Disclosures

Scientific advisor and advisory boards:
Jounce, BMS, GSK, MedImmune

Pre-Surgical Clinical Trial Design for Immune Monitoring

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GU Medical Oncology & Immunology

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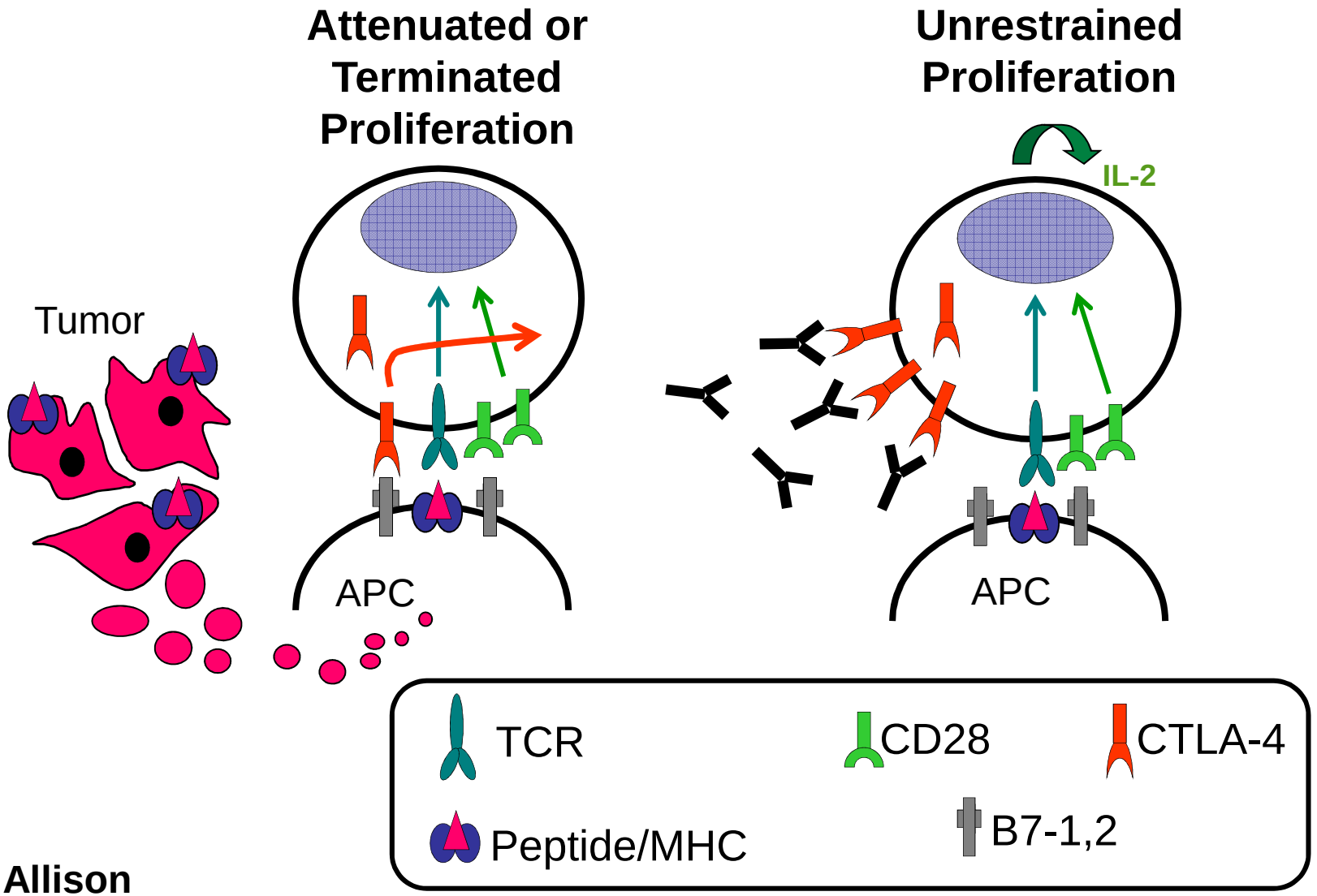
SITC Primer

November 6, 2014

CTLA-4 blockade enhances T cell anti-tumor responses, regardless of tumor type

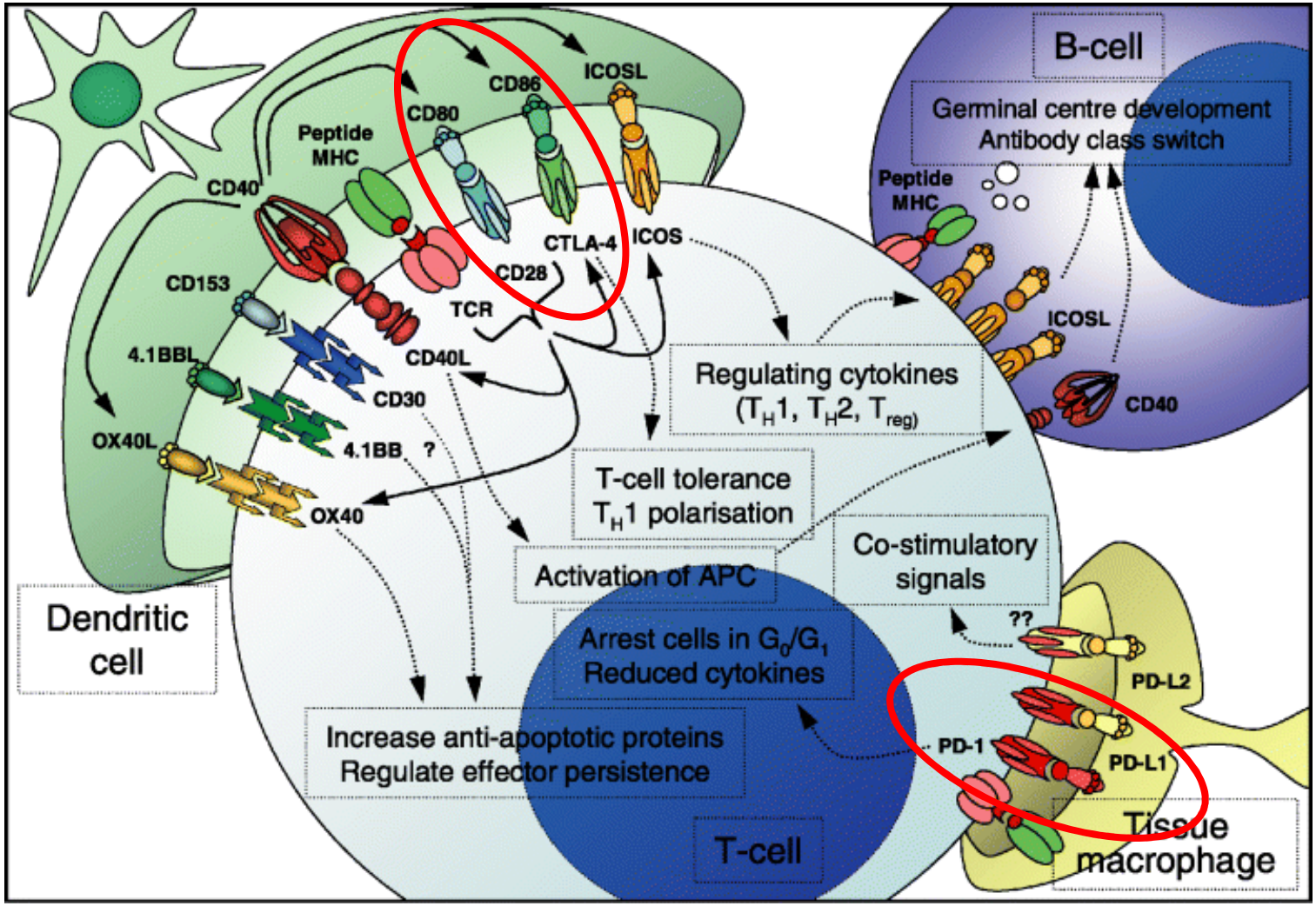


JP Allison

Summary of anti-CTLA-4 antibody (ipilimumab) as treatment for cancer

- **CTLA-4 blockade elicits anti-tumor responses**
- **Durable partial and complete regression of disease observed**
- **Survival benefit in two Phase III clinical trials in patients with metastatic melanoma**
- **FDA-approval for melanoma 2011**

Anti-CTLA-4 opened a new field called immune checkpoint therapy

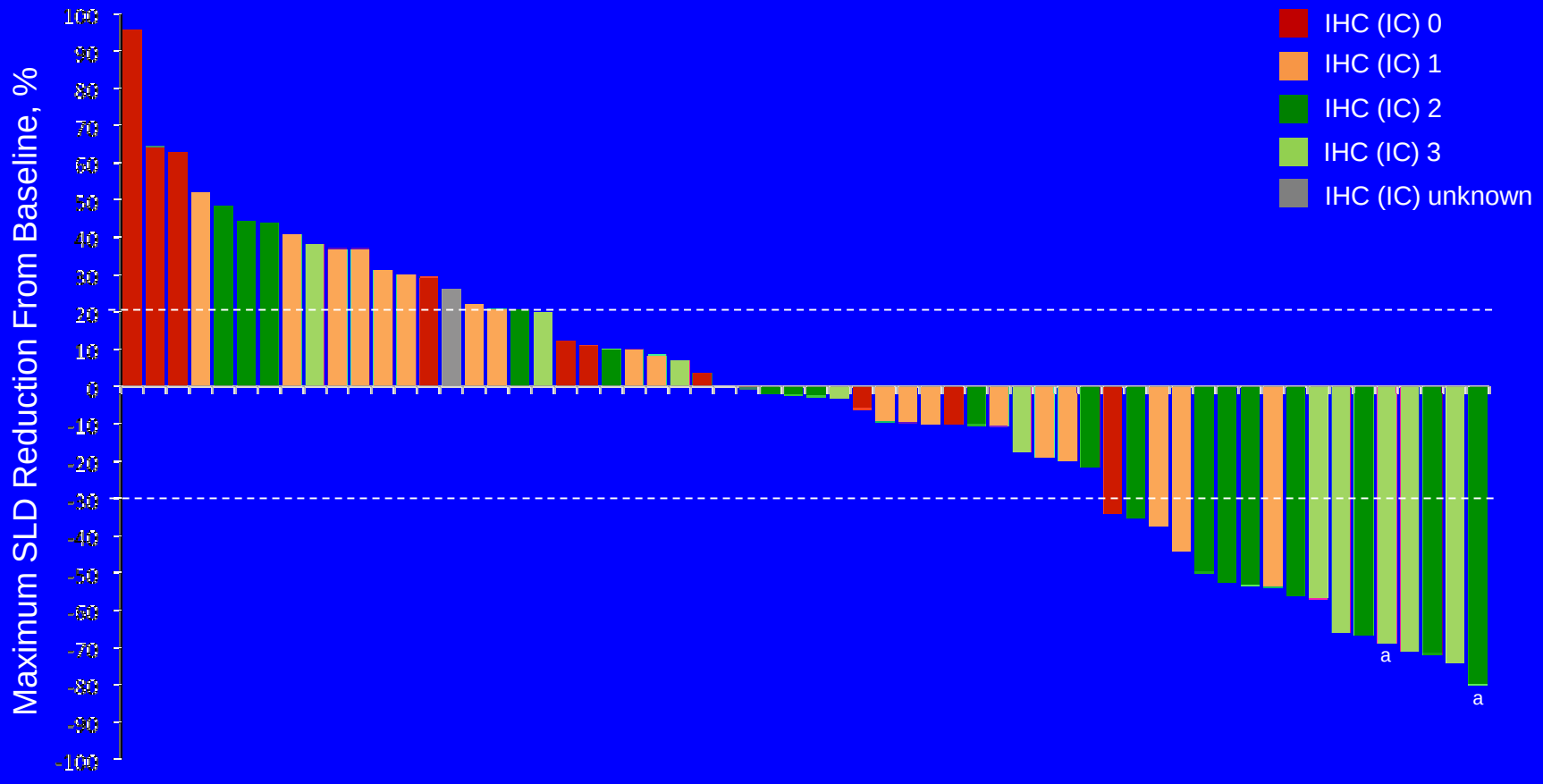


Nivolumab (anti-PD-1; BMS) Monotherapy: Phase I summary data

	Med.OS (months)	% survival 1 YR	2YR
NSCLC	9.6	42	14
(95% CI)	(7.8, 12.4)	(33, 51)	(4,24)
MEL	16.8	62	43
(95% CI)	(12.5, 31.6)	(53, 72)	(32, 53)
RCC	>22	70	50
(95% CI)	(13.6, --)	(55, 86)	(31, 70)

