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A Therapeutic Enzyme for Highly Effective Immune Checkpoint Inhibition in Cancer

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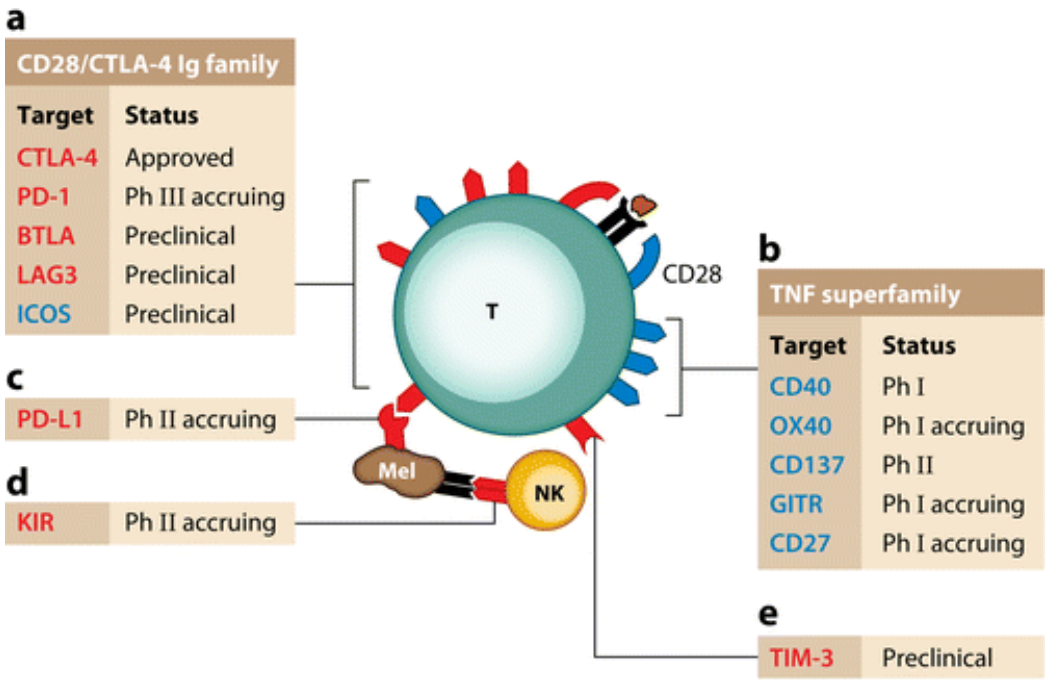
NIH National Institutes of Health

American Cancer Society

COI Declaration: Presenter is the founder and holds equity in Kyn Therapeutics

Modalities of Immune Checkpoint Inhibition

I. Protein Ligand Mediated Signaling

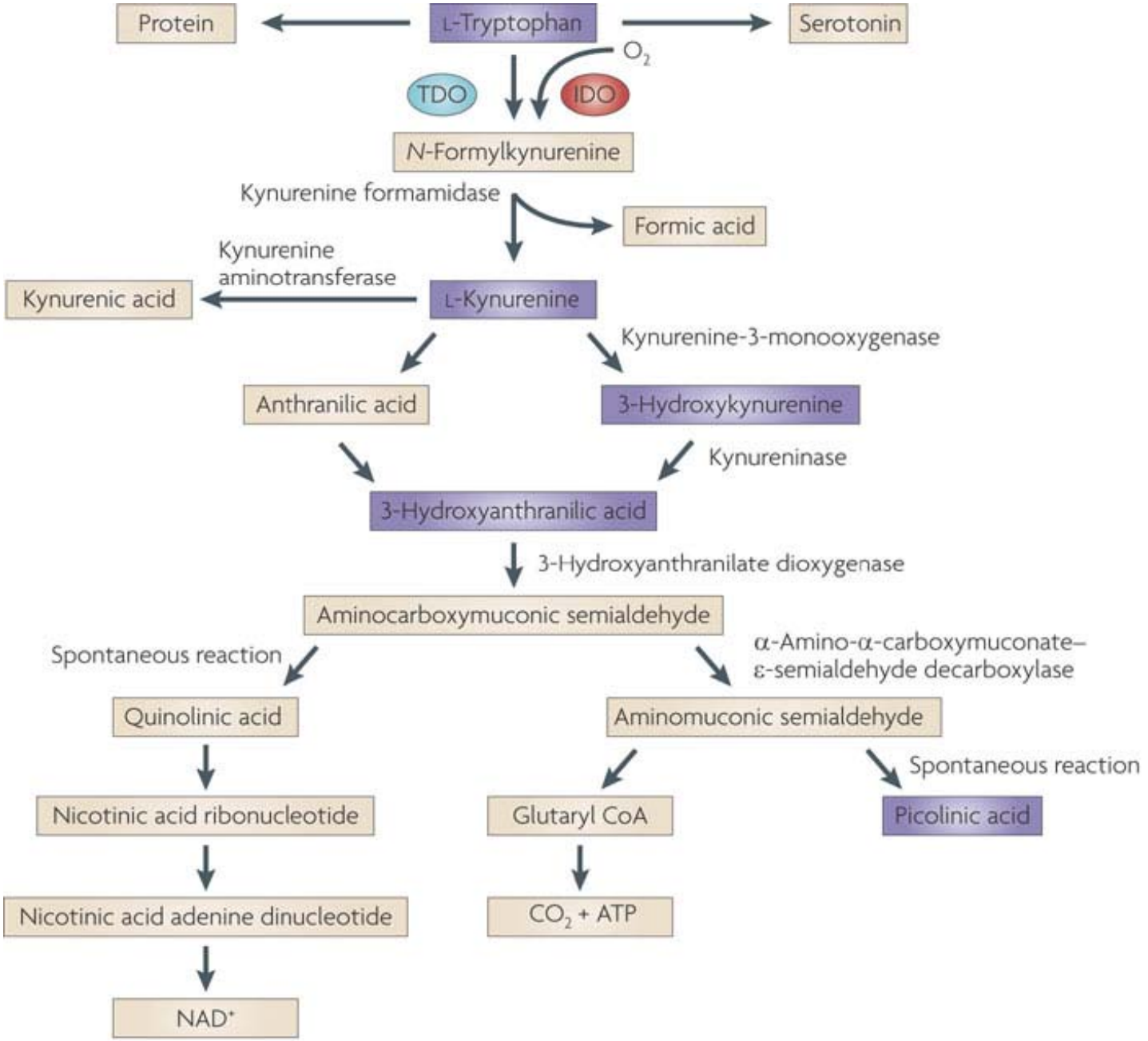


Page DB, et al. 2014.
Annu. Rev. Med. 65:185–202

II. Metabolite-Mediated Immune Modulation

- ***Trp oxidation/ Kynurenine pathway (IDO/TDO pathway***
- **ROS**
- **Adenosine**
- **Arginine**
- ***Lactate?***

