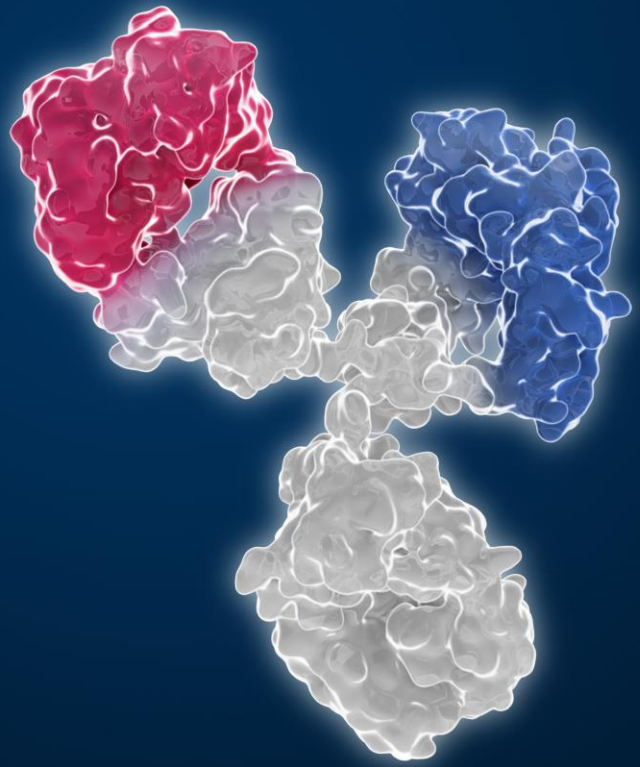


DEVELOPMENT OF NOVEL FULLY HUMAN BISPECIFIC ANTIBODIES FOR ONCOLOGY

Eric Smith

April 10th, 2019

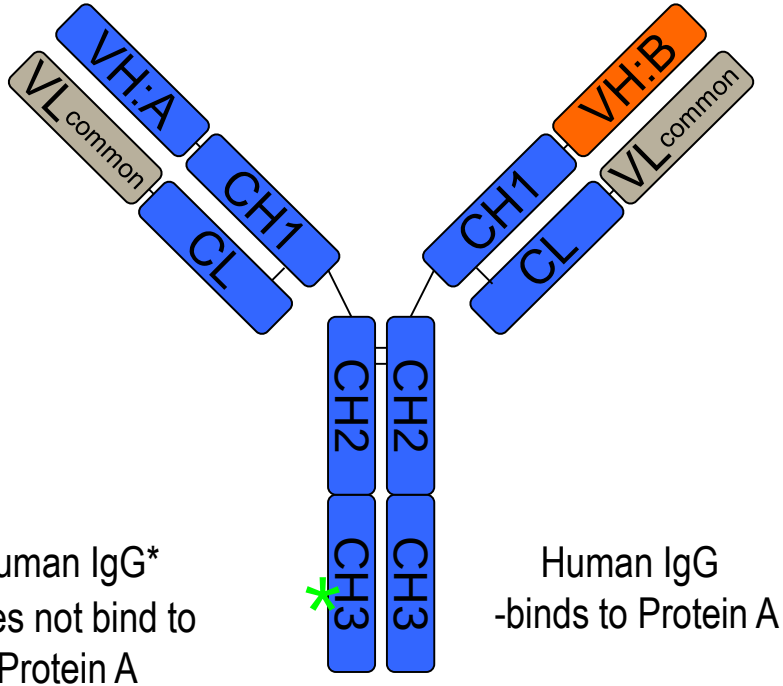
PEGS:Boston

REGENERON

SCIENCE TO MEDICINE®

REGENERON'S BISPECIFIC STRATEGY

- Combine use of a single "common" light chain with a simple substitution (IgG*) that introduces asymmetric protein A binding
  - IgG* substitution allows selective isolation of the bispecific antibody
  - The IgG* is an IgG with two amino acid substitutions that create no new T cell epitopes
  - Common light chain ensures correct light chain pairing
  - The Fc region can be modified to reduce effector function



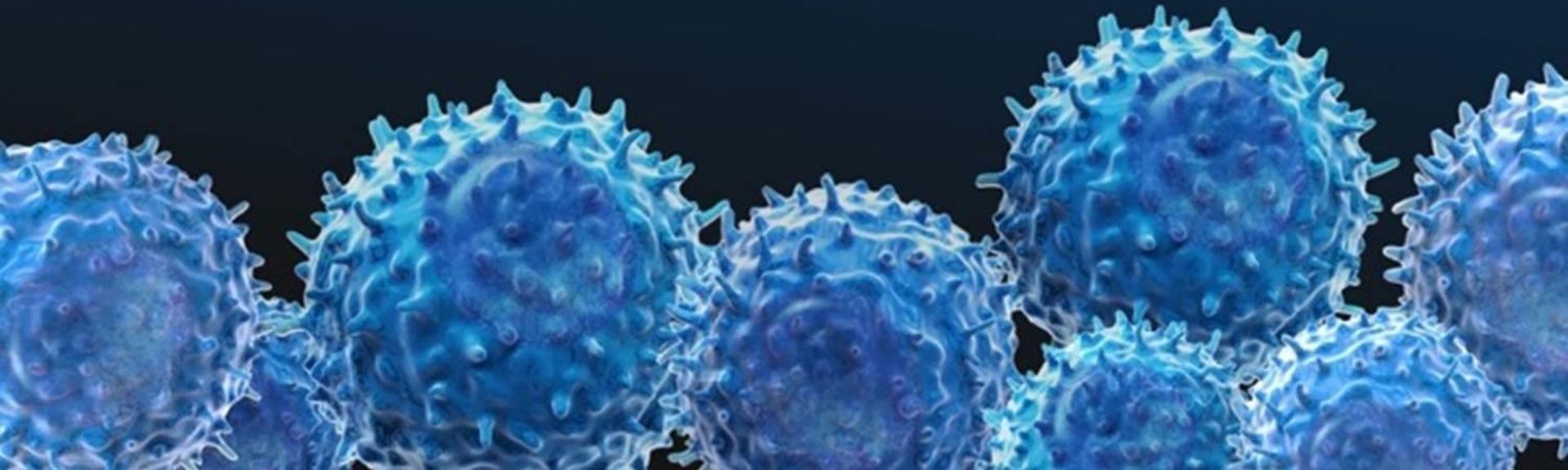
Human IgG*
-does not bind to Protein A

Human IgG
-binds to Protein A

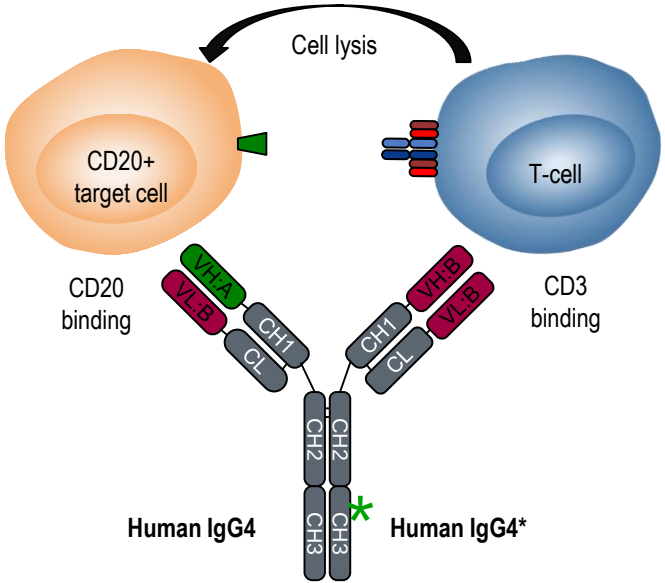
REGENERON CLINICAL STAGE BISPECIFIC PROGRAMS FOR ONCOLOGY:

REGENERON

REGN1979: CD20xCD3
REGN5458: BCMAxCD3
REGN4018: MUC16xCD3



REGN1979: A FULLY HUMAN ANTI-CD20 X ANTI-CD3 BISPECIFIC ANTIBODY FOR B CELL MALIGNANCIES



REGN1979: a Bispecific, hinge-stabilized mAb based on an IgG4 isotype with reduced effector function

"B-cell non-Hodgkin lymphomas as a group comprise one of the most common forms of blood cancer in humans, and they develop from normal B lymphoid progenitor cells."

