Article

Spatial predictors of immunotherapy response in triple-negative breast cancer

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Disclosures

Dr Bianchini has received honoraria for consultancy/advisory role/speaker fee from AstraZeneca, Daichii Sankyo, EISAI, Exact Science, Gilead, Eli Lilly, Menarini/Stemline, MSD, Novartis, Pfizer, Roche, Sanofi, Seagen and Agendia

NeoTRIP trial results

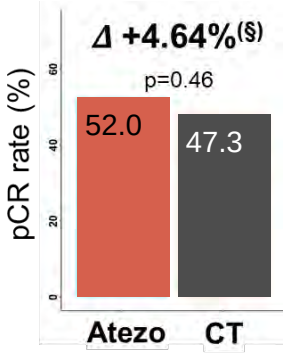
TN high-risk (T1cN1; T2N1; T3N0) or locally advanced

R

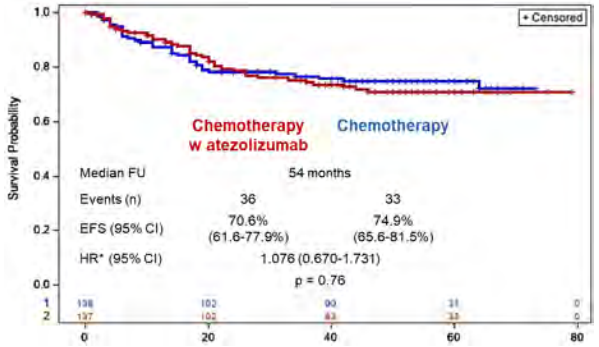
Carboplatin + *nab*-paclitaxel

Carboplatin + *nab*-paclitaxel + Atezolizumab

Surgery



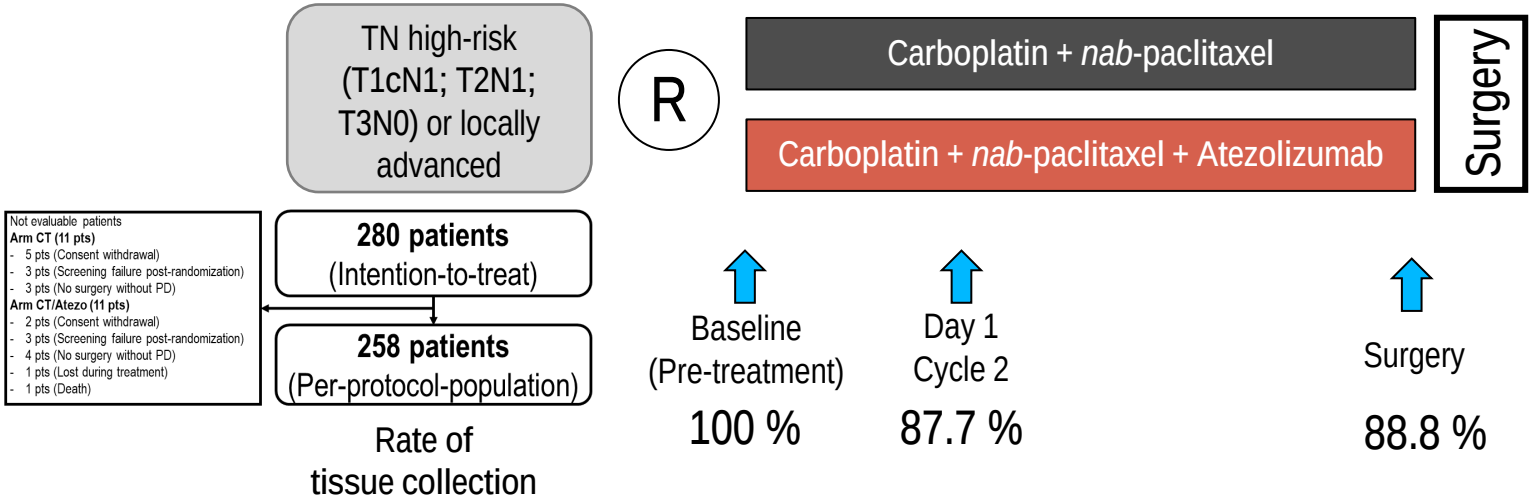
Gianni L Ann Oncol 2022



Gianni L ESMO 2023

Carboplatin (AUC2) + *nab*-paclitaxel (125 mg/m^2) weekly for 2 wks every 3; 8 cy; Atezolizumab (1200 mg) day 1 every 3 wks for 8 cycles

NeoTRIP trial: sample collection for translational studies



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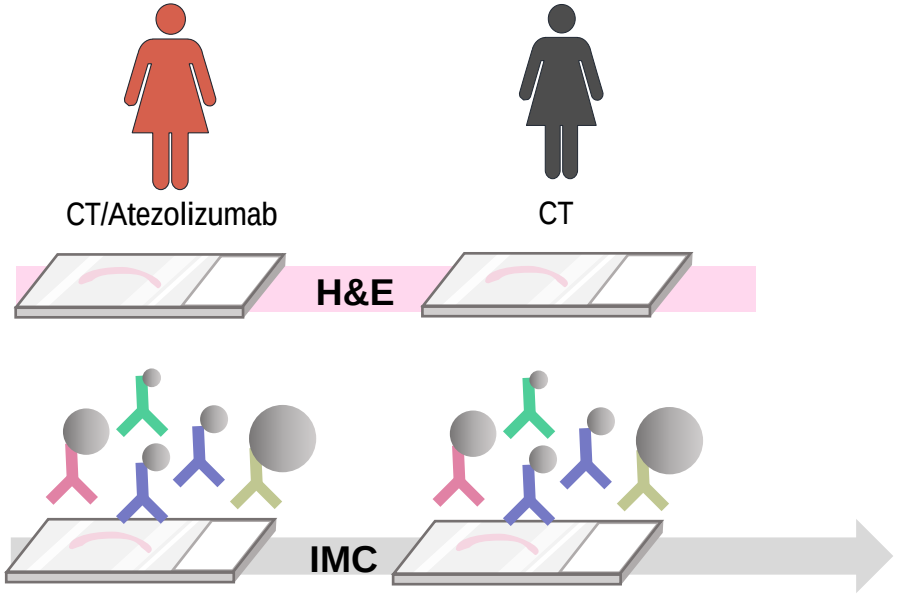
(§) Results in the Per-Protocol-Population (Bianchini G ESMO 2020 LBA13, Bianchini ESMO 2021 LBA11)

IMC: Imaging Mass Cytometry; PPP: Per-protocol-population (patients evaluable for pCR)