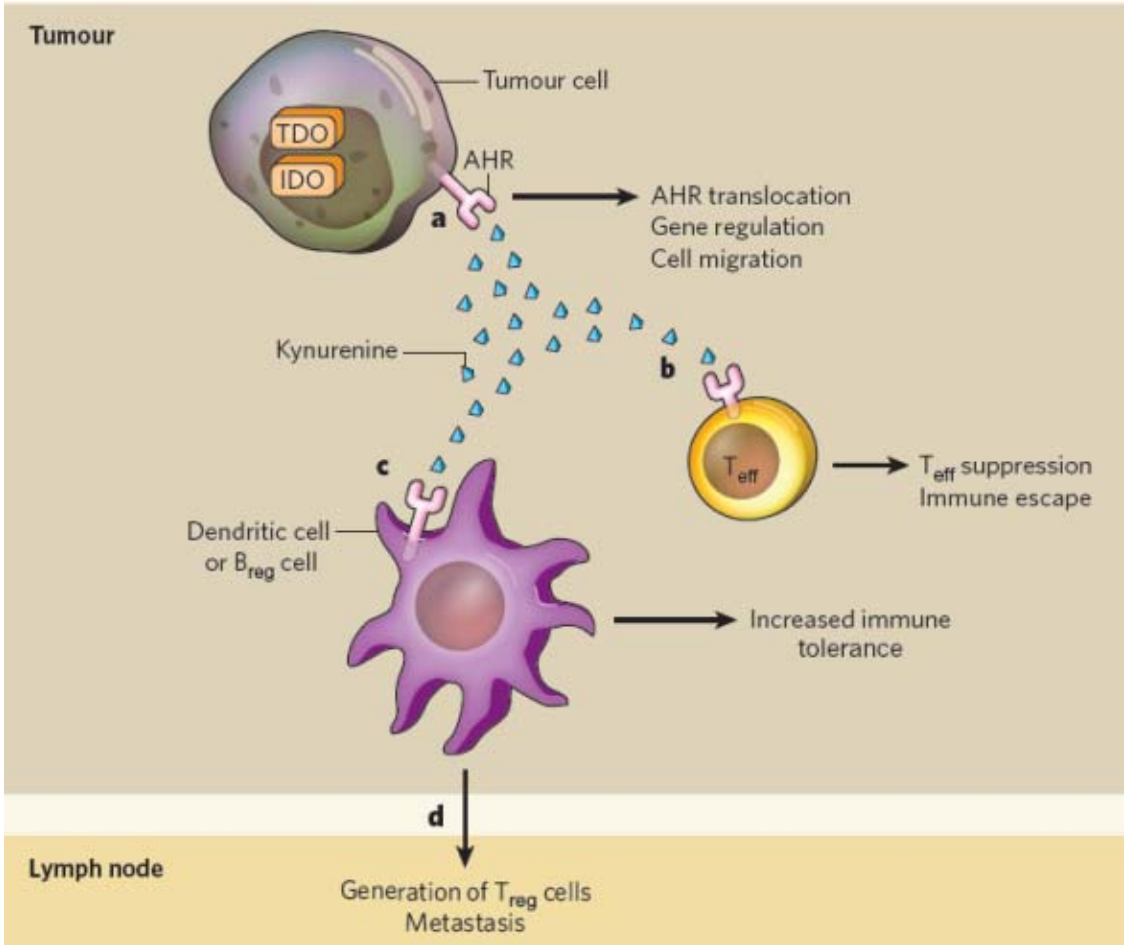
Tryptophan Metabolism



Nature Reviews | Cancer

Immunosuppressive & Tumor Promoting Effects of Kynurenine and its Downstream Products

- ***Exerts its effects though Activation of AhR (Aryl Hydrocarbon Receptor)***
- **T cell apoptosis**
- **Induces Tregs *in vitro***
- **Increases MDSC infiltration**
- **Decreased NK and T cell activation**
- **Induction of tolerogenic dendritic and B cells**
- **Enhances tumor growth**



It is widely thought that immune suppression by the Trp catabolism pathway is due to the depletion of serum Trp. However detailed quantitative analysis indicates that this is not the case:

- *For Trp to be limiting its concentration has to decrease >200 fold relative to serum*
- *Direct immune suppressive effects of Kyn at micromolar concentrtions are well established*

Inhibition of the Kyn Pathway for Cancer Immunotherapy

> 12 clinical trials of IDO1 inhibitors on-going

- **Only IDO1 inhibitors currently in the clinic**

  - ***IDO1: IFNγ inducible, expressed in numerous tumors***
  - ***Contribution and role of the IDO2 isoform have not been established***
  - ***TDO inhibition likely associated with toxicities***

- **IDO1 inhibitors in clinical/preclinical development**

  - ***Incyte therapeutics, INCB24360: Phase II/III (8 clinical trials)***
  - ***Roche, GDC-0919 (acquired from NLG for $175 mil): Phase I***
  - ***BMS, F001287 (acquired from NLG for $800 mil): Preclinical***
  - ***New Link Genetics, Indoximod; Phase II (2 clinical trials)***
  - ***Roche IDO/TDO inhibitor (acquired from Curadev $25 mil); preclinical***

Limitations of Small Molecule IDO1/TDO Inhibitors

1. **No clear PD biomarker for *IDO1 inhibitors*; serum Kyn level impacted by IDO1 inhibitors only when tumor burden is high**

2. **Weak or no anti-tumor effects as monotherapy in published preclinical models**

   e.g. Spranger et al. J. Immunother. Cancer (2014)

