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Mechanisms of ADC Resistance

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Currently FDA-approved ADCs for breast cancer

Approved ADC	Mechanism of Action	Important Clinical Trials
Trastuzumab Deruxtecan (T-DXd)	Anti-HER2 mAb linked to a topoisomerase I inhibitor (Deruxtecan)	DESTINY-Breast01 DESTINY-Breast02 DESTINY-Breast03 DESTINY-Breast04
Trastuzumab Emtansine (T-DM1)	Anti-HER2 mAb linked to a microtubule inhibitor (DM1)	EMILIA MARIANNE TH3RESA KATHERINE
Sacituzumab govitecan (SG)	Antitrophoblast cell-surface antigen 2 (Trop-2) directed antibody linked to a topoisomerase I inhibitor (SN 38, active metabolite of irinotecan)	IMMU-132–01 ASCENT TROPiCS-02
Datopotomab Deruxtecan	Anti-TROP2 IgG1 mAb linked to a topoisomerase I inhibitor (Deruxtecan)	TROPION-Breast01 TROPION-Breast02

Some investigational ADCs in breast cancer

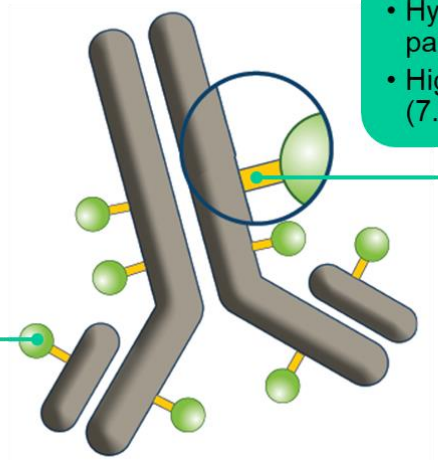
Investigational ADC	Mechanism of Action	Important Clinical Trials
Patritumab Deruxtecan	Anti-HER3 IgG1 mAb linked to a topoisomerase I inhibitor (Deruxtecan)	SOLTI TOT-HER3 A Phase II Study of U3-1402 (Patritumab Deruxtecan) in Patients With Metastatic Breast Cancer
Disitamab Vedotin	Anti-HER2 mAb linked to a microtubule inhibitor (monomethyl auristain E)	Ongoing clinical trials for breast cancer in China
ARX-788	Anti-HER2-targeted mAb linked to AS269	ACE-Breast-01 ACE-Breast-02 I-SPY2
Ladiratuzumab Vedotin	LIV-1 zinc transporter mAb linked to a microtubule inhibitor (monomethyl auristatin E)	Ongoing Phase I trial
Trastuzumab Duocarmazine	Anti-HER2-targeted mAb linked to a DNA alkylating agent (duocarmycin)	TULIP trial

Sacituzumab govitecan (SG) has unique features including a hydrolysable pH-sensitive linker

Features of SG

Humanized anti-Trop-2 antibody
- Targets Trop-2, an epithelial antigen expressed on many solid cancers, including mTNBC

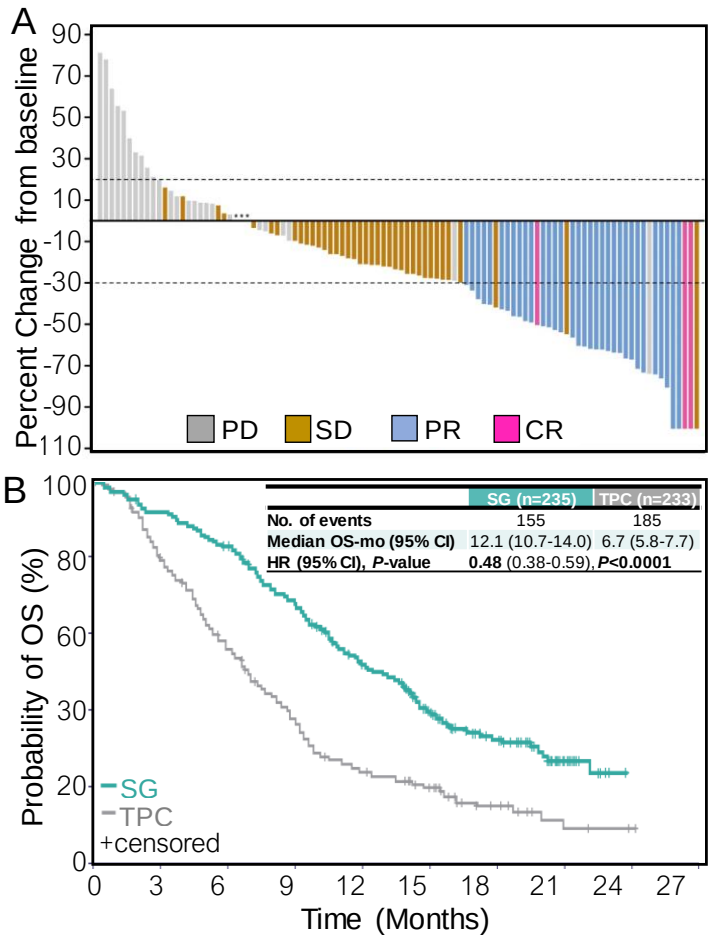
Linker for SN-38
- Hydrolysable linker for payload release
- High drug-to-antibody ratio (7.5:1)



SN-38 (TOP1i) payload
- SN-38 more potent than parent compound, irinotecan
- ADC delivers up to 136-fold more SN-38 than irinotecan in vivo

Full FDA Approval in 2021 for Advanced/Metastatic TNBC
FDA Approval in 2023 for HR+/HER2- Breast Cancer

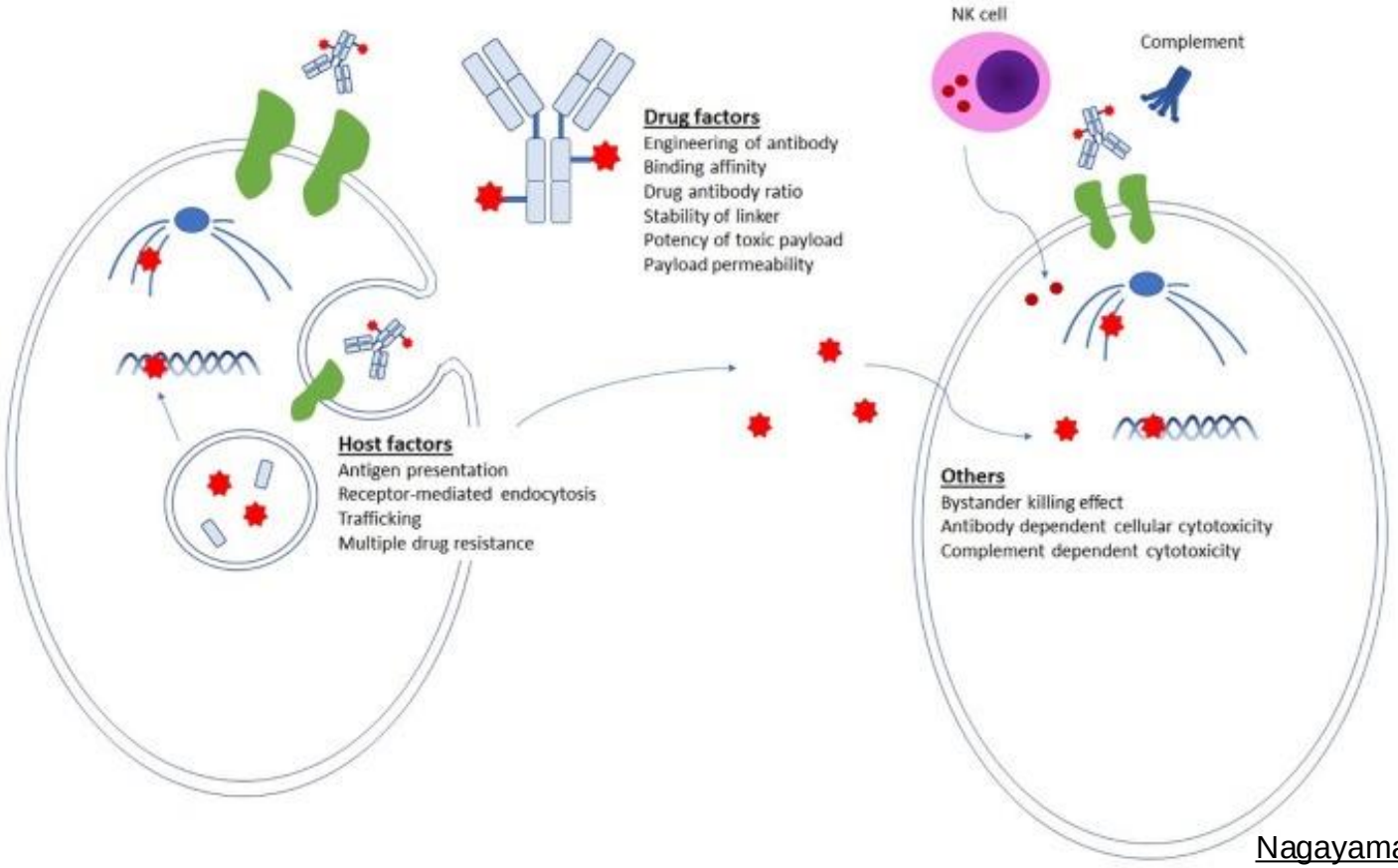
Doubling of Overall Survival in Advanced TNBC



Bardia et al, N Engl J Med. 2021;384(16):1529-1541.
Bardia et al, N Engl J Med. 2019 Feb 21;380(8):741-751

ADC resistance involves target, linker and payload-associated mechanisms

Target	Antibody	Linker	Payload	Tumor
Expression	Target Affinity	Stability	Mechanism	Payload Sensitivity
Trafficking	Internalization Rate	Cleavage Mech.	Potency	Lysosome Integrity
Signaling (Ex/In)	Fc Affinity/ADCC		Cell permeability	MDR/PGP Level
			Drug/Ab ratio	Target Addiction



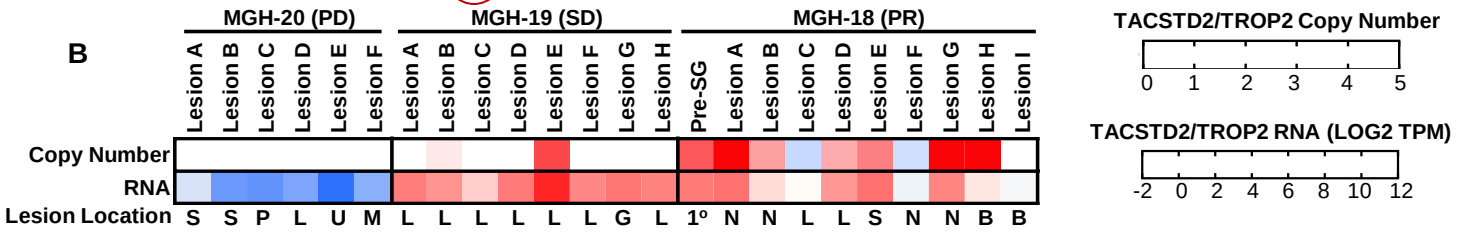
Nagayama et al, Ther Adv Med Oncol. 2020; 12

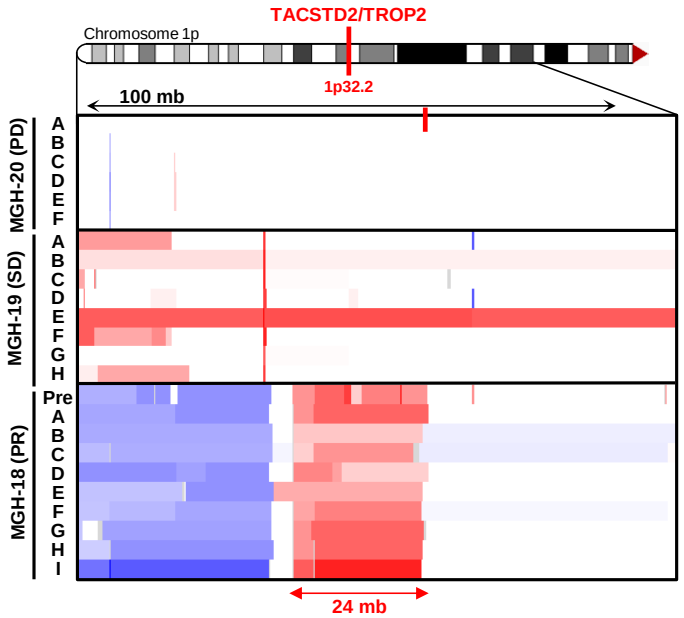
Clinical response to SG associated with TROP2 levels

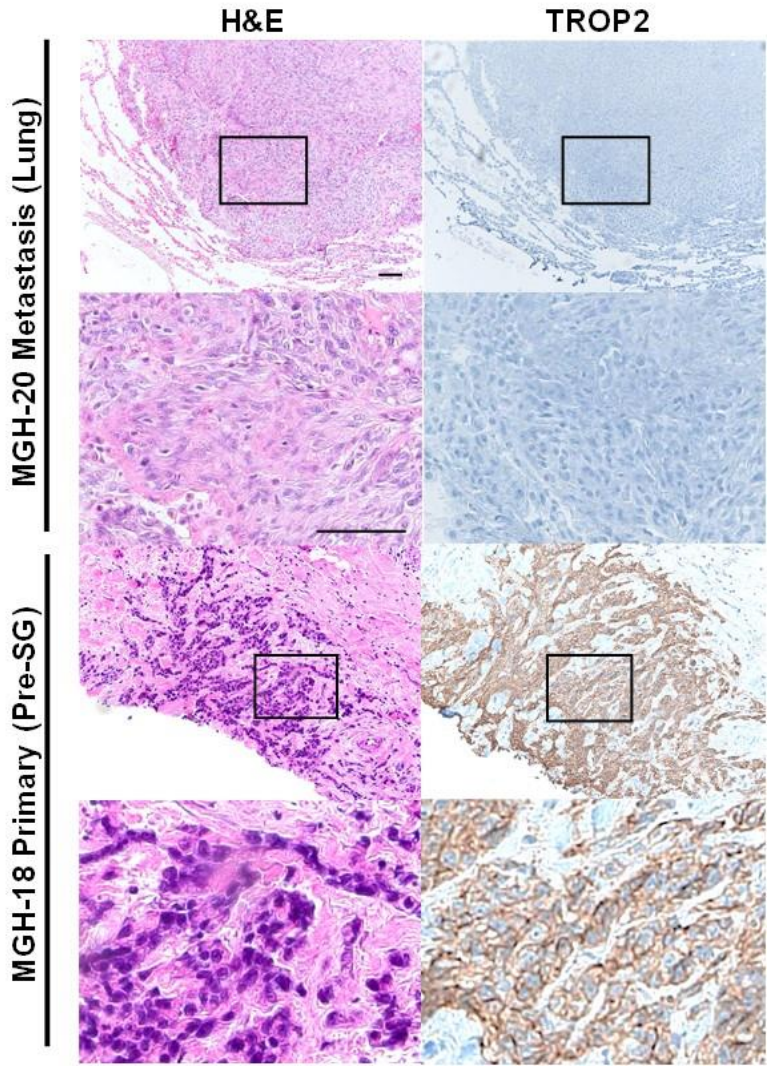
A

Participant ID	Molecular Subtype	Age at Diagnosis	Days on IMMU-132	Days from Last Dose SG to Death	Treatments Before SG	Treatments After SG	Lesions Sequenced at Autopsy	Best Response (per RECIST)	Extent of Best Response (%)
MGH-18	TNBC	41	253	138	2	2	9	PR	-45.0
MGH-19	TNBC	59	150	305	5	4	8	SD	-21.9
MGH-20	TNBC	62	34	56	4	1	6	PD	+78.0

