

Genetic Biomarkers

9p21 Loss Drives Immune-Cold, Checkpoint Inhibitor Resistance in HPV⁻ Head and Neck Squamous Cancer

Teresa Davoli, PhD
Assistant Professor
Institute for Systems Genetics
Department of Biochemistry and Molecular Pharmacology

Disclosure Information

I have the following financial relationships to disclose:

- ▶ **io9**

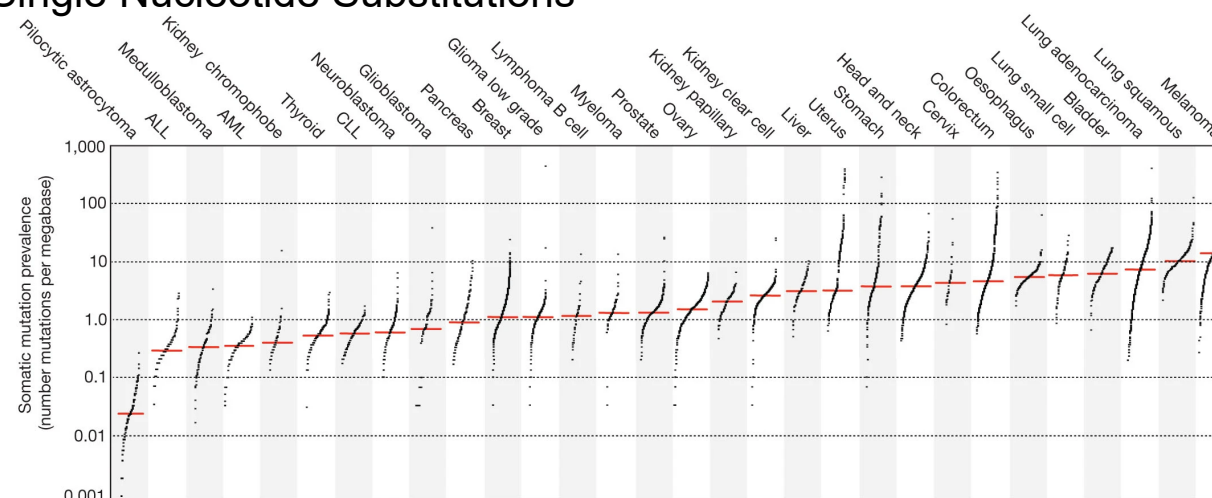
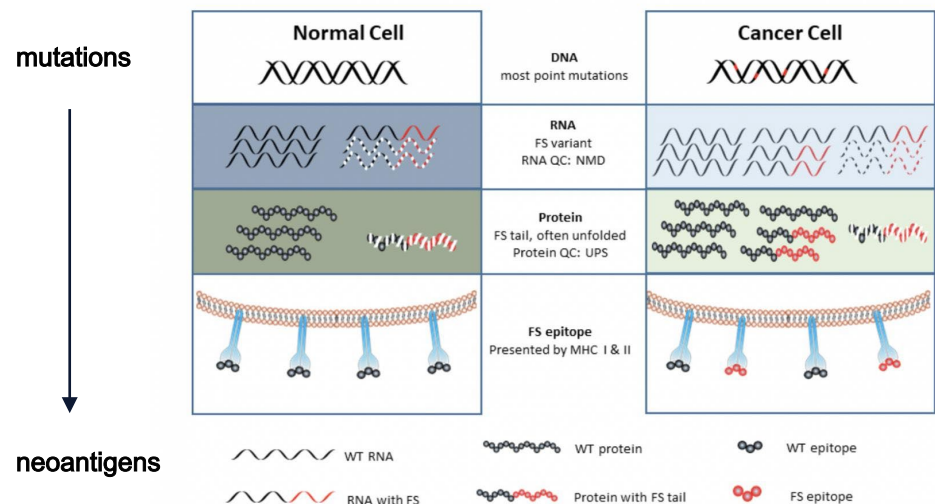
SAB member, equity holder in LLC

- ▶ **Inventor**

patents of 9p hotspots in cancer & immunotherapy

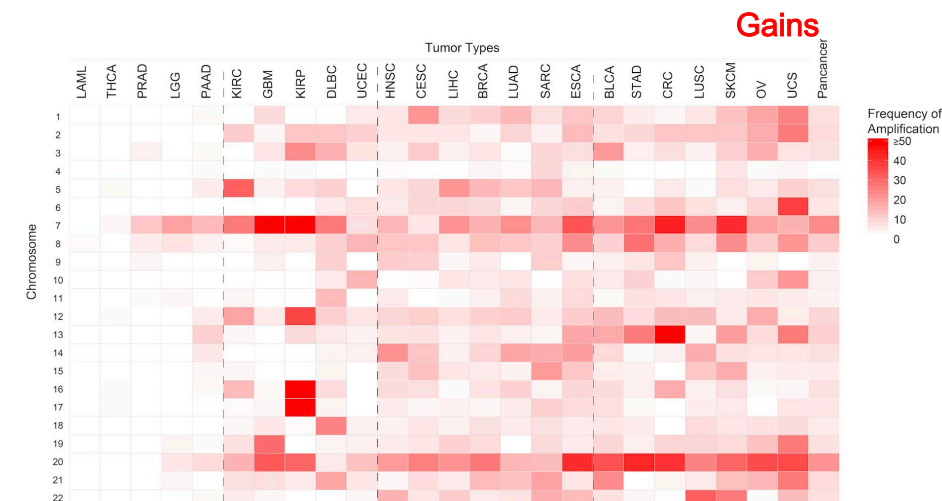
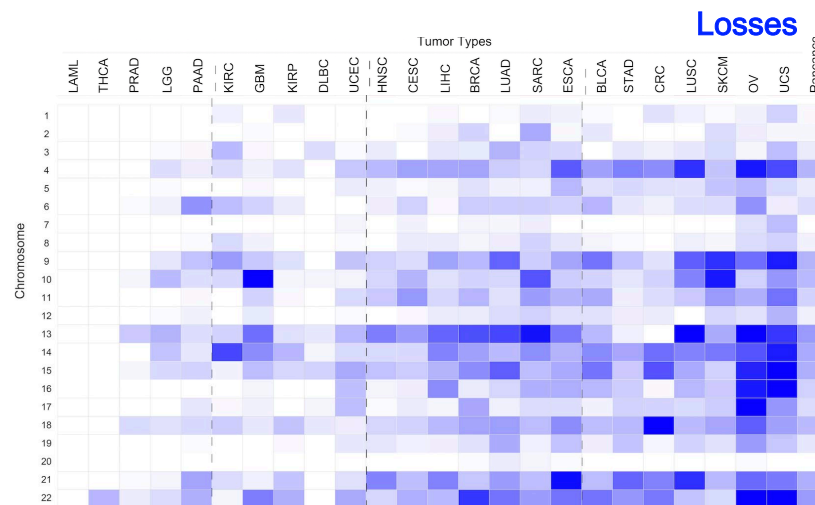
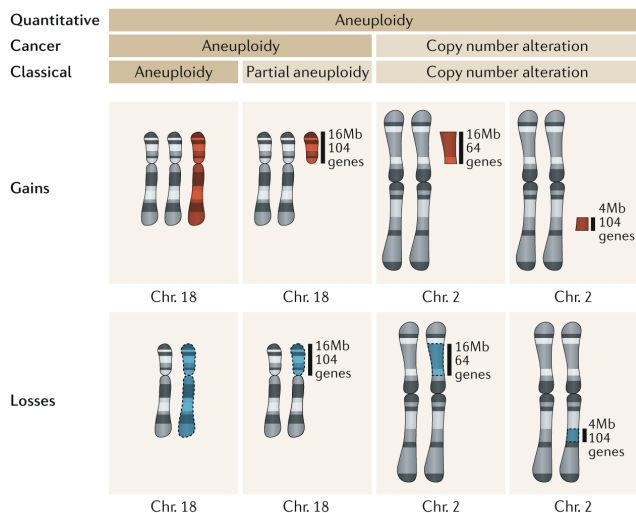
Two distinct genomic alterations in cancer