REGN1979 was designed to eliminate CD20+ B Cell lymphomas by engaging T cells to directly kill the CD20 expressing B Cell

CLINICAL SUMMARY FROM ASH 2018: REGN1979 (CD20XCD3) DISPLAYS EFFICACY AND AN ACCEPTABLE SAFETY PROFILE IN PATIENTS WITH R/R B-NHL

Best Overall Responses[1]

*Relapsed/Refractory Follicular Lymphoma grade 1–3a**

	REGN1979 dose groups		
	<5 mg (n=7)	≥5–≤12 mg (n=5)	≥18–≤40 mg (n=5)
ORR, n (%)	1 (14.3)	5 (100.0)	5 (100.0)
CR, n (%)	1 (14.3)	4 (80.0)	4 (80.0)
PR, n (%)	0	1 (20.0)	1 (20.0)
Responding patients who did not progress during study treatment, n/N (% of responders)	1 (100.0)	4 (80.0)	5 (100.0)

Relapsed/Refractory Diffuse Large B Cell Lymphoma

	REGN1979 dose groups		
	<5 mg (n=15)	≥5–≤12 mg (n=11)	≥18–≤40 mg (n=10)
ORR, n (%)	3 (20.0)	2 (18.2)	6 (60.0)
CR, n (%)	0	1 (9.1)	2 (20.0)
PR, n (%)	3 (20.0)	1 (9.1)	4 (40.0)
Responding patients who did not progress during study treatment, n/N (% of responders)	1 (33.3)	1 (50.0)	3 (50.0)

[1]Cheson et al, J Clin Oncol. 2007;25:579-86.

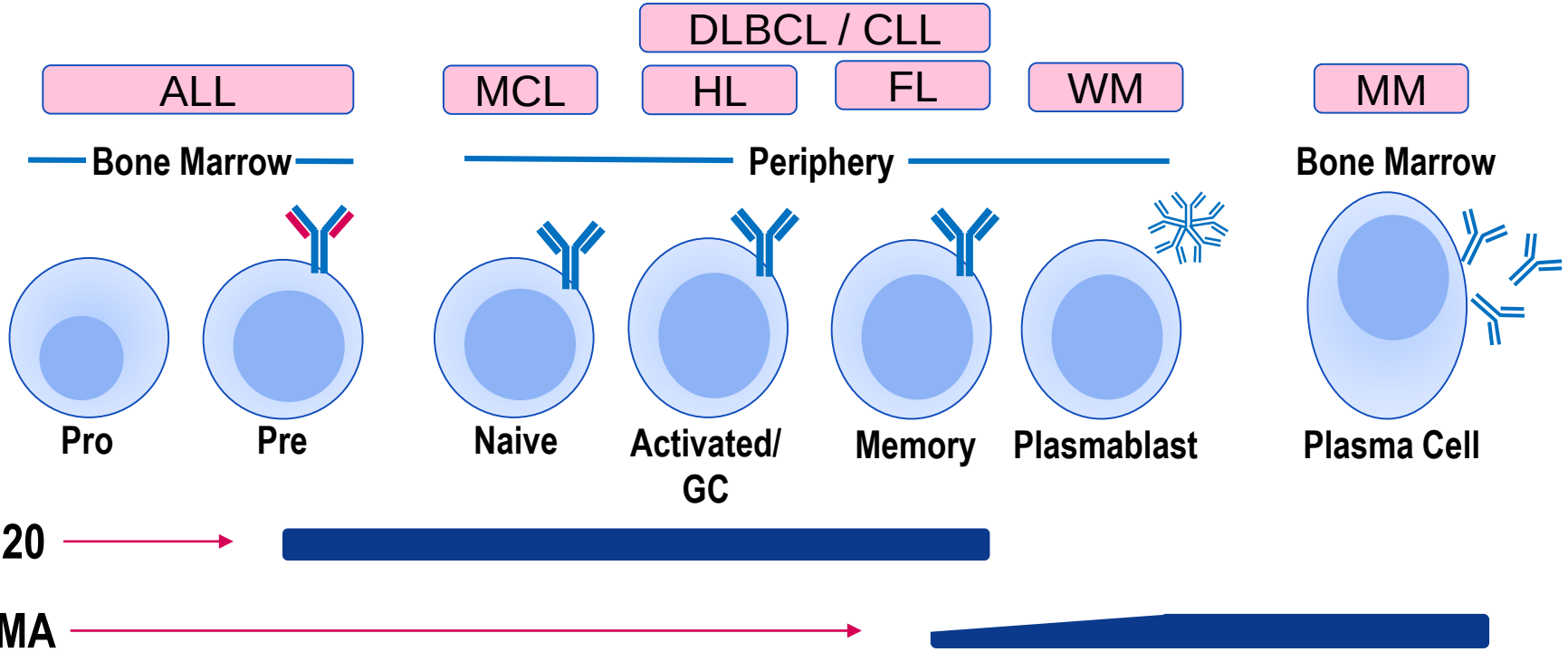
- **Most treatment emergent adverse events were CRS/IRR and associated signs and symptoms, which have been managed with supportive care.**
- **No clinically significant neurological toxicity has been observed.**
- **At doses of 5-40 mg, the preliminary ORR was 100% in pts with FL Grade 1-3a and 60.0% in pts with DLBCL. This promising efficacy at lower dose levels warrants further clinical investigation and dose escalation is currently ongoing.**

BISPECIFIC xCD3 ANTIBODIES FOR OTHER HEMATOPOIETIC MALIGNANCIES: MULTIPLE MYELOMA

- Multiple Myeloma (MM) represents a significant unmet medical need with ~30,000 new cases and 13,000 deaths in the US / year
- Recent advances/approvals include new-generation immunomodulatory drugs, new-generation proteasome inhibitors, and CD38 Ab
- But, despite these advances, **MM remains a generally incurable cancer:** there remains significant unmet need as many patients do not respond and most will eventually relapse; thus, MM-targeted therapies are needed

→ Both bispecific antibodies and CAR T cells are being developed in clinical studies targeting MM

THE RESTRICTED EXPRESSION OF BCMA MAKES IT AN ATTRACTIVE TARGET FOR MM



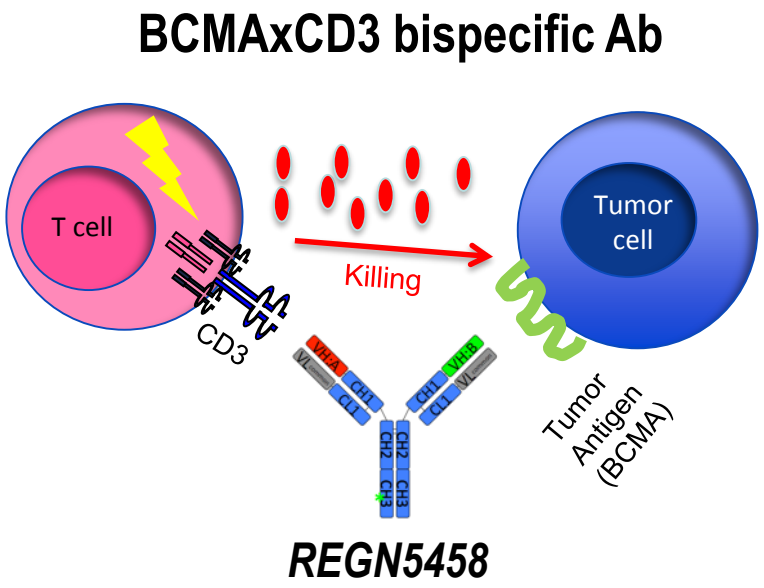
REGN1979 (CD20xCD3) displayed safety/efficacy in patients with CD20+ lymphomas; However CD20 is not generally expressed in multiple myeloma

REGN5458: A BCMAxCD3 BISPECIFIC ANTIBODY FOR THE POTENTIAL TREATMENT OF MULTIPLE MYELOMA

- **B-cell maturation antigen (BCMA)** is also known as tumor necrosis factor receptor superfamily member 17 (TNFRSF17)
  - BCMA is a receptor for **BAFF** and **APRIL**, which are known to promote B cell and plasma cell survival

- Expression patterns
  - Normal tissue: Restricted to **plasma cells** (antibody-secreting subset of B cell lineage) and some activated B cells
  - In Tumors: BCMA is expressed on most **multiple myeloma** cells (malignant plasma cells) in most multiple myeloma patients

- **Opportunity:**
  Develop BCMAxCD3 bispecific antibody targeting BCMA that can be used to treat Multiple Myeloma