B



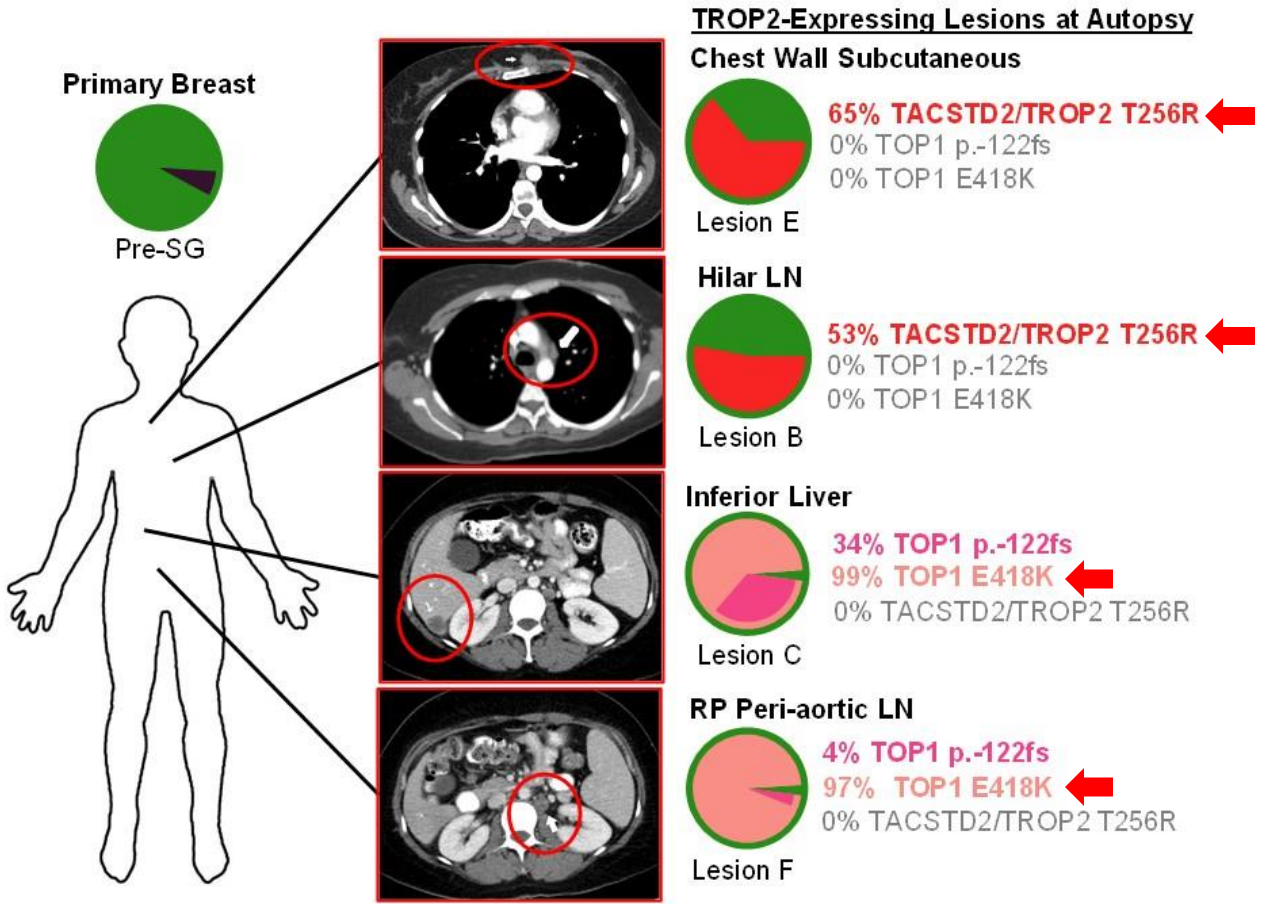


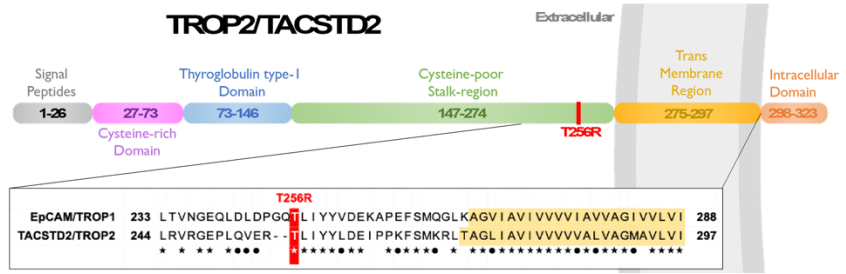


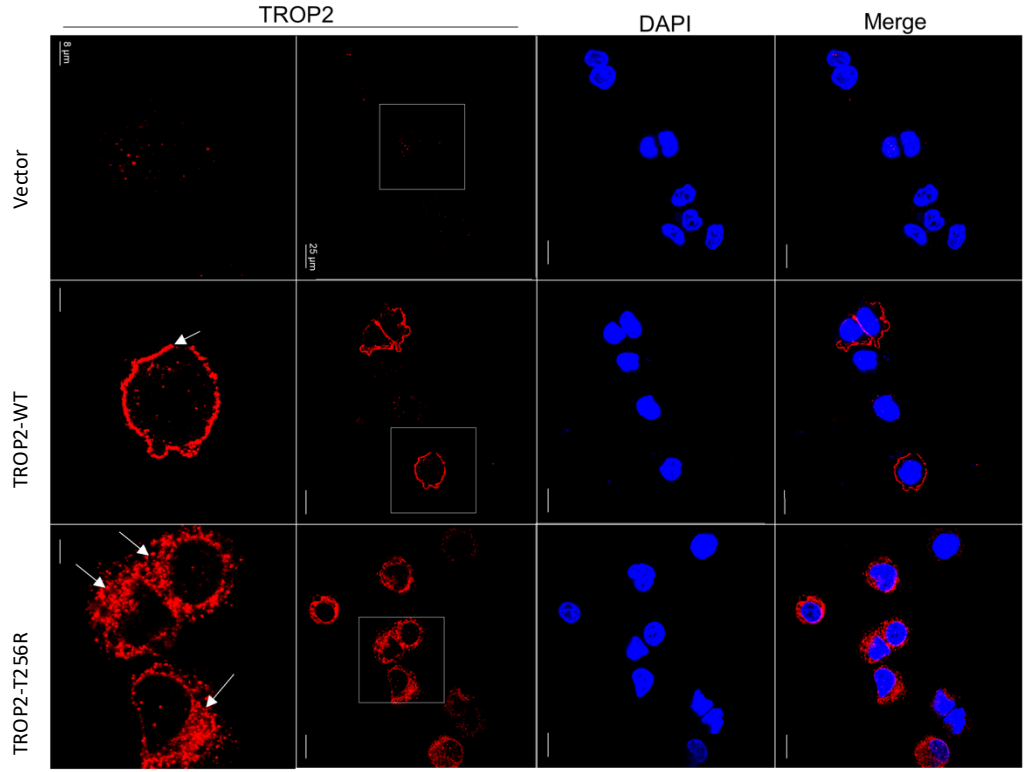
Coates et al, Cancer Discovery 2021 11:2436

Acquired resistance to SG associated with mutations in TROP2 and TOP1

Functional TROP2 and TOP1 mutations in distinct metastases

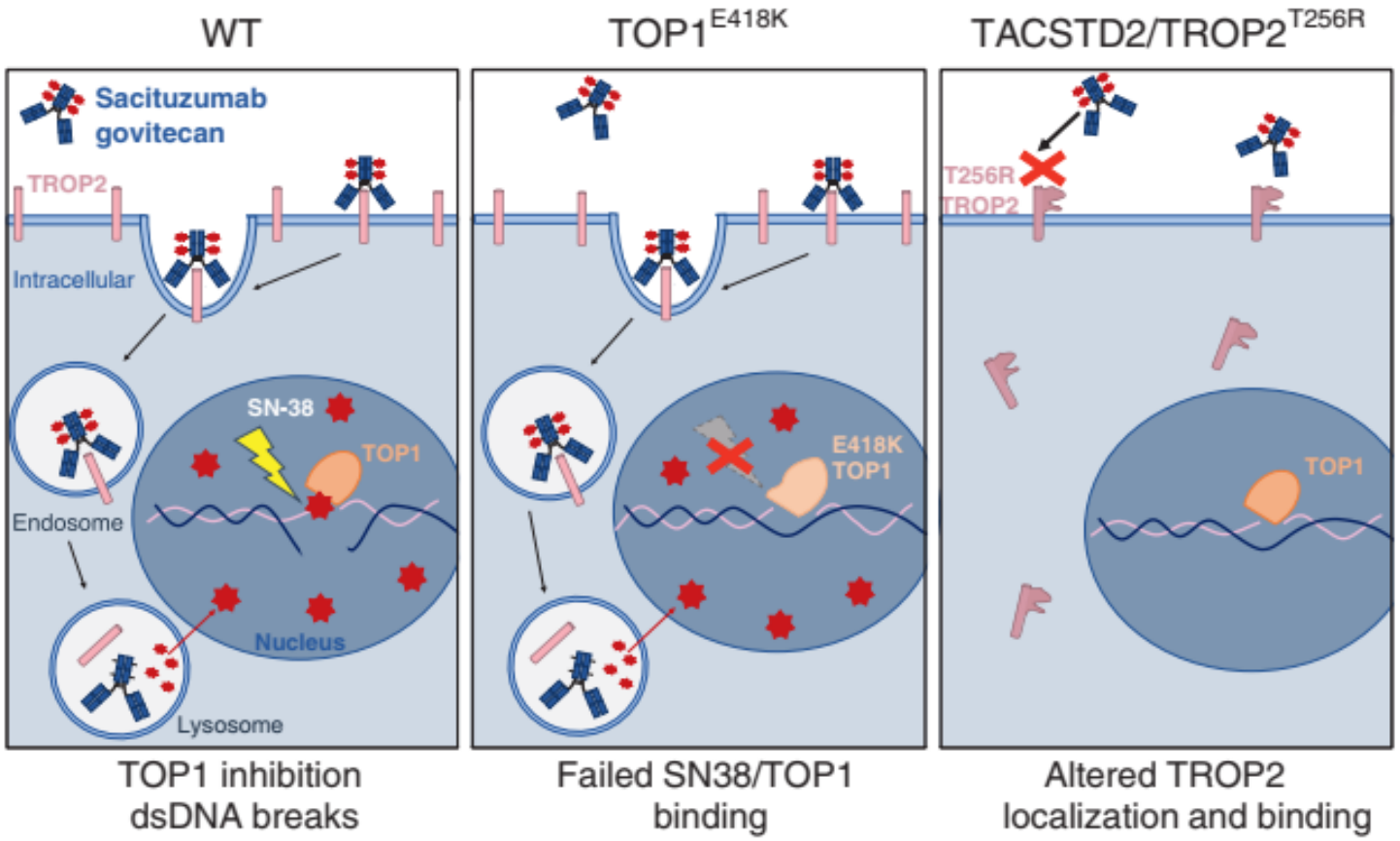






Coates et al, Cancer Discovery 2021 11:2436

Clinical implications of TROP2 and TOP1 somatic mutations for sequential use of ADCs

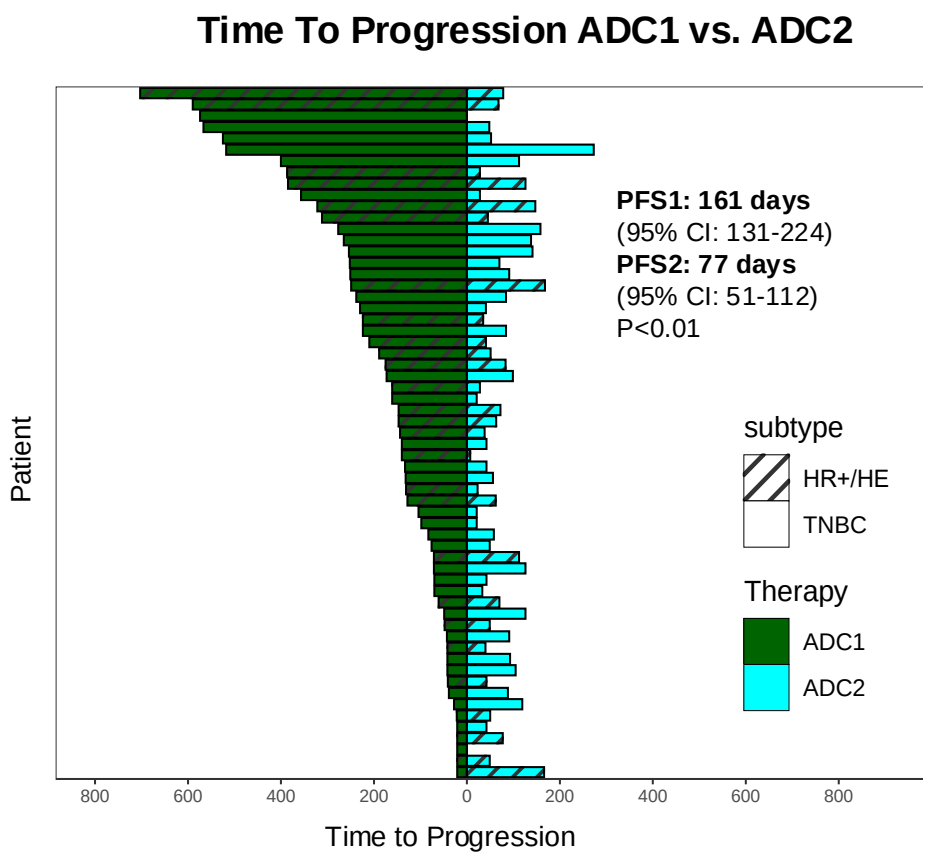


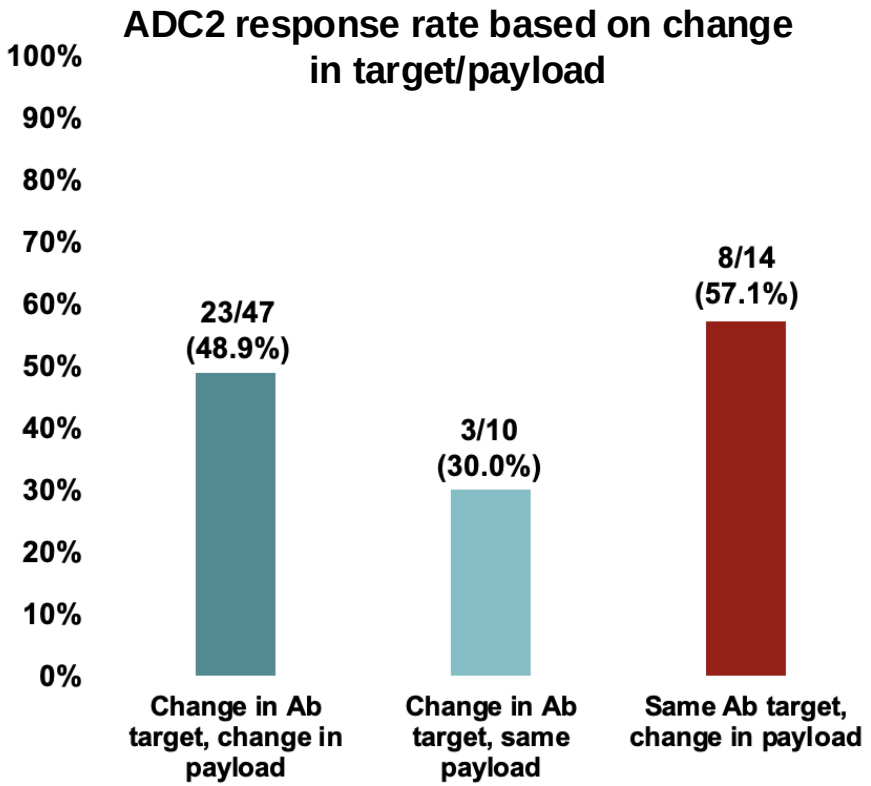
Failed DNA/TOP1i Engagement → Resistance to TOP1 Inhibitor ADC payloads

Failed TROP2 Binding → Resistance to TROP2-Directed ADC

Coates et al, Cancer Discovery 2021 11:2436

Disappointing results with sequential ADC use in MBC

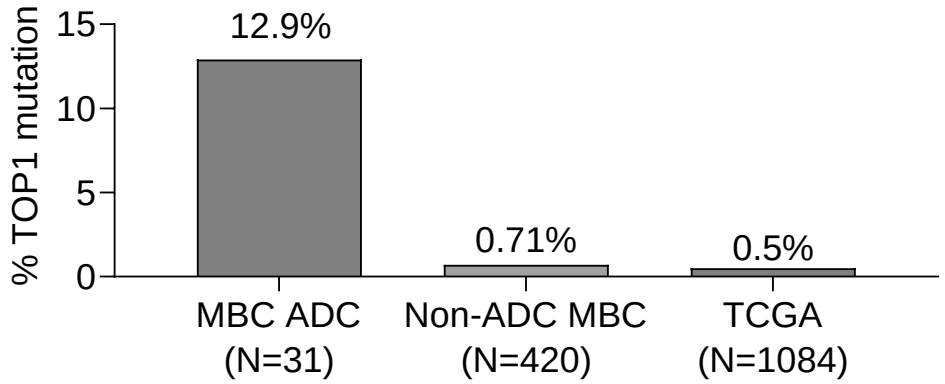




Abelman et al. SABCS 2023

Circulating TOP1 mutations in post-ADC breast cancer patients

Circulating TOP1 mutation incidence

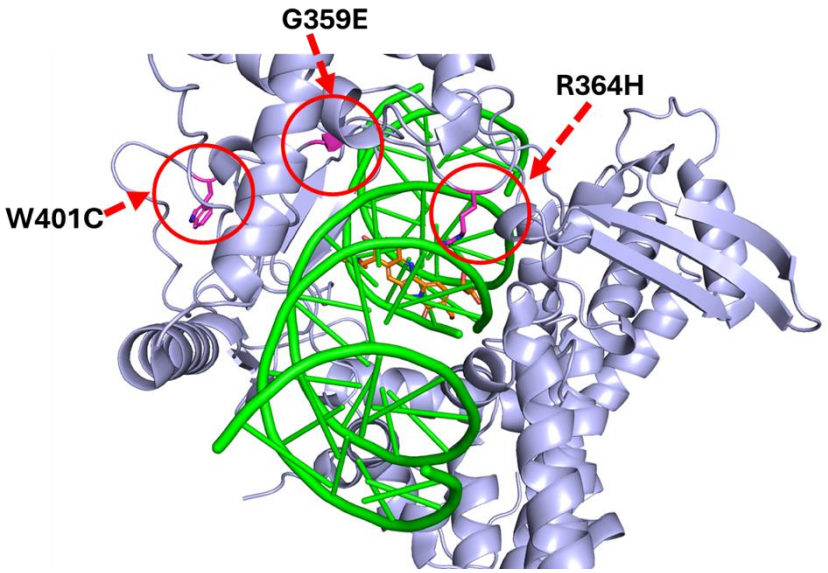


Time on ADC1 (days)	Patient ID	Time on ADC2 (days)	TOP1 Mutation
312	MGH-1	45	R364H
385	MGH-2	126	S57C
574	MGH-3	1	G359E
525	MGH-4	52	W401C

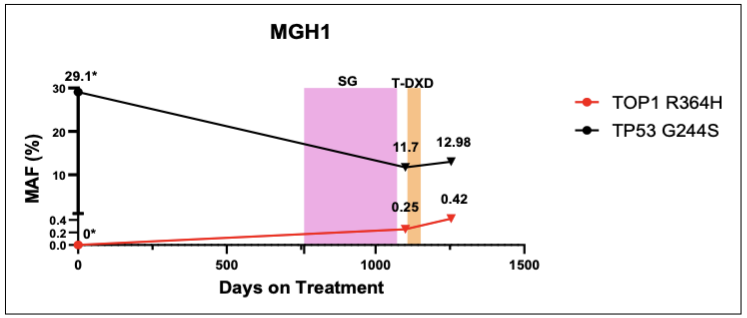
Abelman et al. Clinical Cancer Research 2025

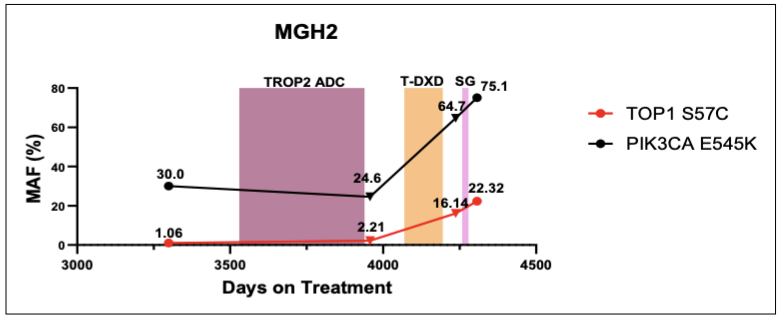
Circulating TOP1 mutation prevalence tracks with disease progression

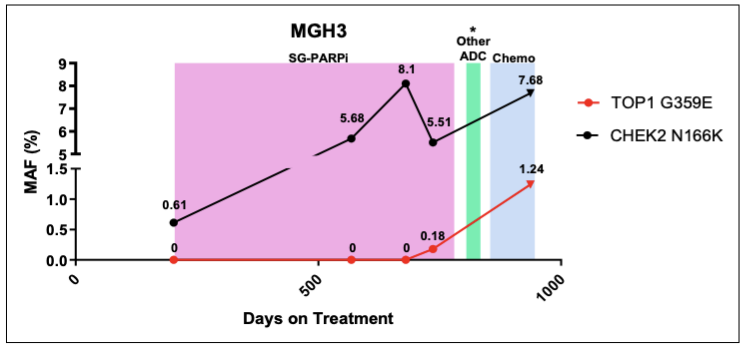
Mutations localized to core TOP1 domain

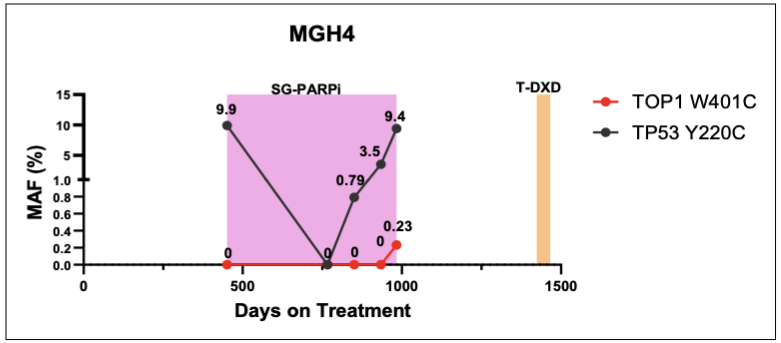


Blood-based tracking of mutation prevalence (ddPCR)