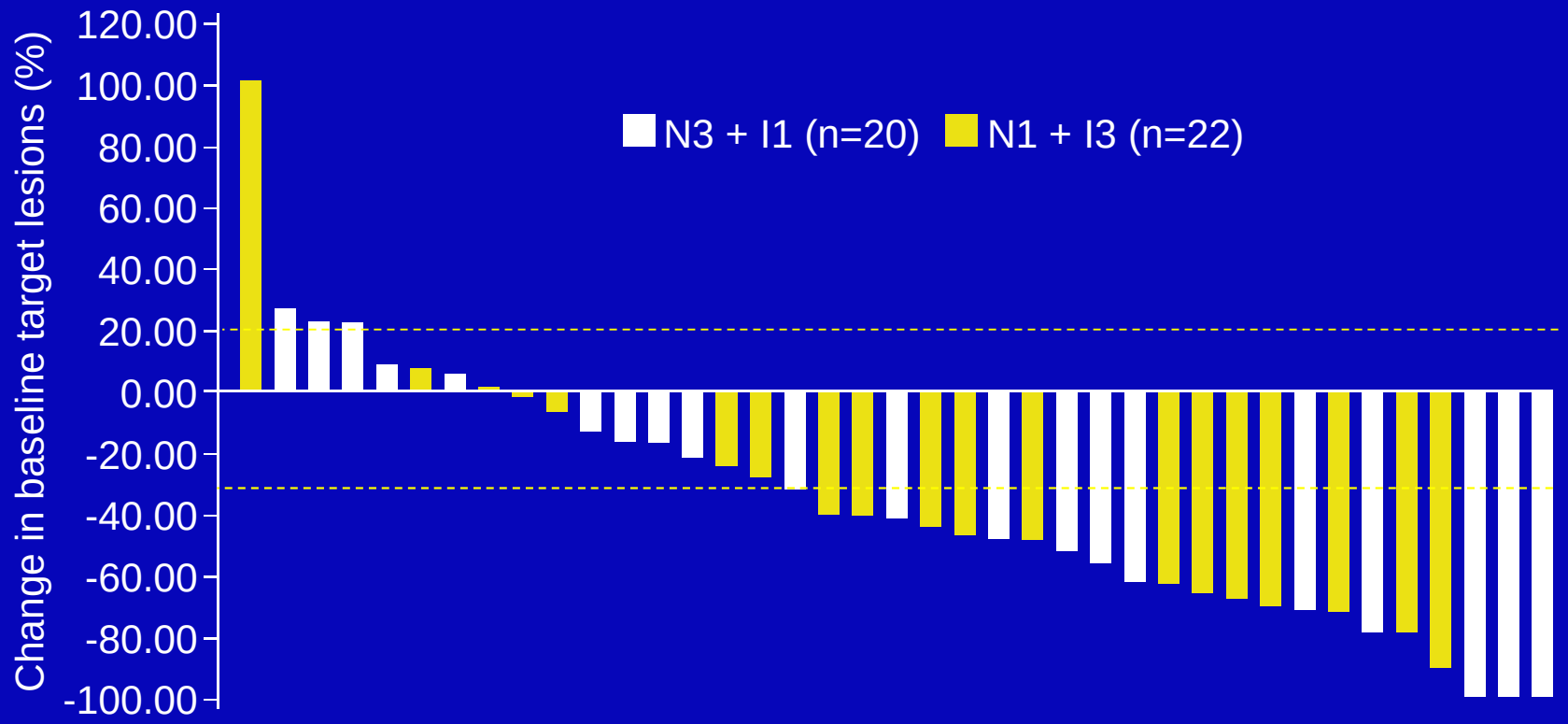
Topalian et al, ASCO 2013

Anti-PD-L1 (MPDL3280A) Monotherapy: Phase I trial in Bladder Cancer



Powles et al, ASCO 2014

Anti-CTLA-4 + Anti-PD-1 Combination: Phase I trial in mRCC



Hammers et al, ASCO 2014

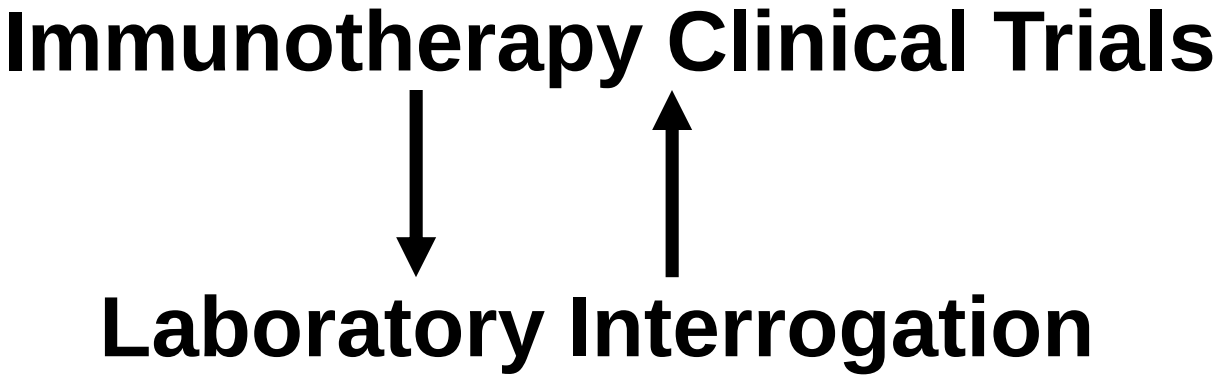
Critical issues for further clinical development

- **What are the cellular and molecular mechanisms involved in the anti-tumor effect? Toxicities?**

- **Can we identify predictive, prognostic or pharmacodynamic biomarkers?**

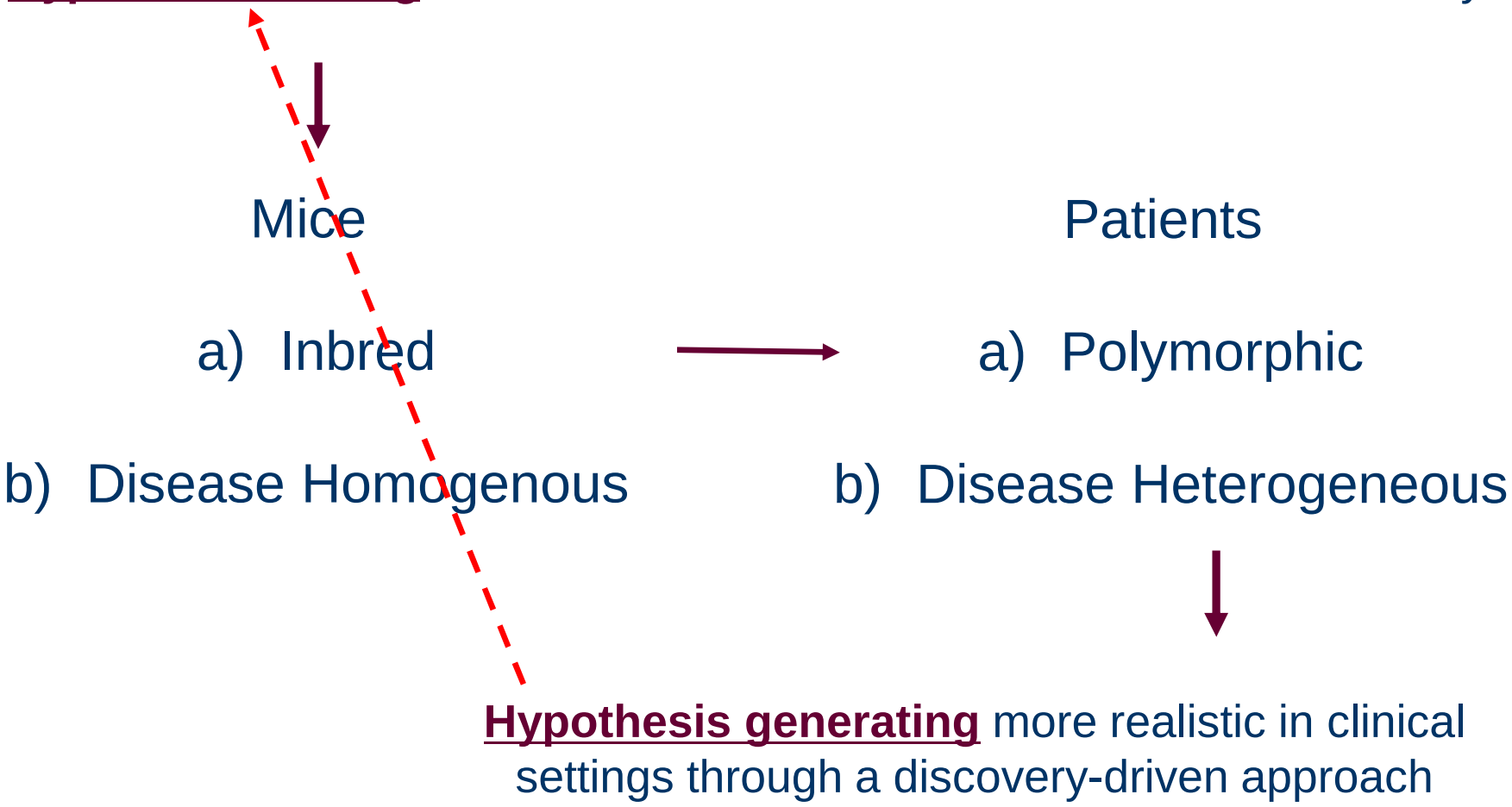
- **What are the best standard-of-care therapies to combine with immune checkpoint blockade and how do we combine these agents?**

- **Can we identify new targets to help improve the numbers of patients who benefit?**


Immunotherapy Clinical Trials
Laboratory Interrogation

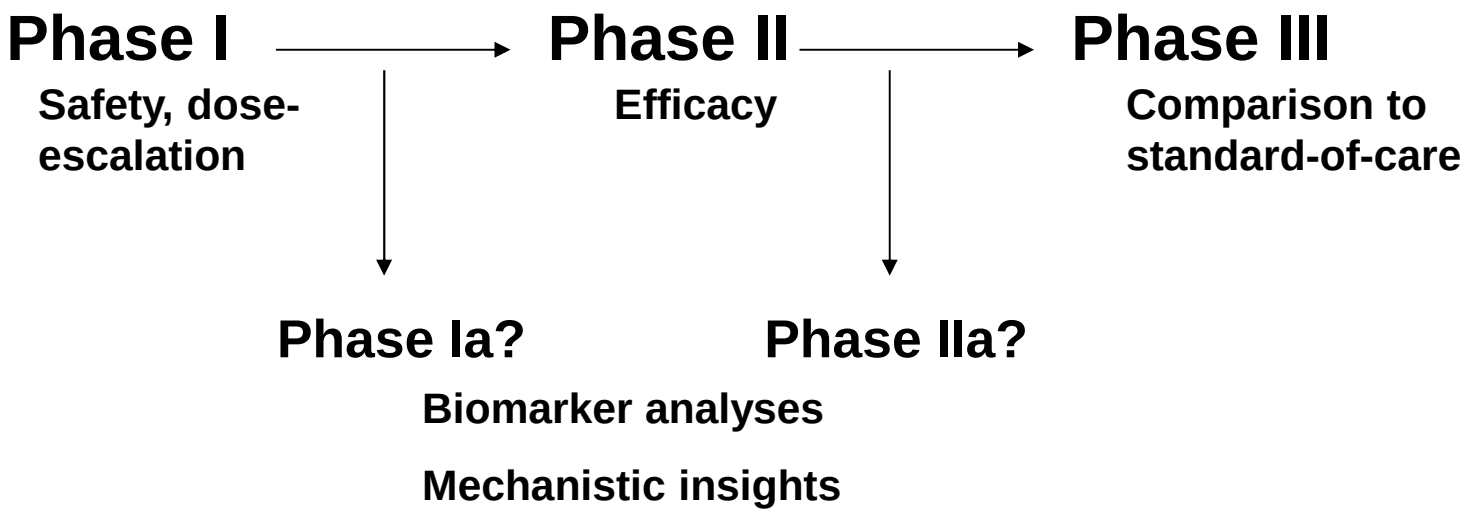
Integrating laboratory and clinical research

Hypothesis testing most efficient when one variable at the time is analyzed

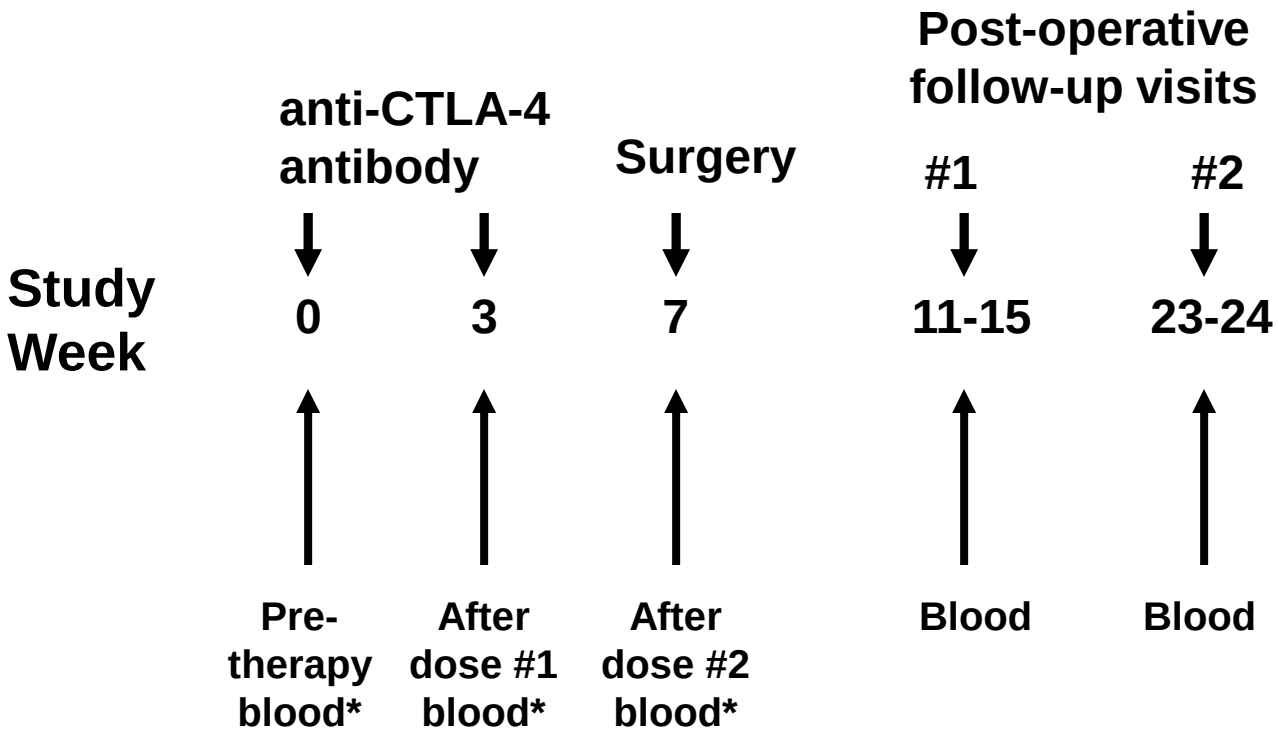


Hörig et al. Nat Med 2005

Re-thinking clinical trial design to obtain appropriate samples for laboratory studies



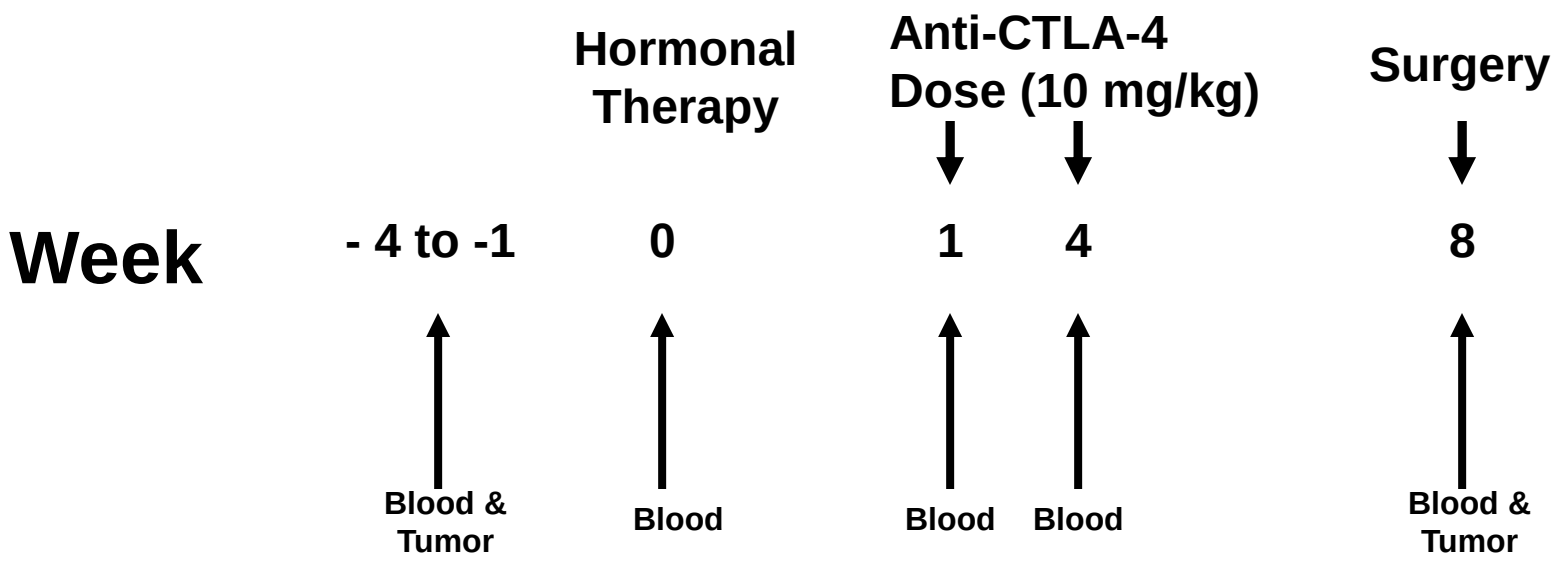
Phase Ia pre-surgical clinical trial with anti-CTLA-4 therapy