Bianchini G SABCS 2021

Forty-three proteins panel for IMC

- Forty-three proteins spanning cancer cells and the tumor microenvironment (TME) were assessed on FFPE samples with 43 antibodies conjugated to isotopically pure rare earth metal reporters and profiled by imaging mass cytometry (IMC)

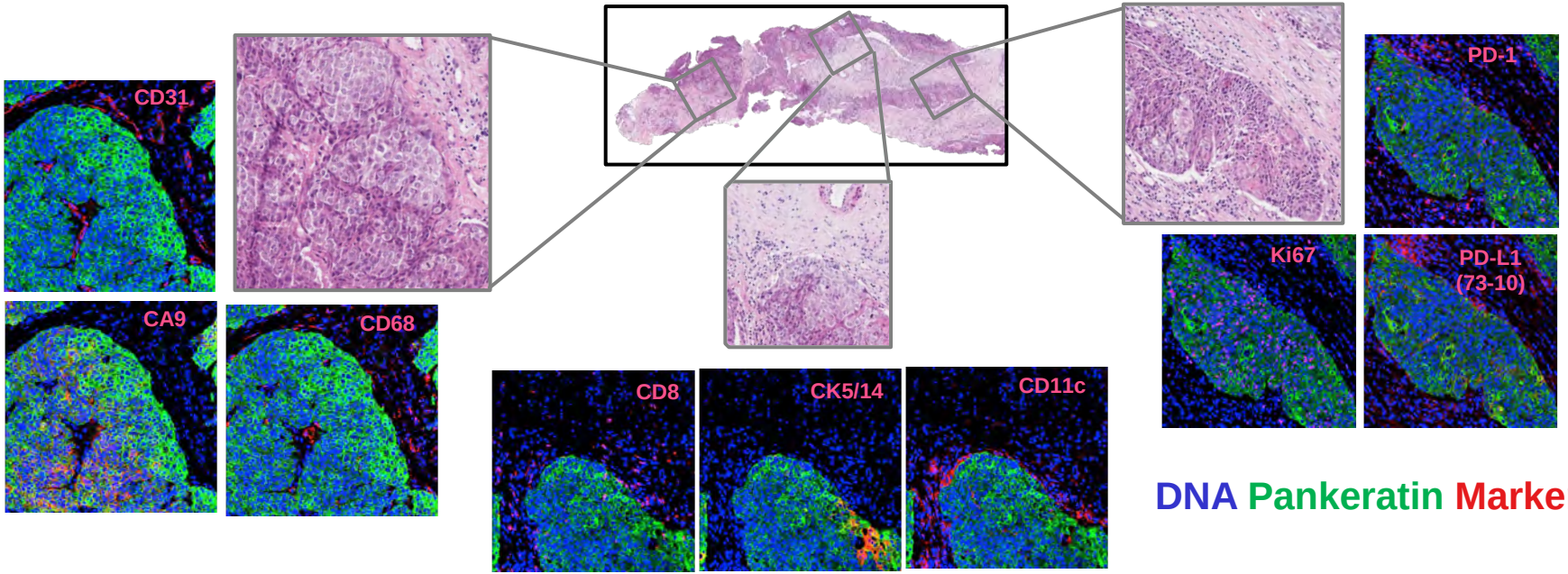


Checkpoint	Lymphoid	Epithelial	Life & Death
PD-L1 (SP142)	CD56	CK5/14	c-PARP
PD-L1 (73-10)	CD20	CK8/18	pH2AX
IDO	CD79a	PanCK	Ki67
PD-1	CD3	**Heterogeneity**	**DNA**
OX40	CD4	AR	H3
ICOS	CD8	GATA3	Ir
Myeloid	FOXP3	CD15	
CD11c	GATA3	**Mesenchymal**	
CD15	Helios	Caveolin-1	
CD163	T-bet	CD31	
CD68	TCF	PDPN	
MPO	TOX	PDGFRB	
MHC I&II	GZMB	SMA	
HLA-ABC	**Pan-immune**	Vimentin	
HLA-DR	CD45	Calponin	

Bianchini G SABCS 2021; Wang XQ Nature 2023

Definition of most informative microscopic fields

- For each sample, we have generated high dimensional images for <u>three microscopic fields</u> that encompass the tumor and tumor-stroma interface.



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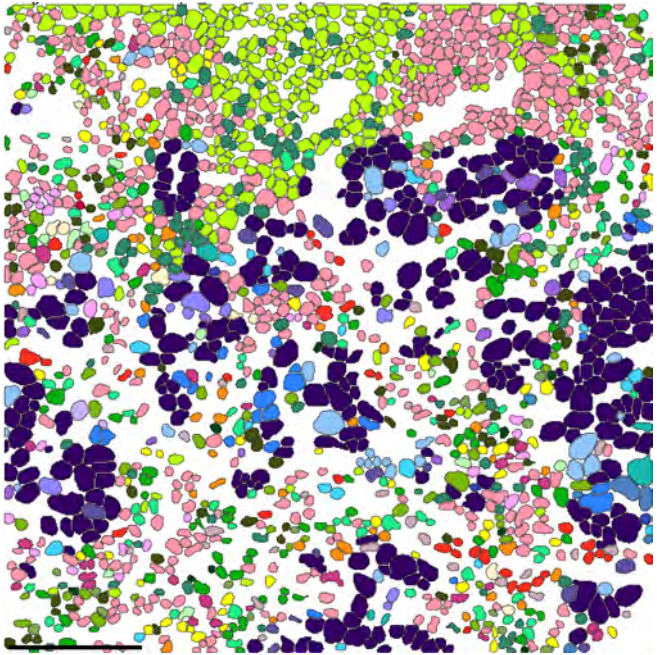
Single-cell information with spatial resolution