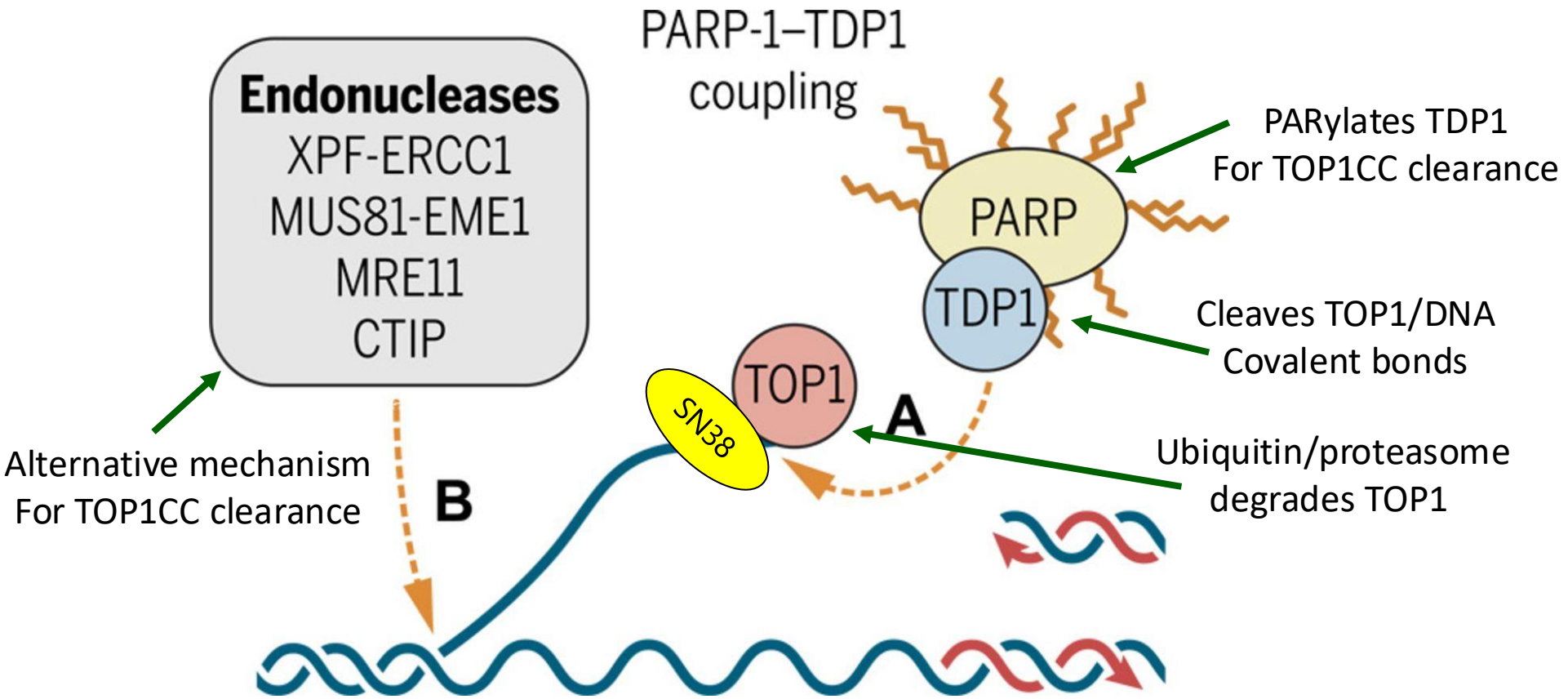






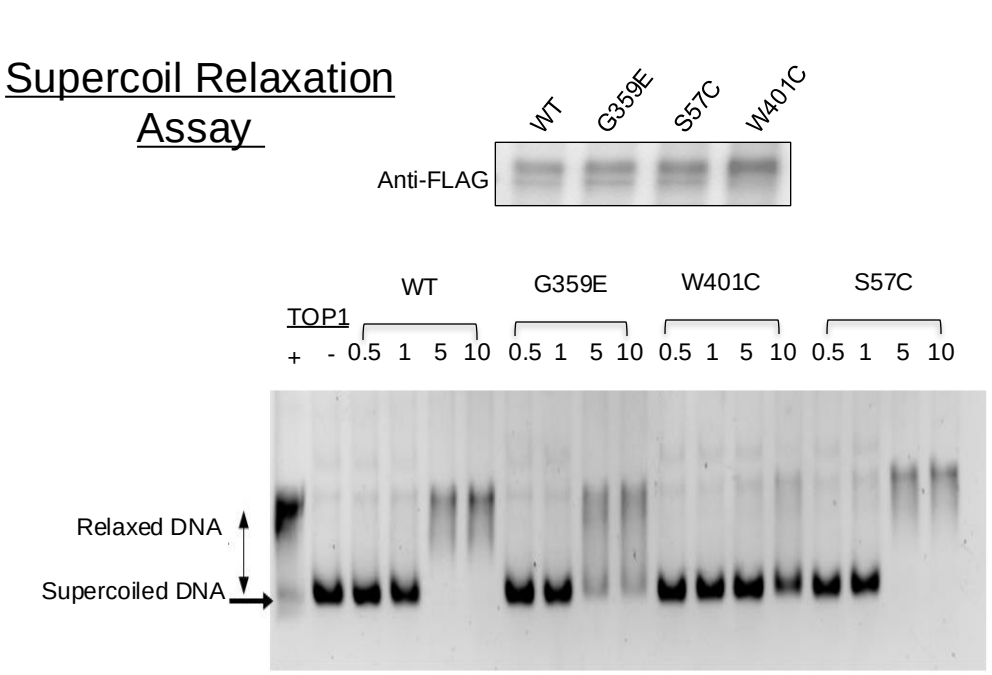


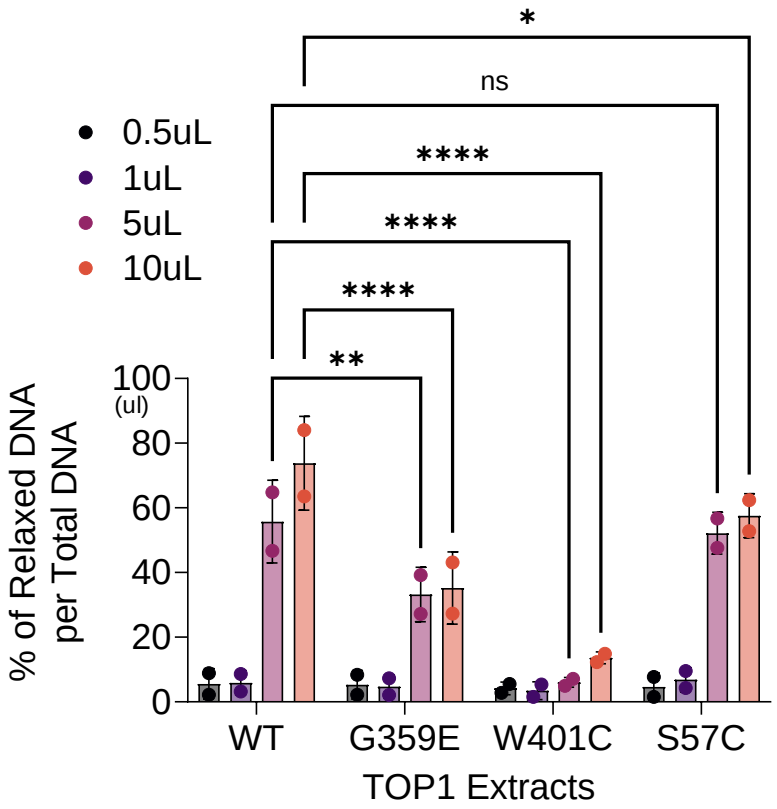
Pathways and mechanism for TOP1 and clearance of TOP1CC



Adapted from scitranslmed.aaf9246

Decreased enzymatic activity of patient-associated TOP1 mutants





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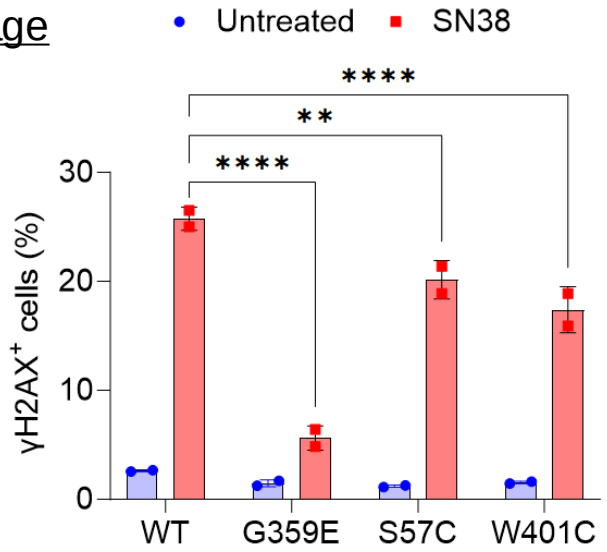
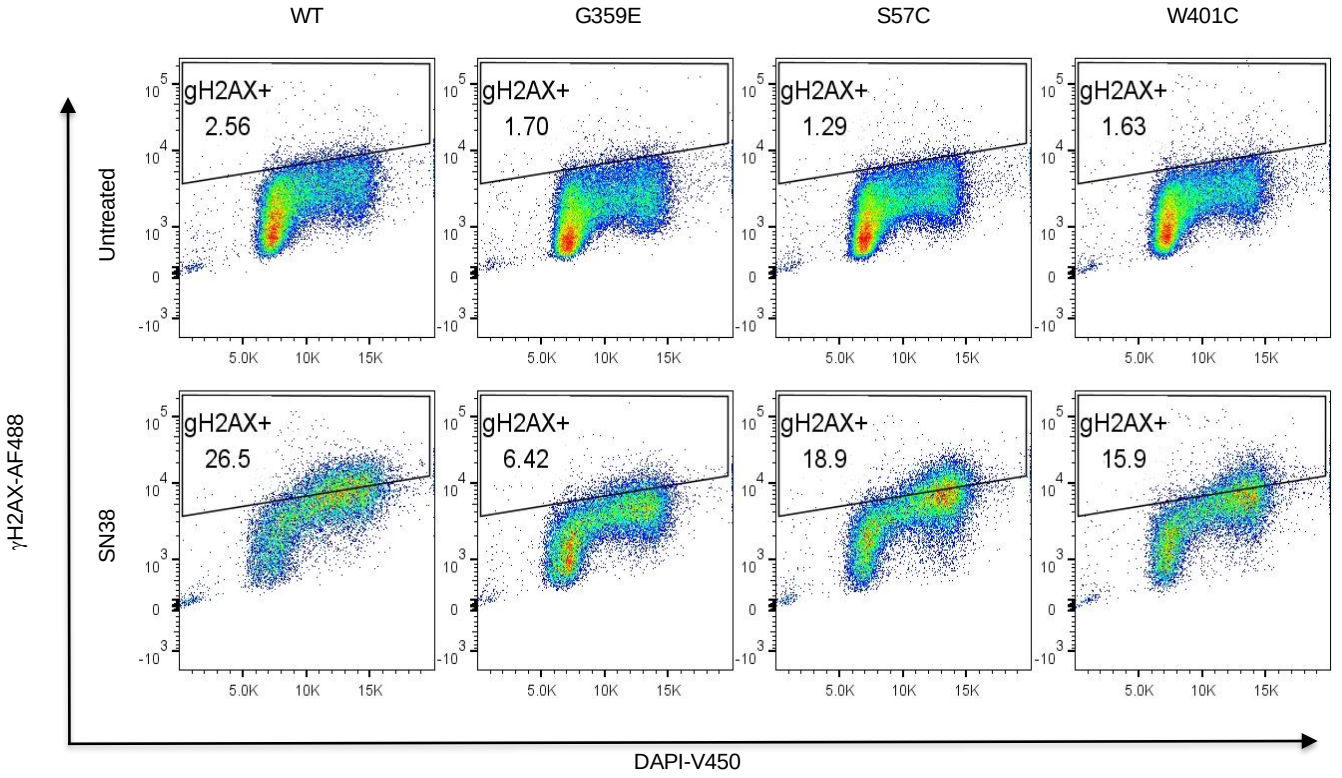
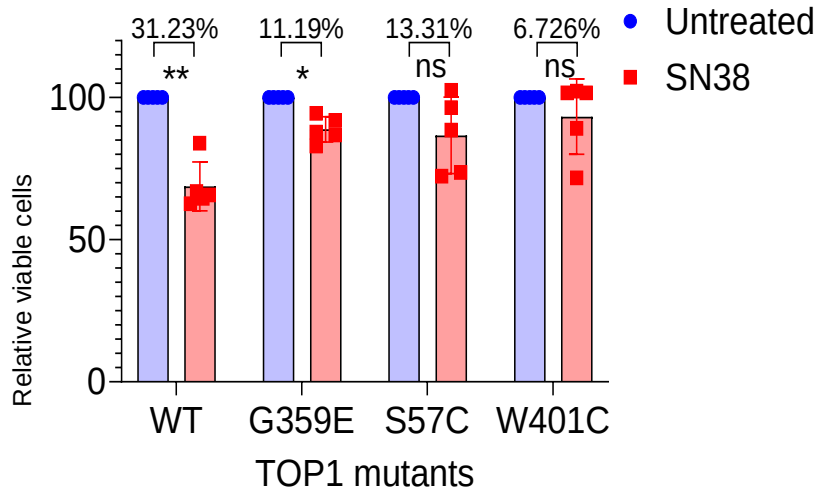
TOP1 mutants are associated with decreased DNA damage and resistance to TOP1 inhibitor in TNBC cells

DNA Damage





Drug Resistance



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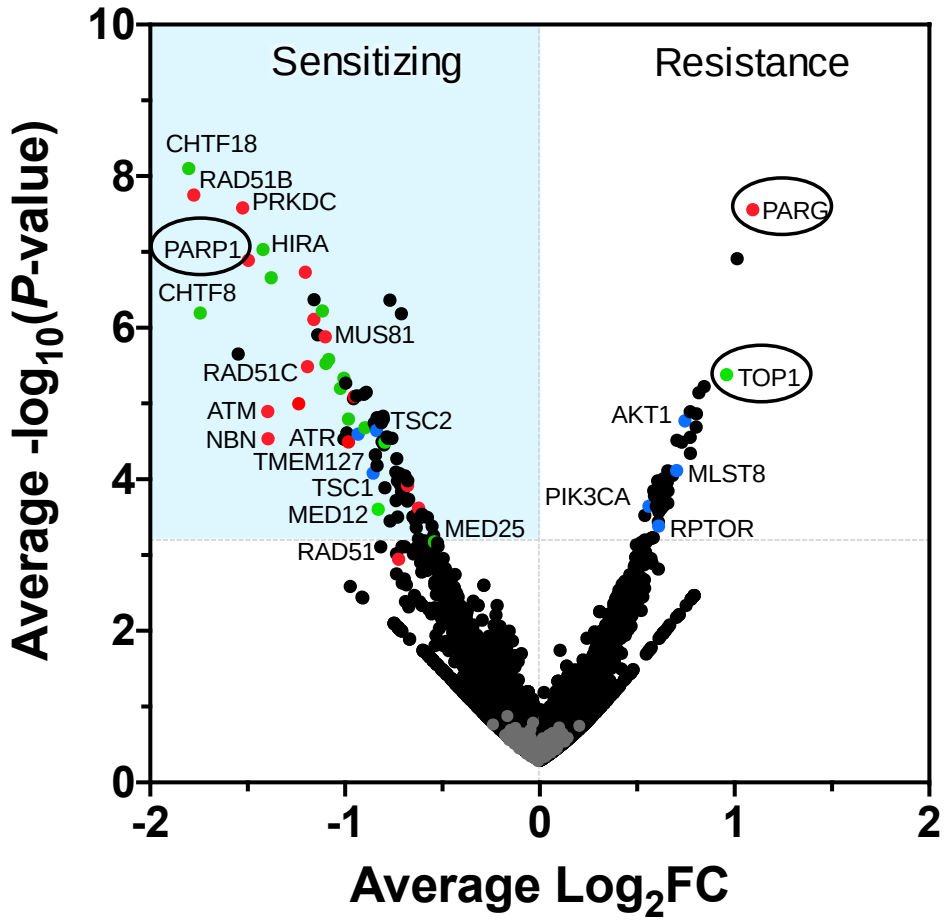
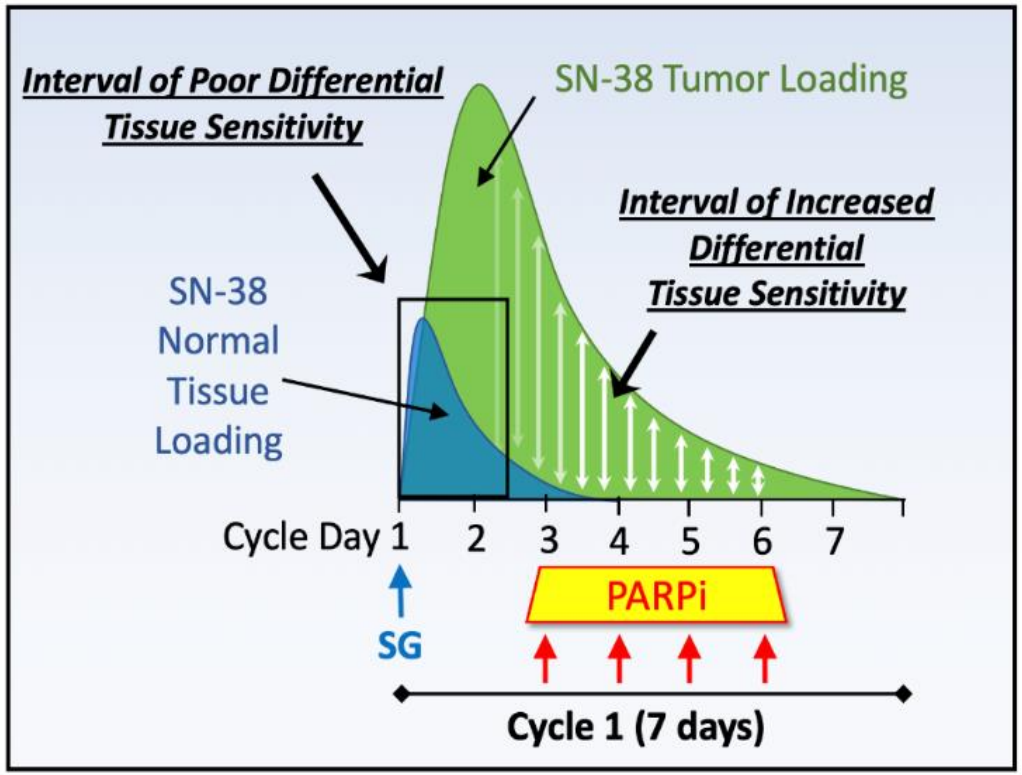
Systematic screens to unveil ADC sensitizing pathways for combination therapy

