TNFR2 Targeting: Cancer Treatment Through Direct Tumor Killing and Treg elimination

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THE TNFR2 TARGET (TNFRSF1B)

TNFR2 is *not* widely expressed on normal cells, but highly expressed in the tumor micro-environment

-on highly activated and potent **Tregs**
-on **oncogene** expressing tumors
-on **MDSC**

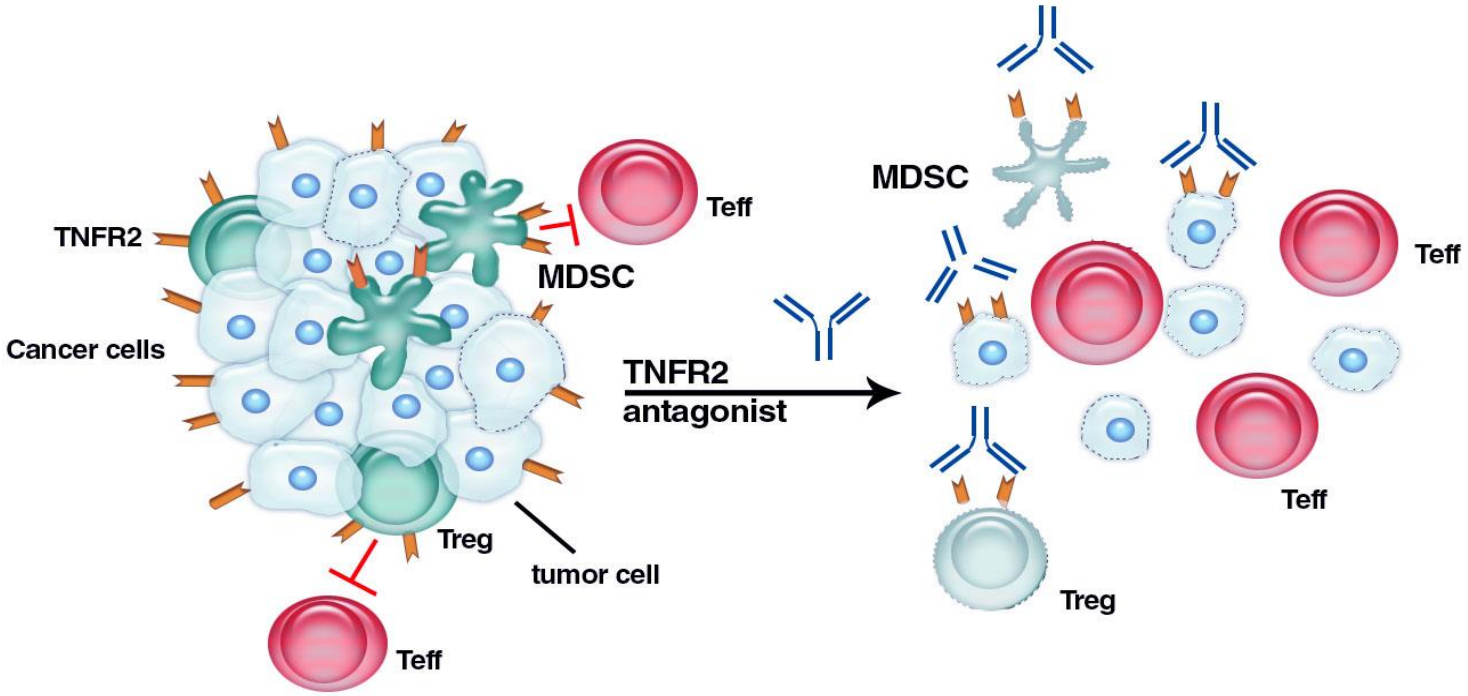
TNFR2 is a signaling pathway most over expressed protein of the cancer micro-environment

- Analysis of over 5,000 subjects with more than 20 tumors shows TNFR2 is the most abundant protein on the infiltrating T cells of tumors is TNFRSF1B (TNFR2)