Point Mutations: Single Nucleotide Substitutions



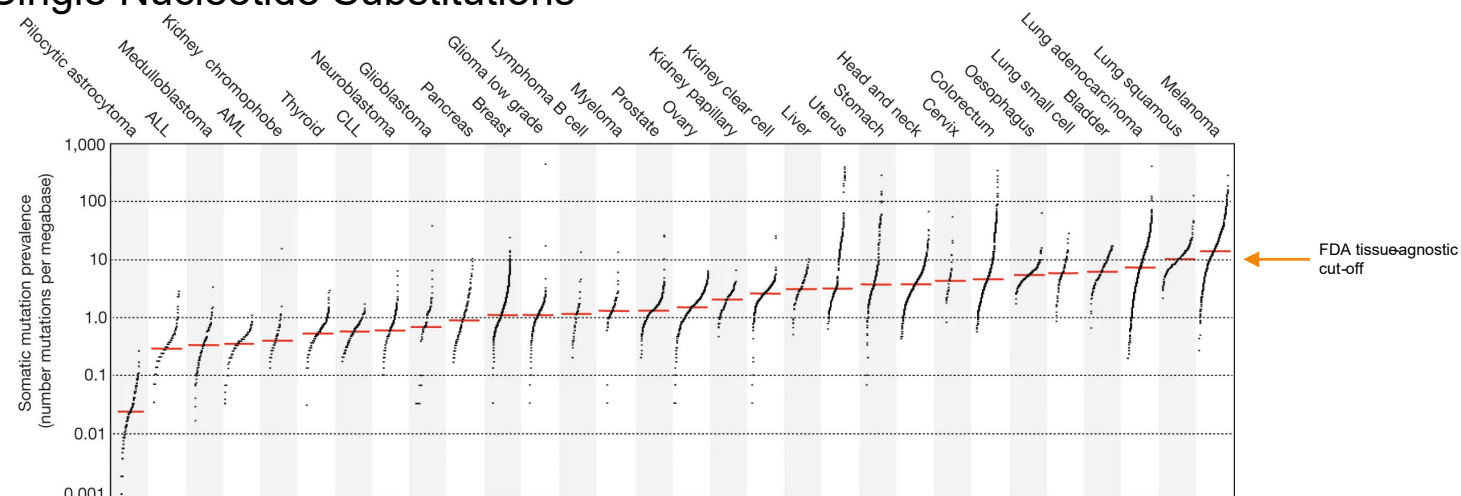
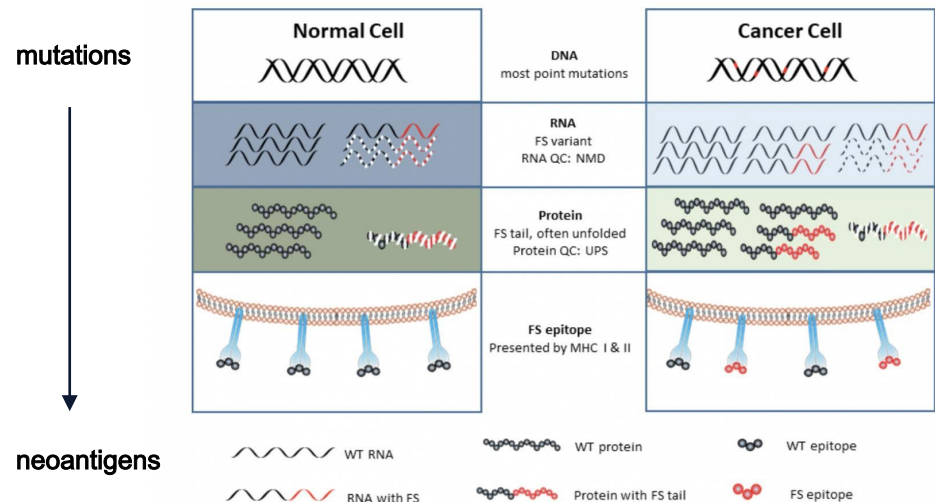
Alexandrov, *Nature* 2013

Somatic Copy Number Alterations (SCNAs) or Aneuploidy



Two distinct genomic alterations in cancer, IO effects

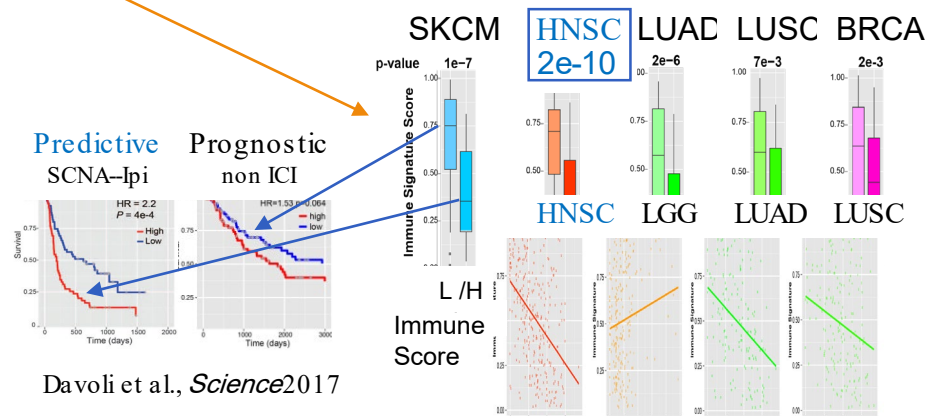
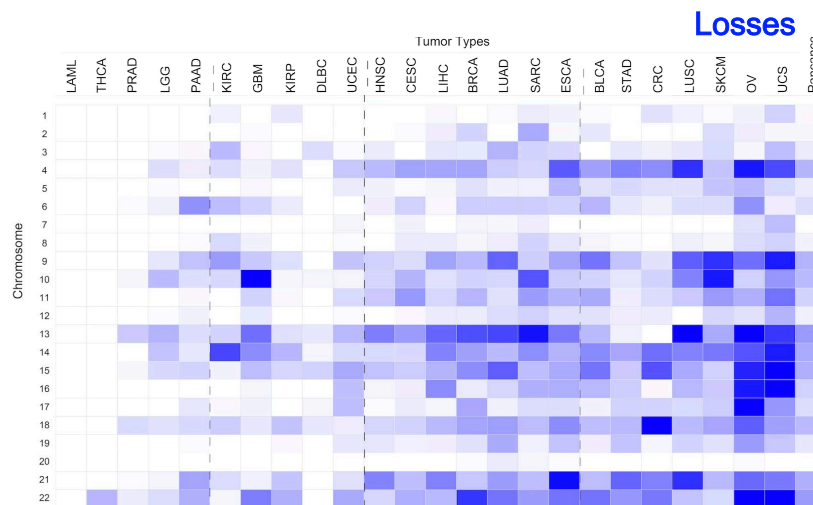
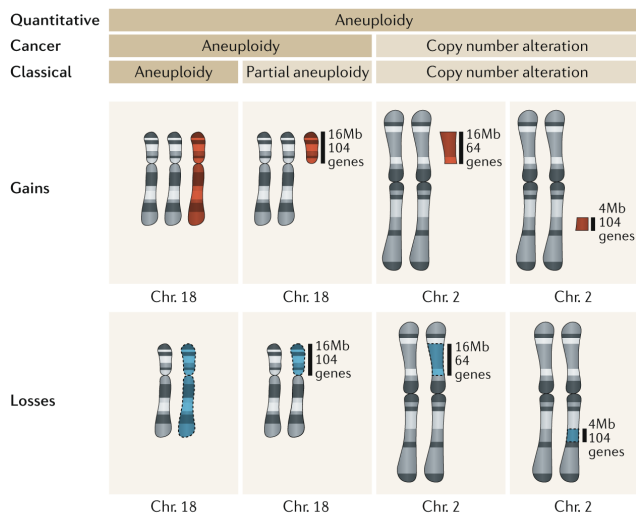
Point Mutations: Single Nucleotide Substitutions



Alexandrov, *Nature* 2013

Somatic Copy Number Alterations (SCNAs) or Aneuploidy

Overall SCNA Level → Immune Cold:
Strongest for HNSC (and PAAD)



HEAD AND NECK CANCER

6th worldwide; 900K/300K, case/death/yr

HPV⁺ rapidly increasing in the U.S.

HPV⁻ most common, lethal U.S., globally

Striking differences in etiology, biology, genetics, staging – ICI outcomes similar

ICI monotherapy ‘cure’, recurrent/metastatic: ~15%*

Precision therapy, predictive biomarkers

Identifying 85% Primary ICI resistance:

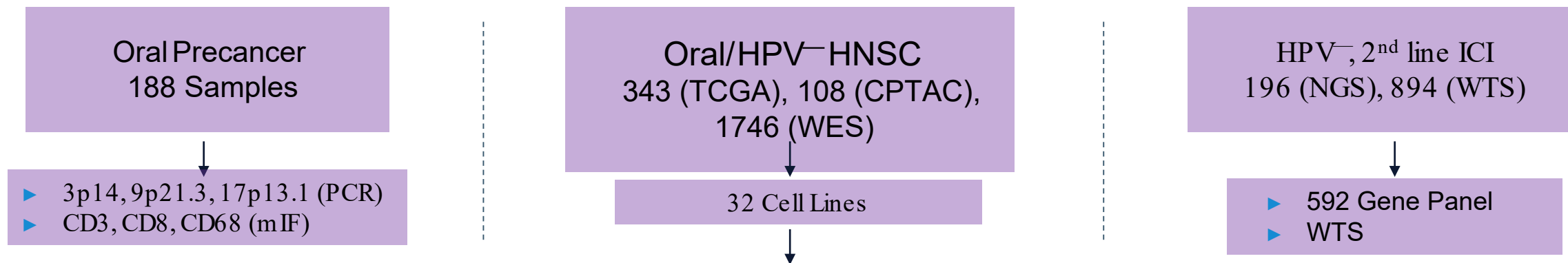
Huge financial and QOL costs
10% severe immune-related AEs
Limited survival time

Most PD-L1+ unresponsive (not CDX);
TMB not predictive

Hypothesis:

As with TKI/targeted, genetic lesions could provide predictive information.

