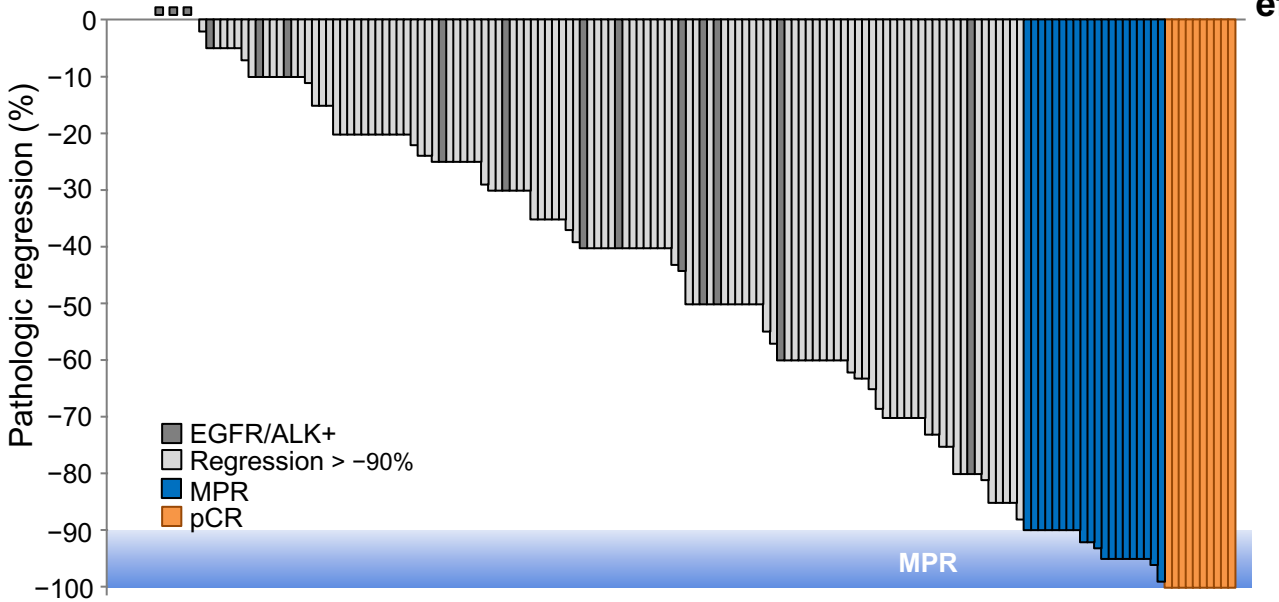


Primary endpoint:

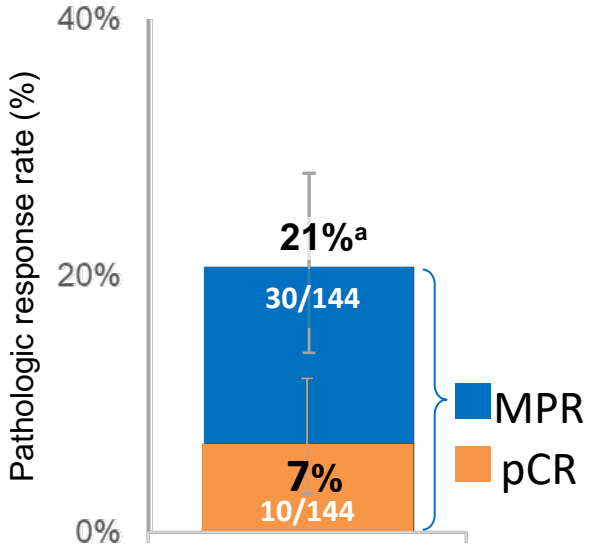
- MPR (≤10% viable tumor cells)

- LCMC3 is the largest reported study of anti–PD-L1 neoadjuvant monotherapy conducted to date (n=181)
- Biomarkers studied include: WES/TMB, bulk RNAseq pre- and post IO, scRNAseq, multiplex IF of tumor pre- and post-IO, TCRseq, cytokines, antigen-specific T-cell analysis, pre- and post- immunophenotyping of peripheral blood and nodal tissue, ctDNA, pathologic and radiographic response, radiomics, and AI analysis of tissue sections.
- Primary manuscript is “accepted in principle” by Nature Medicine, >5 additional manuscripts ready to submit.

LCMC3: Pathologic response in surgery population



Pathologic response in the primary efficacy population (n=144)

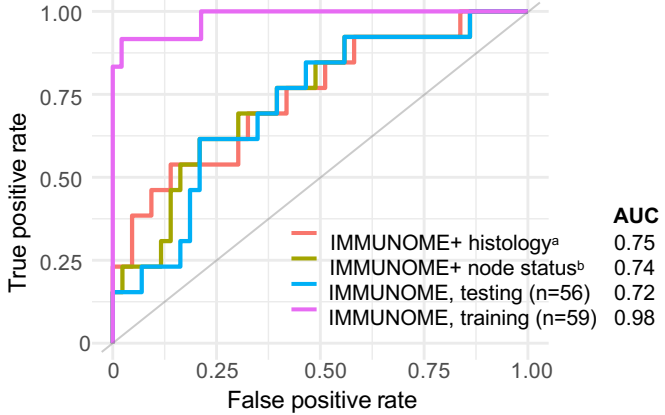


Pathologic regression defined as % viable tumor cells – 100%. MPR, major pathologic response; pCR, pathologic complete response.

[a] Error bars indicate 95% CI.

Baseline peripheral blood immunophenotypes predict MPR

ROC curves for immune cell subsets (n=115)