TNFR2 is may be a primary escape mechanism for αPD1/PDL1 and αCTLA4 failures

TNFR2 is the most broadly expressed surface oncogene on many human cancers

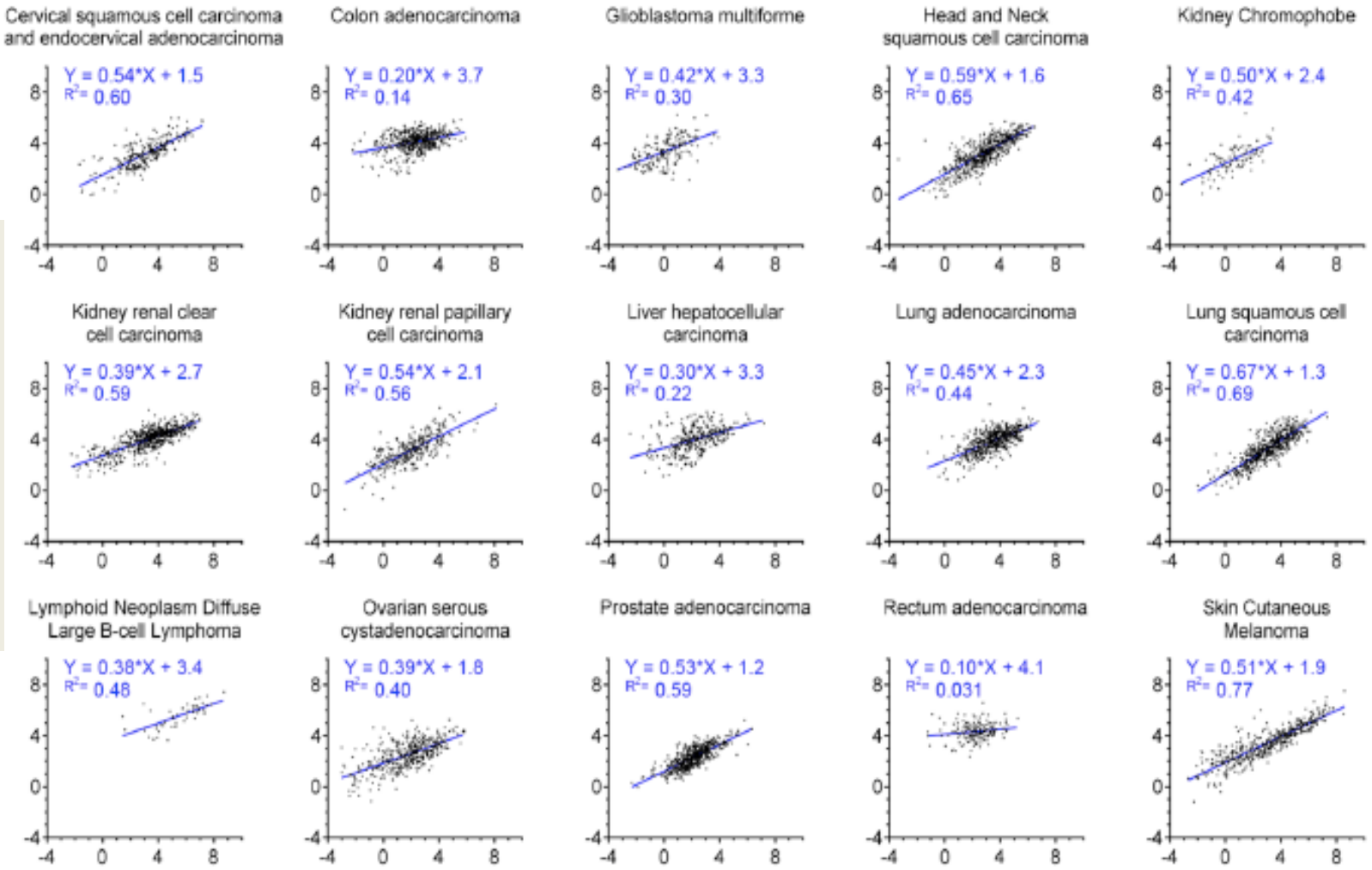
TNFR2 Promotes tumor progression and preserves immunosuppression in the tumor microenvironment



Note: Live Cells, Cancer Cells, Treg, MDSC, Dead Cells, Injured Cells, Teff

Note: Live Cells, Teff, Dead Cells, Cancer Cells Treg,

Diverse human tumors have high TNFR2 expression associated with T cell infiltrates