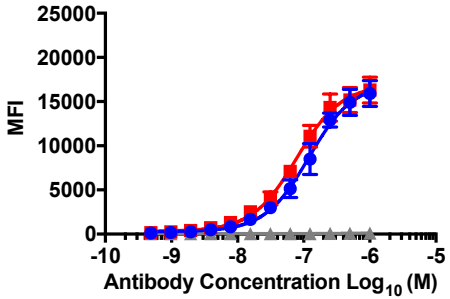
REGN5458 (BCMAXCD3) BINDS TO HUMAN T CELLS AND MYELOMA CELL LINES

Flow cytometry-based binding assay

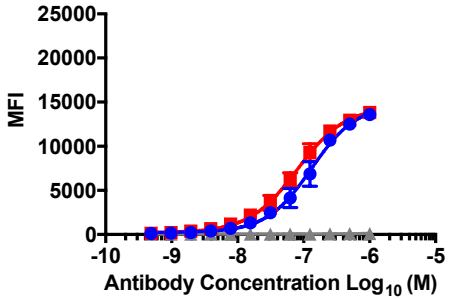
BCMAxCD3 bsAb
CD3-binding Control bsAb
Parental anti-BCMA mAb

BCMAxCD3 binds to T cells

Human CD4 T Cells

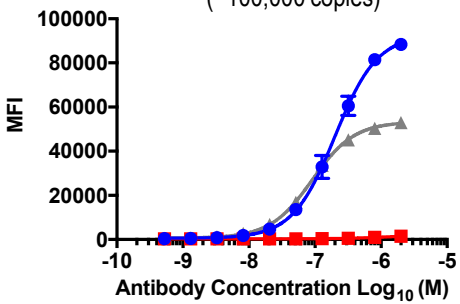


Human CD8 T Cells

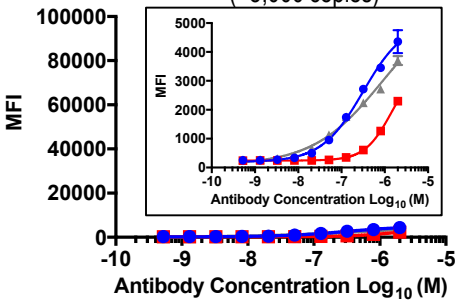


BCMAxCD3 binds to MM cell lines

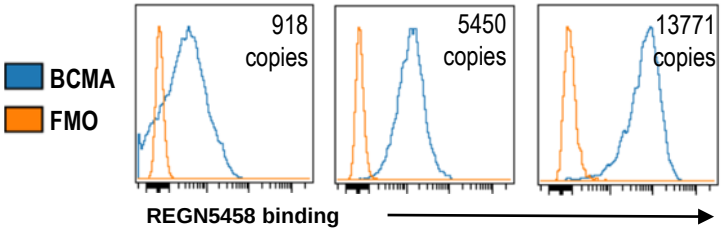
NCI-H929 MM Cells
(~100,000 copies)



MOLP-8 MM Cells
(~5,000 copies)



BCMAxCD3 binds to patient MM cells



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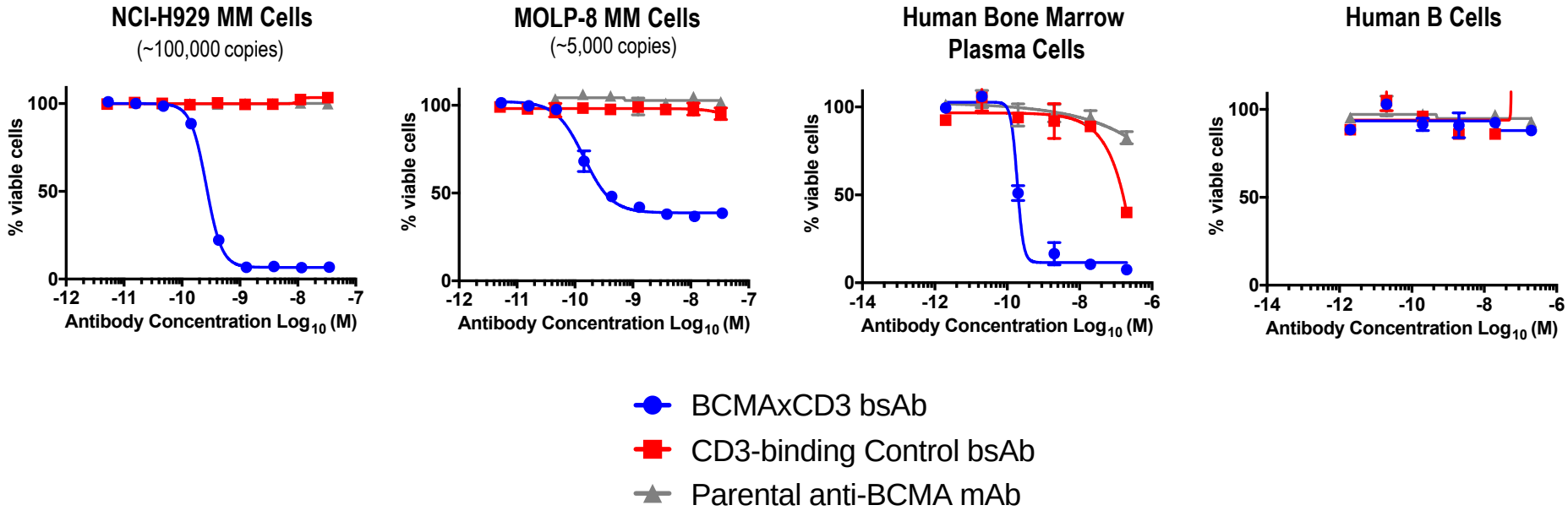
REGN5458 (BCMAxCD3) MEDIATES HUMAN T CELL ACTIVATION AND REDIRECTED KILLING OF MM CELL LINES AND HUMAN PLASMA CELLS, BUT NOT B CELLS

48-hour flow cytometry-based cytotoxicity assay



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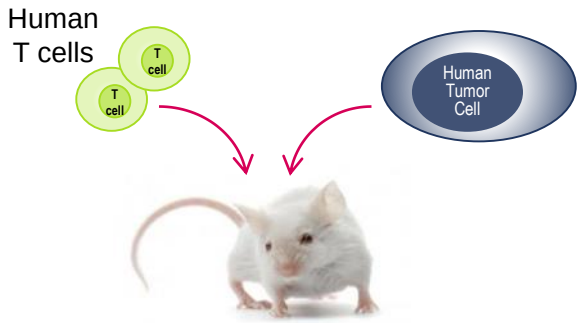
TWO APPROACHES TO TEST IMMUNOTHERAPIES IN MICE

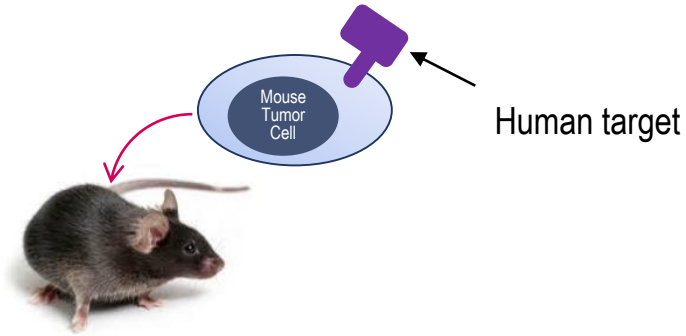
Need for multiple models: *using both immuno-compromised mice with engrafted human immune system, and immuno-competent mice with genetically modified targets*

Xenogenic: Immuno-compromised mice with human effector cells and human tumor cell lines



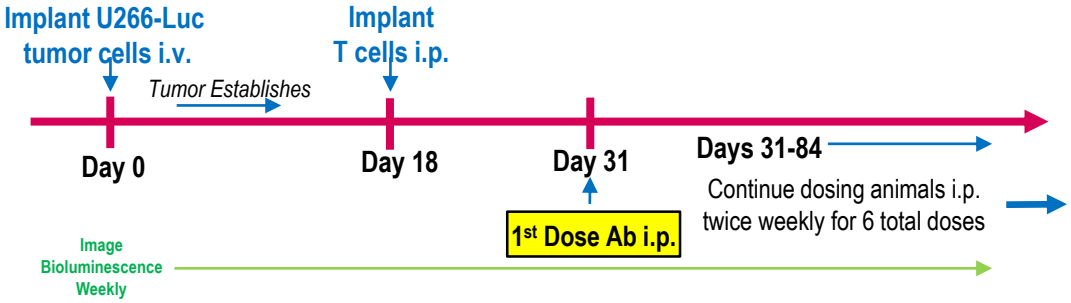
- Mice lack T cells, B cells, NK cells
- This allows a human tumor to grow in these mice without rejection
- Mouse myeloid cells still abundant: monocytes, DCs and granulocytes
- Efficient human T cell engraftment: Both CD4 and CD8 T cells present

Syngeneic: Immuno-competent mice in which host T cells express human CD3 implanted with mouse tumor cell lines expressing the human target