* Lung, liver, cervical, bladder, esophageal, gastric, ovarian, TNBC, ST sarcoma, salivary, endometrial, cancer



Computational Analyses

- ▶ SCNA Level: Total # arms gain/lost; GISTIC2 arm/focal events
- ▶ Purity correction: Pathology & ABSOLUTE
- ▶ CD Thresholds: 35%, 50%, 65%; binary/continuous; CIBERSORT
- ▶ Linear Mixed Effect, logistic regression: stage, *TP53* 5- & 10-fold cross-validation, size effect; binary, continuous; multivariate, FDRs
- ▶ Immune Marker/metrics (e.g., IS), RNAseq
- ▶ Chr9p IO gene dissection studies

9p21 CN Loss Drives Immune-Cold in HPV[−] HNSC

PNAS

Proceedings of the
National Academy of Sciences
of the United States of America

Immune evasion in HPV[−] head and neck precancer-cancer transition is driven by an aneuploid switch involving chromosome 9p loss

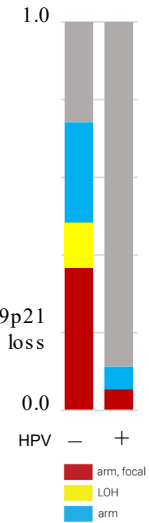
William, Zhao, Bianchi, et al., 2021 (May)
(sent for review November 21, 2020)

Independent of SCNA, immune metrics

Prediction of CD8+ (Pathology)				
	β -coefficient	p-value	size-effect	
3p14 Loss	-0.345	0.373	9.49%	binary
9p21.3 Loss	-1.285	0.0004	37.41%	binary
17p13.1 Loss	-0.486	0.301	11.02%	binary
Chr 7 Gain	-0.430	0.373	9.49%	binary
SCNA level	-0.592	0.002	32.59%	continuous

↓ Immune pathways/genes

- ▶ IFNG $P=1.2E-10$ IFNE $P=5.1e-10$ JAK2 $9E-16$
- ▶ NKG7 $5E-11$ CD4 $P=0.0001$; DC (LILRA4, ITGAE)
- ▶ SASP, TNFA via NF- κ B $P<0.0001$



9p21 CN Loss Drives Immune-Cold in HPV[−] HNSC

PNAS

Proceedings of the
National Academy of Sciences
of the United States of America

Immune evasion in HPV[−] head and neck precancer-cancer transition is driven by an aneuploid switch involving chromosome 9p loss

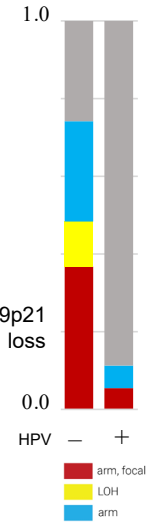
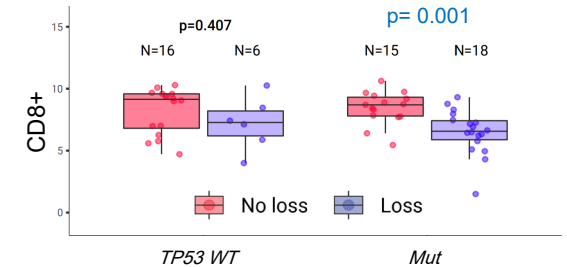
William, Zhao, Bianchi, et al., 2021 (May)
(sent for review November 21, 2020)

Independent of SCNA, immune metrics

Prediction of CD8+ (Pathology)				
	β -coefficient	p-value	size-effect	
3p14 Loss	-0.345	0.373	9.49%	binary
9p21.3 Loss	-1.285	0.0004	37.41%	binary
17p13.1 Loss	-0.486	0.301	11.02%	binary
Chr 7 Gain	-0.430	0.373	9.49%	binary
SCNA level	-0.592	0.002	32.59%	continuous

Immune pathways/genes

- IFNG $P=1.2E-10$ IFNE $P=5.1e-10$ JAK2 $9E-16$
- NKG7 $5E-11$ CD4 $P=.0001$; DC (LILRA4, ITGAE)
- SASP, TNFA via NF- κ B $P<0.0001$
- 9p21 LOH, TP53 epistatic (Stage I-II)



9p21 CN Loss Drives Immune-Cold in HPV[−] HNSC

PNAS

Proceedings of the National Academy of Sciences of the United States of America

Immune evasion in HPV[−] head and neck precancer-cancer transition is driven by an aneuploid switch involving chromosome 9p loss

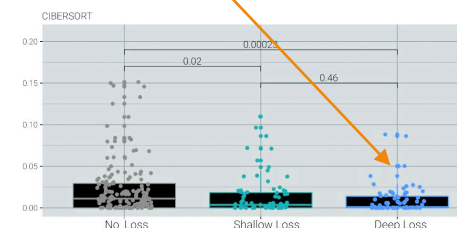
William, Zhao, Bianchi, et al., 2021 (May)
(sent for review November 21, 2020)

Independent of SCNA, immune metrics

Prediction of CD8+ (Pathology)				
	β -coefficient	p-value	size-effect	
3p14 Loss	-0.345	0.373	9.49%	binary
9p21.3 Loss	-1.285	0.0004	37.41%	binary
17p13.1 Loss	-0.486	0.301	11.02%	binary
Chr 7 Gain	-0.430	0.373	9.49%	binary
SCNA level	-0.592	0.002	32.59%	continuous

↓ Immune pathways/genes

- IFNG $P=1.2E-10$ IFNE $P=5.1e-10$ JAK2 $9E-16$
- NKG7 $5E-11$ CD4 $P=.0001$; DC (LILRA4, ITGAE)
- SASP, TNFA via NF- κ B $P<0.0001$
- CD8/9p21 deep loss:



Pan-cancer: ↓ / ↑ immune activation/suppression

- 9p21.3 HD $\approx$ LOH($\approx$ arm event) $>$ WT

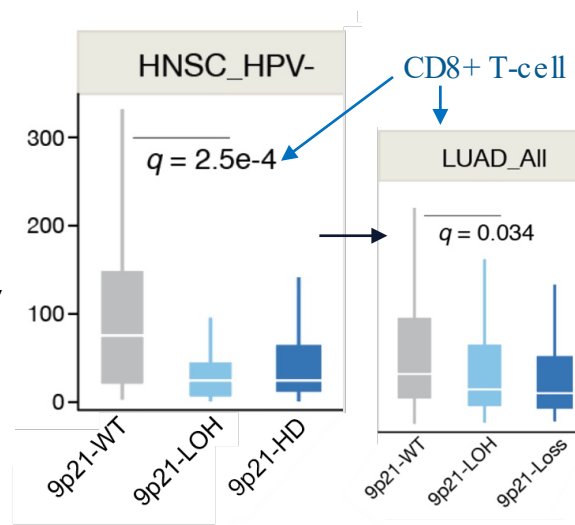
nature communications

2021 (September)

9p21 loss confers a cold tumor immune microenvironment and primary resistance to immune checkpoint therapy

Guangchun Han^{1,17}, Guoliang Yang^{2,17}, Dapeng Hao¹, Yang Lu³, Kyaw Thein⁴, Benjamin S. Simpson⁵, Jianfeng Chen², Ryan Sun⁶, Omar Alhalabi², Ruiping Wang¹, Minghao Dang¹, Enyu Dai¹, Shaojun Zhang¹, Fengqi Nie², Shuangtao Zhao¹, Charles Guo⁷, Ameer Hamza⁷, Bogdan Czerniak⁷, Chao Cheng⁸, Arlene Siefker-Radtke², Krishna Bhat⁹, Andrew Futreal¹, Guang Peng¹⁰, Jennifer Wargo¹¹, Weiyi Peng¹², Humam Kadara⁹, Jaffer Ajani¹³, Charles Swanton^{14,15}, Kevin Litchfield^{5,15}, Jordi Rodon Ahnert^{1,4,18}, Jianjun Gao^{2,18} & Linghua Wang^{1,16,18}

(received: 23 November 2020)