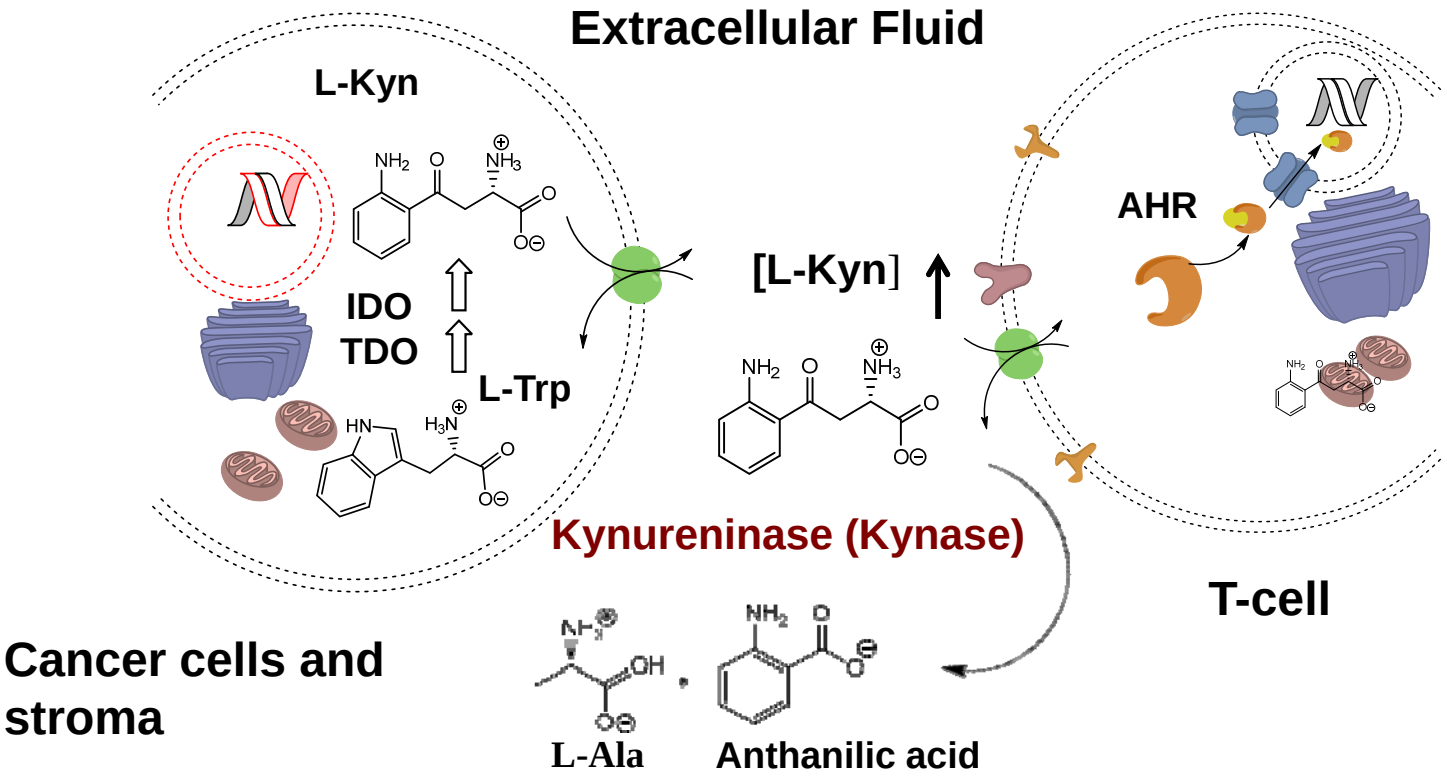
3. **Redundancy in Kyn synthesis pathways in tumors:**
   IDO1 +ve: 16% TDO: +ve: 19% IDO1+TDO: 15%

   e.g. Pilotte et al. PNAS (2012)

4. **Toxicity concerns with TDO or IDO1+TDO inhibition: >10-fold elevation in serum Trp levels, elevated Seretonin, blockade of nicotinamide synthesis bladder-generated carcinogens**

5. ***Highly competitive landscape, no clear differentiator***

Hypothesis: Enzyme-mediated elimination of Kynurenine (Kyn) into non-toxic can offer significant therapeutic advantages relative to IDO/TDO inhibitors



Kynureninase: A Checkpoint Inhibitor Therapeutic Enzyme

- **<u>Effect of Kyn is paracrine</u>: degradation of extracellular Kyn blocks immunosuppressive effects independent on IDO/TDO expression status**
- **<u>Sensitive, readily observable PD effect</u> i.e. monitoring serum Kyn concentration**
- **Intracellular, homeostatic Kyn pool, esp. liver, not perturbed**
- **Enzyme metabolites (L-Ala, anthanilic acid) inert and excreted in urine**
- **Minimal risk for off-site toxicities**
- **Whereas with IDO1/TDO small molecule inhibitors resistance can develop, cannot envision resistance to Kynase unless the entire pathway is bypassed**

Kynase Mechanistic Validation Studies

Enzyme	K_{cat}/K_m for Kyn (M^{-1}/s^{-1})	K_{cat}/K_m for DL 3' OH-Kyn (M^{-1}/s^{-1})
Homo sapiens **Kynase**	$4.6x10^2$	$1.2x10^5$
Mus musculus **Kynase**	$8x10^2$	NT

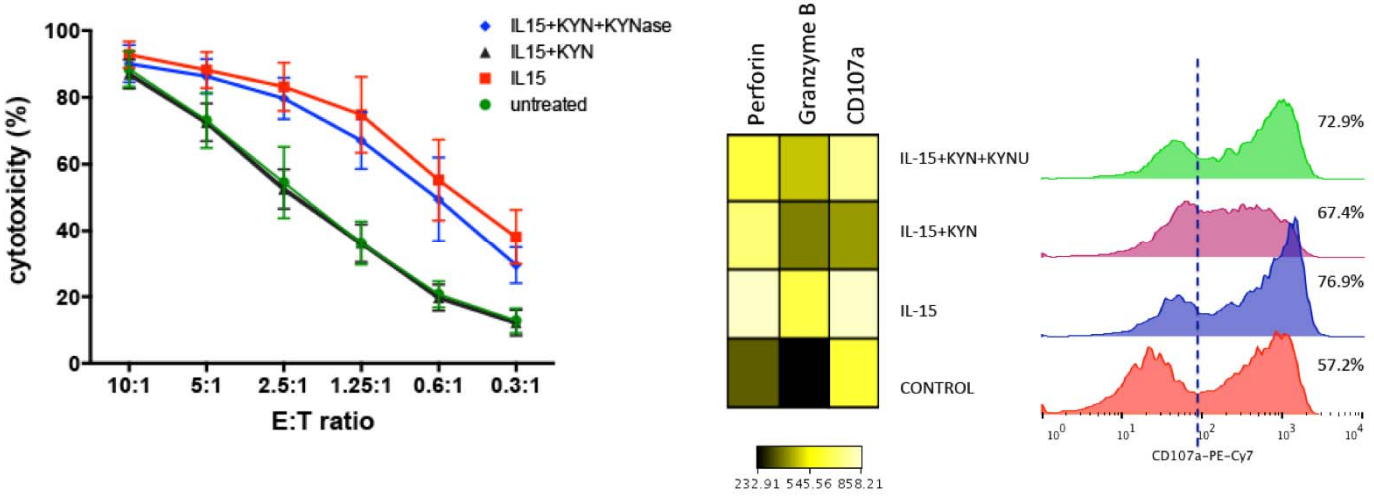
Many prokaryotic Kynases display high activity towards Kyn vs 3'OH Kyn