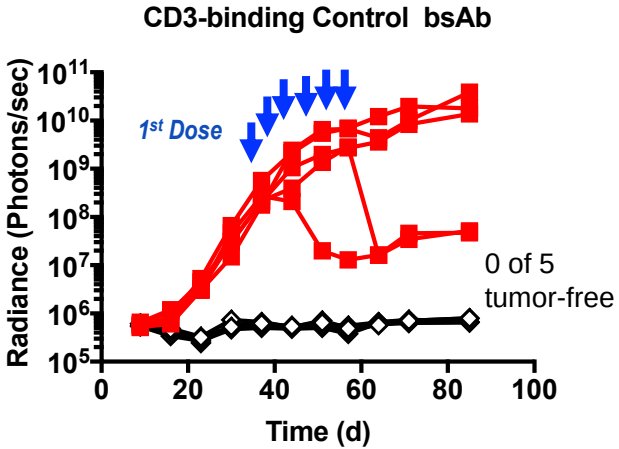


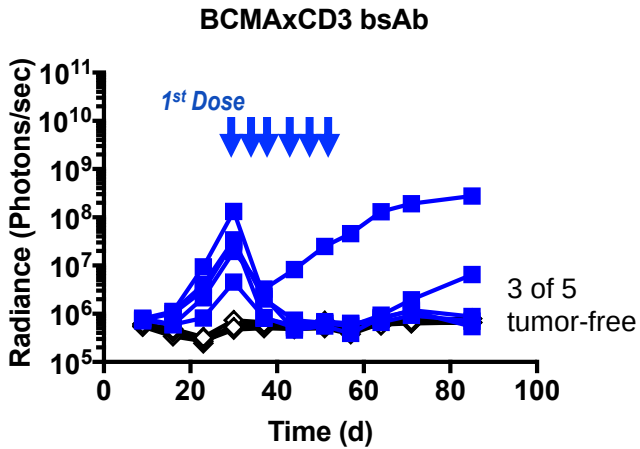
- Must transfect human target onto mouse tumor cell line
- Mice are genetically modified to express human target
- Mice express human effector molecule on T cells, e.g., CD3
- Full murine immune system present, allowing examination of immunotherapy combinations as well as toxicity

MURINE XENOGENIC MODEL: REGN5458 (BCMAxCD3) DEMONSTRATES DOSE-DEPENDENT ANTI-TUMOR EFFICACY AGAINST DISSEMINATED HUMAN MM TUMORS



Dosing was at 0.4 mg/kg





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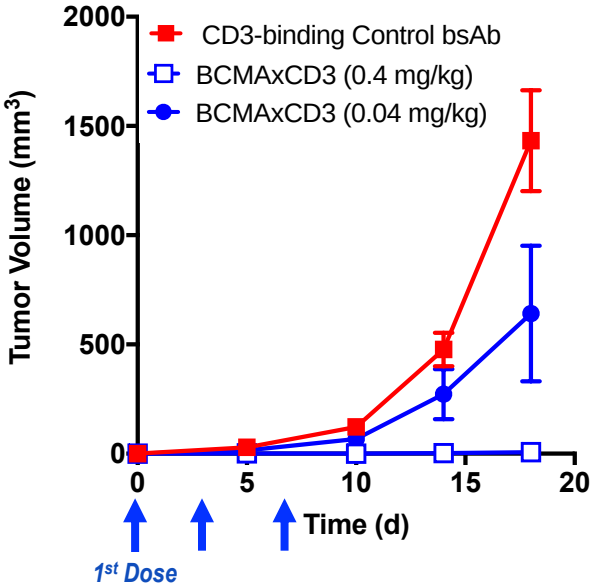
SYNGENEIC MODEL: REGN5458 (BCMAxCD3) DEMONSTRATES ANTI-TUMOR EFFICACY IN MICE GENETICALLY HUMANIZED FOR CD3

Subcutaneous MC38/hBCMA Model

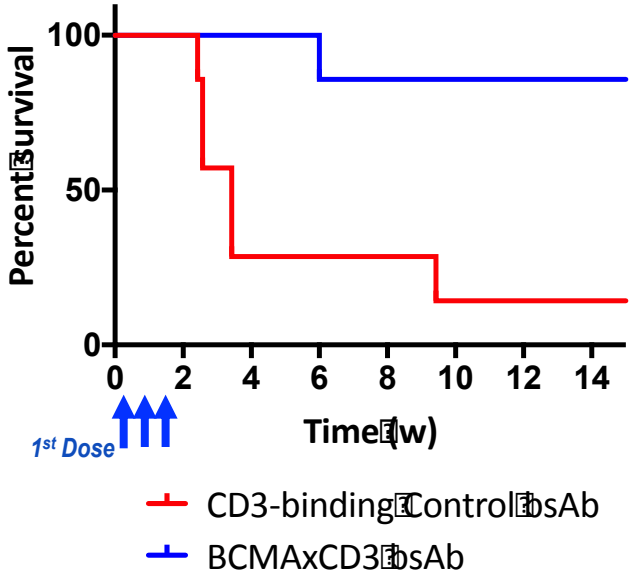


Immunocompetent CD3-humanized Mice:

-Genetically modified to express human CD3 in place of mouse CD3;

-Implanted with mouse tumors that express human BCMA

Systemic (i.v.) EL4/hBCMA Model (0.4 mg/kg)



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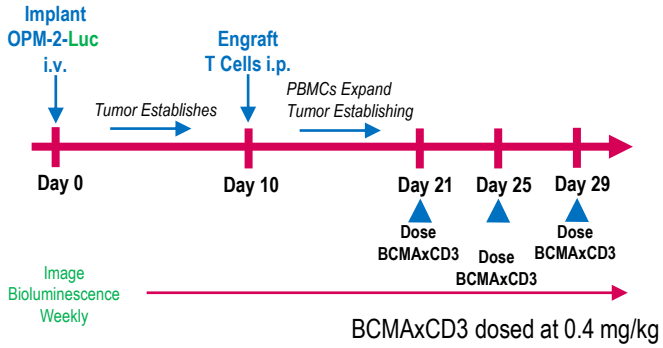
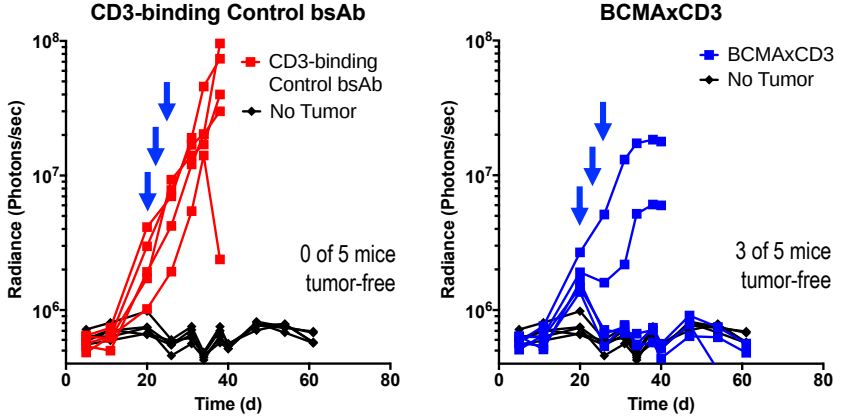
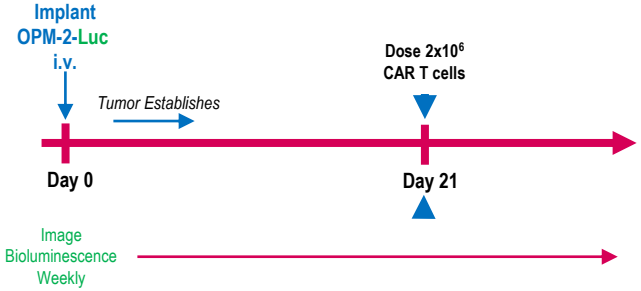
XENOGENIC MODEL: BENCHMARKING BISPECIFIC ANTIBODY TO CAR T IN VIVO

BCMAxCD3 BISPECIFIC ANTIBODY CAN RAPIDLY CLEAR ESTABLISHED SYSTEMIC BCMA+ OPM-2 TUMORS IN VIVO

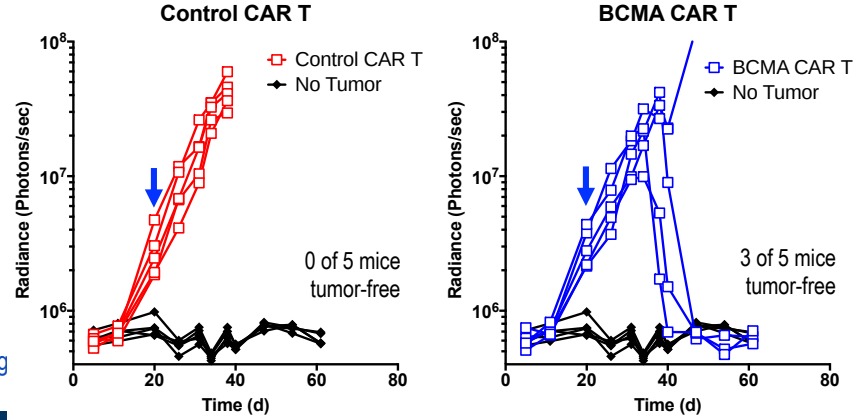




BCMA CAR T CAN CLEAR ESTABLISHED SYSTEMIC BCMA+ OPM-2 TUMORS IN VIVO; SLOWER KINETICS THAN BISPECIFICS



Anti-BCMA CAR T constructs were designed using an scFv derived from the BCMA binding arm of REGN5458 (BCMAxCD3), with 4-1BB and CD3 intracellular signaling domains.