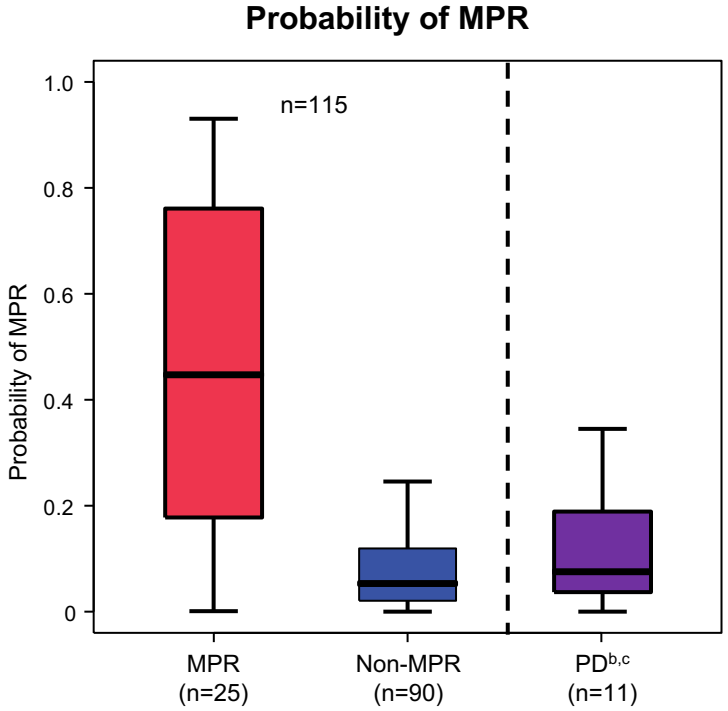
	Cell type	CD45⁺ immunophenotype
Positively associated with MPR	Non-T/ non-NK cells	**ILT2⁺NKG2A⁻**CD63⁻CD3⁻CD158e1⁻CD158b⁺CD56⁻**KIR2DL1⁺**CD16⁻
		NKG2A⁻CD94⁻NKG2D⁺CD3⁻CD56⁻CD117⁻CD127⁻CD161⁻CD16⁻
	NK cell	**NKG2A⁺**HLA-DR⁺CD69⁺CD3⁻CD158e1⁻CD158b⁻CD56⁻**KIR2DL1⁻**CD16⁺
Negatively associated with MPR	NK cells	CD16⁺CD336⁻CD3⁻CD244⁻CD335⁺NKG2D⁻CD56⁻CD161⁻CD337⁺
		ILT2⁺NKG2A⁺CD63⁻CD3⁻CD158e1⁻CD158b⁻CD56⁺**KIR2DL1⁻**CD16⁻
	NK-like T cells	**NKG2A⁺**HLA-DR⁻CD69⁺CD3⁺CD158e1⁻CD158b⁻CD56⁺**KIR2DL1⁻**CD16⁻
		HLA-DR⁺CD69⁻CD19⁻CD56⁺CD16⁻CD134⁻CD4⁻CD3⁺CD8⁺
		NKG2A⁻CD94⁻NKG2D⁺CD3⁺CD56⁺CD117⁻ CD127⁻CD161⁻CD16⁺
		ILT2⁺NKG2A⁺CD63⁻CD3⁺CD158e1⁻CD158b⁻CD56⁺**KIR2DL1⁻**CD16⁻
		γ/δ⁻ α/β⁺CD19⁻CD56⁺CD16⁻CD13/14⁻CD4⁺CD3⁺CD8⁺
		γ/δ⁺α/β⁻CD19⁻CD56⁺CD16⁺CD13/14⁻CD4⁻CD3⁺CD8⁻
		γ/δ⁻α/β⁻CD19⁻CD56⁺CD16⁺CD13/14⁻CD4⁻CD3⁺CD8⁻
	Naive T cell	CD62⁻CD27⁺CD56/16⁻CD45RO⁻CCR7⁻CD45RA⁺CD4⁺CD3⁺CD8⁺

- IMMUNOME flow cytometry data from pre-treatment peripheral blood samples (n=115) were divided into training and testing sets and used to build and test an immune cell model predictive of MPR
- Pre-treatment peripheral blood samples were placed into training or testing sets and analyzed using an approach based on generalized additive models and regularized regression (LASSO). Immune cell subsets detected in fewer than 50% of samples were excluded
- 13 immune cell subsets in the baseline peripheral blood sample predicted MPR, including NK-cell and NK-like T-cell subtypes expressing
  ILT2 and NKG2A

Oezkan et al. WCLC 2021

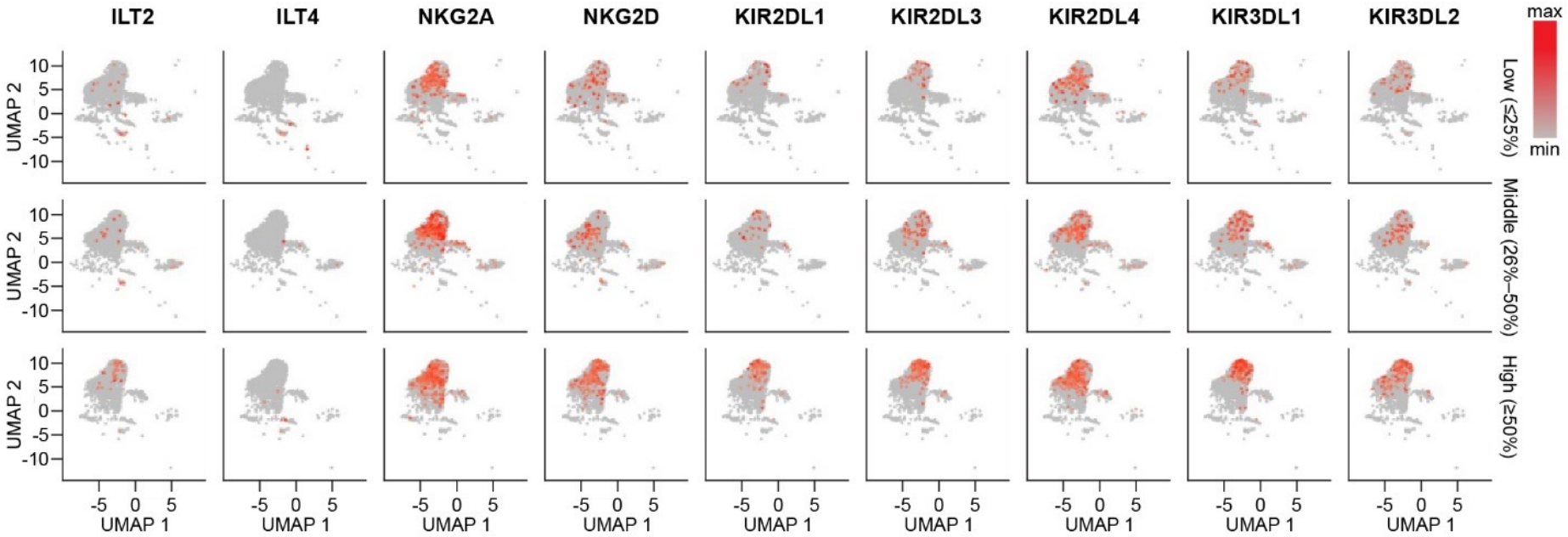
LCMC3: Prediction of MPR via immune cells in pre-treatment peripheral blood

- Using our model, the probability of achieving MPR was calculated for each patient based on the pre-treatment peripheral blood immunophenotypes
- As a second testing cohort, another set of patients with primary PD were evaluated

