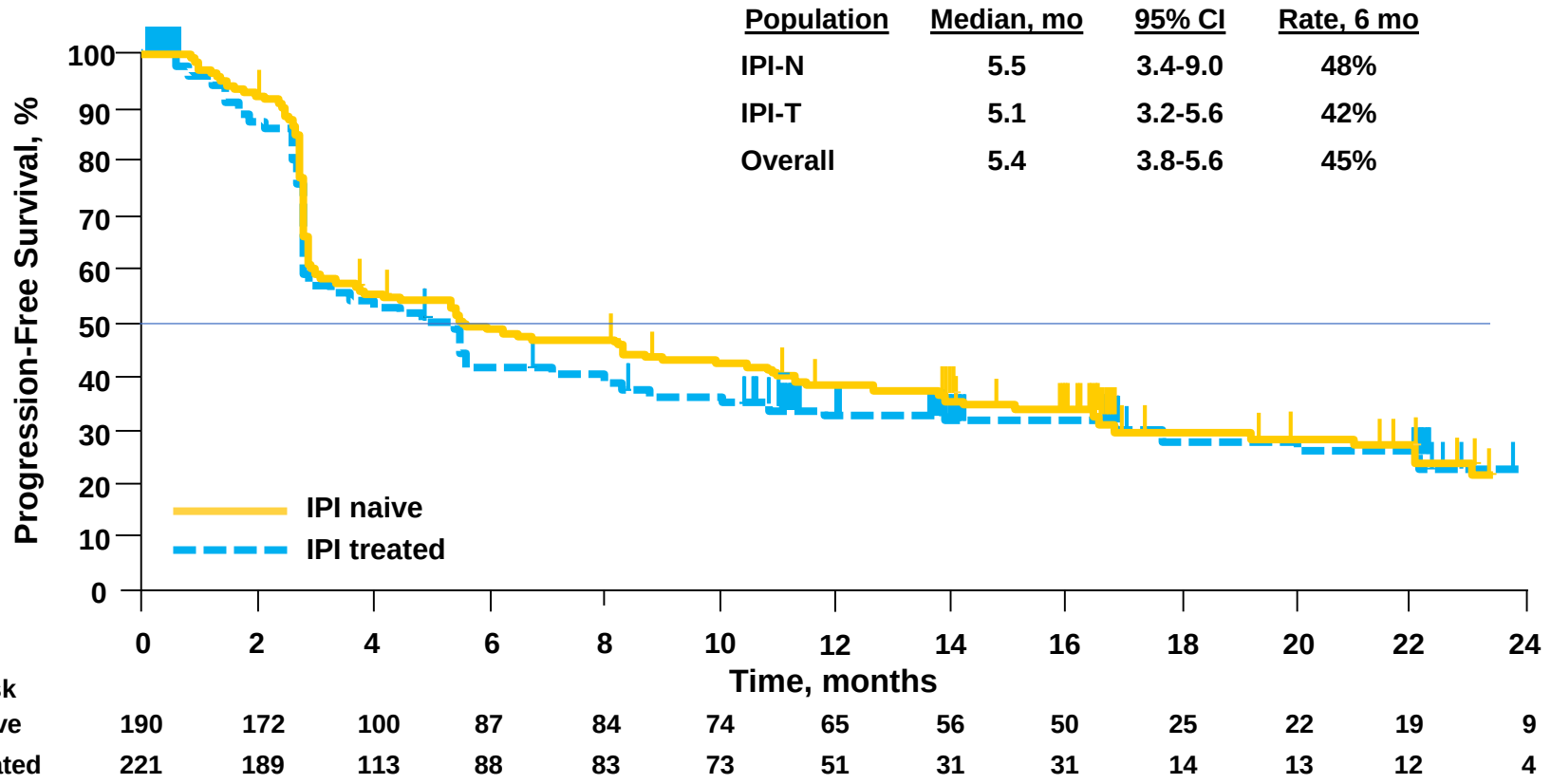
Immunotherapy Response and Biomarkers

Adil Daud MBBS
Professor of Medicine and Dermatology
Director Melanoma Program
University of California, San Francisco

Keynote 001: Kaplan-Meier Estimate of PFS

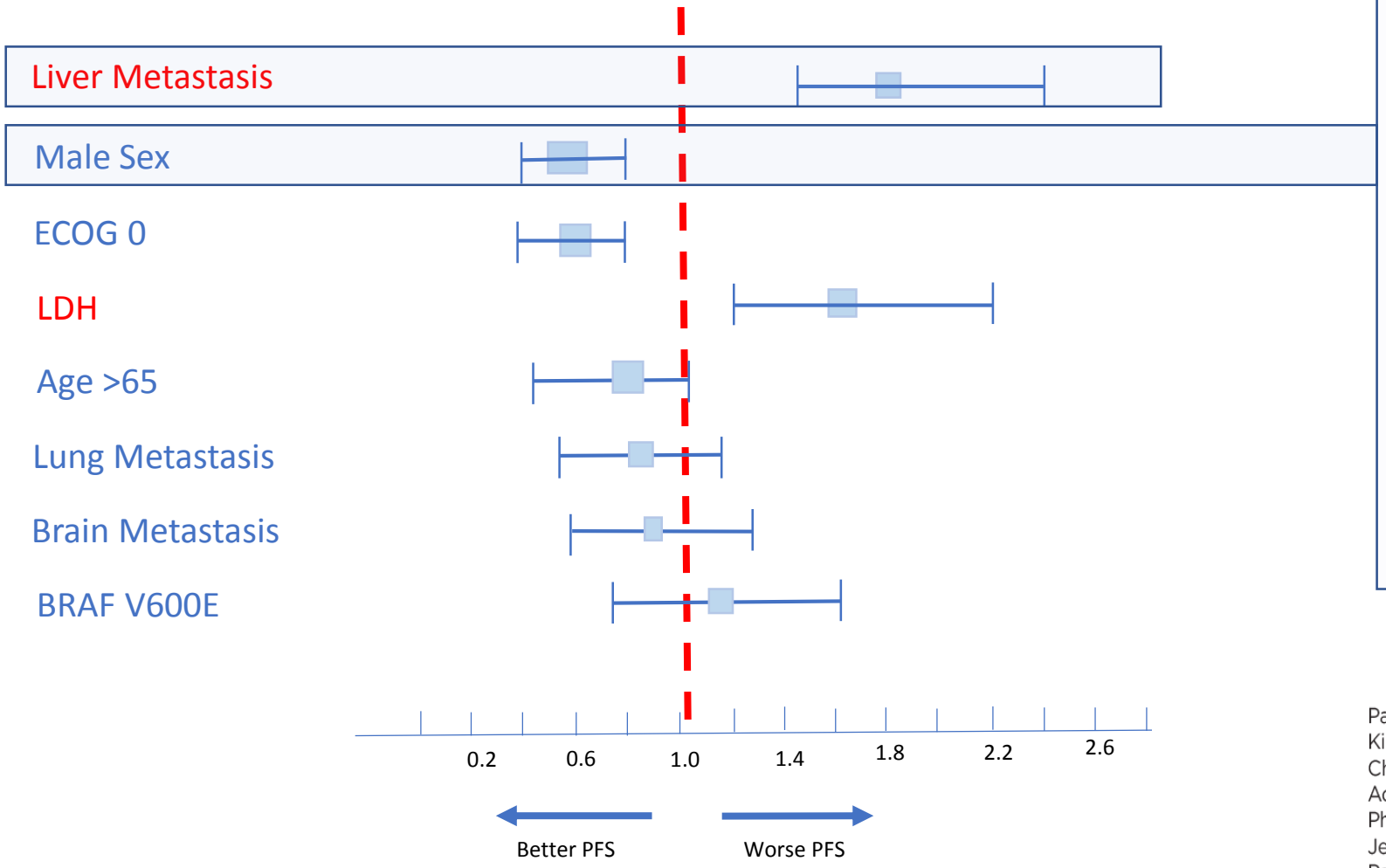


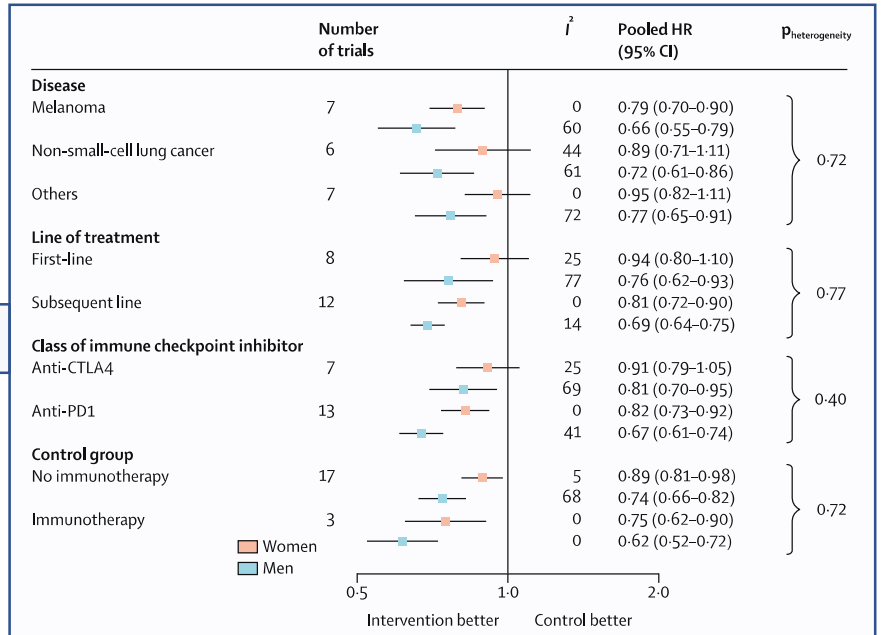
Population	Median, mo	95% CI	Rate, 6 mo
IPI-N	5.5	3.4-9.0	48%
IPI-T	5.1	3.2-5.6	42%
Overall	5.4	3.8-5.6	45%

n at risk													
IPI naive	190	172	100	87	84	74	65	56	50	25	22	19	9
IPI treated	221	189	113	88	83	73	51	31	31	14	13	12	4

Analysis cut-off date: April 18, 2014.

Liver Metastasis and Treatment Outcome with Anti-PD-1 Monoclonal Antibody in Patients with Melanoma and NSCLC



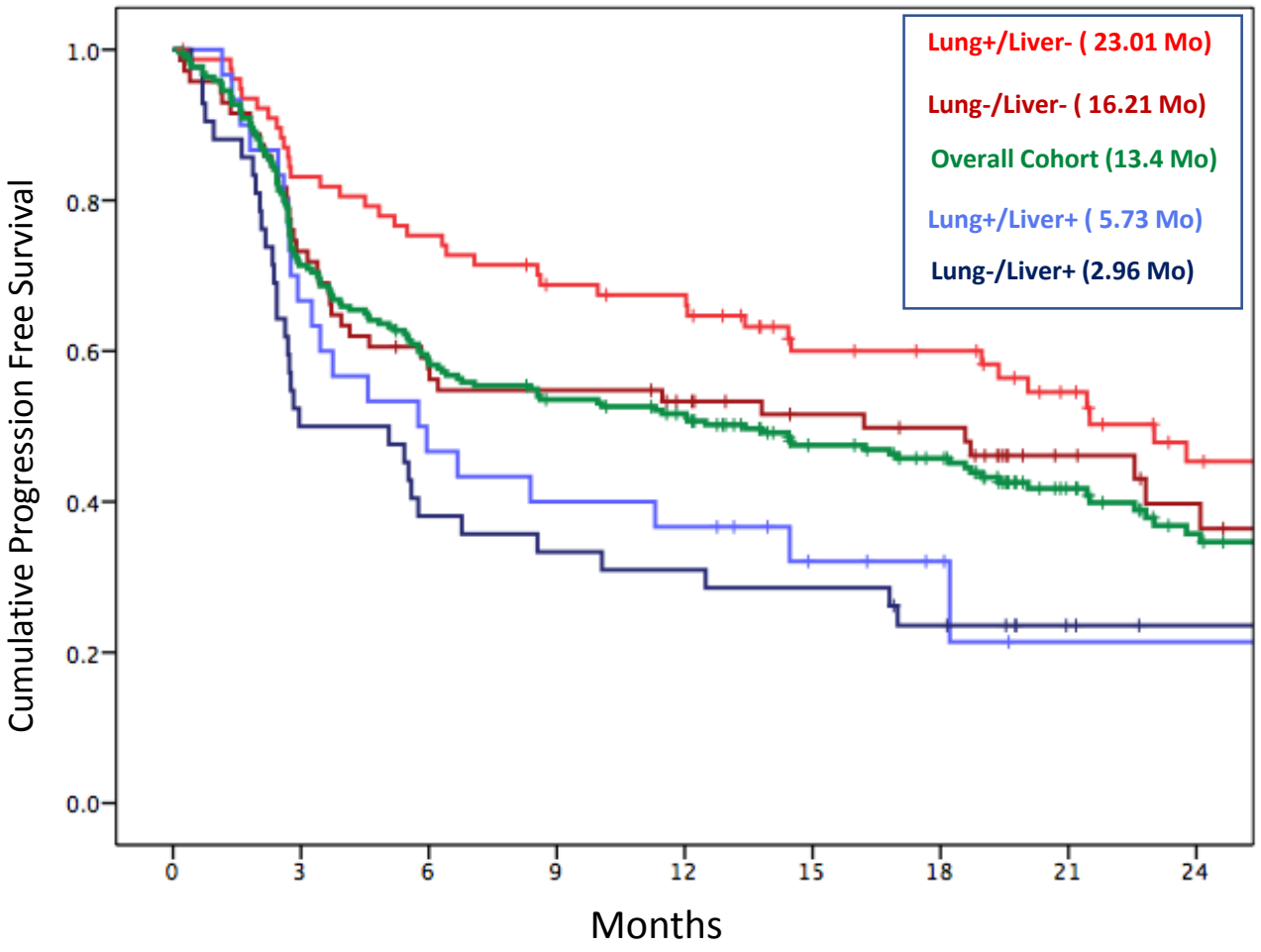


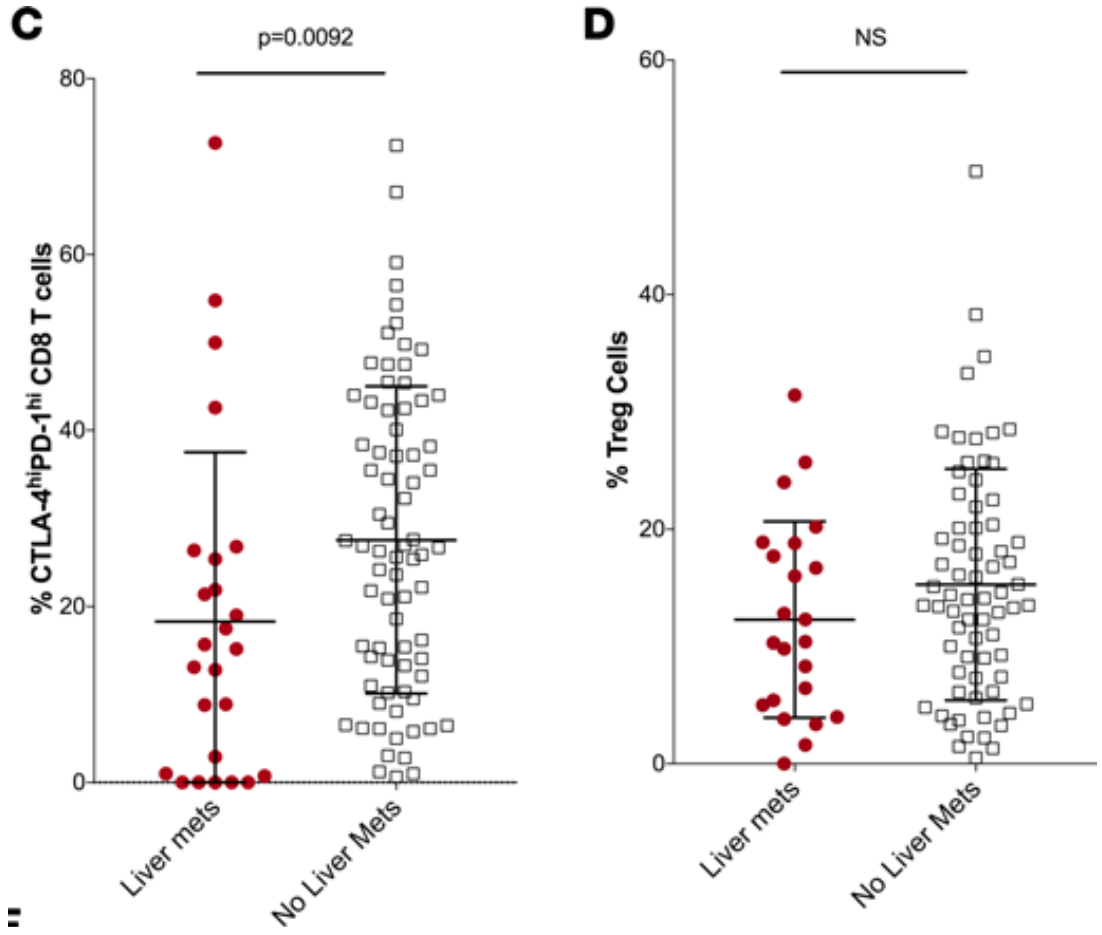
Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis

Fabio Conforti, Laura Pala, Vincenzo Bagnardi, Tommaso De Pas, Marco Martinetti, Giuseppe Viale, Richard D Gelber, Aron Goldhirsch

Paul C. Tumeh[1], Matthew D. Hellmann[2], Omid Hamid[3], Katy K. Tsai[4], Kimberly L. Loo[4], Matthew A. Gubens[4], Michael Rosenblum[4], Christina L. Harview[1], Janis M. Taube[5], Nathan Handley[4], Neharika Khurana[4], Adi Nosrati[4], Matthew F. Krummel[4], Andrew Tucker[1], Eduardo V. Sosa[4], Phillip J. Sanchez[1], Nooriel Banayan[1], Juan C. Osorio[2], Dan L. Nguyen-Kim[5], Jeremy Chang[1], I. Peter Shintaku[1], Peter D. Boasberg[3], Emma J. Taylor[1], Pamela N. Munster[4], Alain P. Algazi[4], Bartosz Chmielowski[1], Reinhard Dummer[5], Tristan R. Grogan[1], David Elashoff[1], Jimmy Hwang[4], Simone M. Goldinger[6], Edward B. Garon[1], Robert H. Pierce[7], and Adil Daud[4]

Cancer Immunology Research

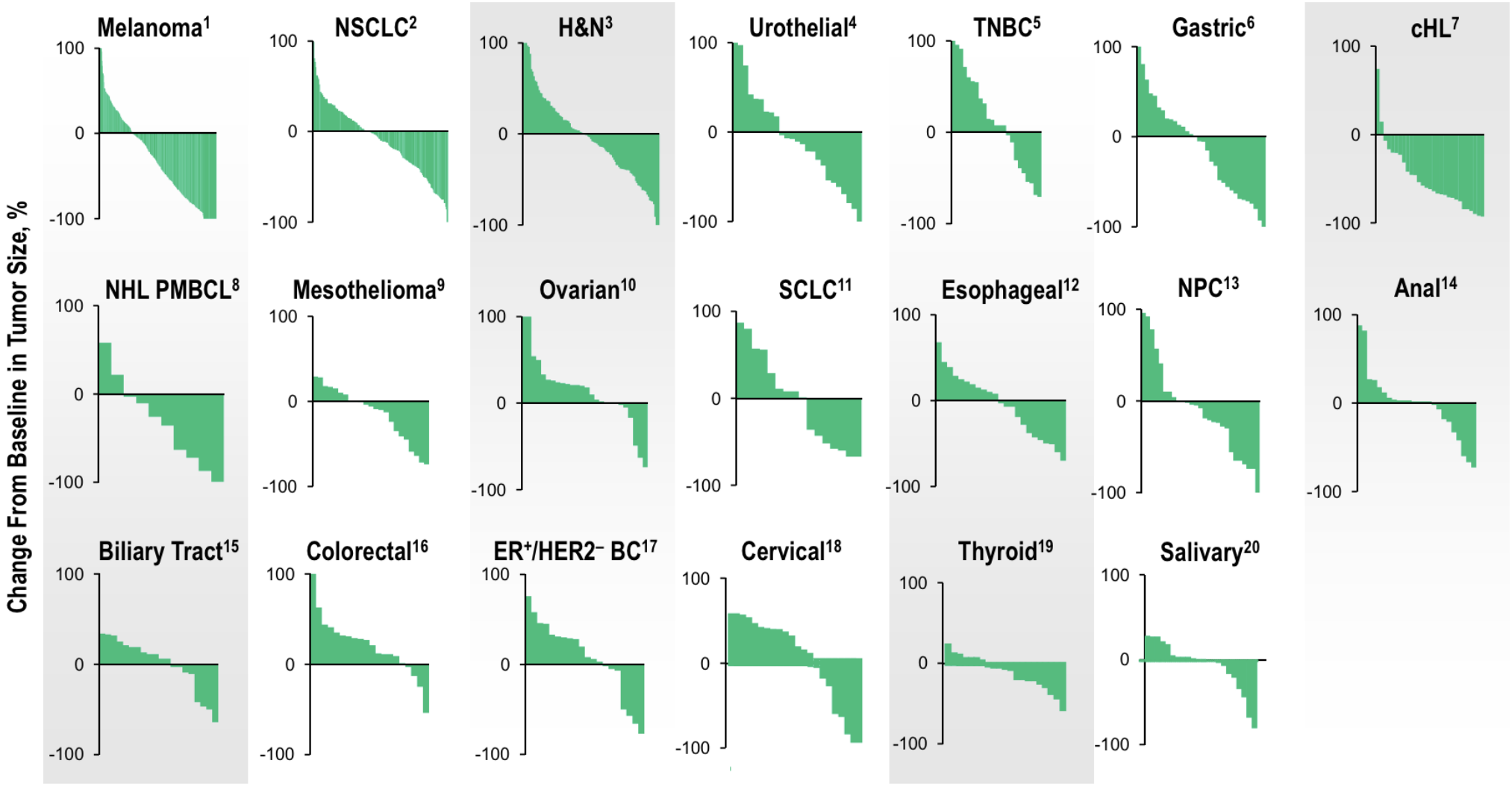




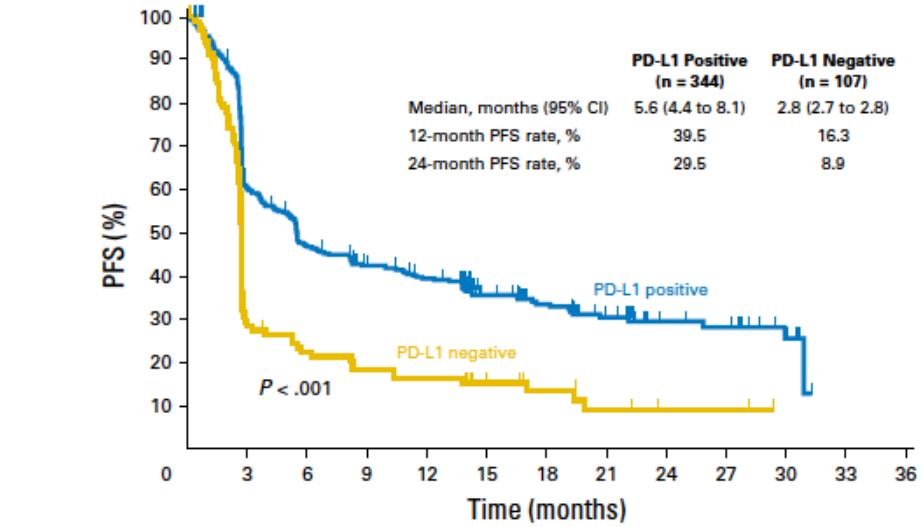
Partially exhausted tumor-infiltrating lymphocytes predict response to combination immunotherapy

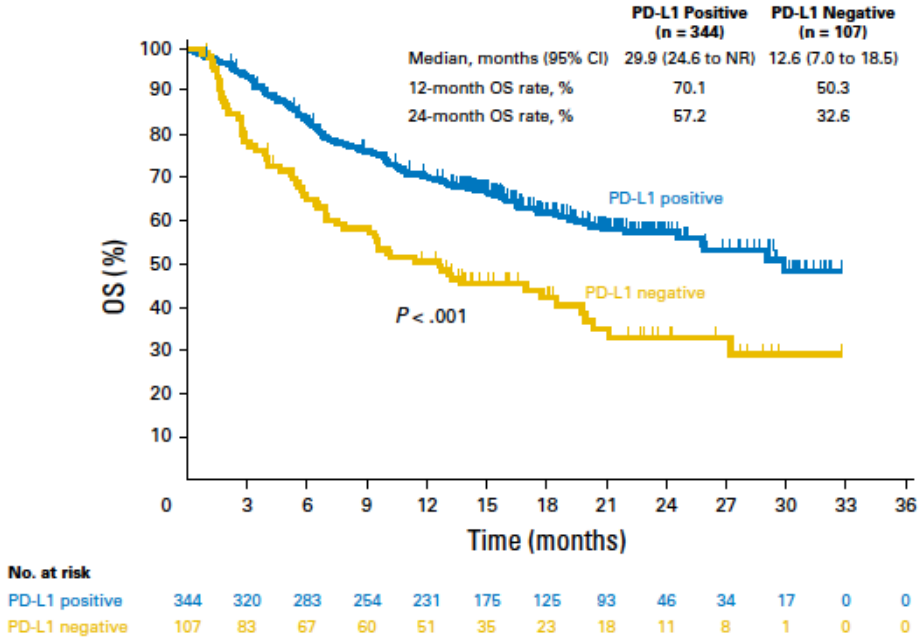
Kimberly Loo,[1] Katy K. Tsai,[1] Kelly Mahuron,[2] Jacqueline Liu,[1] Mariela L. Pauli,[3] Priscila M. Sandoval,[3] Adi Nosrati,[1] James Lee,[1] Lawrence Chen,[1] Jimmy Hwang,[5] Lauren S. Levine,[1] Matthew F. Krummel,[4] Alain P. Algazi,[1] Michael D Alvarado,[2] Michael D. Rosenblum,[3] and Adil I. Daud[1,3]