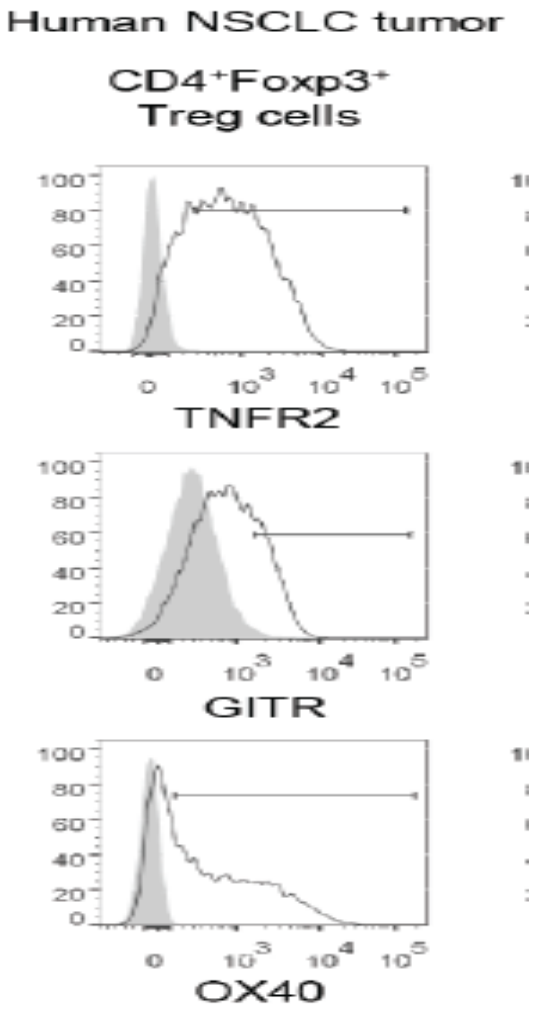


Oncotarget 2016, Medimmune/AstraZeneca

The Cancer Genome Atlas

TNFR2 Tregs Compared to Popular Targets

- TNFR2 compared to other targets in the infiltrate
  - GITR and OX40
  - Tregs expressing each of the targets
  - TNFR2 has more expression in the tumor microenvironment
- TNFR2 is the dominant protein expressed in the tumor microenvironment



Oncotarget 2016, Medimmune/AstraZeneca

Science 2016, Broad/DFCI Institute

RESEARCH ARTICLES

CANCER GENOMICS

Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq

Itay Tirosh,[1*] Benjamin Izar,[1,2,3*†‡] Sanjay M. Prakadan,[1,4,5,6] Marc H. Wadsworth II,[1,4,5,6] Daniel Treacy,[1] John J. Trombetta,[1] Asaf Rotem,[1,2,3] Christopher Rodman,[1] Christine Lian,[7] George Murphy,[7] Mohammad Fallahi-Sichani,[8] Ken Dutton-Regester,[1,2,9] Jia-Ren Lin,[10] Ofir Cohen,[1] Parin Shah,[2] Diana Lu,[1] Alex S. Genshaft,[1,4,5,6] Travis K. Hughes,[1,4,6,11] Carly G. K. Ziegler,[1,4,6,11]

Result: A very abundant gene expressed in the Tregs of melanoma is **TNFSF1B** (TNFR2).

TNFR2 ONCOGENE PUBLICATIONS

Nature Genetics 2015

Genomic analysis of mycosis fungoides and Sézary syndrome identifies recurrent alterations in TNFR2

Alexander Ungewickell[1,2,12], Aparna Bhaduri[1,12], Eon Rios[1], Jason Reuter[3], Carolyn S Lee[1], Angela Mah[1], Ashley Zehnder[1], Robert Ohgami[4], Shashikant Kulkarni[5–7], Randall Armstrong[8], Wen-Kai Weng[8], Dita Gratzinger[4], Mahkam Tavallaee[9], Alain Rook[10], Michael Snyder[3], Youn Kim[9] & Paul A Khavari[1,11]

Mycosis fungoides and Sézary syndrome comprise the majority of cutaneous T cell lymphomas (CTCLs), disorders notable for their clinical heterogeneity that can present in skin or peripheral blood. Effective treatment options for CTCL are limited, and the genetic basis of these T cell lymphomas remains incompletely characterized[1]. Here we report recurrent point mutations and genomic gains of *TNFRSF1B*, encoding the tumor necrosis factor receptor TNFR2, in 18% of patients with mycosis fungoides and Sézary syndrome. Expression of the recurrent TNFR2 Thr377Ile mutant in T cells leads to enhanced non-canonical NF-κB signaling that is sensitive to

frequent recurrent point mutation in our data set occurred at codon 377 of *TNFRSF1B* (5%; 4/73), encoding tumor necrosis factor receptor 2 (TNFR2), resulting in a recurrent TNFR2 Thr377Ile mutant. TNFR2 is a receptor that regulates key T cell signaling pathways and has not previously been implicated in cancer (**Fig. 1a**)[4].