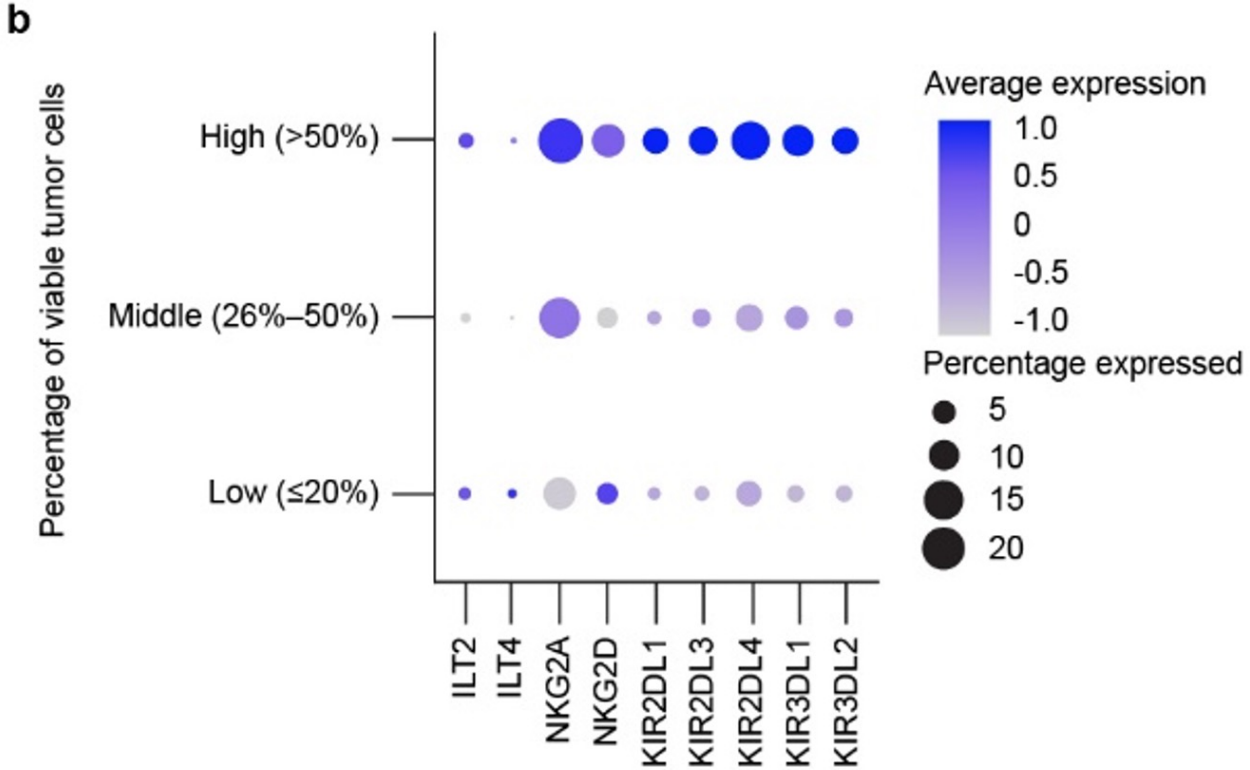


Oezkan et al, WCLC 2021

Association of tumor NK markers with response by scRNAseq

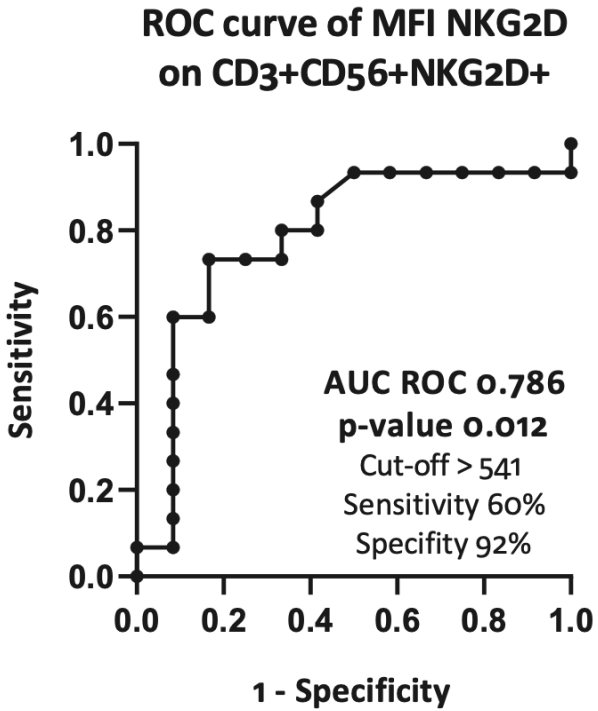


Association of tumor NK markers with response by scRNAseq



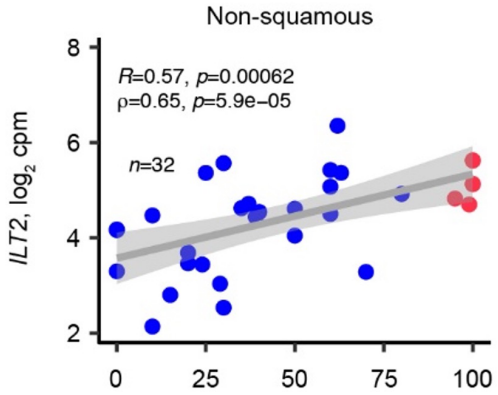
Higher expression of NK cell markers in tumor associated with lack of response