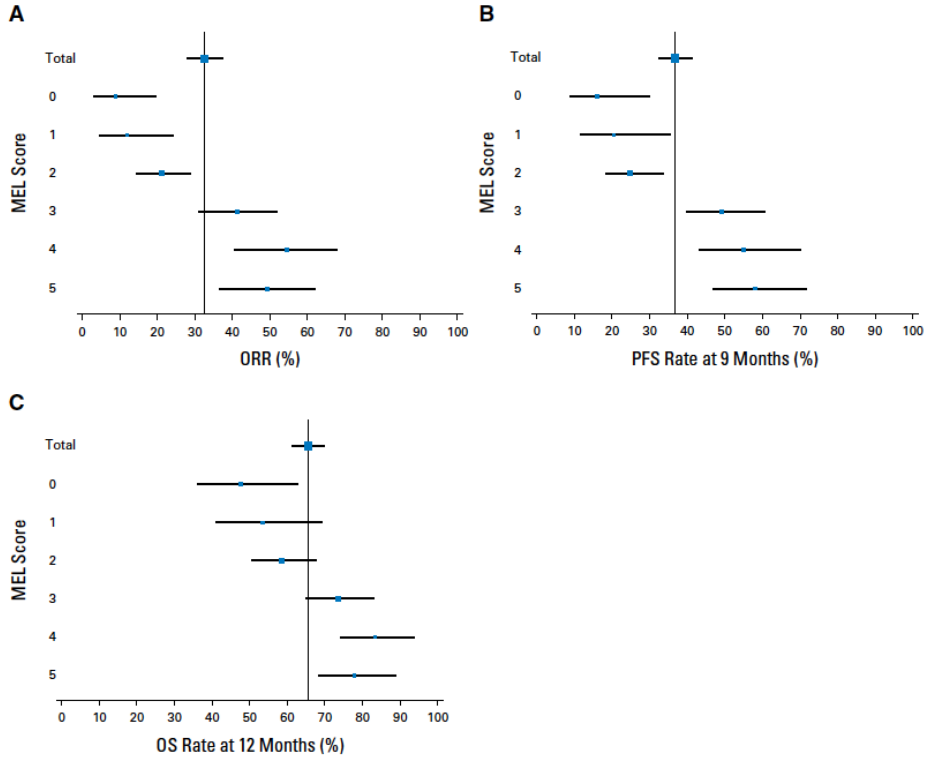
Activity of Pembrolizumab in Cancer

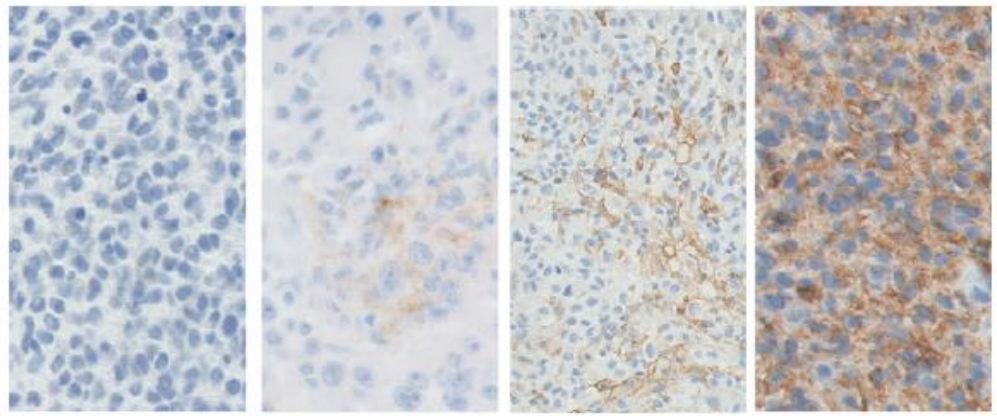


1. Daud A et al. ASCO 2015; 2. Garon EB et al. ESMO 2014; 3. Seiwert T et al. ASCO 2015; 4. Plimack E et al. ASCO 2015; 5. Nanda R et al. SABCS 2014; 6. Bang YJ et al. ASCO 2015 ; 7. Moskowitz C et al. ASH 2014; 8. Zinzani PL et al. ASH 2015; 9. Alley EA et al. AACR 2015; 10. Varga A et al. ASCO 2015; 11. Ott PA et al. 2015 ASCO; 12. Doi T et al. ASCO 2015; 13. Hsu C et al. ECC 2015; 14. Ott PA et al. ECC 2015; 15. Bang Y-J et al. ECC 2015; 16. O'Neil B et al. ECC 2015; 17. Rugo HS et al. SABCS 2015;









PD-L1 Negative
0% staining
MEL score, 0

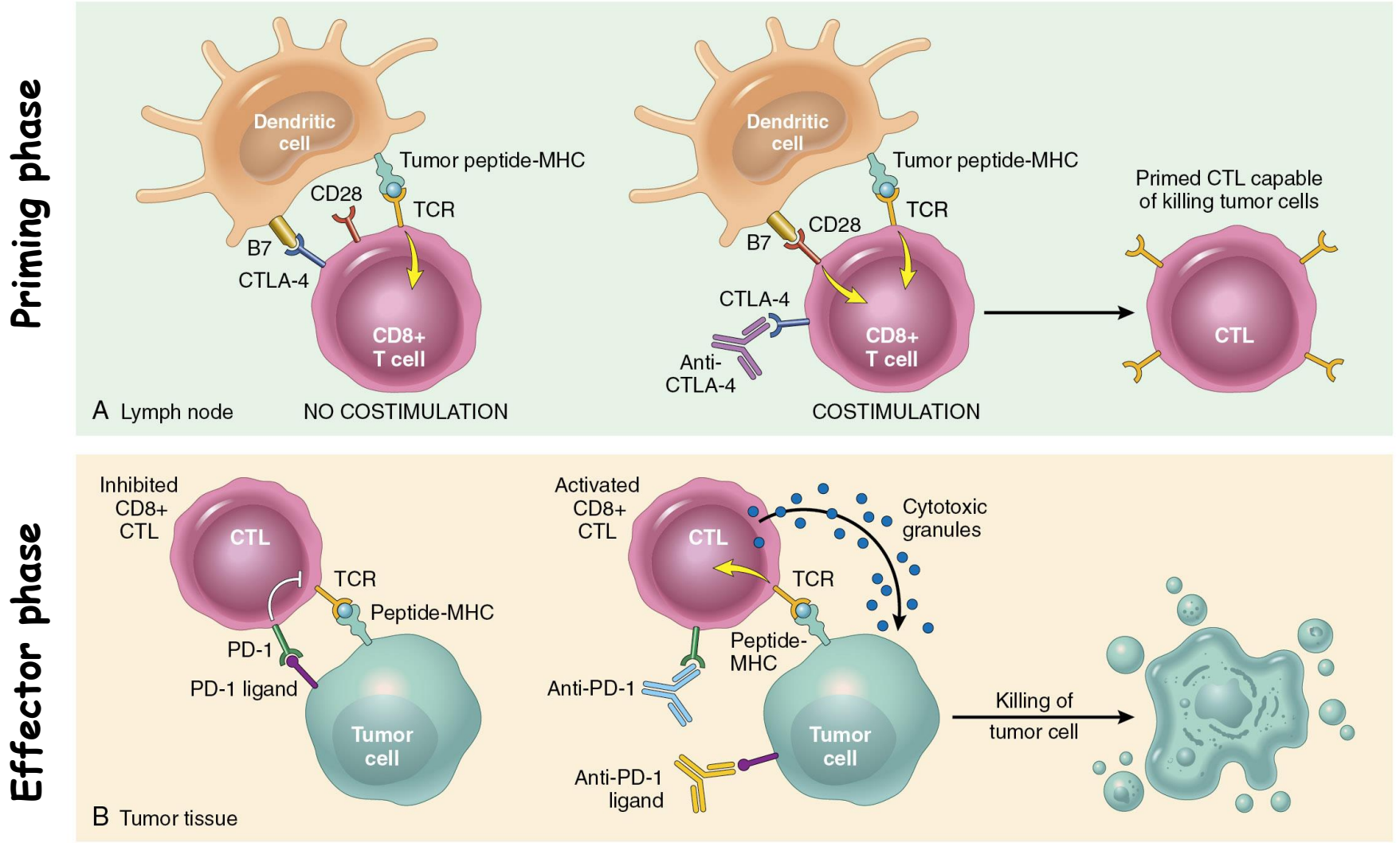
PD-L1 Positive
1%-9% staining
MEL score, 2

PD-L1 Positive
10%-32% staining
MEL score, 3

PD-L1 Positive
66%-100% staining
MEL score, 5

Daud et al, JCO, Oct 2016

Checkpoint blockade for cancer immunotherapy

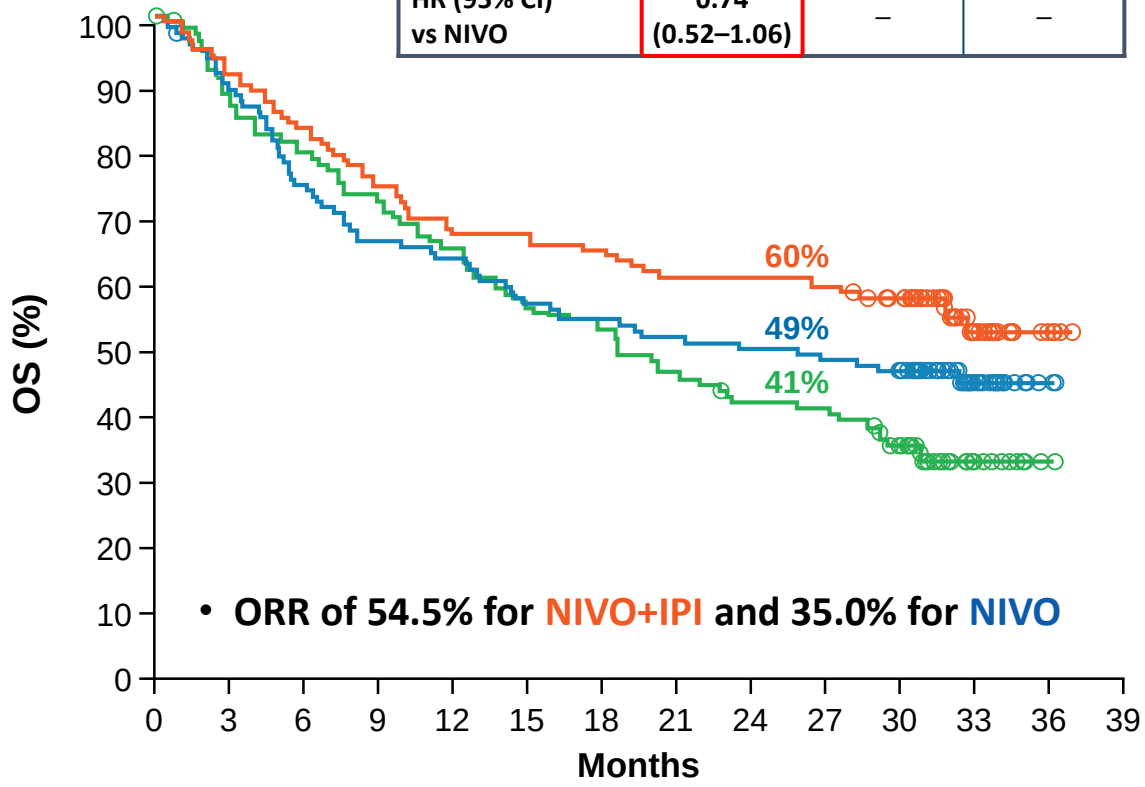


Courtesy of Abul K Abbas, MD

OS by Tumor PD-L1 Expression: 1% Cutoff

PD-L1 Expression Level <1%

<1% PD-L1	NIVO+IPI	NIVO	IPI
Median OS, mo (95% CI)	NR (26.5–NR)	23.5 (13.0–NR)	18.6 (13.7–23.2)
HR (95% CI) vs NIVO	0.74 (0.52–1.06)	–	–

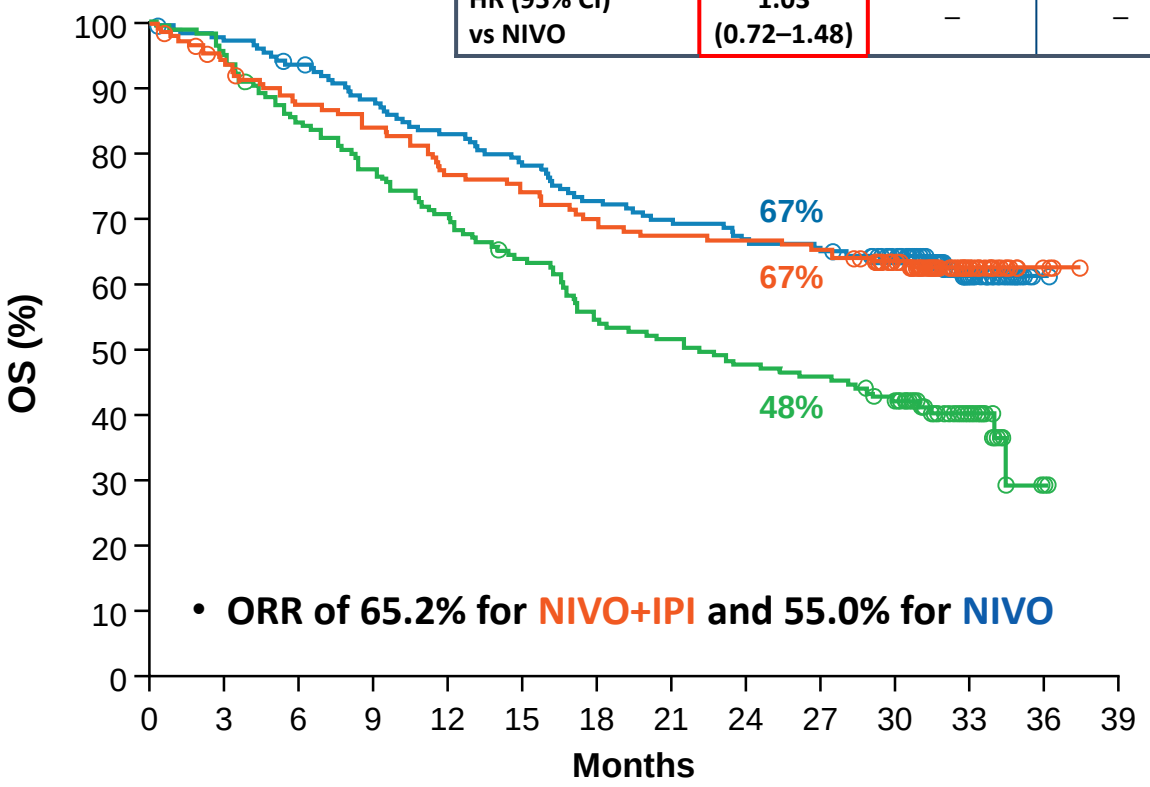


Patients at risk:

NIVO+IPI	123	113	102	91	82	82	79	74	74	72	66	18	4	0
NIVO	117	103	86	76	73	65	62	59	57	55	50	16	2	0
IPI	113	96	87	79	71	61	57	50	44	43	32	10	1	0

PD-L1 Expression Level ≥1%

≥1% PD-L1	NIVO+IPI	NIVO	IPI
Median OS, mo (95% CI)	NR	NR	22.1 (17.1–29.7)
HR (95% CI) vs NIVO	1.03 (0.72–1.48)	–	–



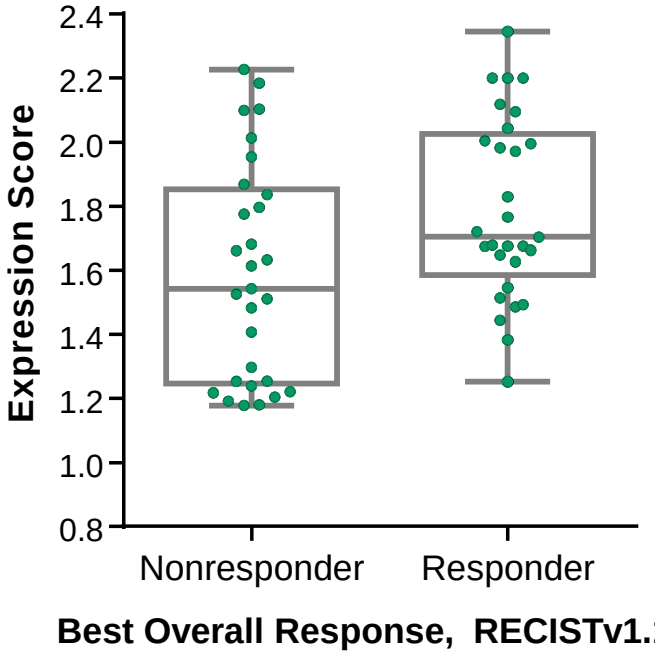
Patients at risk:

NIVO+IPI	155	144	132	127	116	112	105	102	101	99	85	27	3	0
NIVO	171	165	158	148	139	131	122	117	112	109	98	36	1	0
IPI	164	155	138	126	115	102	89	83	77	74	64	21	2	0

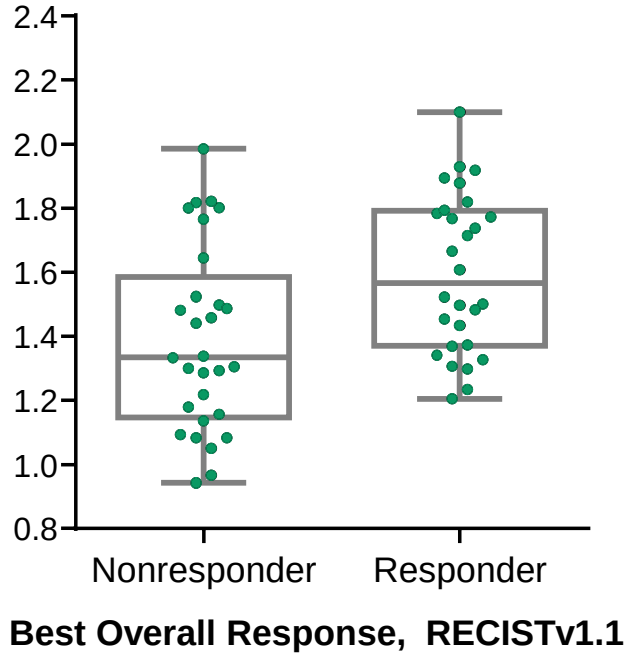
Slide from Larkin et al. AACR Annual Meeting 2017 CT075

IFNγ and Expanded Immune Signatures Correlate With Response to Pembrolizumab in Melanoma

Preliminary IFNγ (10 gene)



Preliminary Expanded Immune (28 gene)



Correlation With Response in the Validation Set[a]

Signature	BOR by RECIST N = 51	PFS by RECIST N = 62	OS N = 62
Preliminary IFNγ	*P* = 0.047	*P* = 0.016	*P* = 0.090
Preliminary expanded immune	*P* = 0.027	*P* = 0.015	*P* = 0.105

[a]Development of the expanded immune signature was performed in an unsupervised manner by individuals blinded to response data.
Nominal one-sided *P* value from logistic regression (for best overall response per RECIST v1.1) or Cox regression (for PFS and OS).

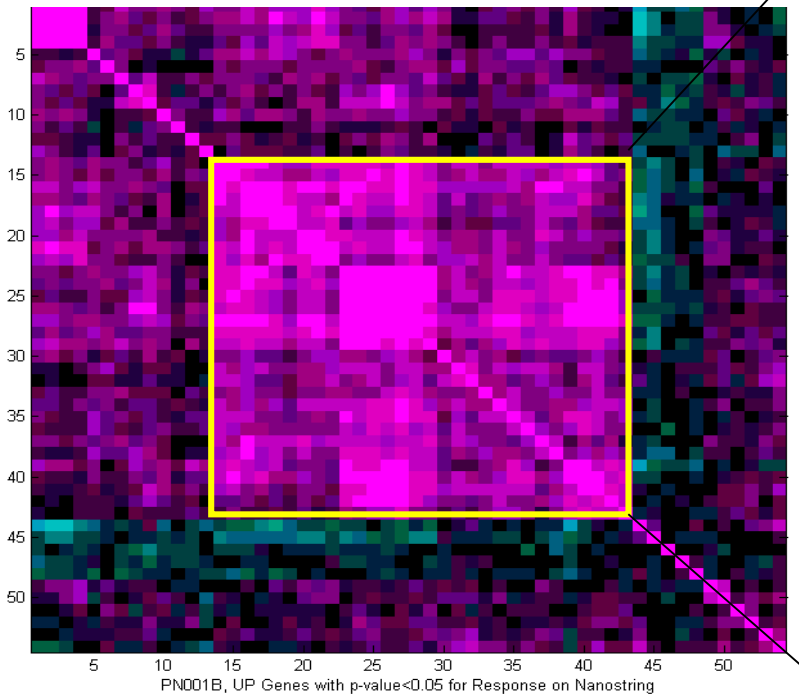
IFNγ Signature validated with clinical outcome

Melanoma Discovery Set

19 Patients

Melanoma Validation Set

62 Patients Independent Data Set



Correlation Matrix of Top Significant Genes in the Discovery Set Evaluated in the Validation Set

IFNγ Signature

IL2Rg
CXCR6
CD3d
CD2
ITGAL
TAGAP
CIITA
HLA-DRA
PTPRC
CXCL9
CCL5
NKG7
GZMA
PRF1
CCR5
CD3e
GZMK
IFNγ
HLA-E
GZMB
PDCD1
SLAMF6
CXCL13
CXCL10
IDO1
LAG3
STAT1
CXCL11

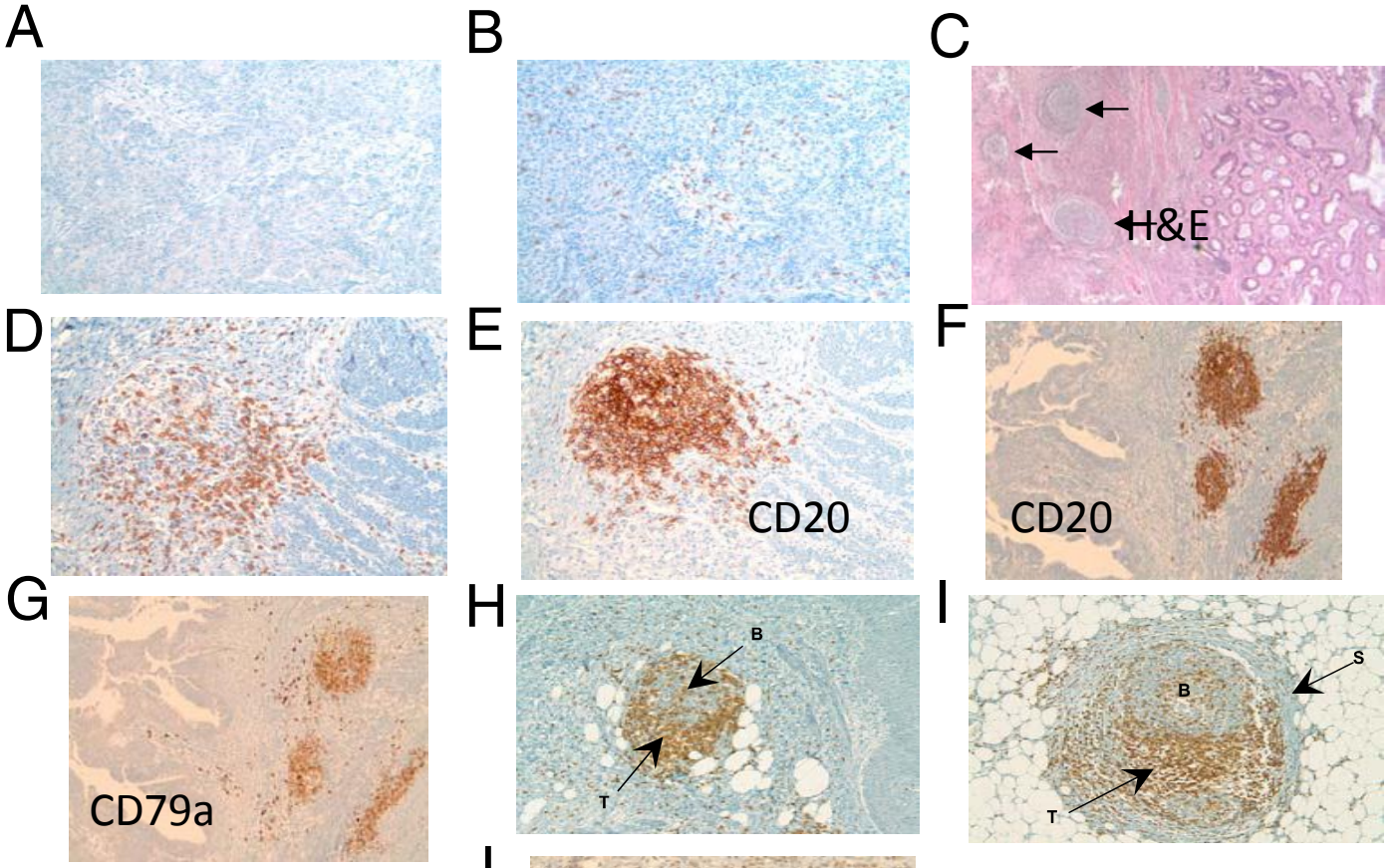
Tertiary Lymphoid structure signature

CCL2
CCL3
CCL4
CCL5
CCL8
CCL18
CCL19
CXCL9
CXCL10
CXCL11
CXCL13

IFN-γ Signature or Tertiary Lymphoid Structure Signature?

