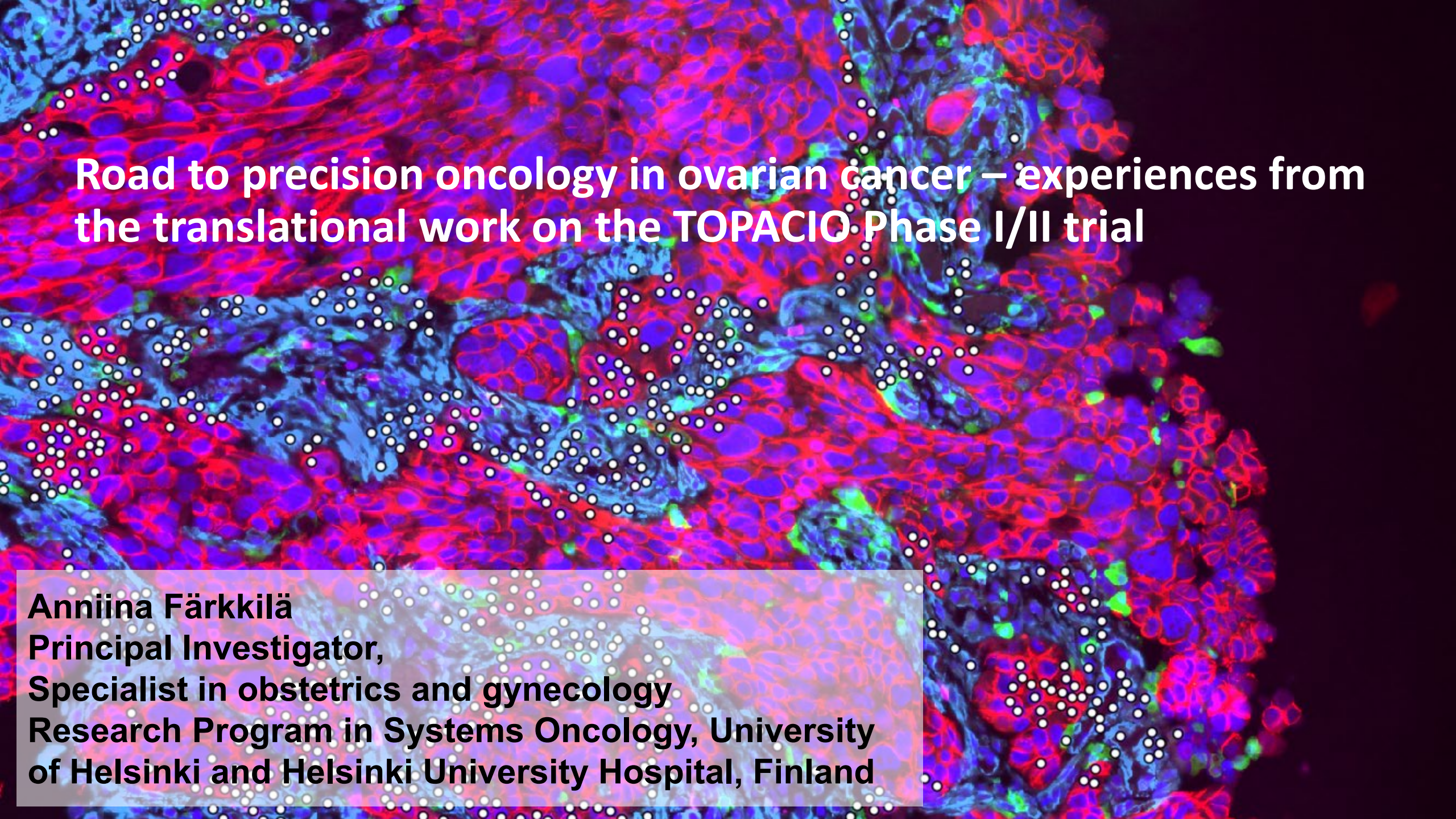


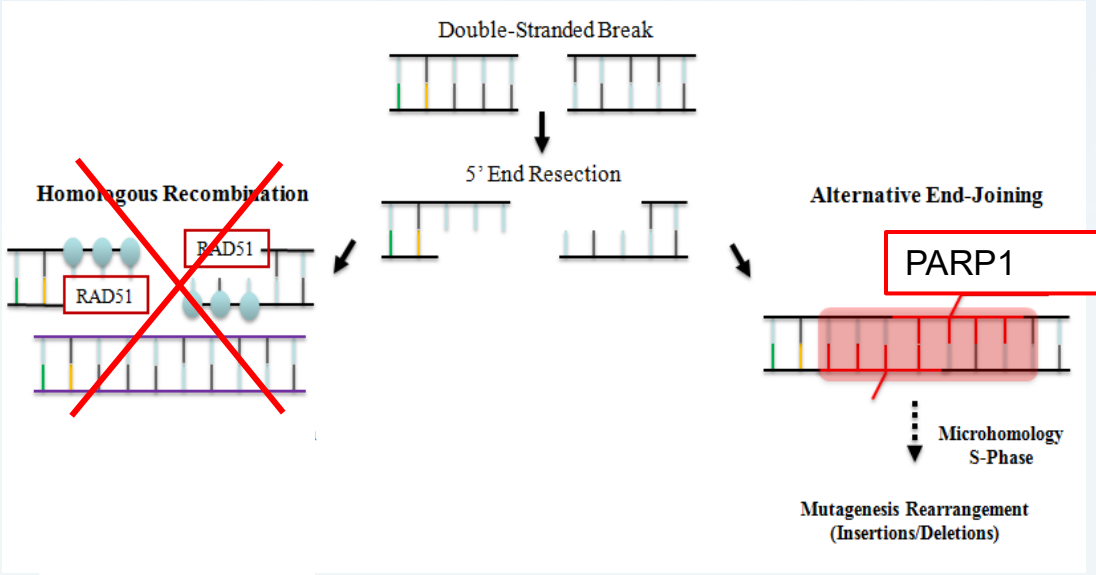
Road to precision oncology in ovarian cancer – experiences from the translational work on the TOPACIO Phase I/II trial

Anniina Färkkilä
Principal Investigator,
Specialist in obstetrics and gynecology
Research Program in Systems Oncology, University of Helsinki and Helsinki University Hospital, Finland

Disclosures

- Principal Investigator, Systems Oncology Research Program, University of Helsinki
- Specialist in Obstetrics and Gynecology, Helsinki University Hospital, Helsinki, Finland
- Board member: Nordic Society of Gynecologic Oncology (NSGO)
- Translational working groups; NSGO, European Network for Gynecologic Cancer trials (ENGOT), Gynecologic Cancer Intergroup (GCIG)
- Molecular tumor board: FINPROVE, Finnish national cancer institute (FICAN)
- Speaker's honoraria; Ferring Pharmaceuticals, Astra Zeneca, GSK
- Grant support: Astra Zeneca, GSK

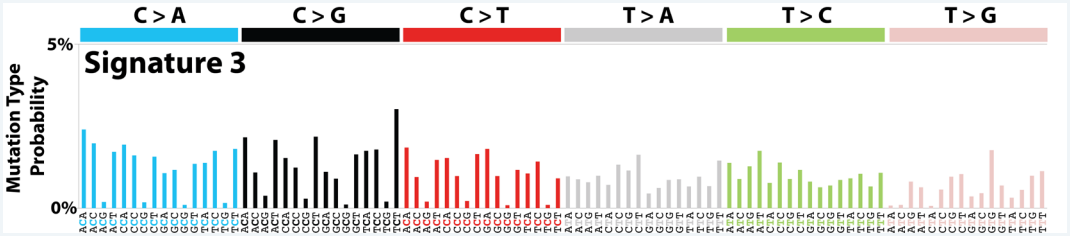
HR-deficient HGSOCs are dependent of error-prone DNA repair mechanisms



Synthetic lethality with PARP inhibitors

Chromosomal instability

Mutational Signature 3



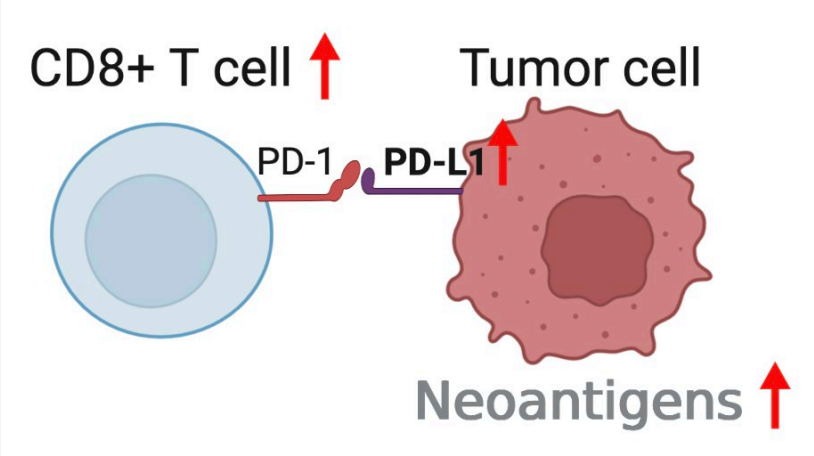
Alexandrov LB, et al. Nature. 2013;500:415–412; Ceccaldi R, et al. Nature. 2015;518:258–262; Konstantinopoulos PA, et al. *Cancer Discov*. 2015;5(11):1137–54.
HGSOC, high-grade serous ovarian cancer; HR, homologous recombination; PARP, poly(ADP-ribose) polymerase.