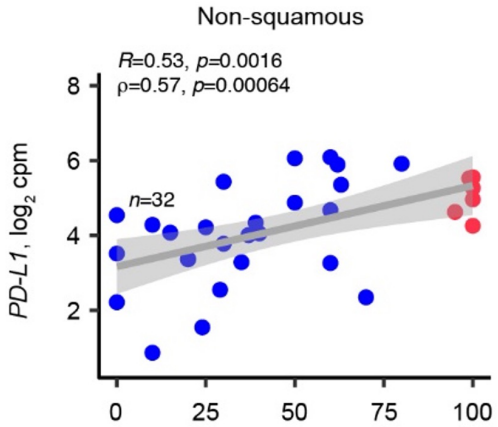
NKG2D+ cells and PCR in NADIM



Laza-Briviesca et al, CTM 2021

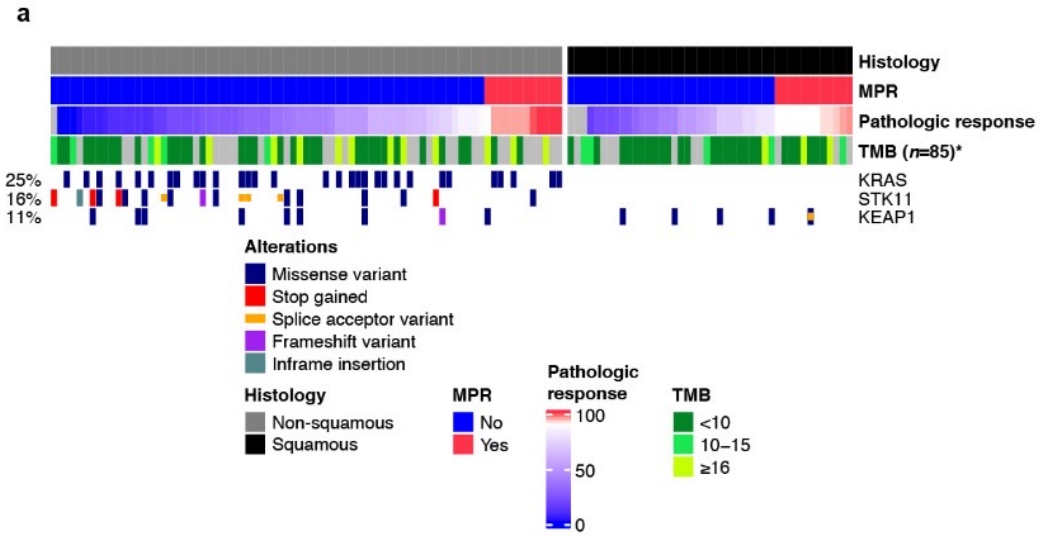
Association of baseline tumor RNAseq ILT2 and PD-L1 with response

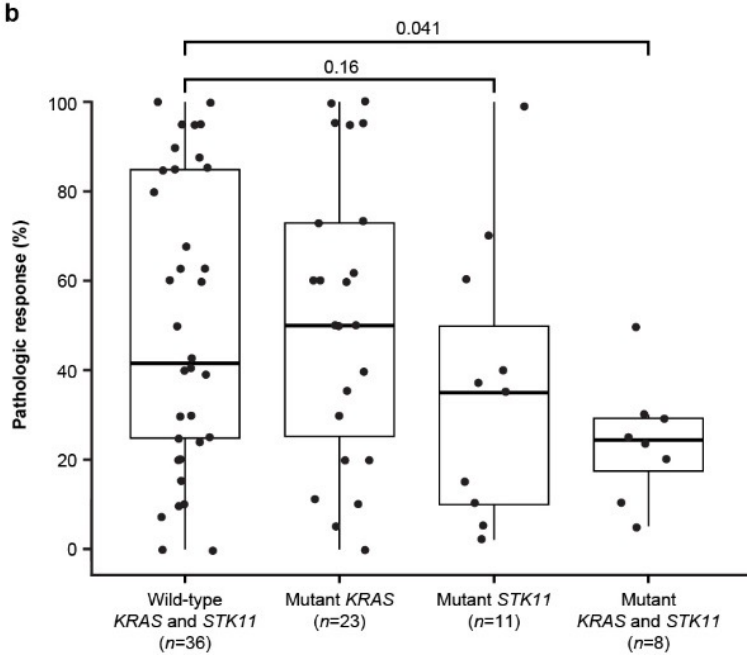




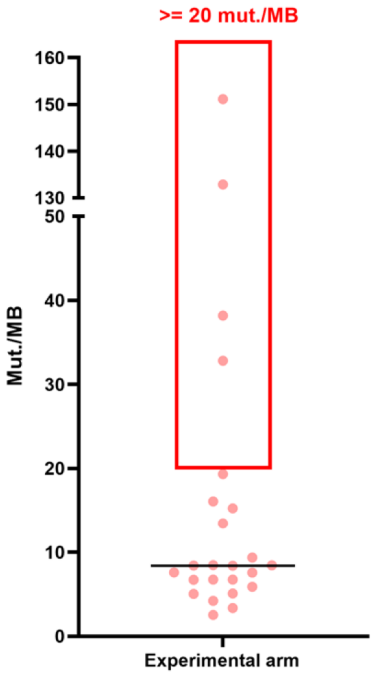
ILT2 expression is at least as strong as PD-L1 in baseline tumor

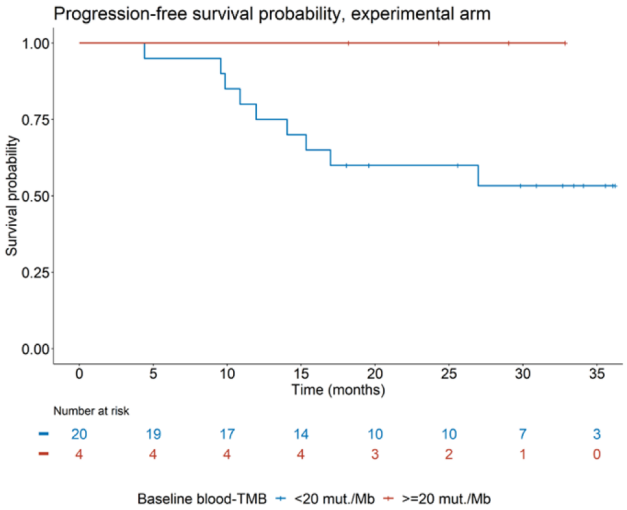
Association of specific mutations and TMB with pathologic response