Genome-wide CRISPR Screen with SG

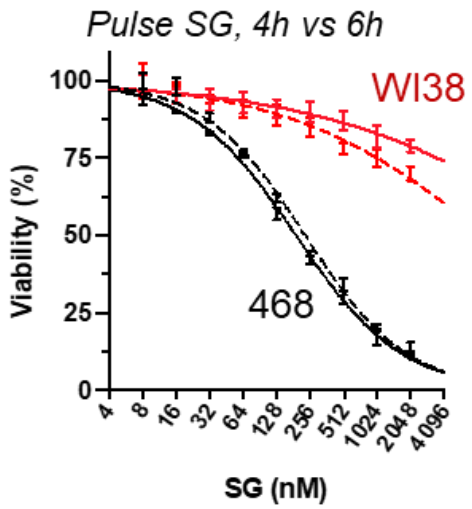


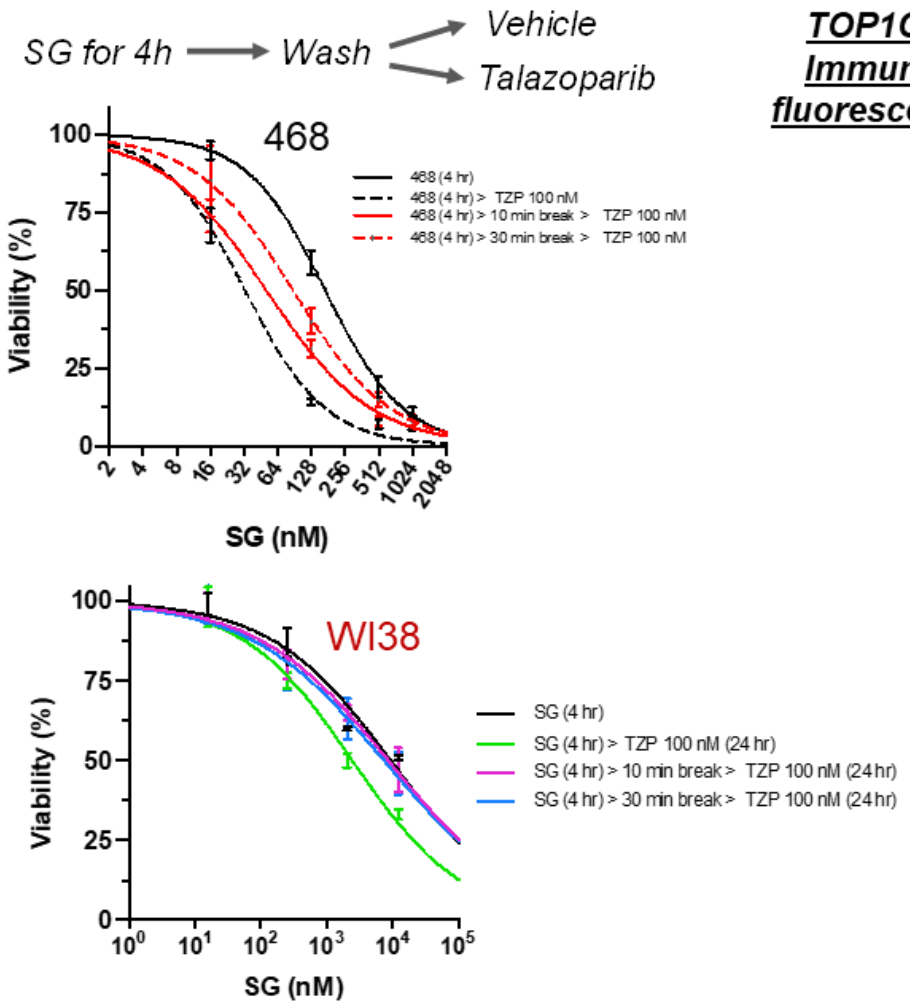
Sequential dosing to enhance the therapeutic window



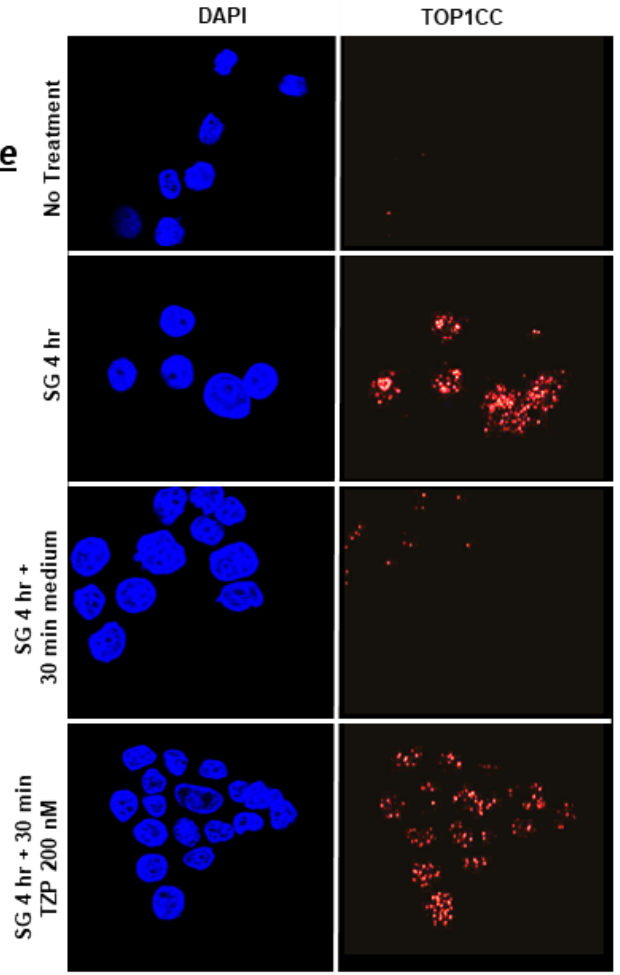
Sequential dosing of SG and PARP inhibitor preserves TOP1CC stabilization and synergistic toxicity

MDA-MB468 (TROP2+)
vs. WI38 (TROP2-)





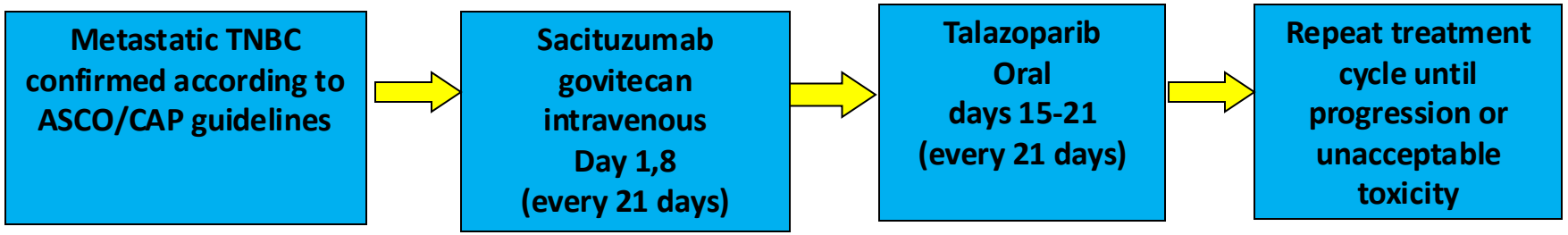
TOP1CC Immuno-fluorescence



Bardia A et al, Clinical Cancer Research 2024

Phase 1b/2 study of sacituzumab and talazoparib in metastatic TNBC

Aditya Bardia



Key eligibility criteria

- Female or male, ≥18 years of age
- No limit on prior therapy
- Measurable disease

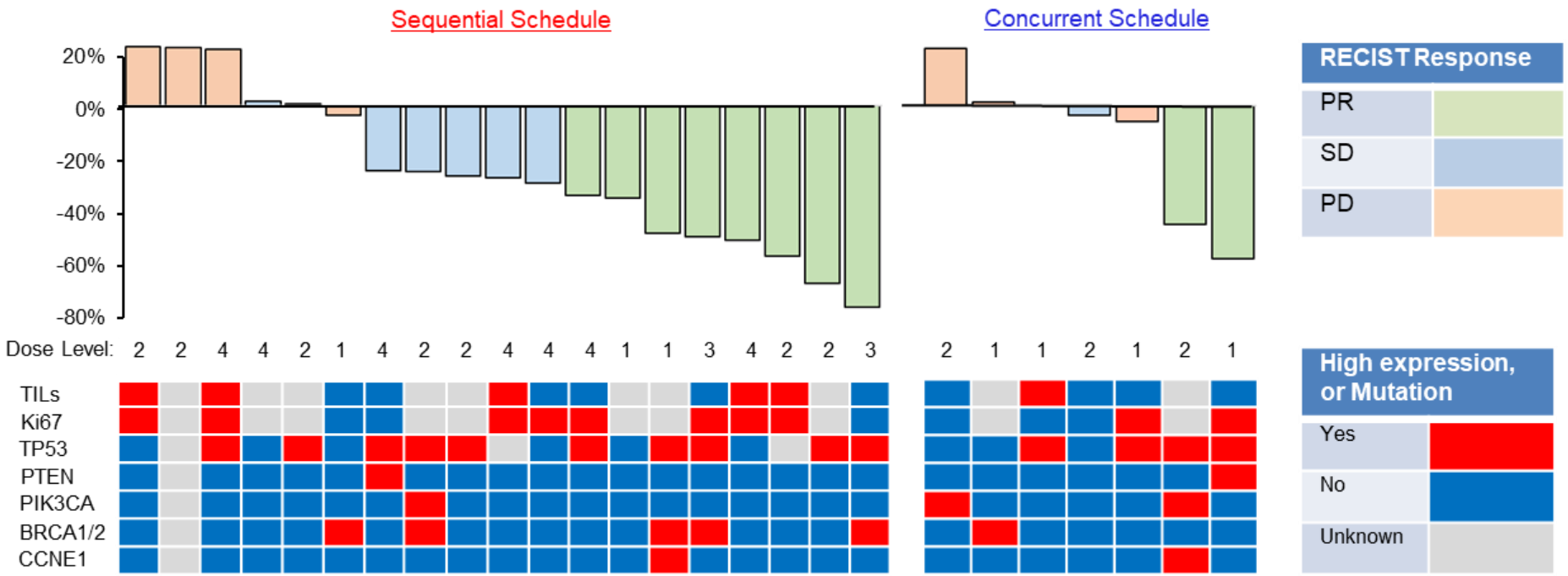
Evaluations

- Response evaluation by investigators
- Other evaluations: safety
- Biomarker evaluation,

DF/HCC Protocol #: 19-239
NCT04039230

Response and biomarkers in Phase 1b study of SG and talazoparib in metastatic TNBC

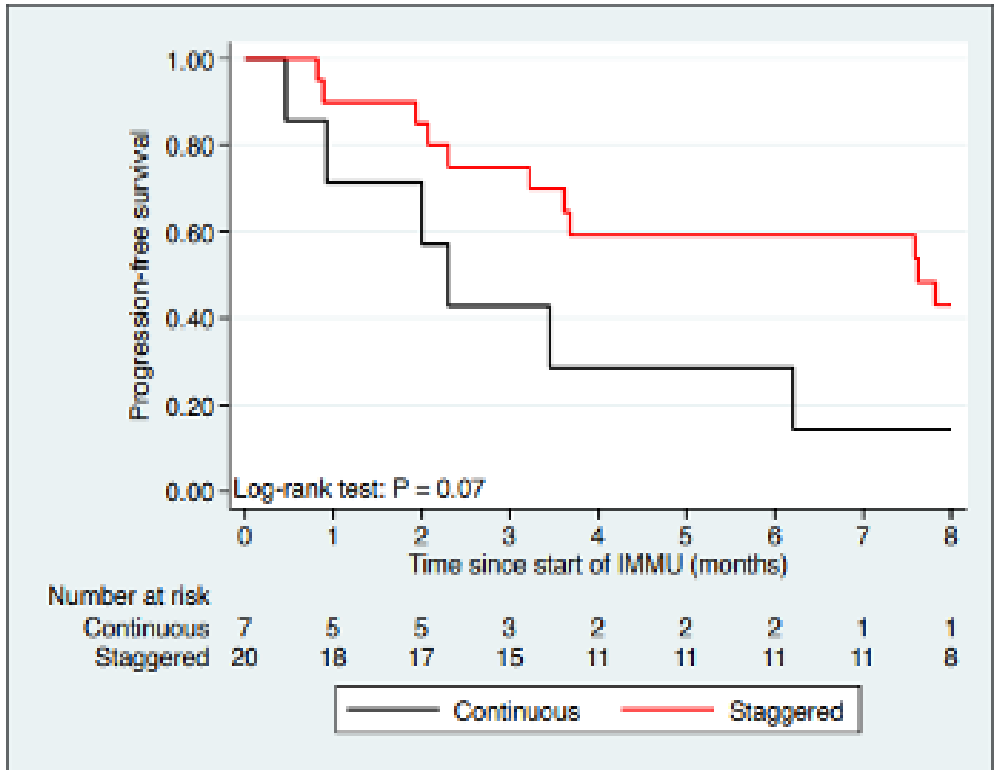
Response and Biomarkers



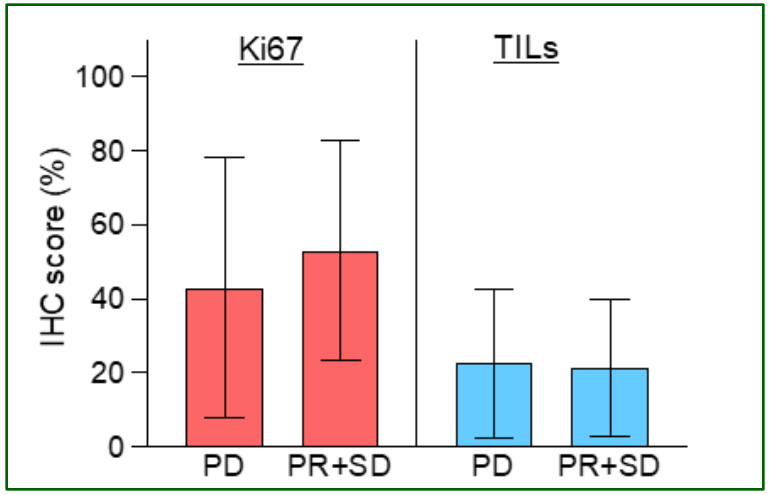
Bardia A et al, Clinical Cancer Research 2024

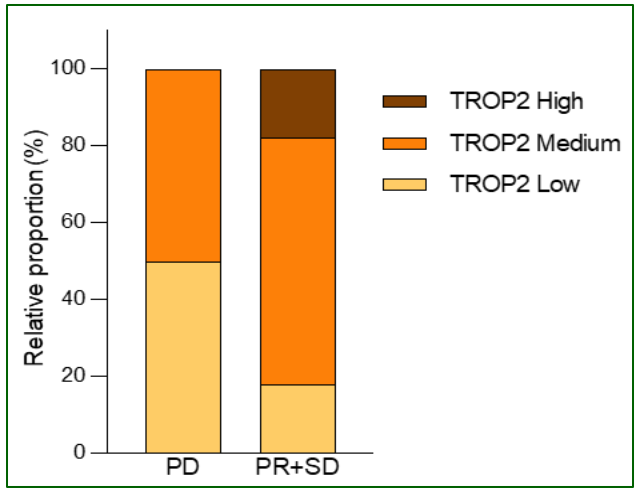
PFS and histological correlates in Phase 1b study of SG and talazoparib in metastatic TNBC

Progression-Free Survival



Clinical Correlates of Response





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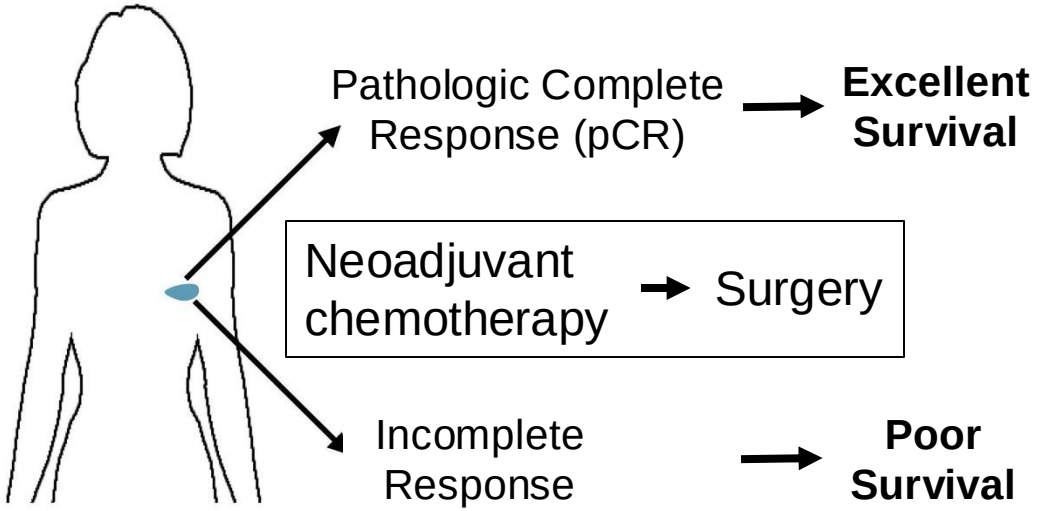
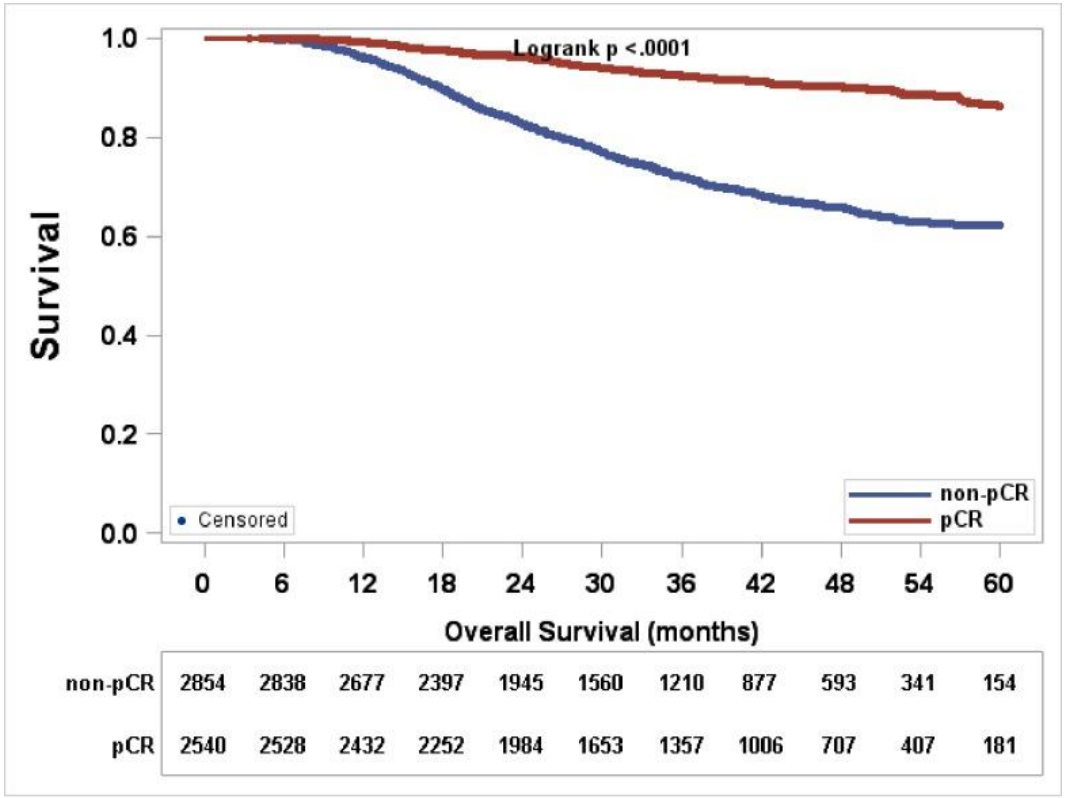
Pre-operative therapy of TNBC as a platform to understand response, resistance and long-term outcomes