Cell phenotypes

Epithelial

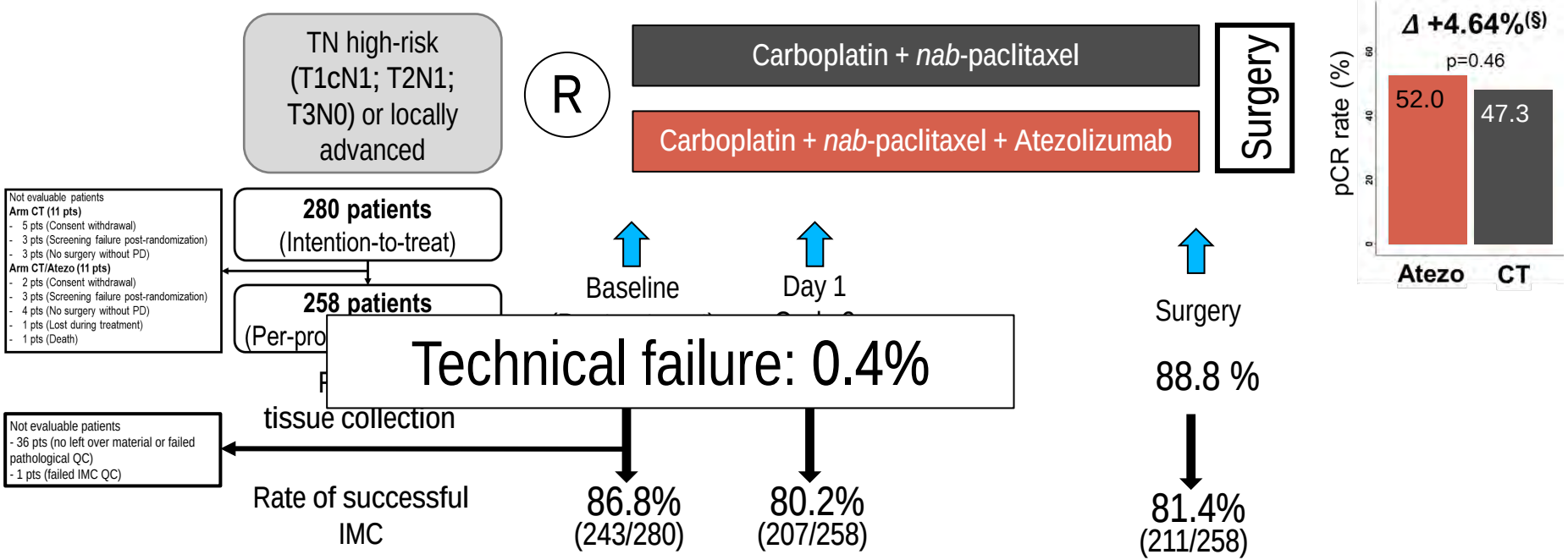
- CK8/18^{med}
- CK^{lo}GATA3^{+}
- panCK^{med}
- Basal
- Vimentin^{+} EMT
- CD15^{+}
- pH2AX^{+} DSB
- Apoptosis
- MHC I&II^{hi}
- PD–L1^{+} IDO^{+}
- PD–L1^{+} GZMB^{+}
- TCF1^{+}

TME

- CD8^{+} T
- CD8^{+} GZMB^{+} T
- CD8^{+} TCF1^{+} T
- CD8^{+} PD1^{+} T$_{Ex}$
- CD4^{+} TCF1^{+} T
- CD4^{+} PD1^{+} T
- Treg
- CD20^{+} B
- CD79a^{+} Plasma
- CD56^{+} NK
- M2 Mac
- DCs
- Neutrophils
- PD–L1^{+} APCs
- PD–L1^{+} IDO^{+} APCs
- Endothelial
- Fibroblasts
- Myofibroblasts
- PDPN^{+} Stromal

Bianchini G SABCS 2021; Wang XQ Nature 2023

NeoTRIP trial: rate of imaging mass cytometry (IMC) success



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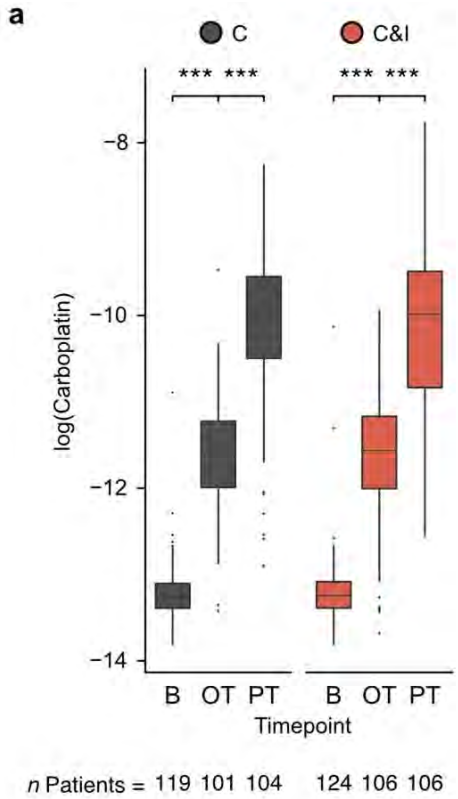
In situ carboplatin (on-treatment and at surgery)

Associations between cell phenotypes and TNBC subtypes



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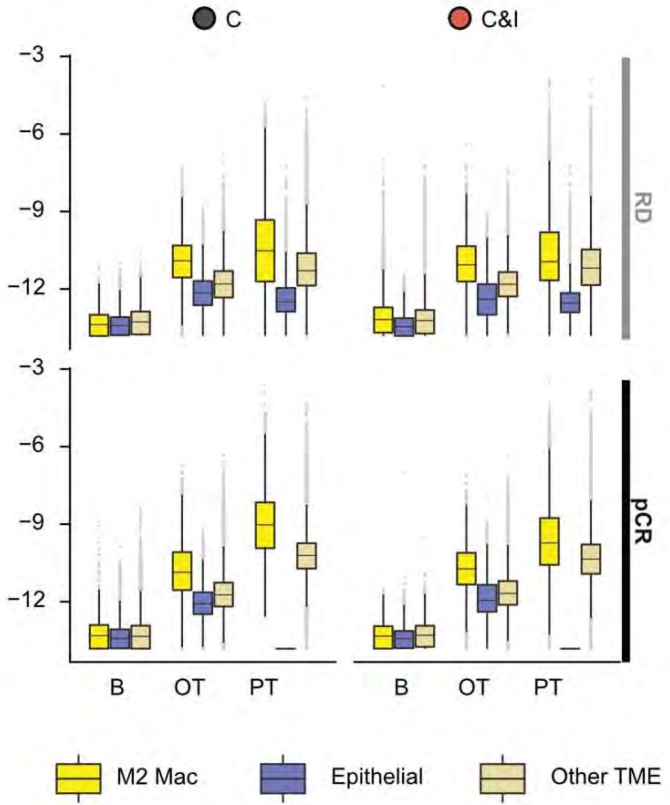
Associations between cell phenotypes and TNBC subtypes