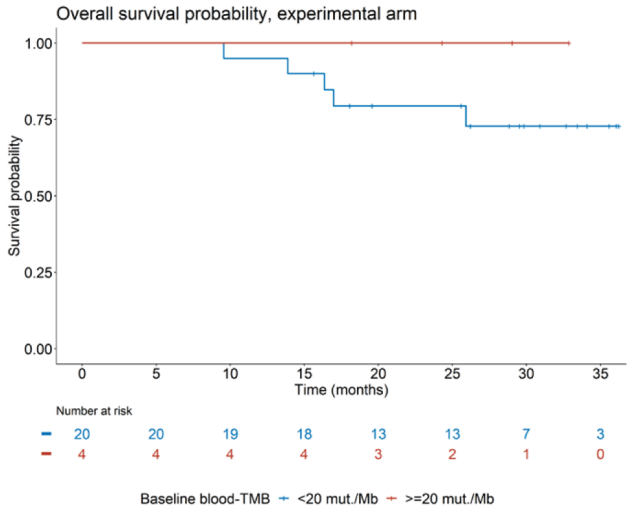




Very high TMB and good outcomes

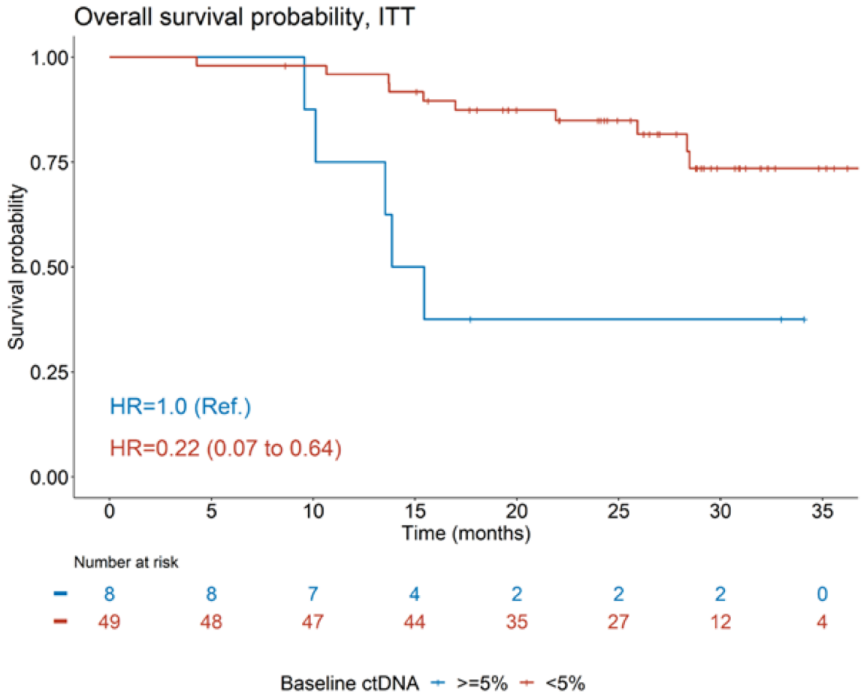




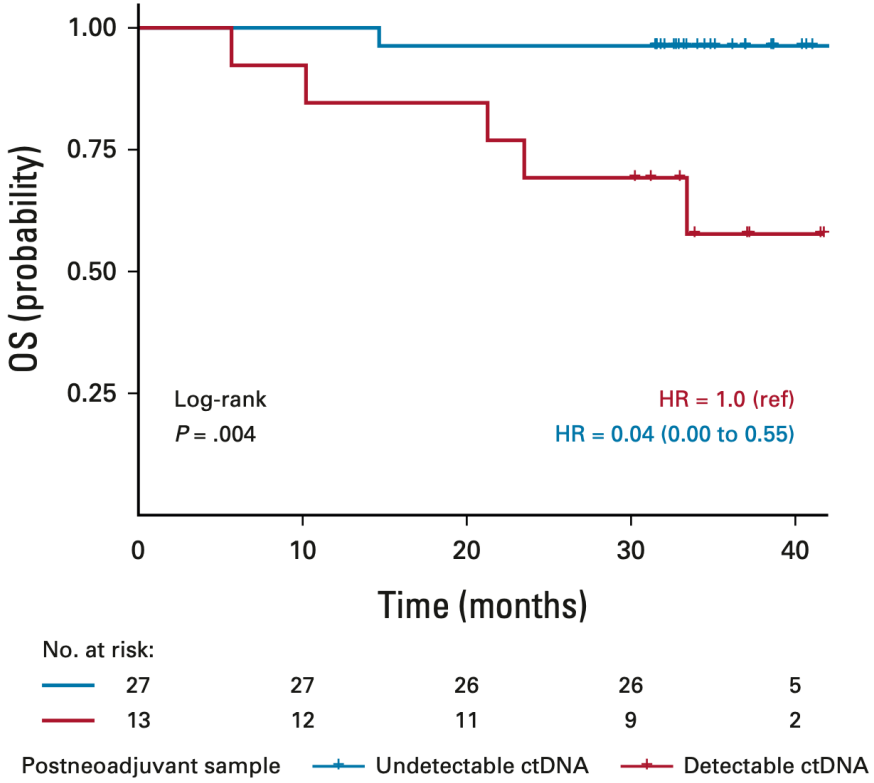


Provencio et al, NEJM 2023

Outcomes by baseline and post neoadjuvant ctDNA



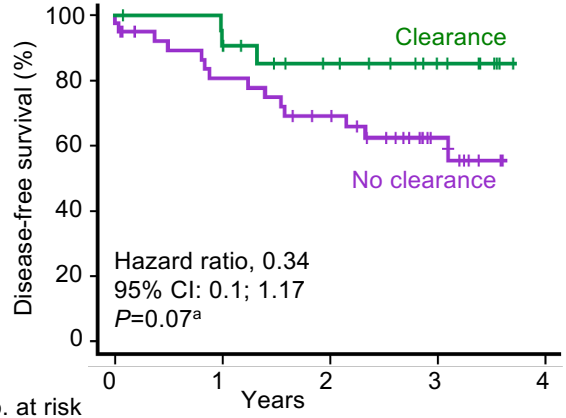
Provencio et al, NEJM 2023



Provencio et al, JCO 2022

Preliminary results showed improved disease-free survival in patients with ctDNA clearance

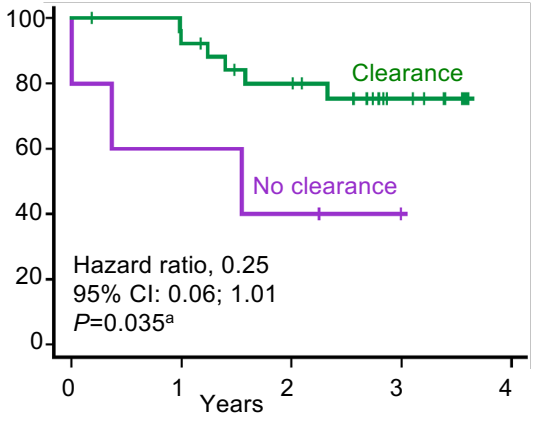
Disease-free survival by baseline to post-atezo clearance



No. at risk					
Clearance	22	19	13	7	0
No clearance	39	28	22	9	0

Years	Clearance		No clearance	
	DFS rate, %	At risk, n	DFS rate, %	At risk, n
1	91	19	81	28
2	85	13	69	22
3	85	7	62	9