5-year Overall survival rate and 95%CI:

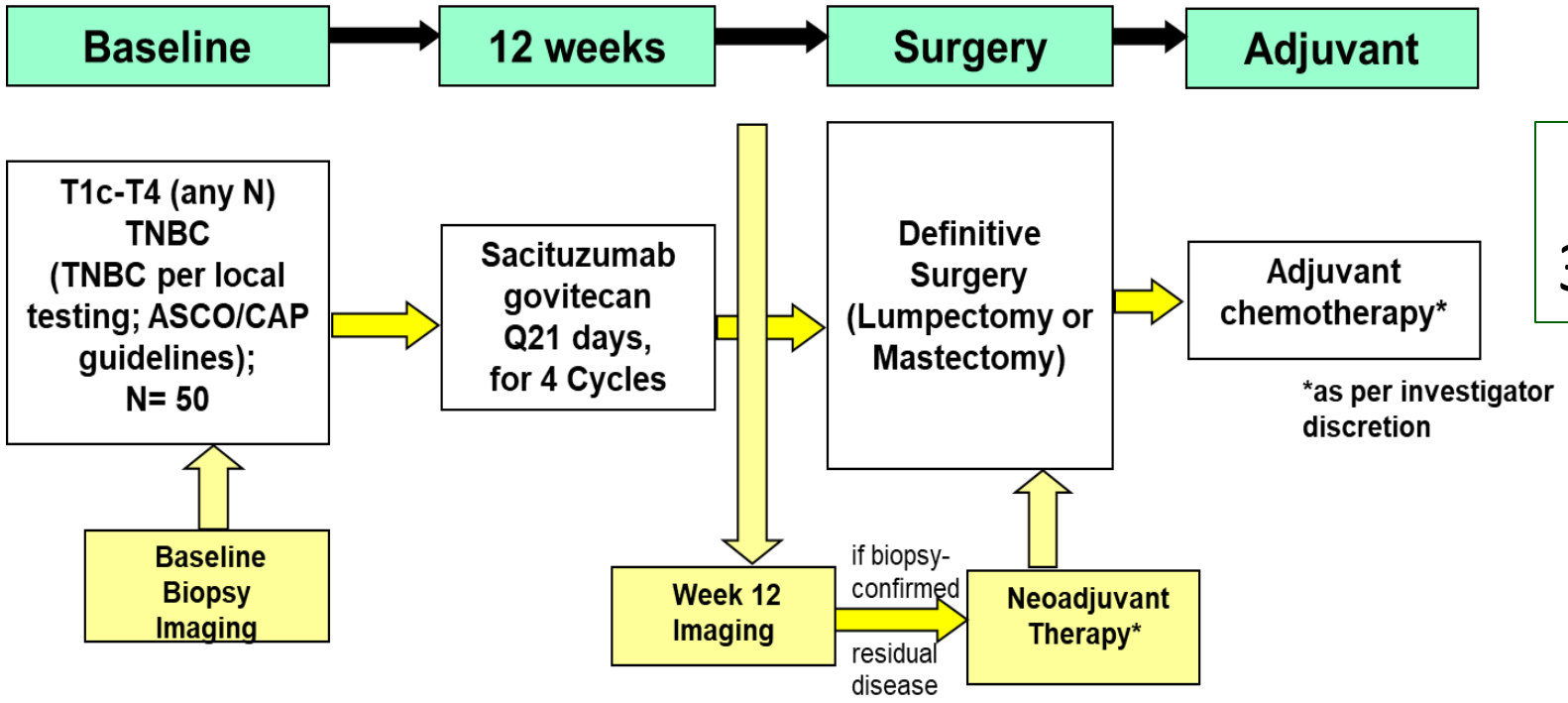
pCR: 86.2% (83.6 – 88.5%)

Non-pCR: 62.3% (59.8 – 64.7%)

PLoS One. 2019; 14(9): e0222358

Neoadjuvant SG for TNBC (NeoSTAR) including pre/post-treatment tumor analysis

Laura Spring



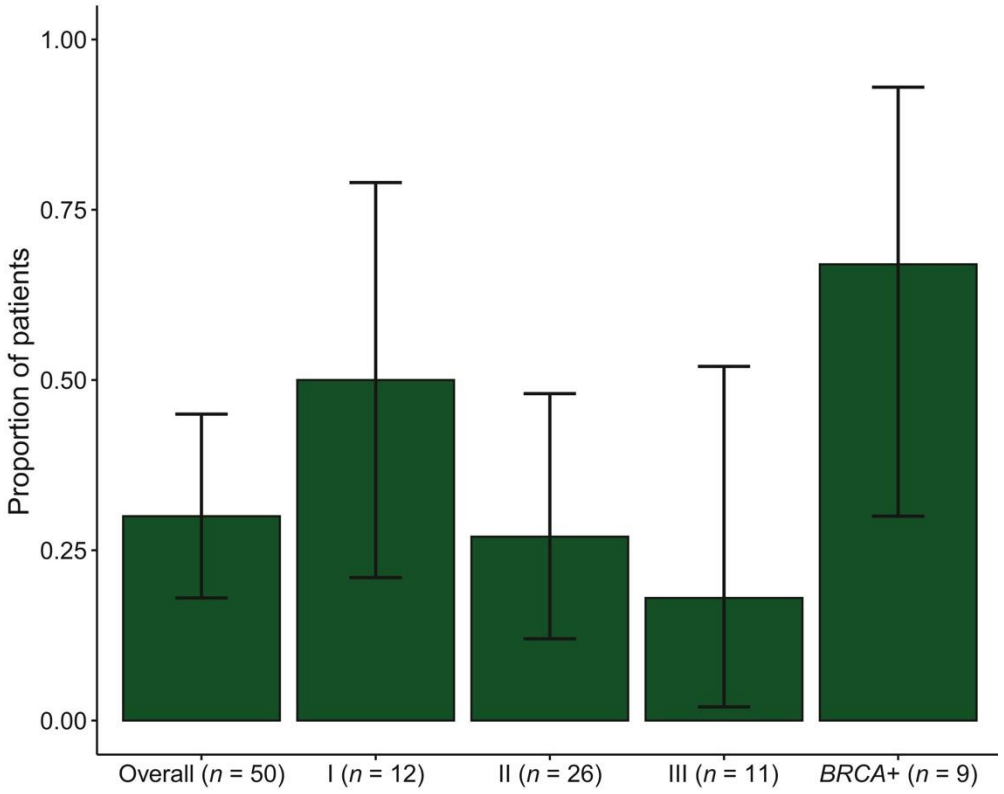
pCR Rate:
30% to SG alone

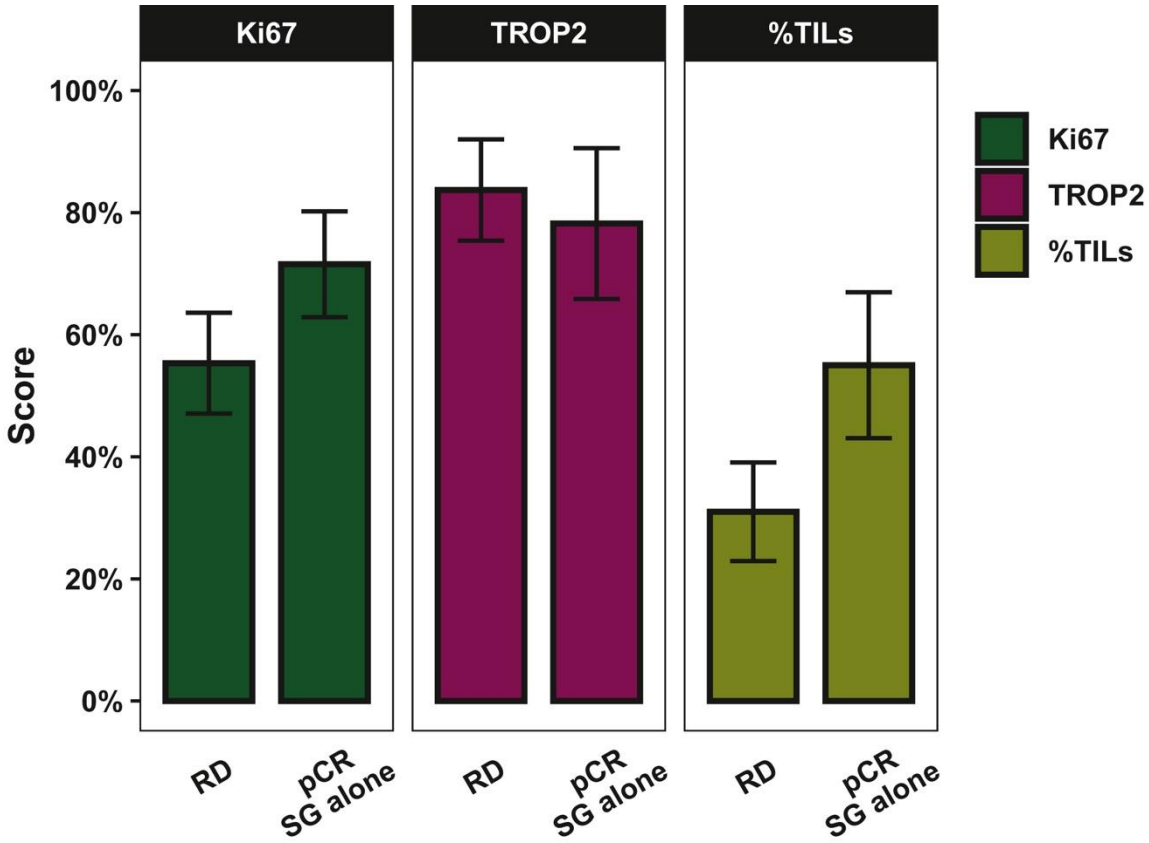
Similar trial in combination with immunotherapy as well as for HR+ breast cancer

Spring LM et al. *Annals of Oncology* 2024

NeoSTAR response and histologic correlates

pCR by Stage and BRCA1/2 Status

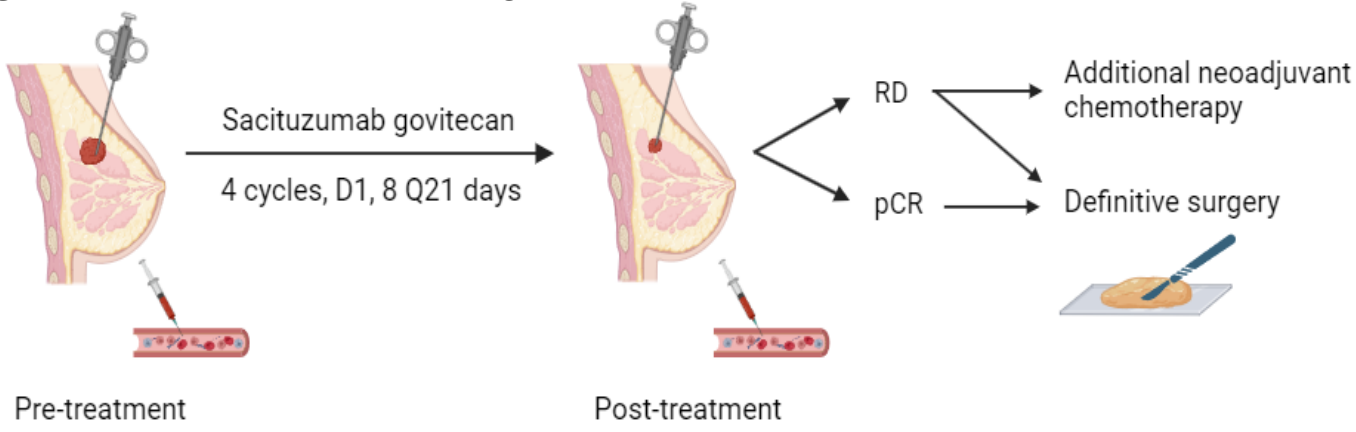




Spring LM et al. *Annals of Oncology* 2024

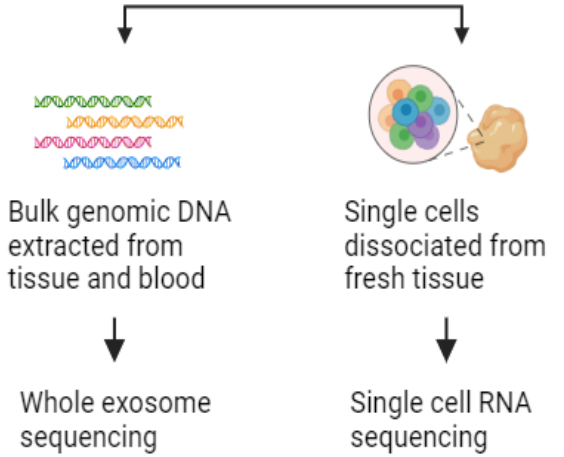
Translational schema and workflow for NeoSTAR

Ting Liu, *James Coates, Siang Boon Koh, Nicole Peiris*



Non-Responder (NR)

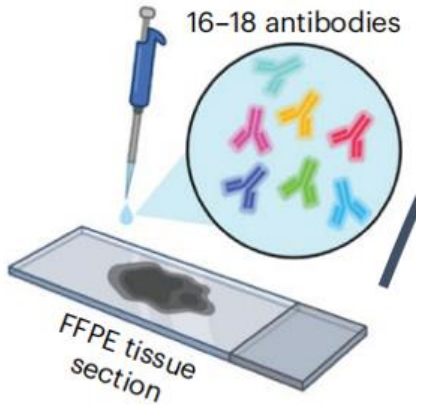
Responder (R)



Sample Numbers:

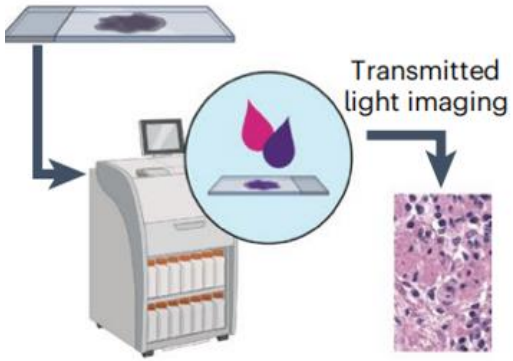
	Responder (R): pCR after SG	Non-responder (NR): RD after SG
Pre	scRNA: n=9 WES: n=12	scRNA: n=16 WES: n=24
Post	scRNA: n=1 WES: n=2	scRNA: n=11 WES: n=15

1. One-shot immunostaining



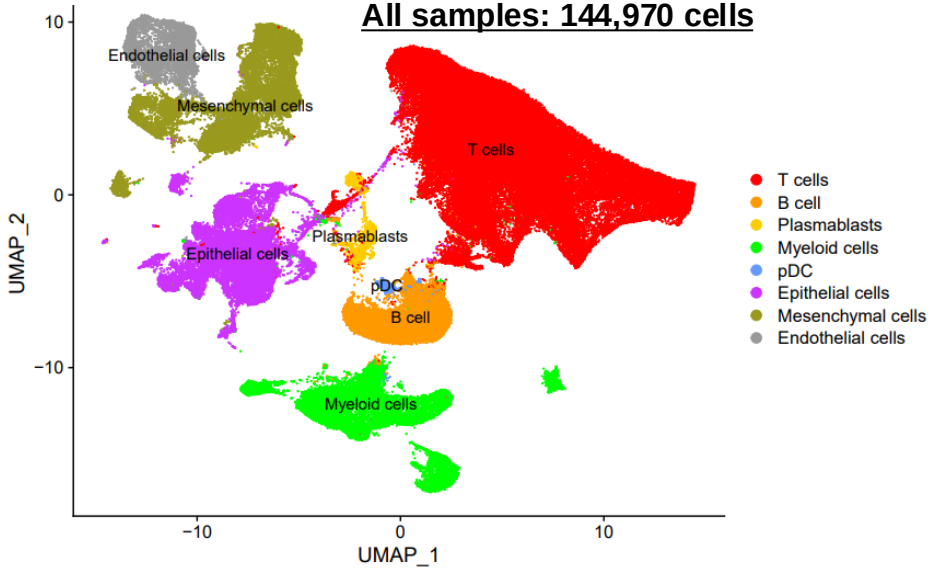
Epithelial	Lymphoid	Myeloid
Pan-CK	CD3e	CD68
E-Cad	CD4	CD163
TROP2	CD8a	CD11c
Proliferation	FOXP3	**Stromal**
KI67	CD20	CD31
Immune	**Checkpoint**	SMA
CD45	PD-1	