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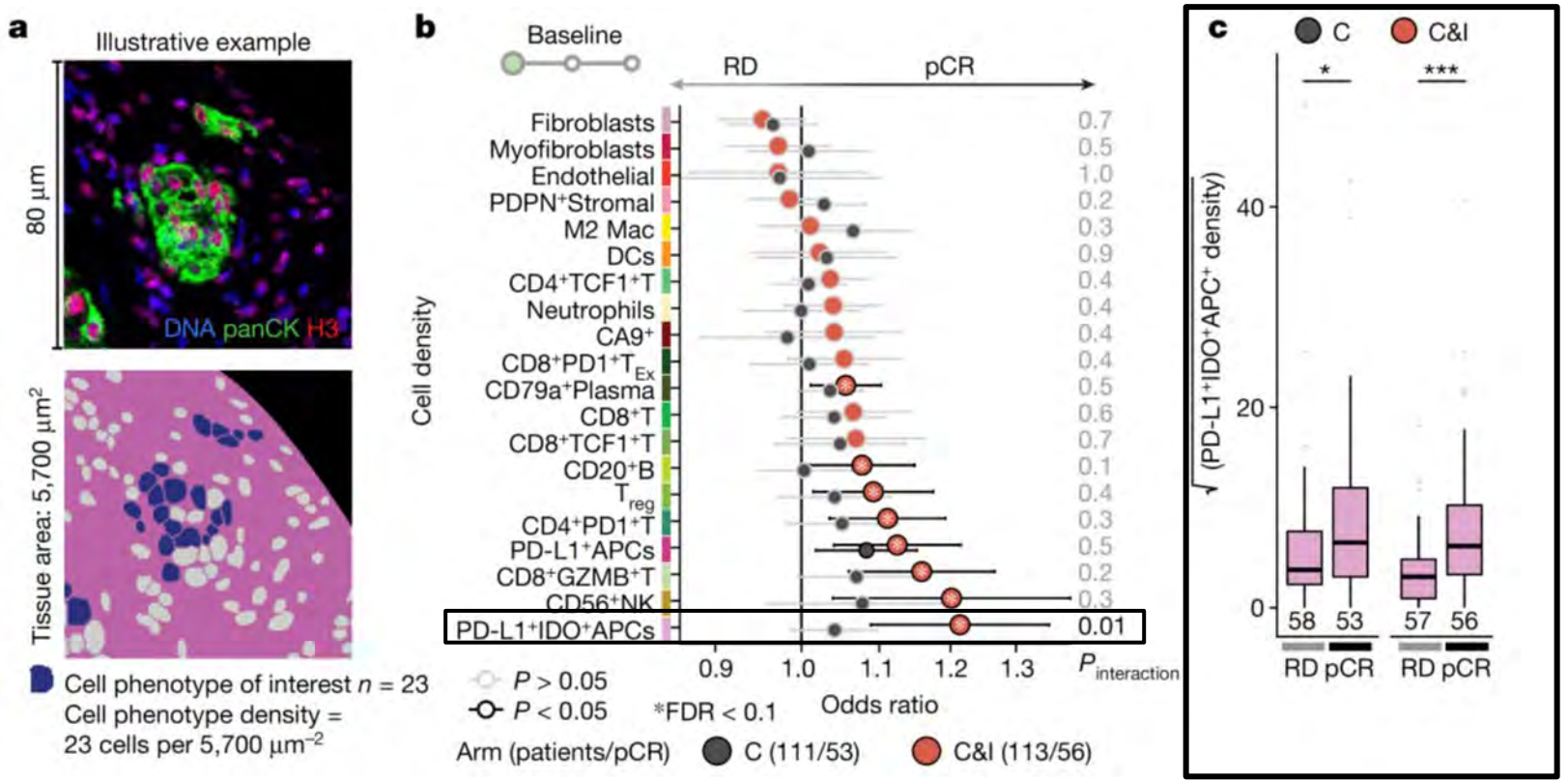
Baseline biomarkers

Cell phenotypes

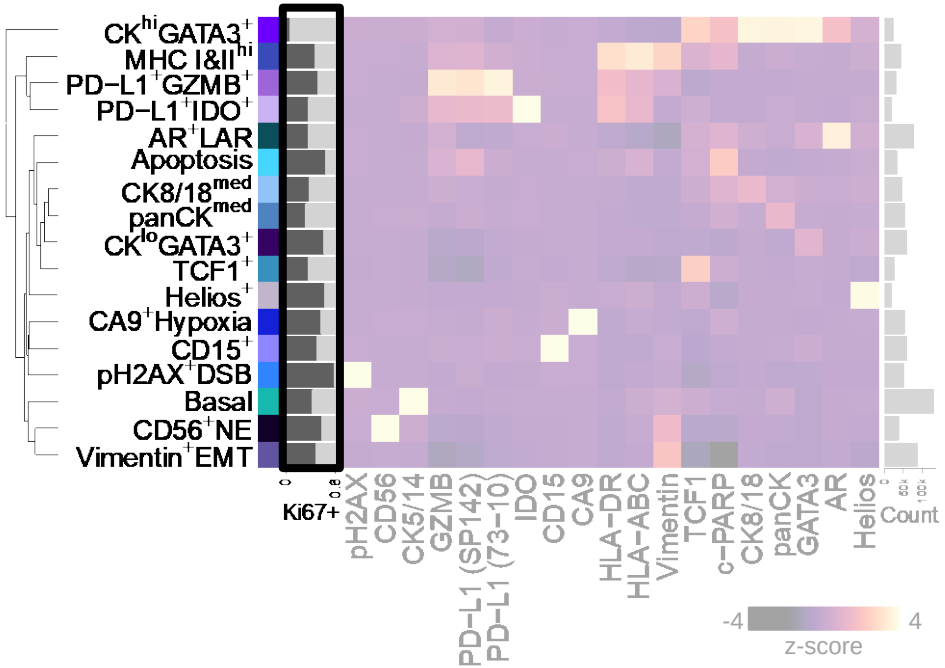
Association between *cell phenotypes density* and pCR by arm