***Blood drawn prior to antibody dose administered and prior to surgery**

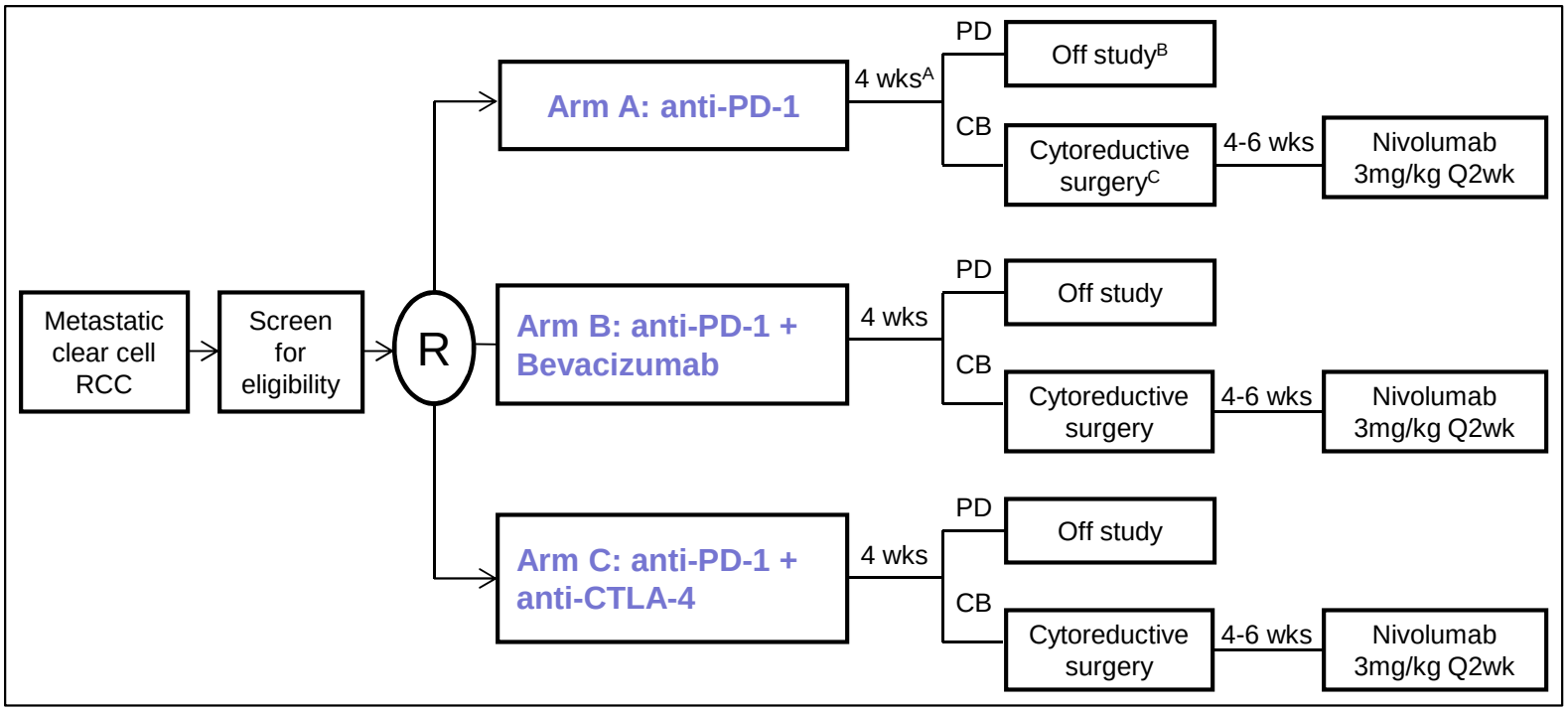
Clinical Trial for Patients with Localized Bladder Cancer (N=12)

Prostate cancer pre-surgical combination study: αCTLA-4 plus hormonal therapy



Clinical Trial for Patients with Localized Prostate Cancer (N=20)

Pre-Surgical Trial in RCC:
Anti-PD-1 + [Bevacizumab or Anti-CTLA-4]
in mRCC Patients Eligible for Cytoreductive Surgery

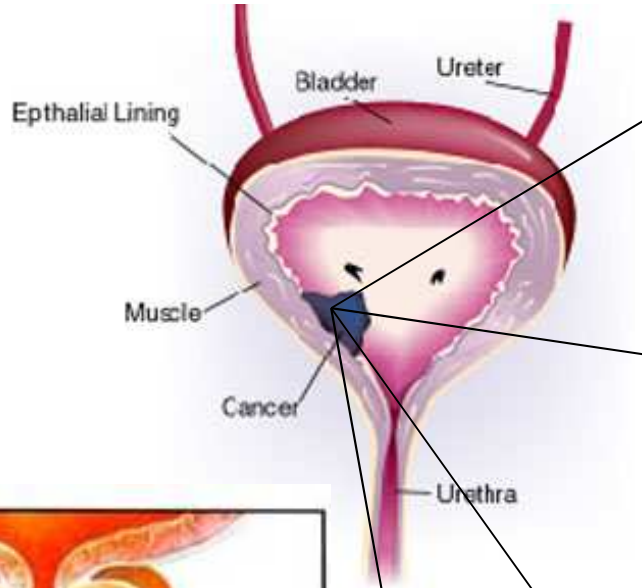


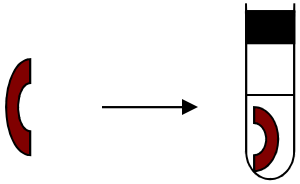
A. Eligible patients will undergo cytoreductive nephrectomy 4 weeks after the last cycle of therapy (i.e. 6 weeks after the last infusion).

B. A small portion of patients with progression of disease (PD) may still be qualified for cytoreductive nephrectomy per discretion of urologists and medical oncologists. However, these patients will be treated post-operatively with other agents rather than clinical study drugs of this protocol.

C. For patients who have clinical benefits (CB) including clinical response and stable disease pre-operatively, they will continue nivolumab monotherapy, starting 4-6 weeks post-surgery until disease progression or intolerable toxicity. Patients who have progression of disease pre-operatively may be considered for other standard or investigational agents.

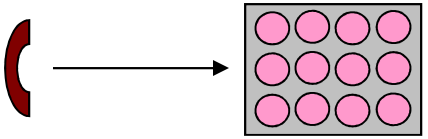
Tissue Procurement





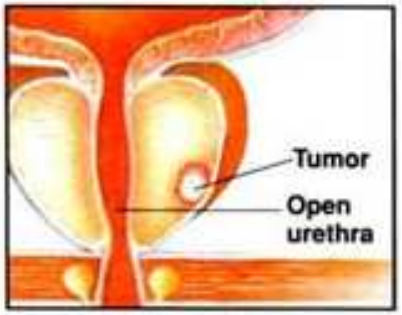
RNA later

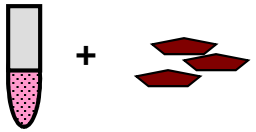
Core biopsy (~ 1 cm x 1mm x 1mm)

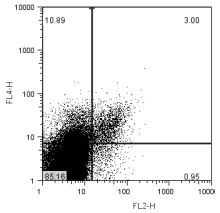


Core biopsy

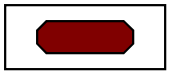
TIL expansion

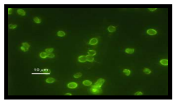






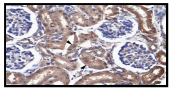
Fine needle aspiration and chunks





IF in frozen sections

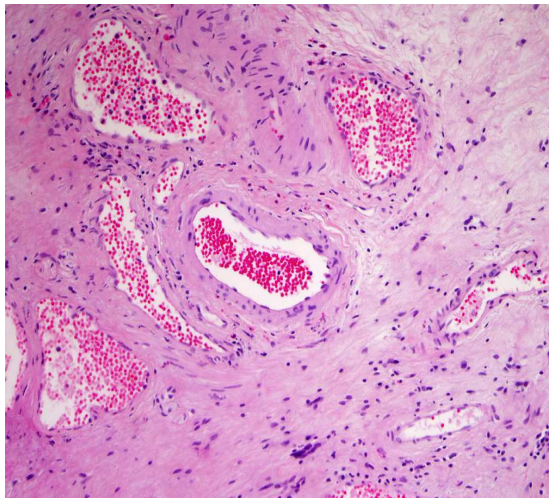
Histology sections



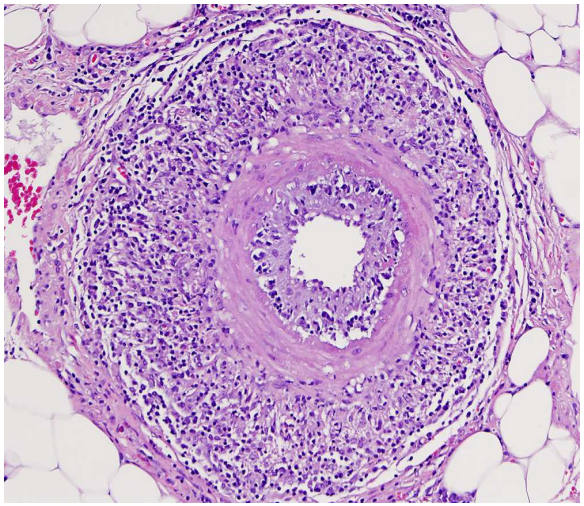
IHC in paraffin sections

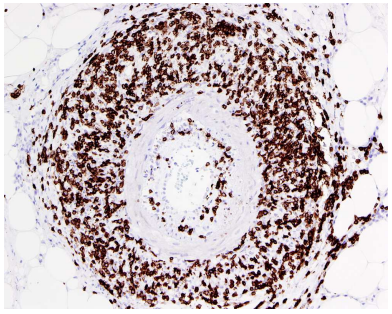
T cell infiltration into tumor tissues after anti-CTLA-4 therapy

Pre-therapy

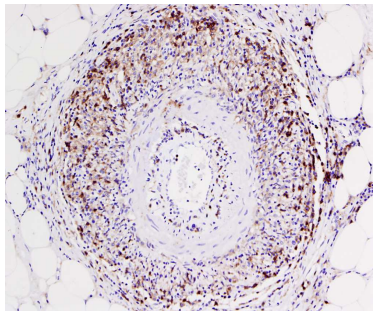


Post-therapy

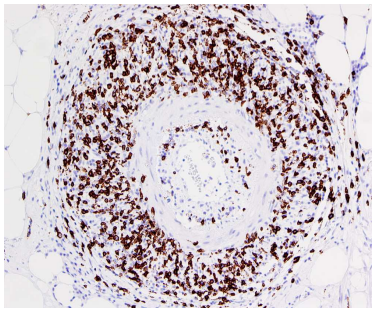




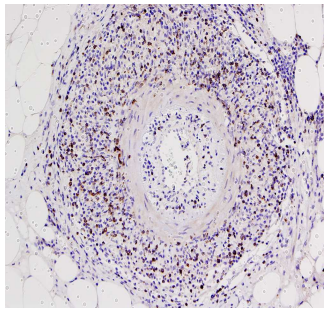
CD3



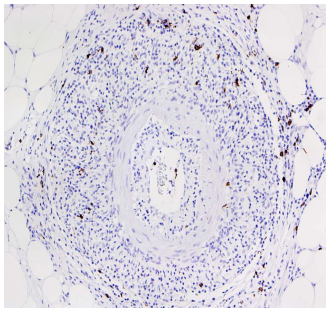
CD4



CD8



Granzyme

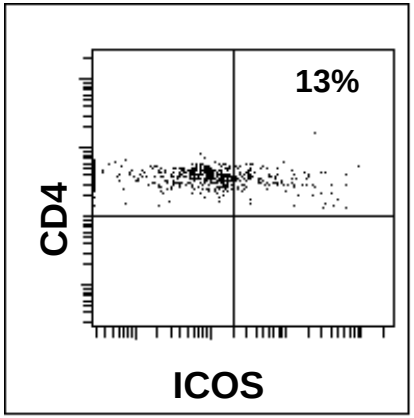


CD20

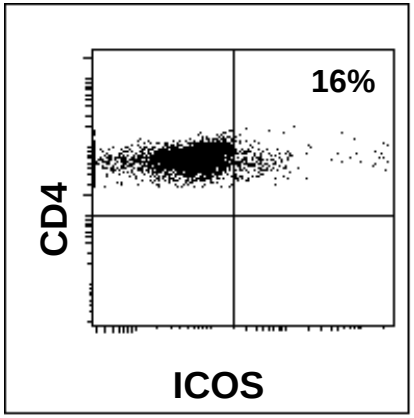
Increased frequency of $CD4^{+}ICOS^{+}$ T cells in tumors from anti-CTLA-4 treated patients

BLADDER

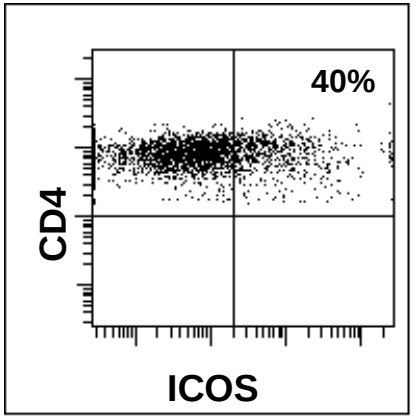
Non-malignant tissues



Tumor tissues: untreated

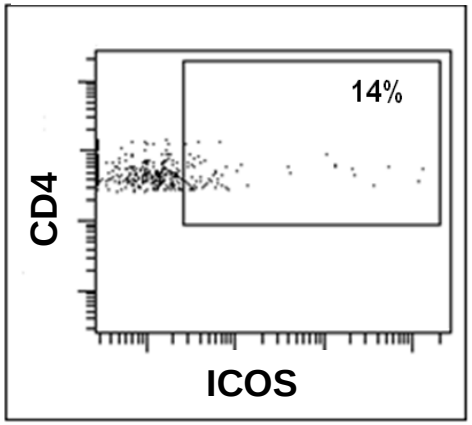


Tumor tissues: anti-CTLA-4 treated

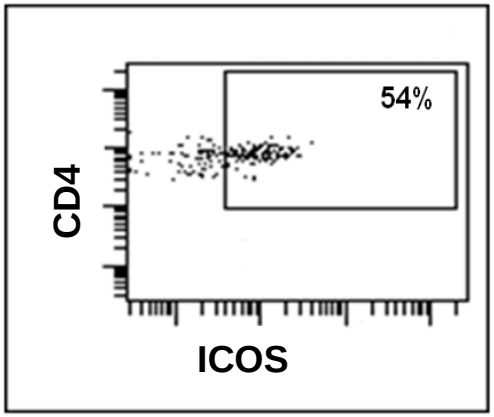


PROSTATE

Tumor tissues: untreated

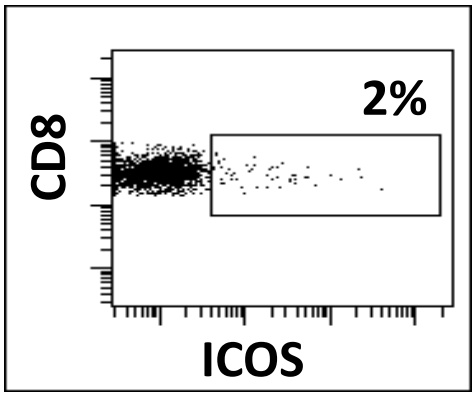


Tumor tissues: anti-CTLA-4 plus Lupron

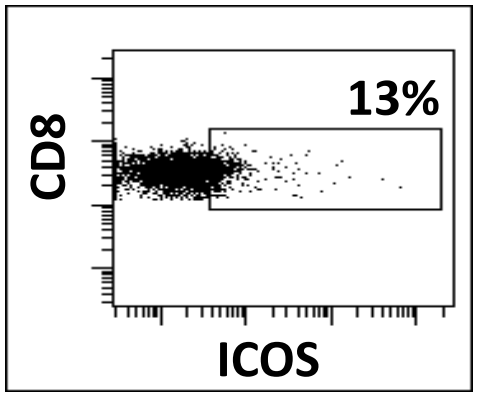


Increased frequency of $CD8^{+}ICOS^{+}$ T cells in tumors from anti-CTLA-4 treated patients