Enzyme	K_{cat}/K_m for Kyn (M^{-1}/s^{-1})	K_{cat}/K_m for DL 3' OH-Kyn (M^{-1}/s^{-1})
Pf-Kynase	$6.0x10^5$	$1.8x10^2$
Mp-Kynase	$3.7x10^4$	$5.2x10^2$
Ca-Kynase	1.2	1.5
Fs-Kynase	9.9	13.8
Cp-Kynase	$2.7x10^4$	$4.2x10^2$
Cm-Kynase	$1.4x10^2$	$1.2x10^5$

In vitro Reversal of Kyn-mediated Immune Suppression by Kynase

Kynase:

1. **Prevents T cell apoptosis by Kyn**
2. **Restores activation of T cells incubated with Kyn**
3. **Reverses Kyn induced NK cell anergy**



Data by Professor Dean Lee MD Anderson

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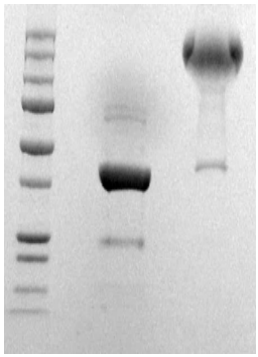
Pharmacodynamics

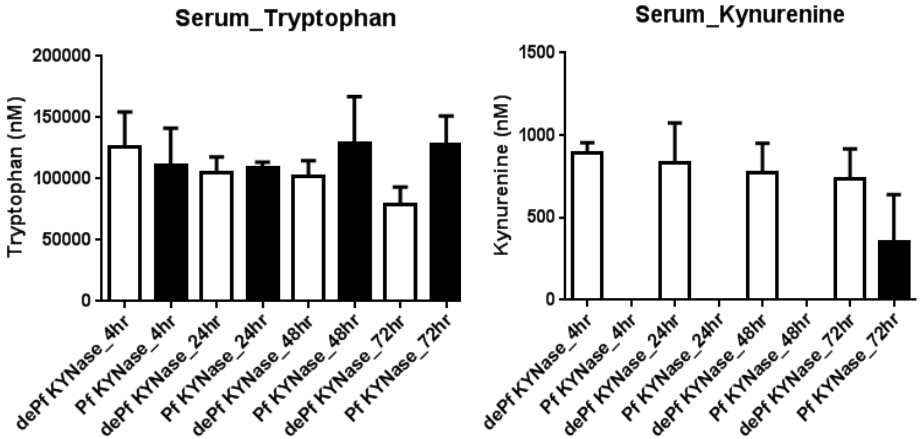


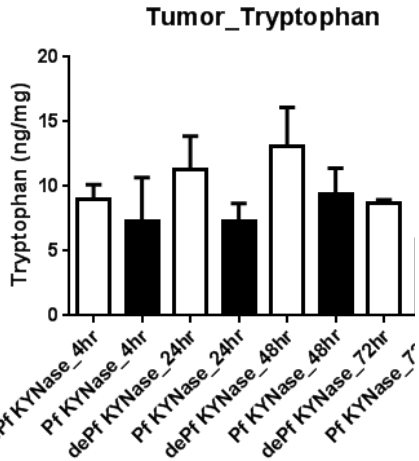
Pf-Kyn-Peg 5K

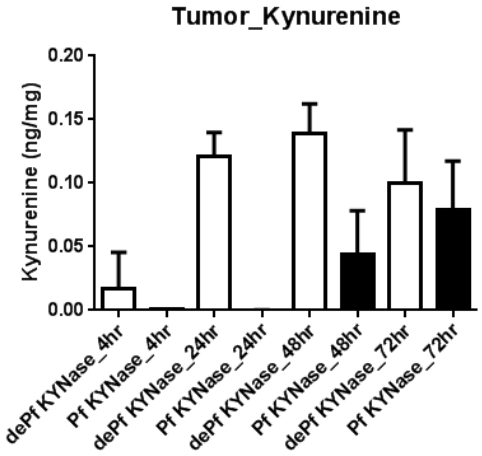
Kynase

- Pf-Kynase (bacterial) expressed in *E.coli*
- Purified to 95% homogeneity, endotoxin <20 EU/mg,
- PEGylated by NHS-PEG 5 K