We noted that many somatic alterations involved pathways related to TNFR2 and non-canonical nuclear factor (NF)-κB signaling, primarily regulating T cell survival and proliferation (**Fig. 1a**), including the TNFR2 pathway itself, as well as the T cell receptor (TCR) and CD28 pathways. This finding is consistent with previous transcriptome sequencing indicating differential expression of genes in the

Oncogene mutations drive TNFR2 expression in CTCL

Science Signaling 2017

RESEARCH ARTICLE | CANCER IMMUNOTHERAPY

Targeting TNFR2 with antagonistic antibodies inhibits proliferation of ovarian cancer cells and tumor-associated T_{regs}

Heather Torrey[1], John Butterworth[1], Toshiyuki Mera[1], Yoshiaki Okubo[1], Limei Wang[1], Danielle Baum[1], Audrey Defusco[1], Sara Plager[1], Sarah Warden[1], Daniel Huang[1], Eva Vanamee[1], Rosemary Foster[2], and Denise L. Faustman[1,*]

+ Author Affiliations

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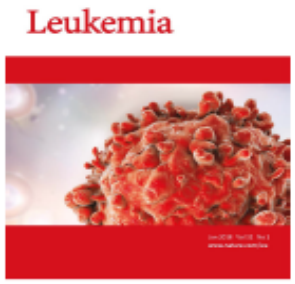
Sci. Signal. 17 Jan 2017:
Vol. 10, Issue 462,
DOI: 10.1126/scisignal.aaf8608

Novel TNFR2 antagonistic antibodies identified



Autocrine Tnf Signaling Favors Malignant Cells in Myelofibrosis in A Tnfr2-Dependent Fashion

TNFR2 pathways drives myelofibrosis through reduced amount of inhibitor Xiap, MAPK8 through JAK2 –V617F and increased cIAP

The Journal of Immunology

Transmembrane TNF-α Promotes Suppressive Activities of Myeloid-Derived Suppressor Cells via TNFR2

frontiers in Immunology

TNF Receptor 2 Makes Tumor Necrosis Factor a Friend of Tumors

Front. Immunol., 28 May 2018

TNFR2: The abundant surface oncogene of many human tumors

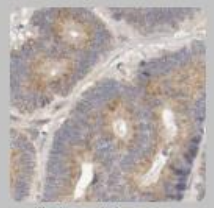
TNFRSF1B

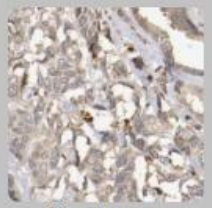
TISSUE | N/A SUBCELL | RNA CELL LINE | CANCER

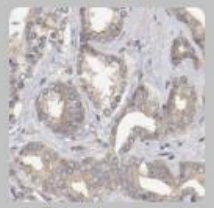
NE/PROTEIN
TIBODY/ANTIGEN
NCER ATLAS
ionary
Dictionary

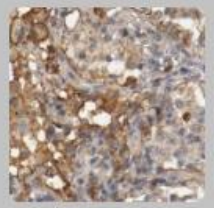
CANCER ATLAS

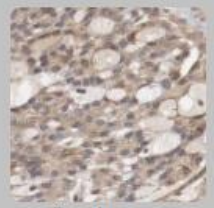
Gene description	Tumor necrosis factor receptor superfamily, member 1B
Protein class	Cancer-related genes, Candidate cardiovascular disease genes, CD markers, Plasma proteins, Predicted membrane proteins, Predicted secreted proteins
Protein evidence	Evidence at protein level