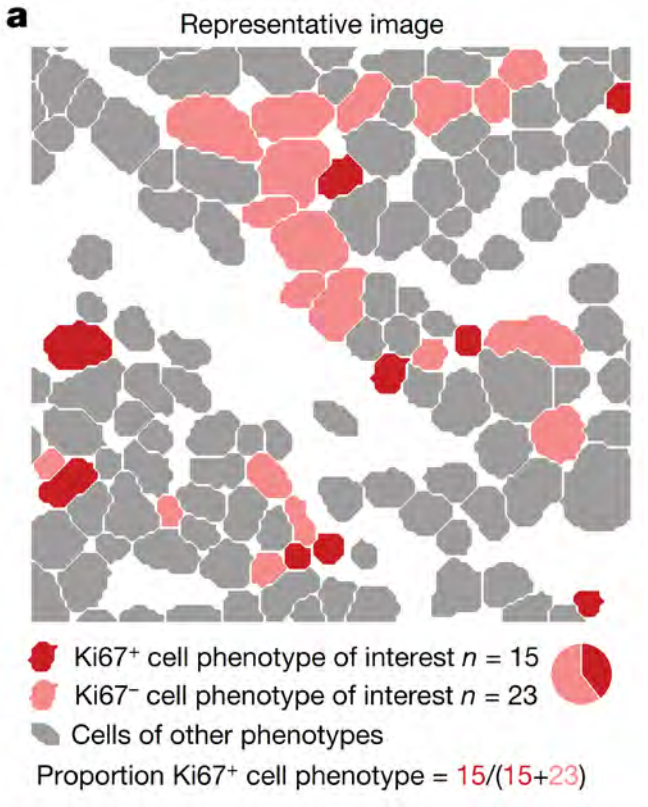


Baseline biomarkers

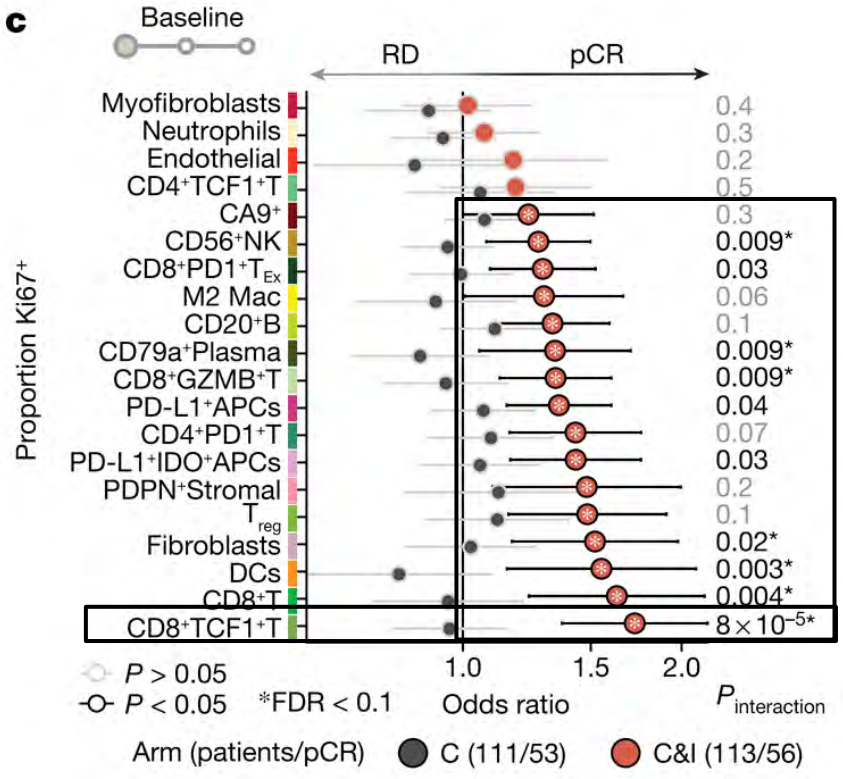
Functional state of cell types

Association between *proliferation fraction of cell phenotypes* and pCR by arm





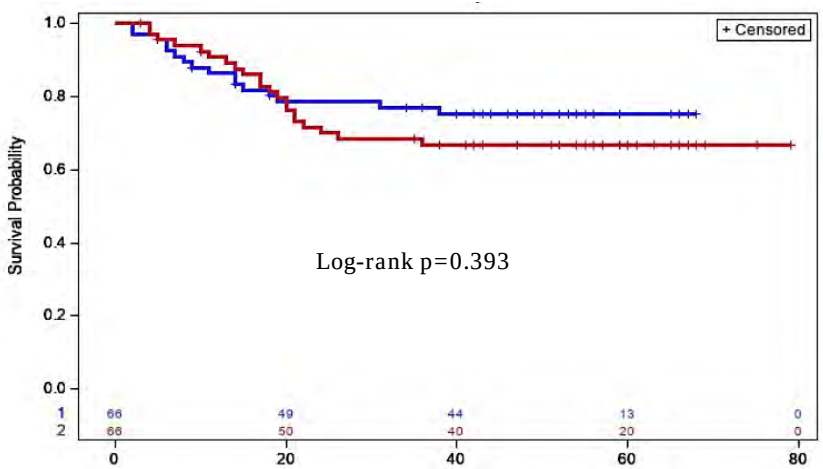
Association between *proliferation fraction of immune cell phenotypes* and pCR by arm



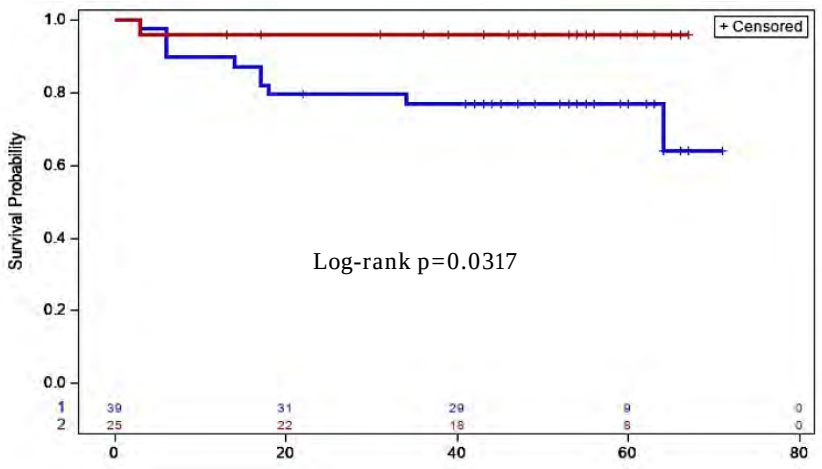
High CD8+TCF1+Ki67+ density linked to increased EFS by atezolizumab/CT combination