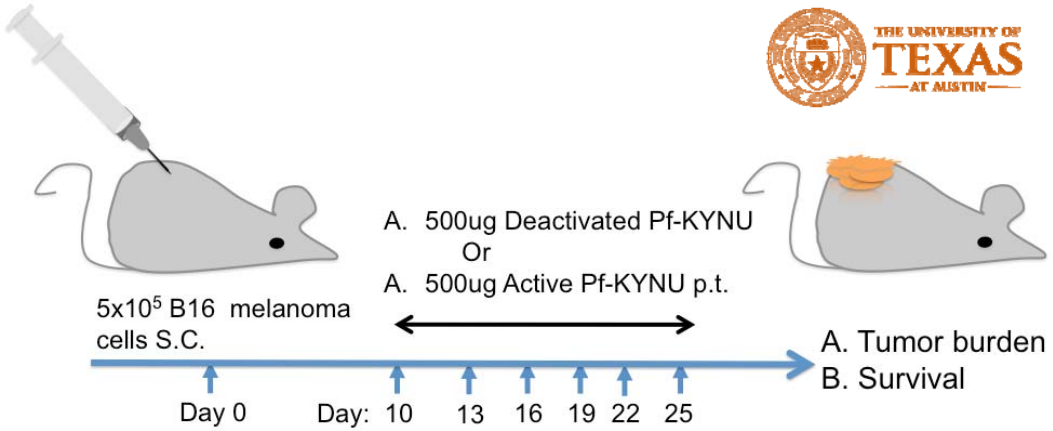
Single Dose PD in B16 Melanoma Model



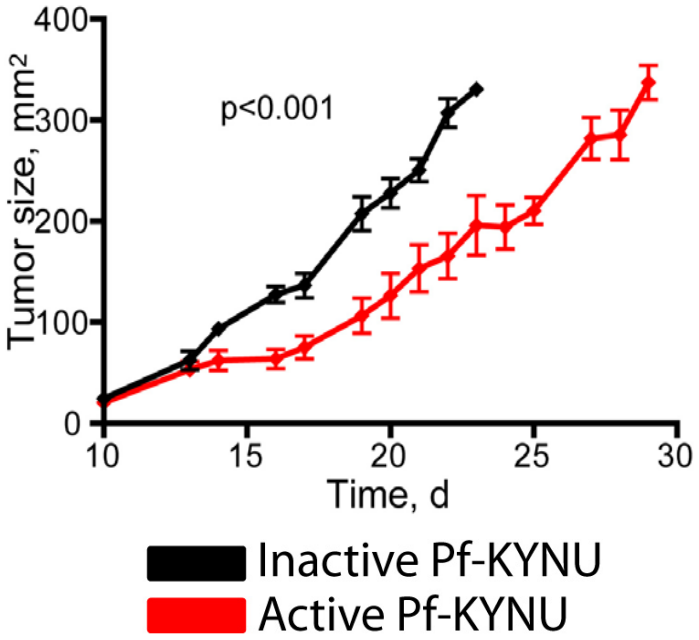


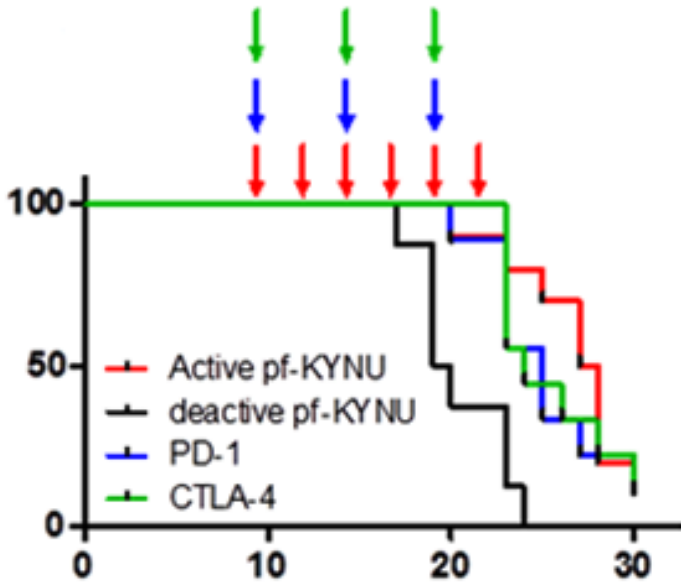


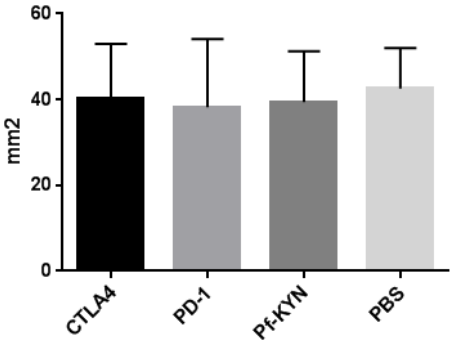
Kynase Monotherapy in B16 Melanoma Model