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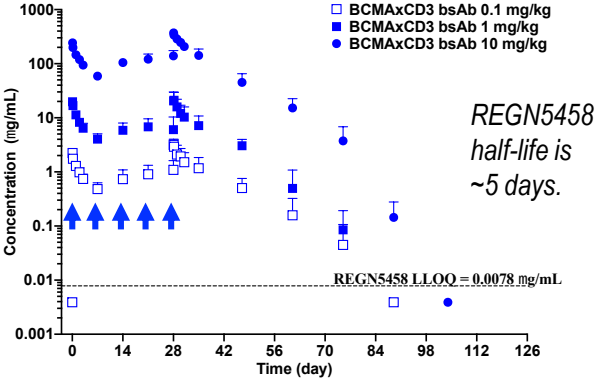
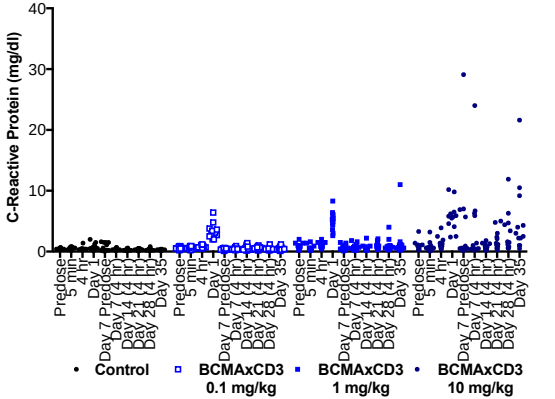
CYNOMOLGUS MONKEY PK/PD: REGN5458 (BCMAxCD3) SHOWS LINEAR PHARMACOKINETICS, TRANSIENT DOSE-PROPORTIONAL CRP ELEVATION, AND BONE MARROW PLASMA CELL DEPLETION

Pharmacokinetic Analysis

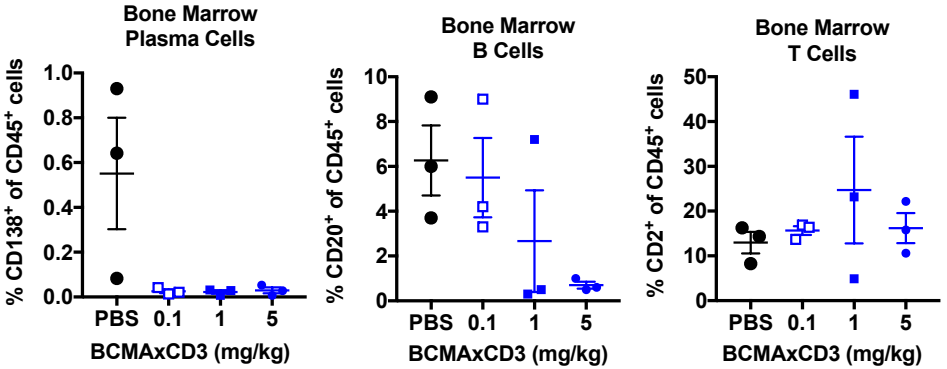


REGN5458 half-life is ~5 days.

Serum C-Reactive Protein (CRP) Levels



Bone marrow harvest 7-days after BCMAxCD3 infusion and analyzed by flow cytometry:



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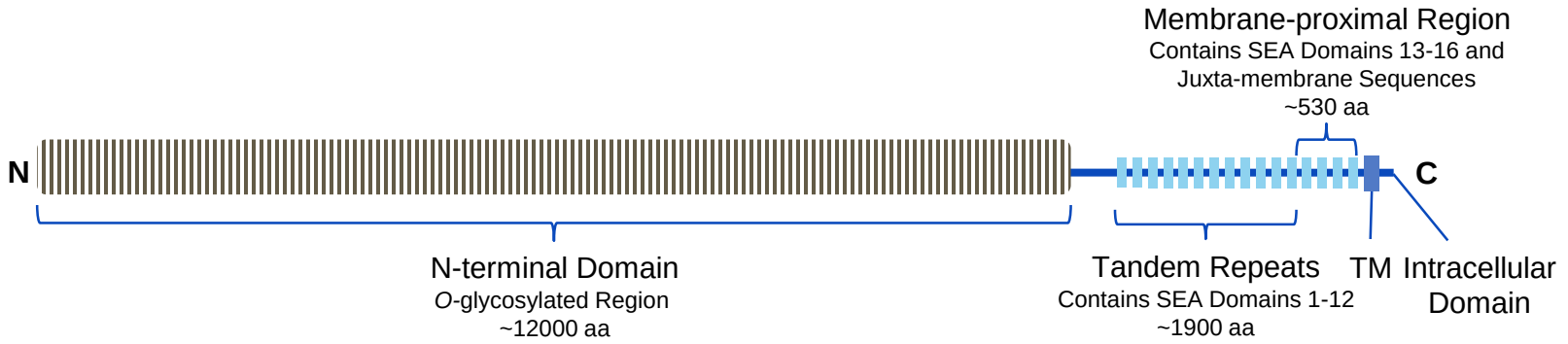
BCMAxCD3 BISPECIFIC ANTIBODY SUMMARY:

- BCMAxCD3 bispecific antibody (REGN5458) shows potent *in vitro* and *in vivo* activity against multiple myeloma cell lines and primary cells
- REGN5458 is well-tolerated and depletes BCMA^{+} plasma cells in cynomolgus monkeys
- Both REGN5458 and anti-BCMA CAR T cells show similar anti-tumor activities in vitro and in vivo

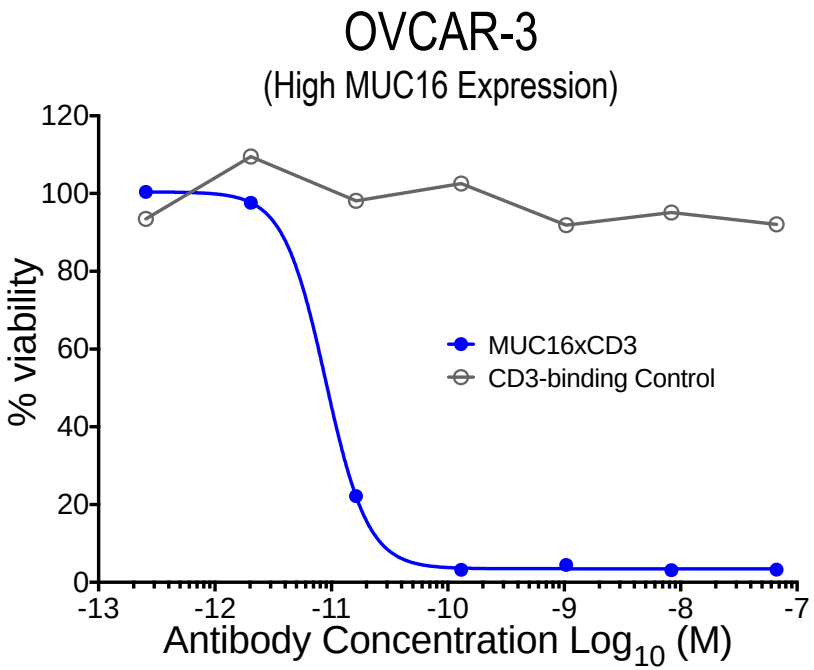
Based on the promising *in vitro*, *in vivo*, and pre-clinical safety evaluations, a Phase 1 trial has been initiated for REGN5458 in Multiple Myeloma