Colorectal cancer


Breast cancer


Prostate cancer


Lung cancer


Renal cancer

STAINING SUMMARY

HPA004796 | CAB013045

Tissue	Cancer staining	Protein expression of normal tissue	Tissue	Cancer staining	Protein expression of normal tissue
Breast cancer			Melanoma		
Carcinoid			Ovarian cancer		
Cervical cancer			Pancreatic cancer		
Colorectal cancer			Prostate cancer		
Endometrial cancer			Renal cancer		
Glioma			Skin cancer		
Head and neck cancer			Stomach cancer		
Liver cancer			Testis cancer		
Lung cancer			Thyroid cancer		
Lymphoma			Urothelial cancer		

Level of antibody staining/expression
High
Medium
Low
Not detected

Staining summary: Most malignant cells showed weak to moderate cytoplasmic staining. Cases of testicular and several cases of renal cancers displayed strong positivity. Distinct nuclear staining was seen in occasional cases of urothelial cancer.

TNFR2: The difficult task of TNFR2 antagonism

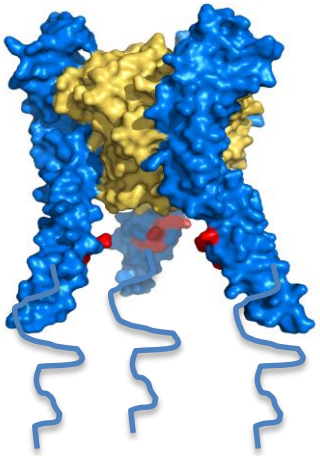
Basic Science of TNFR2 Antagonism

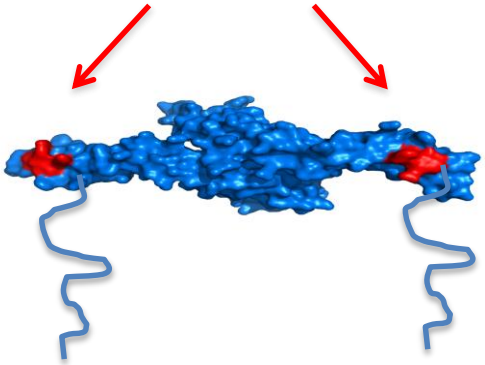
Multiyear program:

- A very small region of TNFR2 receptor is the binding region for antibody antagonism (not like CTLA4/PD1 blocking antibodies)
- It is possible to design TNFR2 antagonistic antibodies that can kill Tregs even in the presence of TNF
- Structural biology of the unique 3D structure of the inhibitory TNFR2 structure and prevention of intracellular signaling.

- Screening of dominant TNFR2 antibodies that have the following three desirable traits:
  1. Heightened tumor microenvironment Treg killing over peripheral Treg killing
  2. Heightened tumor microenvironment T effector proliferation
  3. Direct killing of TNFR2 oncogene expressing human tumors even with diverse underlying mutations for aberrant oncogene expression on parenchymal cells
  4. No cross linking required
  5. Ne Fc receptor needed
  6. Work well even when agonistic ligand, TNF, is present.

Dominant TNFR2 Antagonistic Antibodies
-the unique structural motifs
-the unique stabilization of the non-signaling anti-parallel dimer





Impossible for Dom Abs to bind to trimer

- -remember whole antibodies work
- -remember F(ab')2 antibodies work
- -remember Fab do not work

Dom Abs Produce Anti-parallel Dimers

- -remember whole antibodies work
- -remember F(ab')2 antibodies work
- -remember Fab do not work
- -remember dominant with TNF
- -remember independent of TNF

Binding region of dominant TNFR2 antagonists

Leukemia

Article | OPEN | Published: 24 October 2018

Immunotherapy

Targeted killing of TNFR2-expressing tumor cells and T_{regs} by TNFR2 antagonistic antibodies in advanced Sézary syndrome