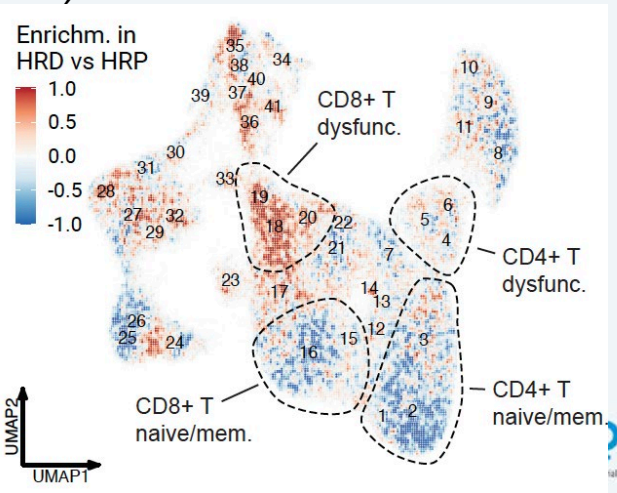
Tumor genotypes shape the tumor microenvironment (TME) in HGSC

- Tumor genotypes associate with distinct TMEs and responses to immunotherapies *(Iyer 2020, Zhang 2018, Vasquez-Garcia BiorXiv 2021)*

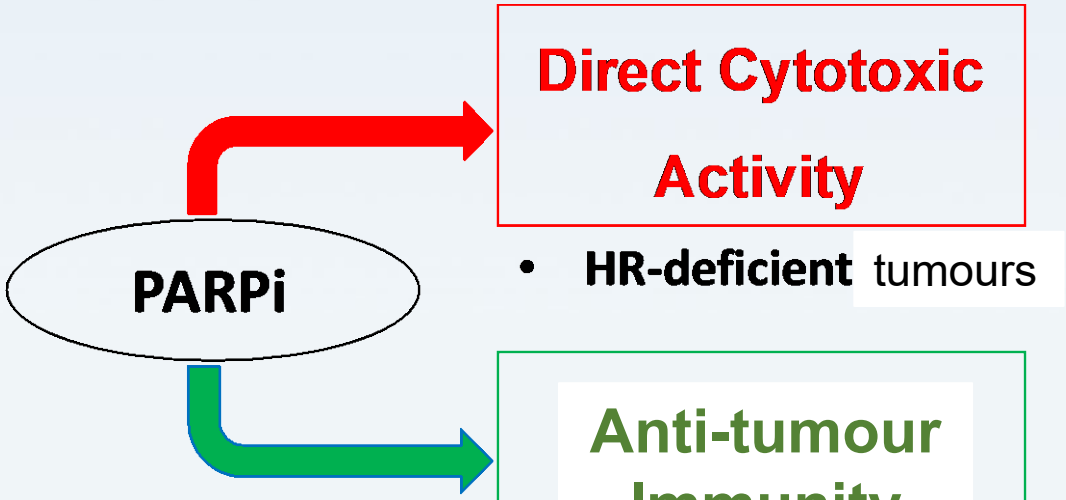
Tumor genotypes shape the tumor microenvironment (TME) in HGSC

- Tumor genotypes associate with distinct TMEs and responses to immunotherapies *(Iyer 2020, Zhang 2018, Vasquez-Garcia BiorXiv 2021)*
- BRCAmut/HRD tumors have suggested to have
  - higher neoantigen load, PD-L1 expression and CD8+T-cell infiltration *(Vasquez-Garcia Biorxiv 2021, Strickland 2016, Bohm 2017, Sato 2017)*
  - Enhanced responses to PARPi + immune checkpoint blockade in preclinical models *(Ding 2018; Jiao 2017; Parkes 2016; Shen 2019, Iyer 2020)*





PARP inhibitors enhance anti-tumour immunity



- **HR-deficient** tumours

- **In HR-deficient and -proficient**
- Mediated by Type I Interferons
- Augmented by PD-1/PD-L1 blockade

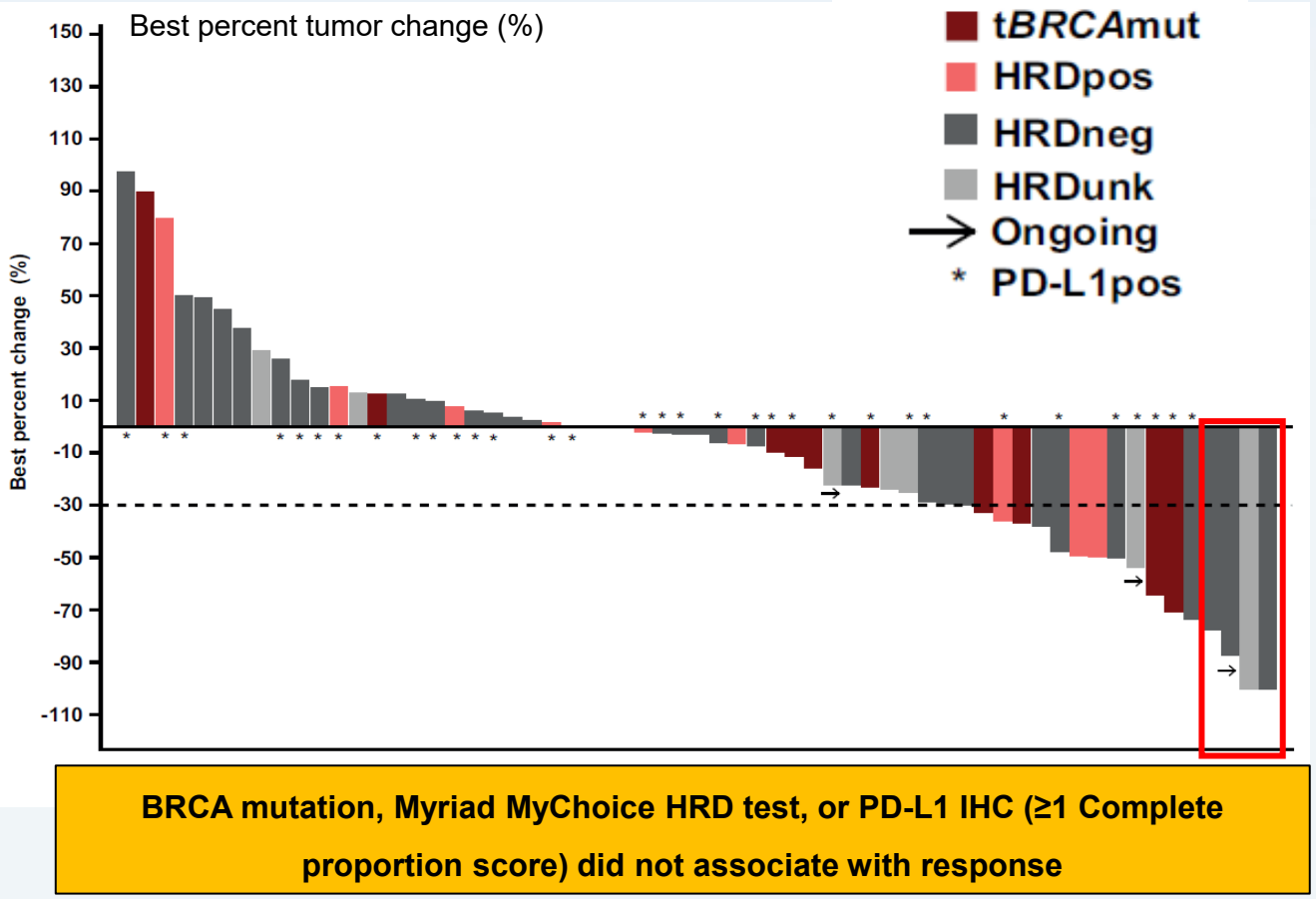
Rationale for combining PARP inhibition with immune checkpoint blockade

Ding L, et al. *Cell Reports.* 2018;25:2972–2980; Jiao S, et al. *Clin Can Res.* 2017;23(14):3711–3720; Parkes EE, et al. *JNCI.* 2016;109(1):djw199; Shen J, et al. *Can Res.* 2019;79(2):311–319.
HR, homologous recombination; PARP(i), poly(ADP-ribose) polymerase (inhibitor).

Combination of PARPi and PD-1i results in clinical activity in platinum-resistant ovarian cancer (OC)

- **TOPACIO/Keynote-162** (NCT02657889) phase I/II trial enrolled 62 patients with relapsed, platinum-resistant OC

Best Objective Response (BOR)	**Integrated efficacy analysis**	
Evaluable patients n=60	n	%
Complete response (CR)	3	5
Partial response (PR)	8	13
Stable disease (SD)	28	47
Progressive disease (PD)	20	33
Objective response rate (ORR)	**11**	**18 %**
Clinical benefit	**39**	**65 %**



BRCA mutation, Myriad MyChoice HRD test, or PD-L1 IHC (≥1 Complete proportion score) did not associate with response

Konstantinopoulos et al. JAMA Oncol. 2019 Jun 13;5(8):1141-9 [t]BRCA(1/2), [tumour] breast cancer (type 1/2) susceptibility gene; HRD(pos/neg/unk), homologous recombination deficient (positive/negative/unknown); mut, mutation; OC, ovarian cancer;
PARP(i), poly(ADP-ribose) polymerase (inhibitor).

Translational work in the TOPACIO trial

- Fundgin from Su2C – Catalyst grant
- Working with Tesaro(GSK), Dana-Farber Cancer Institute, University of Washington
  - 3-way MTA:s
  - monthly teleconferences
- Sharing of results and publications

JAMA Oncol. 2019 Aug; 5(8): 1141–1149.
Published online 2019 Jun 13. doi: 10.1001/jamaoncol.2019.1048
PMCID: PMC6567832
PMID: 31194228

Single-Arm Phases 1 and 2 Trial of Niraparib in Combination With Pembrolizumab in Patients With Recurrent Platinum-Resistant Ovarian Carcinoma

Panagiotis A. Konstantinopoulos, MD, PhD,[1,2] Steven Waggoner, MD,[3] Gregory A. Vidal, MD,[4] Monica Mita, MD,[5] John W. Moroney, MD,[6] Robert Holloway, MD,[7,8] Linda Van Le, MD,[9] Jasgit C. Sachdev, MD,[10,11] Eloise Chapman-Davis, MD,[12] Gerardo Colon-Otero, MD,[13] Richard T. Penson, MD,[14] Ursula A. Matulonis, MD,[15] Young Bae Kim, MD,[16] Kathleen N. Moore, MD,[17,18] Elizabeth M. Swisher, MD,[19] Anniina Färkkilä, MD,[20] Alan D'Andrea, MD,[21] Erica Stringer-Reasor, MD,[22] Jing Wang, PhD,[23] Nathan Buerstatte, MPH,[24] Sujata Arora, MS,[25] Julie R. Graham, PhD,[26] Dmitri Bobilev, MD,[26] Bruce J. Dezube, MD,[26] and Pamela Munster, MD[27]

Nat Commun. 2020; 11: 1459.
Published online 2020 Mar 19. doi: 10.1038/s41467-020-15315-8
PMCID: PMC7081234
PMID: 32193378

Immunogenomic profiling determines responses to combined PARP and PD-1 inhibition in ovarian cancer

Anniina Färkkilä,[1,2,3,4] Doga C. Gulhan,[#2] Julia Casado,[#3] Connor A. Jacobson,[4] Huy Nguyen,[1] Bose Kochupurakkal,[1] Zoltan Maliga,[4] Clarence Yapp,[4] Yu-An Chen,[4] Denis Schapiro,[4] Yinghui Zhou,[5] Julie R. Graham,[5] Bruce J. Dezube,[5] Pamela Munster,[6] Sandro Santagata,[7] Elizabeth Garcia,[8] Scott Rodig,[8] Ana Lako,[8] Dipanjan Chowdhury,[1] Geoffrey I. Shapiro,[1] Ursula A. Matulonis,[1] Peter J. Park,[9] Sampsa Hautaniemi,[3] Peter K. Sorger,[4] Elizabeth M. Swisher,[10] Alan D. D'Andrea,[#1] and Panagiotis A. Konstantinopoulos[#1]