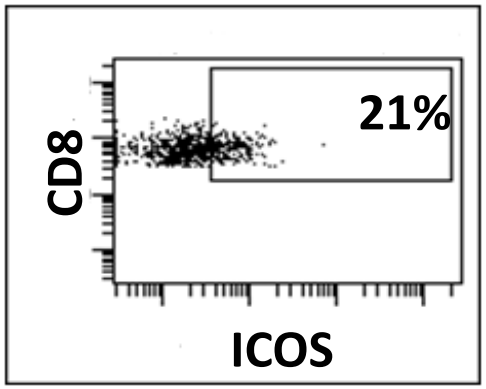
Tumor tissues: untreated



Tumor tissues: anti-CTLA-4 treated



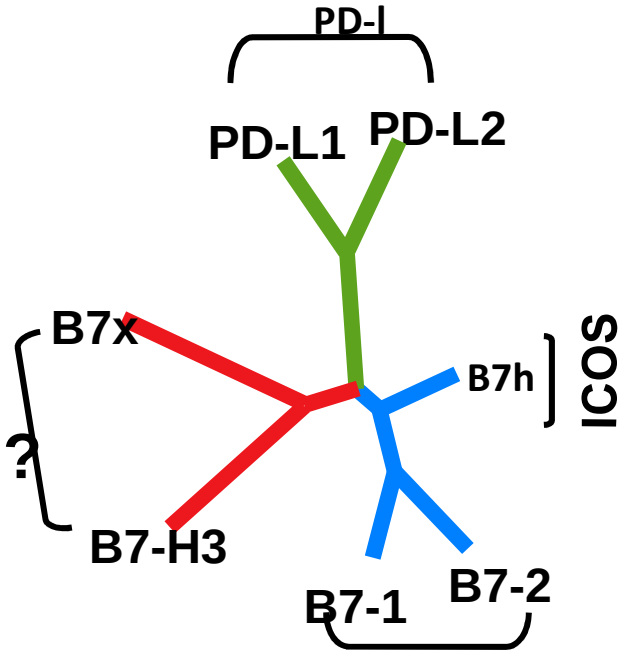
Tumor tissues: anti-CTLA-4 plus Lupron



Inducible Costimulator: ICOS

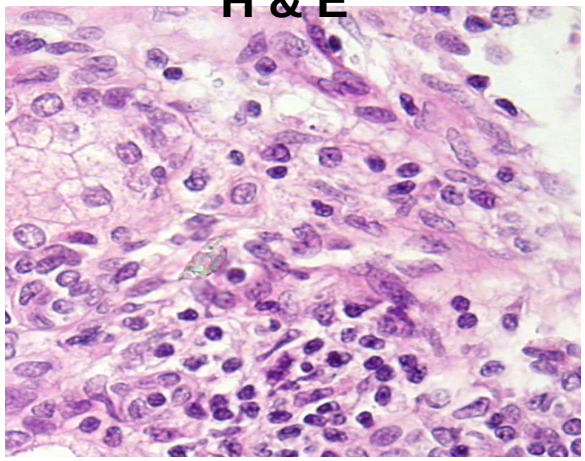
- **Inducible costimulator (ICOS)**
  - **-belongs to CD28/CTLA-4 family**
  - **-expression increased on activated T cells**
  - **-diverse role reported**

- **Role in anti-tumor responses not established**

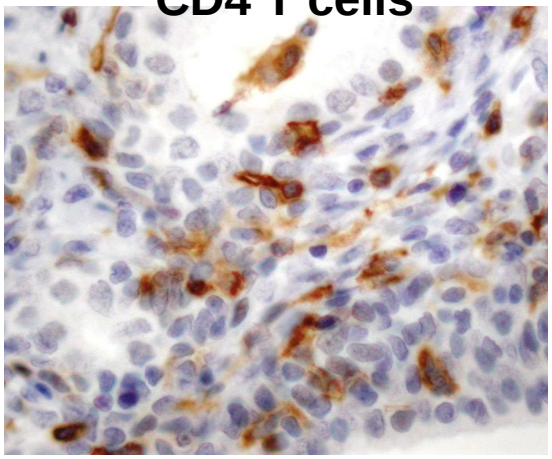


Investigating function: Are ICOS$^+$ T cells from TILs antigen-specific and capable of producing Th1 cytokines?

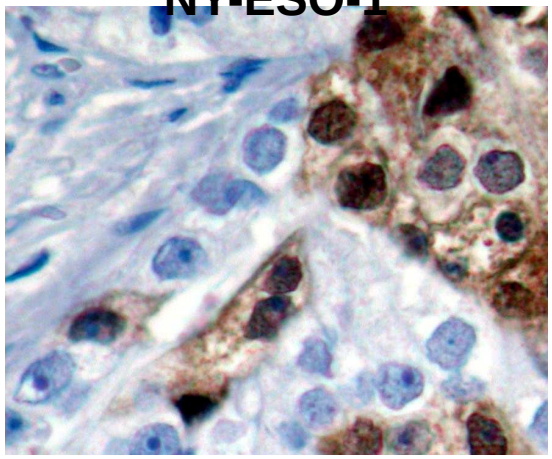
H & E

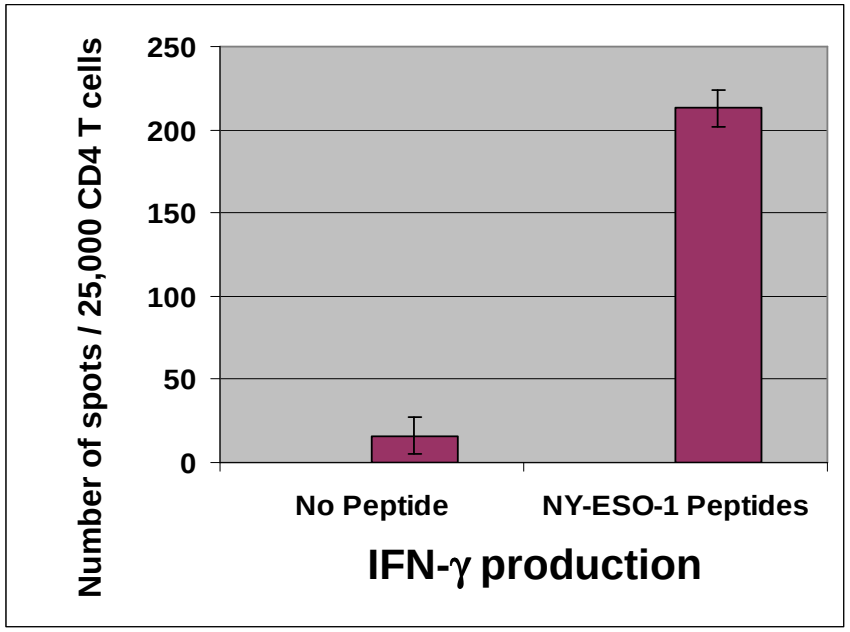


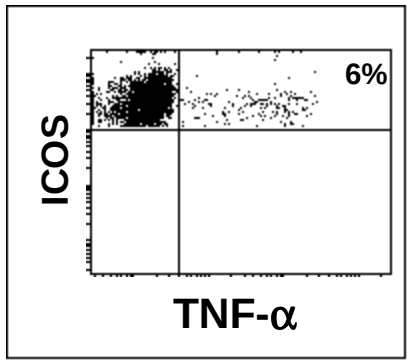
CD4 T cells

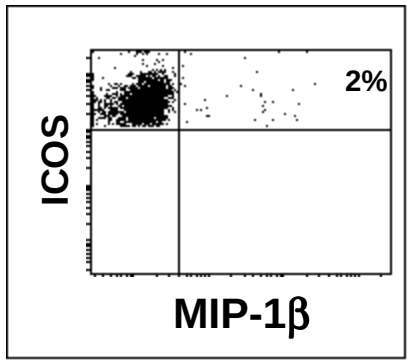


NY-ESO-1









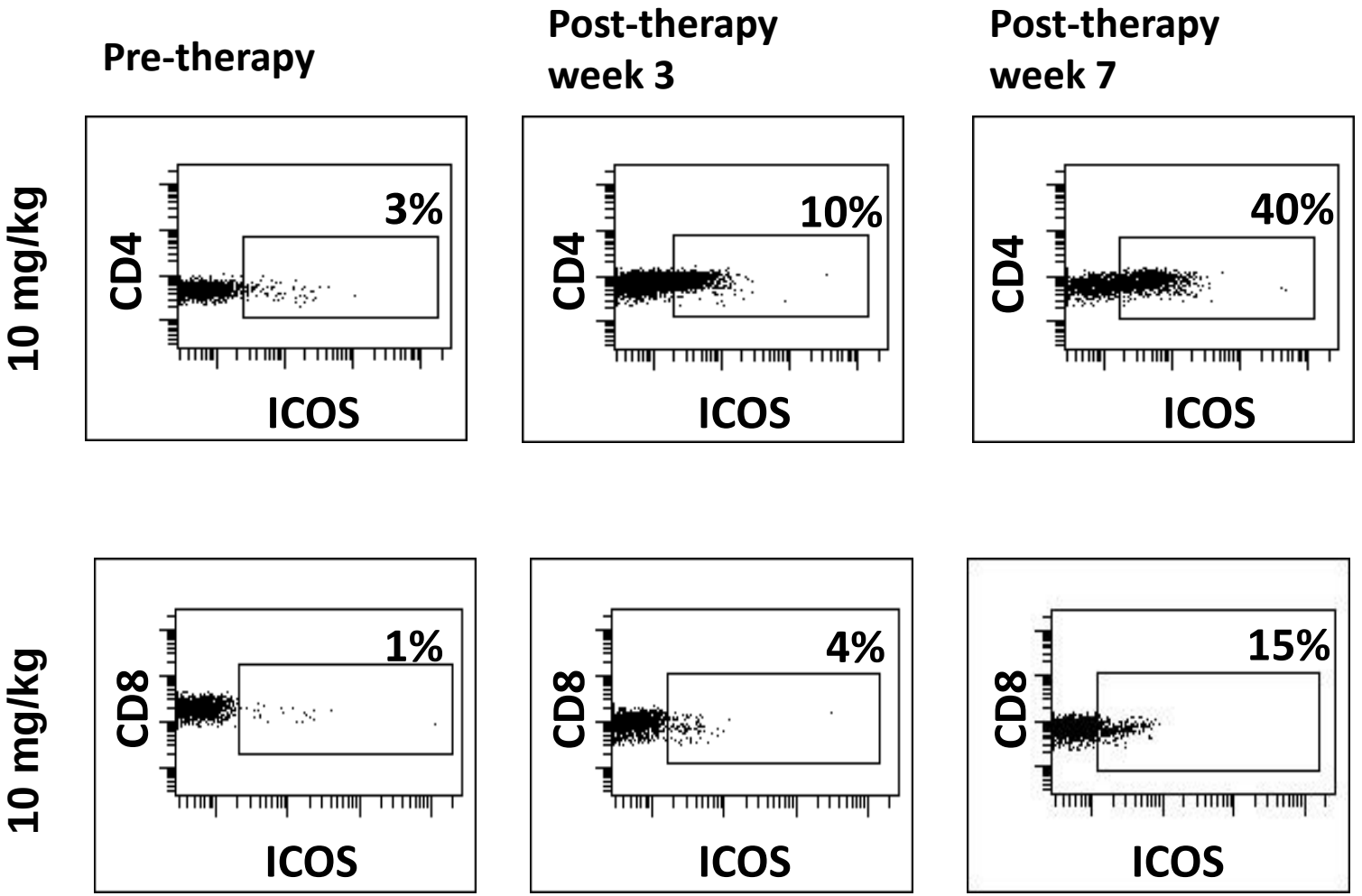
Chen et al., *Proc Natl Acad Sci*, 2009

What about immunologic events in the systemic circulation?

Do they correlate with observed changes in tumor tissues?

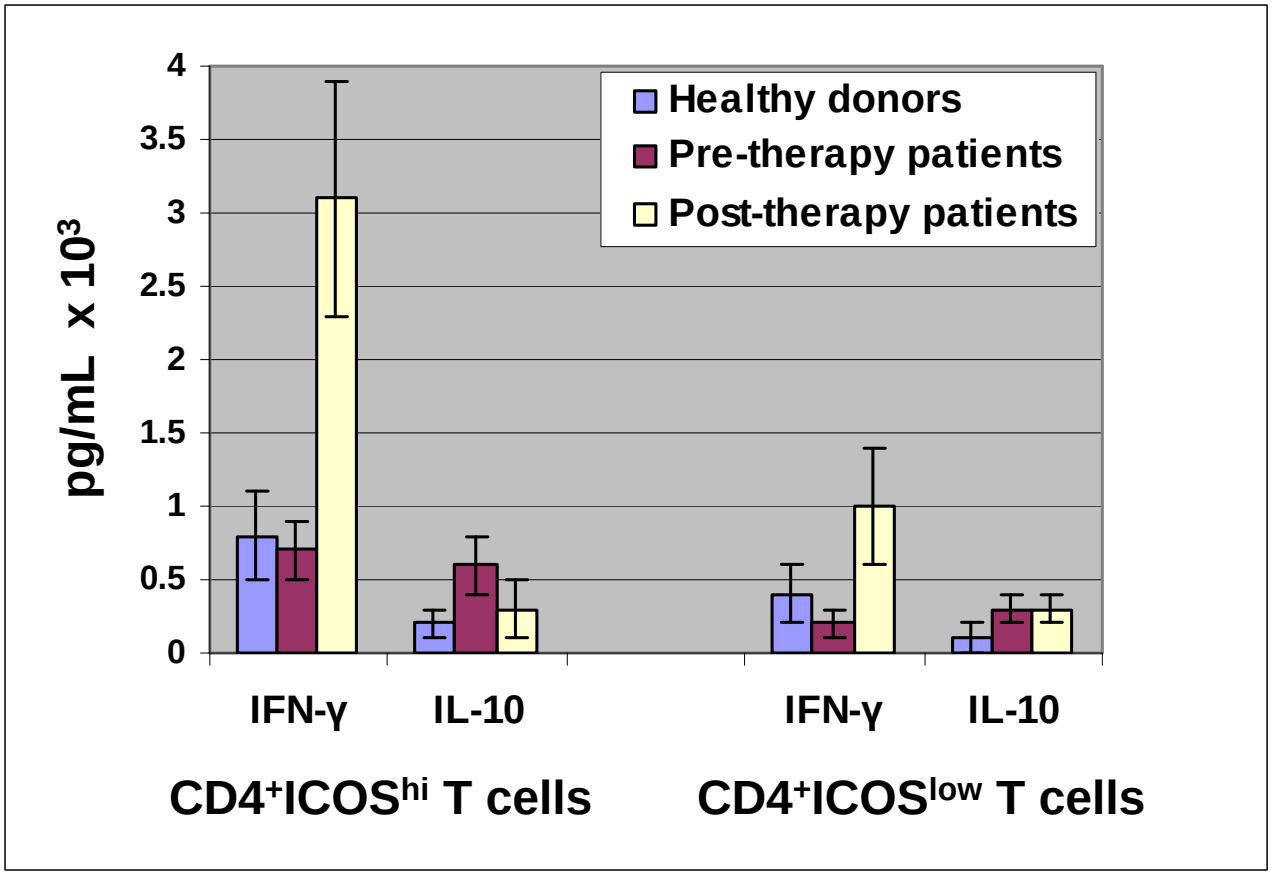
Can we identify an immunologic signature in blood that correlates with the immunologic signature in tumor tissues for ipilimumab therapy?