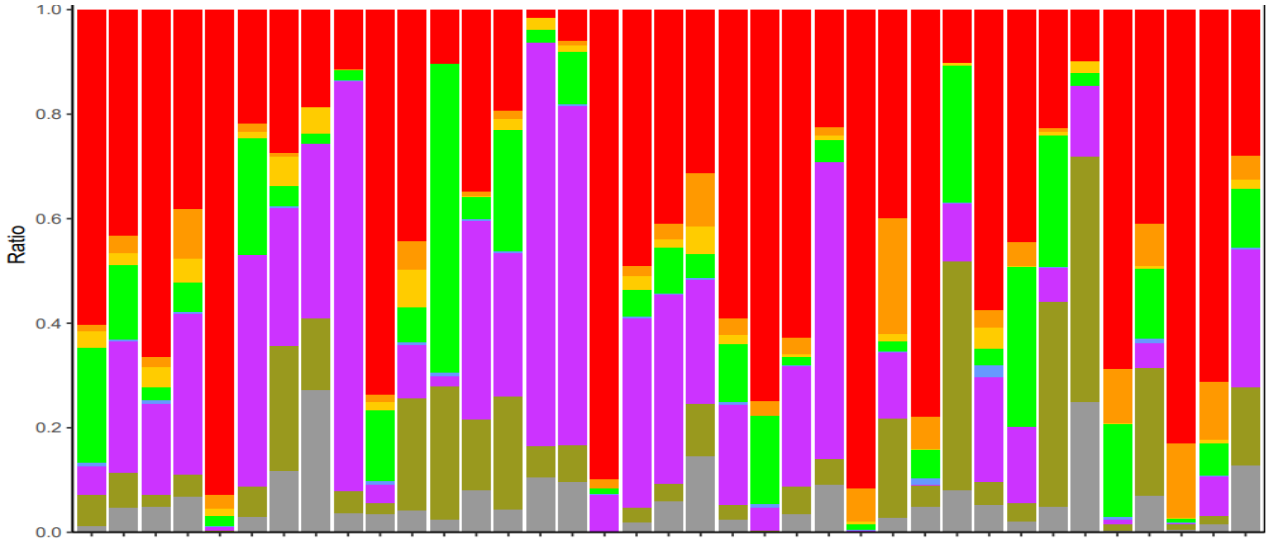
5. H&E staining



6. Same-section H&E image

Cell type assignments by mixed method design





Non-epithelial cell Subtypes

CD8+T cell
- CD8T_c0_Tnaive_CCR7
- CD8T_c1_Teff.mem_PTPRC
- CD8T_c2_Teff.mem_RNF125
- CD8T_c3_Tterm.ex_LAG3
- CD8T_c4_Tprog.ex_PRF1
- CD8T_c5_Tprog.ex_IFNG
- CD8T_c6_Teff.mem_HSPA8
- CD8T_c7_Teff.mem_ZNF683
- CD8T_c8_Tprog.ex_XCL2
- CD8T_c9_Tprog.ex_CCL4
- CD8T_c10_Tcyc_MKI67

Innate lymphoid cell
- ILC_c0_NK_NCAM1
- ILC_c1_NKT_FCGR3A
- ILC_c2_ILC3_KIT
- ILC_c3_NK_HSPA1A
- ILC_c4_NKT_IFNG

CD4+T cell
- CD4T_c0_Teff.mem_GPR183
- CD4T_c1_Tnaive_CCR7
- CD4T_c2_Treg_FOXP3
- CD4T_c3_Th1_JUN
- CD4T_c4_Teff.mem_PTPRC
- CD4T_c5_Tfh_CXCL13
- CD4T_c6_Teff.mem_IFIT3
- CD4T_c7_Teff.mem_GZMB
- CD4T_c8_Teff.mem_NFKB1
- CD4T_c9_Teff.mem_CCL5
- CD4T_c10_Tcyc_MKI67
- CD4T_c11_Teff.mem_S100A4

B cell
- B_c0_Bmem_CD79A
- B_c1_Bmem_CCR7
- B_c2_Bmem_HSPA1A
- B_c3_Bnaive_IGHD
- B_c4_Bmem_BANK1
- B_c5_PlgG_IGHG1
- B_c6_PlgG_IGLC3
- B_c7_PlgG_IGHG2
- B_c8_PlgA_IGHA1
- B_c9_PlgM_IGHM
- B_c10_Bgerm.cen_MYC

Myeloid cell
- M_c0_Neutrophil_FCGR3B
- M_c1_Mono_S100A12
- M_c10_pDC_LILRA4
- M_c11_Cycling_MKI67
- M_c12_Macro_ISG15
- M_c14_mregDC_LAMP3
- M_c2_Macro_APOE
- M_c3_cDC2_FCER1A
- M_c4_Macro_CCL4
- M_c5_cDC1_CLEC9A
- M_c5_Mast_KIT
- M_c6_Macro_SPP1
- M_c7_Macro_SELENOP
- M_c8_Transitional_CTSK
- M_c9_Mono_FCN1

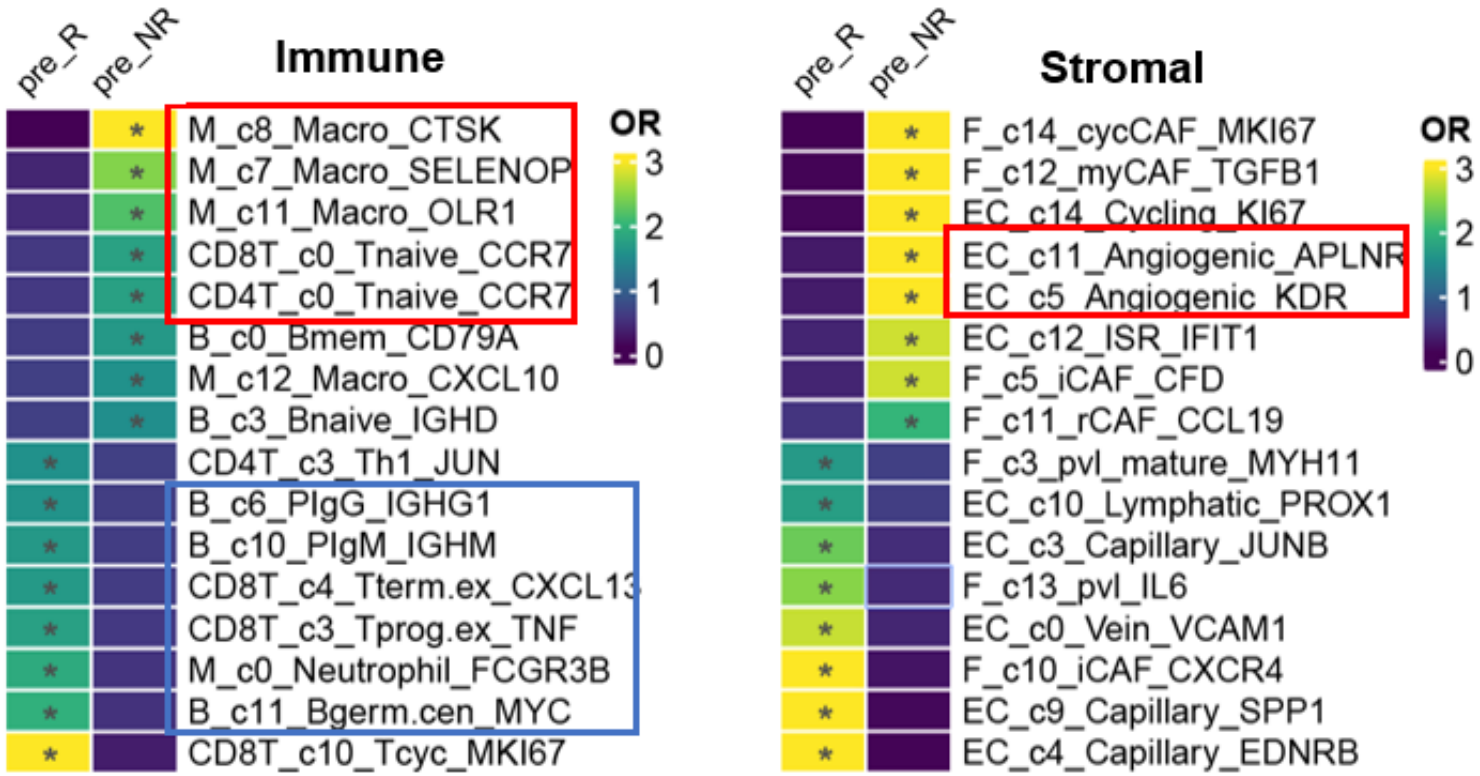
Mesenchymal cell
- F_c0_myCAF_COL5A1
- F_c1_iCAF_SELENOP
- F_c10_rCAF_CCL19
- F_c11_Transitional_TGFB1
- F_c12_imPVL_IL6
- F_c13_Cycling_MKI67
- F_c2_Intermediate_ENO1
- F_c3_mPVL_MYH11
- F_c4_imPVL_RGS5
- F_c5_iCAF_CFD
- F_c6_myCAF_MMP11
- F_c7_myCAF_SFRP4
- F_c8_myCAF_COL11A1
- F_c9_apCAF_HLA-DRA

Endothelial cell
- EC_c0_Vein_S100A13
- EC_c1_Vein_ACKR1
- EC_c2_Angiogenic_INSR
- EC_c3_Vein_VCAM1
- EC_c4_Capillary_JUNB
- EC_c5_Artery_HEY1
- EC_c6_Capillary_EDNRB
- EC_c7_Capillary_CA4
- EC_c8_Vein_HSP90AB1
- EC_c9_Transtional_POSTN
- EC_c10_Lymphatic_PROX1
- EC_c11_Capillary_SPP1
- EC_c12_Cycling_MKI67
- EC_c13_ISR_ISG15

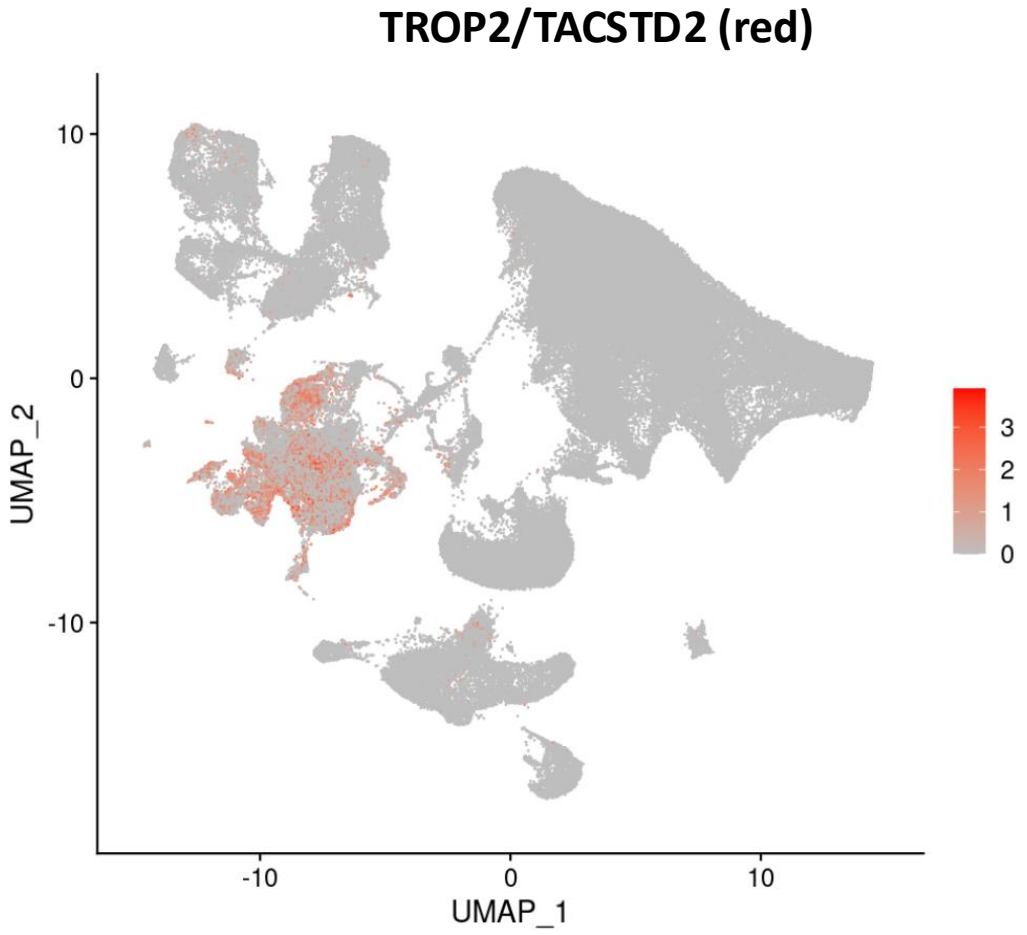
Epithelial cell

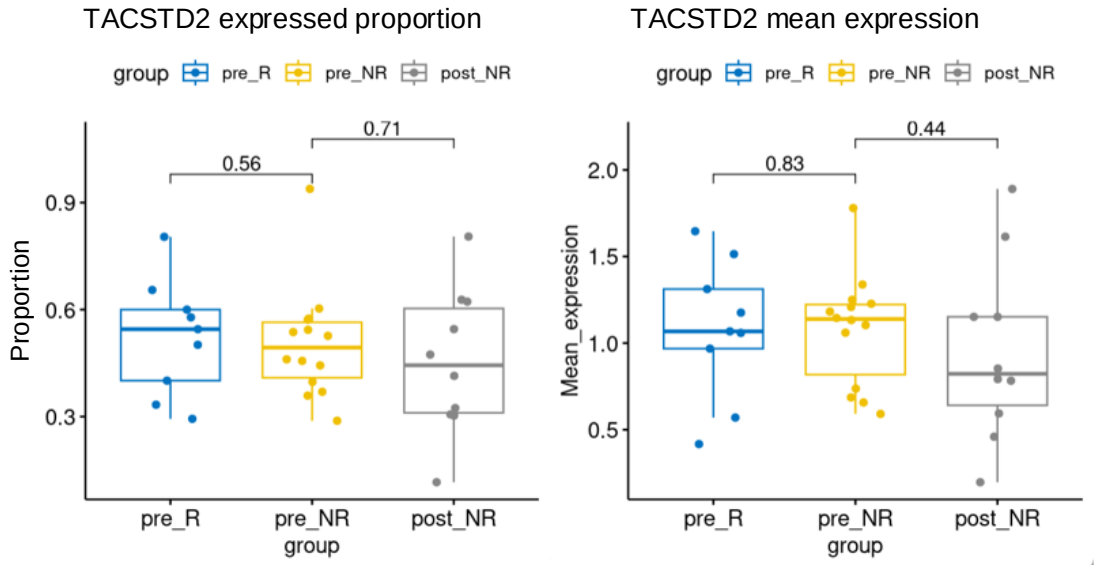
Immune and stromal populations distinguishing pCR tumors

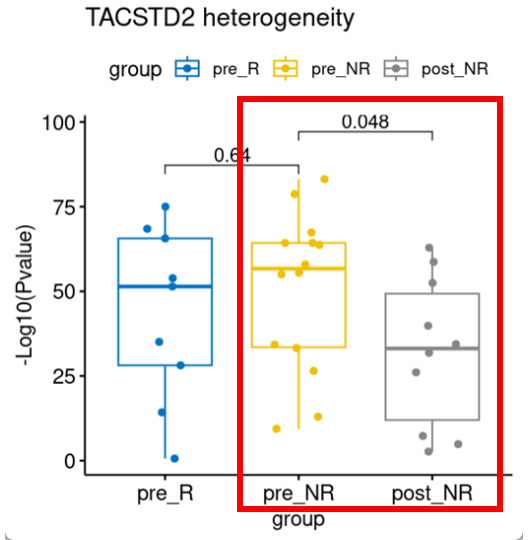
Responders: more activated and mature immune cell subsets.
Non-responders: have more immune suppressive macrophages and angiogenic endothelial cells



TROP2 expression is heterogenous and not associated with treatment response

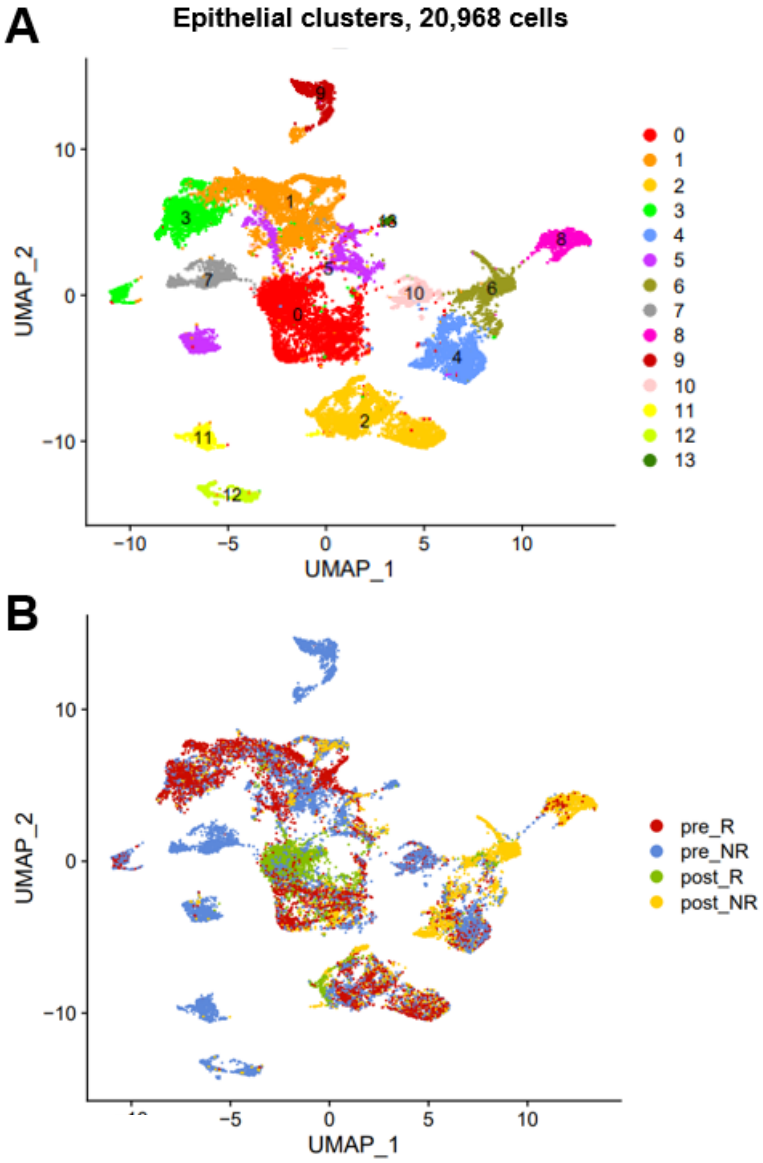






- TROP2 heterogeneity in each sample is measured by the deviation between the actual zero counts of TROP2 and the expectations with Poisson distribution. (ref 10.1038/s41467-022-29358-6)