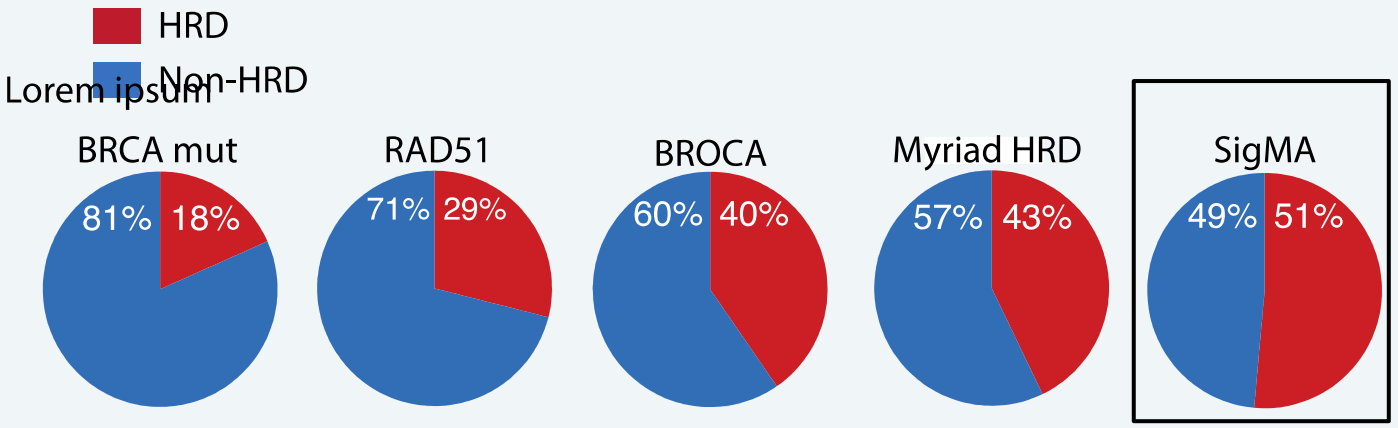
Genomic and immunologic correlative analyses on FFPE tumour samples

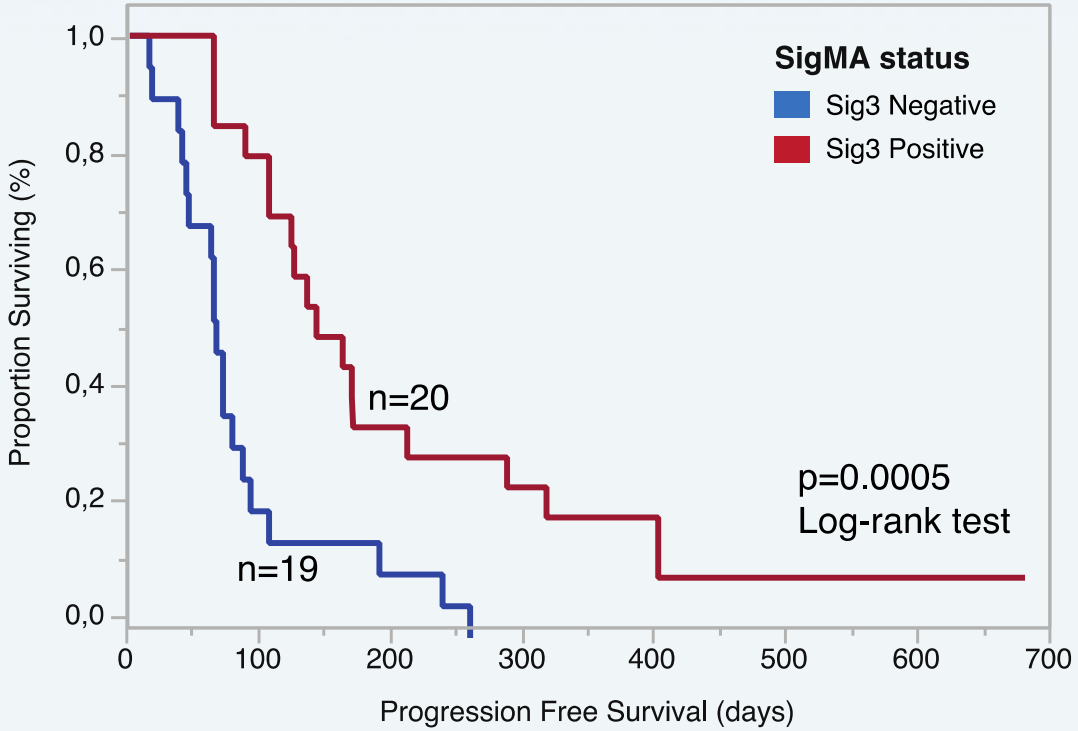
1. **Tumour HRD profiling**
   - Oncopanel targeted sequencing for 350 genes
     - SigMA Mutation signature 3 analysis[1]
   - Targeted sequencing of 85 HR-DNA repair genes (BROCA), and analysis for BRCA1/RAD51 hypermethylation
   - RAD51 IHC
2. **Nanostring mRNA expression profiling**
   - expression of 800 genes
     - Immune cell type and pathway analysis
3. **Single-cell analysis of the tumour microenvironment**
   - tCycIF multiplexed immunofluorescence – Image analysis
     - Immune cell types
     - Functional states
     - Spatial analysis

1. Gulhan DC, et al. *Nat Genet.* 2019;51(5):912–919.
BRCA(1/2), breast cancer (type 1/2) susceptibility gene; HR(D), homologous recombination (deficient).

Mutational signature 3 positivity correlates with clinical benefit

- **BRCAmut, RAD51 IHC, BROCA or Myriad HRD did not associate with response**
- **Sig3 with SigMA identified more HRD compared to other methods**





Median PFS; 2.2 months vs 5.1 months

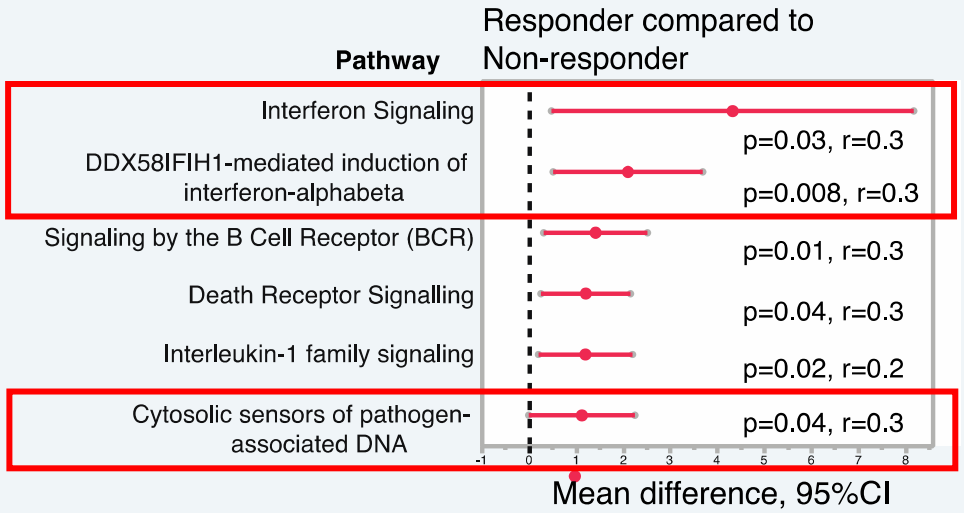
Farkkila A, et al. *Nat Comm.* 2020;11(1):1459.

BRCA(1/2), breast cancer (type 1/2) susceptibility gene; HRD, homologous recombination deficient; mut, mutation; PFS, progression free survival.

Interferon signaling and exhausted CD8+T-cells associate with response

- 800 genes (IO360 + 30 DNA repair genes)
- NSolver Advanced Analysis; Pathway analysis, Cell type scores