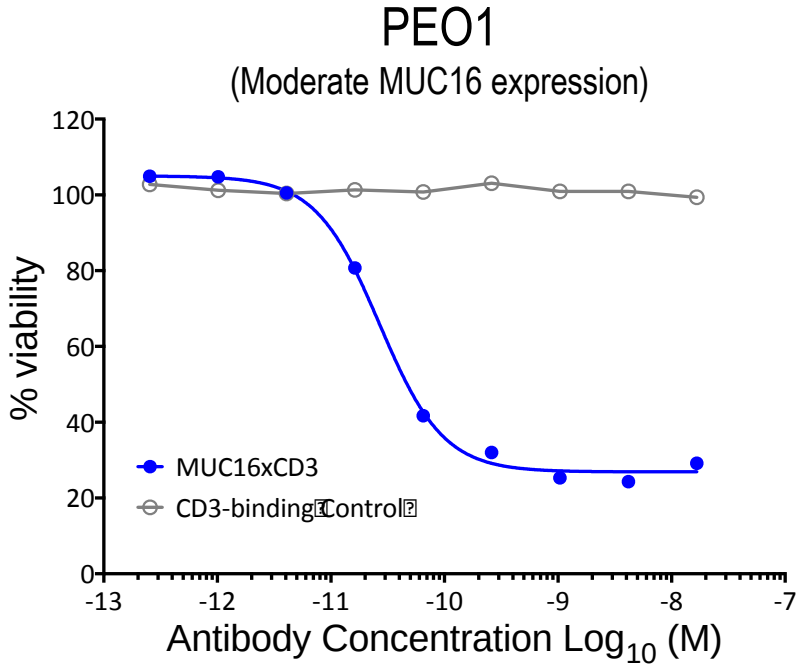
CD3 BISPECIFICS FOR SOLID TUMOR INDICATIONS: MUC16xCD3

- **Rationale: MUC16 is a large transmembrane protein that is expressed in ovarian cancer as well as subsets of pancreatic, breast, uterine and lung cancers**
  - MUC16 contains up to 60 mucin domain repeats (~156 aa each)
  - Expressed in normal: uterine/endometrium, corneal ovarian and tracheal tissue as well as secretions from normal human bronchial epithelial cells
  - Deletion of MUC16 in mice does not produce any obvious phenotype – mice are viable and fertile; function unclear
  - CA-125: shed form of MUC16: Serum protein/antigen elevated in ovarian cancer and used as biomarker for ovarian cancer progression and drug response



REGN4018, A MUC16xCD3 BISPECIFIC, SHOWS *IN VITRO* CYTOTOXICITY AGAINST OVARIAN CELL LINES AT pM CONCENTRATIONS





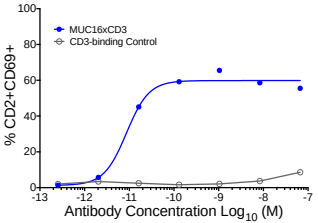
Cells were incubated with adherent cell depleted PBMC (~1:4 ratio) for 48 hours

Crawford et al 2018 Submitted

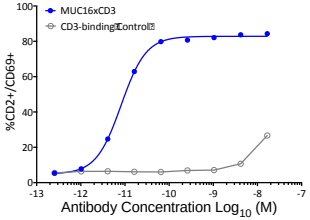
THE OBSERVED *IN VITRO* ACTIVITY CORRELATES WITH T-CELL ACTIVATION AND CYTOKINE RELEASE

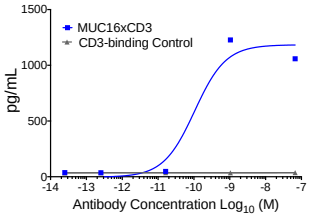
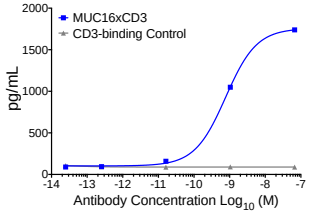
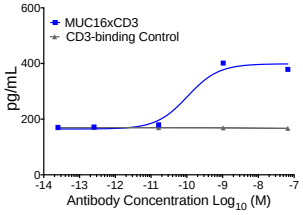
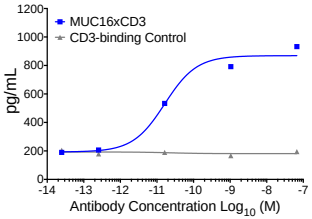
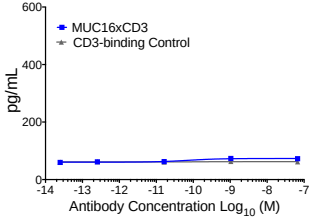
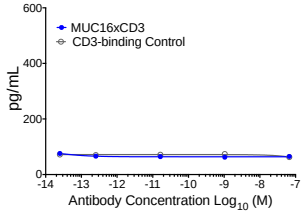
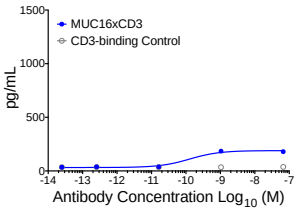
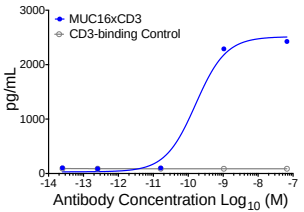
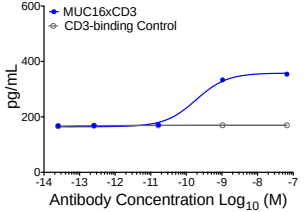
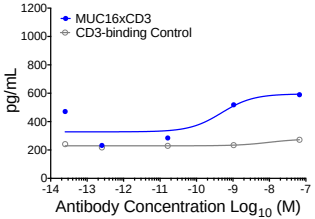
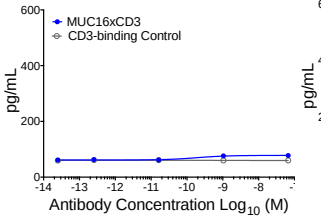
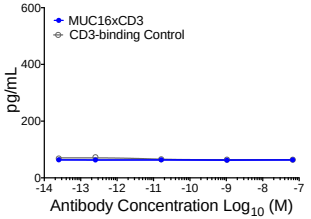
T Cell Activation (CD69)

OVCAR-3



PEO1

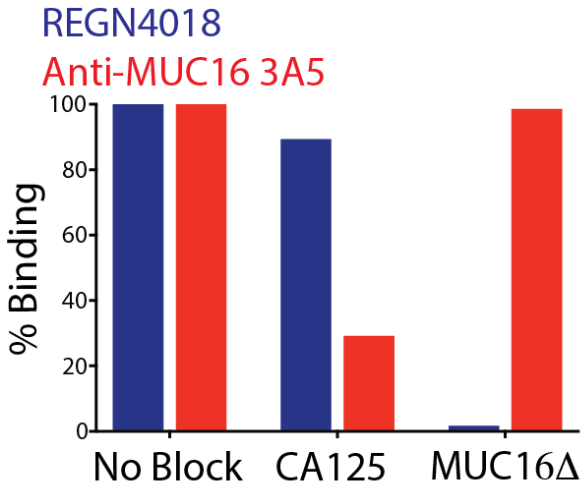


	INFg	TNFa	IL-10	IL-6	IL-4	IL-2
OVCAR-3						
PEO1						

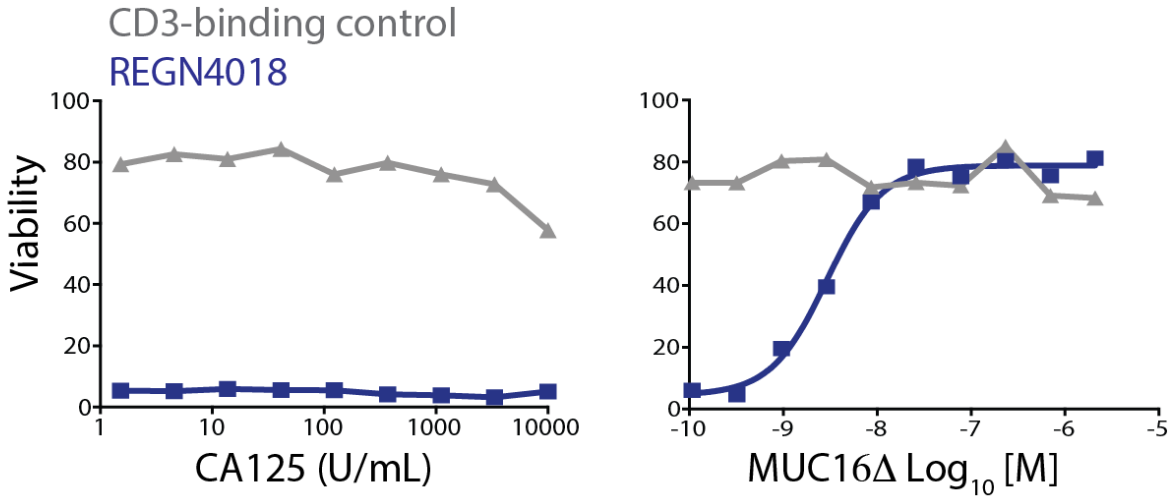
Crawford et al 2018 Submitted

REGN4018 MAINTAINS BINDING AND CYTOTOXICITY OF OVARIAN CANCER CELL LINES IN PRESENCE OF HIGH LEVELS OF CA-125 IN *IN VITRO* BIOASSAYS