Domenico Coppola,* Michael Nebozhyn,† Farah Khalil,* Hongyue Dai,† Timothy Yeatman,‡ Andrey Loboda,† and James J. Mulé§

From the Anatomic Pathology Division, the Gastrointestinal Oncology Program,‡ and the Cutaneous Oncology Program,§ Moffitt Cancer Center, Tampa, Florida; and Oncology Molecular Profiling,† Merck Research Laboratories, West Point, Pennsylvania*

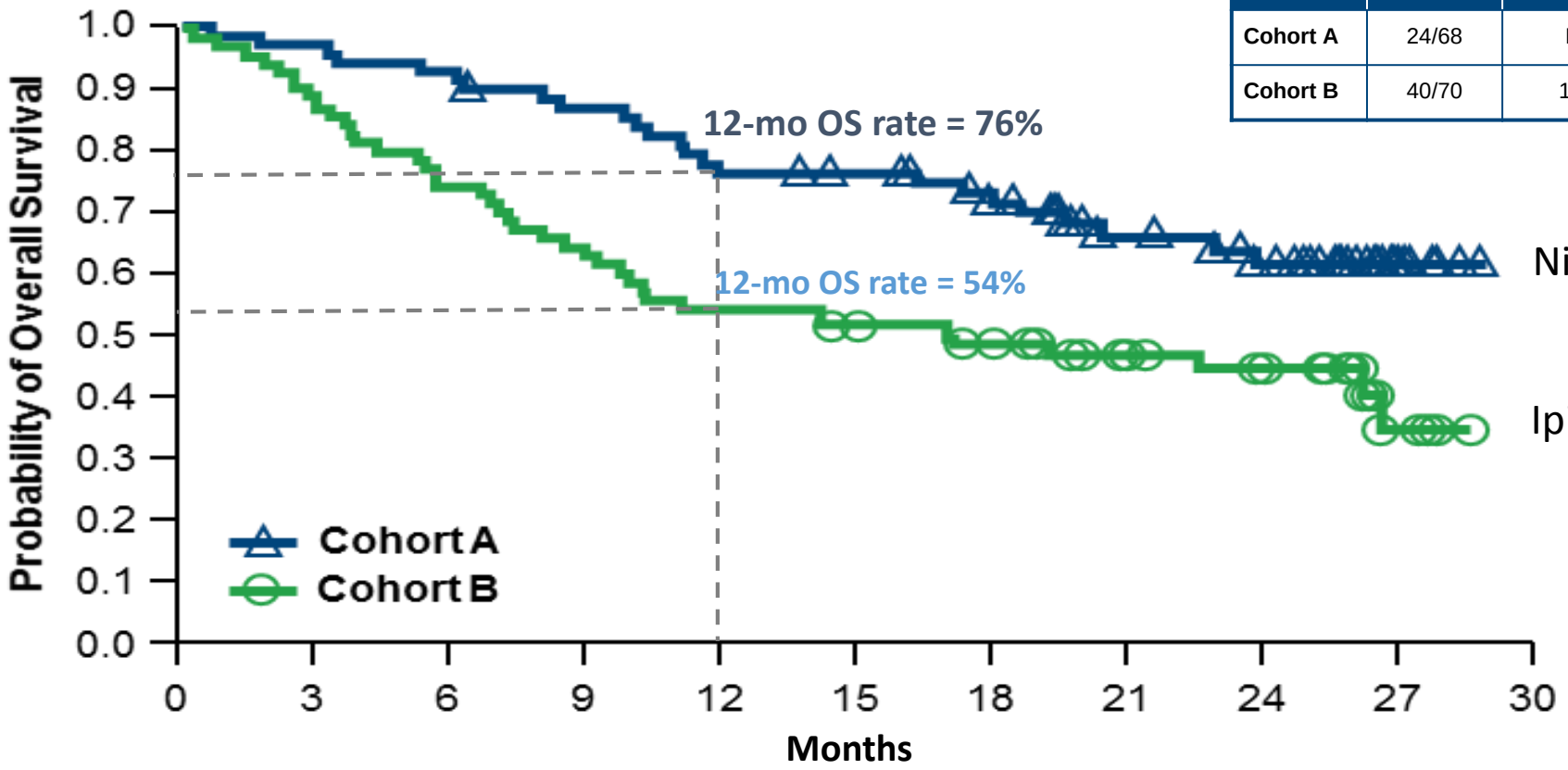


- CCL2
- CCL3
- CCL4
- CCL5
- CCL8
- CCL18
- CCL19
- CXCL9
- CXCL10
- CXCL11
- CXCL13

Unique Ectopic Lymph Node-Like Structures Present in Human Primary Colorectal Carcinoma Are Identified by Immune Gene Array Profiling

Checkmate 064 Overall Survival Analysis

	Events	Median OS (95% CI)
Cohort A	24/68	NA (23.7-NA)
Cohort B	40/70	16.9 (9.2-26.5)



Number of patients at risk

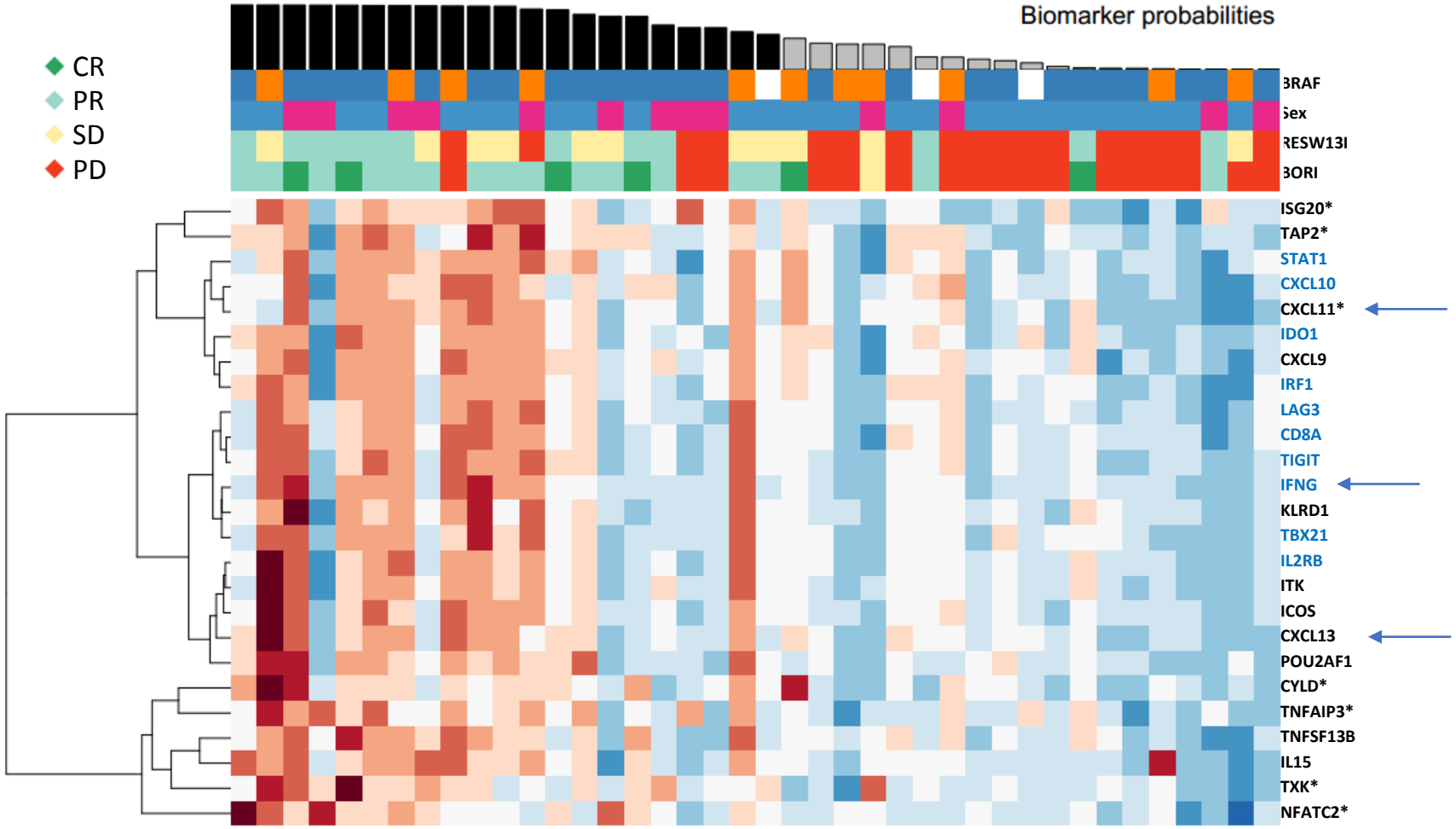
Cohort A	68	66	63	58	51	49	42	31	25	6	0
Cohort B	70	61	52	44	38	34	29	22	17	5	0

Abbreviations: CI, confidence interval; NR, not reached; OS, overall survival.

Weber et al, Checkmate 064

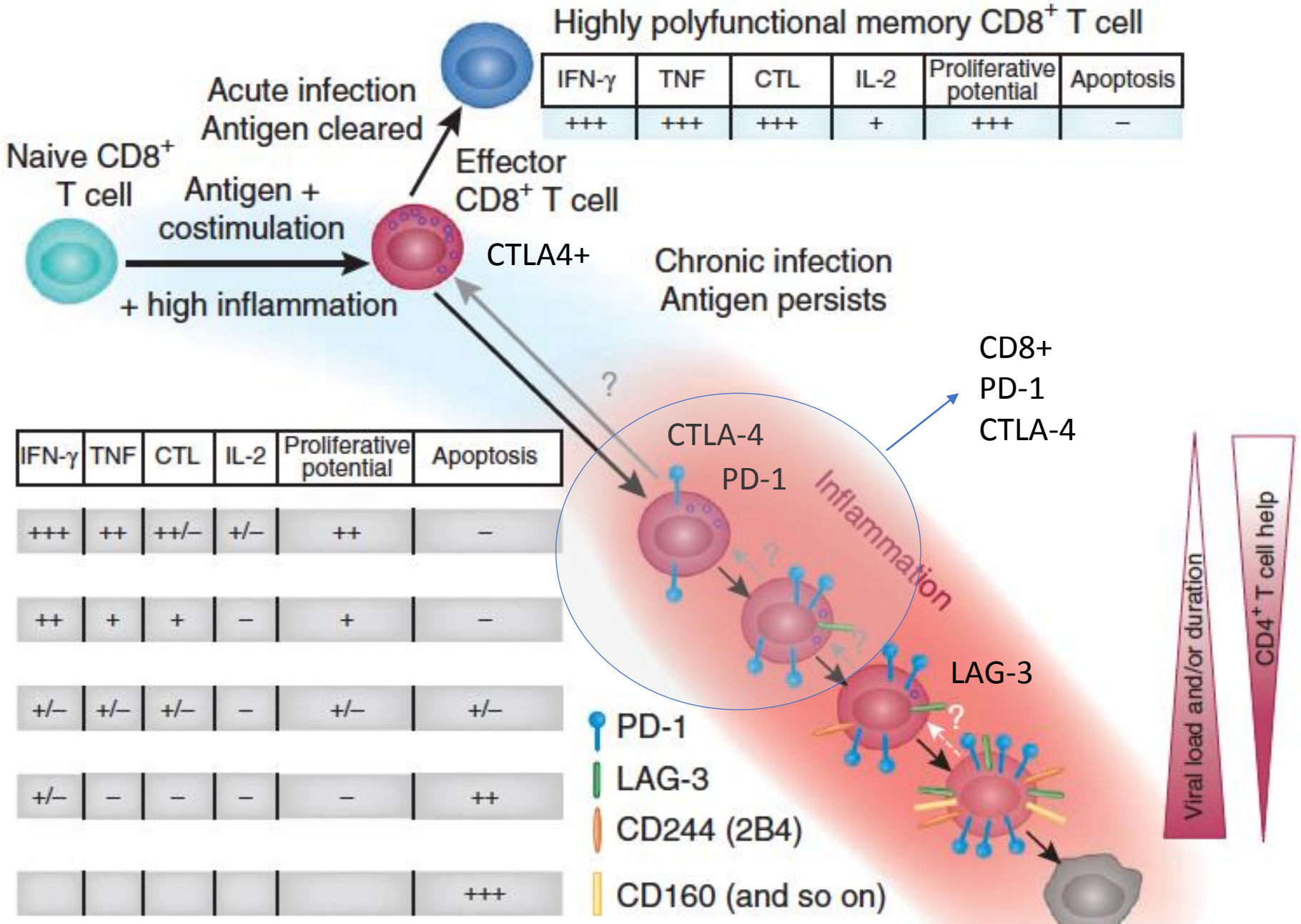
Optimized Gene Classifier of Immune-Related Genes that Predict Response in Cohort A

- Gene classifier includes IFN-γ-related genes and others
- There is no optimal gene signature for Cohort B



Rodig et al, ASCO 2017. Biomarker Analysis Checkmate 064

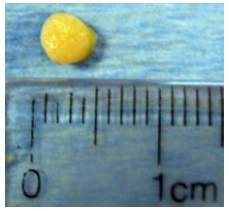
Biomarker	Nivo > Ipi Fisher's Exact P-Value	Ipi > Nivo Fisher's Exact P-Value
MHC Class I IHC, %	0.53	**0.02**
MHC Class II IHC, %	**0.05**	0.99
CD3 (cell/mm^2)	**0.05**	0.19
CD4 (cell/mm^2)	0.07	0.19
CD8 (cell/mm^2)	0.08	0.16
PD-1+ (cell/mm^2)	**0.02**	0.32
Tumor PD-L1+, %	**0.03**	0.56
Tumor PD-L1+, % (using external reference)	0.23	0.77
Tumor PD-L1+, % (using external reference)	0.06	0.64
Tumor PD-L2+, %	0.28	0.43
Missense mutations (N)	**0.004**	**0.01***



Modified from E John Wherry, Nature Immunology 12: 492-499, 2011

Functional Immuno-Flow Cytometry of tumor tissue

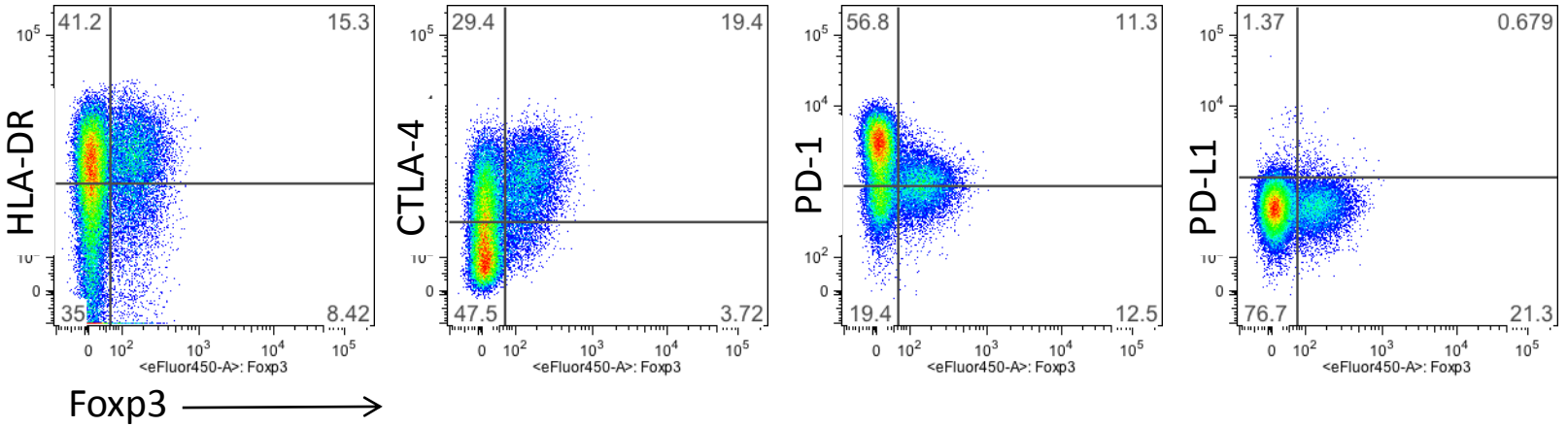
Core or Punch Biopsy



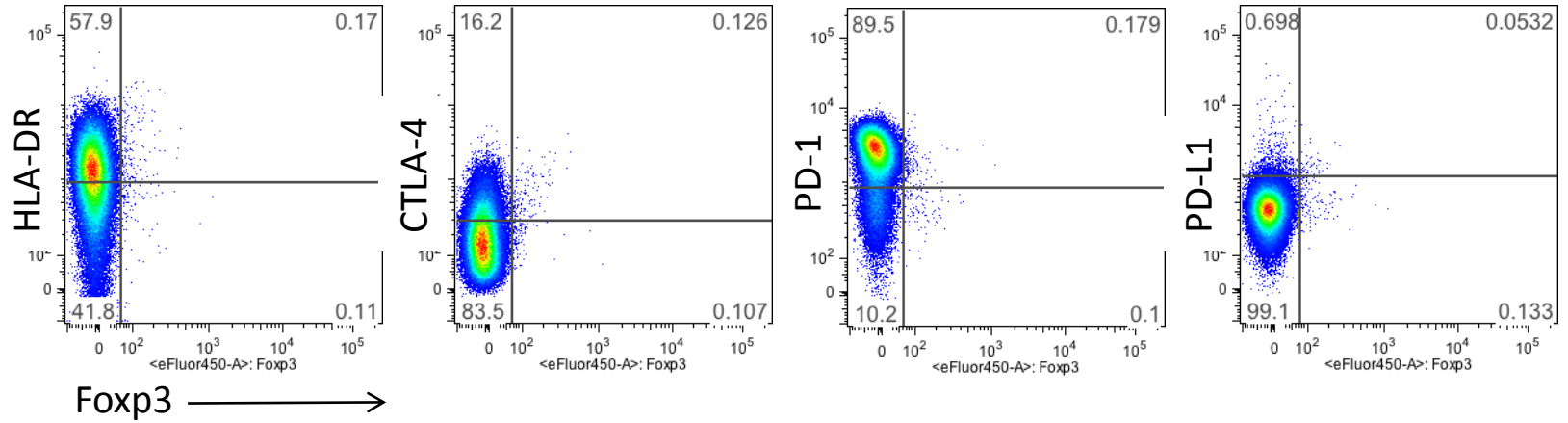
overnight digestion

multicolor flow cytometry

CD4+



CD8+

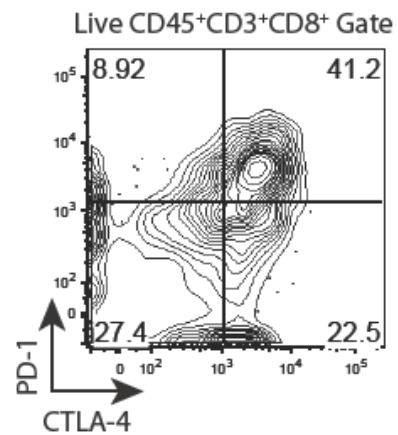


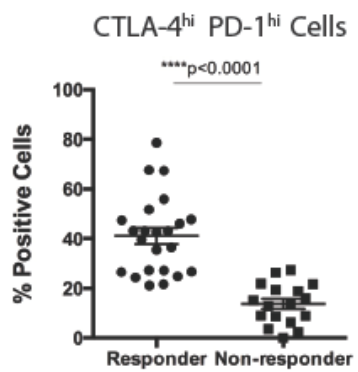
The Journal of Clinical Investigation CLINICAL MEDICINE

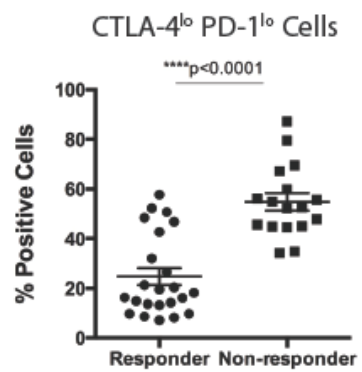
Tumor immune profiling predicts response to anti–PD-1 therapy in human melanoma

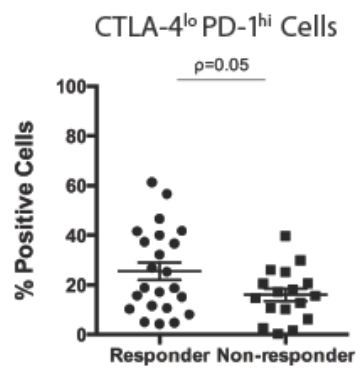
Adil I. Daud,[1] Kimberly Loo,[1] Mariela L. Pauli,[2] Robert Sanchez-Rodriguez,[2] Priscila Munoz Sandoval,[2] Keyon Taravati,[2] Katy Tsai,[1] Adi Nosrati,[1] Lorenzo Nardo,[3] Michael D. Alvarado,[1] Alain P. Algazi,[1] Miguel H. Pampaloni,[4] Iryna V. Lobach,[1] Jimmy Hwang,[1] Robert H. Pierce,[5] Iris K. Gratz,[6] Matthew F. Krummel,[4] and Michael D. Rosenblum[2]

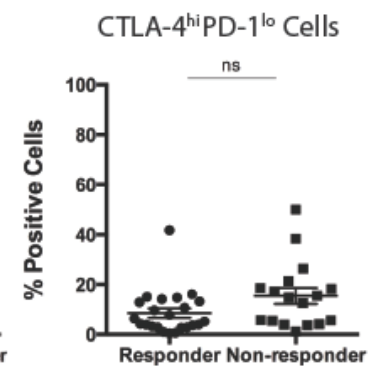
[1]Helen Diller Comprehensive Cancer Center, [2]Department of Dermatology, [3]Department of Radiology, and [4]Department of Pathology, UCSF, San Francisco, California, USA. [5]Oncosec Inc., San Diego, California, USA. [6]Department of Molecular Biology, University of Salzburg, Salzburg, Austria.