Exploratory analysis

CD8+TCF1+Ki67+ low



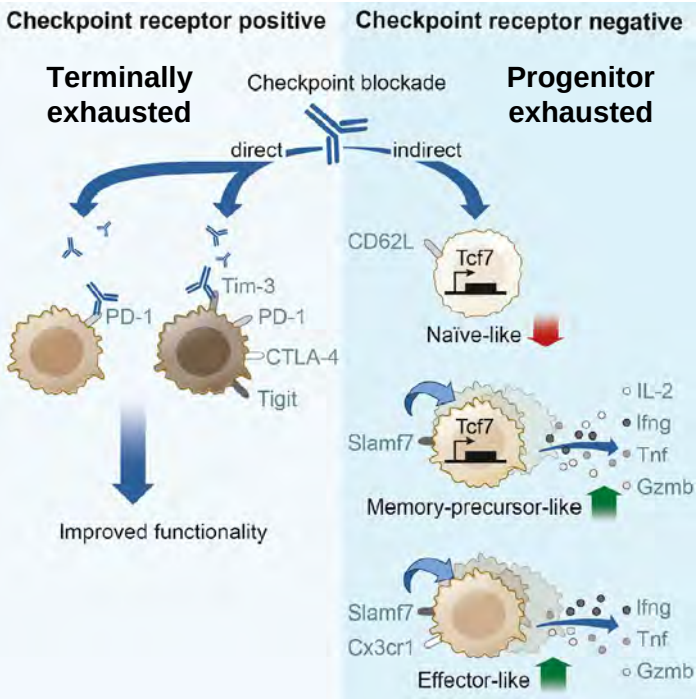
CD8+TCF1+Ki67+ high



Test for interaction p = 0.046

Note: analysis by tertiles low (low and intermediate tertiles) and high (higher tertile)

A subset of progenitor exhausted T Cell (Tcf7/Tcf1+ PD1+CD9+) is critical to generate a response to ICI (and vaccine)



- Heterogeneity of exhausted $CD8^+$ T cells populations: **terminally exhausted** (T_{TE} cells) and **progenitor exhausted** (T_{PE} cells)
- The proliferative burst after PD-1 blockade came almost exclusively from the dysfunctional subset of **Tcf7/$Tcf1^+$PD-1^+CD8^+** T cell subset which has **stem-like or progenitor properties**
- T_{PE} cells differentiate into T_{TE} cells in response to TCR stimulation and promote tumor control and correlated with prolonged PFS and OS on therapy

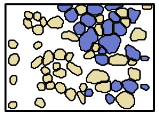
Im SJ Nature 2016, Sade-Feldman Cell 2018, Gettinger SN Nat Commun 2018, Siddiqui I Immunity 2019, Li H Cell 2019, Kurtulus S Immunity 2019; Miller BC *Nat Immunol* 2019; Kumar Vodnala S Nature 2019; Gebhardt T, Nature Reviews Cancer 2023; Gill AL Sci Immunol 2023

Baseline biomarkers

Heterotypic interactions

Spatial connectivity between Epithelial and TME

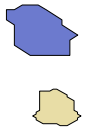
- Heterotypic spatial connectivity between epithelial and TME cells were assessed
- Only tight contacts between epithelial and TME cells were considered interactions



Epithelial cells

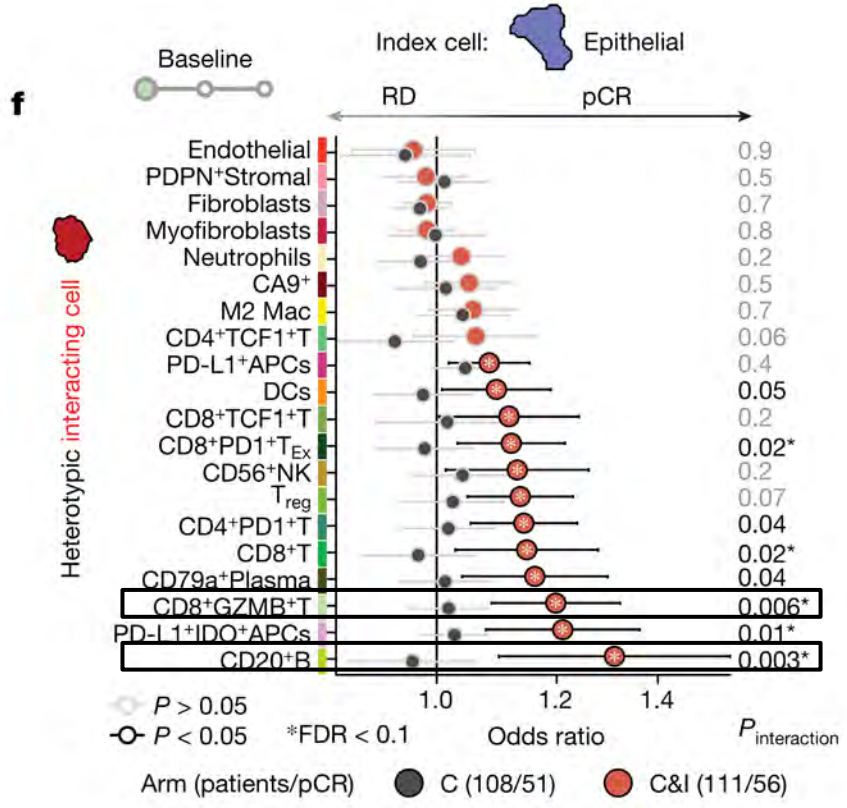
TME cells

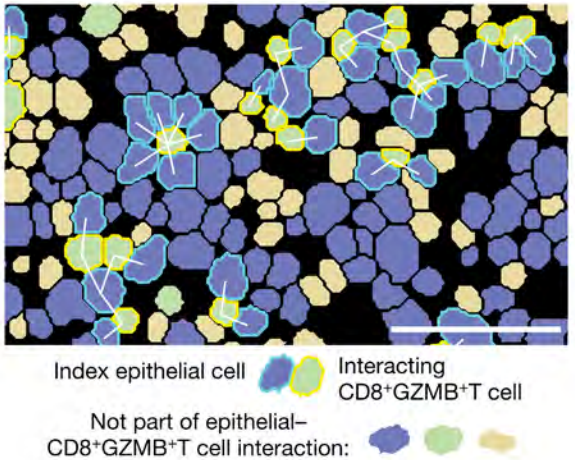
Interaction



NO interaction

Association between *heterotypic spatial interactions* of Epithelial-TME types and pCR by arm