9p21 CN Loss Drives Immune-Cold in HPV⁻ HNSC

PNAS

Proceedings of the
National Academy of Sciences
of the United States of America

Immune evasion in HPV⁻ head and neck precancer-cancer transition is driven by an aneuploid switch involving chromosome 9p loss

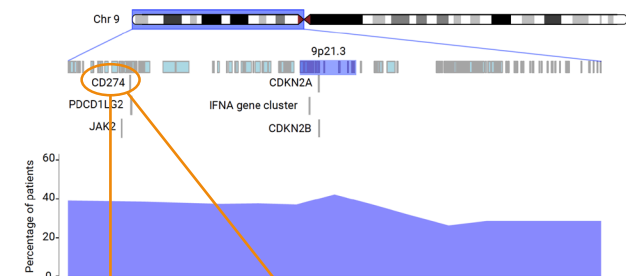
William, Zhao, Bianchi, et al., 2021 (May)
(sent for review November 21, 2020)

Independent of SCNA, immune metrics

Prediction of CD8+ (Pathology)				
	β -coefficient	p-value	size-effect	
3p14 Loss	-0.345	0.373	9.49%	binary
9p21.3 Loss	-1.285	0.0004	37.41%	binary
17p13.1 Loss	-0.486	0.301	11.02%	binary
Chr 7 Gain	-0.430	0.373	9.49%	binary
SCNA level	-0.592	0.002	32.59%	continuous

Immune pathways/genes

- IFNG $P=1.2E-10$ IFNE $P=5.1e-10$ JAK2 $9E-16$
- NKG7 $5E-11$ CD4 $P=.0001$; DC (LILRA4, ITGAE)
- SASP, TNFA via NF- κ B $P<0.0001$
- 9p21/9p24: $P=1.8E-57$ (arm, LOH); $P=2E-07$ (LOH)



Pan-cancer: ↓ / ↑ immune activation/suppression

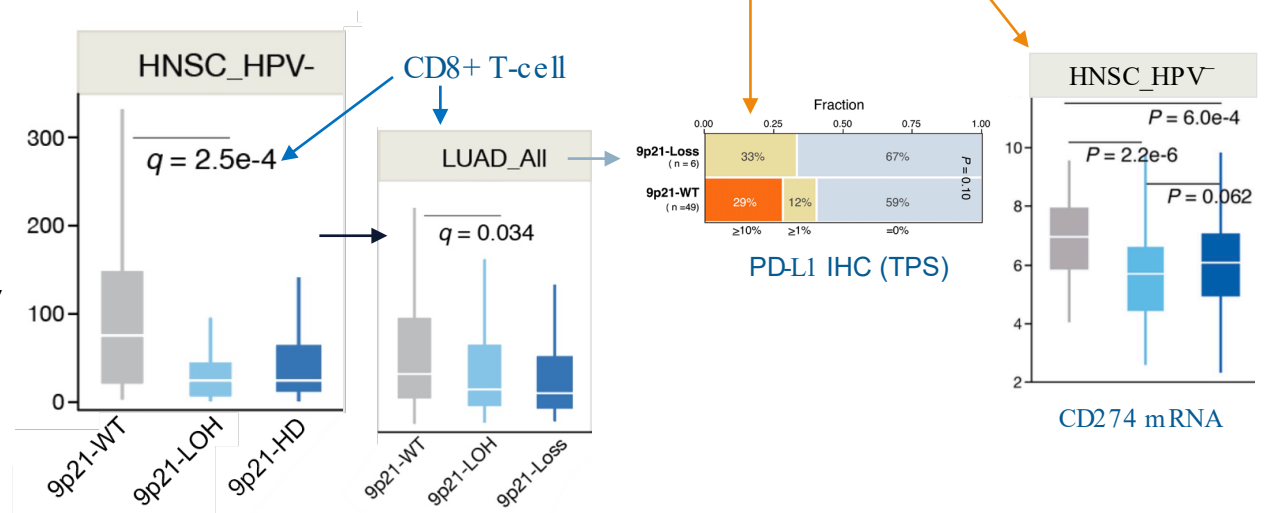
nature
communications

2021 (September)

9p21 loss confers a cold tumor immune microenvironment and primary resistance to immune checkpoint therapy

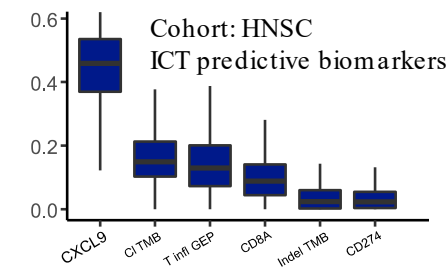
Guangchun Han^{1,17}, Guoliang Yang^{2,17}, Dapeng Hao¹, Yang Lu³, Kyaw Thein⁴, Benjamin S. Simpson⁵, Jianfeng Chen², Ryan Sun⁶, Omar Alhalabi², Ruiping Wang¹, Minghao Dang¹, Enyu Dai¹, Shaojun Zhang¹, Fengqi Nie², Shuangtao Zhao¹, Charles Guo⁷, Ameer Hamza⁷, Bogdan Czerniak⁷, Chao Cheng⁸, Arlene Siefker-Radtke², Krishna Bhat⁹, Andrew Futreal¹, Guang Peng¹⁰, Jennifer Wargo¹¹, Weiyi Peng¹², Humam Kadara⁹, Jaffer Ajani¹³, Charles Swanton^{14,15}, Kevin Litchfield^{5,15}, Jordi Rodon Ahnert^{1,4,18}, Jianjun Gao^{2,18} & Linghua Wang^{1,16,18}

(received: 23 November 2020)



9p21 CN Loss Drives Immune-Cold in HPV⁻ HNSC: CXCL9/10

CXCL9/10 (2021)



Litchfield et al., *Cell*, Feb. 2021

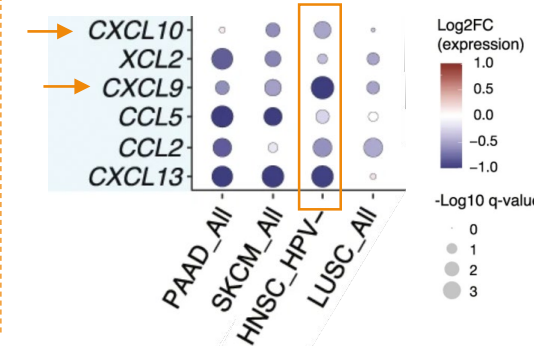
9p21 Loss → CXCL9/10 depletion

	CXCL9	β -coefficient	q-value	
→ 9p21.3 Loss		-1.463	0.0001	binary
17p13.1 Loss		-0.570	0.242	binary
SCNA level		-0.354	0.049	continuous

▶ CXCL9/10: **FDR=1.46E-15/1.44E-20**

→ CXCL9/10 (4q21.1): **$\rho=1.0$ (DNA), $\rho=0.86$ (RNA)**

William et al., *PNAS* May 2021 (Zhao et al., in press & unpublished)



Han et al., *Nat. Commun.*, Sept. 2021

Prediction of CD8+ (Pathology)

	β -coefficient	p-value	size-effect	
3p14 Loss	-0.345	0.373	9.49%	binary
→ 9p21.3 Loss	-1.285	0.0004	37.41%	binary
17p13.1 Loss	-0.486	0.301	11.02%	binary
Chr 7 Gain	-0.430	0.373	9.49%	binary
SCNA level	-0.592	0.002	32.59%	continuous

PNAS

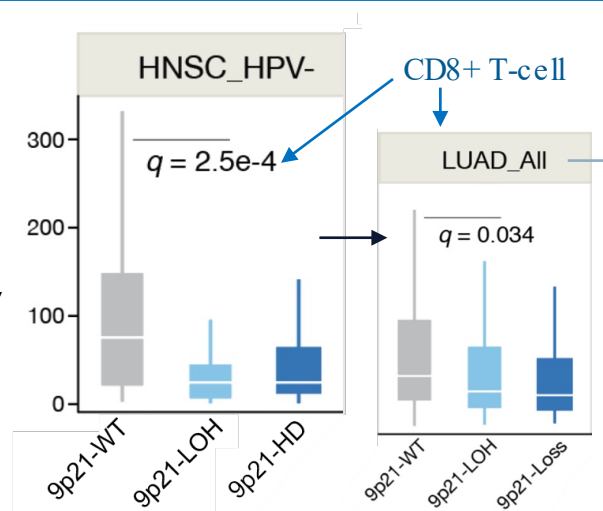
Proceedings of the
National Academy of Sciences
of the United States of America

Immune evasion in HPV⁻ head and neck precancer-cancer transition is driven by an aneuploid switch involving chromosome 9p loss

William, Zhao, Bianchi, et al., 2021 (May)
(sent for review **November 21, 2020**)