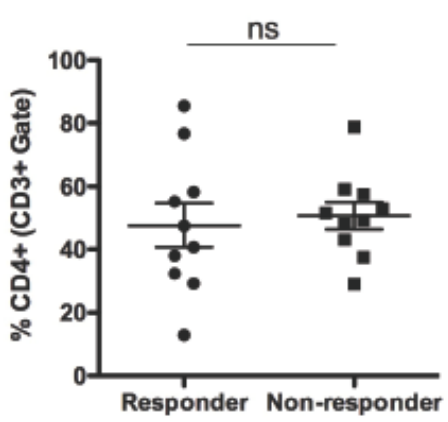


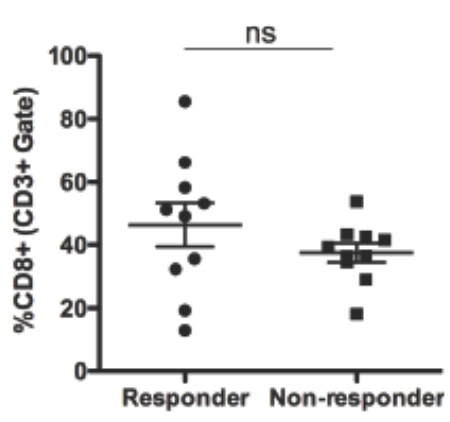


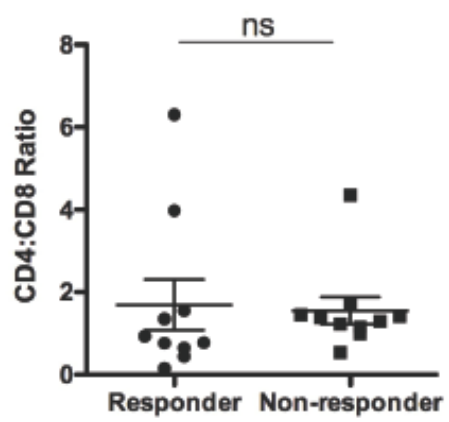


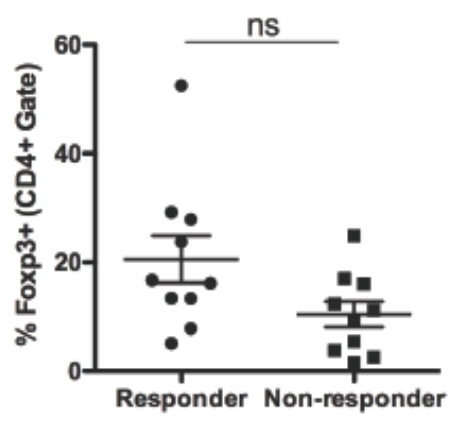


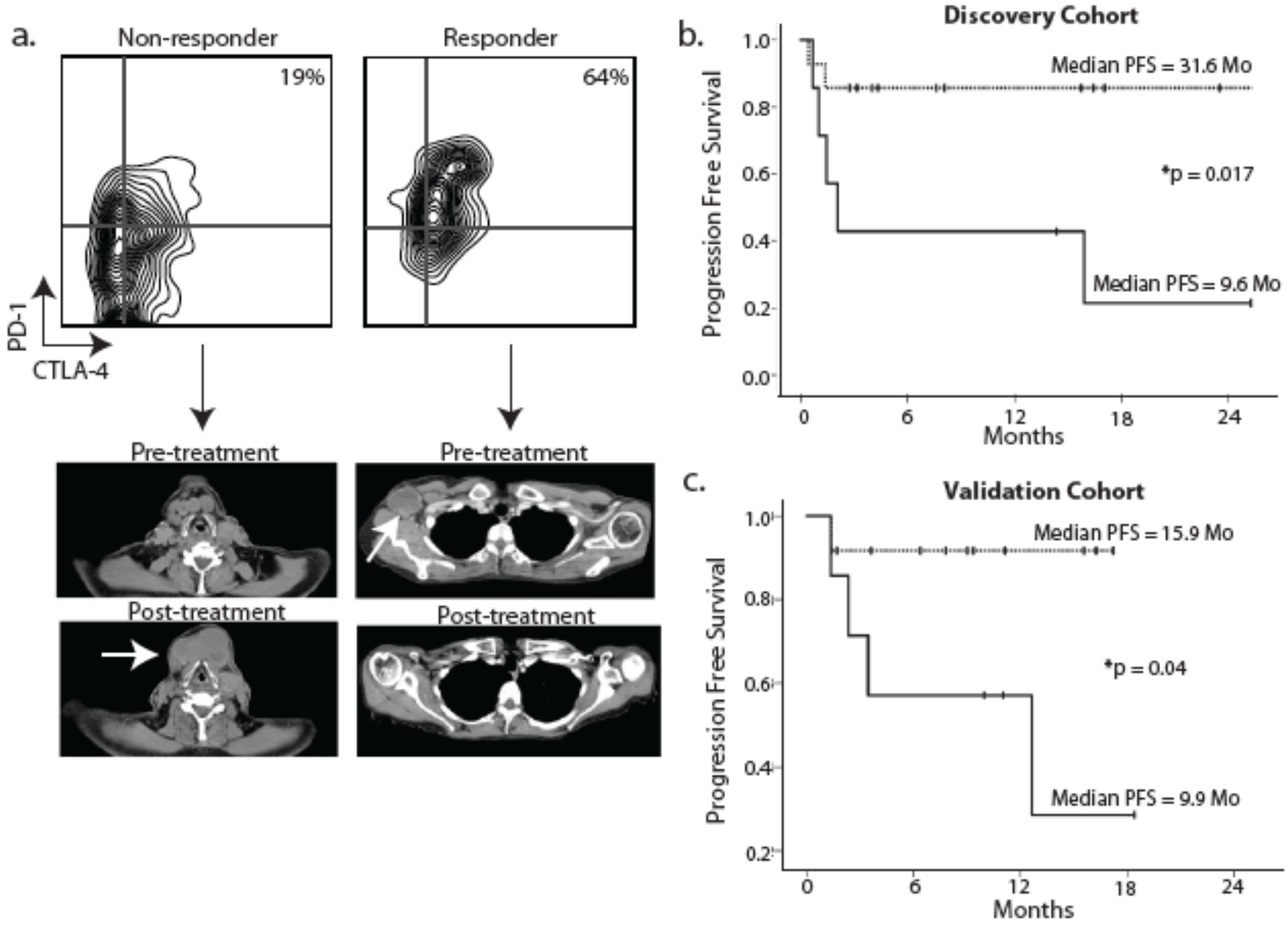


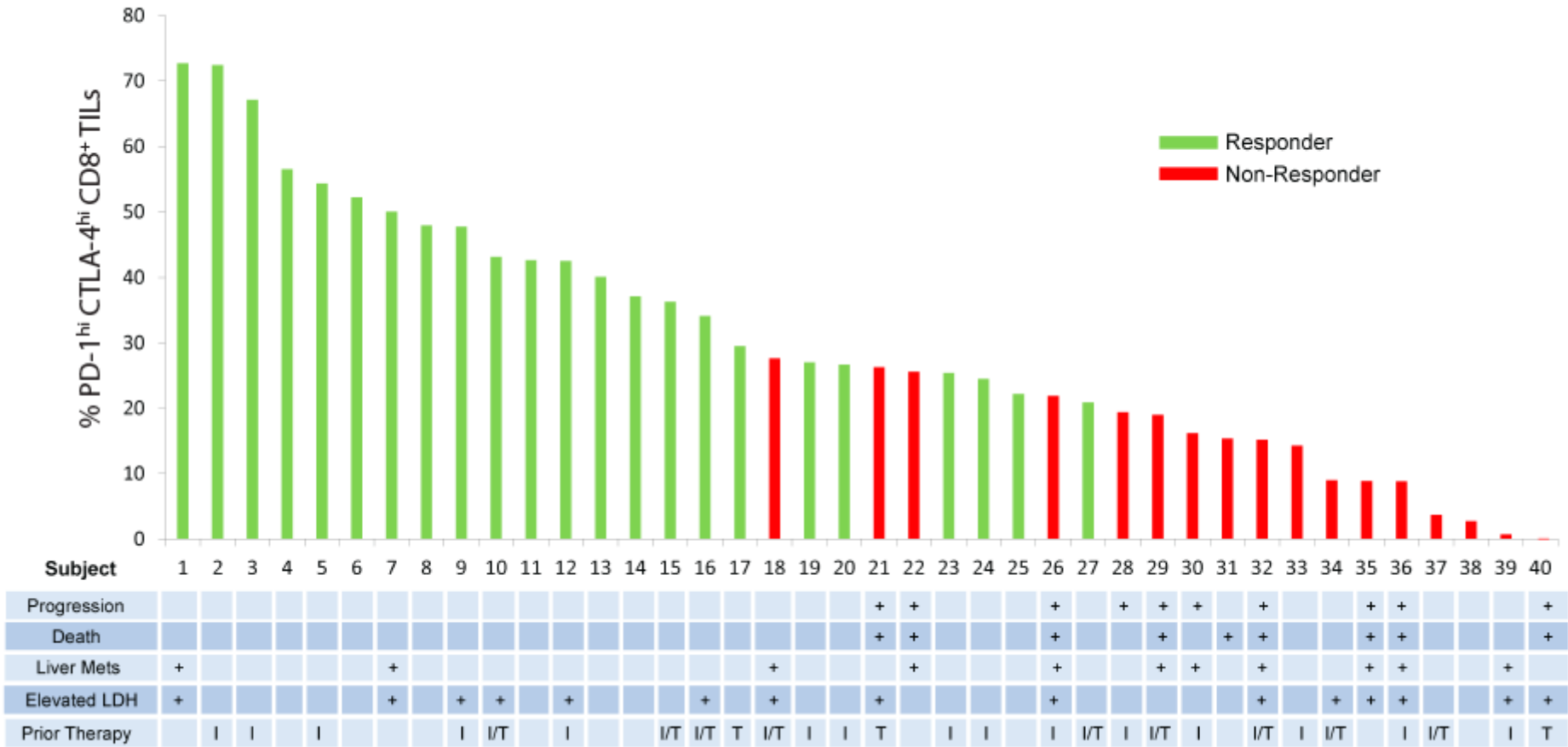




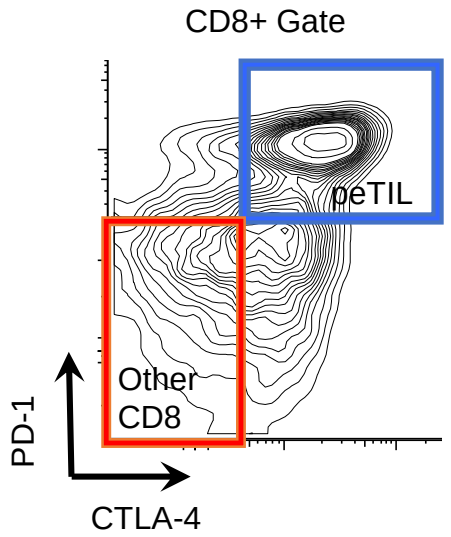





a.
Non-responder
Responder
19%
64%
PD-1
CTLA-4
Pre-treatment
Pre-treatment
Post-treatment
Post-treatment
b.
Discovery Cohort
Median PFS = 31.6 Mo
*p = 0.017
Median PFS = 9.6 Mo
Progression Free Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
6
12
18
24
Months
c.
Validation Cohort
Median PFS = 15.9 Mo
*p = 0.04
Median PFS = 9.9 Mo
Progression Free Survival
1.0
0.8
0.6
0.4
0.2
0
6
12
18
24
Months


% PD-1hi CTLA-4hi CD8+ TILs
80
70
60
50
40
30
20
10
0
Responder
Non-Responder
Subject
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
Progression + + +

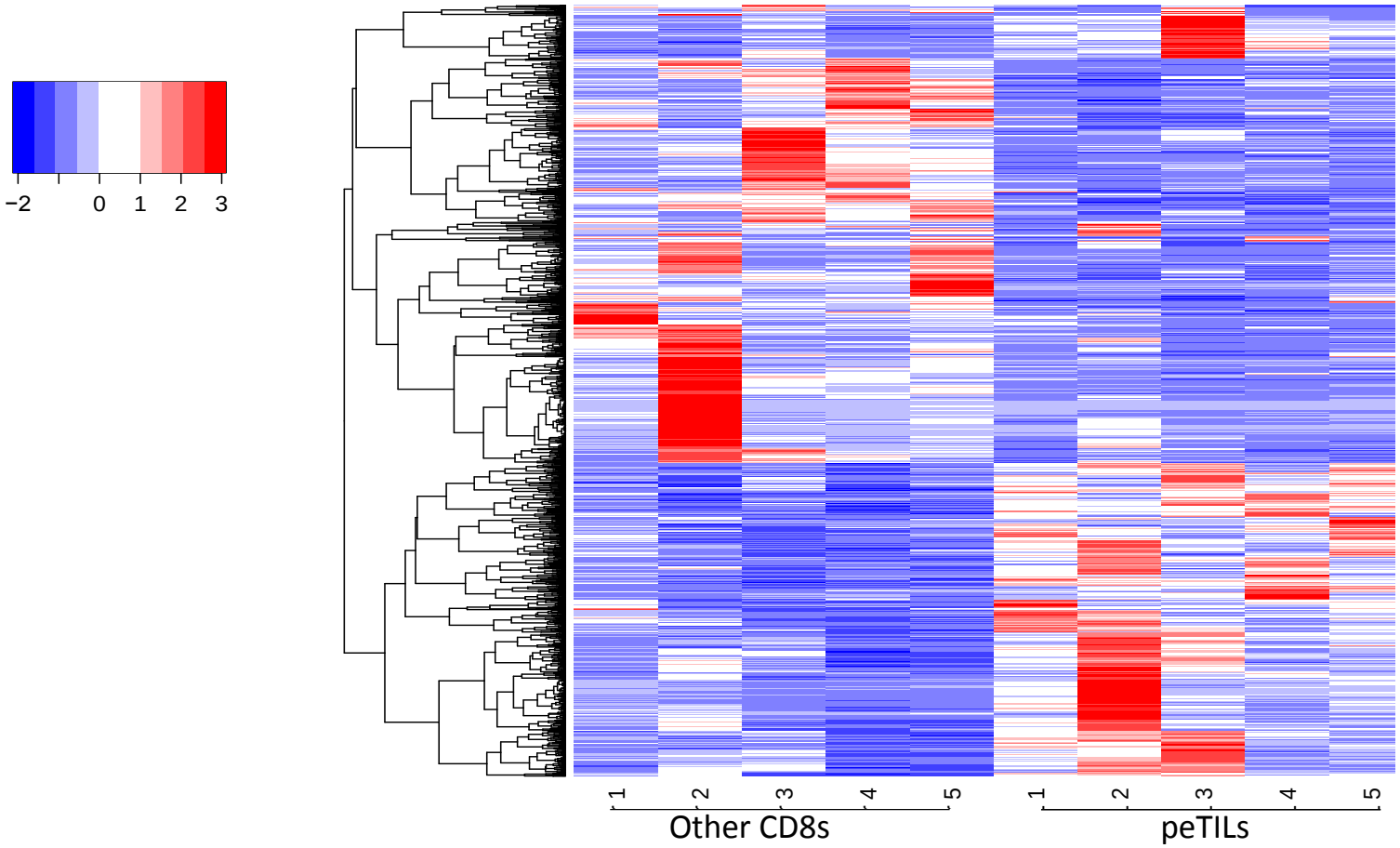
Exhaustion markers in our sorted peTILs compared to other CD8s

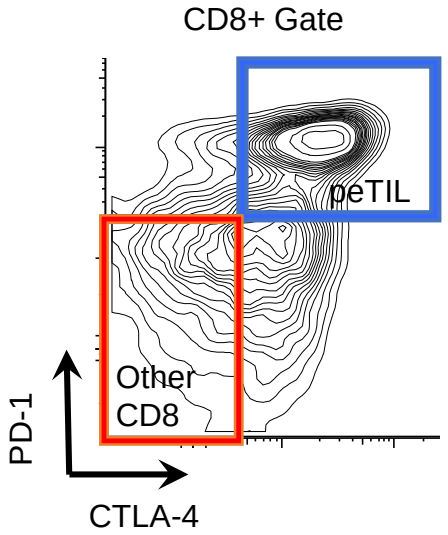


Gene	log2FoldChange	Padj	Other CD8s – ave counts	peTILs – ave counts
PD-1	5.69	6.16E-47	127.5	5582.4
Tim-3	4.32	2.41E-18	698.2	8053.4
CTLA-4	3.38	9.19E-12	1852.5	16417.1
LAG-3	2.70	2.65E-09	2446.6	13518.6
TIGIT	2.42	5.96E-11	1207.9	6715.7
Ki67	4.09	9.89E-11	341.8	2953.1

Of Interest:
Laylin (LAYN)
CXCR6
CXCL13

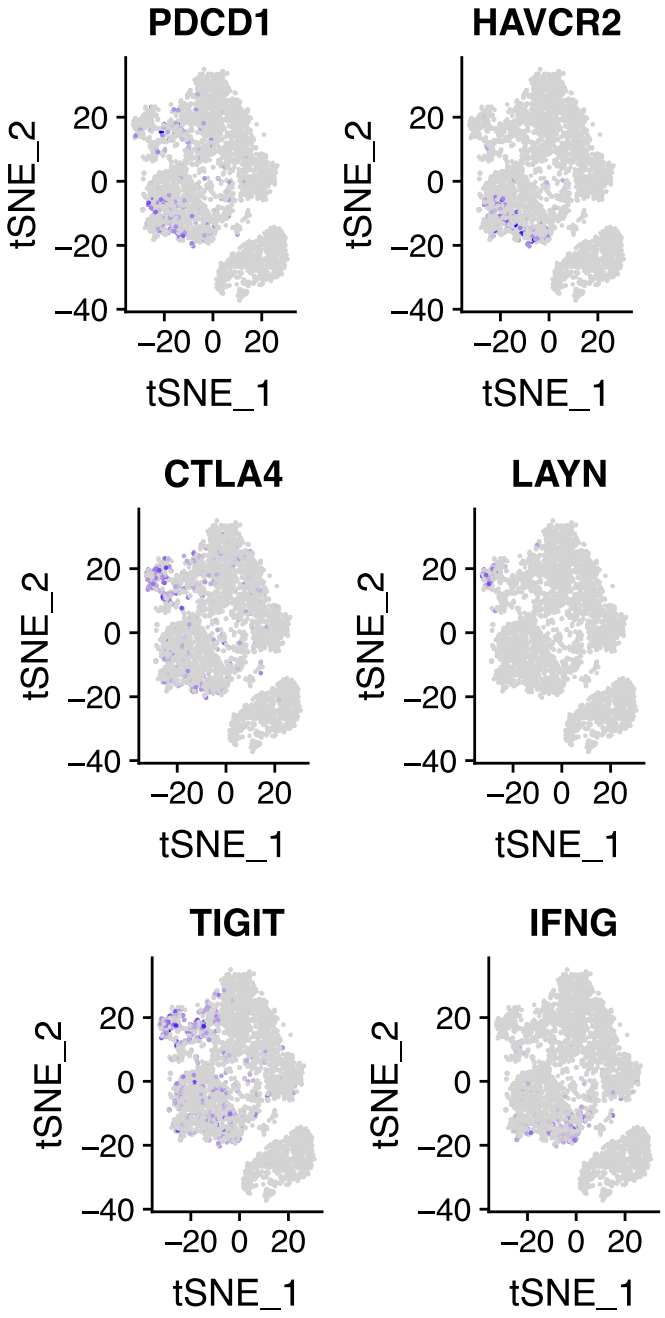
Numerous genes are differentially expressed between PD-1^{+}CTLA-4^{+} peTILs and other CD8+ T cells in the tumor microenvironment in human melanoma

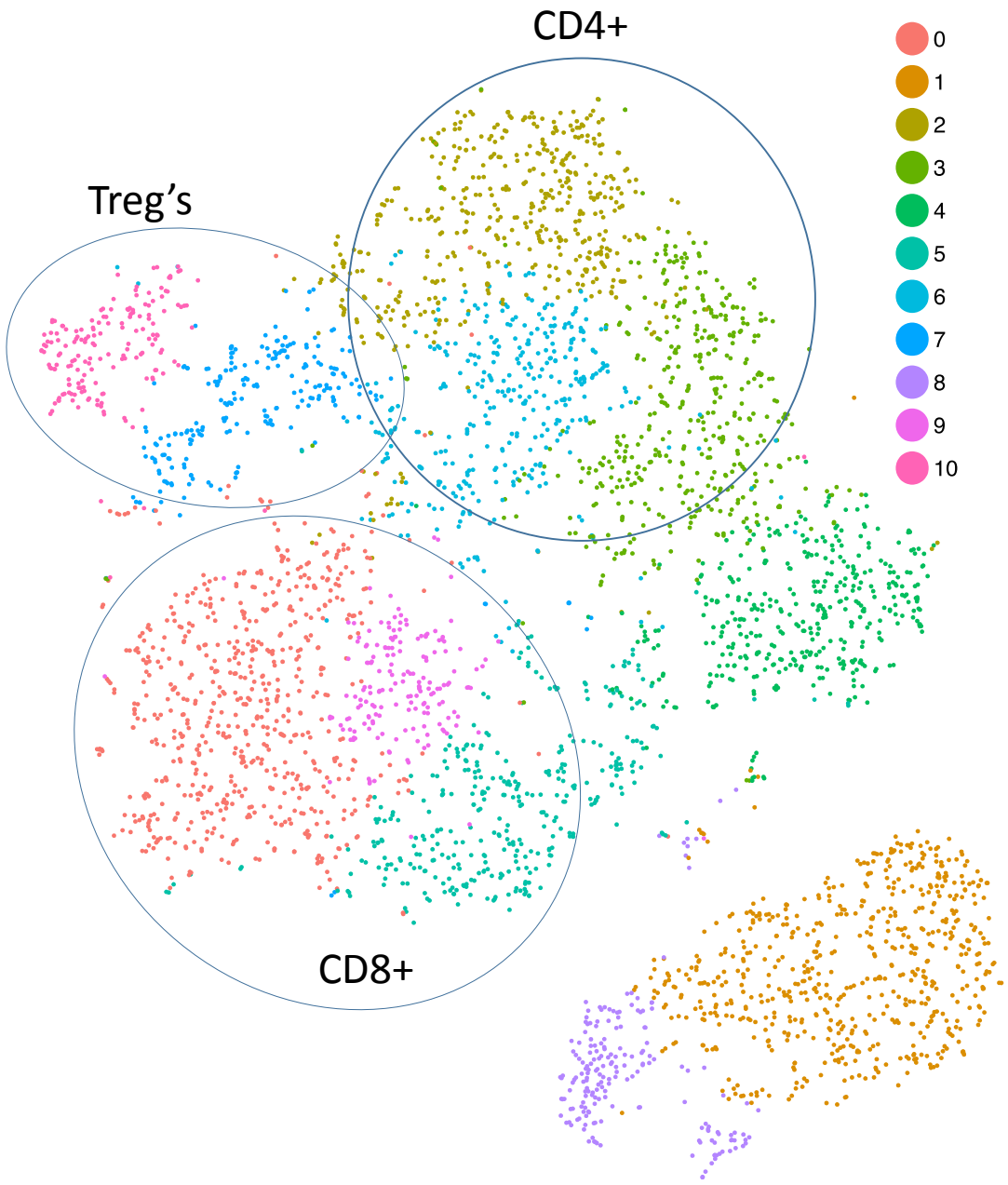


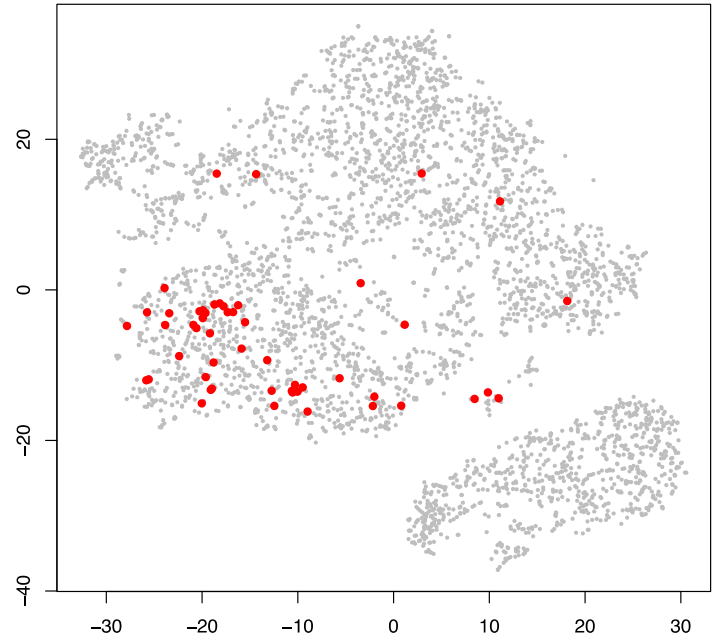


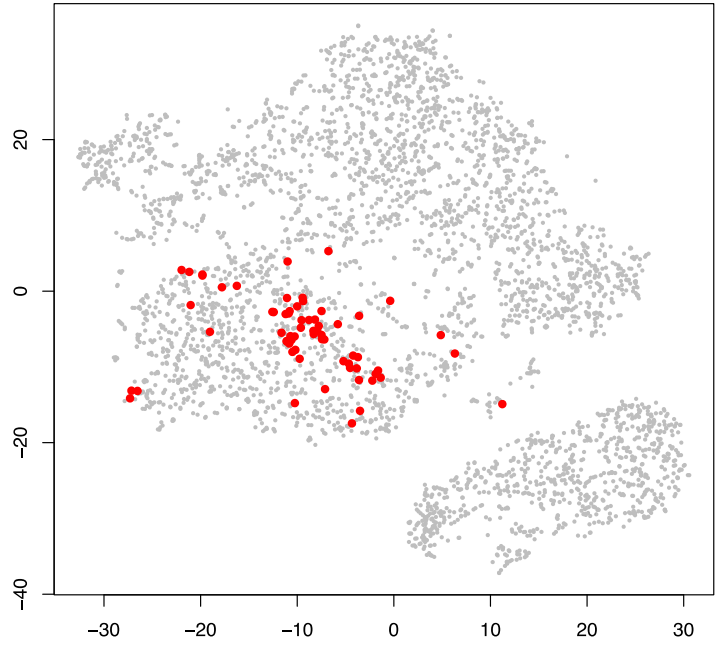
*Data from whole RNA transcriptome sequencing of sorted human melanoma tumor infiltrating T cells in the UCSF Rosenblum Lab

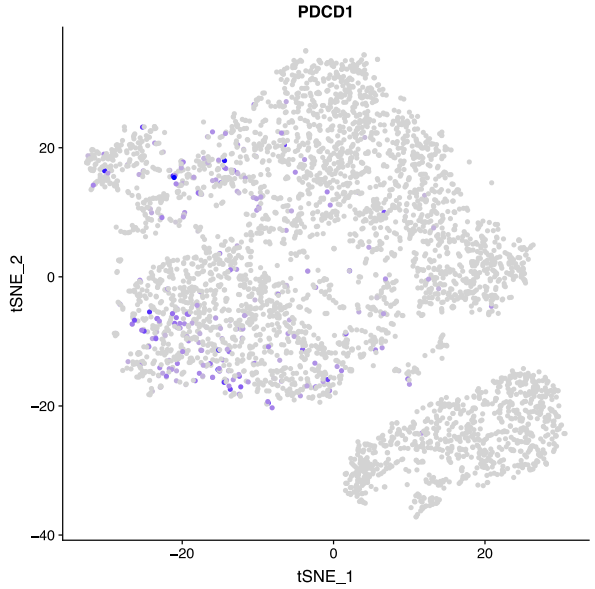
Single cell seq of CD45+ sorted cells-Mel K-252