Disease-free survival by baseline to post-surgery clearance

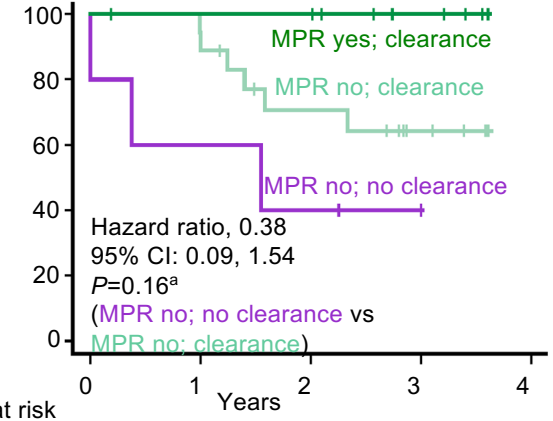


No. at risk					
Clearance	27	24	19	10	0
No clearance	5	3	2	0	0

Years	Clearance		No clearance	
	DFS rate, %	At risk, n	DFS rate, %	At risk, n
1	92	24	60	3
2	80	19	40	2
3	75	10	–	–

Stratify by MPR

Disease-free survival by baseline to post-surgery clearance and MPR



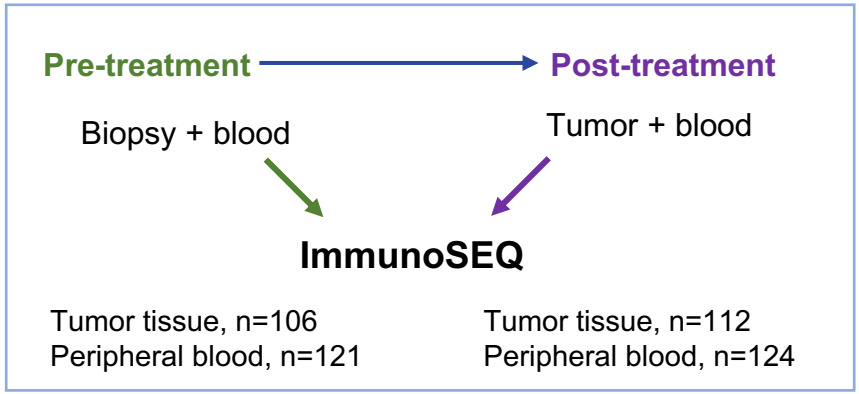
No. at risk					
MPR yes; clearance	8	8	8	4	0
MPR no; clearance	19	16	11	6	0
MPR no; no clearance	5	3	2	0	0

Years	MPR yes; clearance		MPR no; clearance		MPR no; no clearance	
	DFS rate, %	At risk, n	DFS rate, %	At risk, n	DFS rate, %	At risk, n
1	100	8	89	16	60	3
2	100	8	71	11	40	2
3	100	4	64	6	–	–

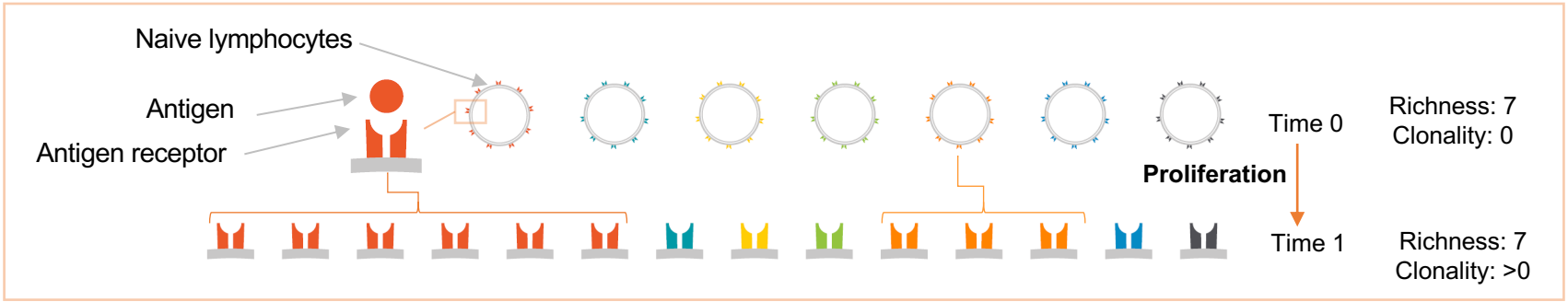
[a] Log-rank test.

Kris et al. LCMC3 ctDNA
https://bit.ly/3wvlmKn

Assessment of T cell dynamics in peripheral blood and tumor tissue



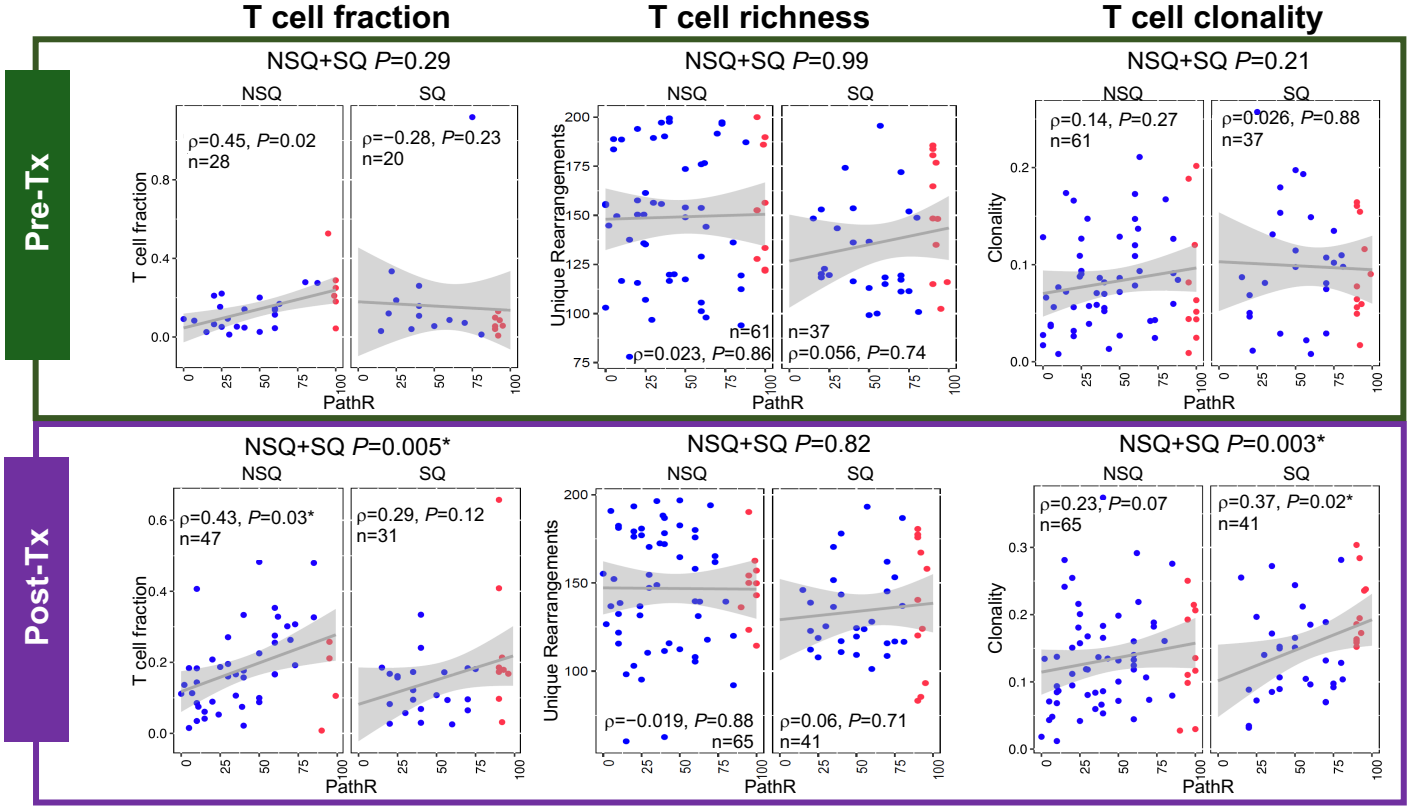
- ImmunoSEQ[a] was used to evaluate
  - **T cell fraction**: fraction of T cells as a proportion of total nucleated cells
  - **T cell richness**[b]: number of total unique T cell clones
  - **T cell clonality**[c]: higher number indicates predominance of specific clones in a sample