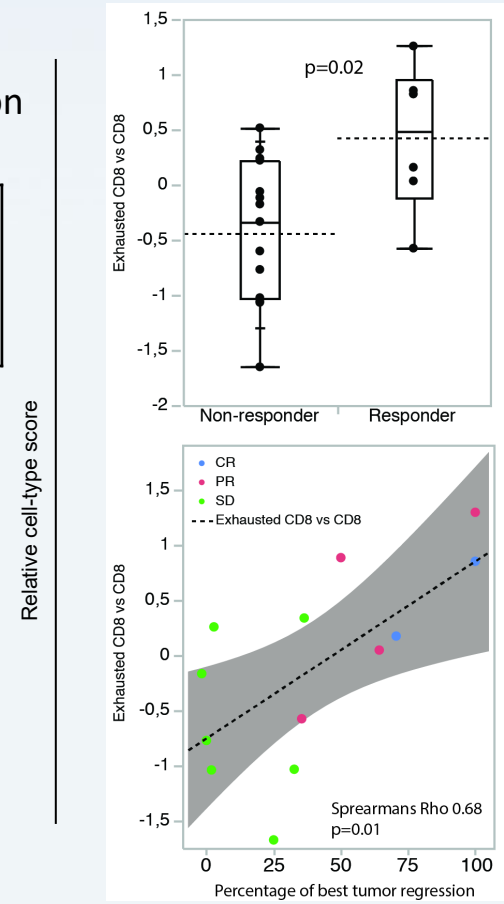
Interferon Signalling



Exhausted CD8+T-cells

Exhaustion Markers

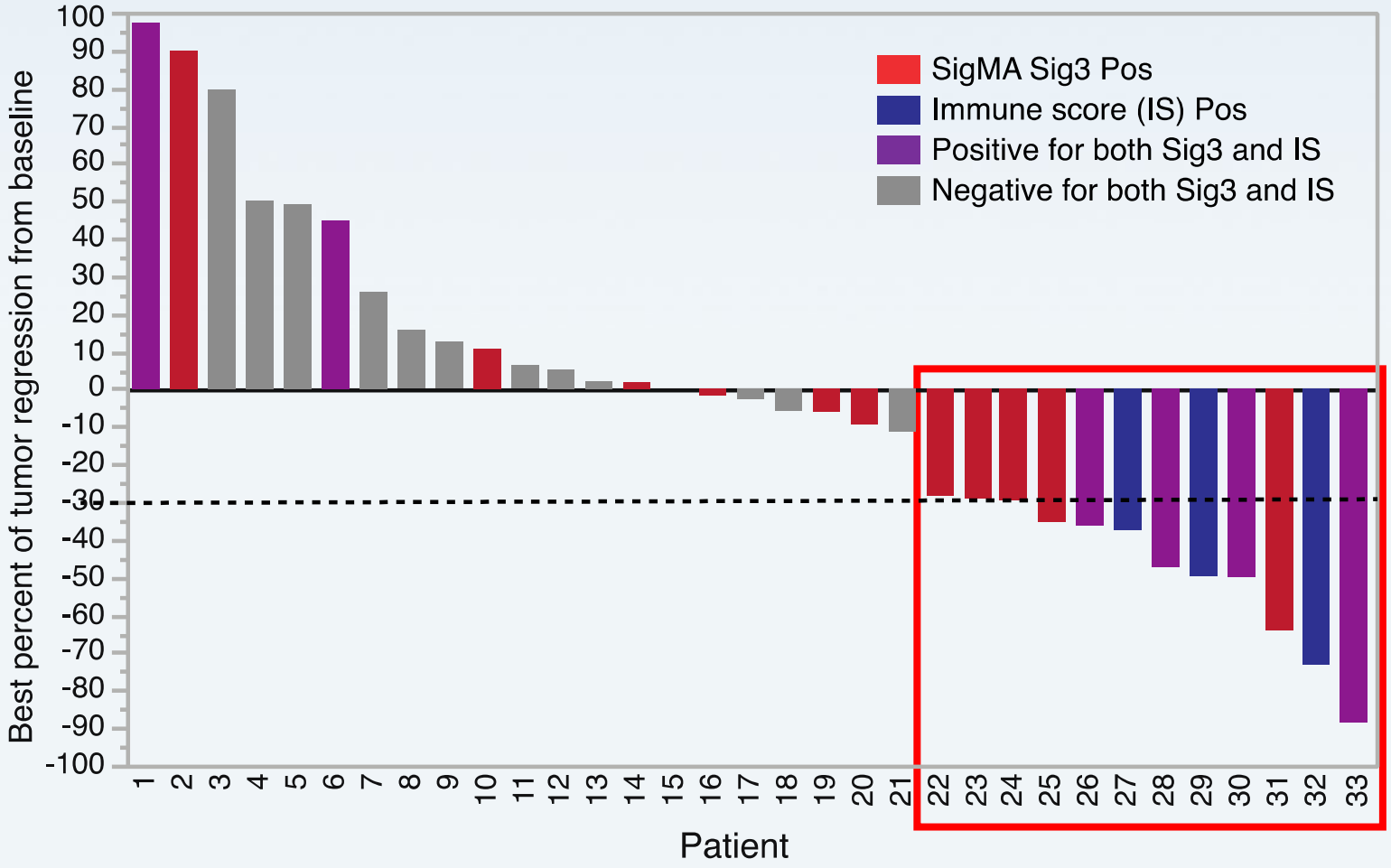
CD244
EOMES
LAG3
PTGER4



Farkkila A, et al. *Nat Comm.* 2020;11(1):1459.
CI, confidence interval.

Mutational signature 3 and immune score identify all responders

Immune Score (IS)
Positive; >75% score
Interferon/DDX58/Cytosolic DNA
Exhausted CD8/CD8 T-cell score



-> ORR 29% in the biomarker positive patients

0% in the biomarker negative patients

Farkkila A, et al. *Nat Comm.* 2020;11(1):1459.
IS, immune score; ORR, overall response rate.

Can we learn more about the mechanisms of response associated with these biomarkers?

What is the cell type in the TME that is responsible for the high interferon signalling?

How do exhausted CD8+T-cells associate with response?

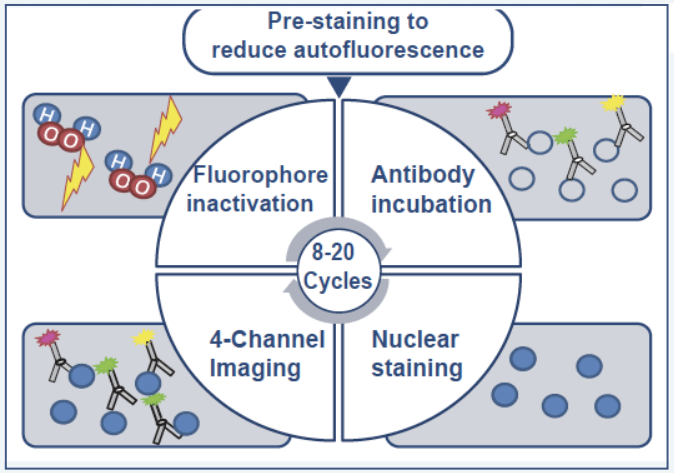
Is mutational signature 3 related to the immune microenvironment?

Are there spatial interactions in the TME that associate with response?

TME, tumour microenvironment.

Single-cell spatial proteomic profiling of the TME

- Multiplexed tissue cyclic immunofluorescence (tCycIF)
  - Formalin Fixed Paraffin Embedded (FFPE) tissue samples
  - up to 60-100 antibodies for cell types and functional states



Staining and image acquisition

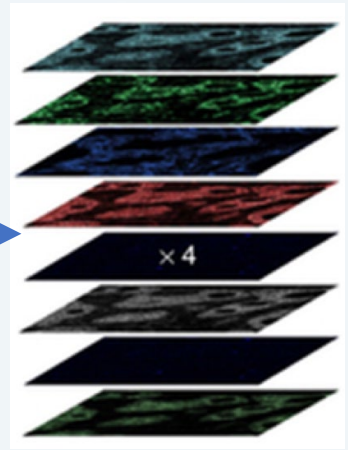
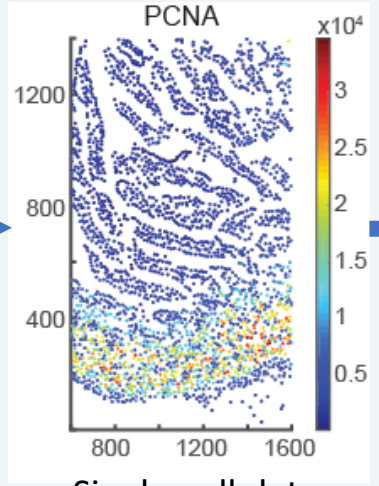
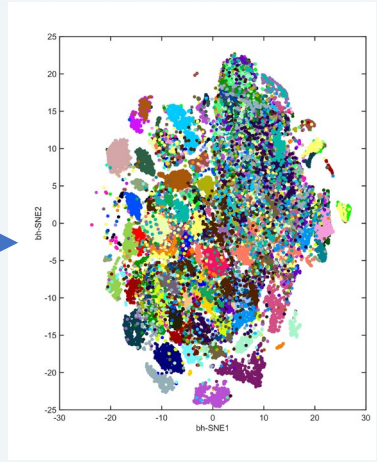


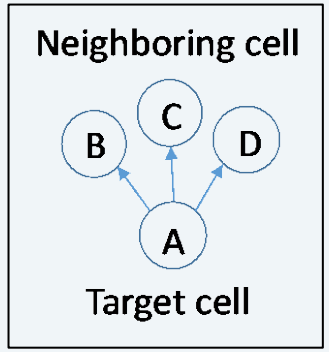
Image processing



Single-cell data quantification



Bioinformatics



Spatial analysis

Farkkila A, et al. *Nat Comm.* 2020;11(1):1459.
TME, tumour microenvironment.

tCycIF maker panel of cell types and states in the TME

Cell types

Tumor cells
PAX8
CK7
E-cadherin

Immune cells	Role
CD3d	T-cells
CD8	Cytotoxic T-cells
CD4	T-cells
CD54RO	Memory T- cells
CD11c	Dendritic cells
CD207	Dendritic cells
CD11b	Myeloid cells
IBA1	Macrophages
CD163	M2 Macrophages
CD20	B-cells
CD57	NK-cells
CD15	Neutrofils

Stroma
Vimentin
SMA/CD31

Cell states

Marker	Role
cCasp3/cPARP	Apoptosis
yH2AX	DNA damage
53BP1	DNA damage
Ki67	Proliferation
pRB	Proliferation
P21	Cell cycle
CyclinA	Cell cycle
pSTAT1	Interferon pahtway
pTBK1	Interferon pahtway

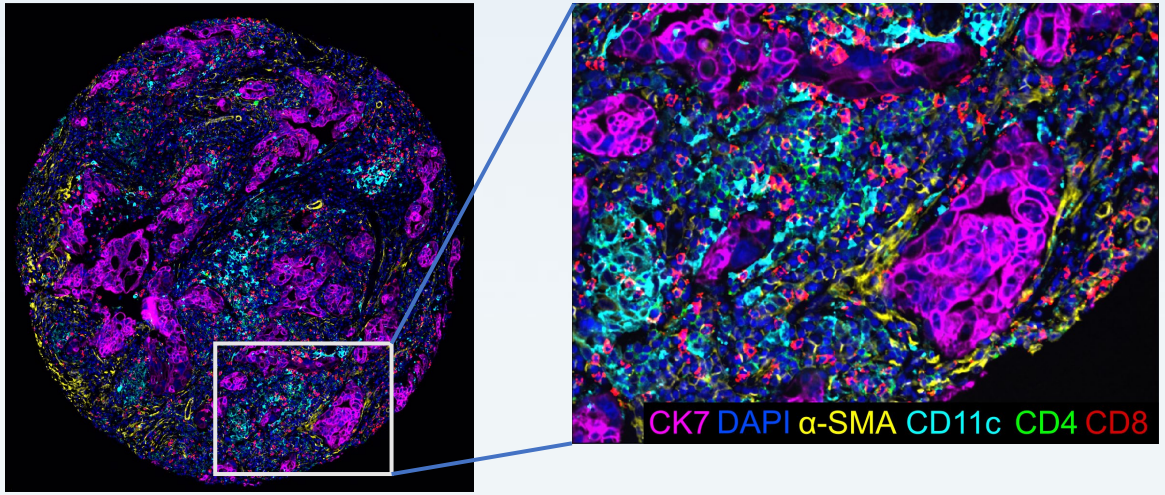
Immune regulation
FOXP3
PD-1
PD-L1
MHCI

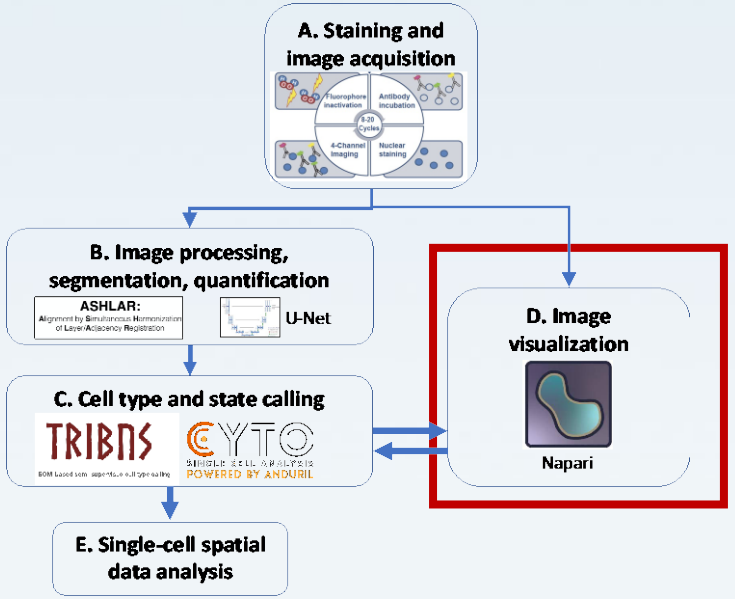
7.2 million single cells
26 patients

Farkkila A, et al. *Nat Comm.* 2020;11(1):1459.
NK-cells, natural killer cells; TME, tumour microenvironment.