CA-125 minimally blocks binding of REGN4018 to MUC16



CA-125 did not inhibit the ability of REGN4018 to induce killing of OVCAR-3 cells

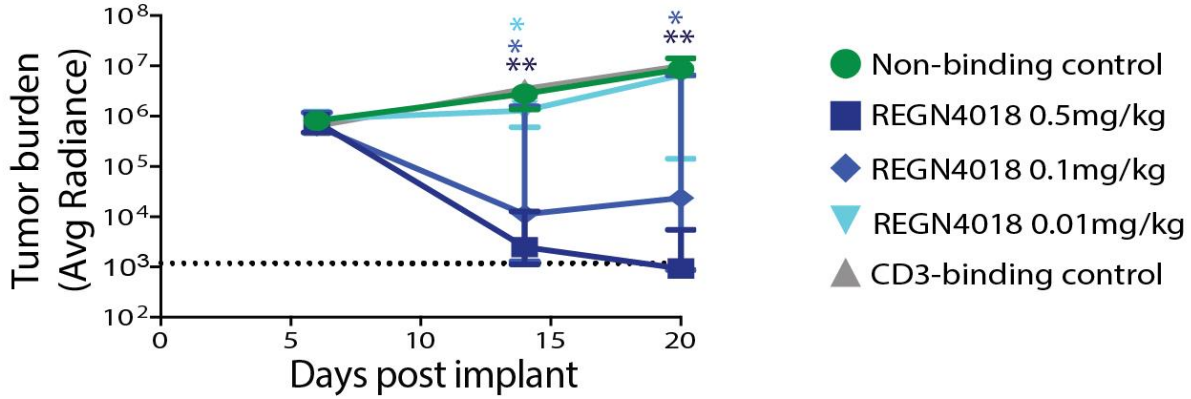


MUC16Δ is a recombinant protein consisting of the membrane proximal domains of MUC16 which was used as the immunogen for REGN4018

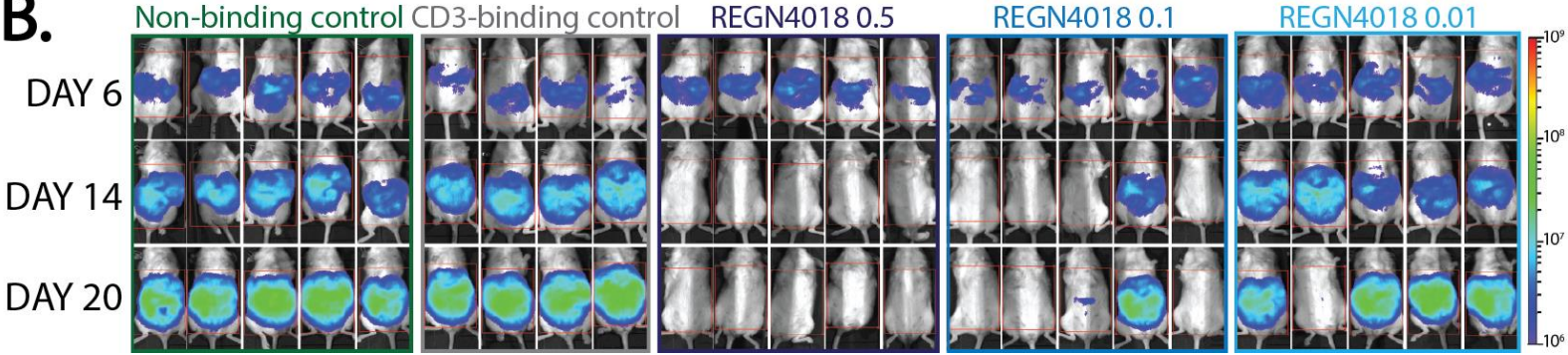
Crawford et al 2018 Submitted

XENOGENIC EFFICACY MODEL: REGN4018 INDUCES POTENT ANTI-TUMOR EFFICACY IN AN OVCAR-3LUC MODEL SYSTEM

A.

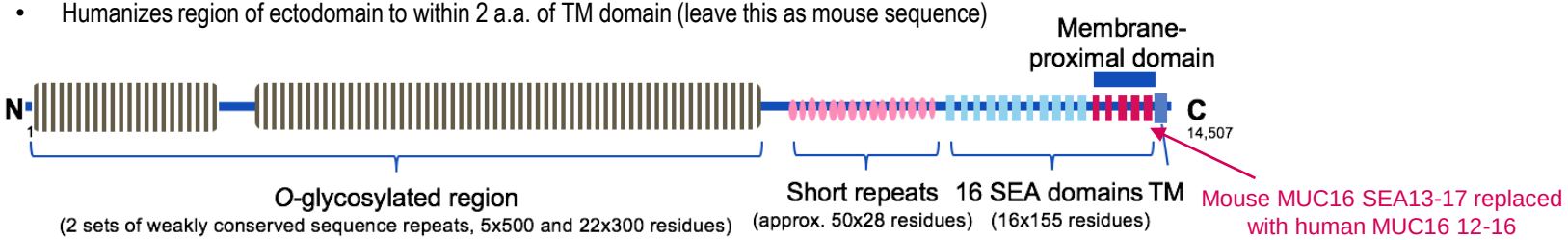


B.

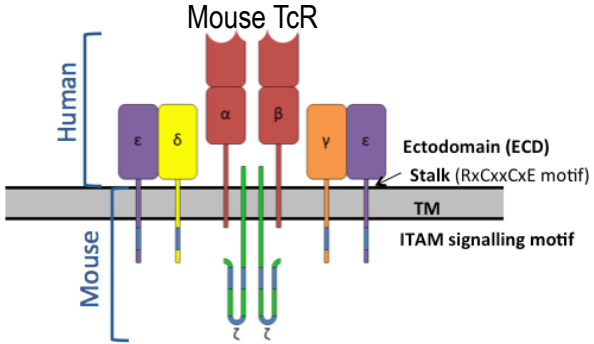


SYNGENEIC EFFICACY STUDIES: HUMANIZATION OF CD3 AND MUC16 IN MICE

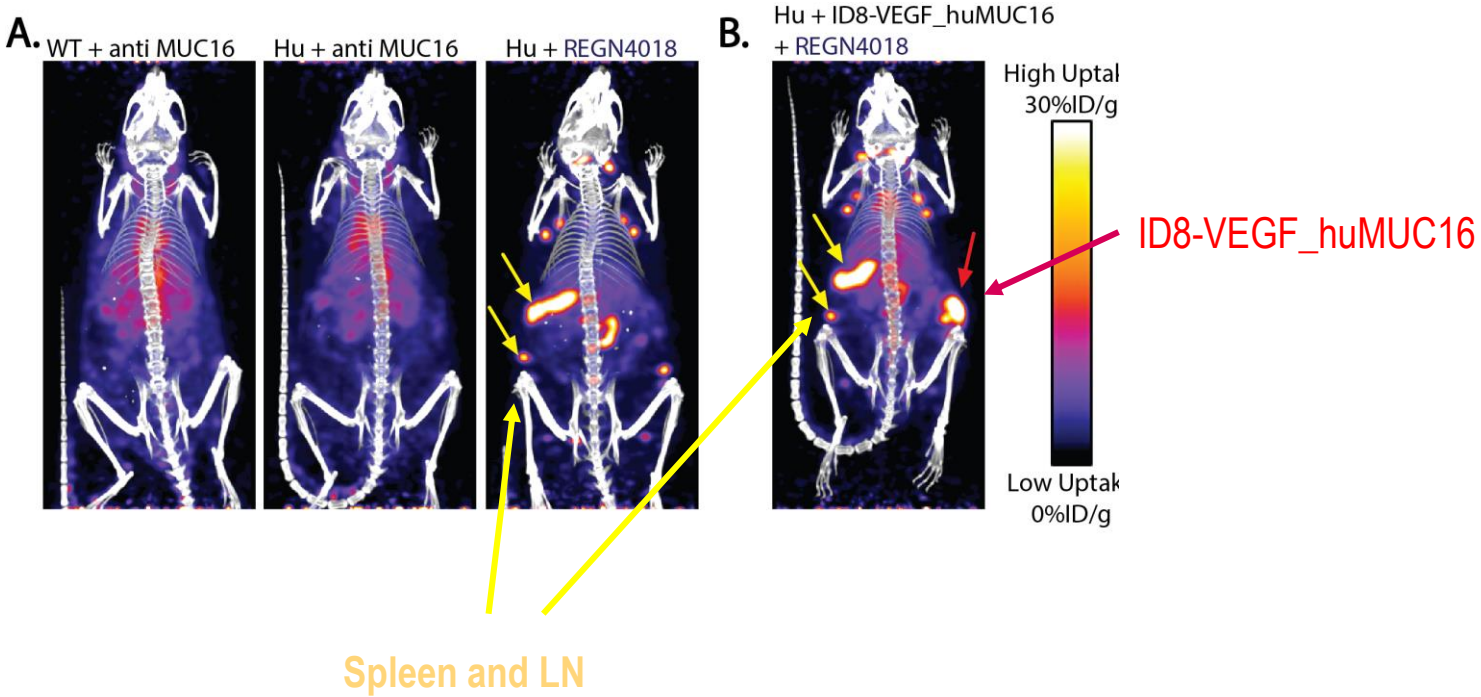
- **MUC16 Strategy:**
  - Replace mouse *Muc16* SEA repeats 13-17 with human *MUC16* SEA repeats 12-16
    - Humanizes region of ectodomain to within 2 a.a. of TM domain (leave this as mouse sequence)