N = 20
P value = 0.003

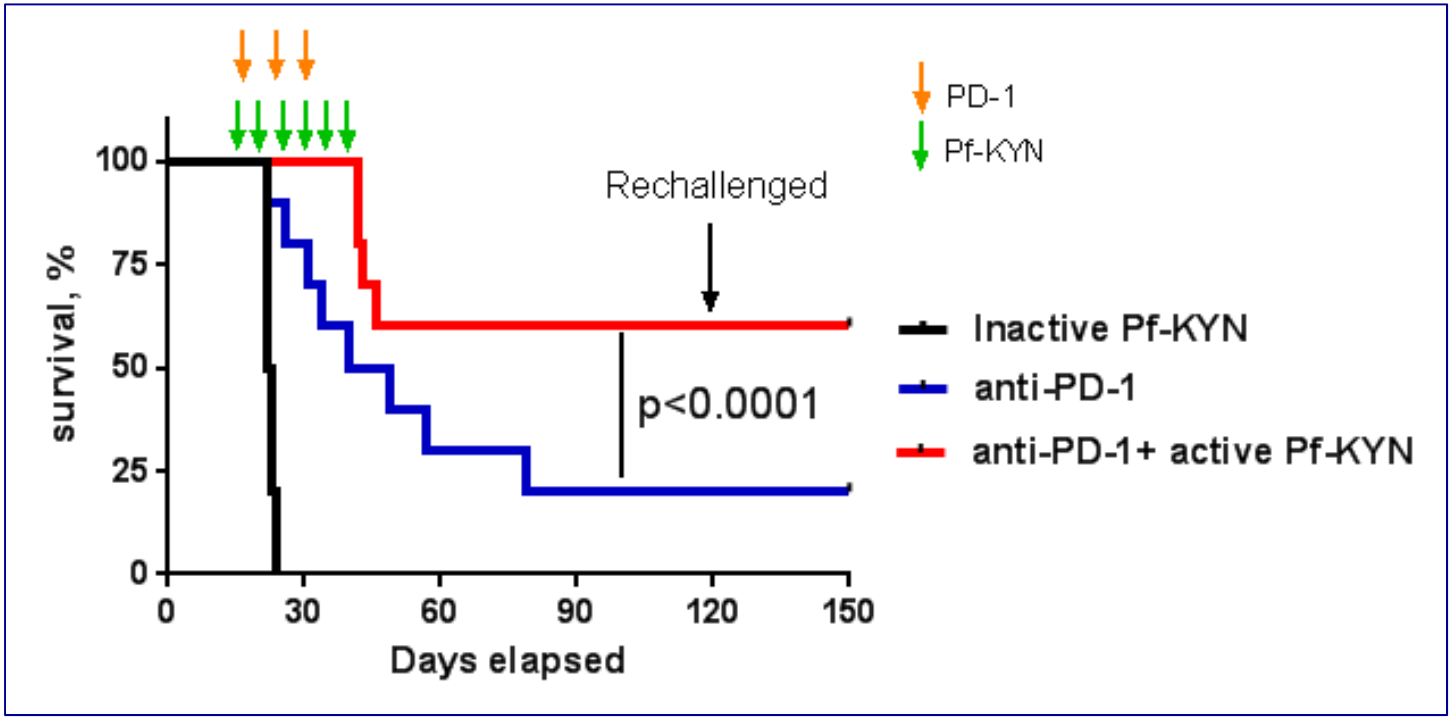


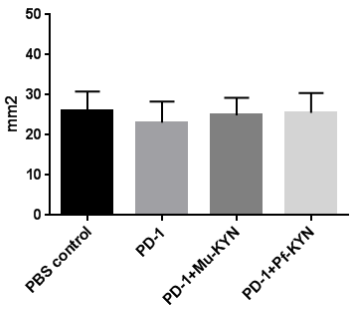




Mean tumor size at treatment start

Combination Therapy with anti-PD-1+Kynase



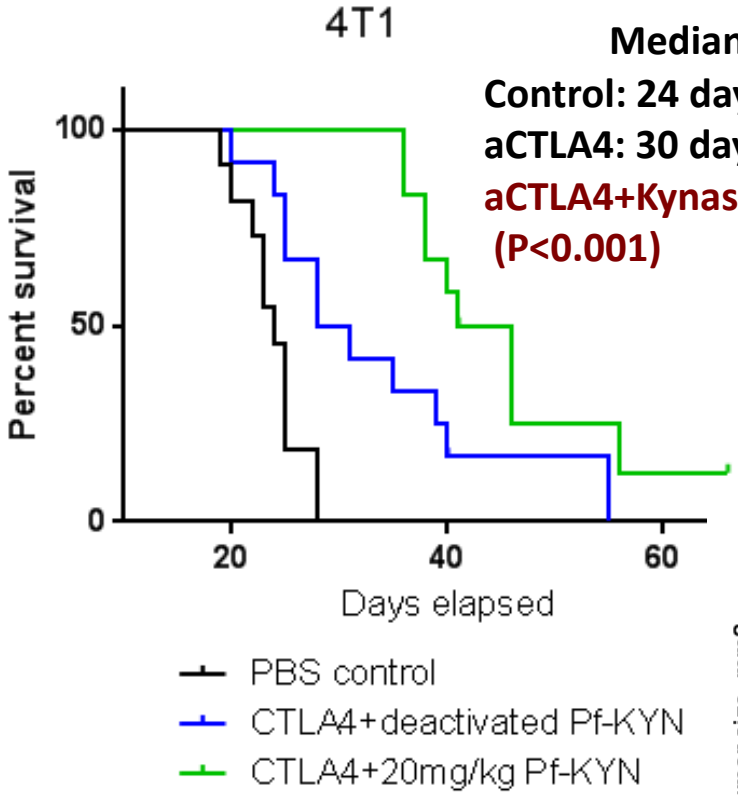


Mean tumor size at onset of treatment= 25 mm^2

aPD-1+Kynase: 6/10 mice survived; immune to re-challenge with B16F10
aPD-1 only: 2/10 mice survived

Tumor model: B16F10 5x10^4 cells injected S.C; n=10 (repeated 2x)
Kynase: 6 doses 20mg/kg weight, every 3 days starting d=10; aPD-1 (clone RMP1-14) at 250 μg/animal dosed on d=10, 14, 18

Combination Therapy with a-CTLA4 in 4T1 Breast Cancer Model

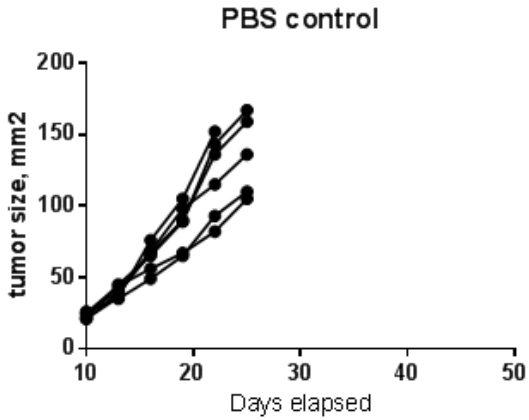


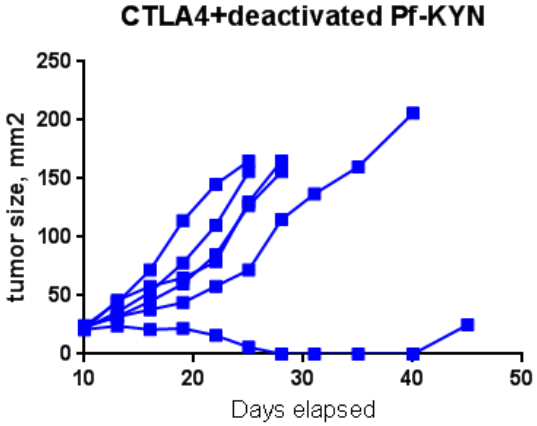
Median survival:

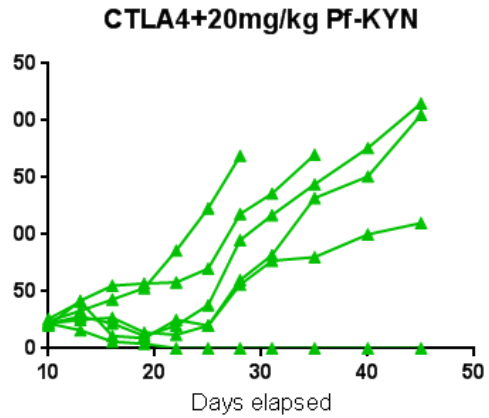
Control: 24 days

aCTLA4: 30 days

aCTLA4+Kynase: 43 days (P<0.001)