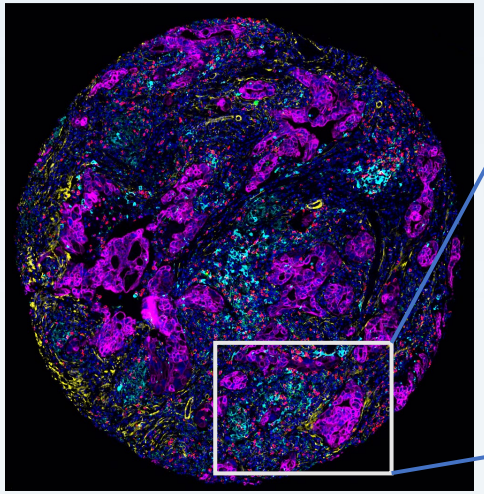
tCycIF tools to define the cell types, functional states, and tissue architecture at single cell resolution

Highly multiplexed imaging

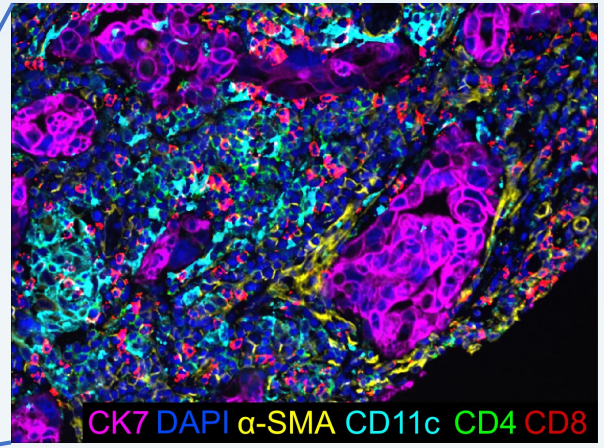


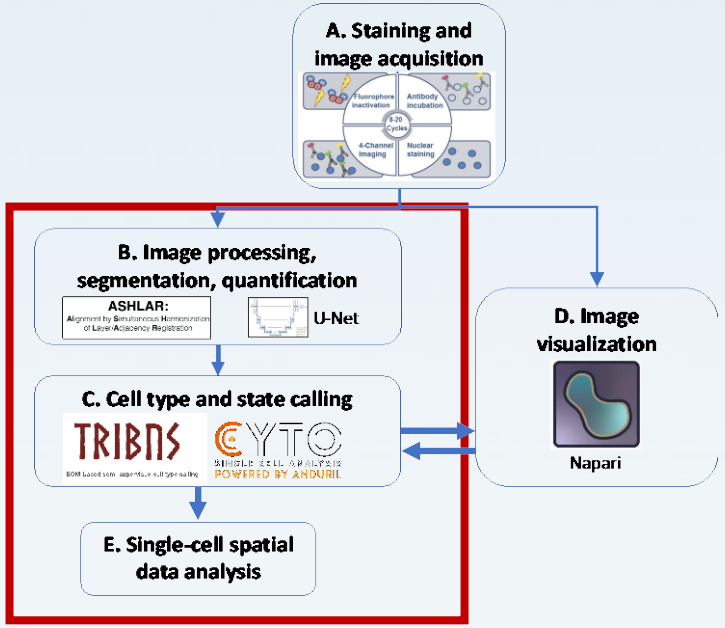


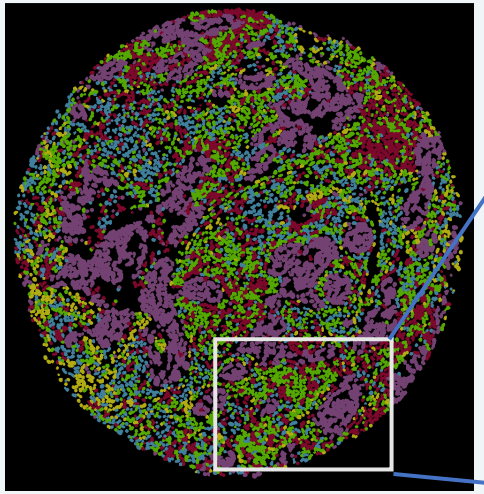
tCycIF tools to define the cell types, functional states, and tissue architecture at single cell resolution