Independent of SCNA, immune metrics

Pan-cancer: ↓ / ↑ immune activation/suppression



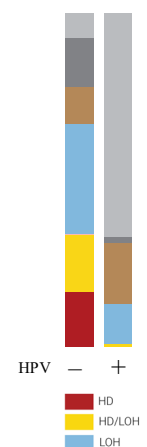
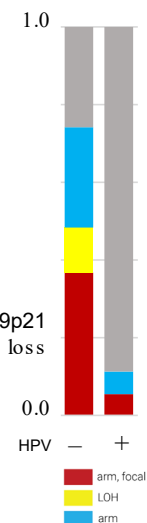
nature
communications

2021 (September)

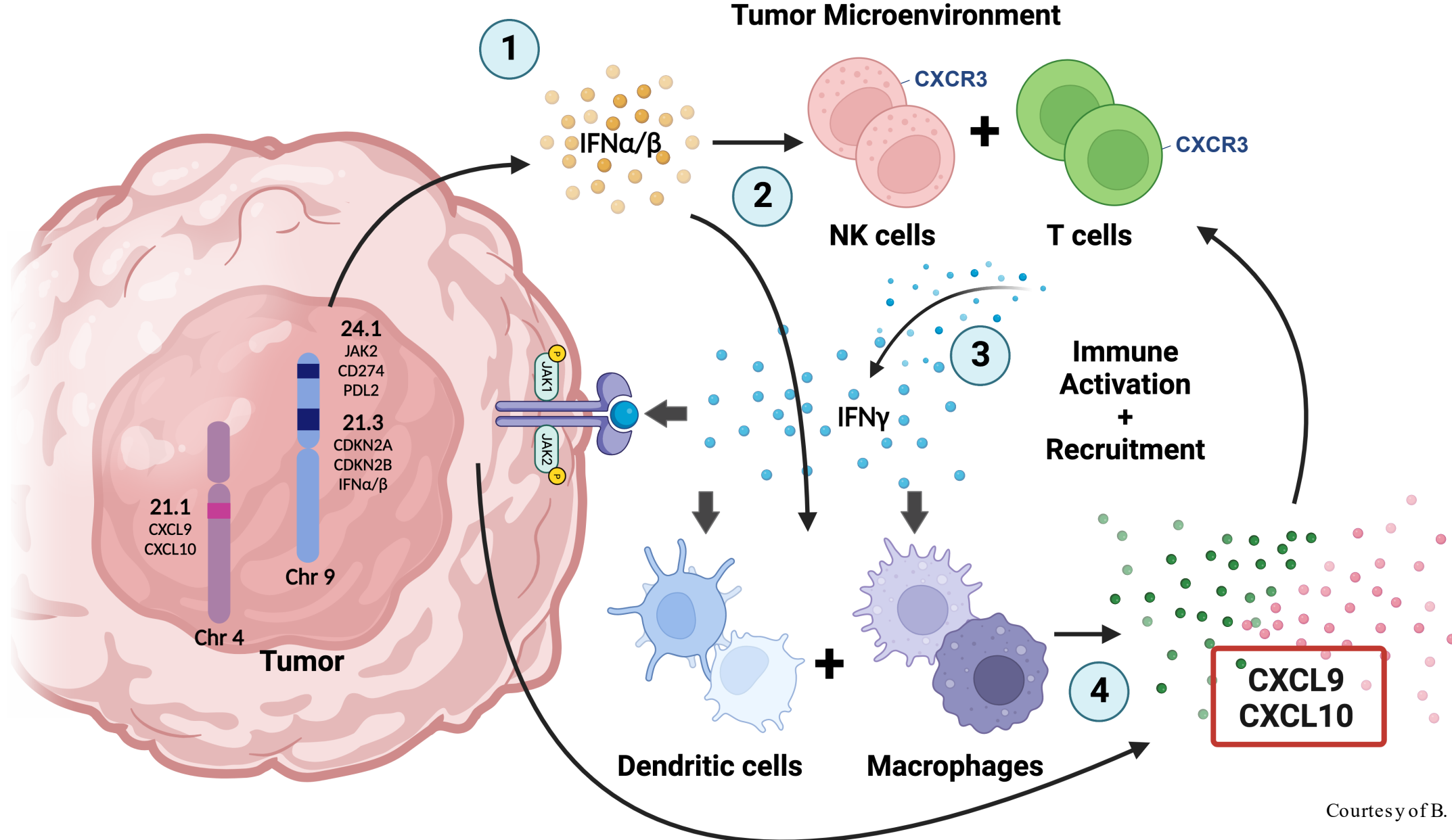
→ 9p21 loss confers a cold tumor immune microenvironment and primary resistance to immune checkpoint therapy

Guangchun Han^{1,17}, Guoliang Yang^{2,17}, Dapeng Hao¹, Yang Lu³, Kyaw Thein⁴, Benjamin S. Simpson⁵, Jianfeng Chen², Ryan Sun⁶, Omar Alhalabi², Ruiping Wang¹, Minghao Dang¹, Enyu Dai¹, Shaojun Zhang¹, Fengqi Nie², Shuangtao Zhao¹, Charles Guo⁷, Ameer Hamza⁷, Bogdan Czerniak⁷, Chao Cheng⁸, Arlene Siefker-Radtke², Krishna Bhat⁹, Andrew Futreal¹, Guang Peng¹⁰, Jennifer Wargo¹¹, Weiyi Peng¹², Humam Kadara⁹, Jaffer Ajani¹³, Charles Swanton^{14,15}, Kevin Litchfield^{5,15}, Jordi Rodon Ahnert^{1,4,18}, Jianjun Gao^{2,18} & Linghua Wang^{1,16,18}

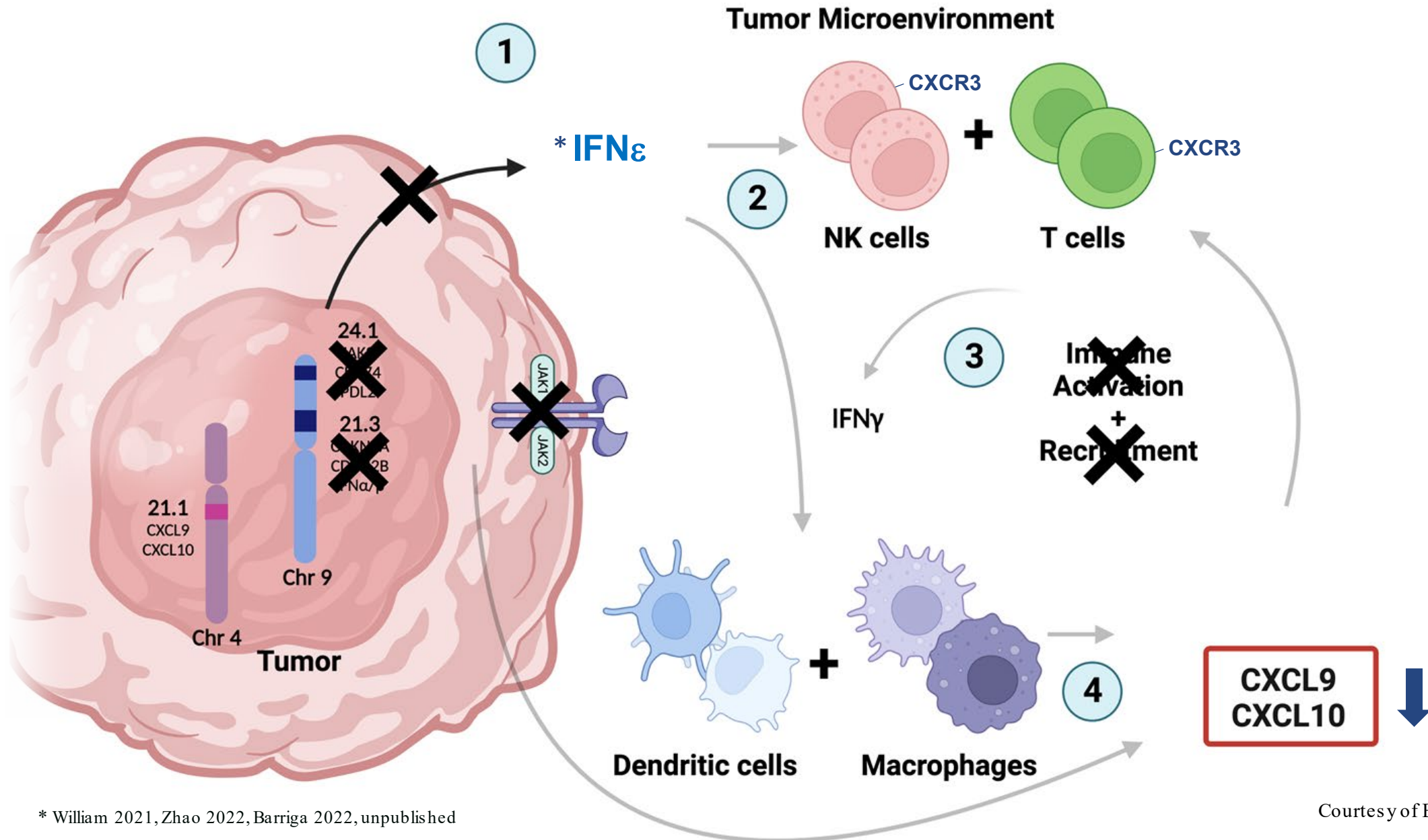
(received: **23 November 2020**)