Frequency of ICOS^{+} T cells increase in peripheral blood after treatment with anti-CTLA-4 antibody



Carthon et al., *Clinical Cancer Research,* 2010

ICOS^{+} T cells in peripheral blood from anti-CTLA-4 treated patients produce IFN-γ



Liakou et al., *Proc Natl Acad Sci*, 2008

ICOS as a pharmacodynamic biomarker of anti-CTLA-4 therapy

		N	Greater than 5.63* (+)	Sensitivity (95%CI)	Specificity (95%CI)
Pre-treatment	All samples	56	2	--	96.4% (87.7%, 99.6%)
After dose #2 of ipilimumab	Cancer Patients	35	25	71% (53.7%, 85.4%)	-

Ng Tang et al., *Cancer Immunology Research*, 2013

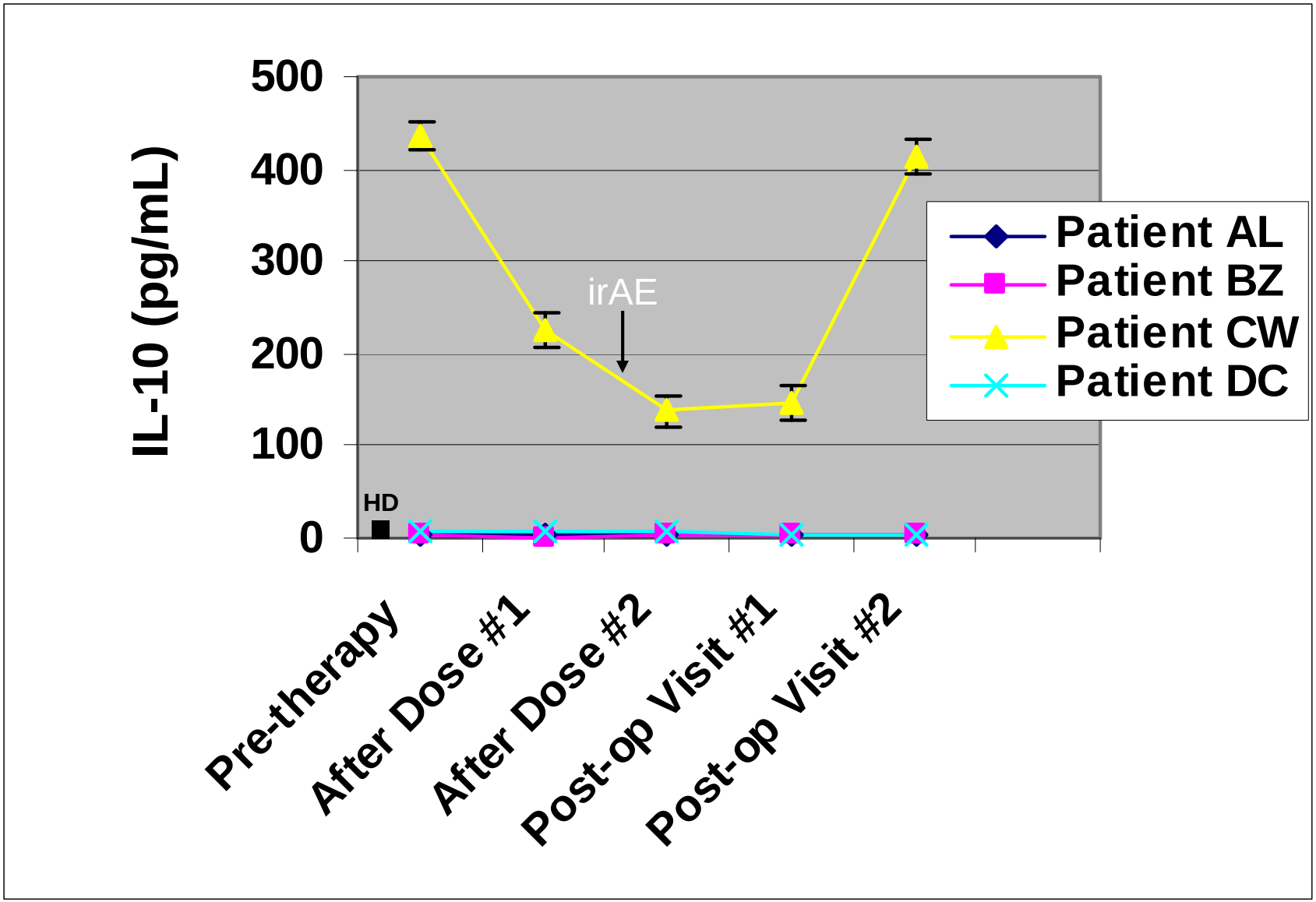
Are changes in ICOS expression associated with immune-related adverse events?

NO

Pre-Operative CTLA-4 Blockade: Patient Characteristics and Tolerability

Patient	Sex	Age	Prior Therapy	Adjuvant Therapy	Drug-Related irAEs	Surgery Delay (wks)	Follow Up (mos)	Status
1	M	66	BCG	None	Rash, Gr 1	None	33.37	NED, Alive
2	M	75	None	CGI chemo	None	5.1 (due to cardiac eval)	32.67	NED, Alive
3	M	71	BCG	None	Amylase & Lipase Increased, Gr 1 Uveitis, Gr 2 Ischemic Papillitis, Gr 3, Diarrhea Gr 1	None	28.83	NED, Alive
4	M	60	None	MVAC chemo	Rash, Gr1	None	27.30	NED, Alive
5	M	55	None	None	Rash, Gr 1	None	24.90	NED, Alive
6	M	75	BCG	None	Rash, Gr 2	None	23.10	NED, Alive
7	M	76	None	None	Rash, Gr 1 Testicular swelling/ Epididymitis, Gr 2	None	7.70	NED, Deceased
8	F	69	None	None	Rash, Gr 1 Transaminitis, Gr 3 Diarrhea, Gr 2	4.0 (due to irAE)	17.50	NED, Alive
9	M	63	None	None	Diarrhea, Gr 2	None	17.03	NED, Alive
10	F	68	None	None	Diarrhea, Gr 3 (Received only one dose of antibody)	10.3 (due to irAE and cardiac & GI eval)	12.23	NED, Alive
11	M	71	BCG	IAG chemo	Rash, Gr 1, Diarrhea, Gr 3	N/A *	9.27	Metastatic Disease, Alive
12	M	66	None	GC chemo	Diarrhea, Gr 2	None	8.33	Metastatic Disease, Alive

Decreased IL-10 in plasma is associated with adverse events



Sun et al., *Cancer Immunity*, 2008

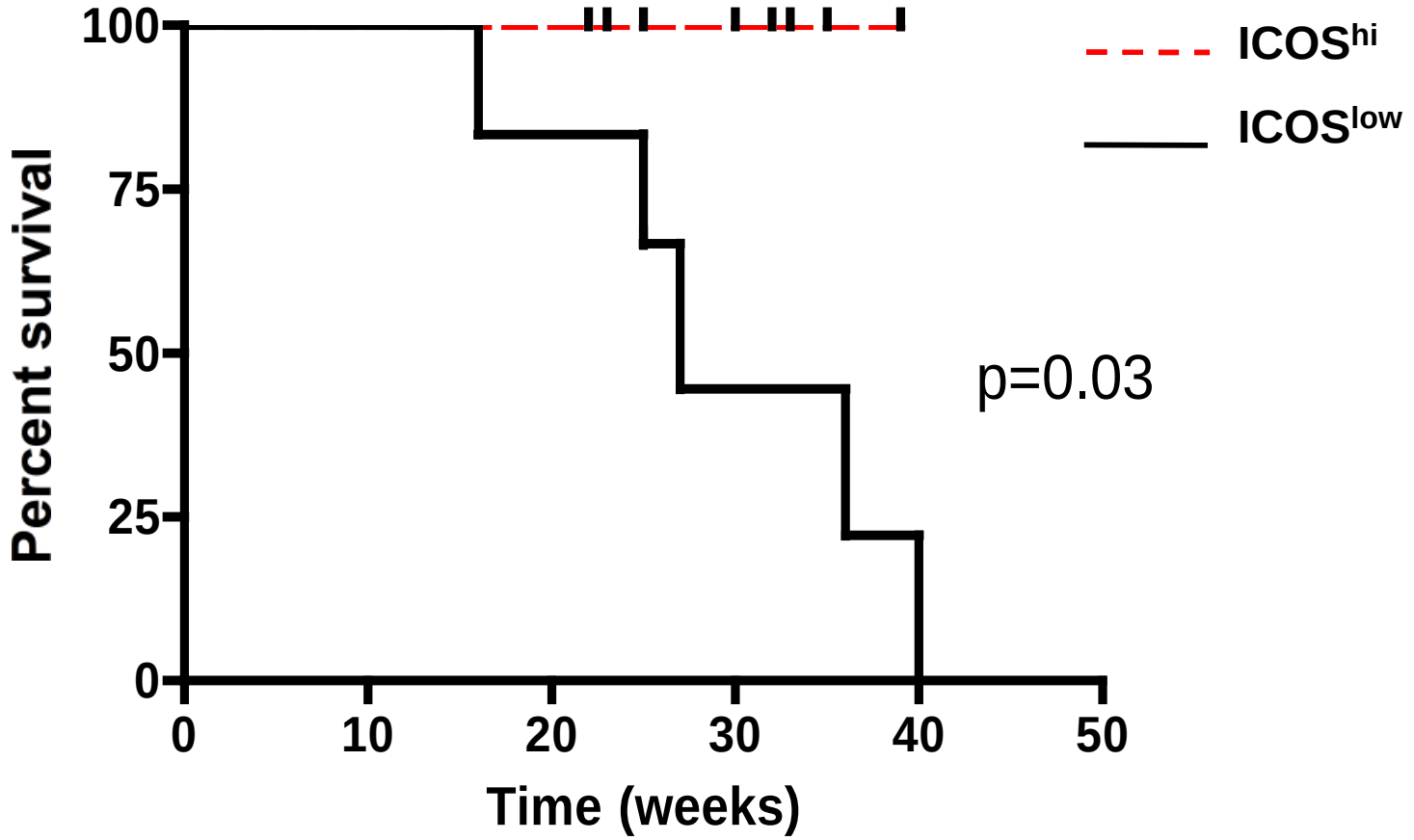
Are changes in ICOS expression associated with clinical benefit?

Pre-Operative CTLA-4 Blockade: Pathology and Cytology Data

Patient Number	Pre-Therapy Pathology (UC, HighGrade)	Post-Therapy Pathology	Pre-Therapy Cytology Fluorescence in situ hybridization (FISH)	Post-Therapy Cytology Fluorescence in situ hybridization (FISH)
1	T1N0M0	T0N0M0	Positive Cytology, Positive FISH	Negative Cytology, Negative FISH
2	T1N0M0	T4N0M0	Positive Cytology, Positive FISH	Positive Cytology, Positive FISH
3	T1N0M0	TisN0M0	Negative Cytology, Positive FISH	Negative Cytology, Negative FISH
4	T2N0M0	T3N1M0	Negative Cytology, Non-contributory FISH	Negative Cytology
5	T1N0M0	TaN0M0	Positive Cytology	Positive Cytology
6	T1N0M0	T0N0M0	Positive Cytology	Negative Cytology, Negative FISH
7	T1N0M0	TaN0M0	Positive Cytology	Positive Cytology
8	T2N0M0	T0N0M0	Negative Cytology, Negative FISH	Negative Cytology, Negative FISH
9	T1N0M0	TisN0M0	Positive Cytology	Positive Cytology
10	T2N0M0	T0N0M0	Positive Cytology	Negative Cytology, Negative FISH
11	T1N0M0	pTXN0M1	Positive Cytology	Positive Cytology
12	T2N0M0, UC & Micropapillary Disease	T2N1M0, UC, Sarcomatoid & Micropapillary Disease	Positive Cytology	Positive Cytology, Positive FISH

Carthon et al., *Clinical Cancer Research,* 2010

Metastatic Melanoma: Sustained elevation of $CD4^{+}ICOS^{hi}$ T cells correlates with survival

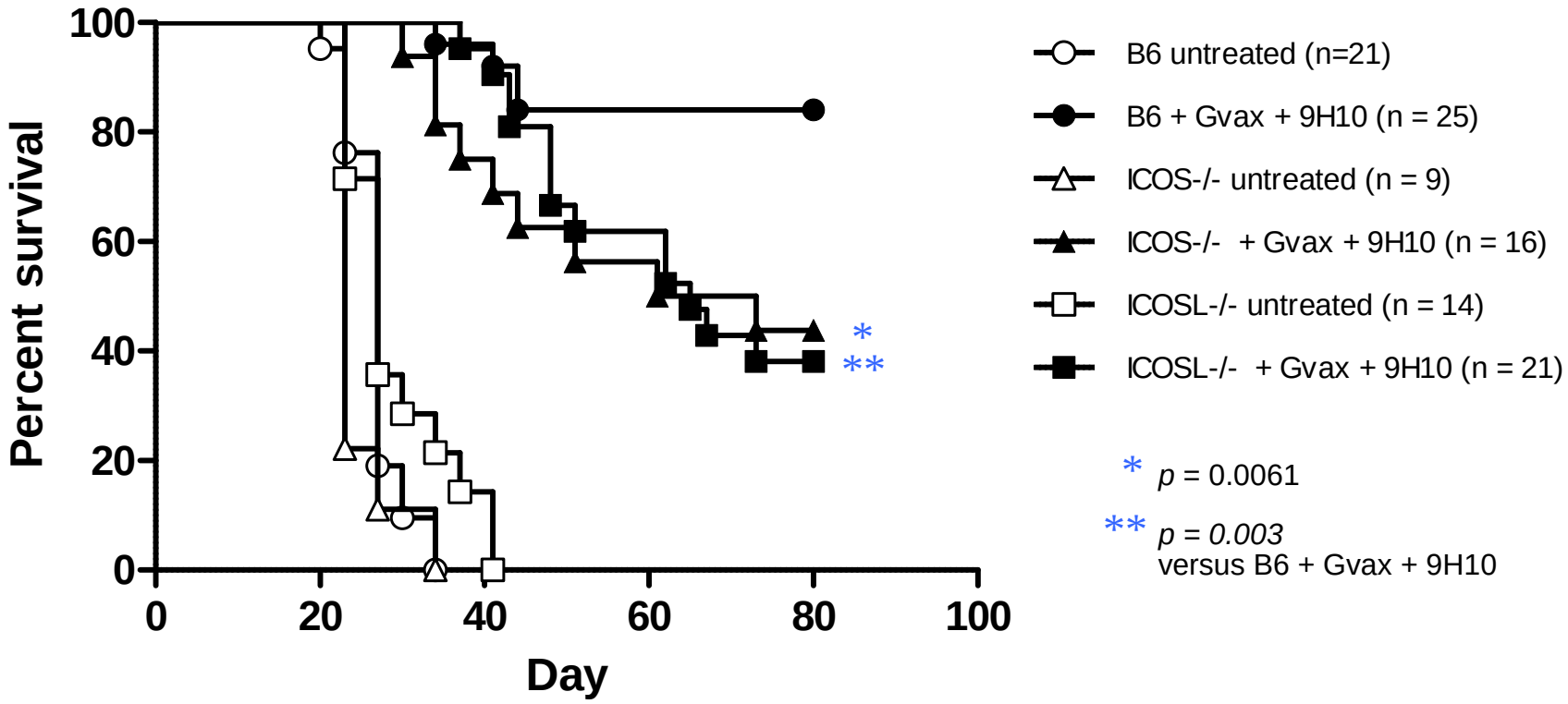


Carthon et al., *Clinical Cancer Research,* 2010

Hypothesis #1

The ICOS/ICOSL pathway is necessary for effective anti-tumor immune responses in the setting of anti-CTLA-4 therapy