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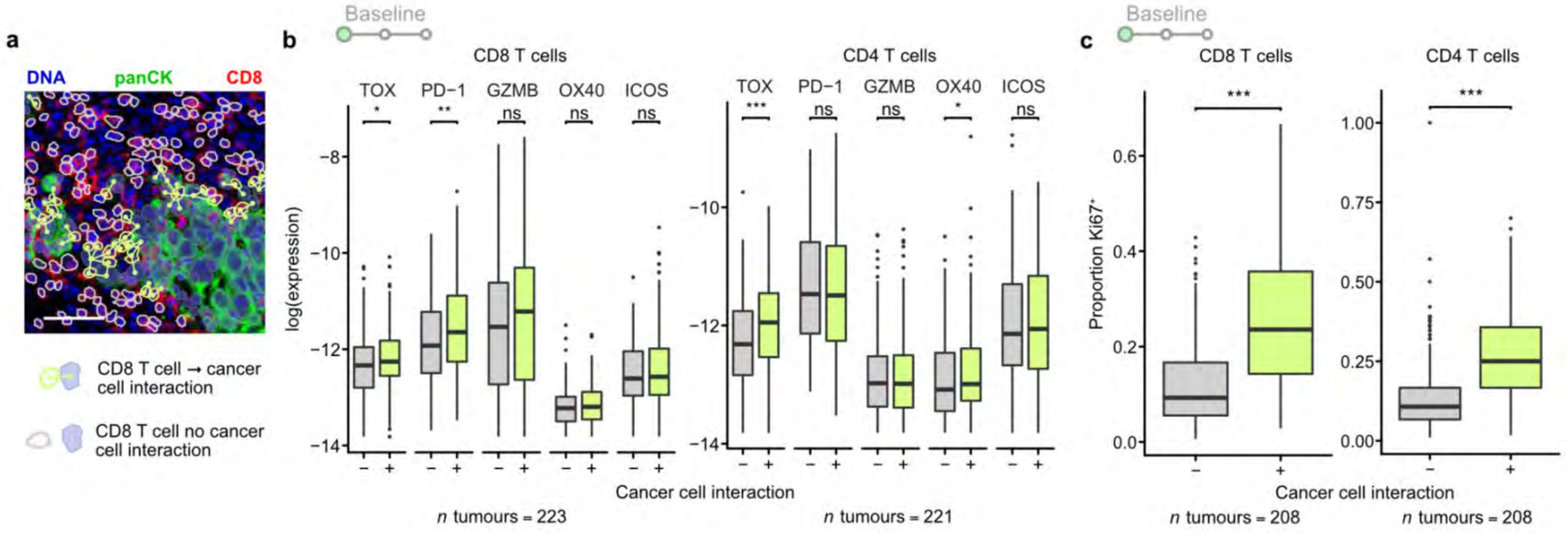
Baseline biomarkers

Functional state of cells with heterotypic interactions

Functional state of “interacting cells”

- T cells in direct contact with cancer cells were functionally distinct:
  ⬆️ activation markers TOX, PD-1, OX40 and ⬆️ proliferating



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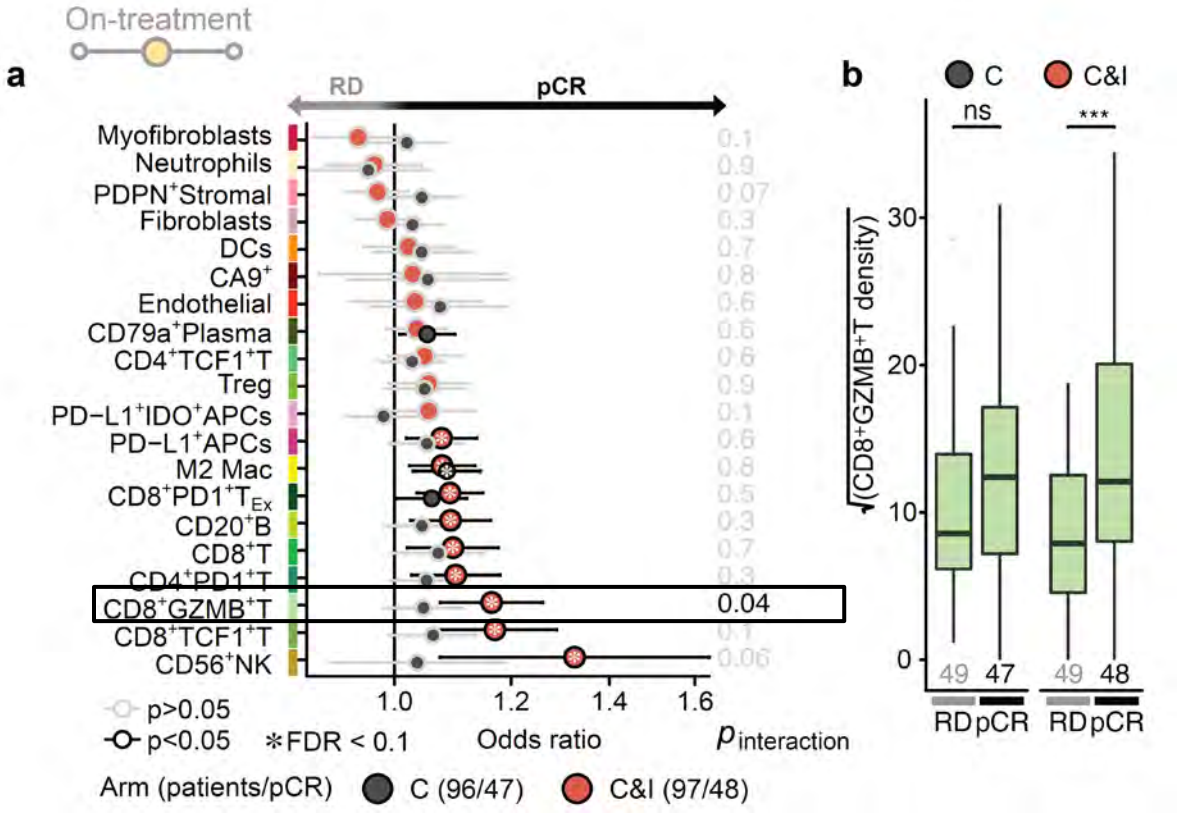
On-treatment biomarkers