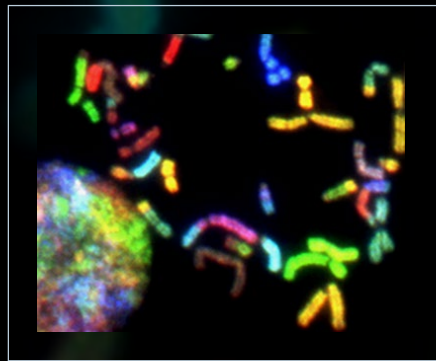
IFNs orchestrate immune activation in the tumor microenvironment



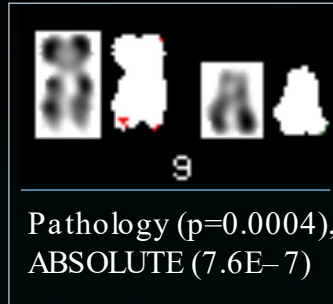
The potential mechanism of 9p loss-mediated immunosuppression via cytokines



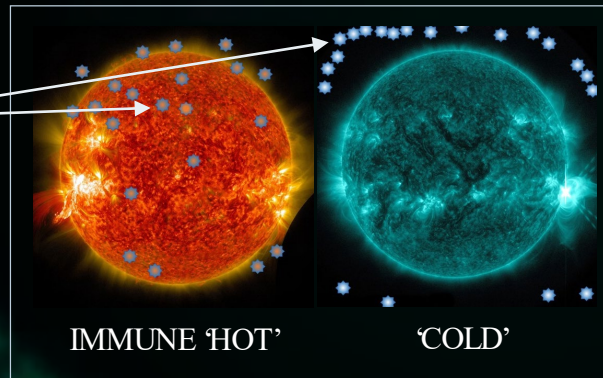
9p copy number loss/immune cold association: > overall SCNA level & all other arm events; immune gene hotbed



SCNA level
($p=0.002^*$)



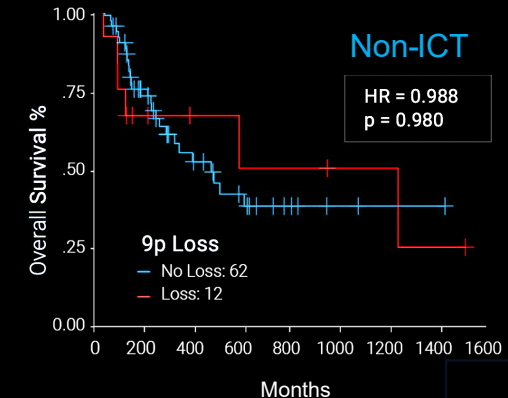
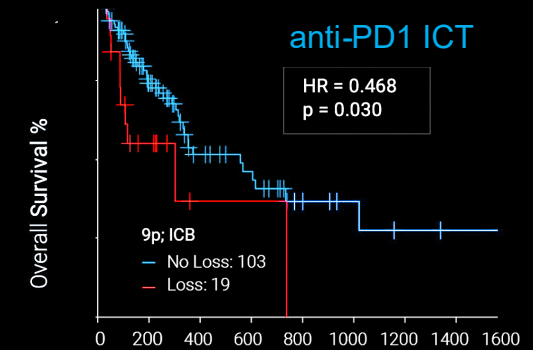
CD8⁺ T Cells



Two types of solid tumors re: immunotherapy

HPV⁻ HNSC

ICT Predictive Biomarker



*c/w Davoli et al., *Science*, 2017

William et al. *PNAS* 2021; Zhao et al. in press

Immune Checkpoint Therapy (ICT) in HPV⁺ HNSC: recurrent, precancer disease

9p21 deletion drives antiPD-1 ICT resistance

7 9p21/IO publications
since May 2021

PNAS

Proceedings of the
National Academy of Sciences
of the United States of America

nature
communications

Trends in
Immunology

npj Precision Oncology

nature

Ebot, et al;
Barriga, et. al*

PNAS

Proceedings of the
National Academy of Sciences
of the United States of America

Hanna et al.,
2022 in prep

William et al.

Han, et al.

Alhalabi, et al.

Spiliopoulou, et al.

Ebot, et al;
Barriga, et. al*

Zhao, et al.

5/2021

9/2021

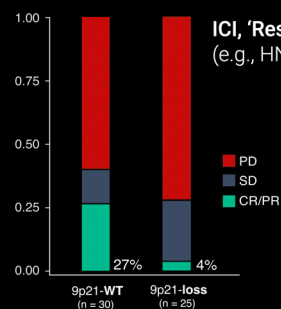
1/2022

4/2022

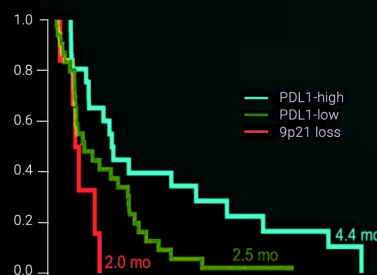
6/2022

9/2022

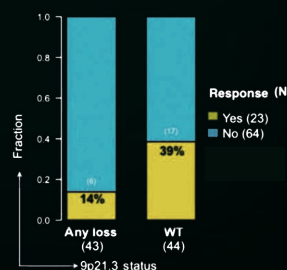
11/2022



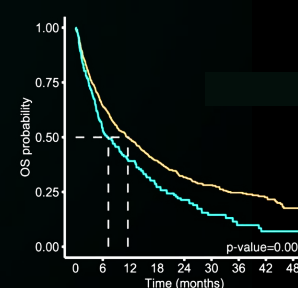
Mixed tumor types



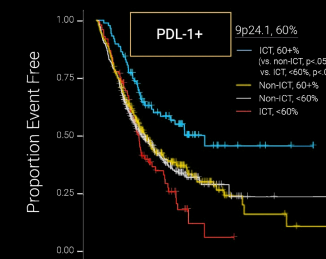
LUAD



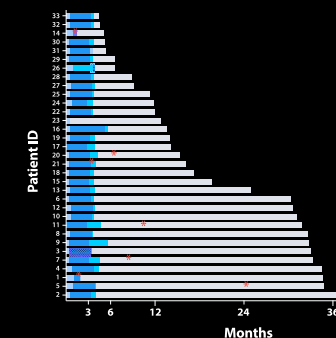
Mixed



NSCLC



HPV⁺ HNSC



HPV⁻ aggressive oral
pre-cancer (PVL)

* Experimental, *Nat. Cancer*, 11/22

Acknowledgements & Collaborators

NYU

Davoli Lab

Xin Zhao
Joy J. Bianchi
Pan Cheng

UCSD

Scott Lippman
Hannah Carter
Ludmil B. Alexandrov, PCGA
Don W. Cleveland
J. Silvio Gutkind
Ezra Cohen
Webster Cavenee



UCLA

Steven M. Dubinett

Caris Life

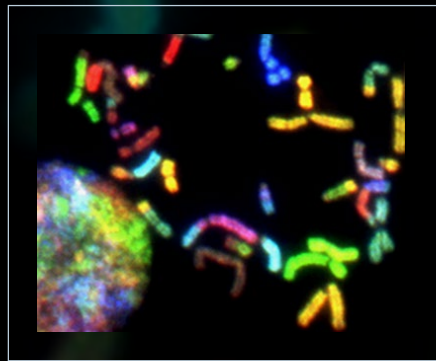
Jim P. Abraham
Daniel McGee
David B. Spetzler

MDACC

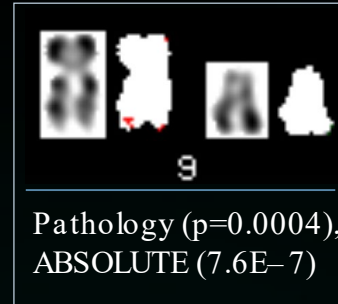
William N. William, Jr.
Heather Y. Lin
J. Jack Lee