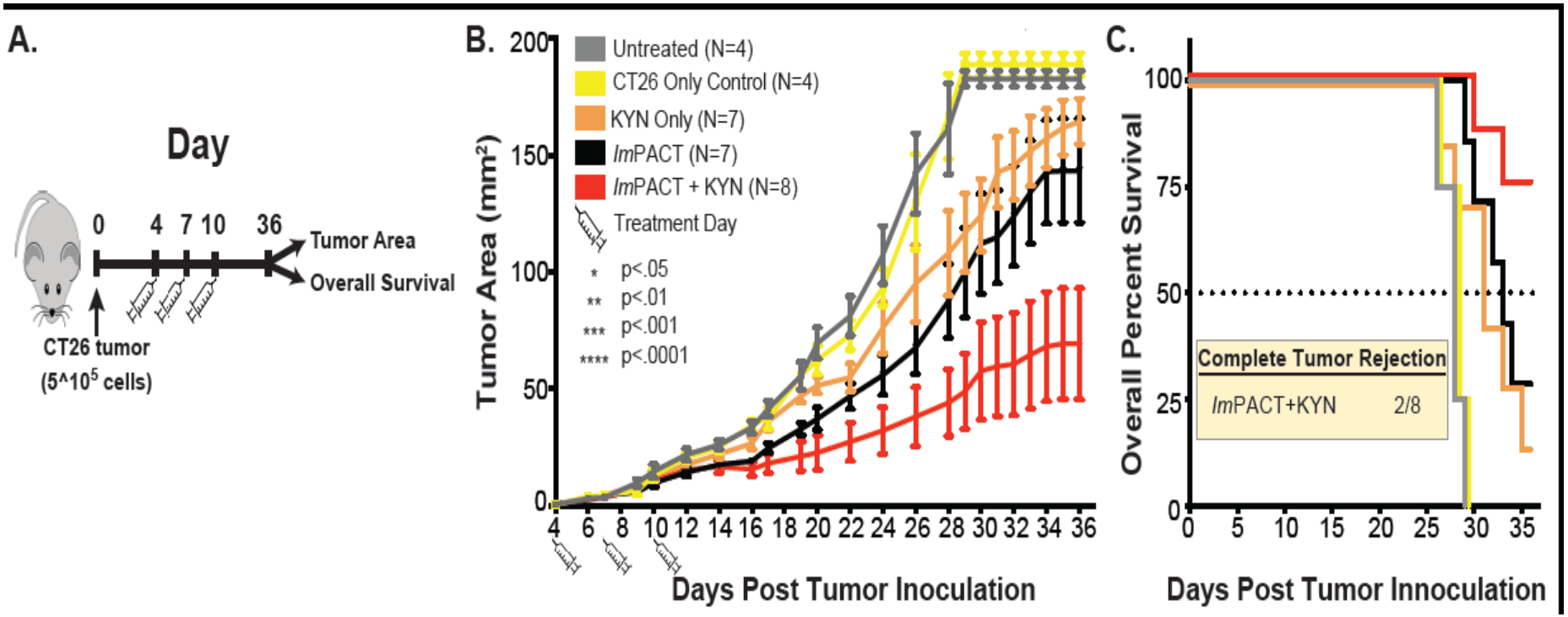
Tumor model: 4T1 5x10^4 cells injected S.C.

Kynase: 6 doses 20mg/kg weight, every 3 days starting d=10

aCTLA4 (clone 9H10): 200 mg/animal, dosed on d=10, 13, 16 (Holmgaard JEM 2013)

Combination with Cancer Vaccine in CT26 Colon Cancer Model

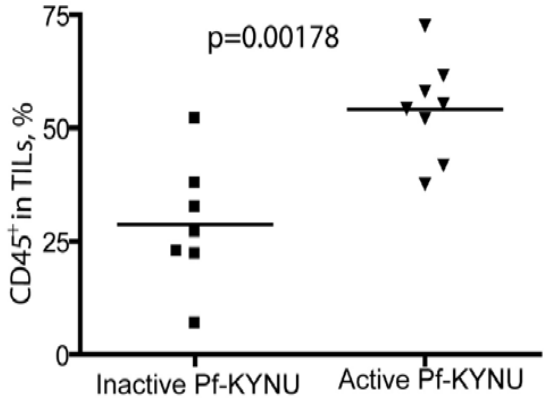
imPACT: Heat Biologics Vacine Technology; Gp96 Expressing Tumor Cells



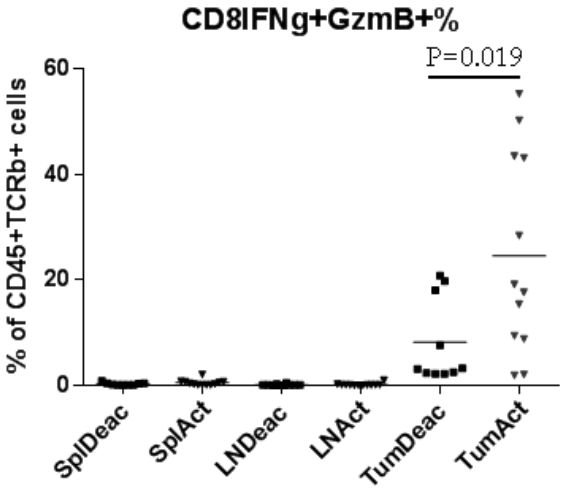
Data by Drs. Taylor Schreiber, George Fromm, Heat Biologics