Sensitive tumours enriched in cytotoxic T cells



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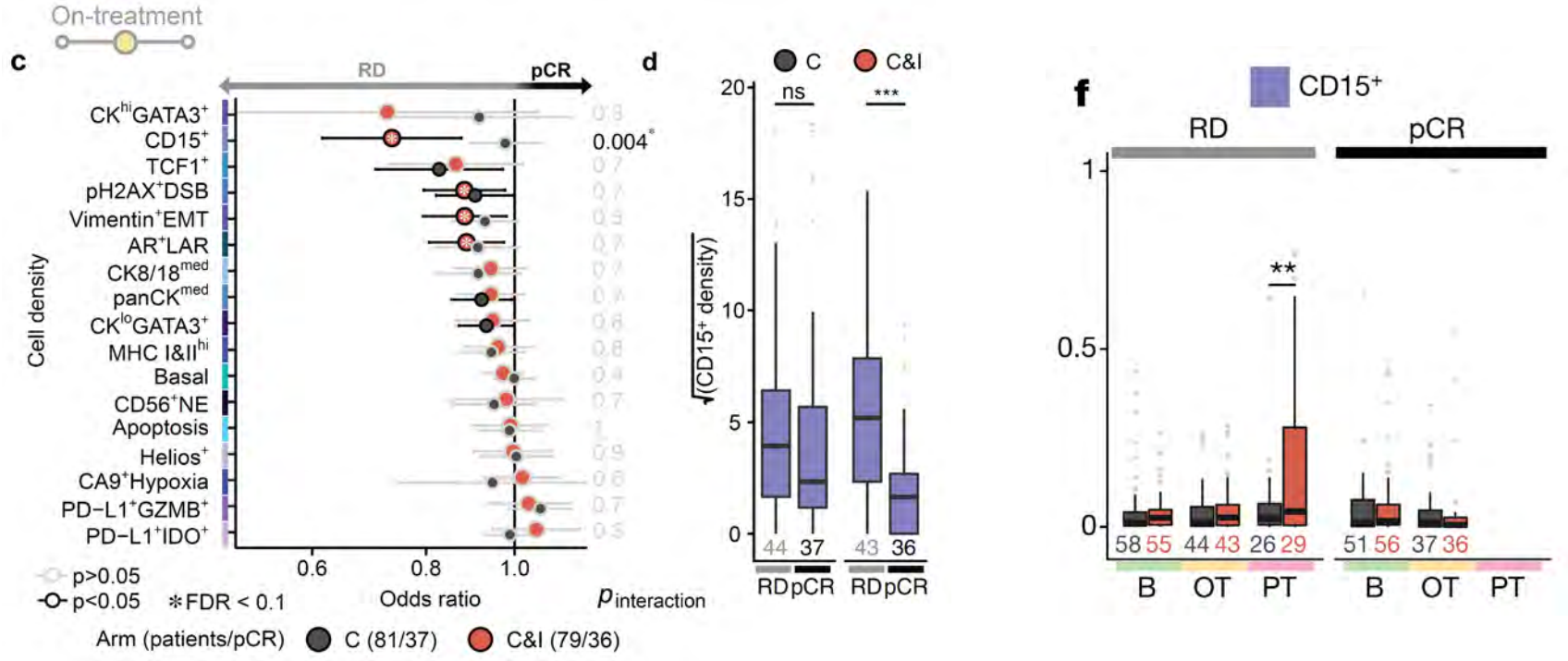
Resistant tumours enriched in CD15+ tumour cells

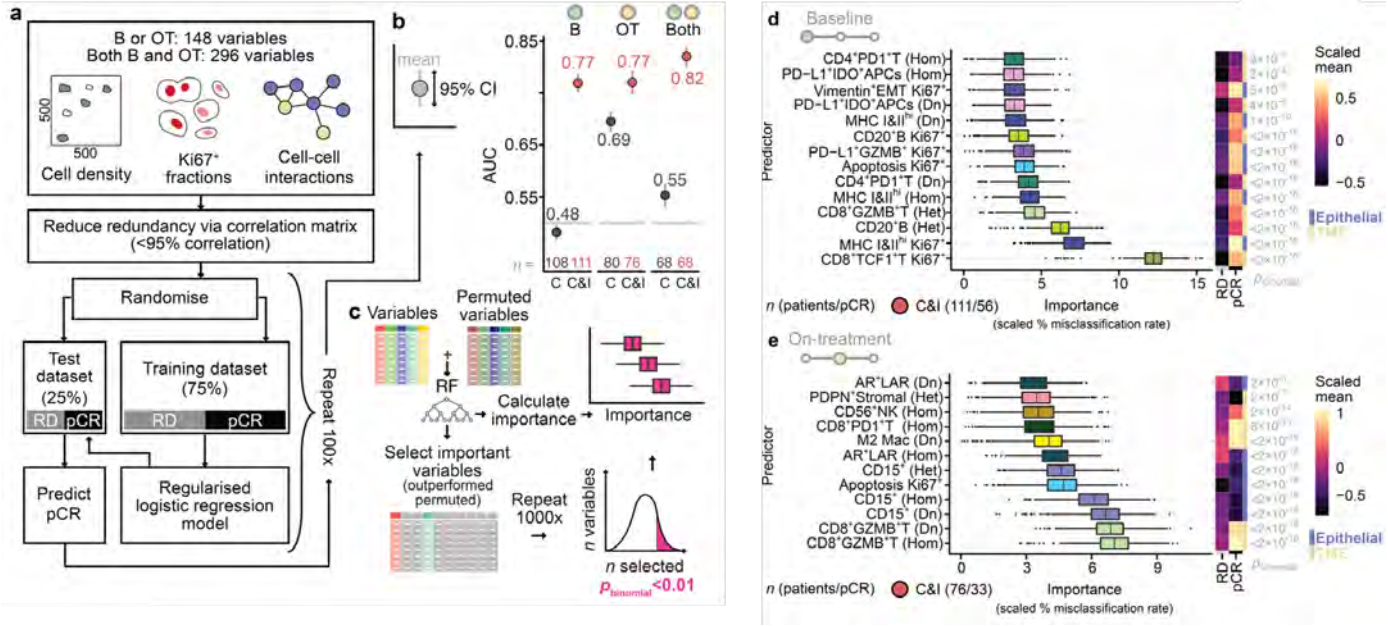


Potential new mechanism of adaptive immune resistance

Multivariate modelling

Dominant ICB response predictors

- Multivariate modeling revealed that TME activation and tumor structure play pivotal roles in predicting treatment responses to immunotherapy
- Additionally, early on-treatment biopsies enhance predictive accuracy



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Data Availability

- All imaging mass cytometry and clinical response data can be accessed via a Zenodo data repository (https://doi.org/10.5281/zenodo.7990870) for academic non-commercial research

Code availability

- Code for image processing is hosted at https://github.com/BodenmillerGroup/ImcSegmentationPipeline and https://github.com/vanvalenlab/deepcell-tf
- All additional analysis code can be accessed via https://doi.org/10.5281/zenodo.7990870 alongside study data.

Conclusions

- IMC is feasible in a large, randomized trial of neoadjuvant therapy and provides a comprehensive overview of TNBC heterogeneity at a single-cell level with spatial resolution

- Immmune cell phenotype, activation state and spatial location are intimately linked, influence ICB effect and differ in sensitive versus resistant tumours early on-treatment

- Systematic enumeration in situ of multicellular spatial organization could help realize precision immuno-oncology

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