ICOS/ICOSL pathway is necessary for optimal anti-tumor responses in the setting of CTLA-4 blockade

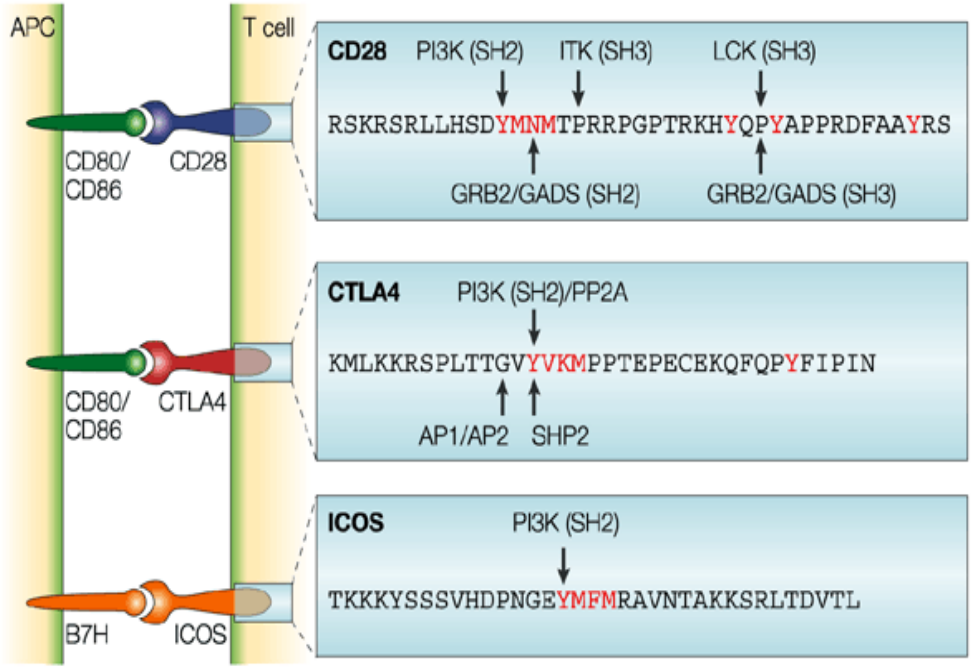


Fu et al., *Cancer Research,* 2011

Why is the ICOS/ICOSL pathway necessary for optimal anti-tumor responses?

Hypothesis #2

ICOS can induce signaling via PI3-kinase to promote T-bet expression with subsequent Th1 responses and tumor rejection



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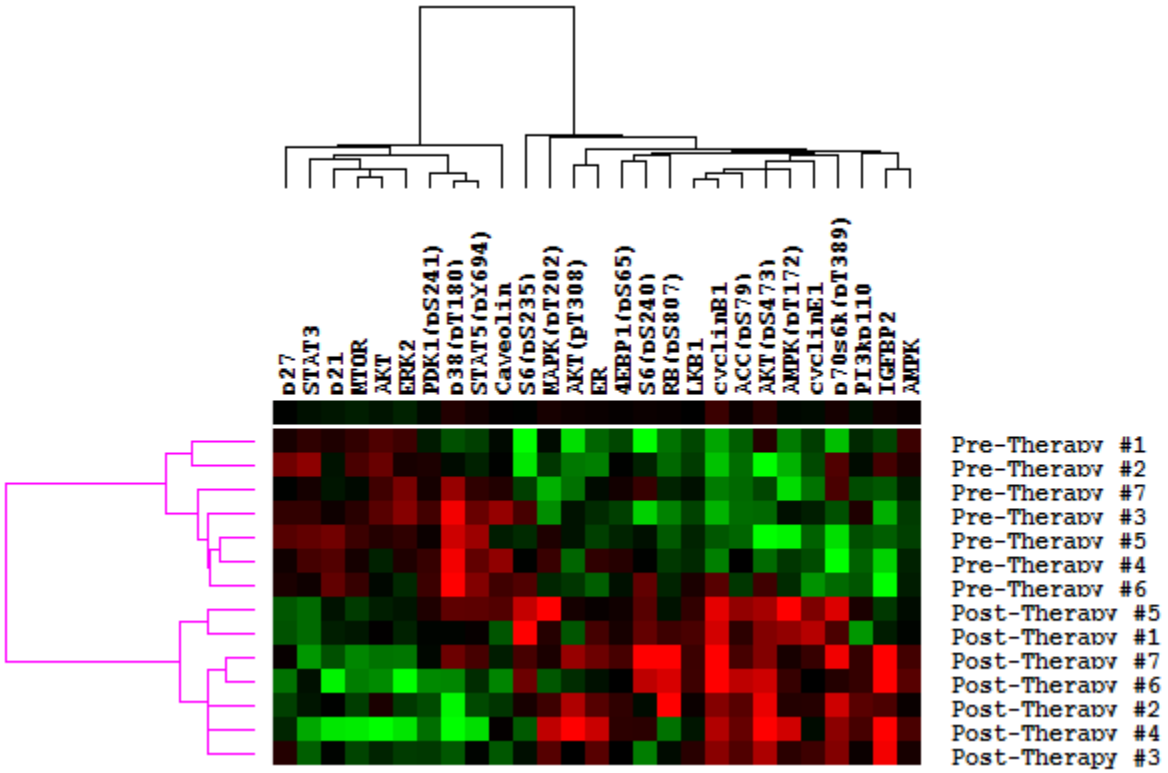
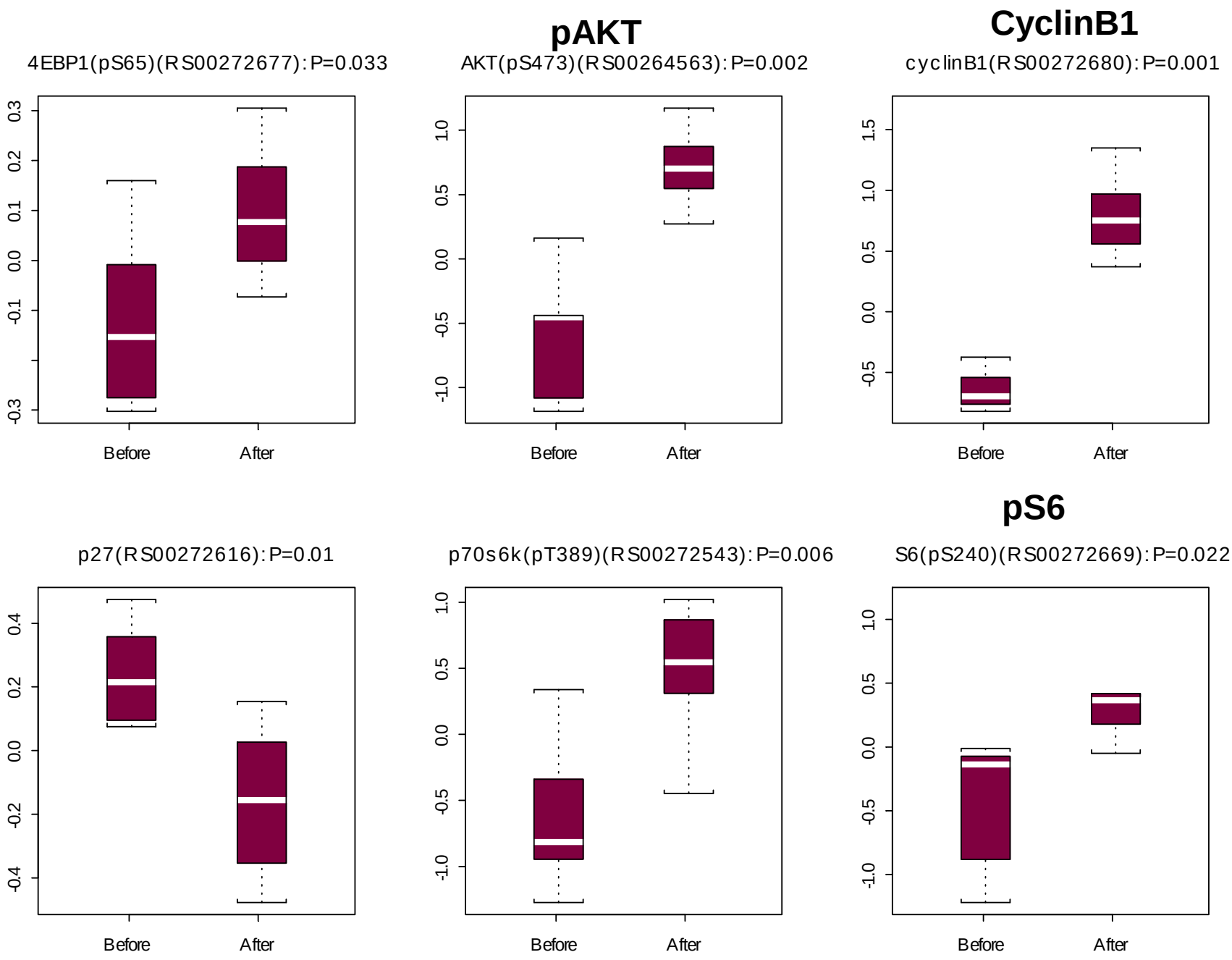
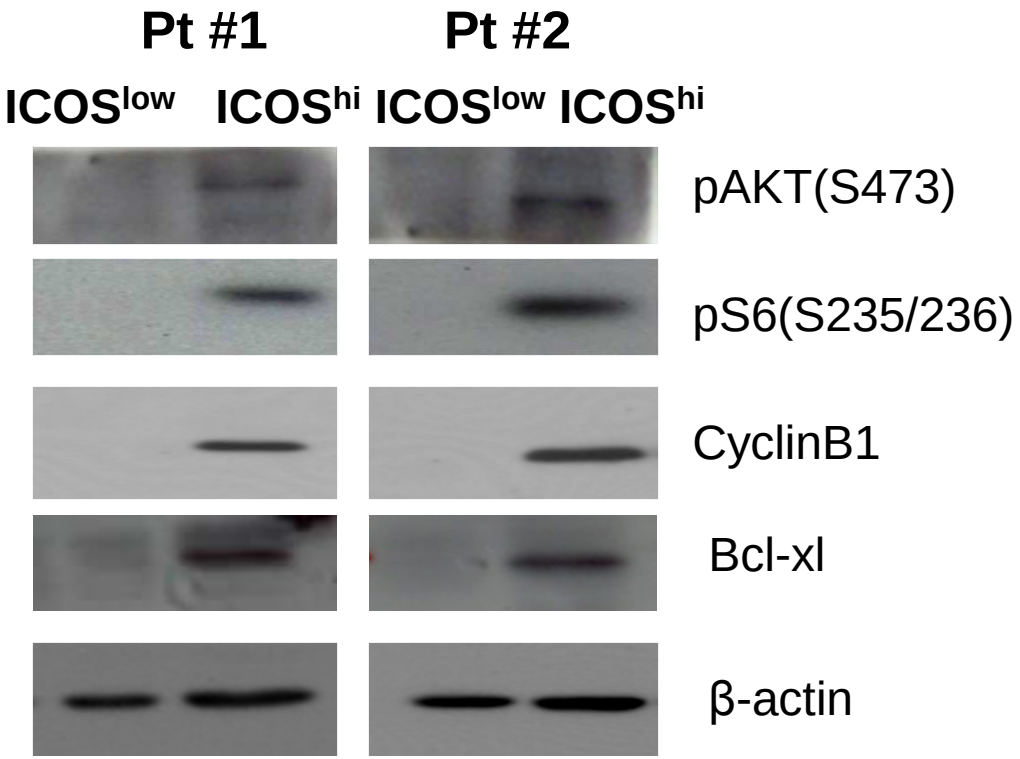
Reverse Phase Protein Array