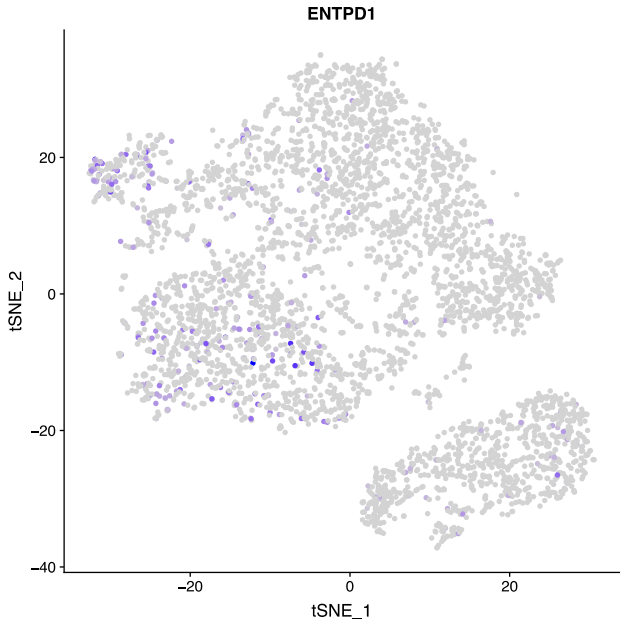


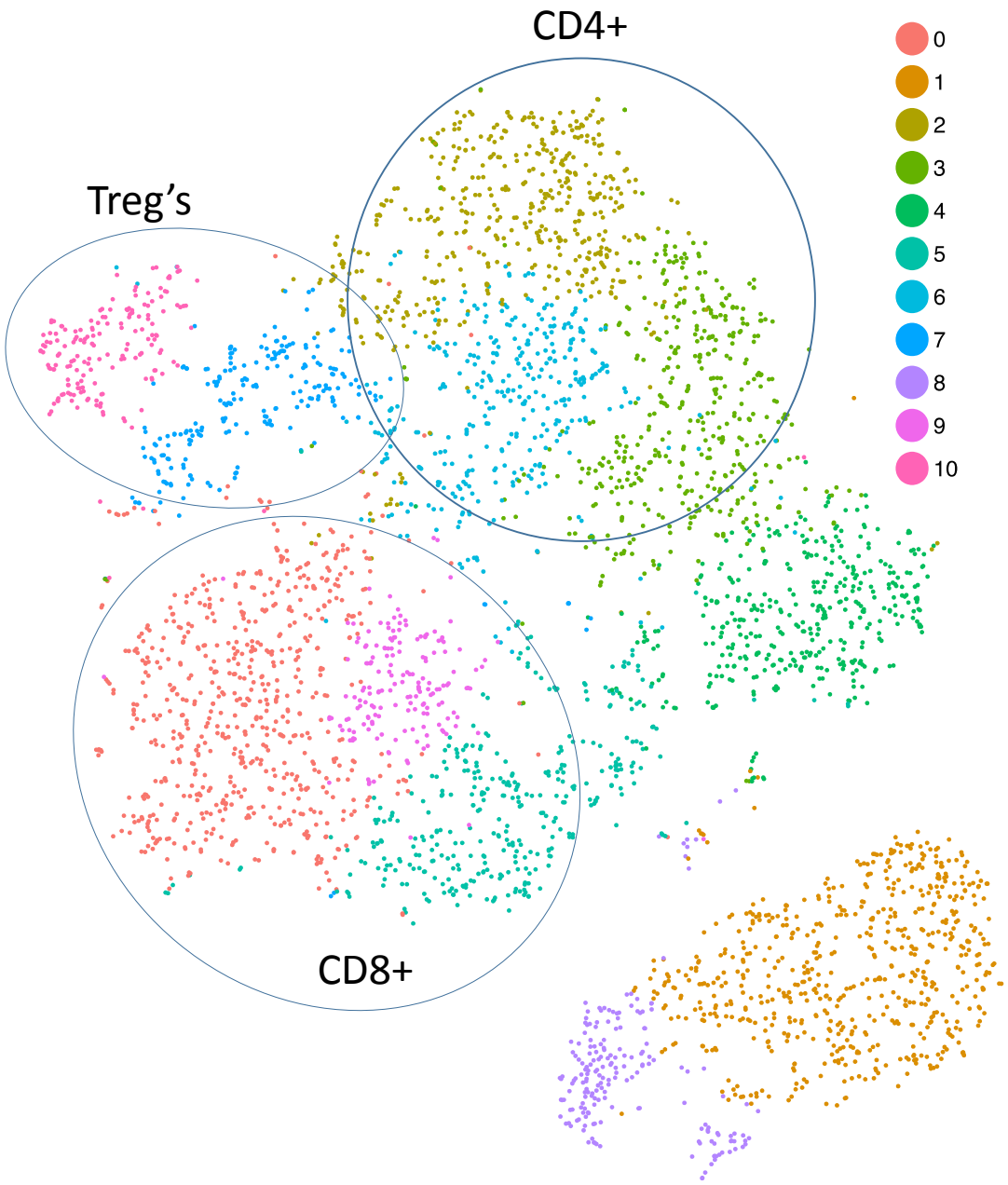


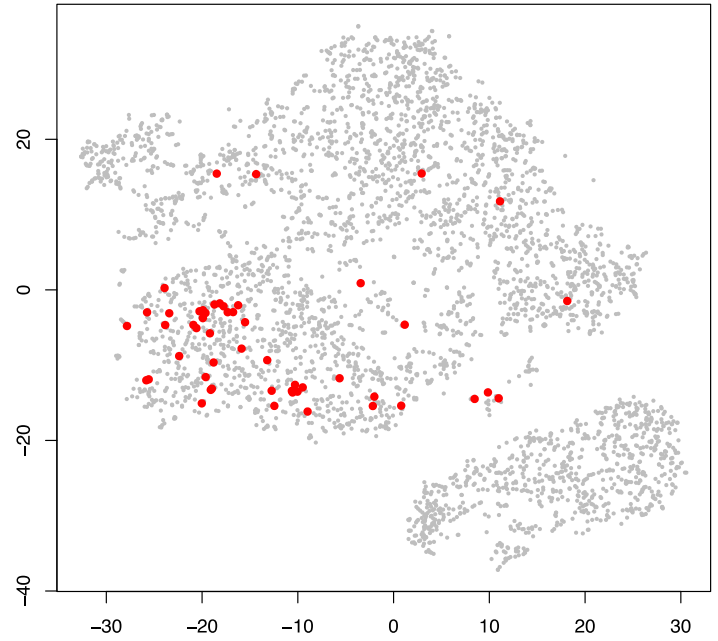


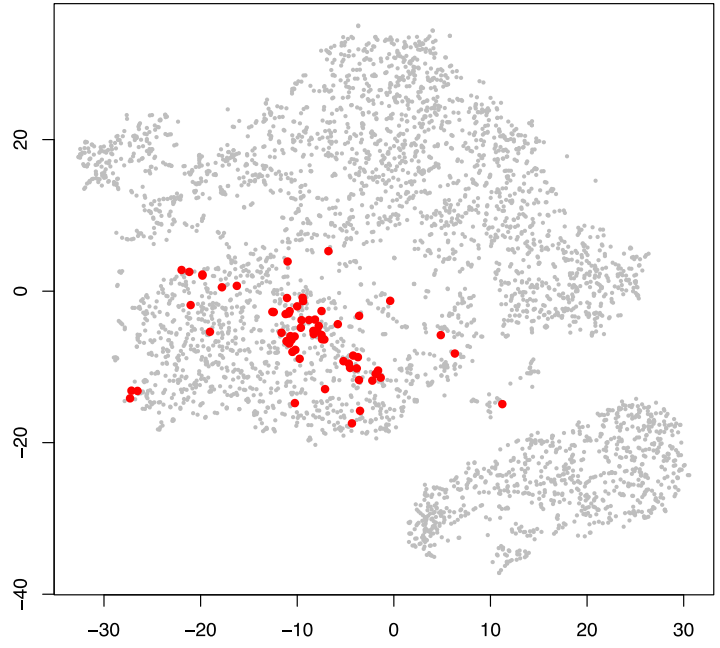



PDCD1
tSNE_2
tSNE_1
20
0
−20
−40
−20
0
20


ENTPD1
tSNE_2
tSNE_1
20
0
−20
−40
−20
0
20

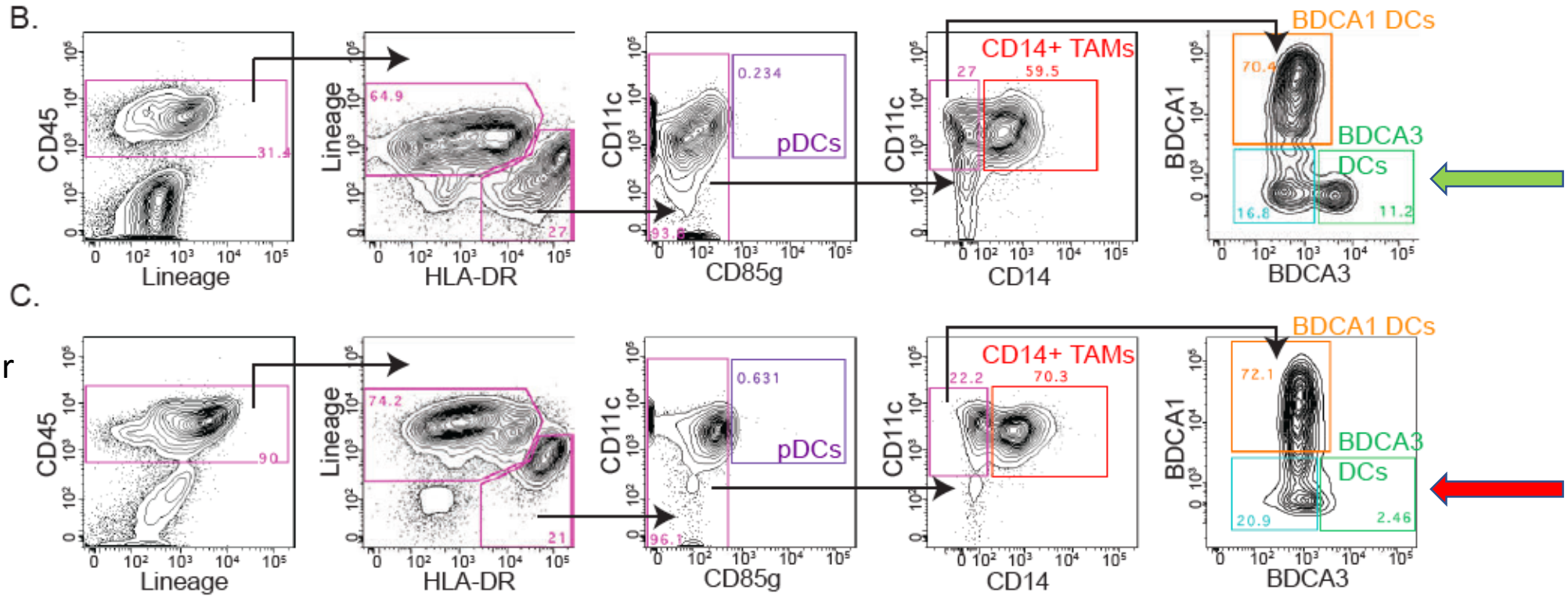

CD4+
Treg's
CD8+
0
1
2
3
4
5
6
7
8
9
10

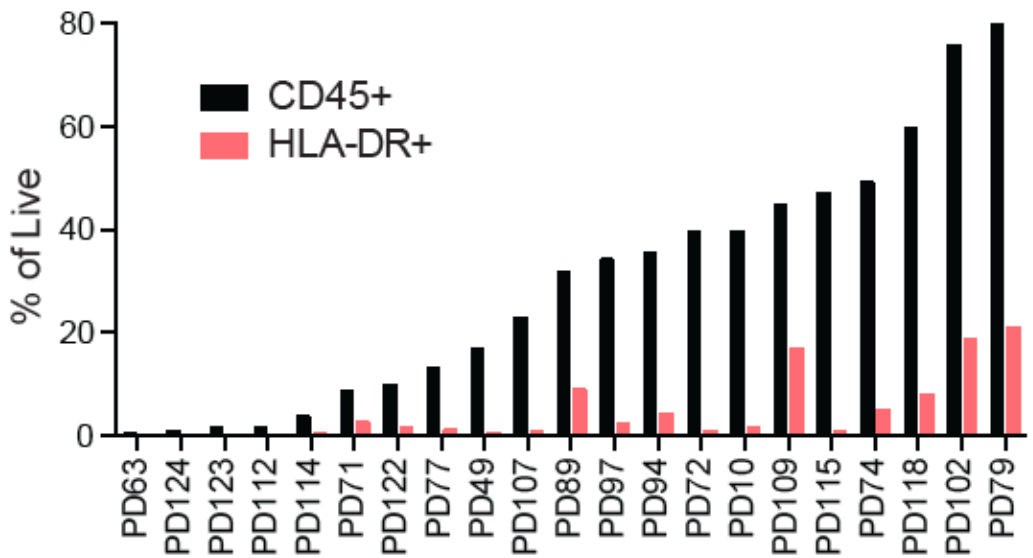

20
0
−20
−40
−30
−20
−10
0
10
20
30

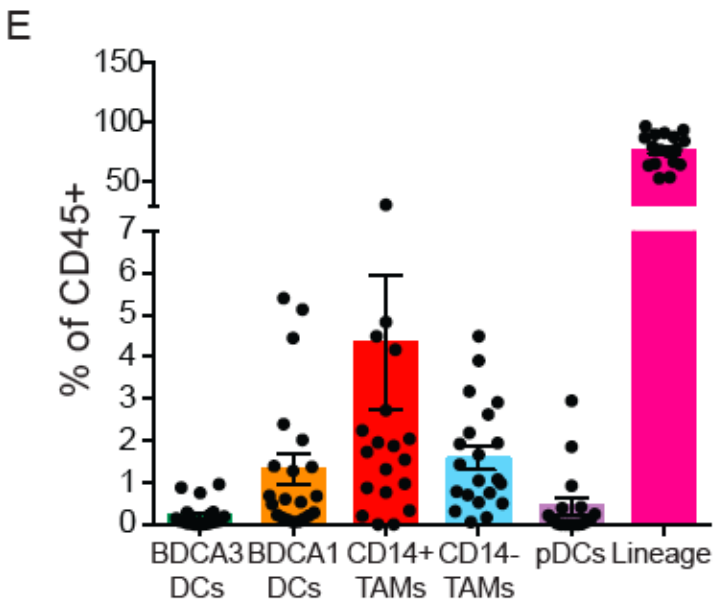

20
0
−20
−40
−30
−20
−10
0
10
20
30

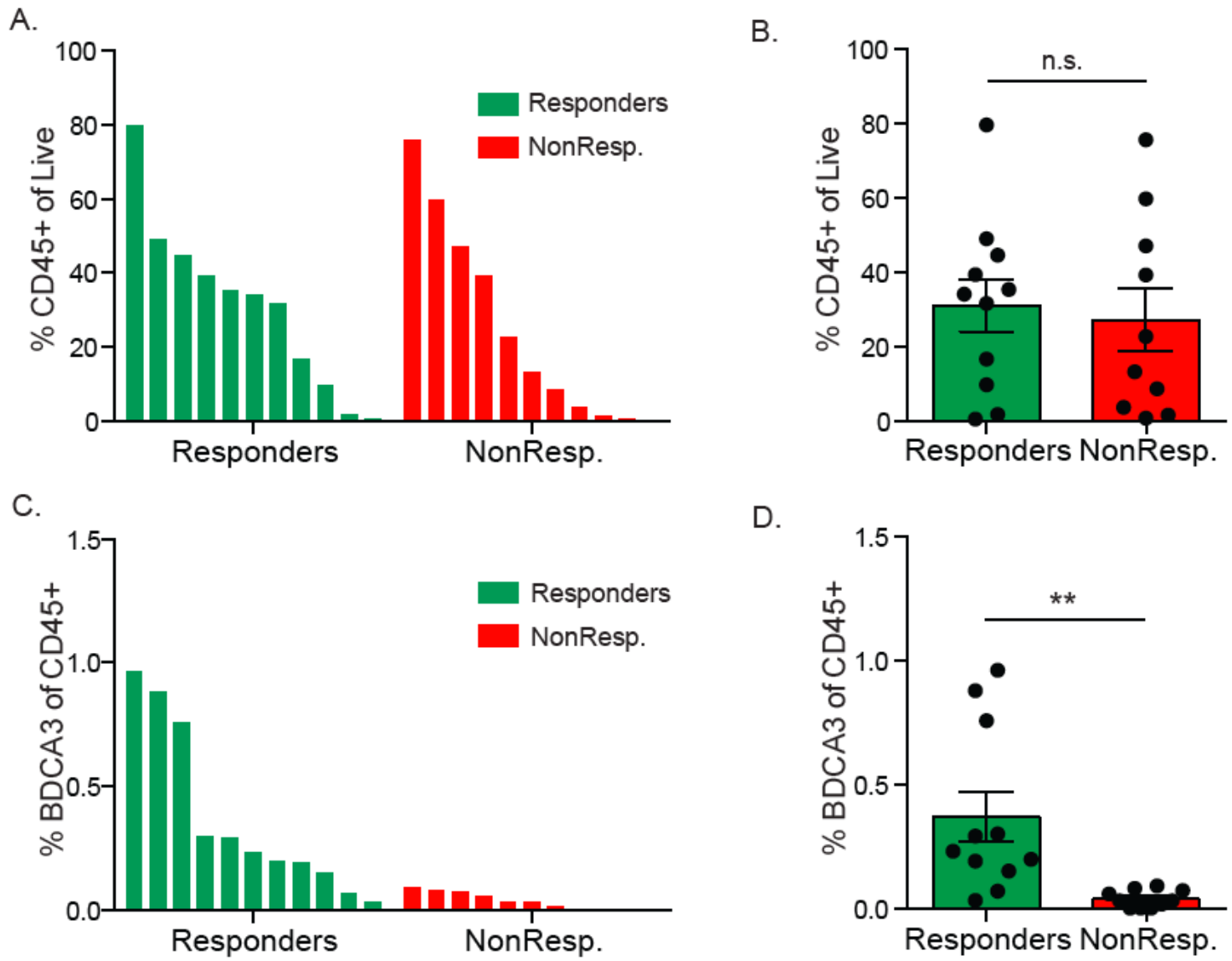
Responder

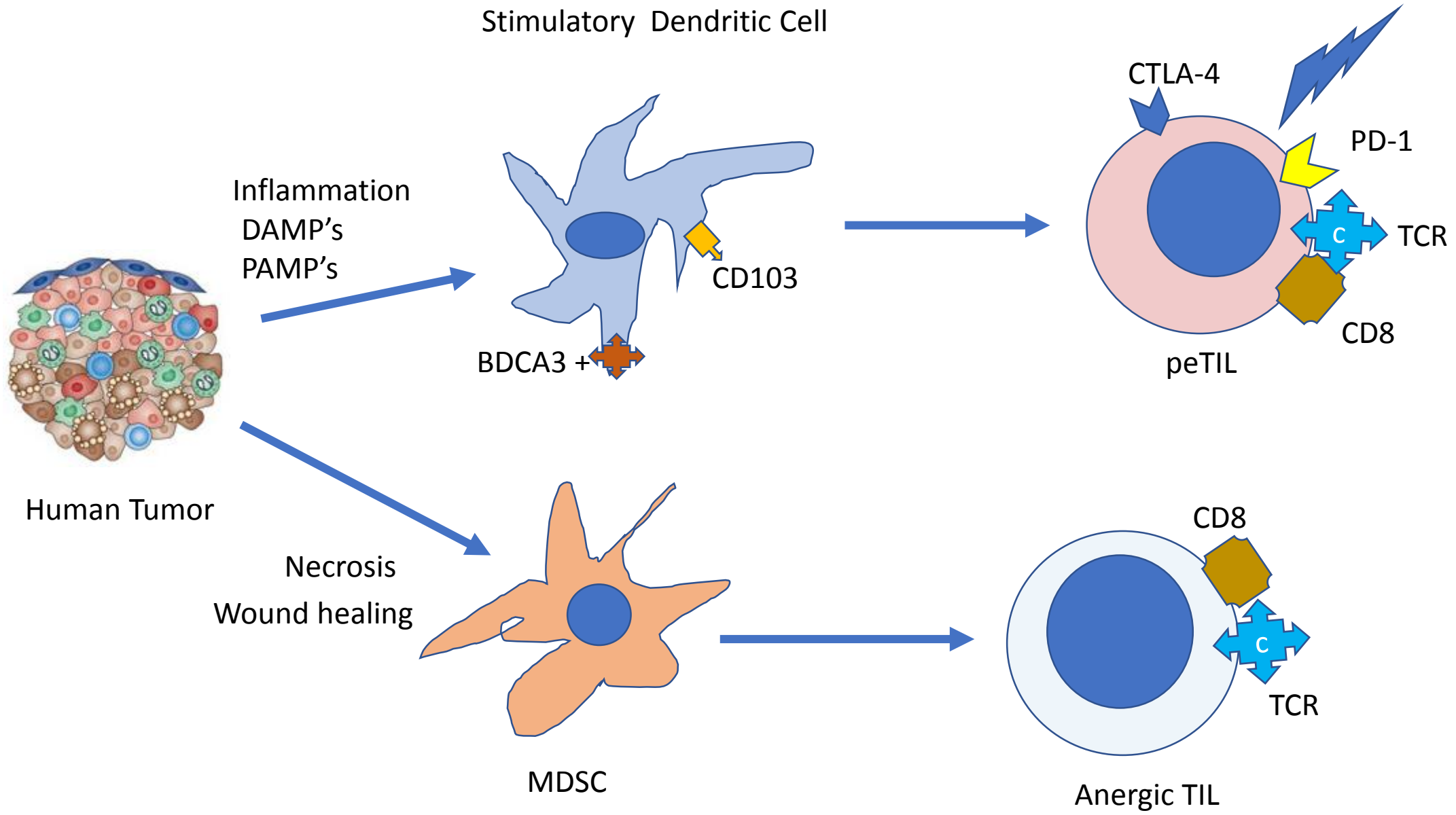
Non-Responder

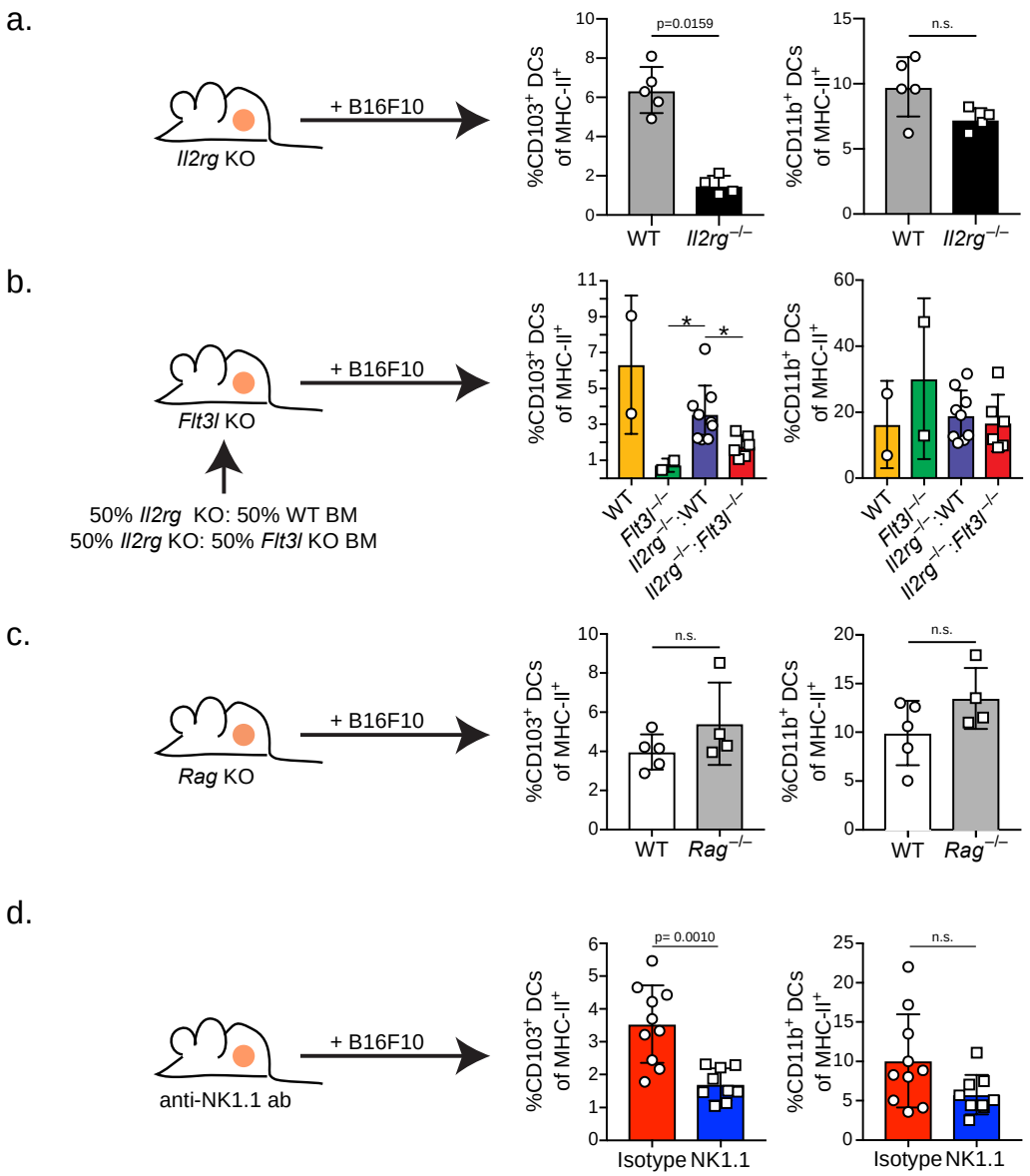


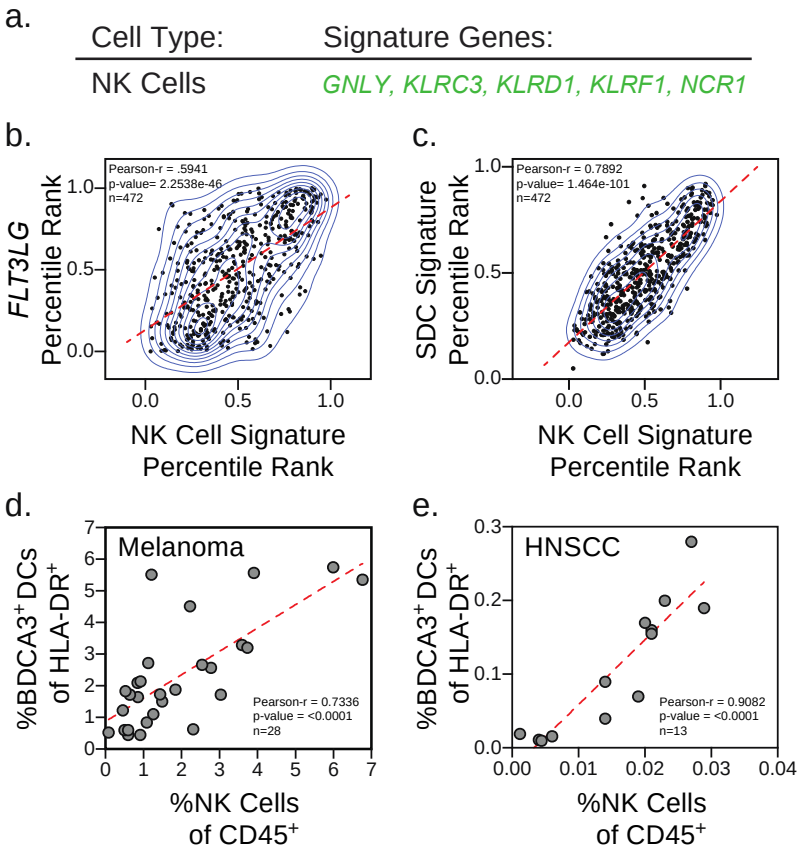





A.
100
80
60
40
20
0
% CD45+ of Live
Responders
NonResp.
Responders
NonResp.
B.
n.s.
100
80
60
40
20
0
% CD45+ of Live
Responders NonResp.
C.
1.5
1.0
0.5
0.0
% BDCA3 of CD45+
Responders
NonResp.
Responders
NonResp.
D.
**
1.5
1.0
0.5
0.0
% BDCA3 of CD45+
Responders NonResp.