NCI P30 CA023100P01 CA106451,
P50 CA097007, R01 DE026644

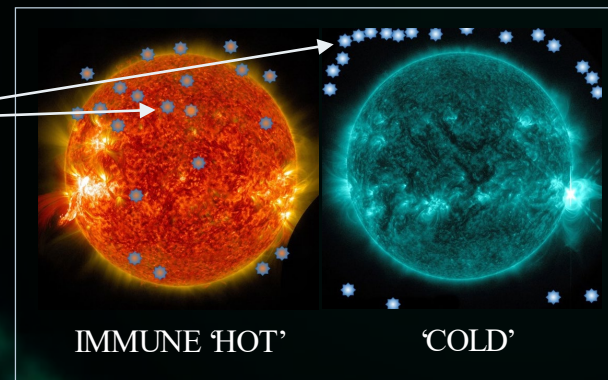
9p copy number loss/immune cold association: > overall SCNA level & all other arm events; immune gene hotbed



SCNA level
($p=0.002^*$)



CD8⁺ T Cells

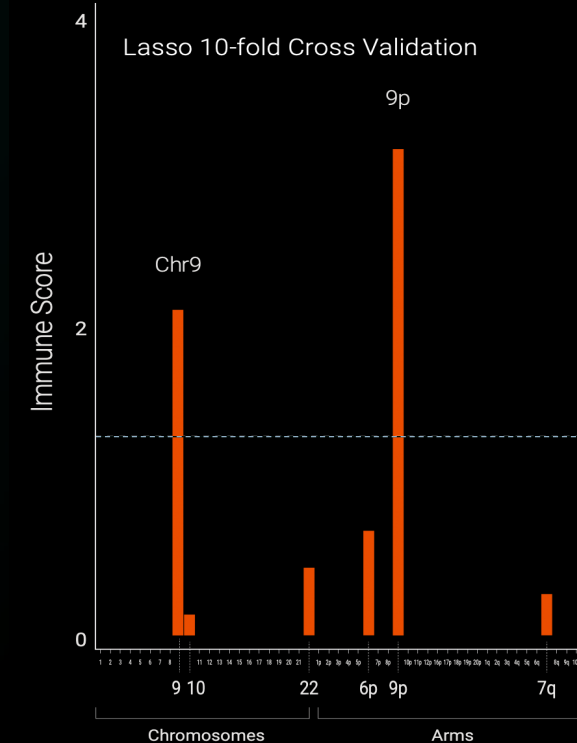


Two types of solid tumors re: immunotherapy

William et al. *PNAS* 2021; Zhao et al. in press

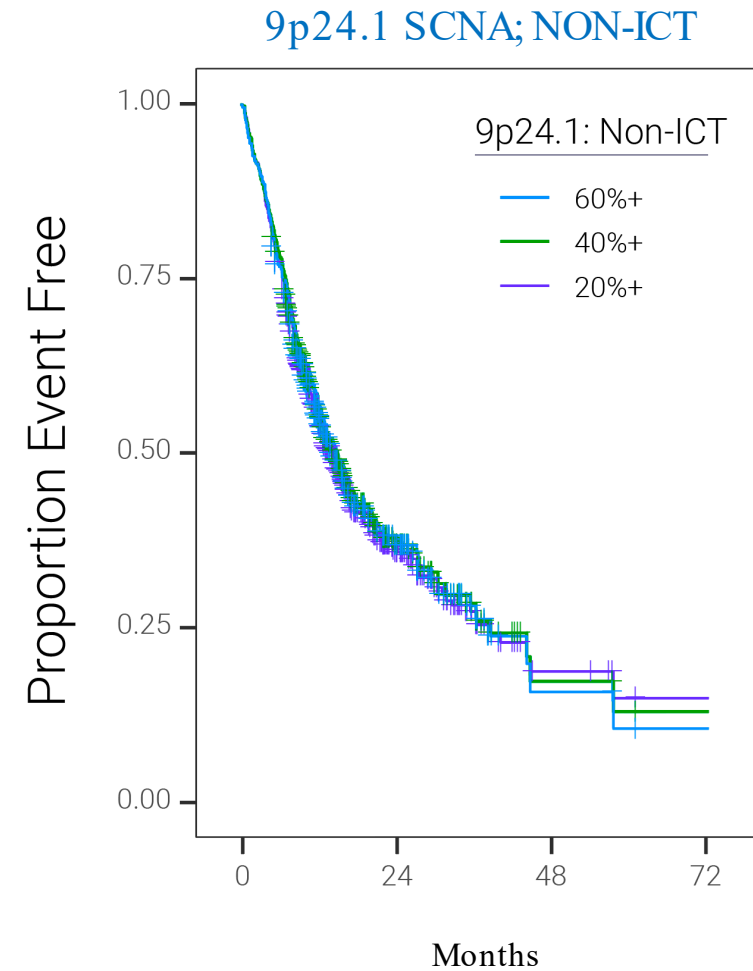
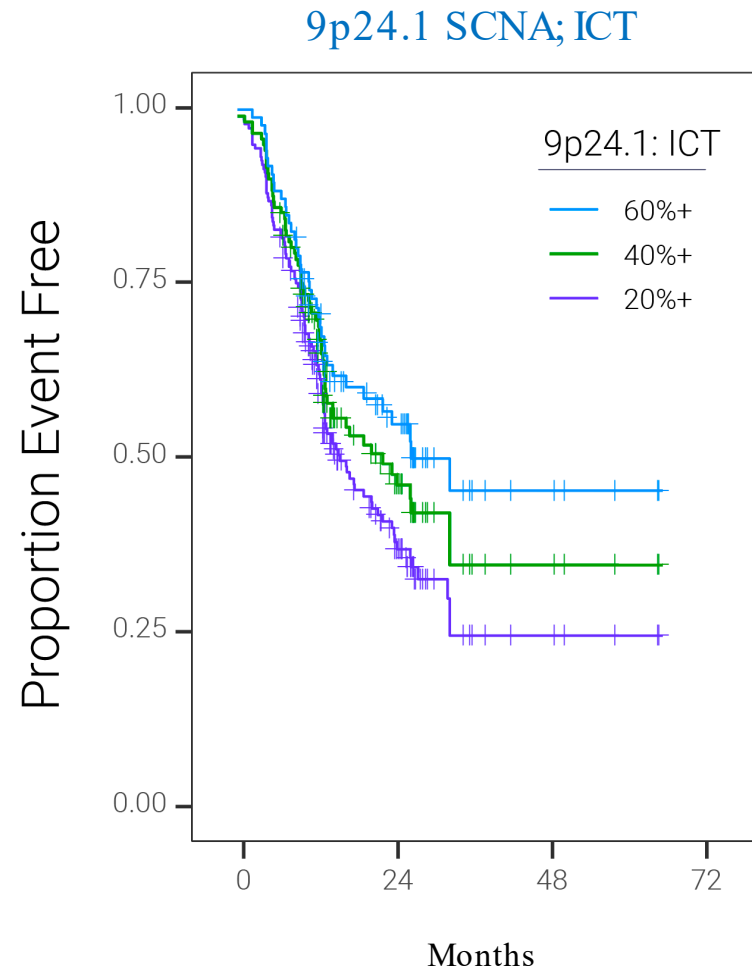
Pathology**			
	β -coefficient	p-value	
9p21.3 Loss (focal; n=40)	-0.831	0.061	binary
9p Loss (arm; n=80)	-1.795	0.0002	binary
9p21 Loss (focal, arm)	-1.285	0.0004	binary