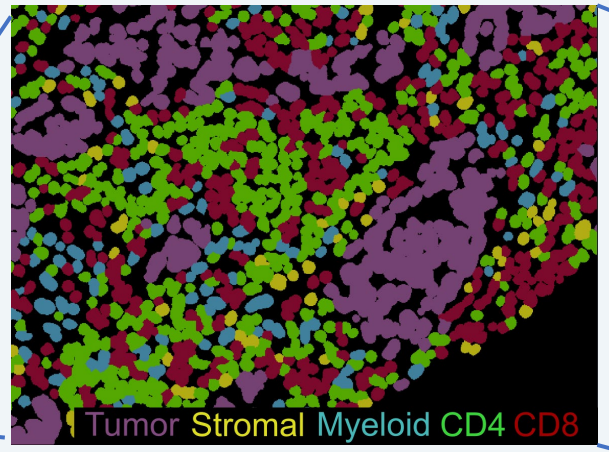
Highly multiplexed imaging



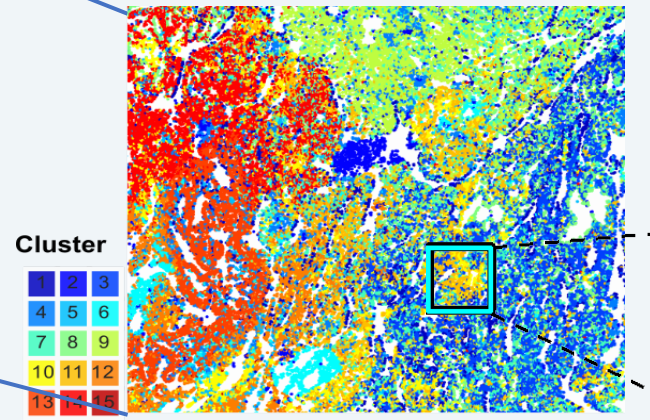


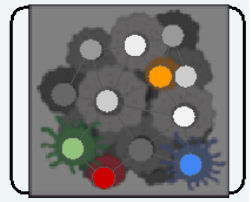


Cell-type annotation

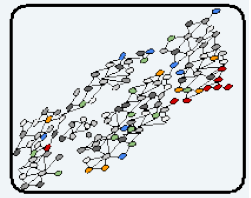


Single-cell spatial phenotypes



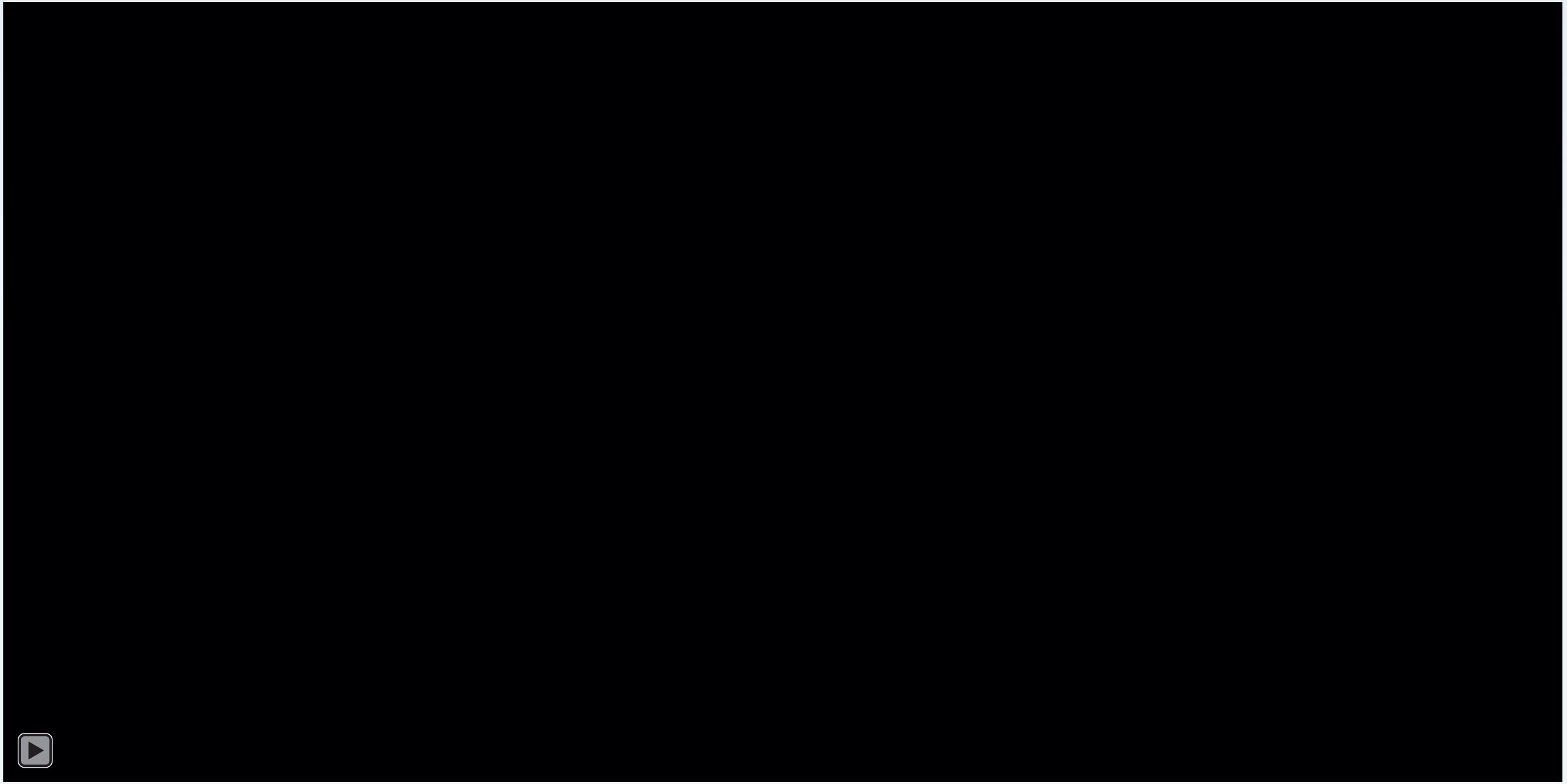


Cellular communities

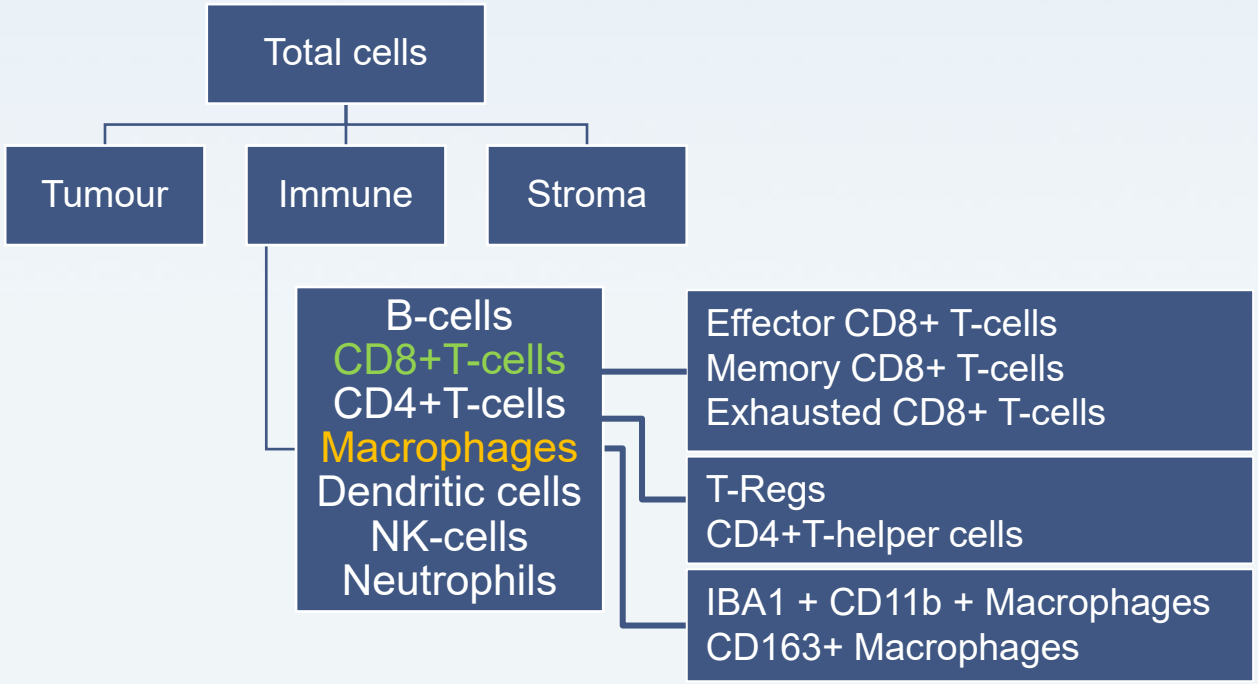


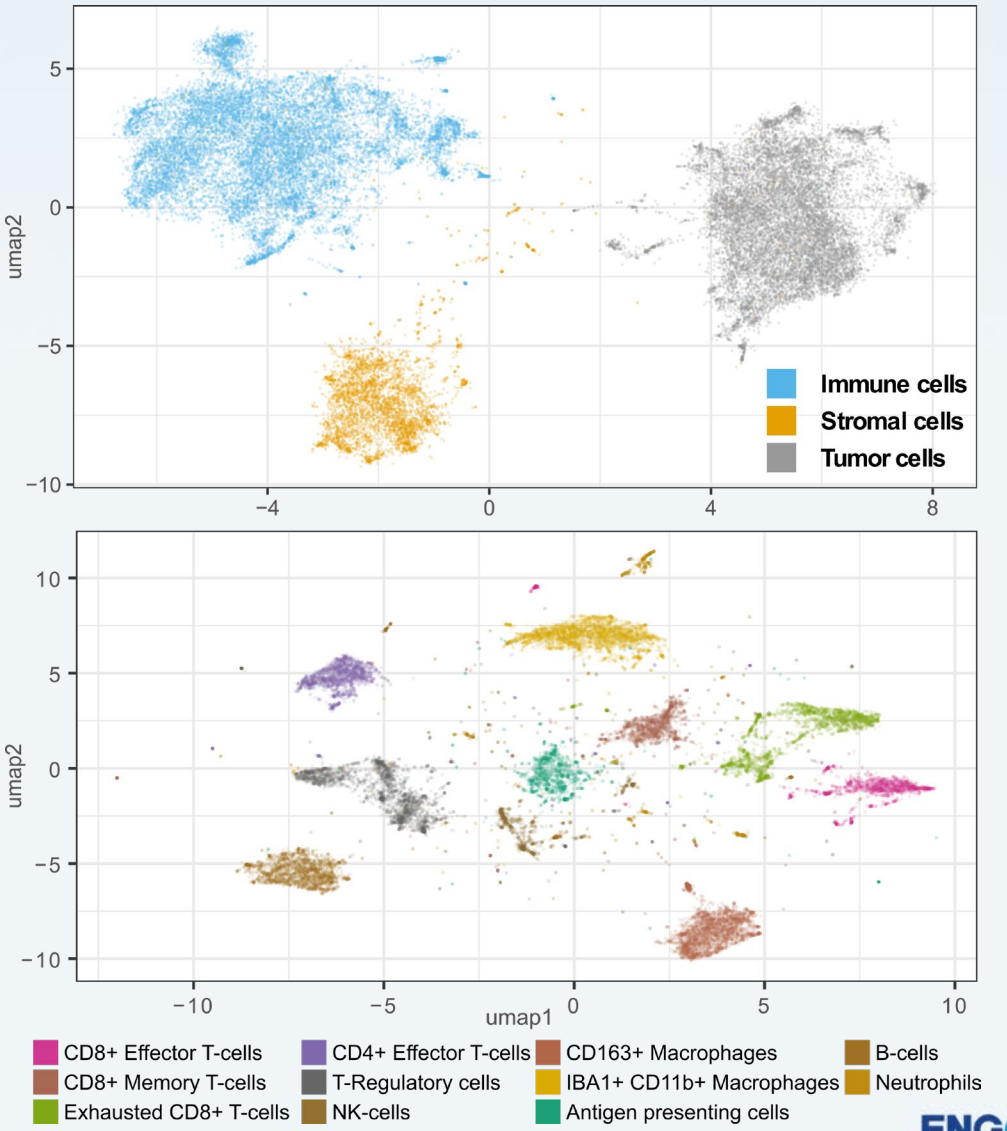
Network structures

Tissue regions



Tribus tool to annotate Immune cell subpopulations

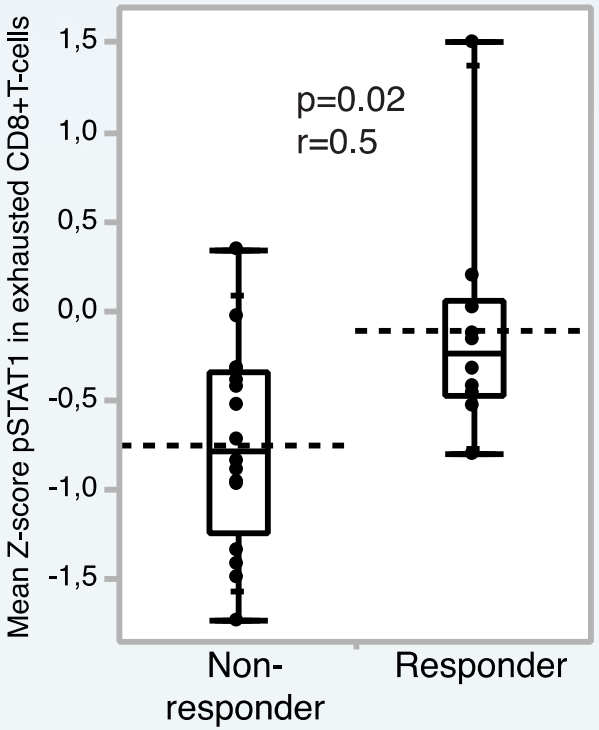


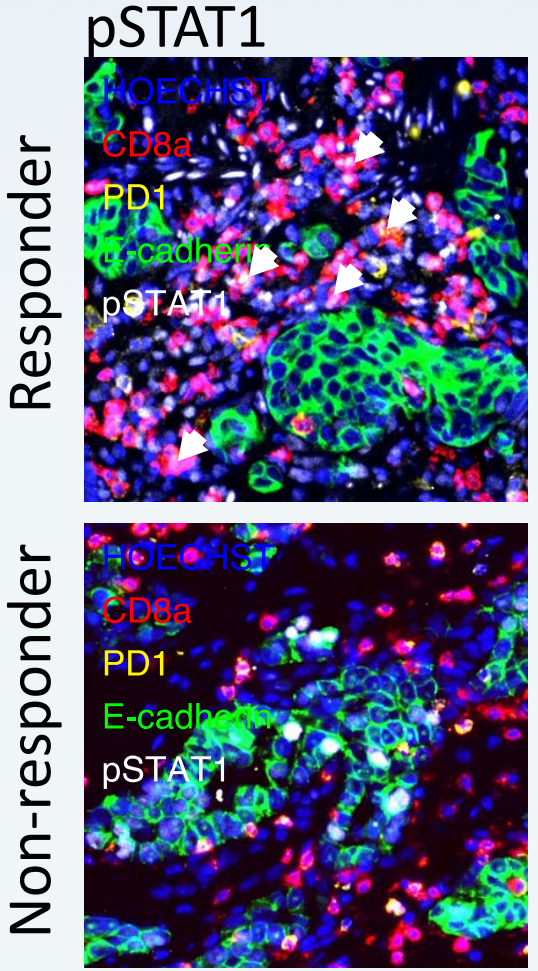


Farkkila A, et al. *Nat Comm.* 2020;11(1):1459.

NK-cells, natural killer cells; T-Regs, T regulatory cells.