[a]CDR3 region of the T cell receptor β-chain. [b]T cell richness was downsampled. [c]Clonality was determined using Simpson's metric.

1. Adaptive Biotechnlogies. immunoSEQ Analyzer: Understanding Clonality. Accessed 26 June 2022 https://www.adaptivebiotech.com/wp-content/uploads/2020/06/immunoSEQ_Analyzer-Tech-Note_Clonality_WEB_MRK-00355.pdf.

T cell dynamics in the tumor associated with response to atezolizumab treatment

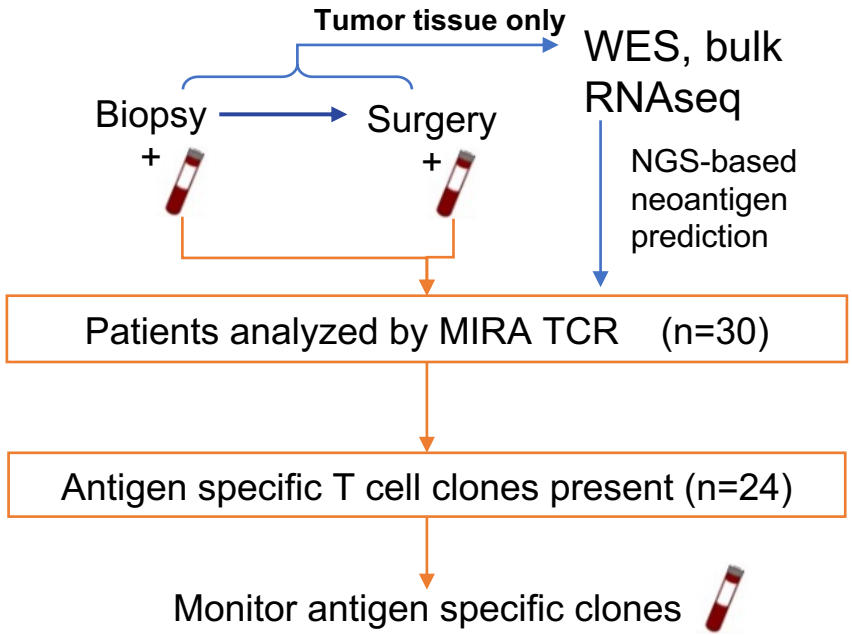


*P<0.05. PathR, pathologic response; Pre-Tx, pre-treatment; Post-Tx, post-treatment.

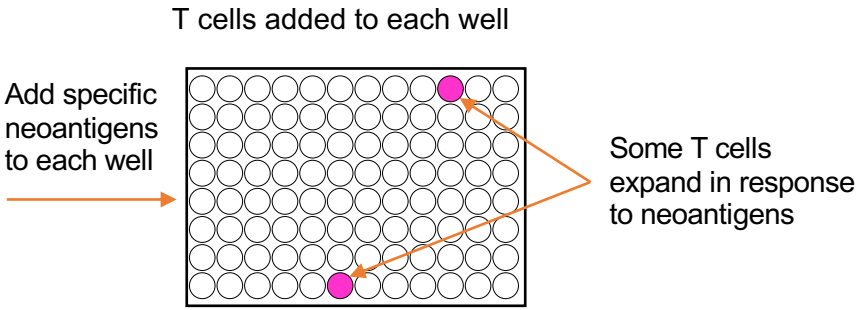
P-values are shown for Spearman (ρ) correlation. Gray line indicates regression line. Shading indicates 95% CI. No multiple test correction was applied.

- Better pathologic response in the non-squamous subtype was associated with **higher pre-treatment T cell fraction** and **higher post-treatment T cell fraction**
- Better pathologic response in the squamous subtype was associated with a **higher post-treatment T cell clonality**

Monitoring T cells using Multiplexed Identification of T cell Receptor Antigen (MIRA)