Identifying shared tumor cell phenotypes across samples



Identifying Meta-Programs Representing Tumor Cell Phenotypes

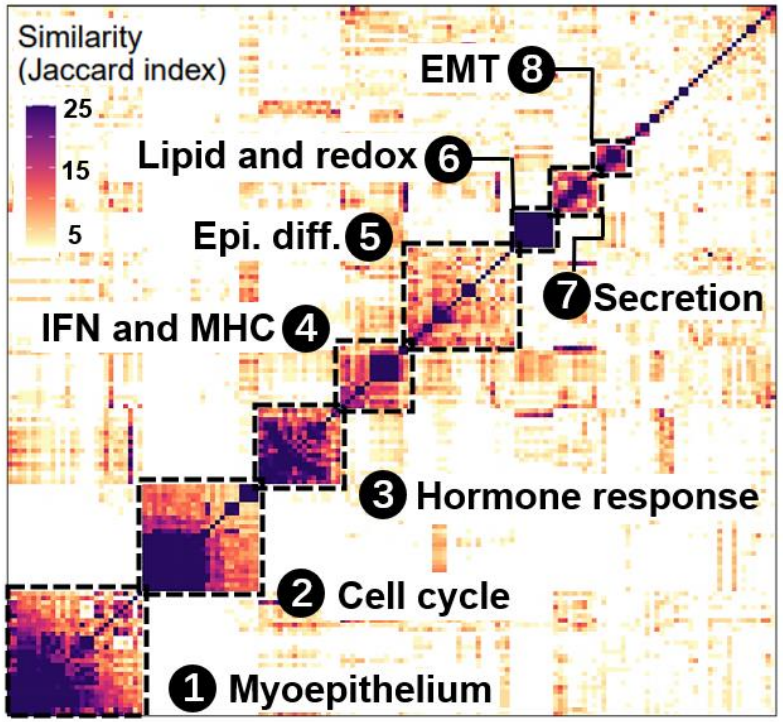
1,365 programs from 35 tumors

↓

170 robust programs

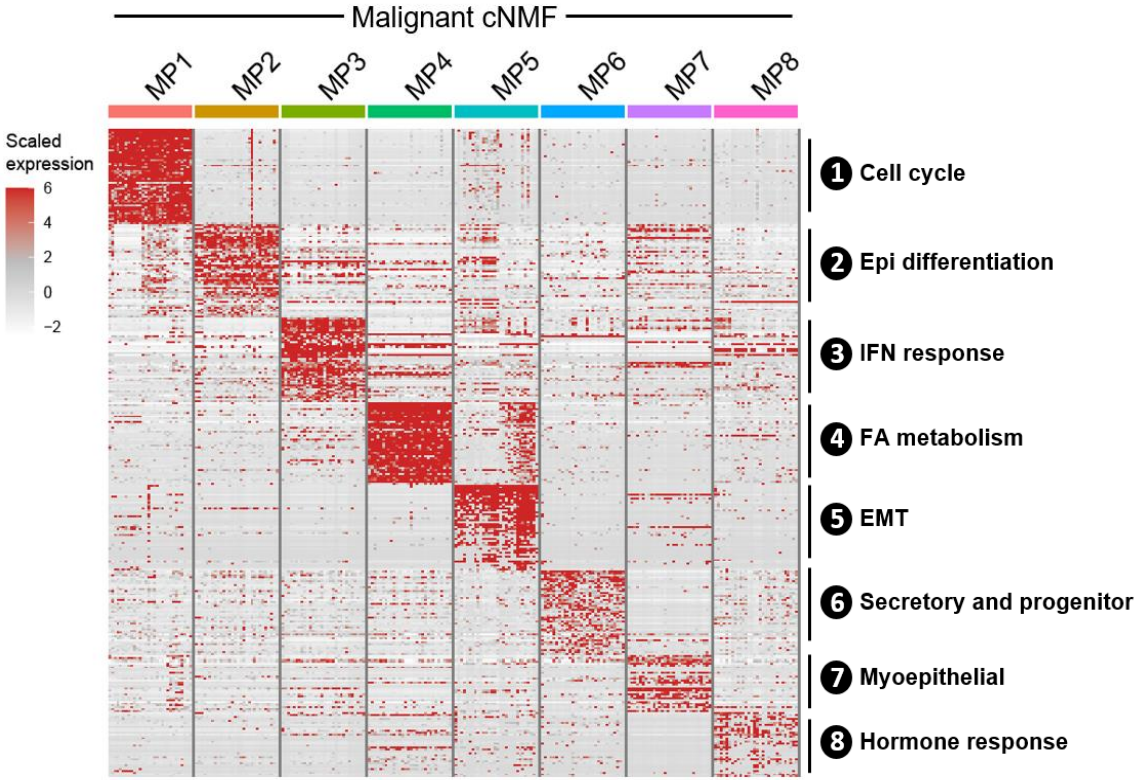
↓

8 meta-programs

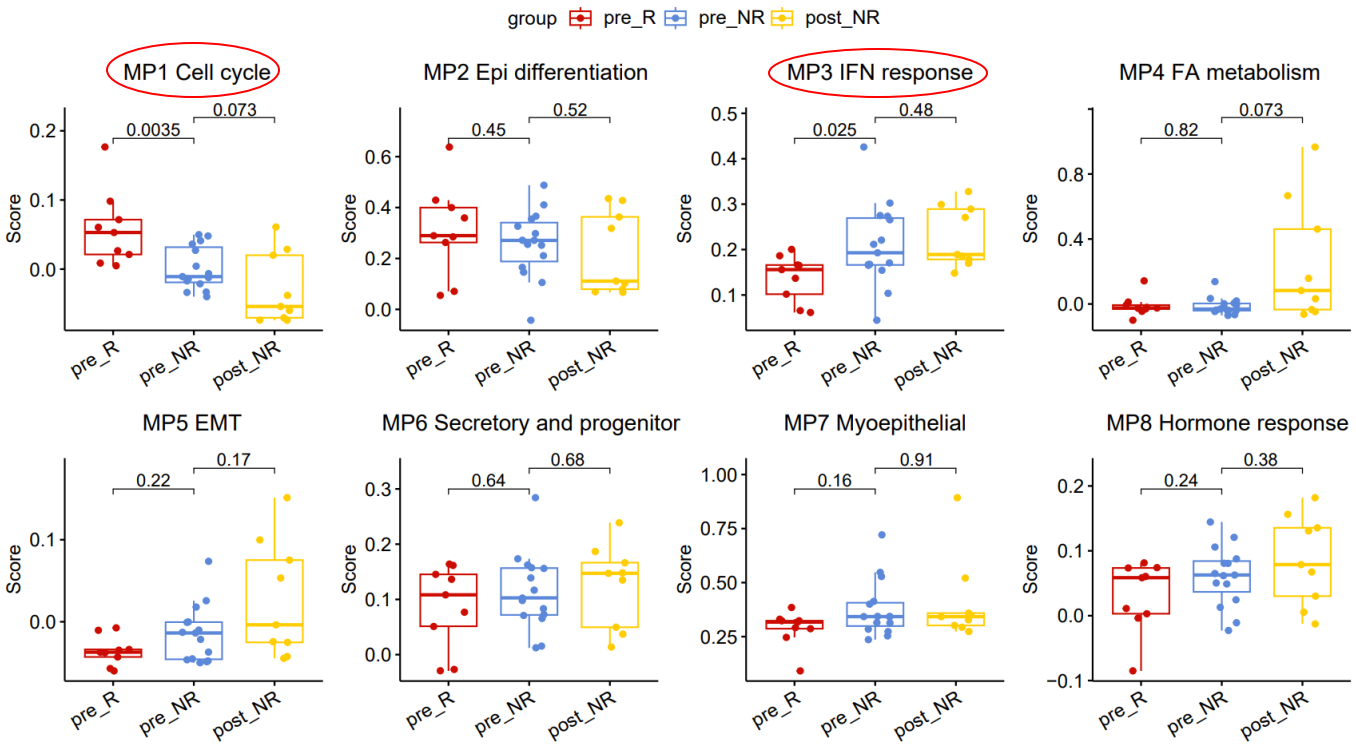


Cell cycle and Interferon response meta-programs are associated with response to SG

Gene expression in Meta-Programs (MPs)

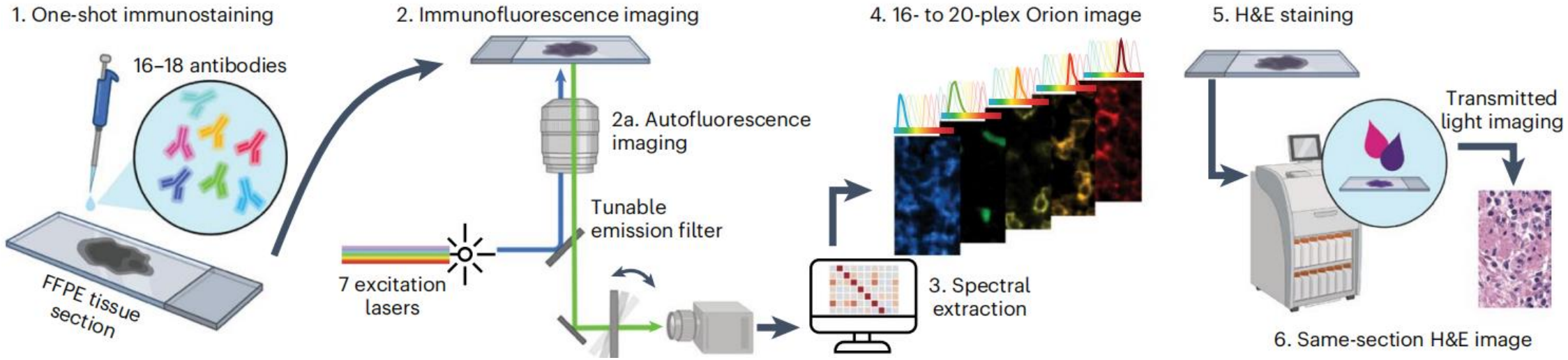


Cell cycle and IFN MPs distinguish R/NR tumors, respectively

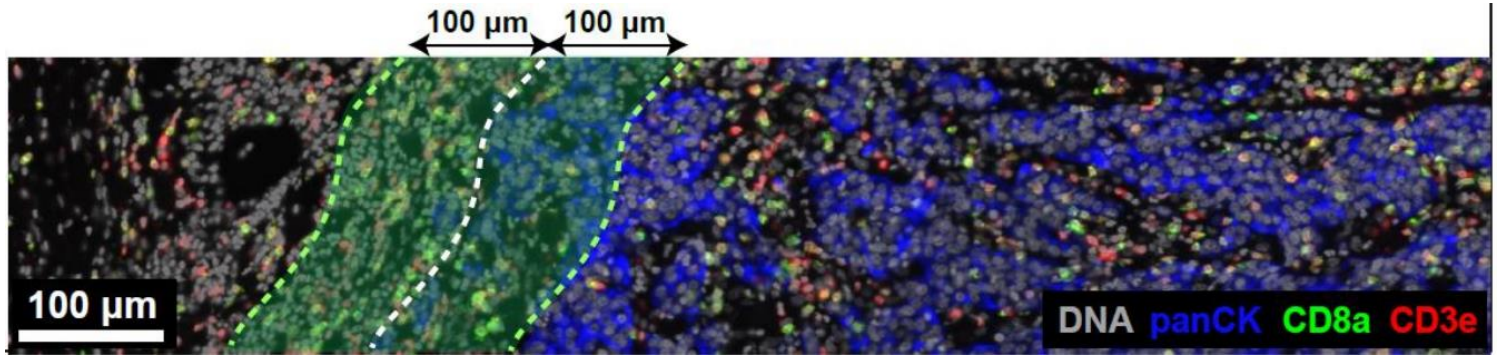


Spatial imaging using Orion™ “one-shot” multiplex IF

Jia-Ren Lin, Sandro Santagata

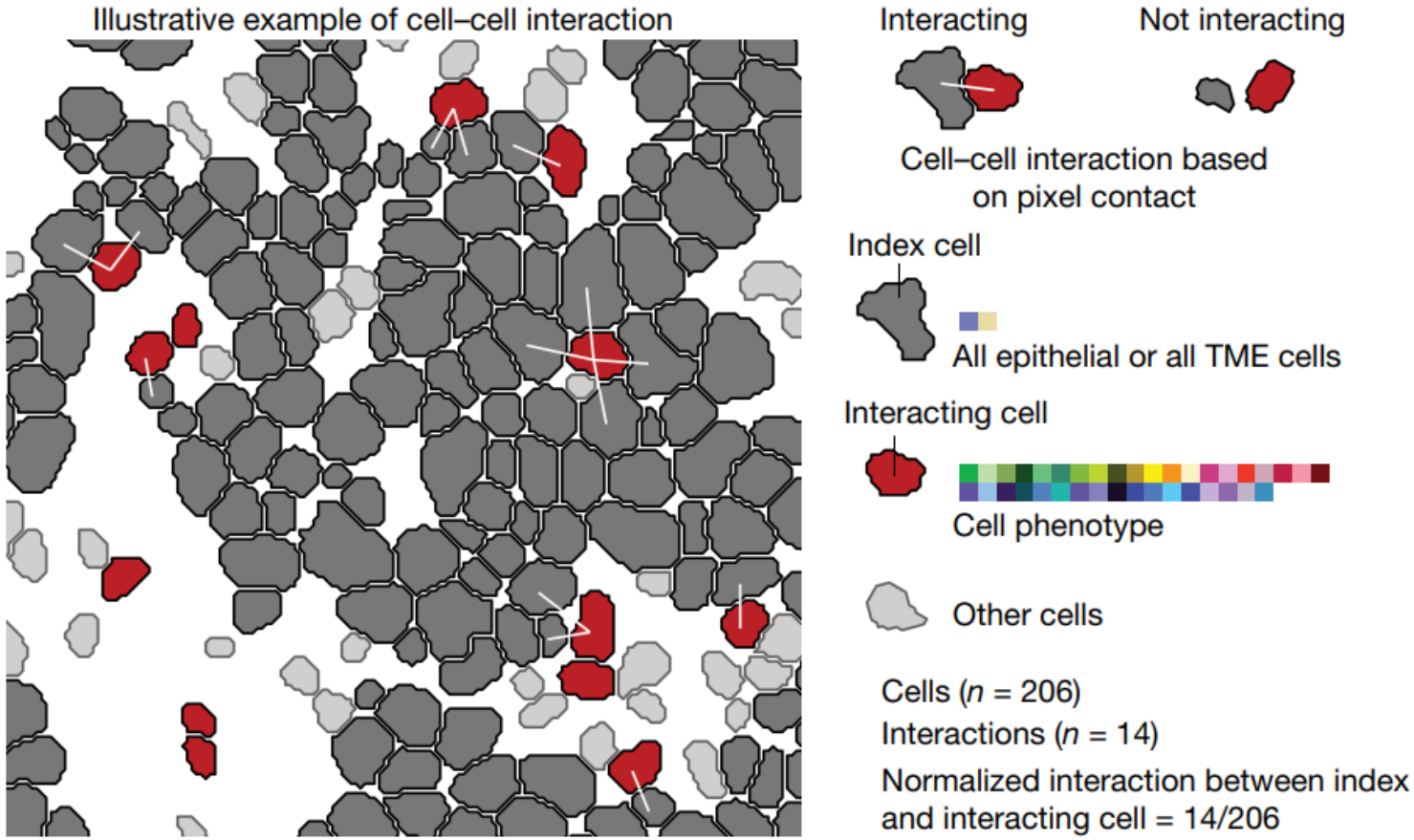


Epithelial	Lymphoid	Myeloid
Pan-CK	CD3e	CD68
E-Cad	CD4	CD163
TROP2	CD8a	CD11c
Proliferation	FOXP3	Stromal
KI67	CD20	CD31
Immune	Checkpoint	SMA
CD45	PD-1	

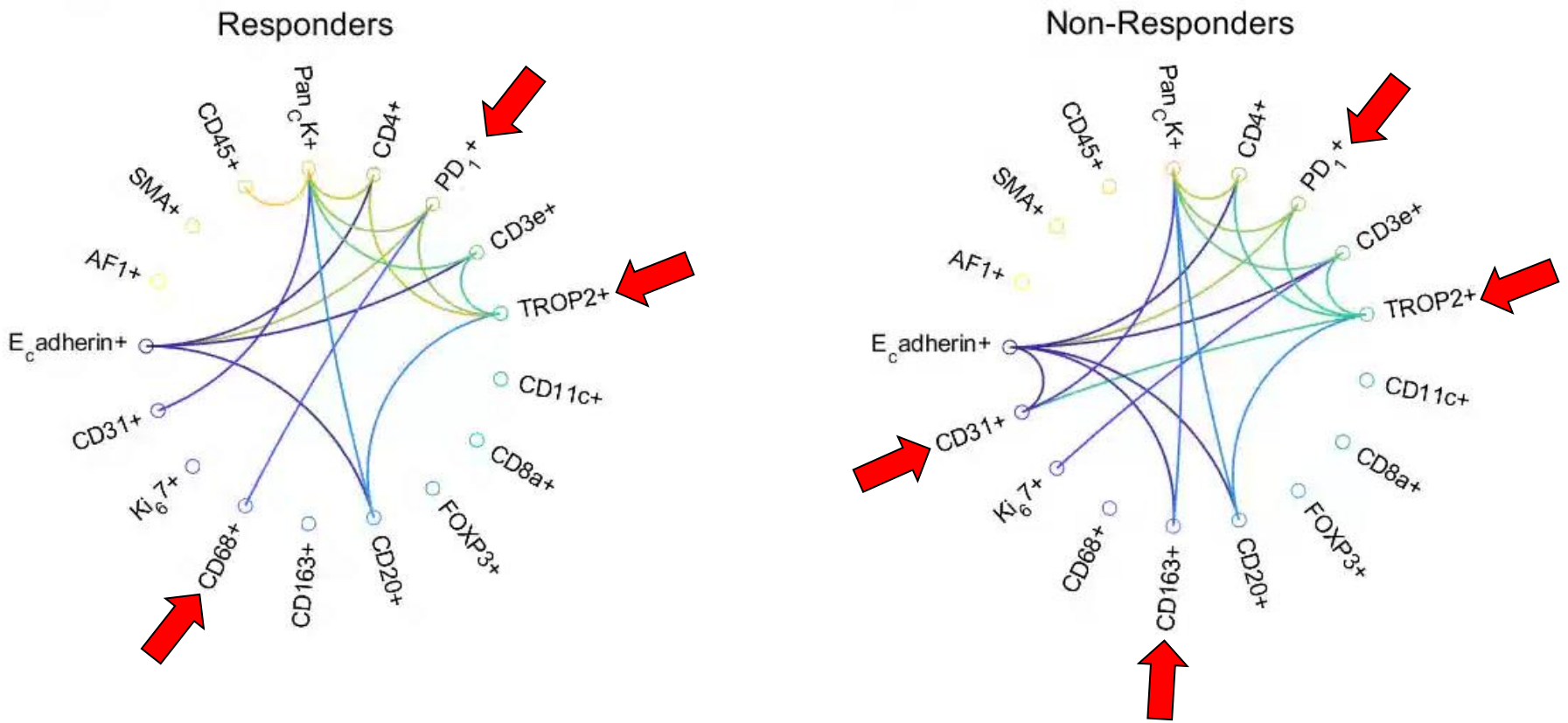


Lin, J-R, Santagata S et al, Nat Cancer. 2023 Jul;4(7):1036-1052

Schematic of cell-cell interaction analysis



Interactions between tumor cells, immune and stromal cells define responses to Sacituzumab govitecan