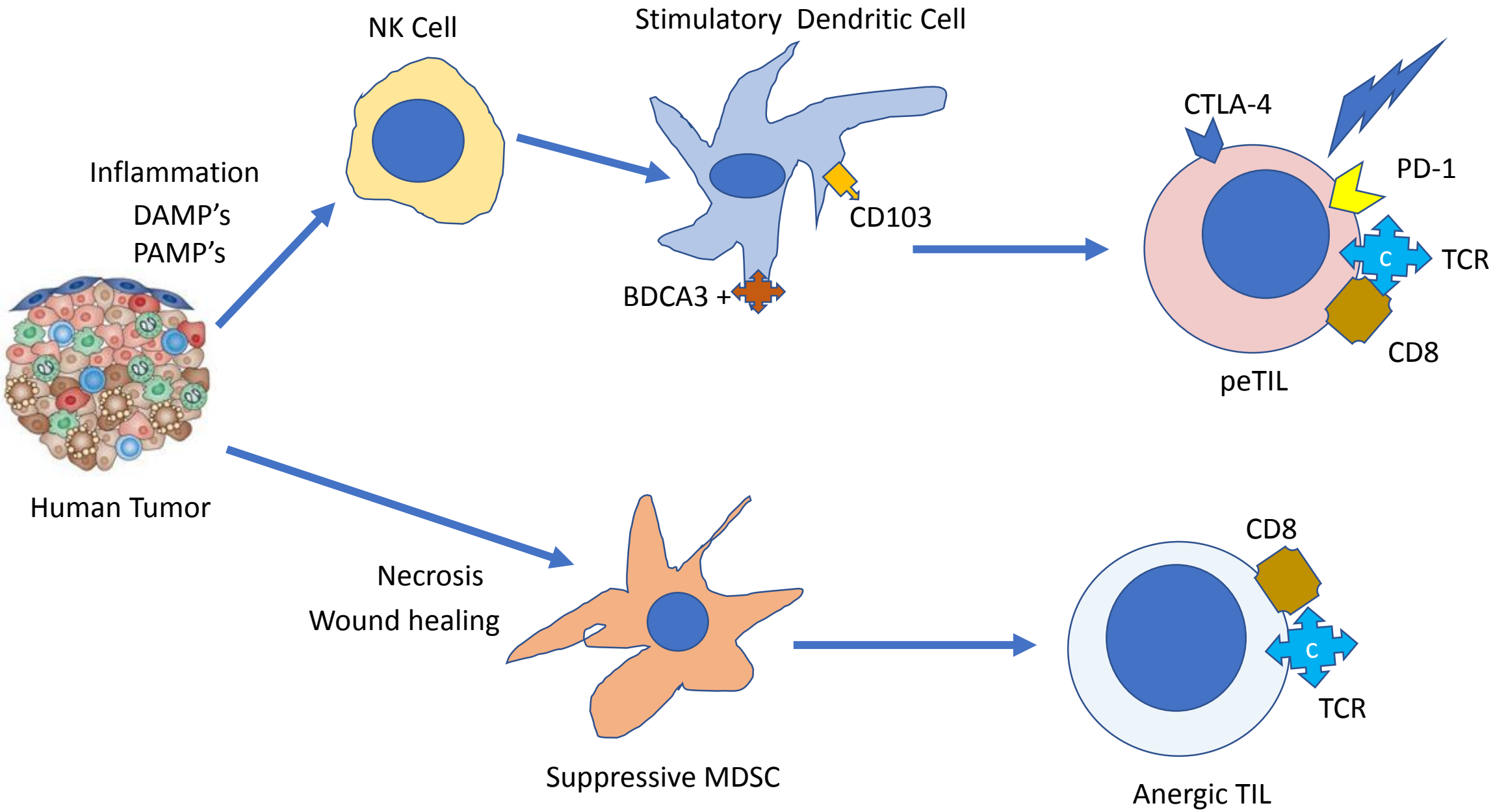

Stimulatory Dendritic Cell
Inflammation
DAMP's
PAMP's
CD103
BDCA3 +
CTLA-4
PD-1
C
TCR
CD8
peTIL
Human Tumor
Necrosis
Wound healing
MDSC
CD8
C
TCR
Anergic TIL

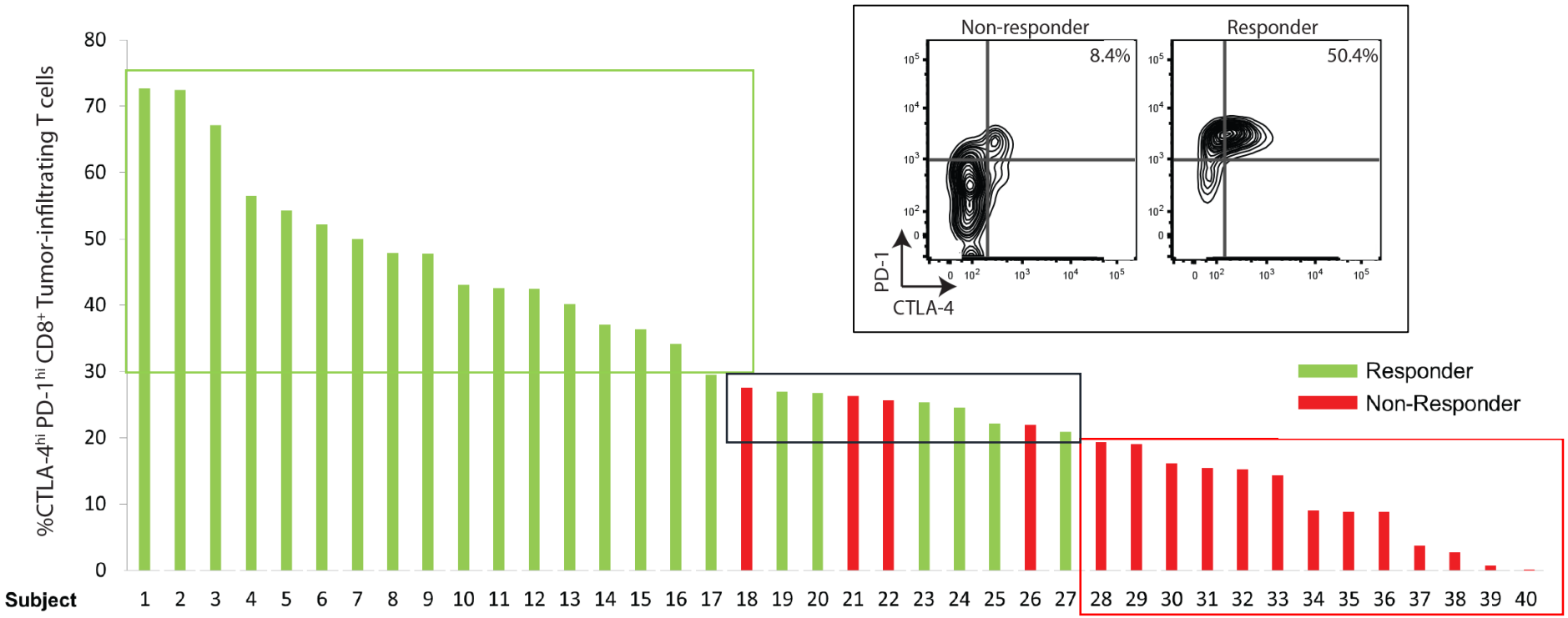




A natural killer–dendritic cell axis defines checkpoint therapy–responsive tumor microenvironments

Kevin C. Barry[1,2], Joy Hsu[1,2], Miranda L. Broz[1,2], Francisco J. Cueto[1,3,4], Mikhail Binnewies[1], Alexis J. Combes[1,2], Amanda E. Nelson[1,2], Kimberly Loo[2,5,6], Raj Kumar[1,2], Michael D. Rosenblum[6], Michael D. Alvarado[6], Denise M. Wolf[7], Dusan Bogunovic[8], Nina Bhardwaj[9], Adil I. Daud[6], Patrick K. Ha[10], William R. Ryan[10], Joshua L. Pollack[11], Bushra Samad[1,2], Saurabh Asthana[2], Vincent Chan[1,2] and Matthew F. Krummel[1,2*]

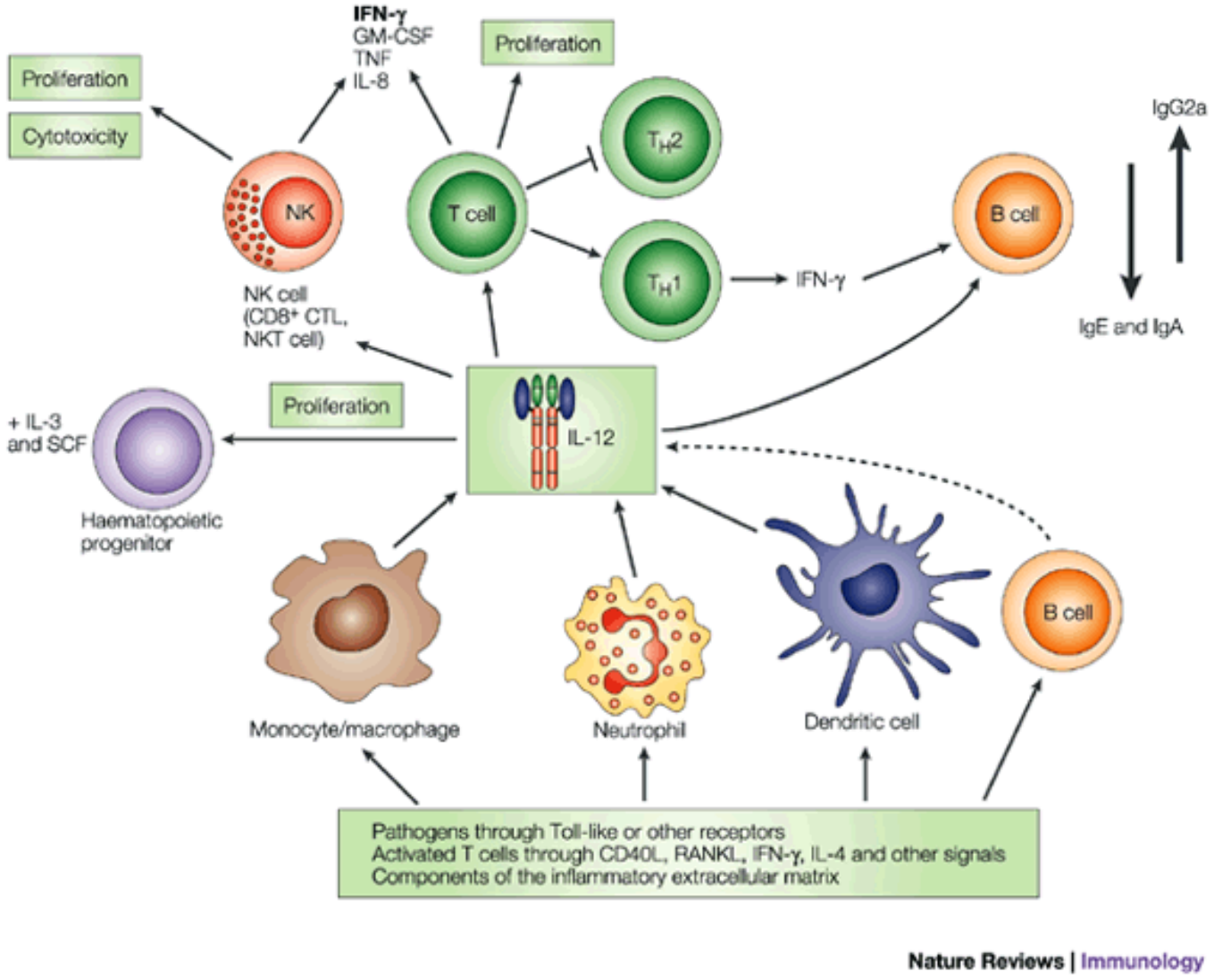

NK Cell
Stimulatory Dendritic Cell
Inflammation
DAMP's
PAMP's
CD103
BDCA3 +
CTLA-4
PD-1
TCR
CD8
peTIL
Human Tumor
Necrosis
Wound healing
Suppressive MDSC
CD8
TCR
Anergic TIL



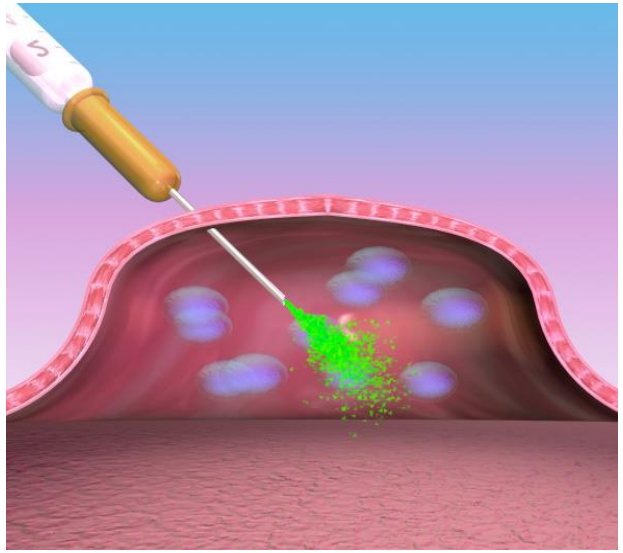
- 100% of patients with >30% of TILs exhibiting CTLA4^{hi}PD-1^{hi} biomarker phenotype went on to respond to anti-PD-1 (PR or CR)
- 100% of patients with <20% of TILs exhibiting CTLA4^{hi}PD-1^{hi} biomarker phenotype failed to respond to anti-PD-1 (SD or PD)
- 60% of patients with 20-30% of TILs exhibiting CTLA4^{hi}PD-1^{hi} biomarker phenotype responded to anti-PD-1 (PR or CR)

Daud (2016) JCI 126 (9): 3447

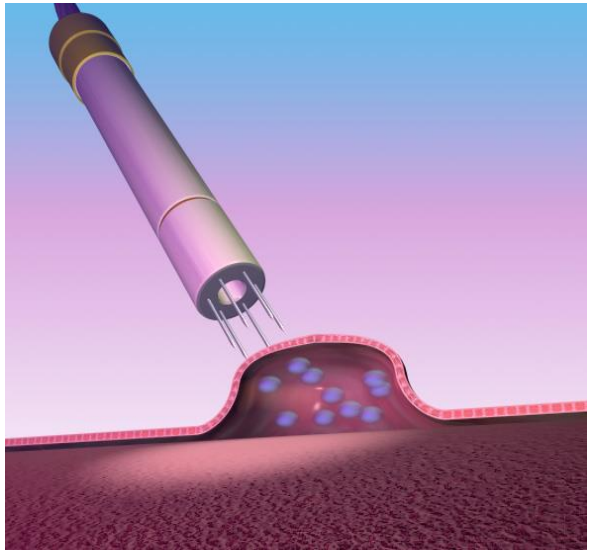
IL-12 is a key mediator of communication between DC/macrophages and effector-T cells and NK Cells



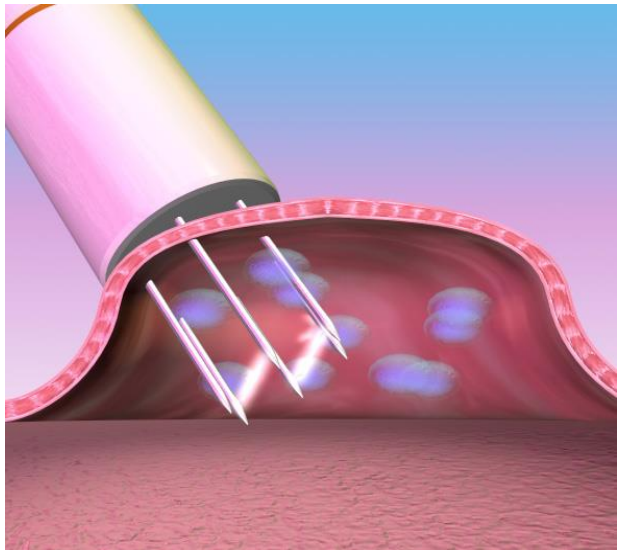
In-vivo Electroporation



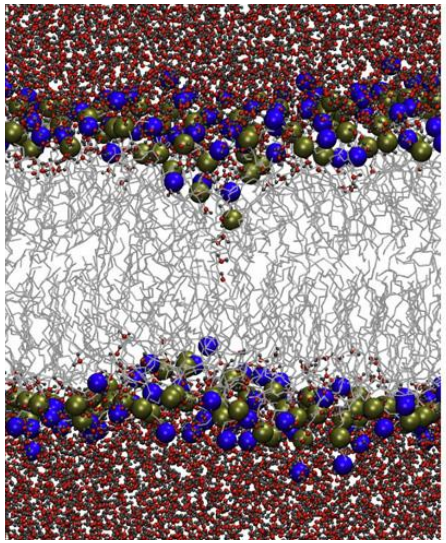
Injection of plasmid

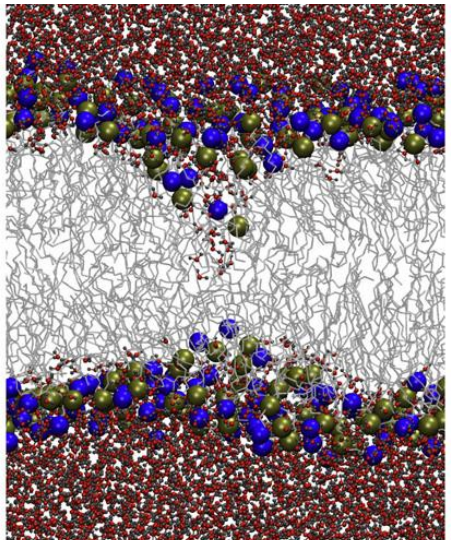


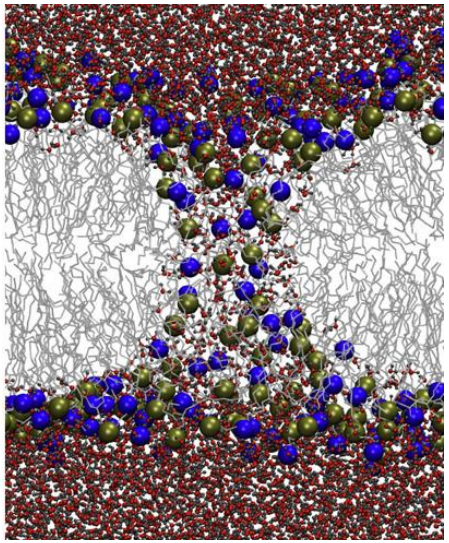
Electrode Insertion



Electroporation







Molecular Dynamics solution of
Hydrophilic channel formation
In membranes under electric fields
Young and Dean 2015
Takman et al 2013