In vivo Mechanistic Effects of Kynase in B16 Melanoma

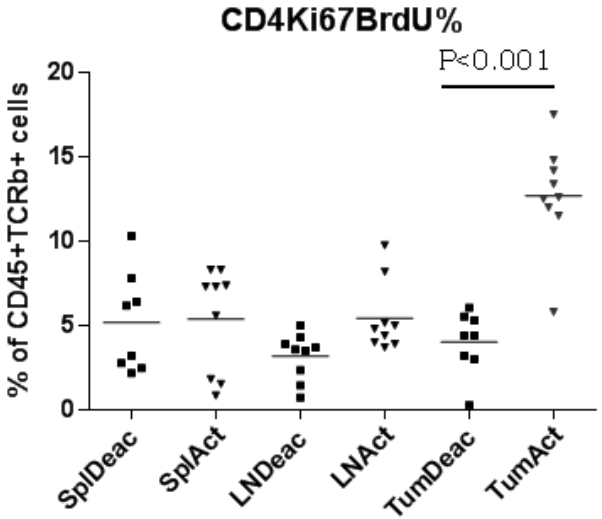
Increased tumor CD45 cell infiltration

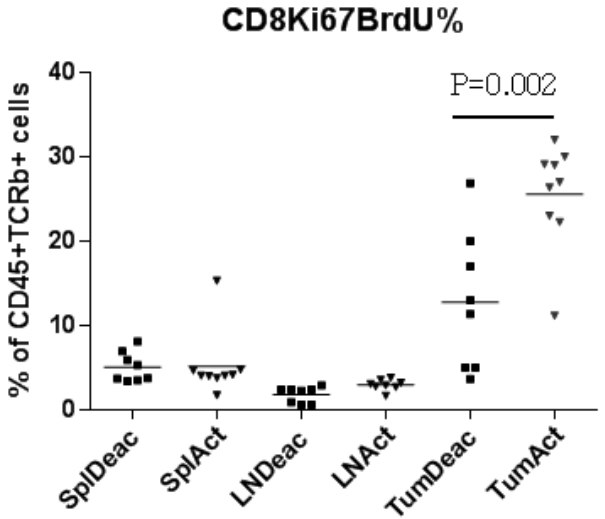


Markedly higher cytotoxic CD8+ TILs



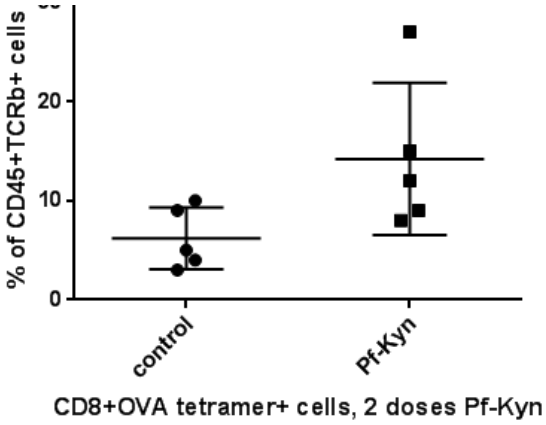
Increased proliferation of tumor CD4+ & CD8+ TILs



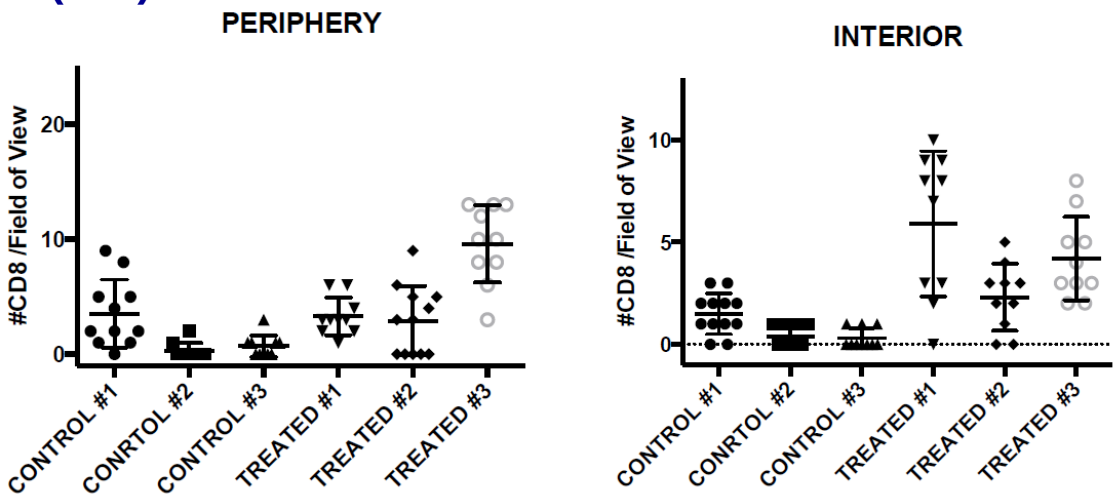


In vivo Mechanistic Effects of Kynase in B16 Melanoma (cont.)

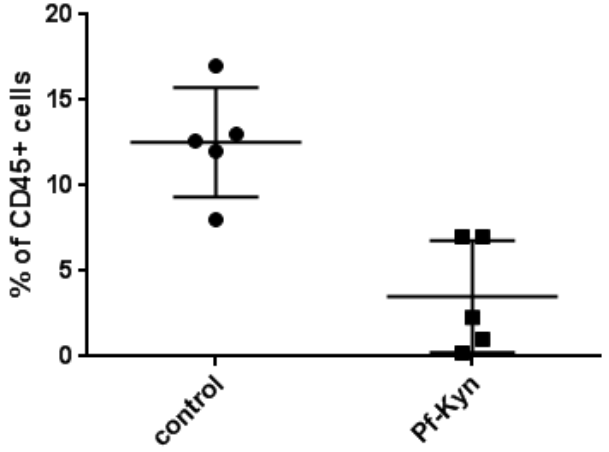
Increased Antigen-Specific TIL CD8+ Cells



Higher CD8+ TIL Penetration in Tumor Interior (IHC)



Decreased MDSCs Infiltration



Engineering a Clinical Candidate Human Kynase

Use of bacterial enzymes for Kyn depletion and immune checkpoint inhibition poses immunogenicity risk

Engineer a human Kynase suitable for clinical development

Deliverables:

- **>500 fold increase in catalytic activity towards Kyn**
- **> PD profile suitable for once a week injection**
- **"Developability": Expression, biophysical stability, solubility**
- **Immnogenicity de-risking**
- **Preliminary CMC and release tests**
- **Rodent and NHP PD and tox (non-GLP)**

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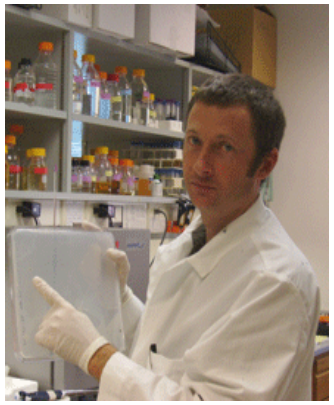

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