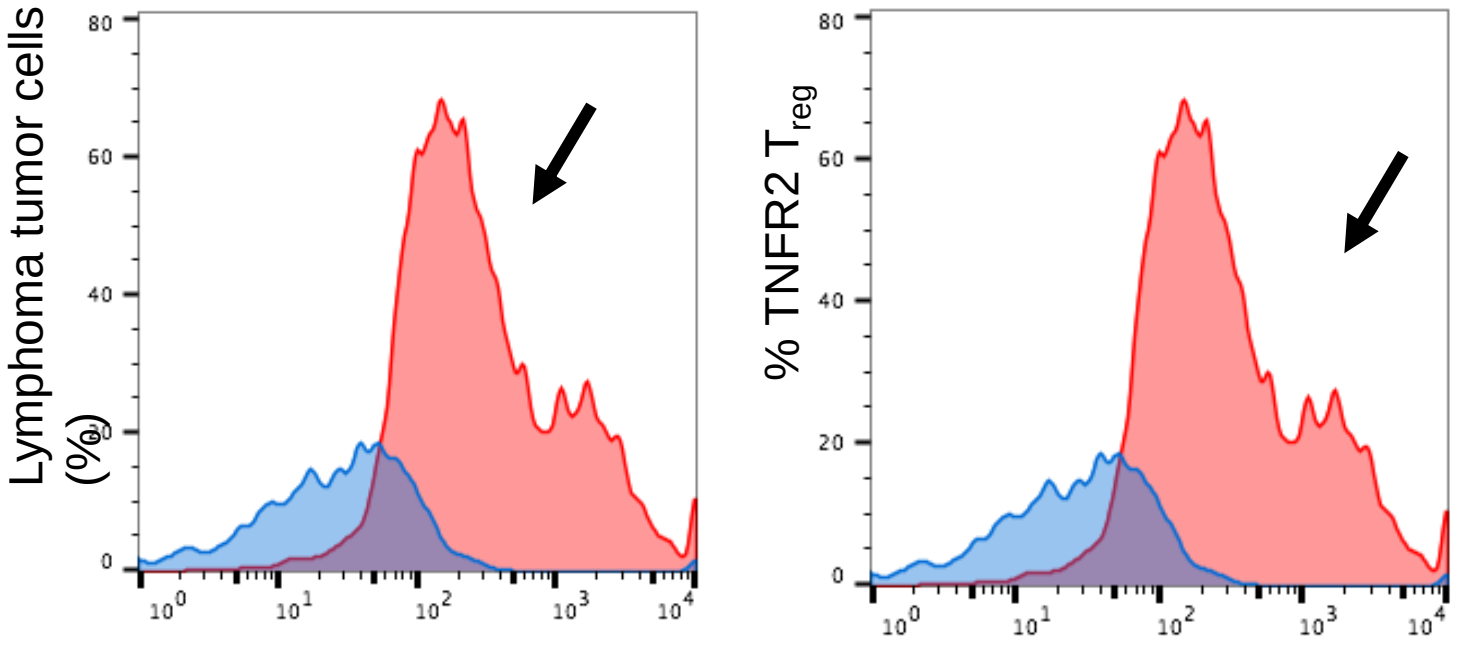
H Torrey, M Khodadoust, L Tran, D Baum, A Defusco, Y H Kim & D L Faustman - Show fewer authors