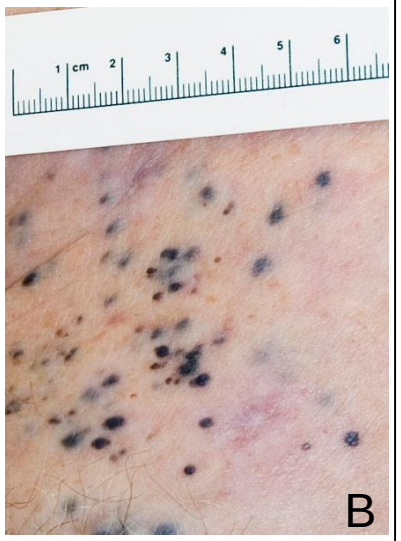
MEDPULSER
VACUTAINER

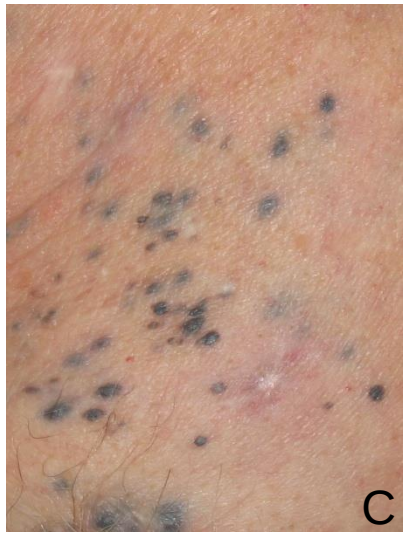
Pre-Tx

D 256

D 637

Patient 9
Cohort 3



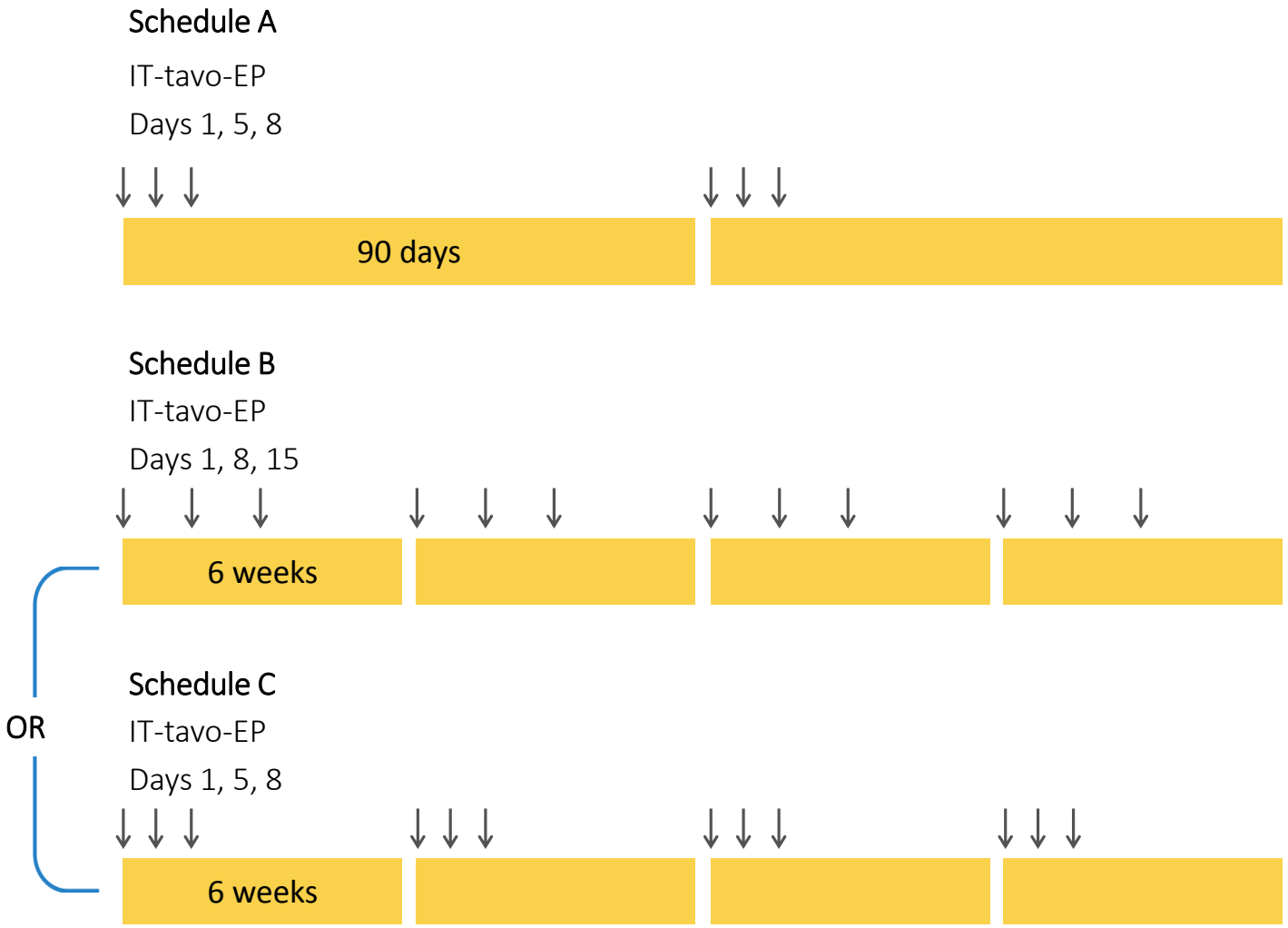


Chest

Back

Daud et al, Phase I trial of pIL-12 electroporation, J Clin Oncol

Phase II clinical Trial of pIL-12 in melanoma



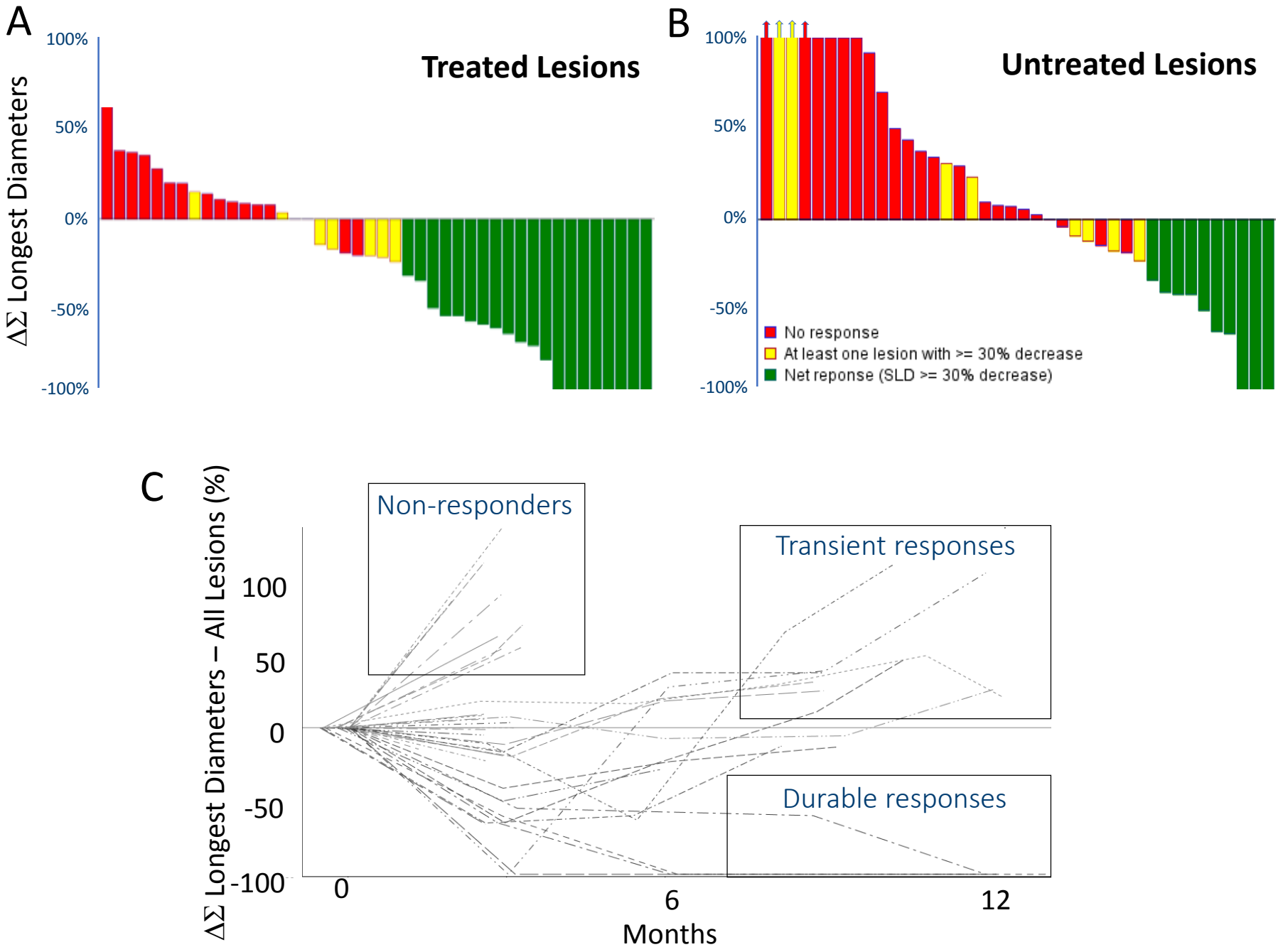
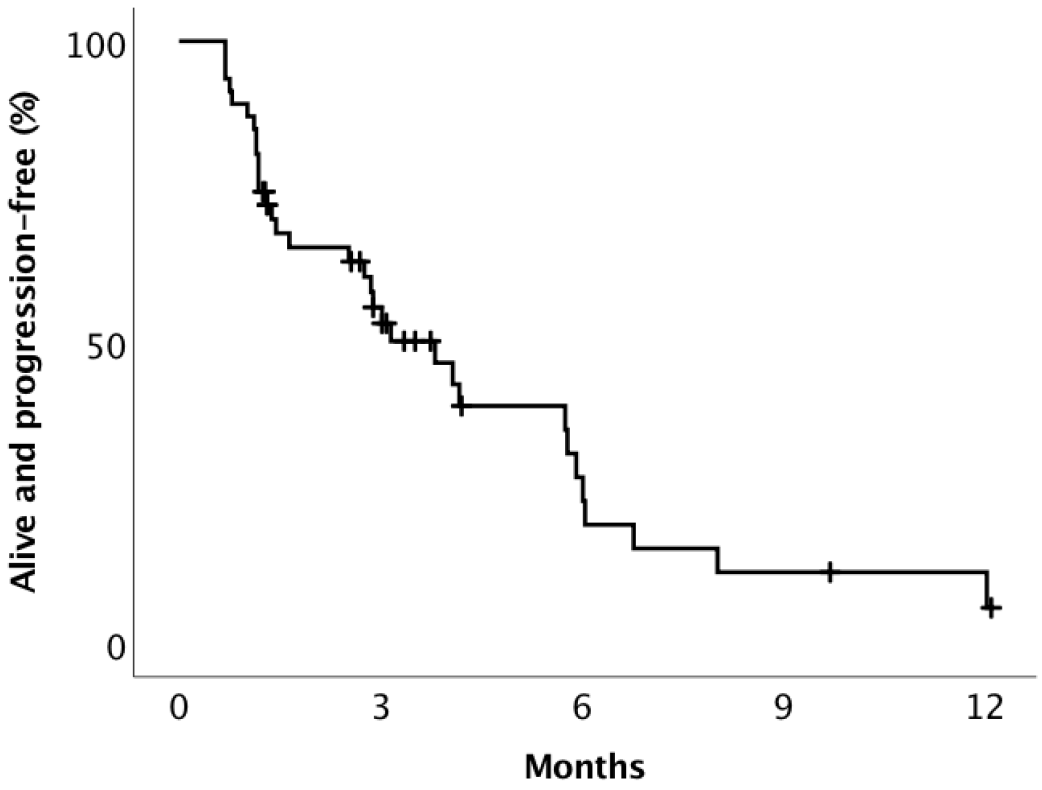
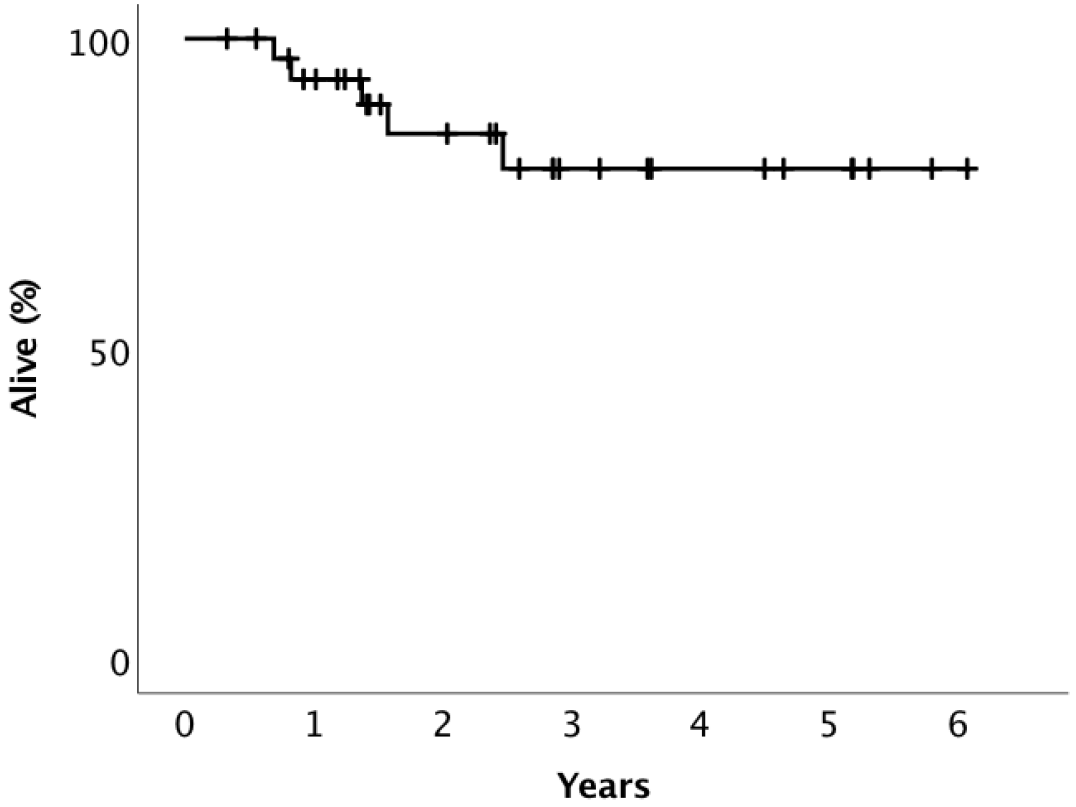


Figure 2. Best overall response in A. Sum of treated lesions and in B. Sum of untreated lesions. C. Overall change in tumor burden over time (N = 48).

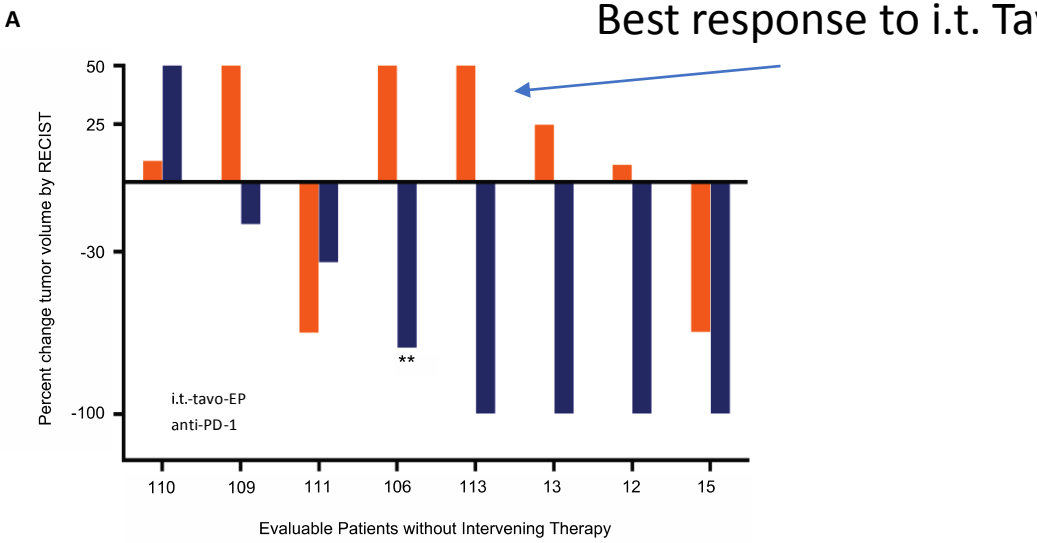
Figure 3. A. Progression-free survival in 48 patients with available data. The median overall survival was 3.8 months (90% C. I. 2.4 to 5.3 months). B. Overall survival in 33 patients with available data. The median overall survival was not reached at a median follow up of 28 months.



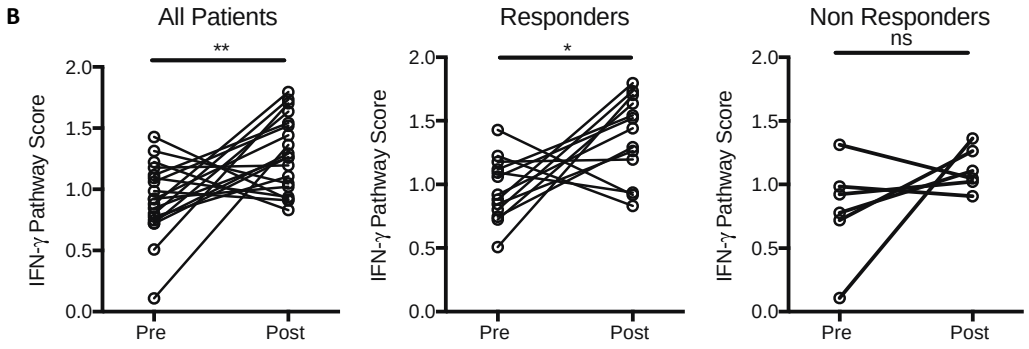


i.t. Tavo Monotherapy leads to increased CD8+ infiltration, PDL-1 and response to PD-1

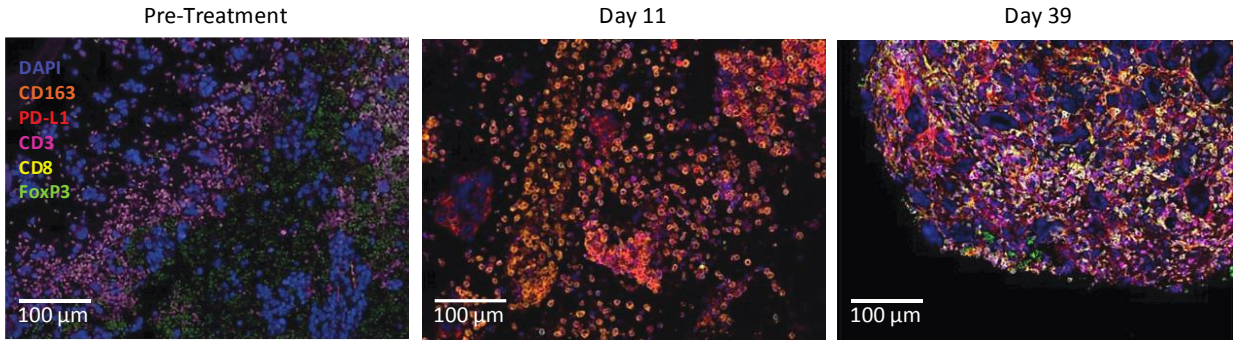
A



B

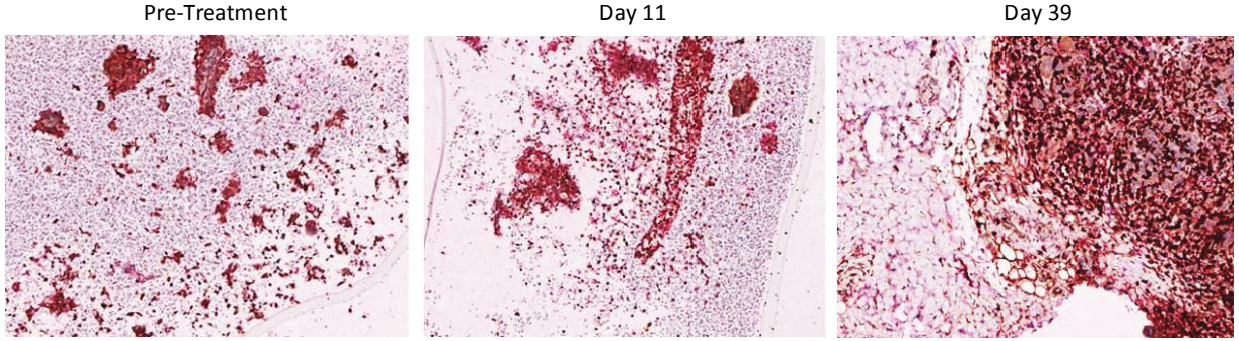


C

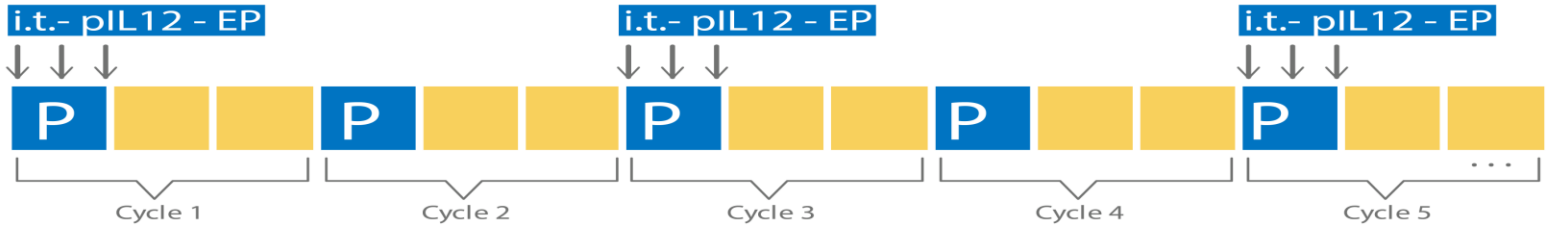


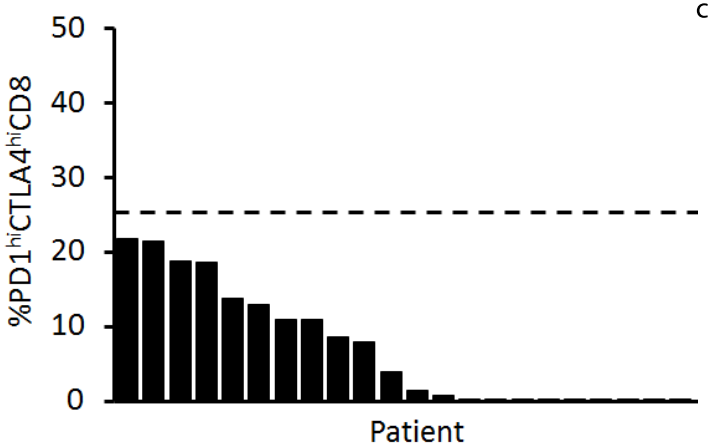
$CD8^+$: $PD\text{-}L1^+$ cell ratio (relative to pre-treatment)	0.08	2.46
$CD8^+$: $FoxP3^+$ cell ratio (relative to pre-treatment)	13.00	41.00

D

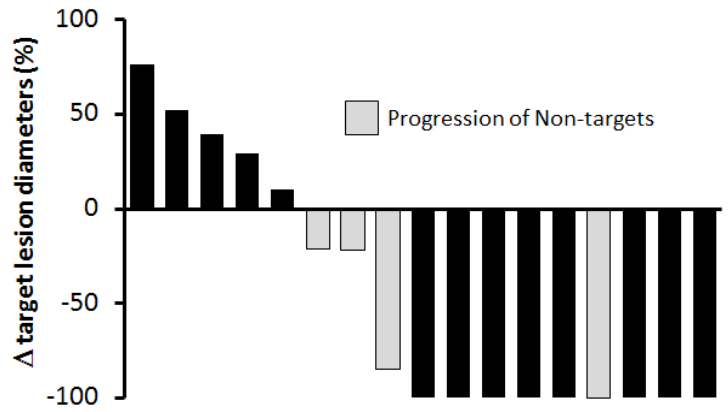


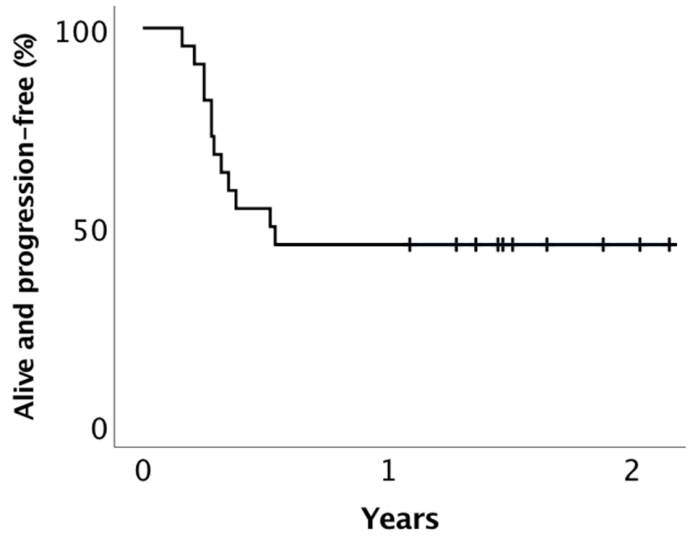
% $CD3^+CD8^+$ Cells	8.7	15	24


i.t.- pIL12 - EP
i.t.- pIL12 - EP
i.t.- pIL12 - EP
P
P
P
P
P
Cycle 1
Cycle 2
Cycle 3
Cycle 4
Cycle 5