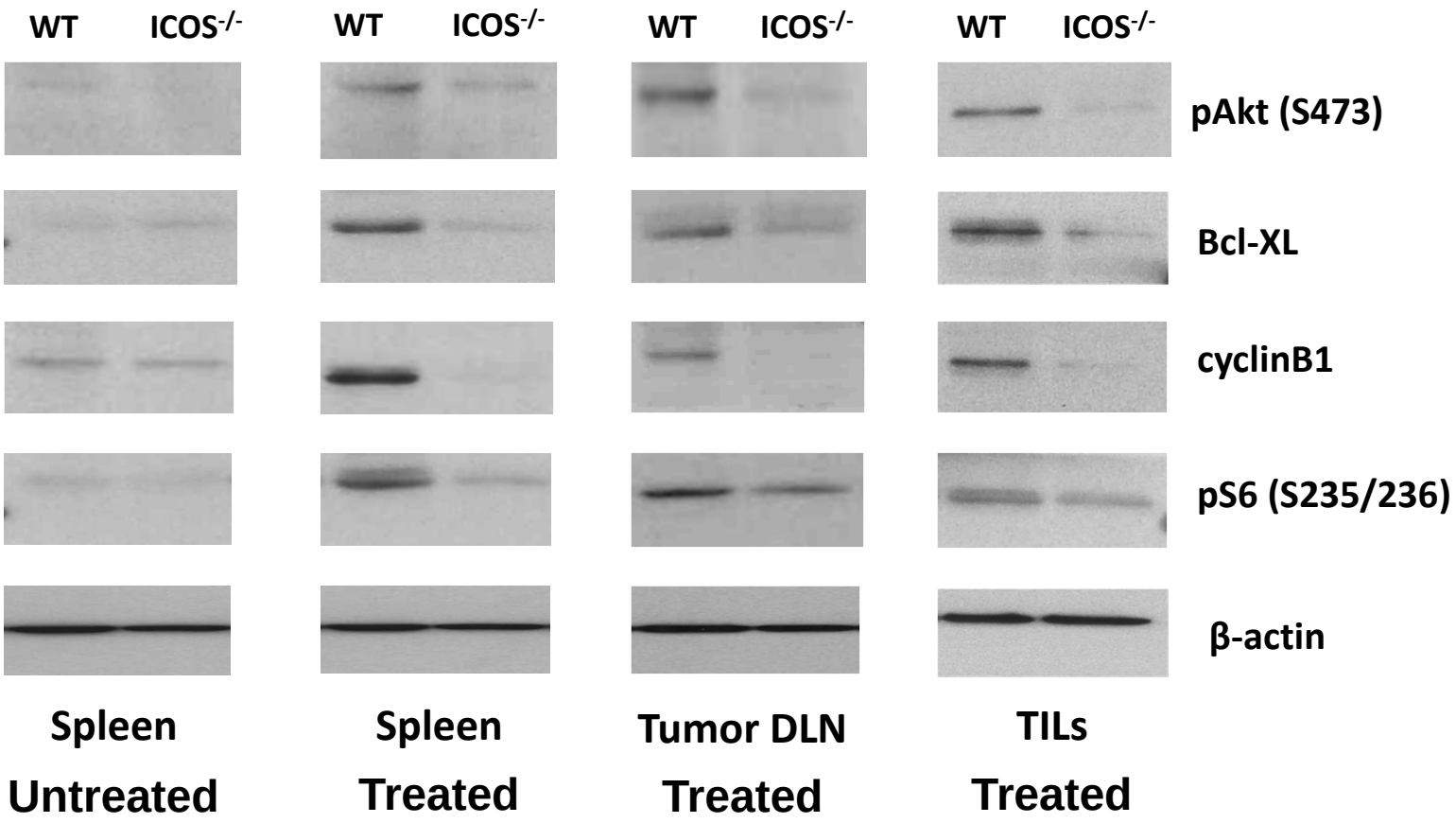



4EBP1(pS65)(RS00272677):P=0.033
-0.3
-0.1
0.0
0.1
0.2
0.3
Before
After
pAKT
AKT(pS473)(RS00264563):P=0.002
-1.0
-0.5
0.0
0.5
1.0
Before
After
CyclinB1
cyclinB1(RS00272680):P=0.001
-0.5
0.0
0.5
1.0
1.5
Before
After
p27(RS00272616):P=0.01
-0.4
-0.2
0.0
0.2
0.4
Before
After
p70s6k(pT389)(RS00272543):P=0.006
-1.0
-0.5
0.0
0.5
1.0
Before
After
pS6
S6(pS240)(RS00272669):P=0.022
-1.0
-0.5
0.0
0.5
1.0
Before
After

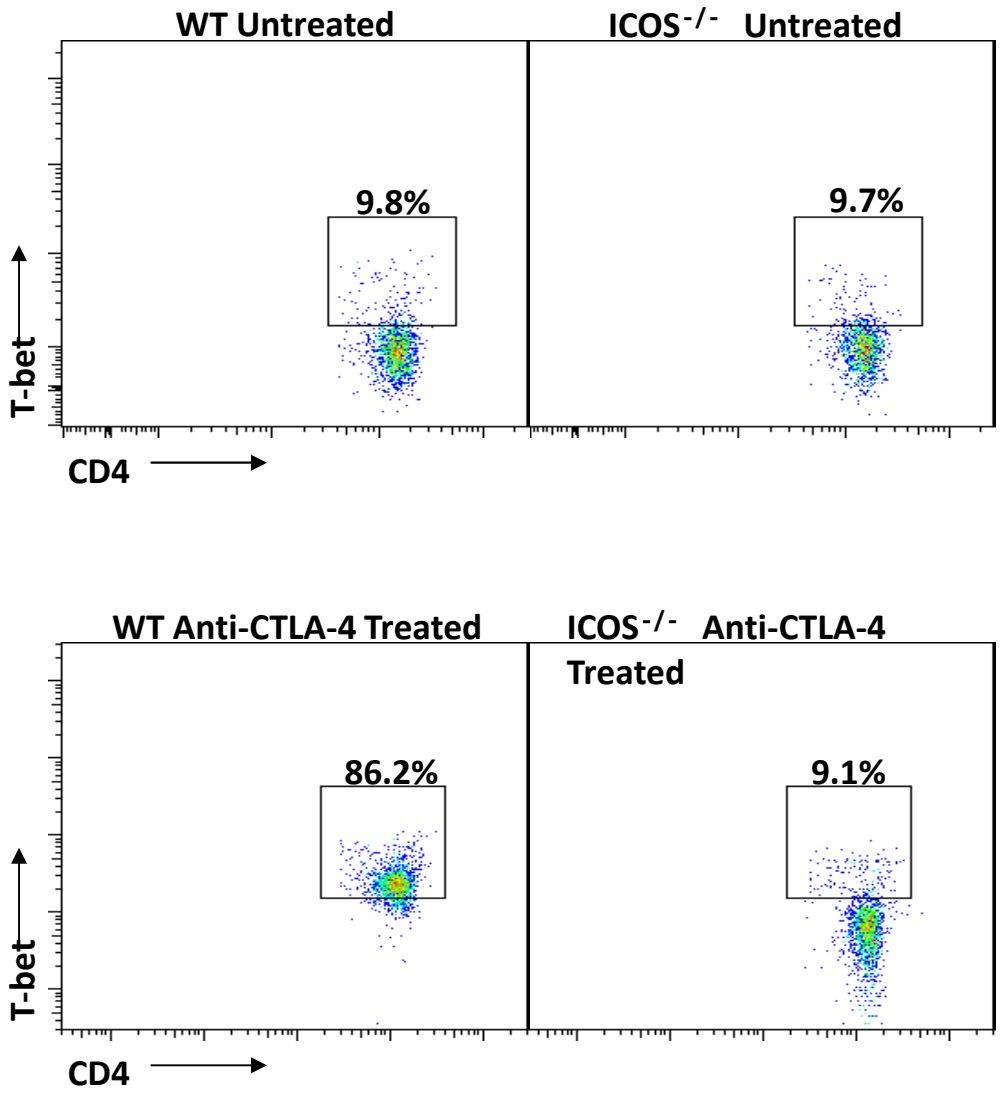
Western blot analyses



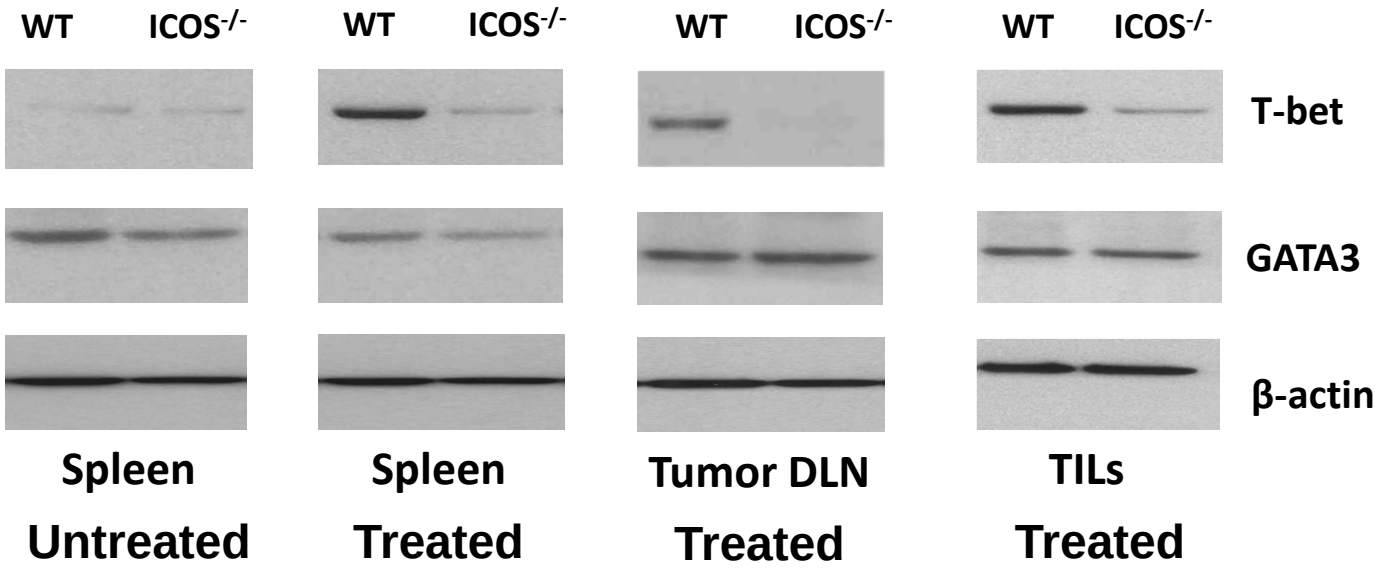
In vivo comparison of PI3K signaling in T cells from WT and ICOS-KO mice



Comparison of T-bet expression in T cells from WT and ICOS-KO mice

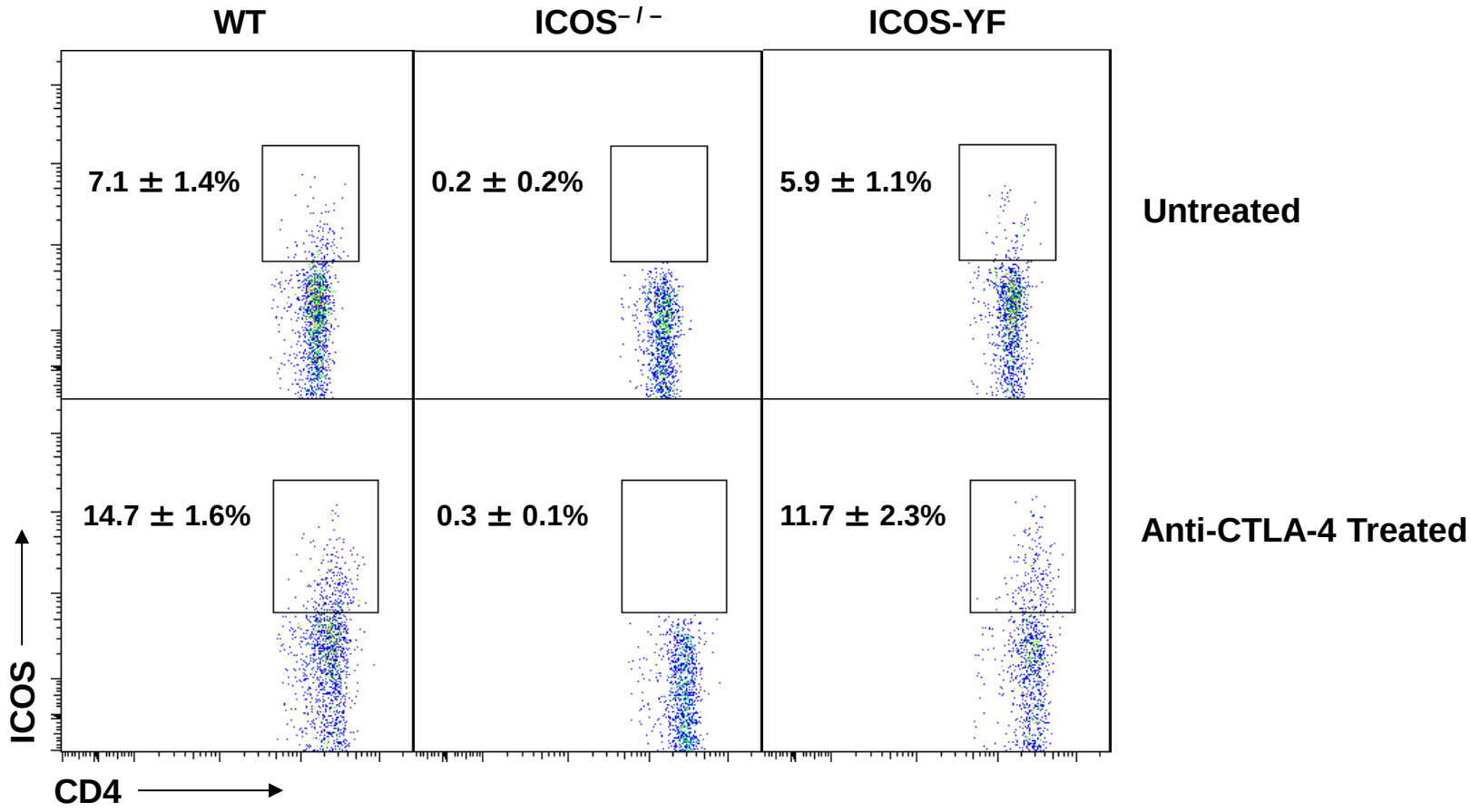


In vivo comparison of T-bet expression in T cells from WT and ICOS-KO mice

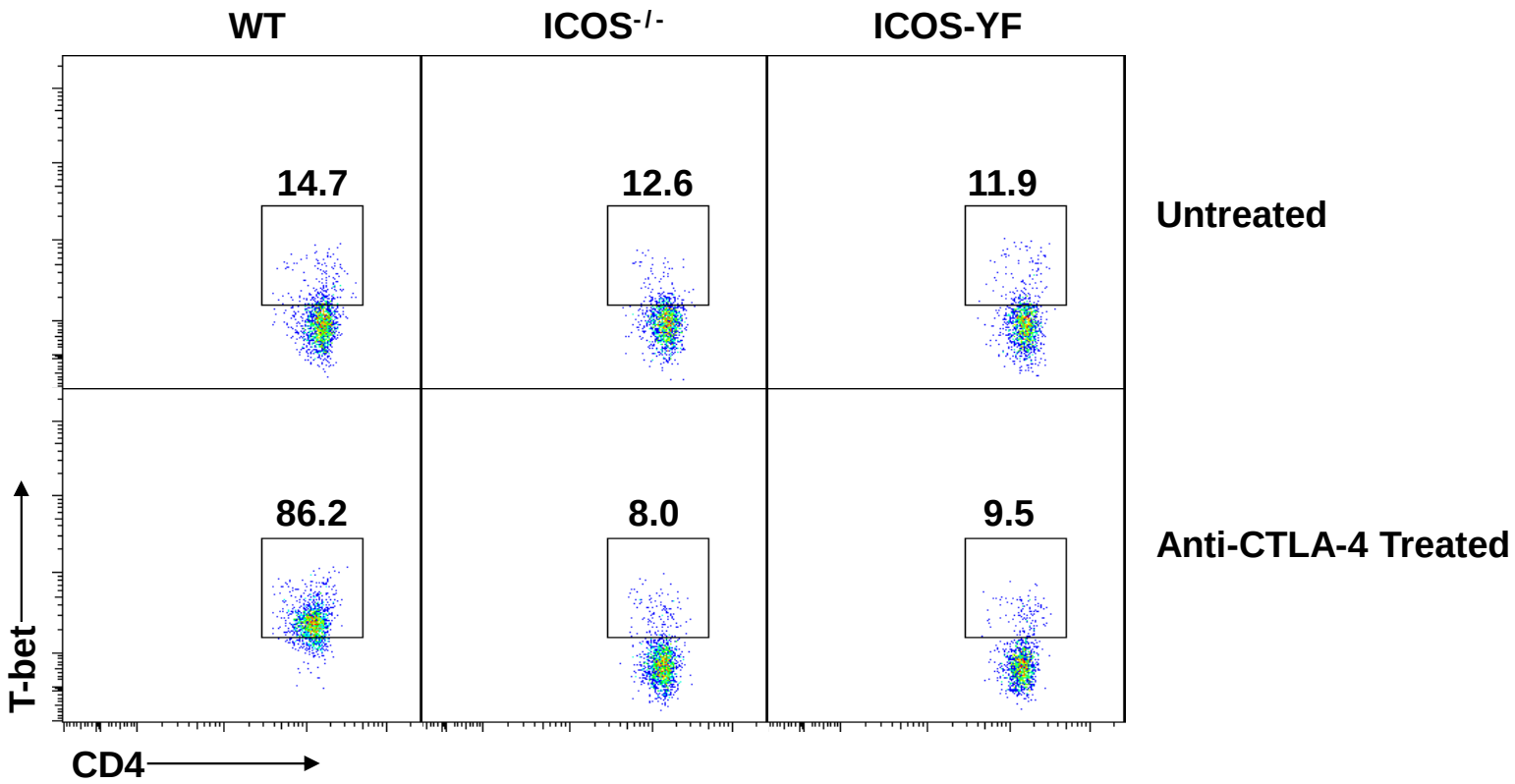


Is ICOS-mediated PI3K-signaling necessary for T-bet expression after treatment with anti-CTLA-4?