** ABSOLUTE (focal / arm); $P=0.051$ / $=0.00004$



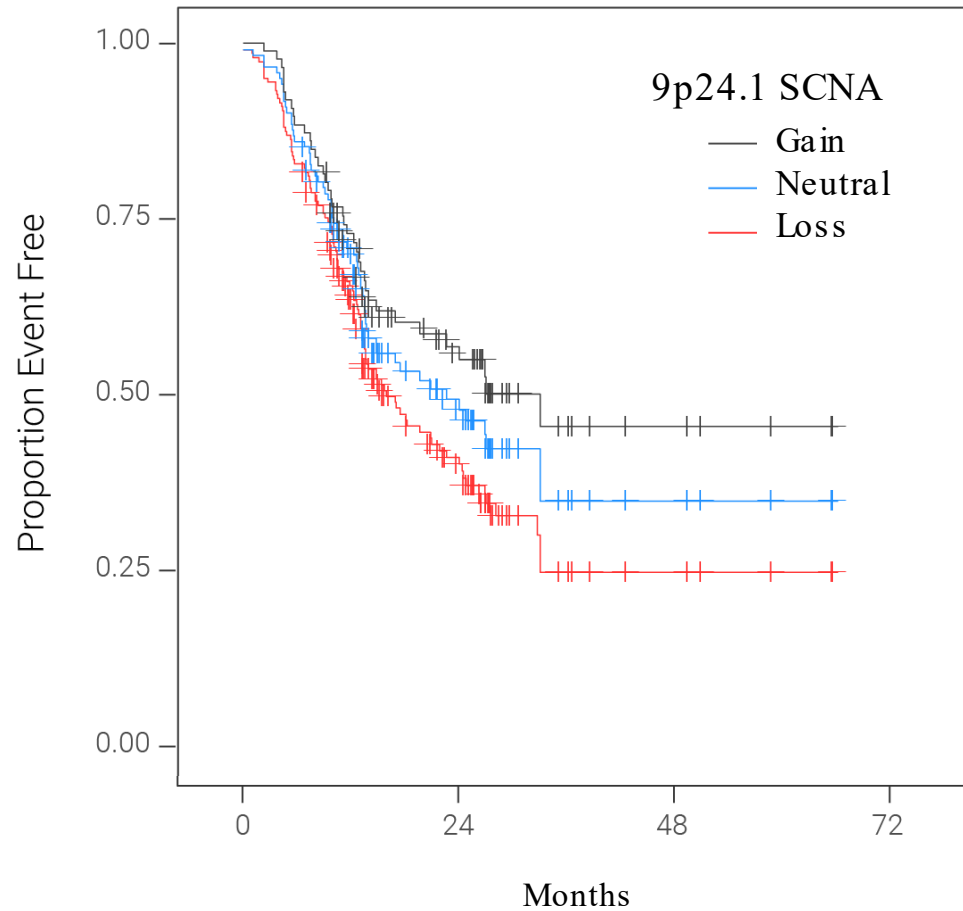
*c/w Davoli et al., *Science*, 2017

9p24.1 Loss (or gain) predicts bad (or good) survival after immunotherapy

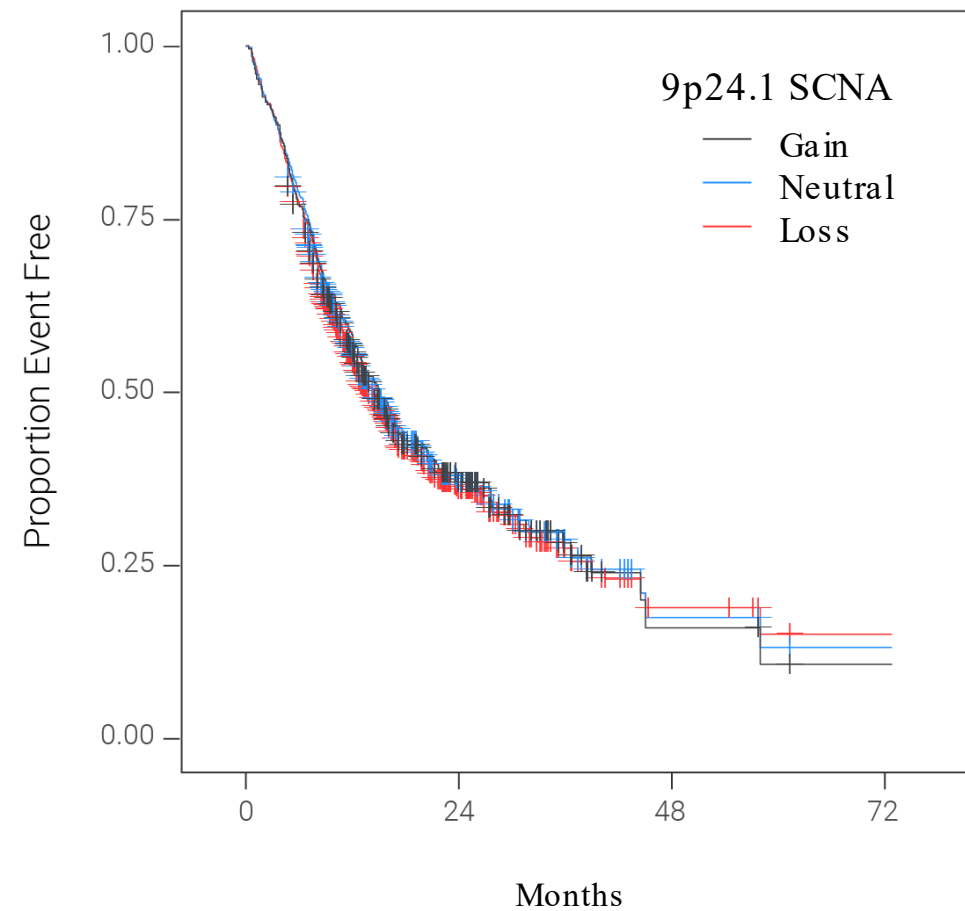


9p24.1 Loss (or gain) predicts bad (or good) survival after immunotherapy

9p24.1 SCNA; ICT

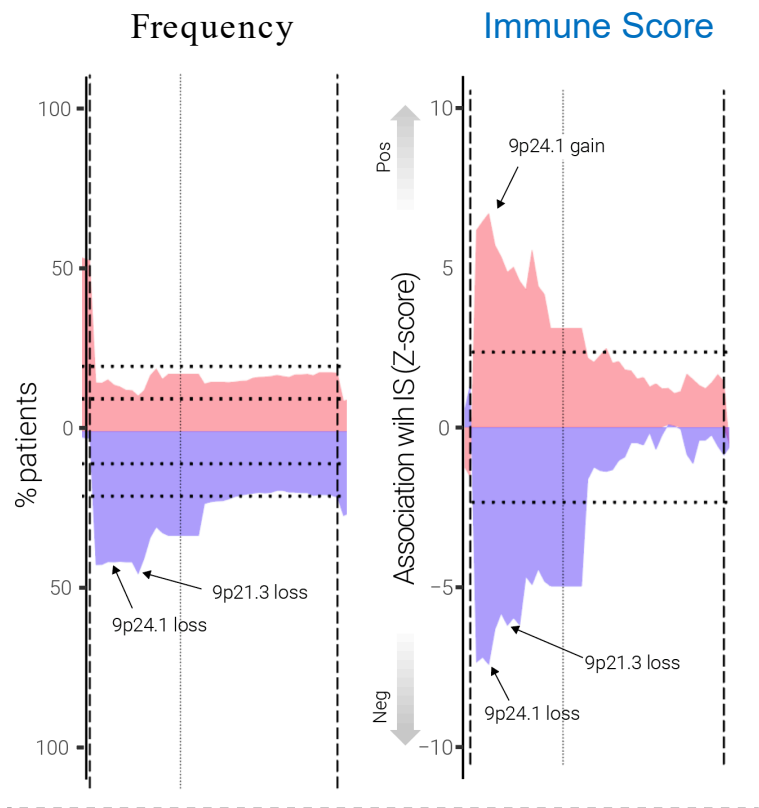


9p24.1 SCNA; NON-ICT



Immune Evasion and 9p across 11 tumor types: Tissue Specificity

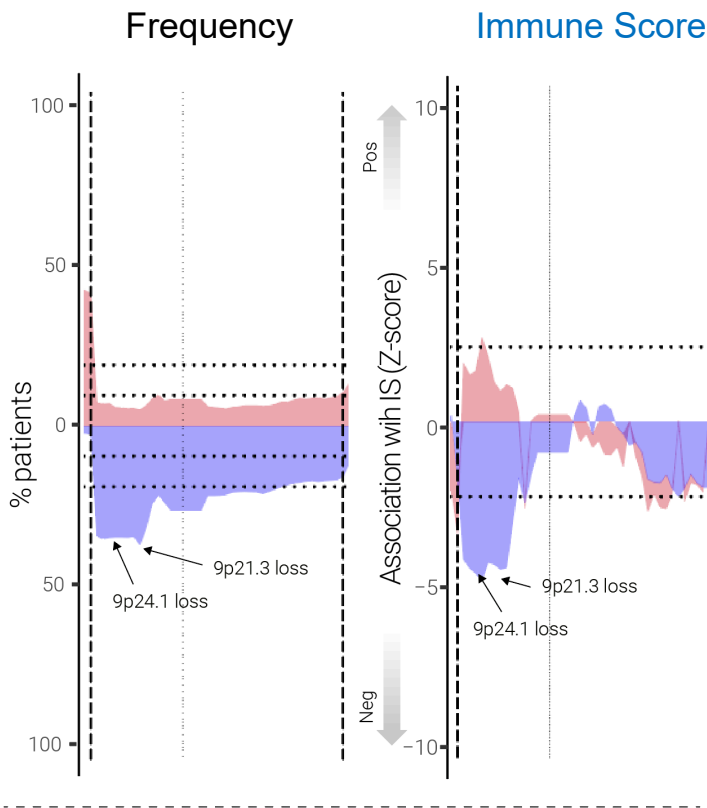
Squamous Cancer (n=1176)



Prediction of IS

	β -coefficient	q-value	
9p24.1 gain	1.26	<0.0001	binary
SCNA level	-0.83	<0.0001	continuous

Adenocarcinoma (n=1229)



	β -coefficient	q-value	
9p24.1 gain	0.38	0.18	binary
SCNA level	-1.04	<0.0001	continuous