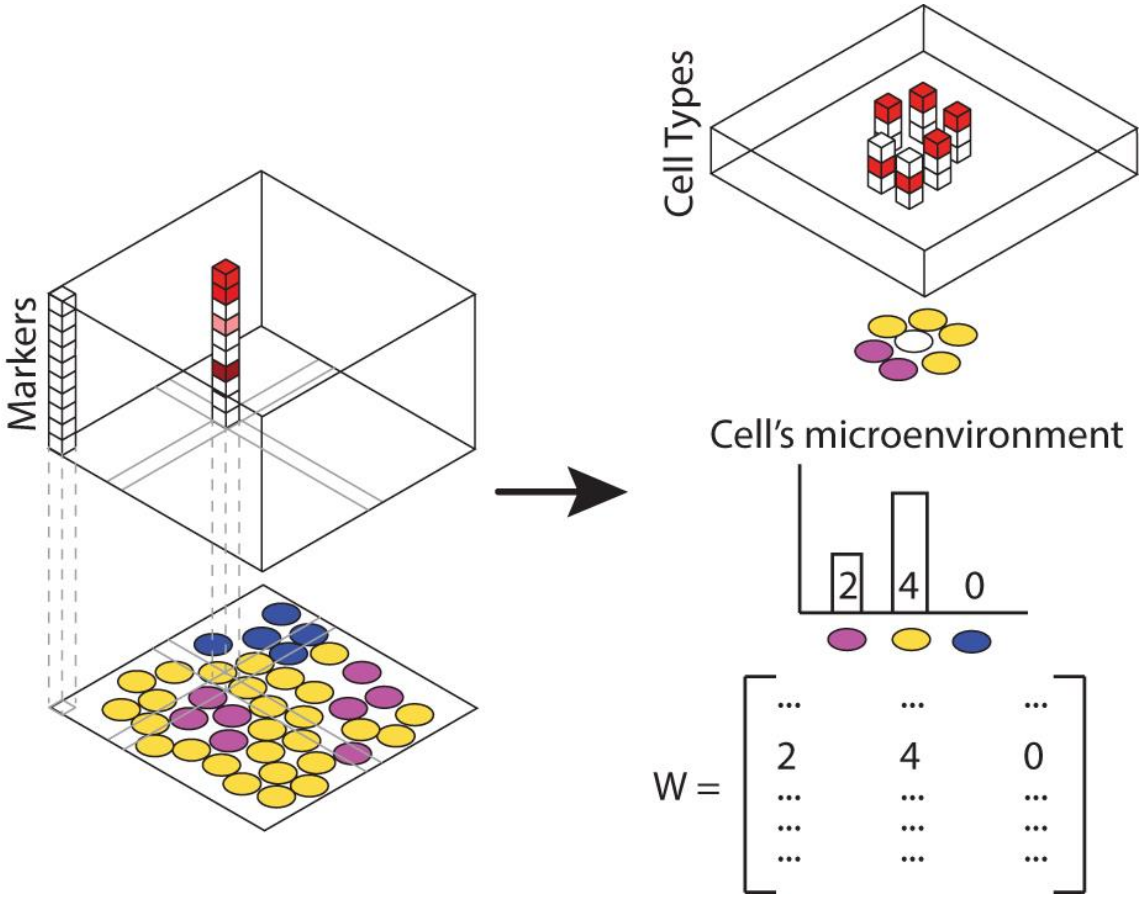


- PD1+ cells have strong interactions with epithelial cells (Pan-CK, E-cad, TROP2) in *both groups.*
- Responders have more CD68-PD1 interactions (M1 antigen presentation with lymphocytes).
- Non-responders have more TROP2-CD163 and TROP2-CD31 interactions (M2 recruitment and angiogenesis induction)

Cellular neighborhood analysis

Identifying spatial neighborhoods ("topics") using latent Drichilet allocation (LDA)

"*Bag of cells*" approach conceptually similar to "*bag of words*" approach in Natural Language Processing (NLP)

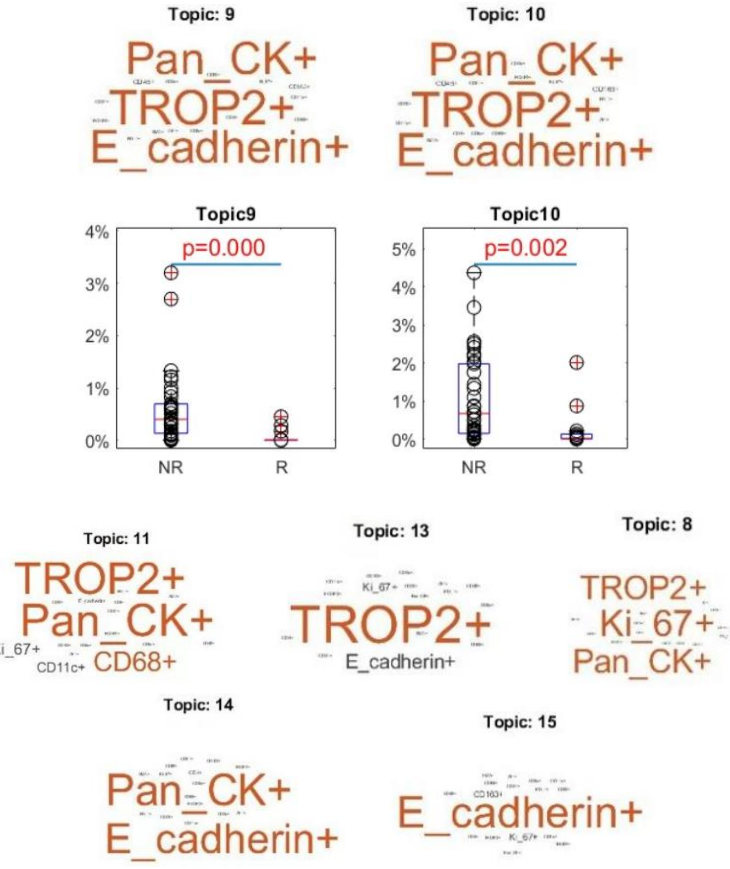


Chen Z, Soifer I, Hilton H, Keren L, Jojic V. Modeling Multiplexed Images with *Spatial-LDA* Reveals Novel Tissue Microenvironments. *J Comput Biol*. 2020;27(8):1204-1218. doi:10.1089/cmb.2019.0340

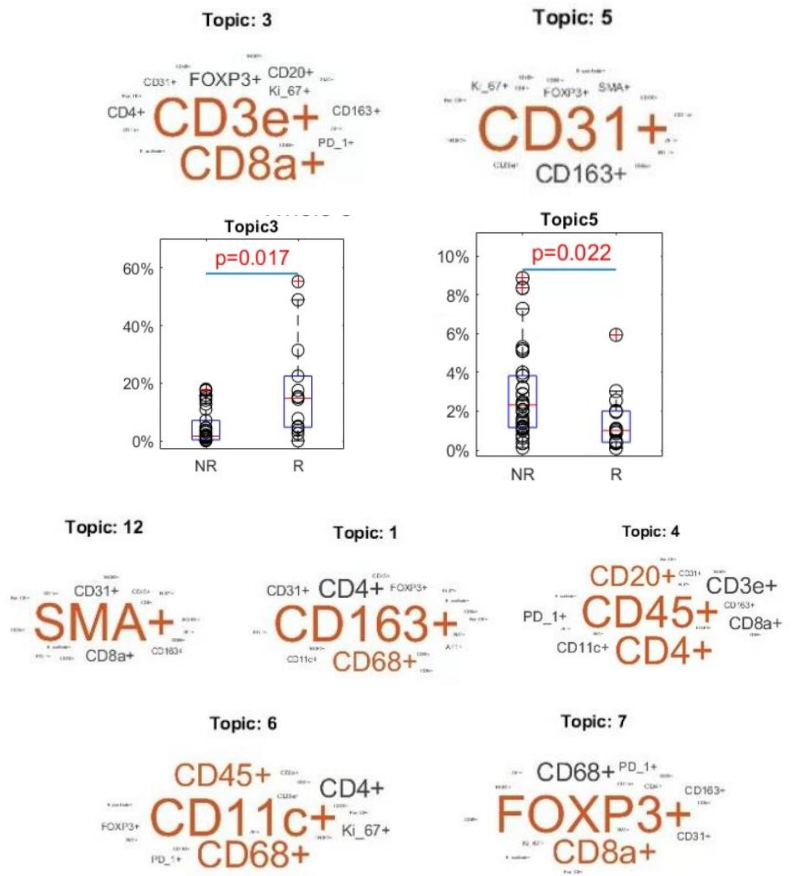
Quiescent, immune-excluded tumor cell clusters identify non-responders.

Jia-Ren Lin, Veerle Bossuyt

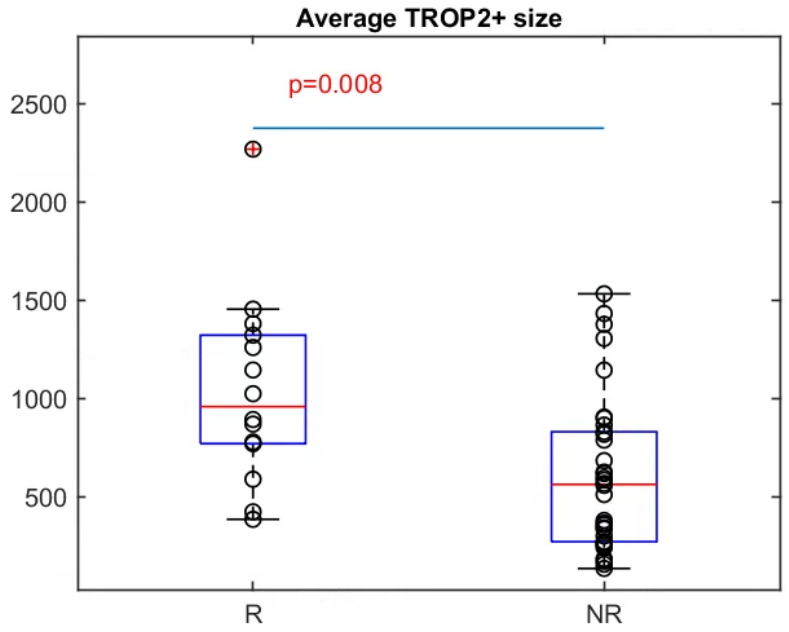
Tumor core topics



TME topics



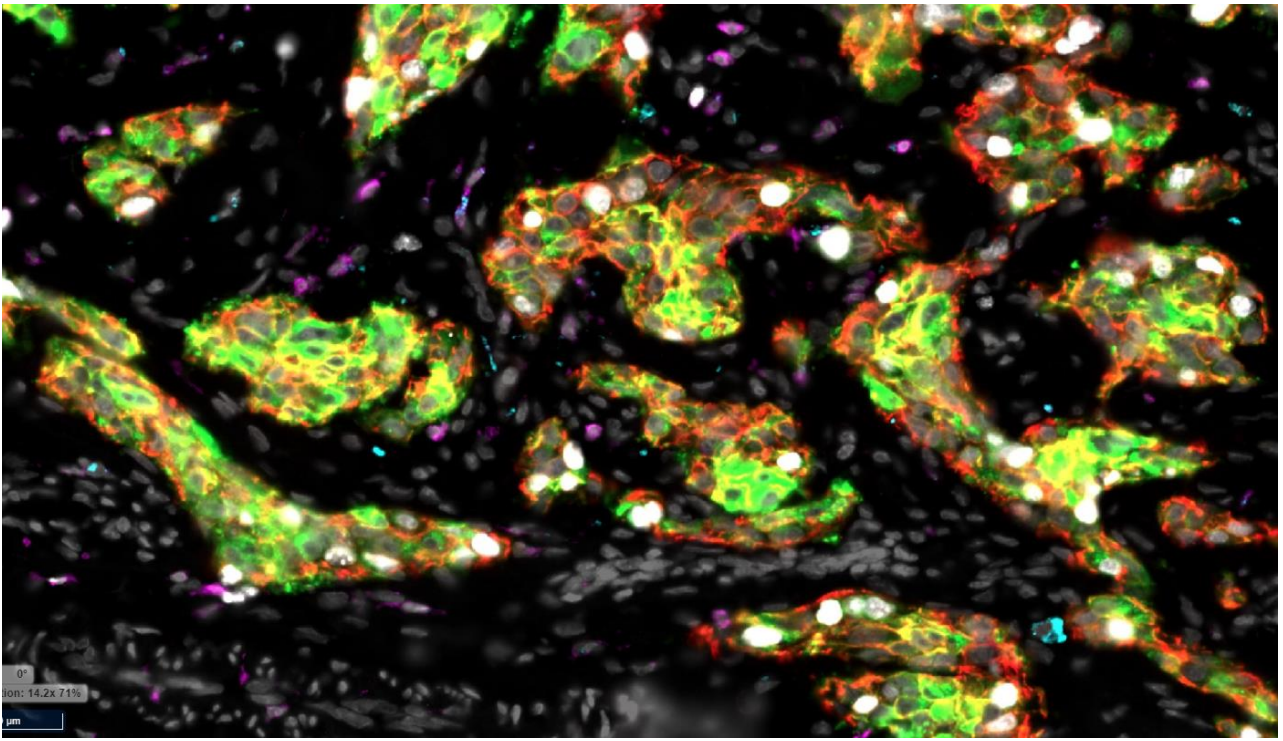
Smaller TROP2+ cell clusters in non-responders



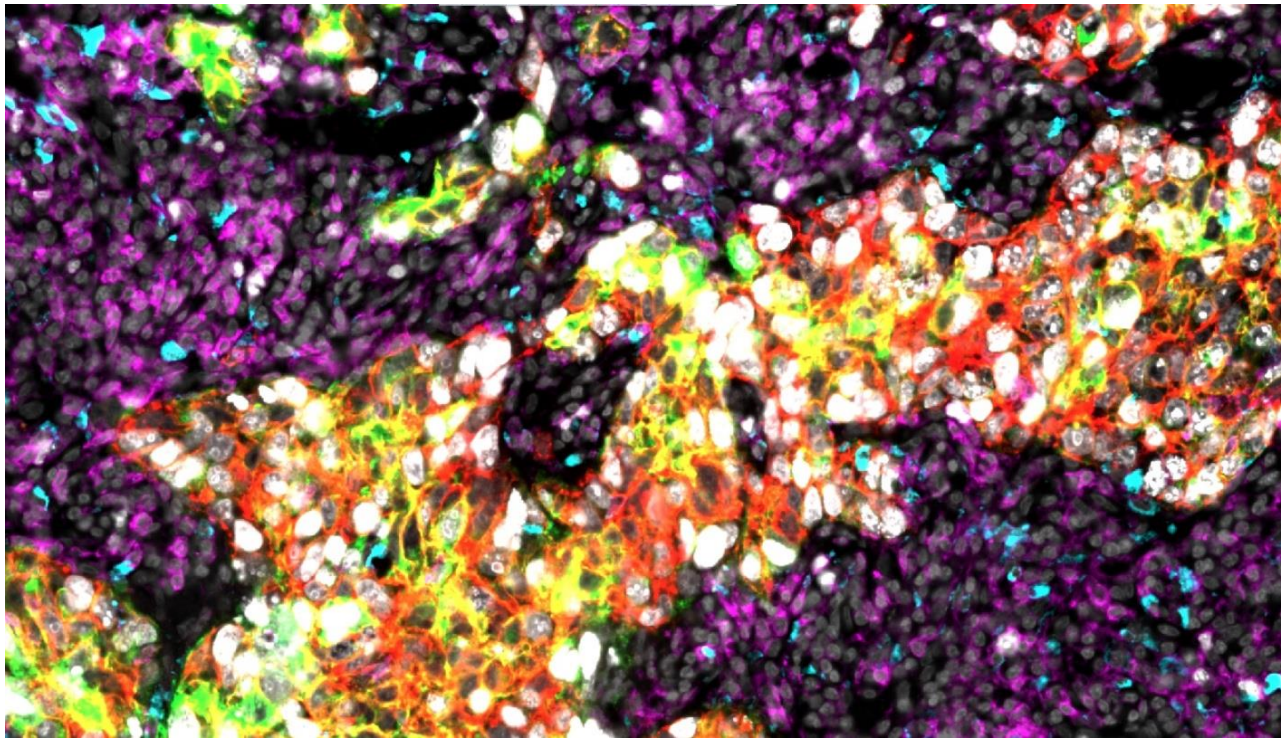
Quiescent, immune-excluded tumor cell clusters identify non-responders.

Jia-Ren Lin, Veerle Bossuyt

Topic 10



Topic 3



DNA E-cadherin TROP2 CD68 CD45 Ki67

Summary

- Resistance to ADCs including SG may involve target and payload-associated mechanisms with near-term clinical implications.
- ADCs represent an exciting platform for mechanism-based therapeutic combinations.
- Tumor cells with hallmarks of chronic Interferon activation are chemo-resistant.
- An activated immune microenvironment is associated with ADC response.
- Systematic integration of clinical and pre-clinical investigation will be required to unravel the complexity of ADC mechanisms and resistance.

Acknowledgements

Ellisen Lab
Ting Liu
Bogang Wu
Elena Bitman
Vincent Guo
Ruby Maharjan
Bryce Ordway
Zuen Ren
Ilze Smidt
Win Thant
Nayana Thimmiah
Isabella Vianna
Akiko Suzuki

Computational Biology
Gad Getz
Ignaty Leshchiner
Mike Lawrence
Esther Rheinbay
Simona Cristea
Franziska Michor

MGH Pathology
Veerle Bossuyt
Dennis Sgroi
Mohammad Miri

Reagents
Immunomedics/Gilead

MGH Breast Program
Laura Spring
Aditya Bardia
Dejan Juric
Steve Isakoff
Sophia Coveno
Aylin Dedeoglu

BWH/HMS
Jia-Ren Lin
Sandro Santagata
Peter Sorger

Former Lab
Po-Han Lin
James Coates
Aiko Nagayama
Siang Boon Koh
Sheng Sun

Funding
NIH: NCI;
DOD/BCRP
Gray Foundation
MGH ECOR Scholars
Ludwig Center at Harvard
Terri Brodeur Foundation
National Cancer Center
Breast Cancer Alliance

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