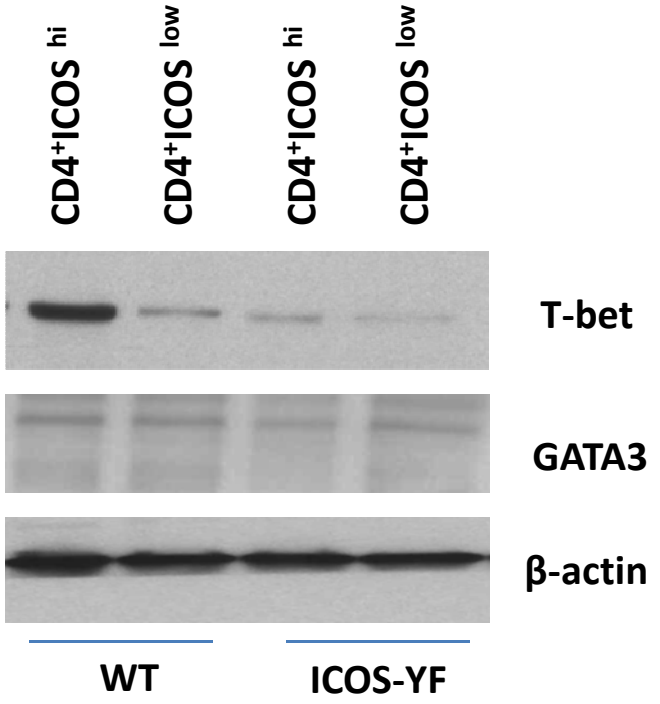
ICOS-YF mice can express ICOS on T-cells but have a single amino acid change that abrogates PI3K-signaling by ICOS

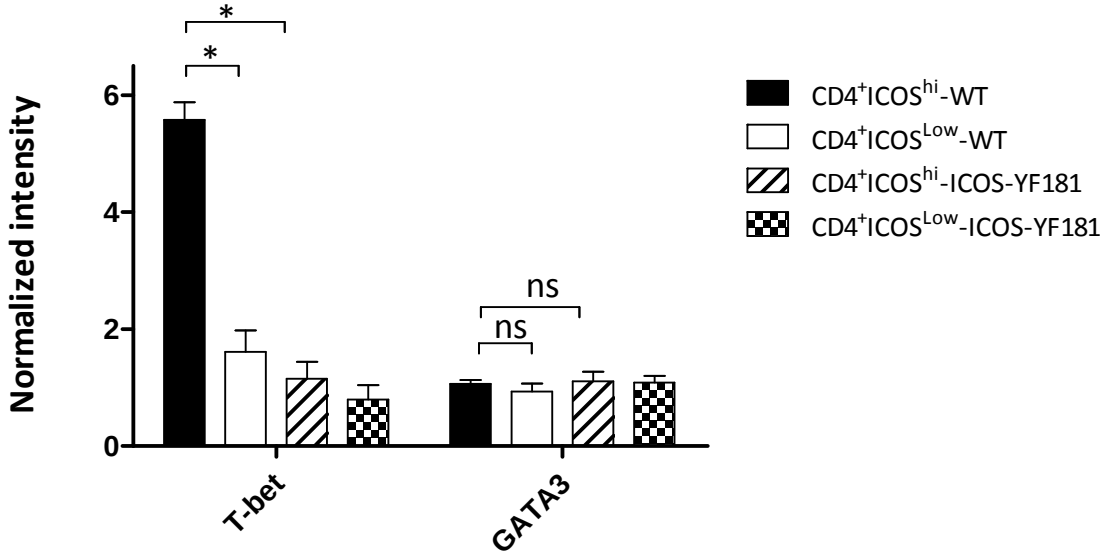


ICOS-YF mice fail to increase T-bet expression in setting of anti-CTLA-4 therapy



ICOS-YF mice fail to increase T-bet expression in setting of anti-CTLA-4 therapy



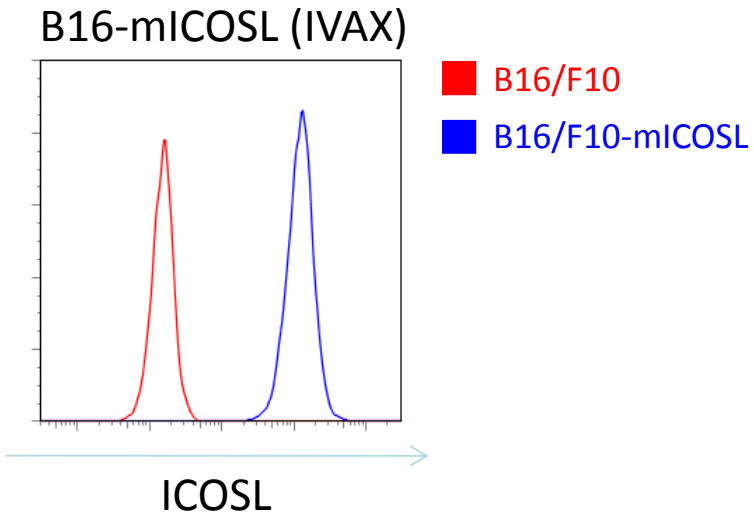


Hypothesis #3

The ICOS/ICOSL pathway can be targeted and developed as a combination therapy strategy with anti-CTLA-4 or other immunotherapies to improve anti-tumor responses

1) **Agonistic anti-ICOS ab**
2) **ICOSL-Ig fusion protein**
3) **Tumor cell vaccine expressing ICOSL**

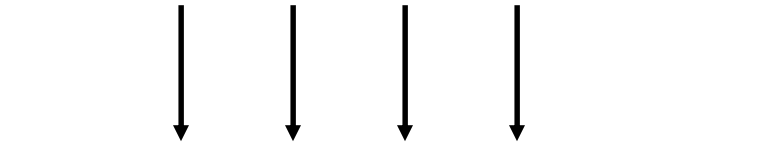
IVAX: tumor cell vaccine expressing ICOSL



Monitor tumor growth
or
Harvest TDLNs, VDLNs, and tumors on day 14, and analyze the phenotype and function of T cells

B16/F10 challenge

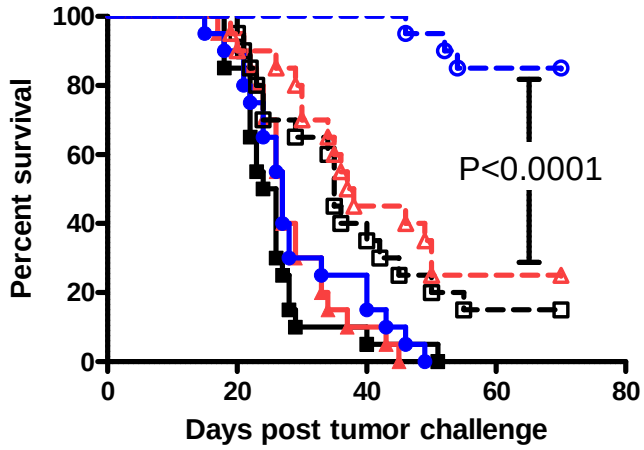
B16/F10 or **B16/F10-mICOSL**
+/- αCTLA-4 on day 3/6/9/12

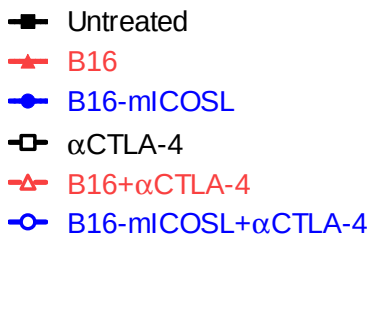


Fan

IVAX plus αCTLA-4 improves anti-tumor responses and requires ICOS/ICOSL interaction

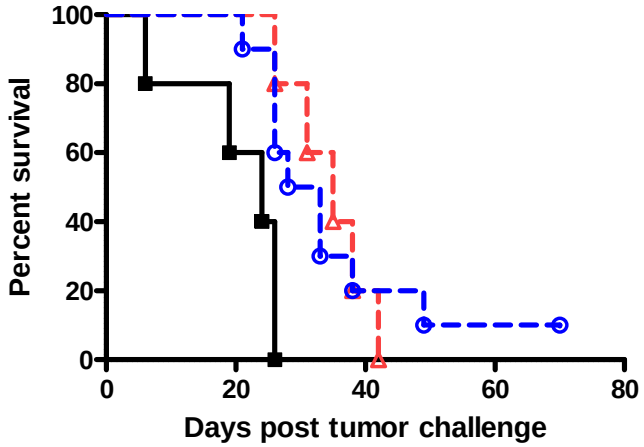
WT

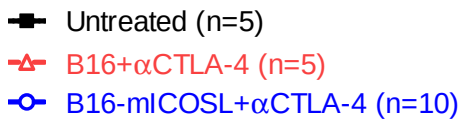




N = 20

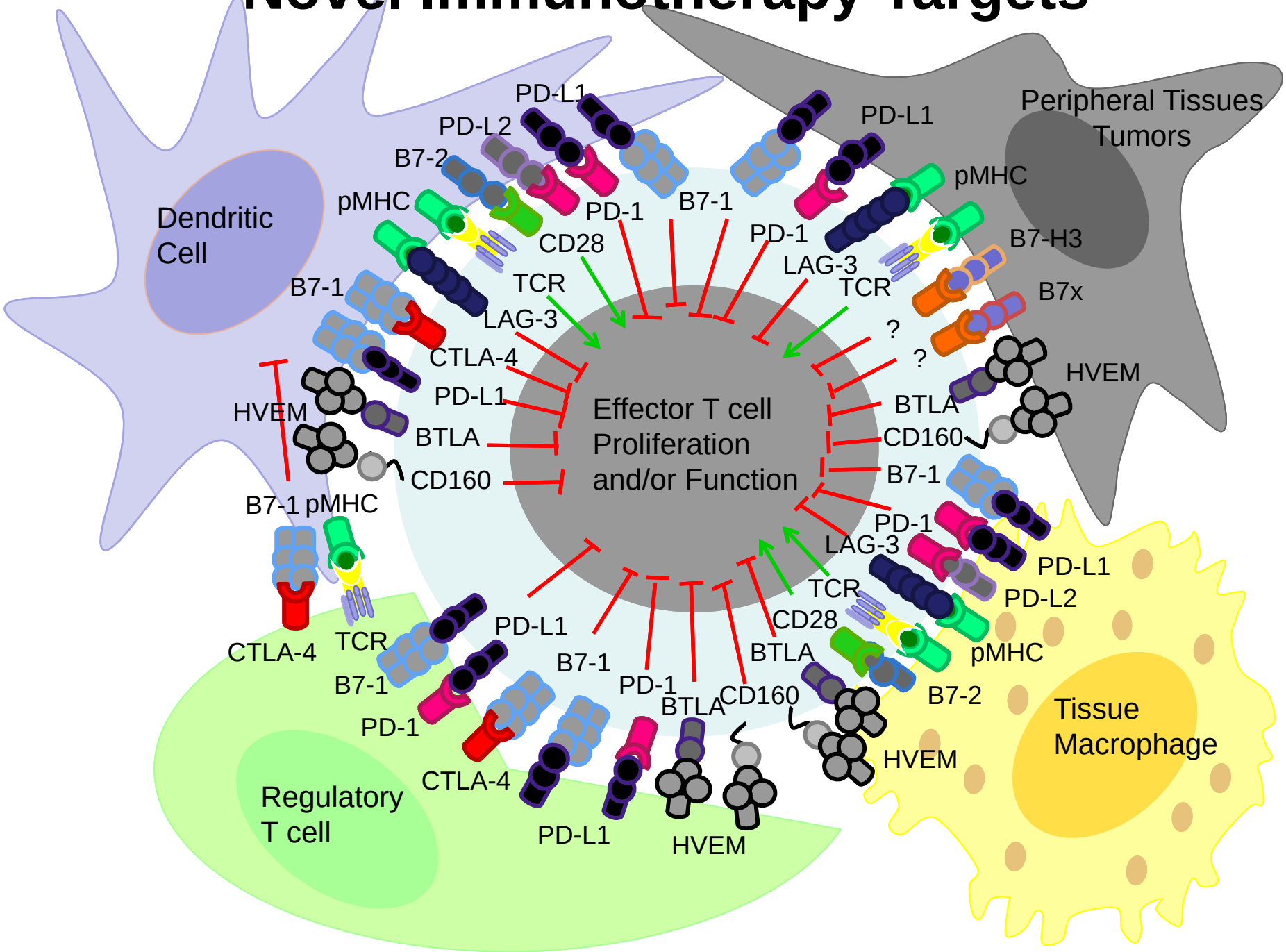
ICOS KO





Fan

Novel Immunotherapy Targets



Conclusions

- **CTLA-4 blockade elicits measurable immunologic changes, which consist of an increase in the frequency of $ICOS^+$ T cells in peripheral blood and tumor tissues**
- **$CD4^+ICOS^{hi}$ T cells from treated patients contain a population that are IFNγ-producing effector T cells and can recognize tumor antigen**
- **ICOS is the first immunologic marker identified in both tumor tissues and the systemic circulation that can be used as a biomarker for monitoring of anti-CTLA-4 treated patients as a possible pharmacodynamic marker of therapy**
- **ICOS appears to mediate signaling via the PI3K pathway to enhance T cell function and anti-tumor immune responses**
- **ICOS/ICOSL pathway may serve as another target for immunotherapy; ongoing studies with IVAX, anti-ICOS abs and ICOSL-Ig fusion protein**
- **Pre-surgical clinical trials provide a feasible platform to study biologic effects within tumor tissues thus leading to meaningful data that can be applied to the metastatic disease setting and provide insights into mechanisms**

- Sharma Lab Team
  - Derek Ng Tang
  - Hong Chen
  - Liangwen Xiong
  - Jingjing Sun
  - Tihui Fu
  - Qiuming He
  - De-Yu Shen
  - JJ Gao
  - Salah Tahir
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- Immunology and Immunotherapy Platform
  - Jim Allison
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- And, the **PATIENTS!**

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MDACC Bladder SPORE
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