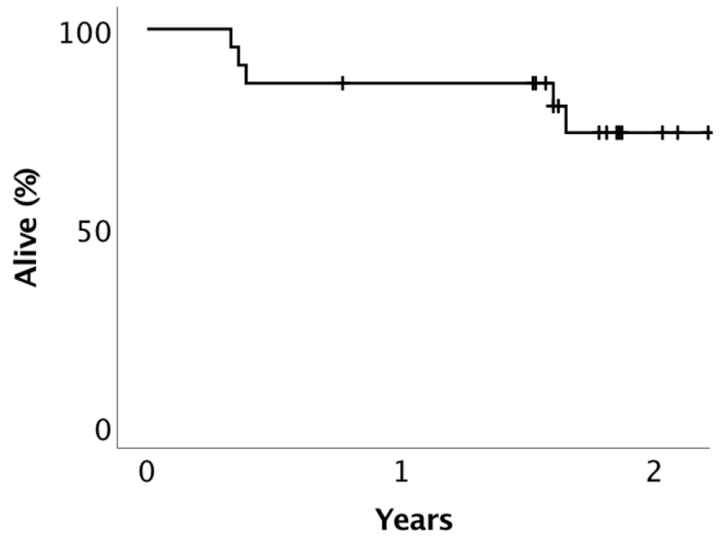

%PD1hiCTLA4hiCD8
50
40
30
20
10
0
Patient

c

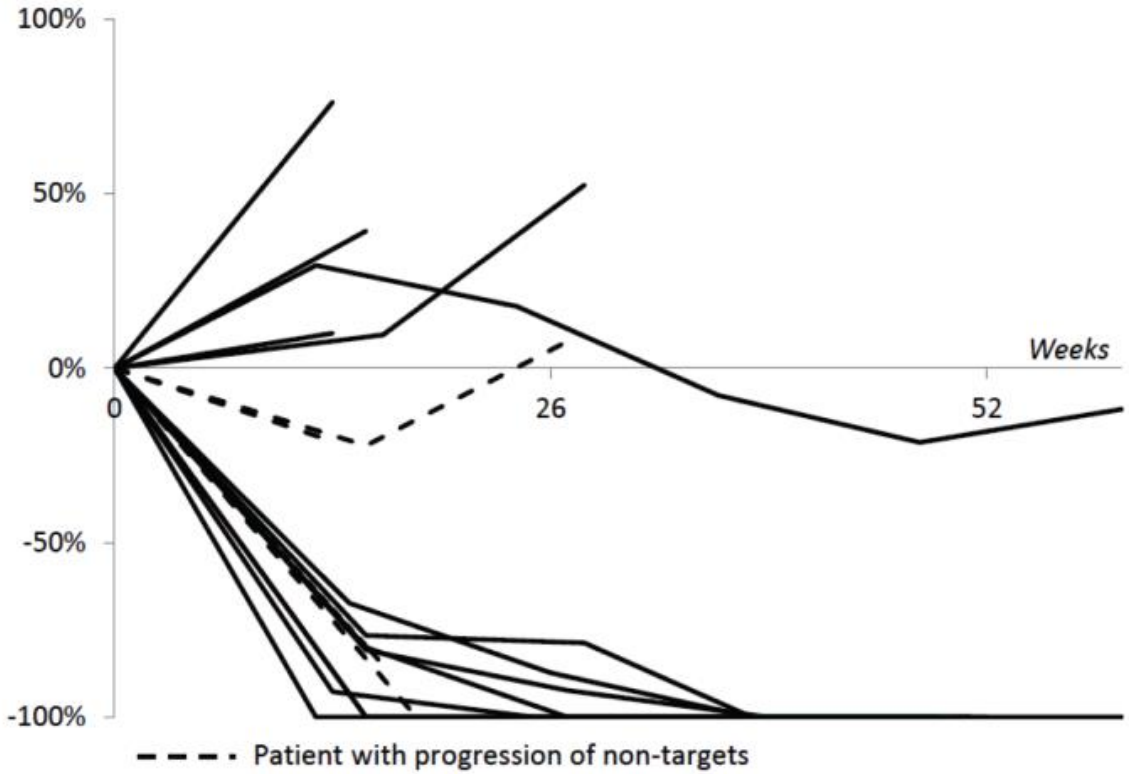

Δ target lesion diameters (%)
100
50
0
-50
-100
Progression of Non-targets


Alive and progression-free (%)
100
50
0
0
1
2
Years

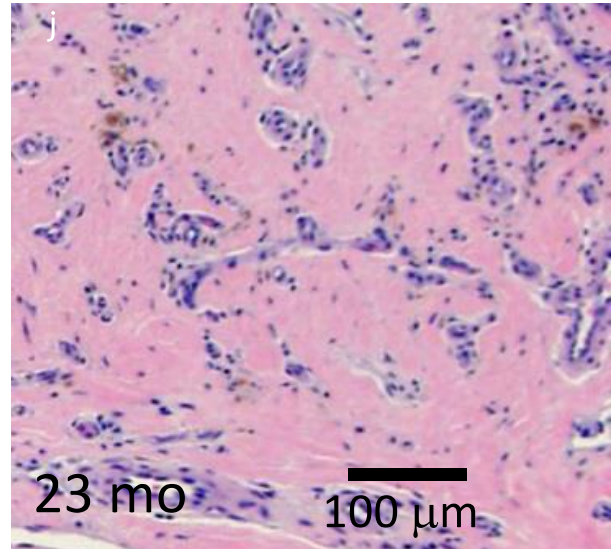
f

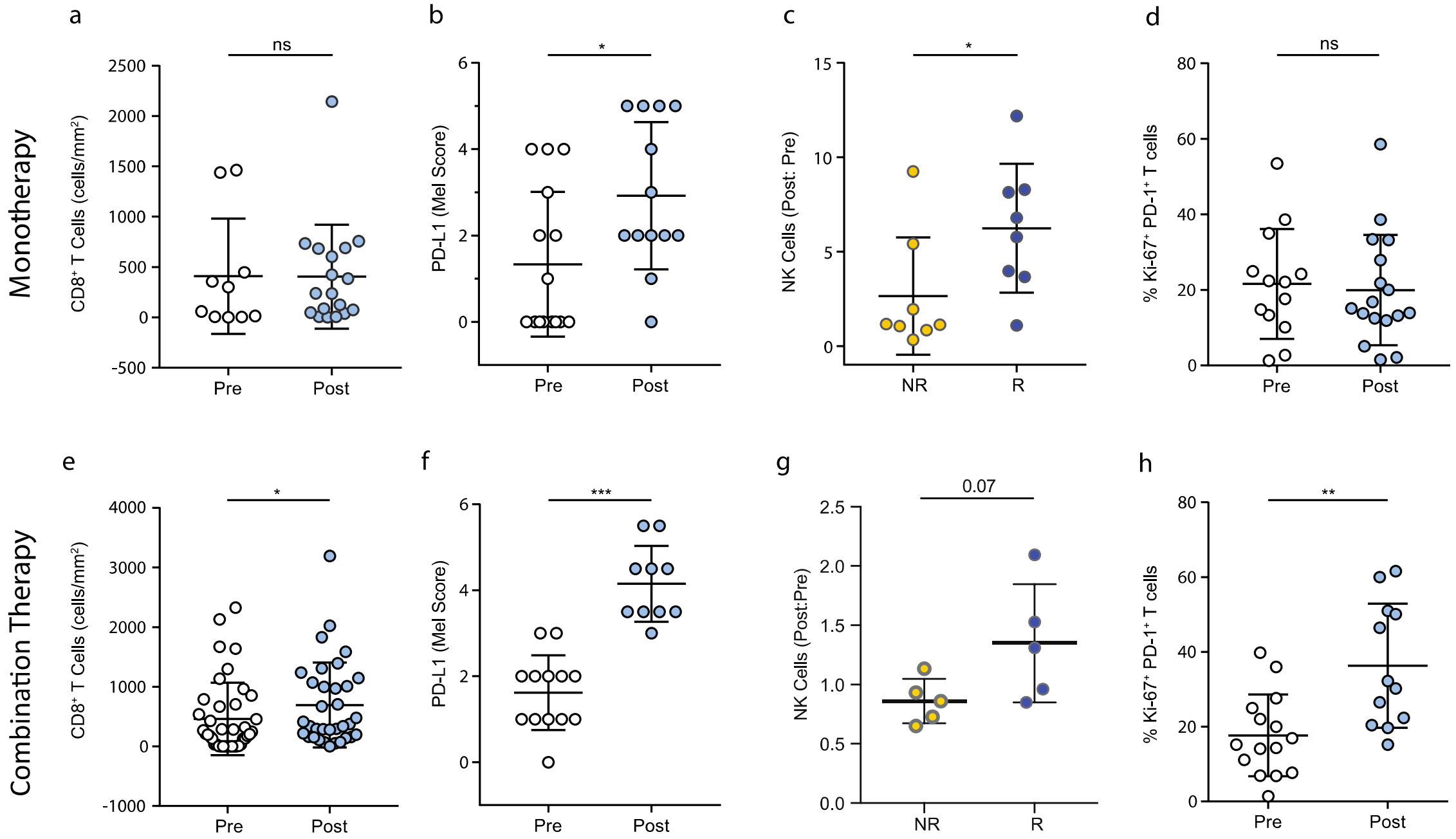

Alive (%)
100
50
0
0
1
2
Years

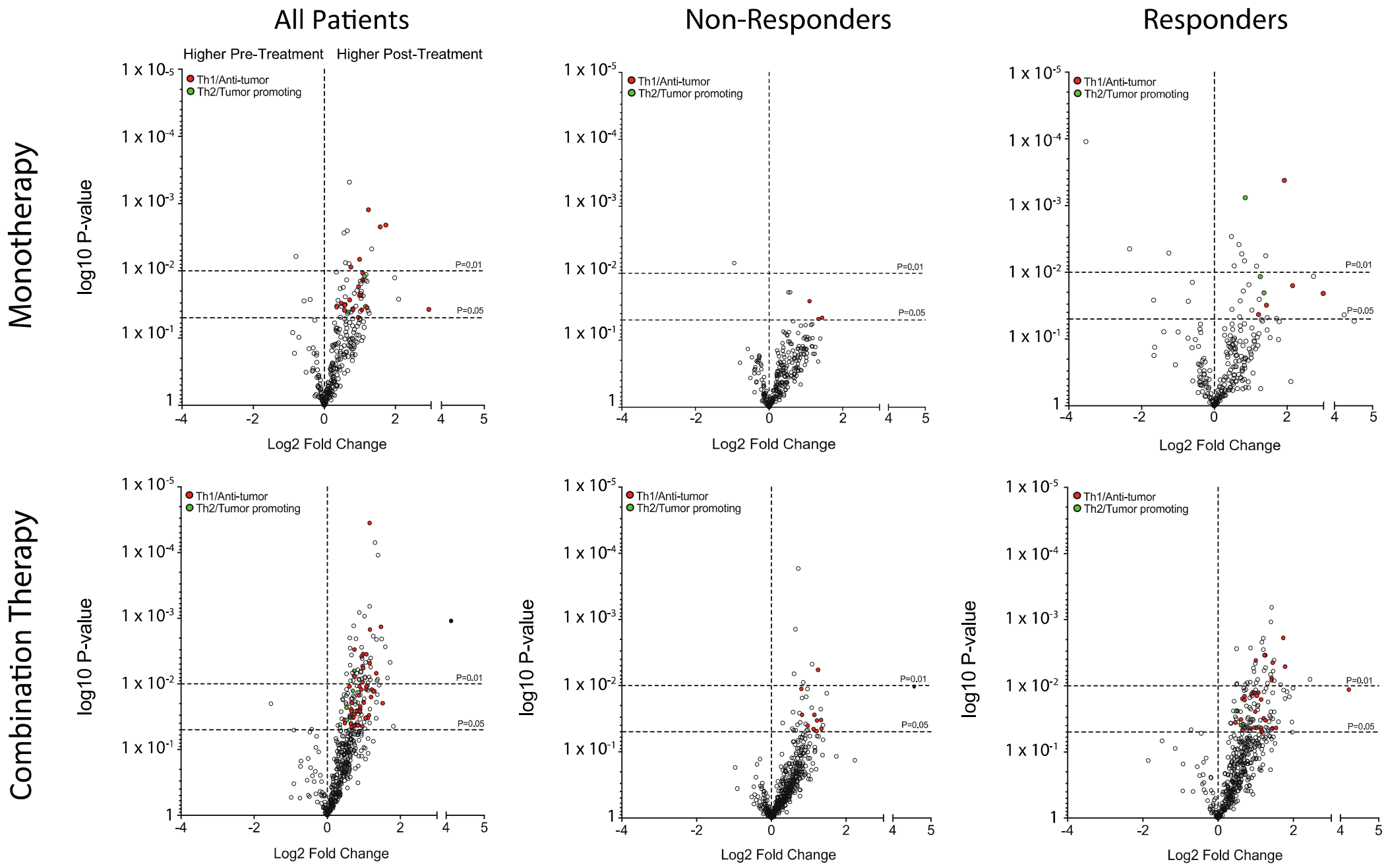
Pre-Tx peCTL%
by RECIST
by Clinical Assessmnet
22 PD PD
11 CR CR
NA PD PR
0 PD PD
8 CR CR
1 PD PD
4 CR CR
9 PD PD
<1 SD SD
2 PD PD
<1 CR CR
22 CR CR
19 PD PD
<1 SD SD
11 CR CR
14 CR CR
13 PD PD
19 CR CR
0 § CR
NA PD PD
NA PR PR
NA PD PD

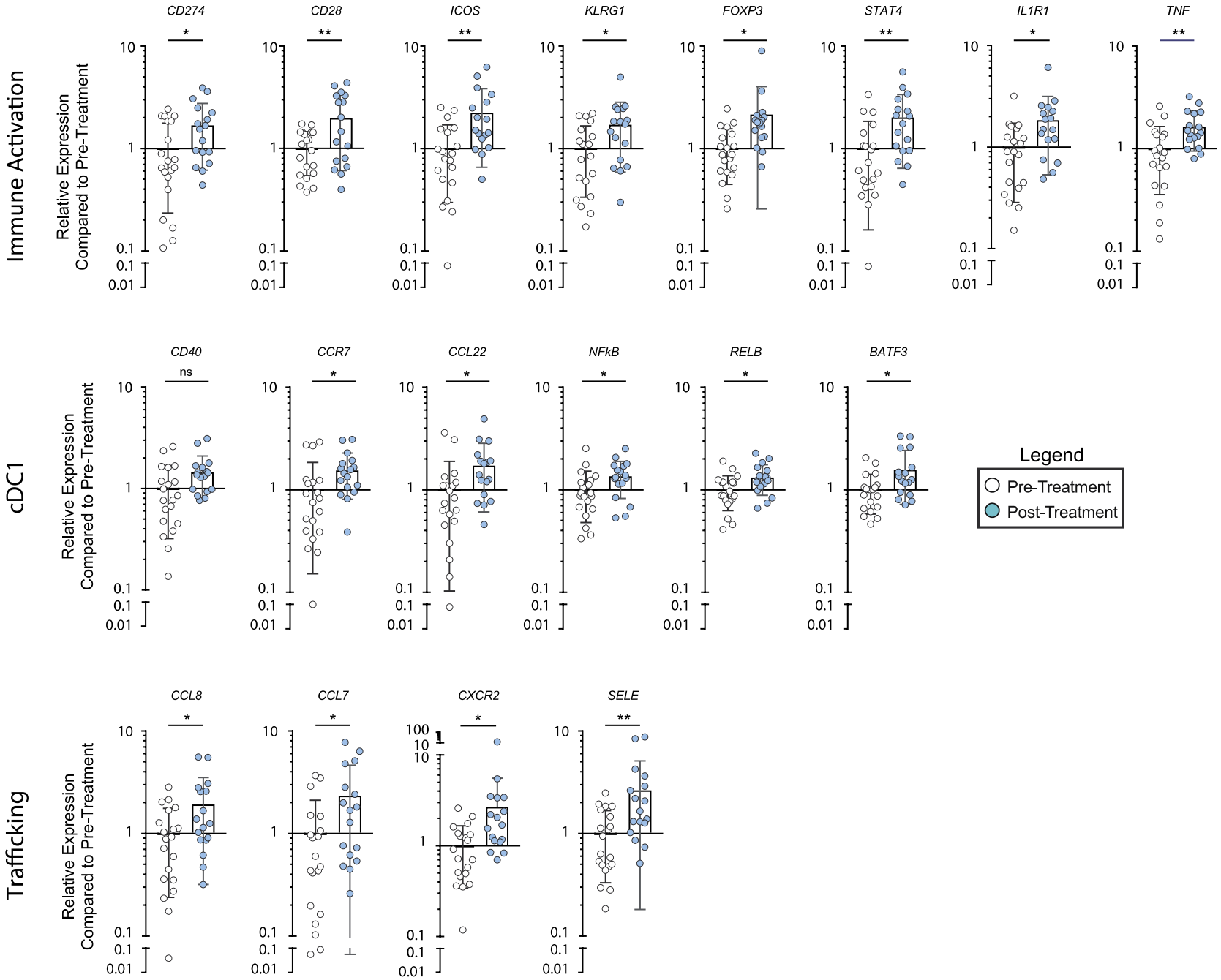


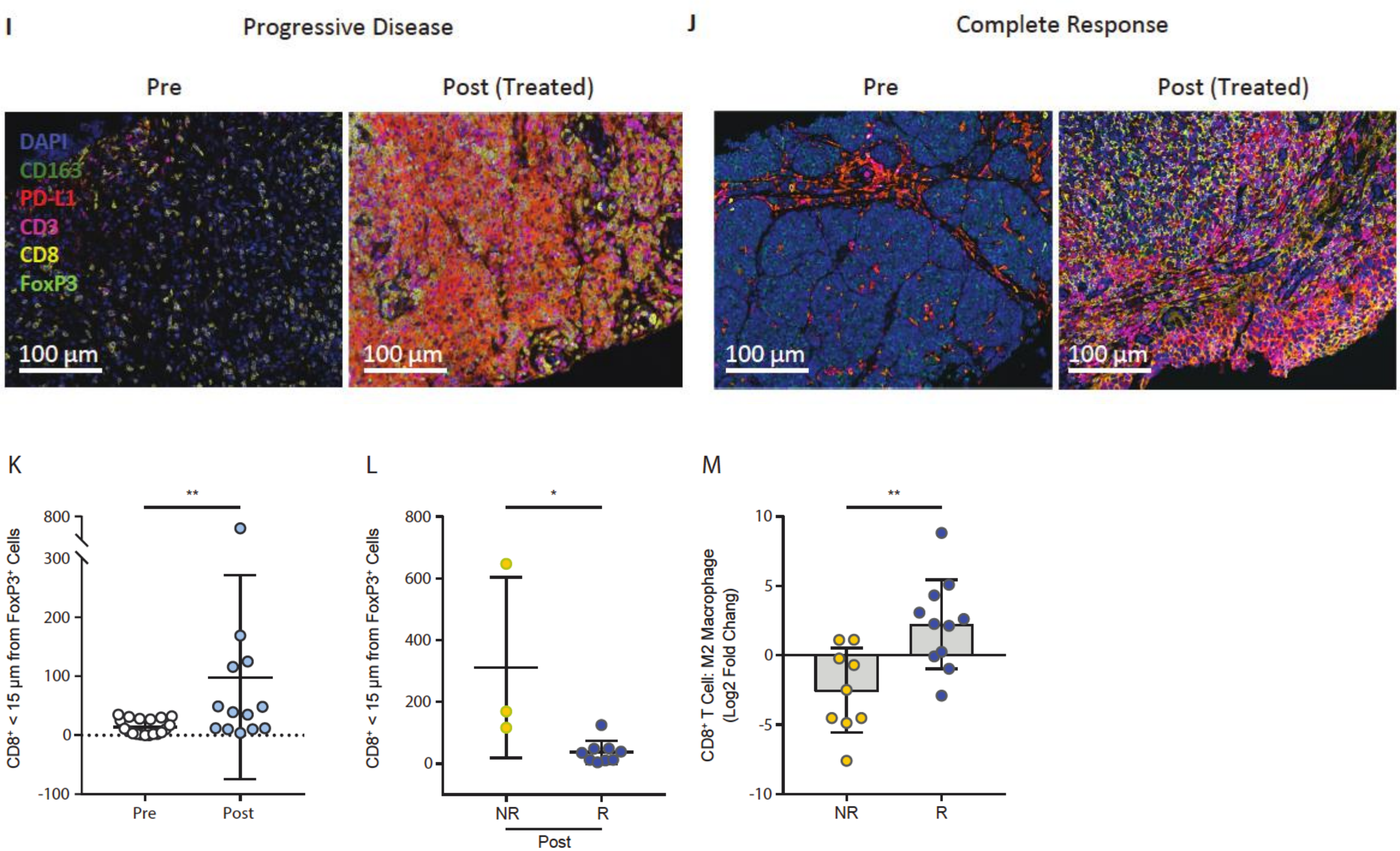
PD by RECIST
s/p ipilimumab
Nivolumab and Pembrolizumab
Extensive disease in the left LE

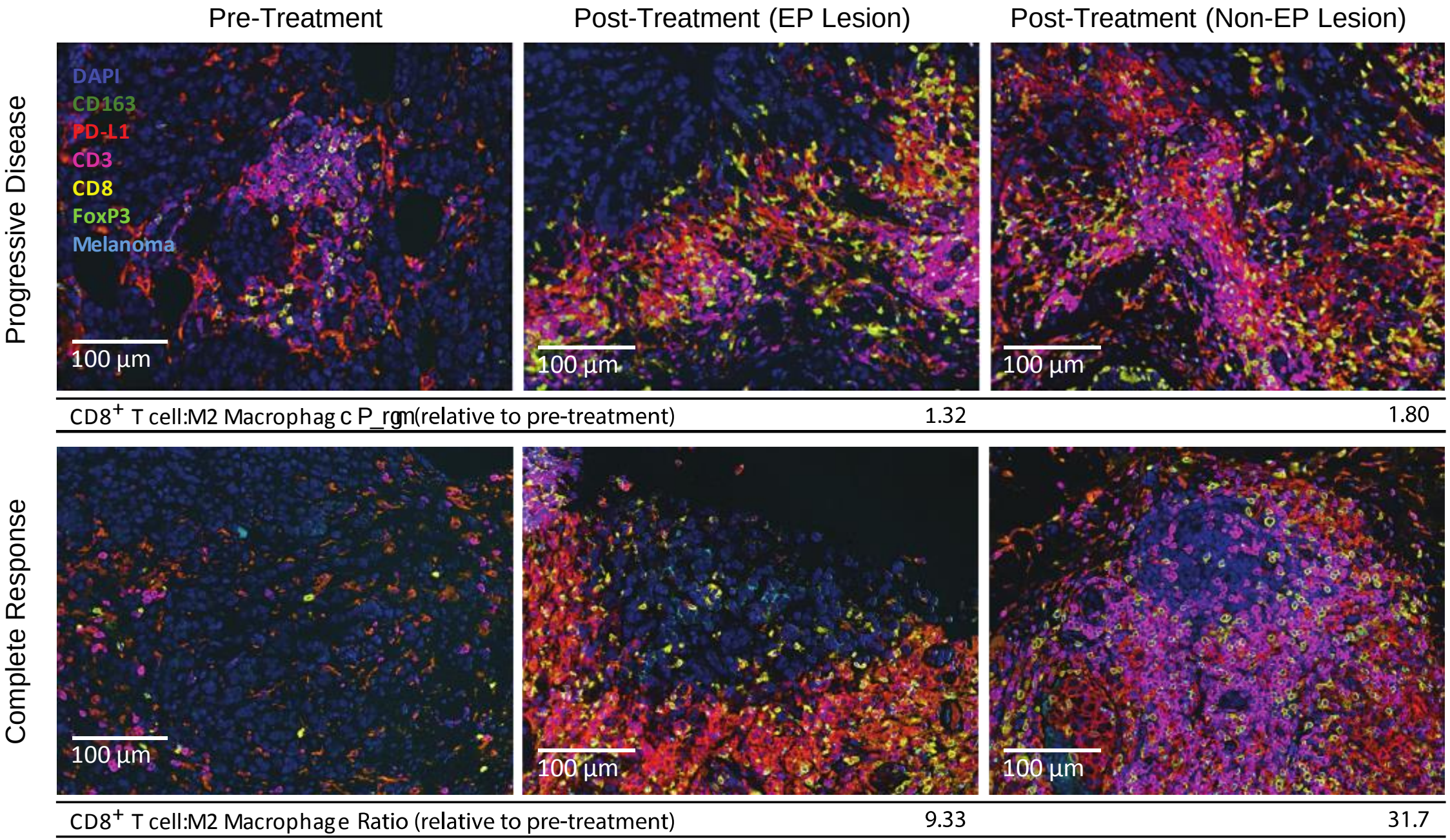


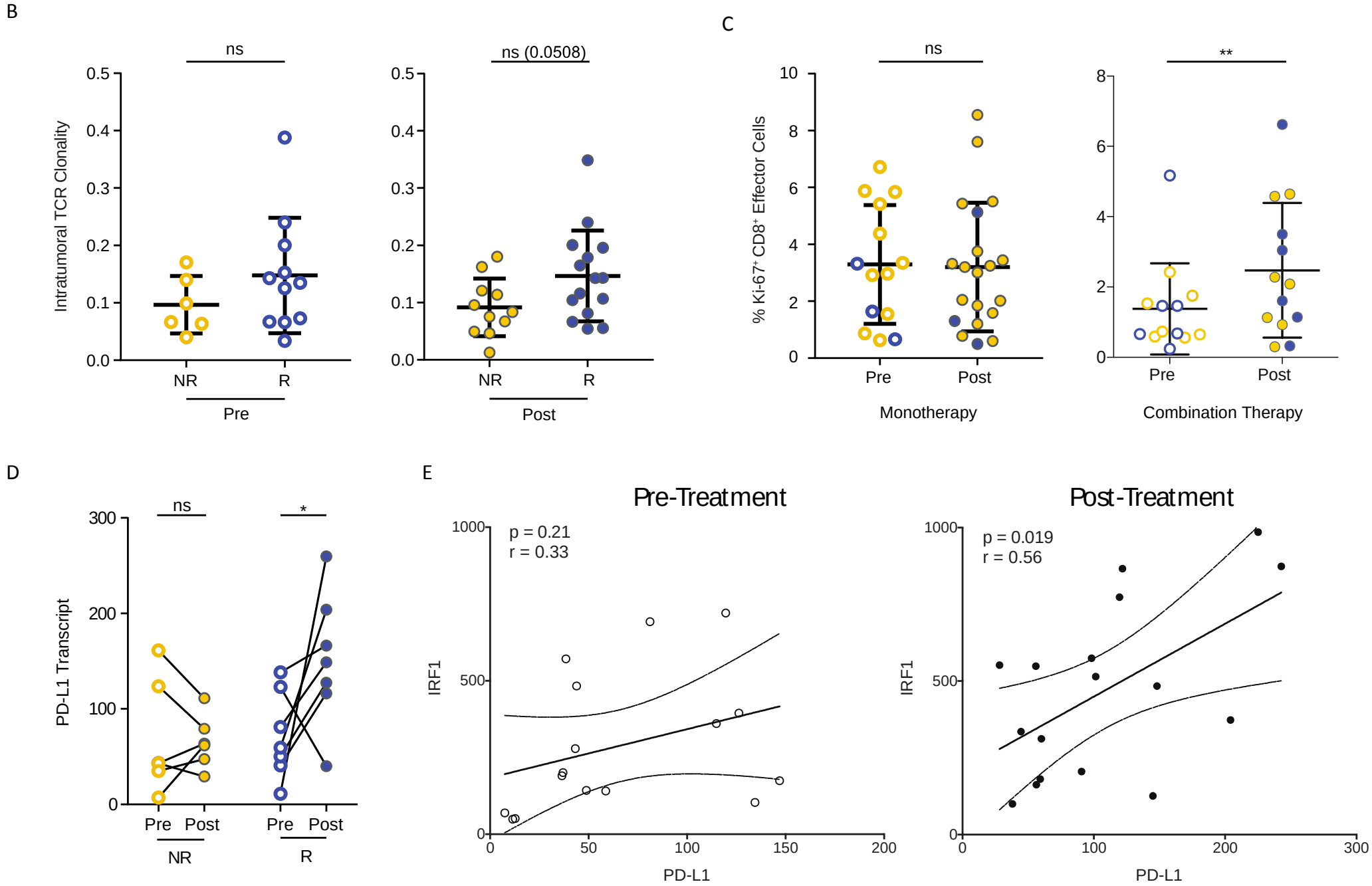

Monotherapy
Combination Therapy
a
b
c
d
e
f
g
h
ns
*
*
ns
*
***
0.07
**
CD8⁺ T Cells (cells/mm²)
PD-L1 (Mel Score)
NK Cells (Post: Pre)
% Ki-67⁺ PD-1⁺ T cells
NK Cells (Post:Pre)
Pre
Post
NR
R


All Patients
Non-Responders
Responders
Monotherapy
Combination Therapy
Higher Pre-Treatment
Higher Post-Treatment
Th1/Anti-tumor
Th2/Tumor promoting
log10 P-value
Log2 Fold Change
P=0.01
P=0.05
1 x 10-5
1 x 10-4
1 x 10-3
1 x 10-2
1 x 10-1
1
-4
-2
0
2
4
5


Immune Activation
cDC1
Trafficking
Relative Expression
Compared to Pre-Treatment
CD274
CD28
ICOS
KLRG1
FOXP3
STAT4
IL1R1
TNF
CD40
CCR7
CCL22
NFkB
RELB
BATF3
CCL8
CCL7
CXCR2
SELE
ns
Legend
Pre-Treatment
Post-Treatment


I
Progressive Disease
Pre
Post (Treated)
DAPI
CD163
PD-L1
CD3
CD8
FoxP3
100 µm
100 µm
J
Complete Response
Pre
Post (Treated)
100 µm
100 µm
K
**
CD8+ < 15 µm from FoxP3+ Cells
800
300
200
100
0
-100
Pre
Post
L
*
CD8+ < 15 µm from FoxP3+ Cells
800
600
400
200
0
NR
R
Post
M
**
CD8+ T Cell: M2 Macrophage (Log2 Fold Chang)
10
5
0
-5
-10
NR
R


Pre-Treatment
Post-Treatment (EP Lesion)
Post-Treatment (Non-EP Lesion)
Progressive Disease
DAPI
CD163
PD-L1
CD3
CD8
FoxP3
Melanoma
100 µm
100 µm
100 µm
CD8+ T cell:M2 Macrophage Ratio (relative to pre-treatment)
1.32
1.80
Complete Response
100 µm
100 µm
100 µm
CD8+ T cell:M2 Macrophage Ratio (relative to pre-treatment)
9.33
31.7


B
ns
ns (0.0508)
Intratumoral TCR Clonality
0.5
0.4
0.3
0.2
0.1
0.0
NR
R
Pre
Post
C
ns
**
% Ki-67+ CD8+ Effector Cells
10
8
6
4
2
0
Pre
Post
Monotherapy
Combination Therapy
D
ns
*
PD-L1 Transcript
300
200
100
0
Pre
Post
NR
R
E
Pre-Treatment
Post-Treatment
p = 0.21
r = 0.33
p = 0.019
r = 0.56
IRF1
PD-L1
1000
500
0
50
100
150
200
300

Conclusions

- PD-1 agents are incredibly active in cancer
  - But resistance develops and non response is a major issue
- Other immunotherapy checkpoints and approaches are being evaluated
- TME is a complex environment that varies by tumor type and mutation burden

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  - Kimberly Loo MD