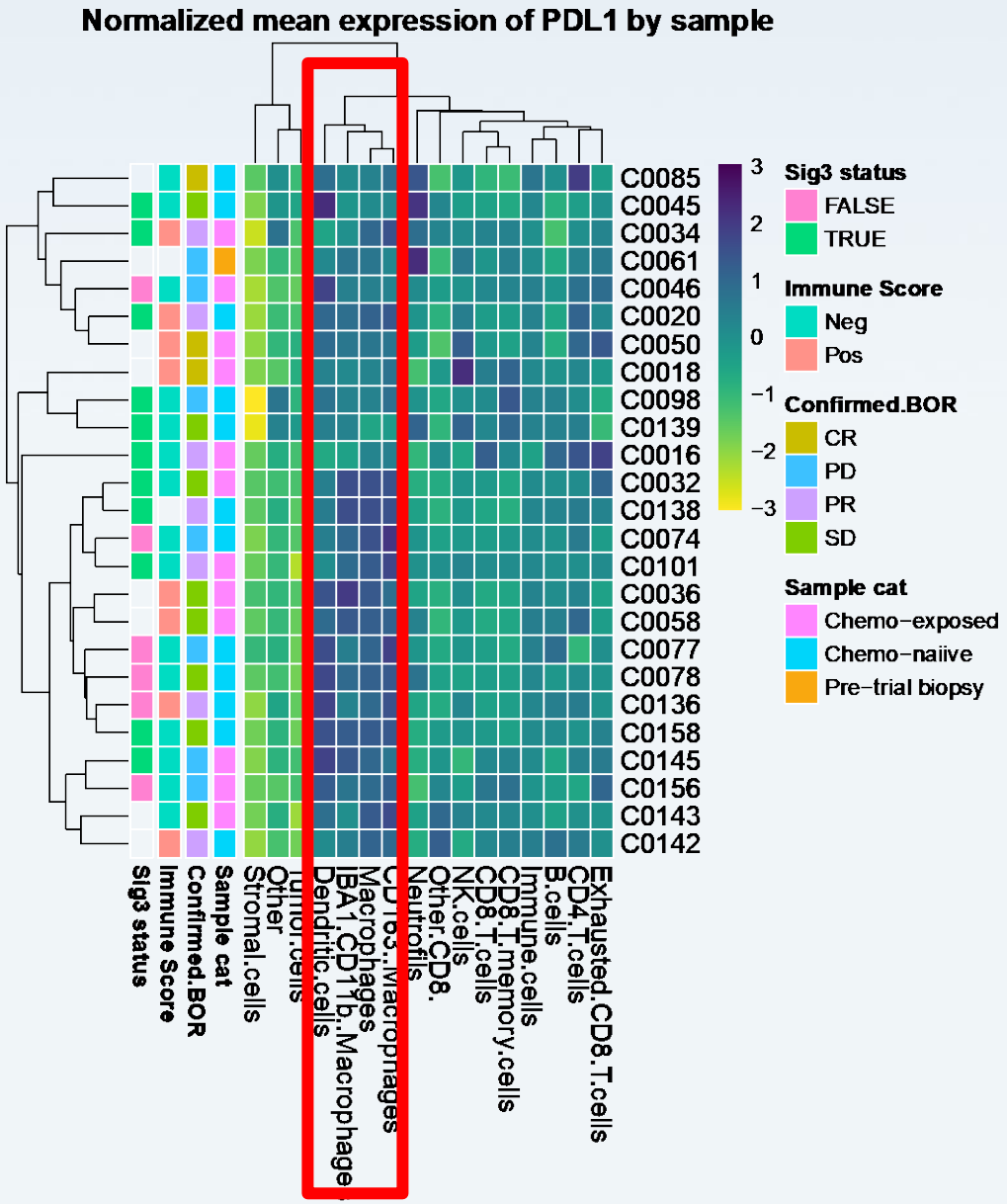
Interferon-activated state of PD-1 positive, exhausted CD8+T-cells associates with response



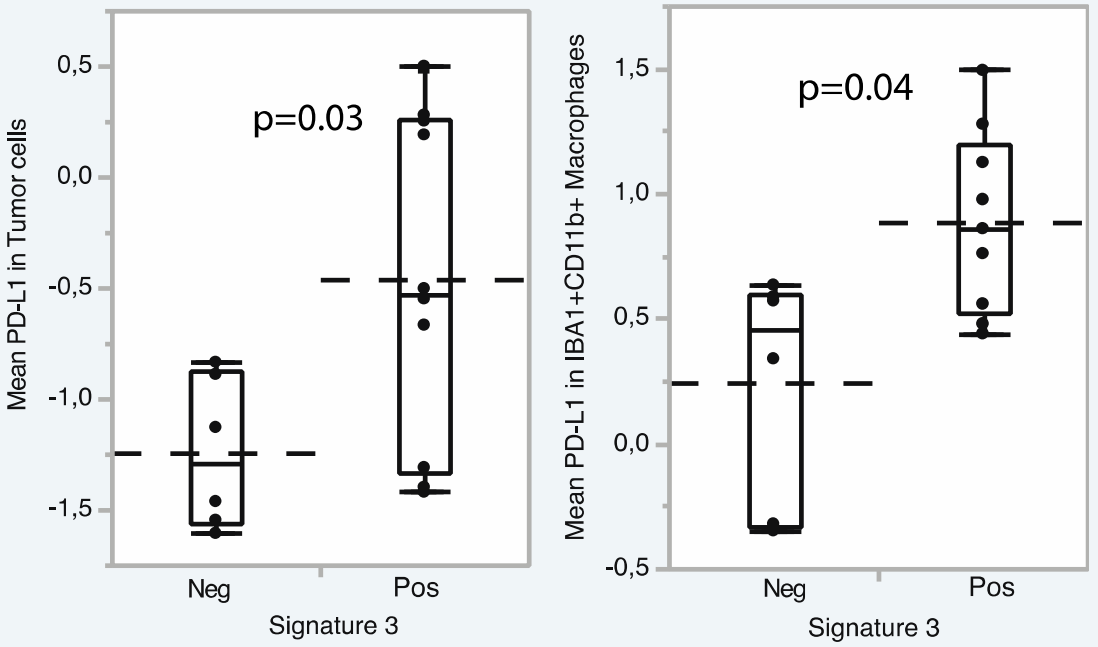


Farkkila A, et al. *Nat Comm.* 2020;11(1):1459.

PD-L1 expression is highest in the macrophages and dendritic cells



- PD-L1 expression highest in the macrophage dendritic cell cluster
- No association to response
- Increased PD-L1 expression in Tumor cells and Macrophages of Sig3 positive tumors

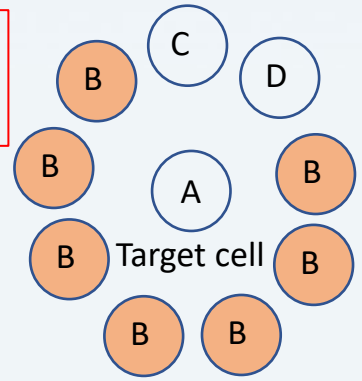


Exhausted CD8+T cells are surrounded by PD-L1 positive tumor cells in the responders

Cellular community

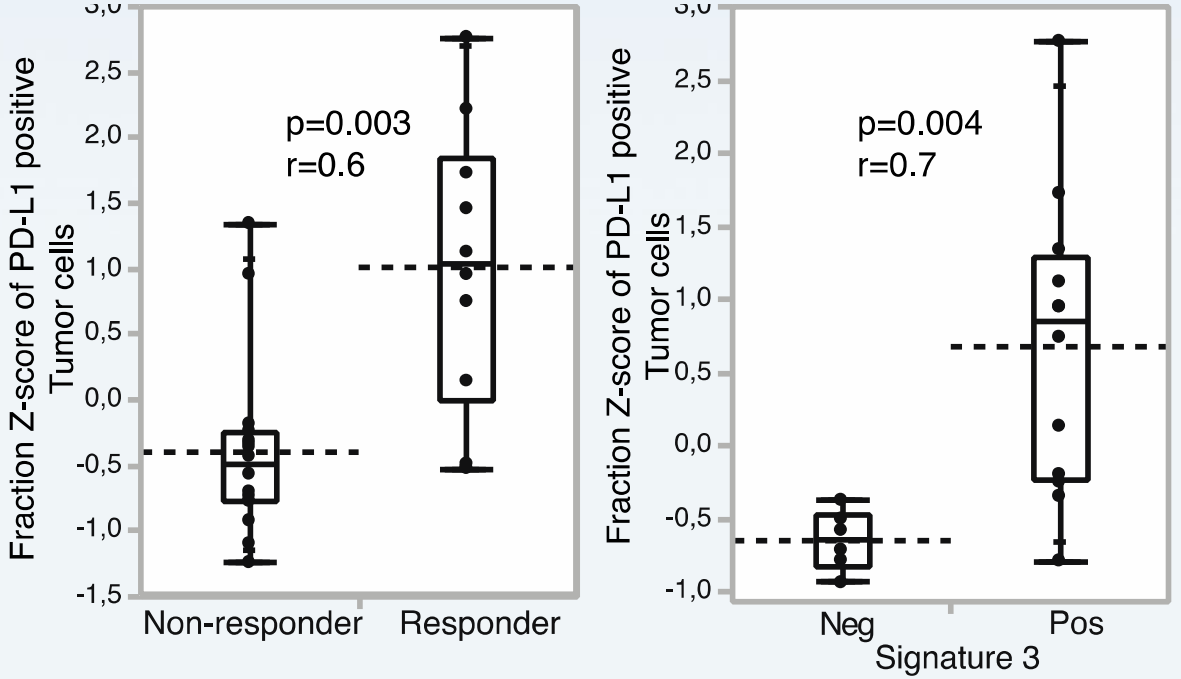
Neighbouring cell

PD-L1 positive Neighbours?