1) T cells in peripheral blood are expanded polyclonally

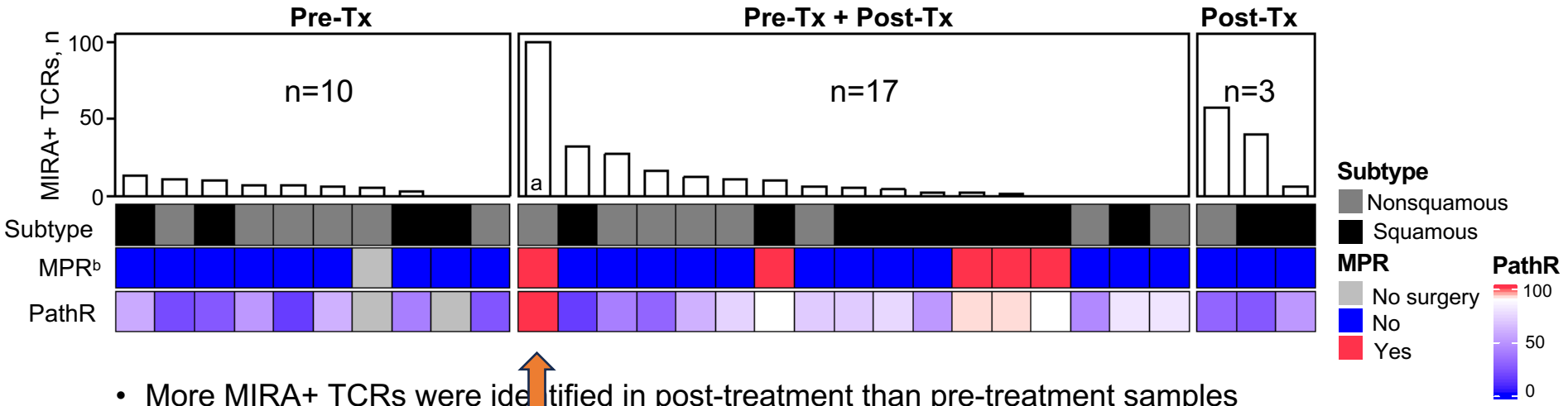


2) Transgenes that encode tumor-specific neoantigens were used to identify TCRs that responded to ≥1 tumor-specific antigen (MIRA+ TCRs)

- **80% (24/30) of MIRA-profiled patient samples had ≥1 MIRA+ TCRs identified and were able to recognize 6% (median, range 1%-21%) of the neoantigens tested**

NGS, next-generation sequencing; RNAseq, RNA sequencing; TCR, T cell receptor; TCRseq, TCR sequencing; WES, whole-exome sequencing.

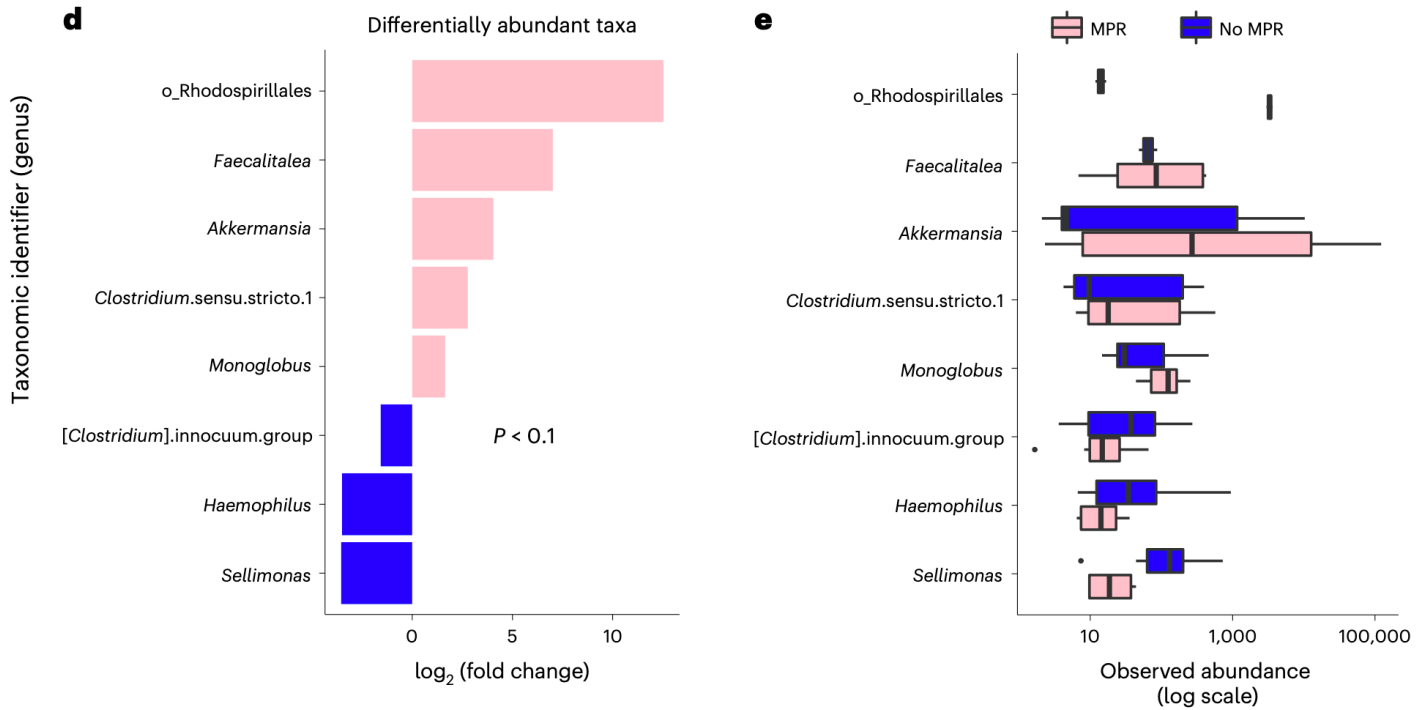
Changes in neoantigen-specific T cells with pathologic response to atezolizumab treatment



- More MIRA+ TCRs were identified in post-treatment than pre-treatment samples
- No difference in the number of MIRA+ TCRs seen in responders vs non-responders to atezolizumab monotherapy
- Highest number of MIRA+ TCRs seen in the only MIRA-profiled complete responder (100% pathologic response)[a]

MIRA, multiplexed identification of T cell receptor antigen; MPR, major pathologic response; PathR, pathologic response; Pre-Tx, pre-treatment; Post-Tx, post-treatment; TCRs, T cell receptors. [a]This patient was profiled as a complete responder (100% pathologic response). [b]One patient had no surgery performed and therefore no pathologic response was assessed.

Fecal microbiome and pathR in NEOSTAR



Cascone et al, NM 2023

Take-home messages

- There is a pressing need to find pre-treatment molecular features of response and potential therapeutically targetable mechanisms of resistance to IO monotherapy
- The neoadjuvant setting is an ideal discovery platform for this
- We have developed a pre-treatment peripheral blood classifier that predicts pathological response
  - This (perhaps surprisingly) identified NK and NKT cell markers as the dominant predictors of poor path response
  - suggests a role for these cells in inhibiting PD-1 pathway responses and their possible utility as selection biomarkers and therapy targets (e.g. ILT2 and NKG2A)
- Combination of ctDNA status and MPR strongly prognostic

The LCMC3 biomarker/clinical teams

Biomarkers	Clinical
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