Leukemia (2018) | Download Citation

https://www.nature.com/articles/s41375-018-0292-9

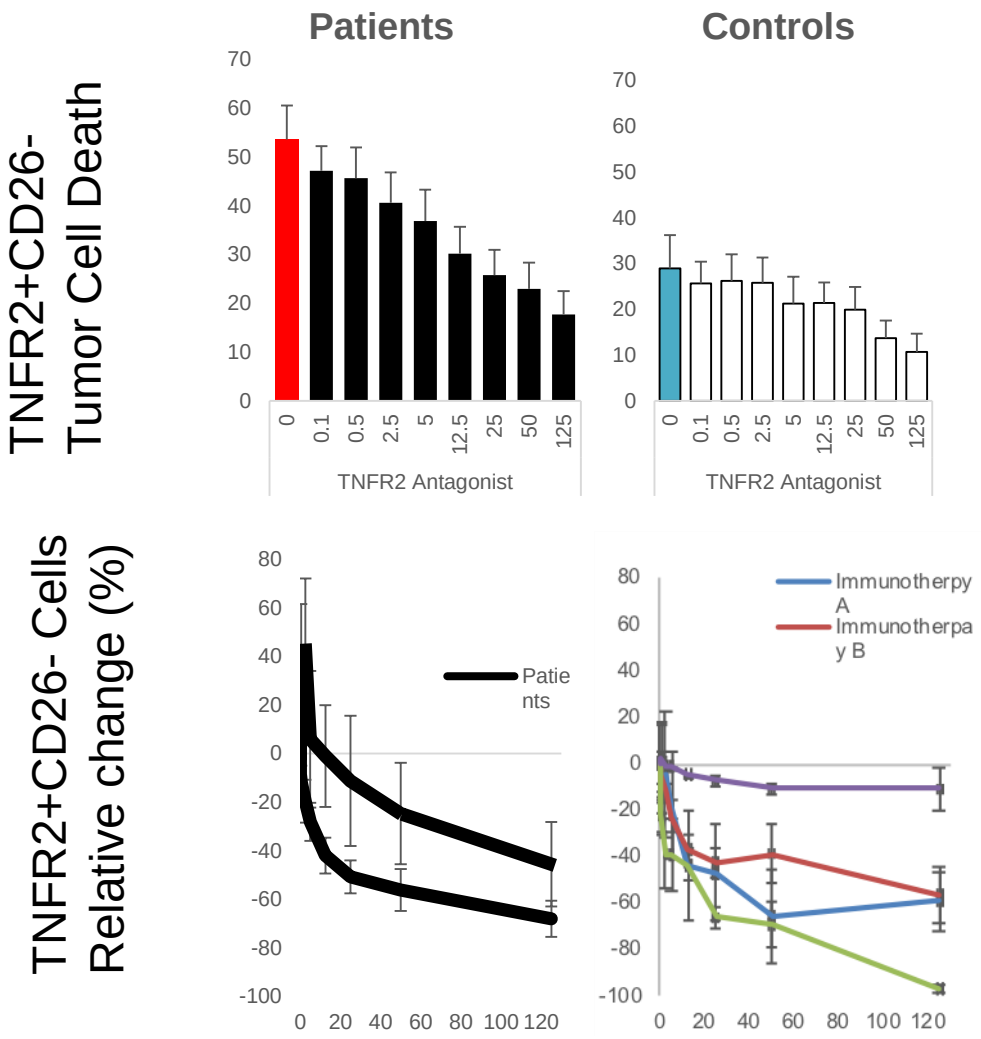
Massive TNFR2 Cancer Oncogene and Treg expression in lymphoma

- Patient Cells

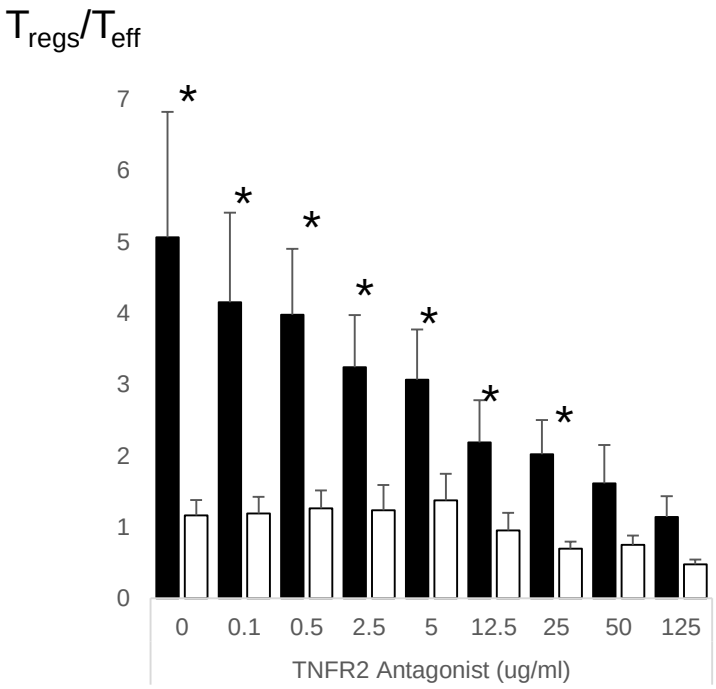


TNFR2 Oncogene Expressing CD26- CTCL

Impressive sensitivity to TNFR2 antagonists



The Severe Immune Dysregulation of CTCL: Correction with TNFR2 Antagonism



TNFR2 Oncology Program

- **Novel target:** TNFR2 (In vivo murine models have recently validated TNFR2 target)
  - TNFR2 receptor upregulated in Tregs of murine and human tumors
  - TNFR2 oncogene expressed on surface of many human tumors
  - TNFR2 is highly expressed protein of myeloid suppressor cells

- **Dominant TNFR2 antagonistic antibodies in human cancers**
  - Corrects the Treg/Teff imbalance
  - Specific to TME
  - Validated in diverse human tumors

- **Goal:** Place TNFR2 antibodies in correct reading frames for maintained antagonism