Exhausted CD8+T-cell

PD-L1+ Tumor cells

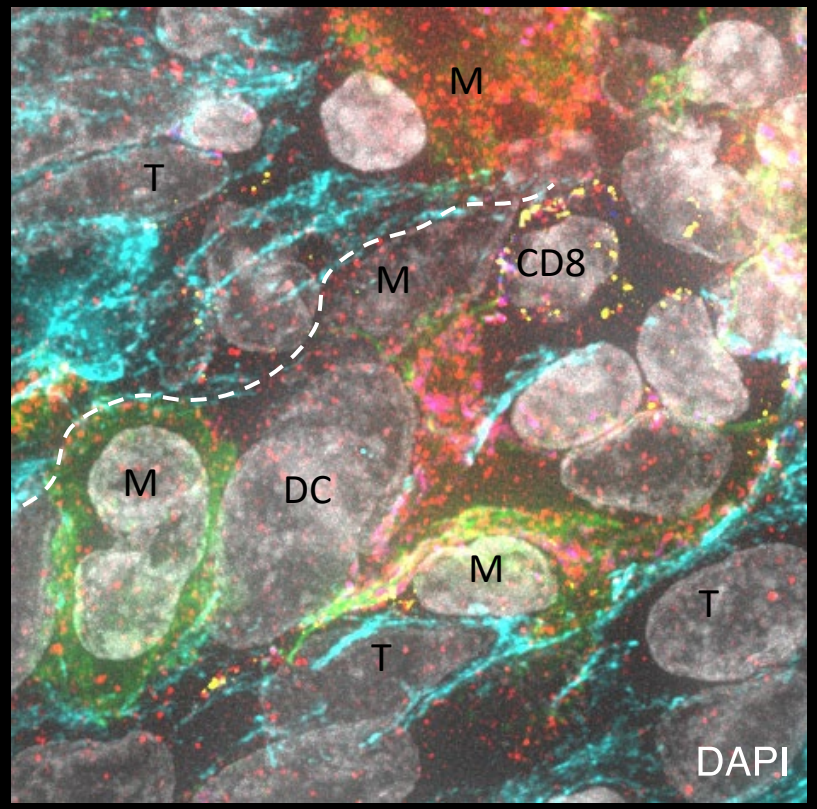


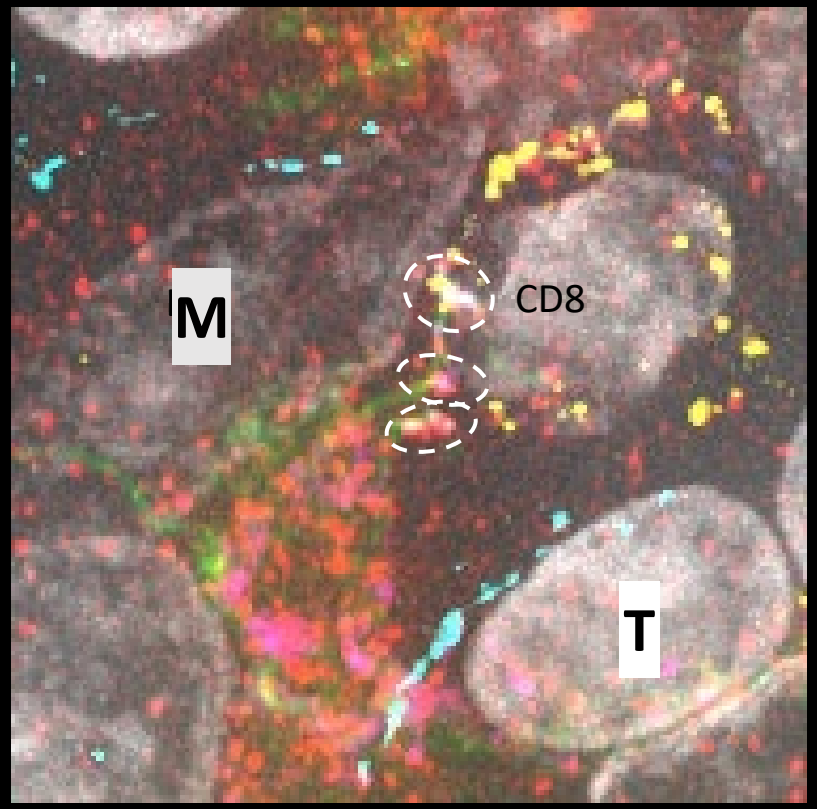
- Exhausted CD8+T-cells reside in PD-L1 positive tumour cell-rich cellular communities in the responders
- These communities are enriched in the Sig3 positive tumors.

Farkkila A, et al. *Nat Comm.* 2020;11(1):1459.

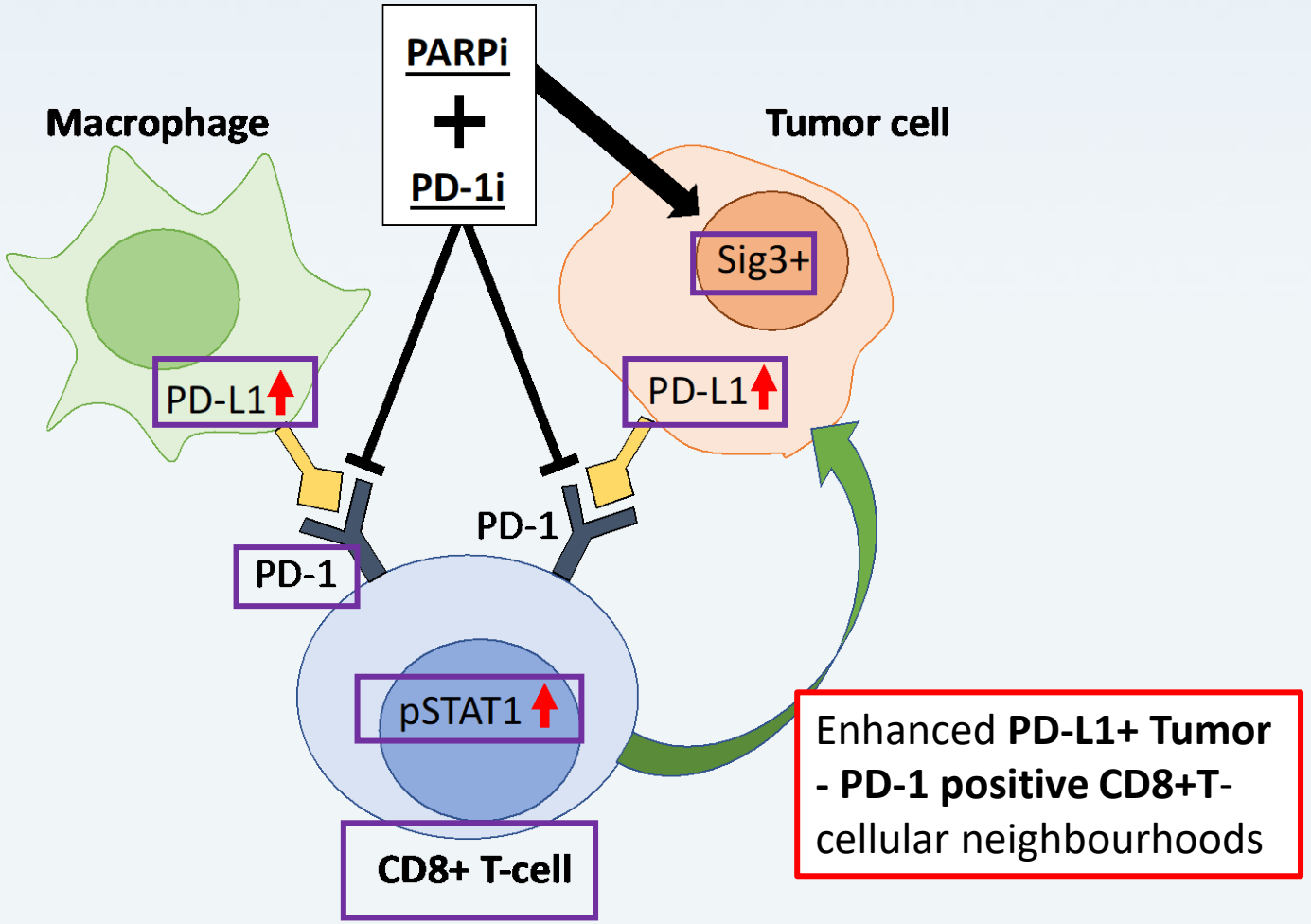
PD-L1, programmed cell death ligand 1.

CD8a
IBA1
CD11c
PD-L1
CK7
PD-1





Determinants of response to the combination of PARP inhibitor and PD-1 inhibitor



Farkkila A, et al. *Nat Comm.* 2020;11(1):1459.

PARP(i), poly(ADP-ribose) polymerase (inhibitor); PD-(L)1, programmed cell death (ligand) 1.

Conclusions

- Tumour mutational signature 3 correlates to clinical activity (SD+PR) to the combination of PARPi plus PD-1 inhibition
  - Indicative of error-prone DNA repair

- mRNA expression analysis identifies increased interferon pathway scores and exhausted T-cell scores in the responders

- tCycIF multiplexed immunofluorescence provides mechanistic insights into response
  - Interferon signaling in PD-1 positive exhausted CD8+T-cells associate with response
  - Sig3/HR deficiency shapes the cellular communities and enhances spatial interactions of PD-L1 positive tumor cells and exhausted CD8+T-cells

PARP(i), poly(ADP-ribose) polymerase (inhibitor); PD-(L)1, programmed cell death (ligand) 1.

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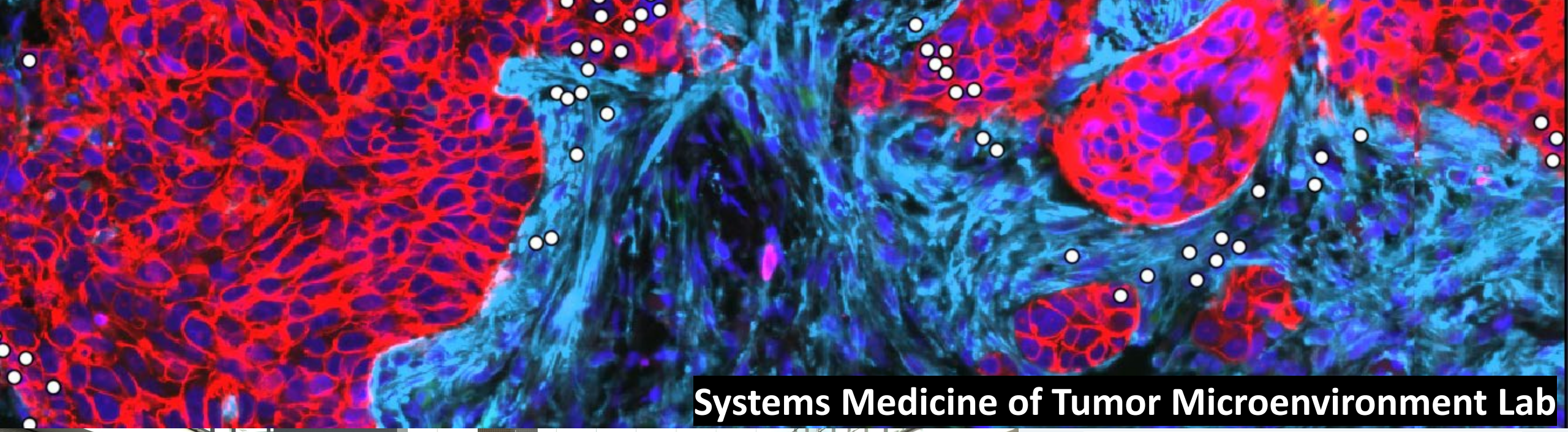
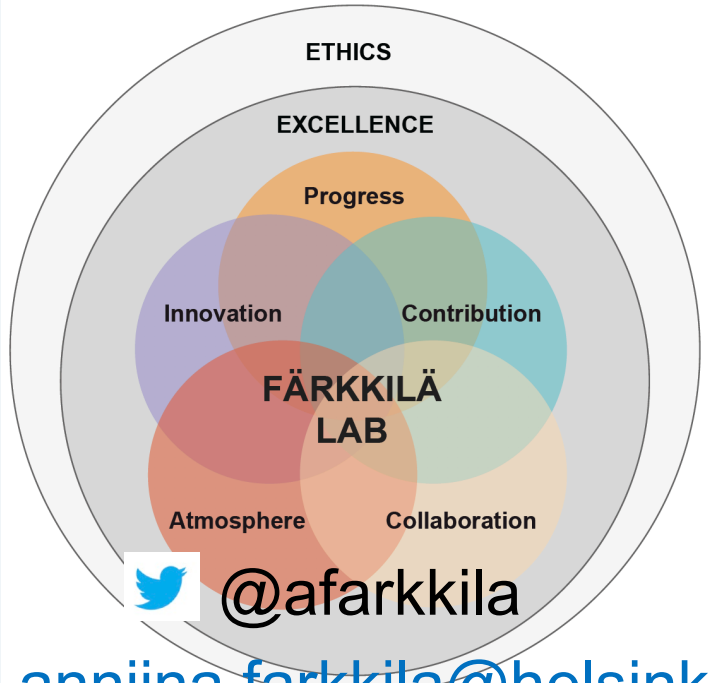


Systems Medicine of Tumor Microenvironment Lab



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