- **CD3 Strategy:**
  - Replace mouse *CD3 delta, epsilon, gamma* with human
    - Mouse T cells now express human CD3 on surface



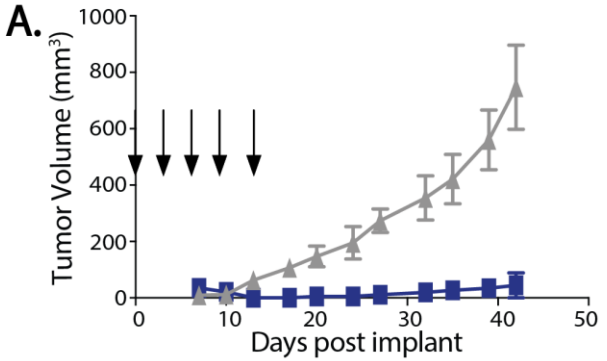
REGN4018 LOCALIZES TO LYMPHOID ORGANS AND MUC16-EXPRESSING TUMORS IN HUMANIZED MUC16/CD3 MICE



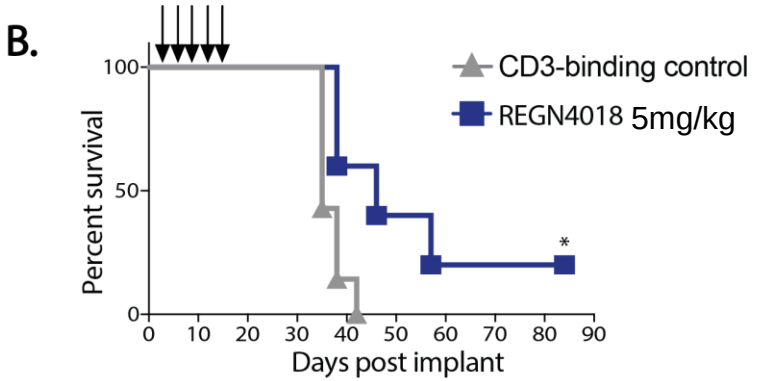
Crawford et al 2018 Submitted

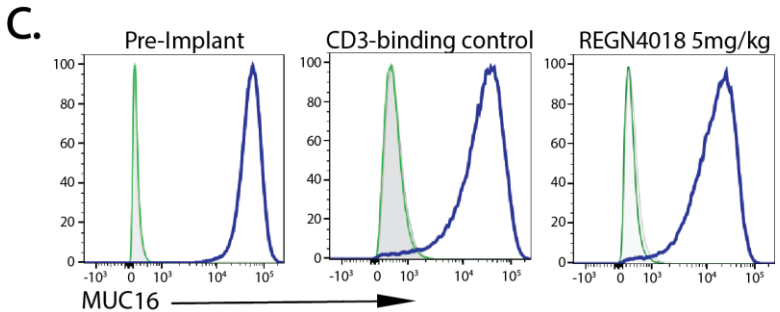
REGN4018 SHOWS EFFICACY IN BOTH IMMEDIATE TREATMENT AND ESTABLISHED SYNGENEIC TUMOR MODELS

ID8-VEGF_HuMUC16 SC model



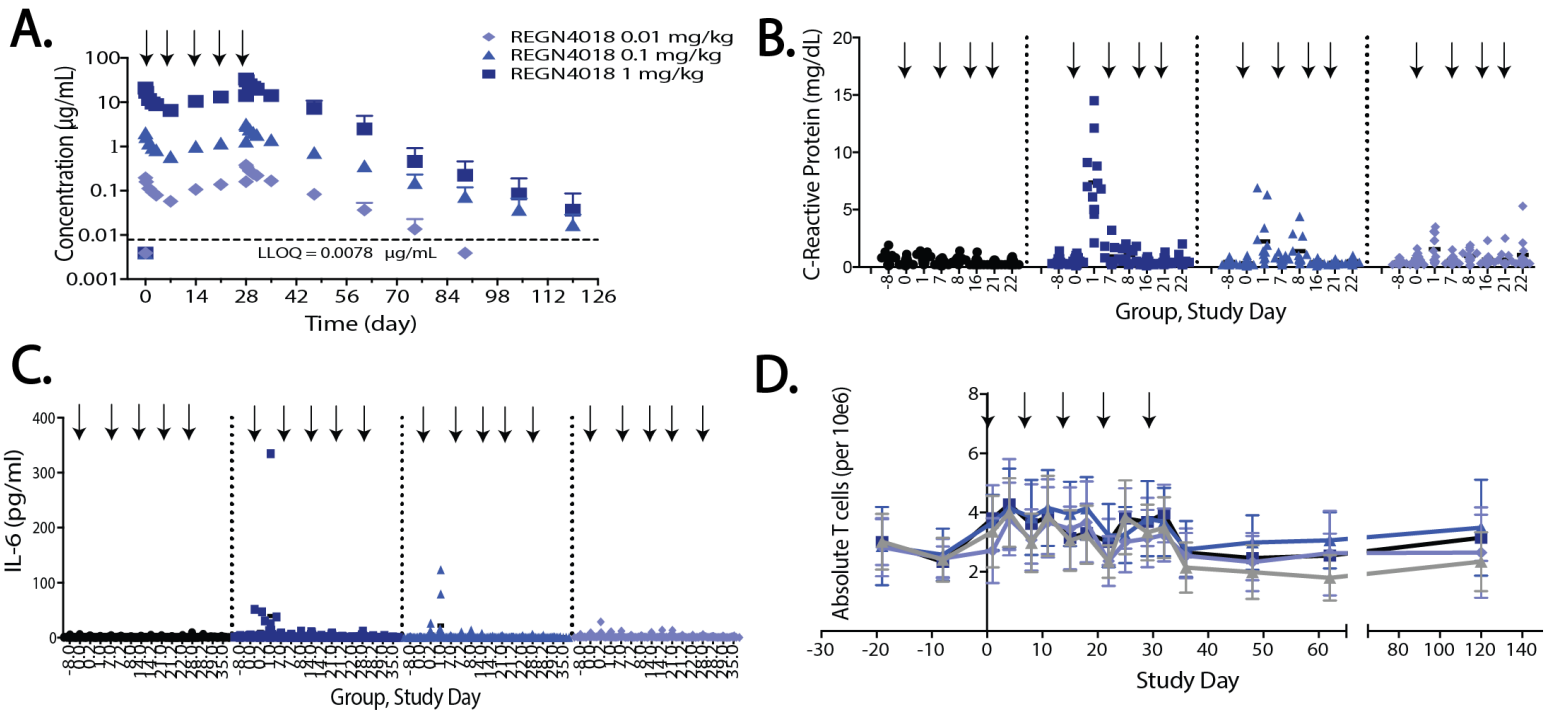
ID8-VEGF_HuMUC16 IP ascites model





Crawford et al 2018 Submitted

REGN4018 SHOWS LINEAR PHARMACOKINETICS AND TRANSIENT DOSE-PROPORTIONAL CRP ELEVATION IN A CYNOMOLGUS MONKEY TOXICITY STUDY



Crawford et al 2018 Submitted

MUC16XCD3: SUMMARY

- MUC16xCD3 bispecific antibodies show potent *in vitro* activity against ovarian cell lines

- High CA-125 levels do not block activity of REGN4018 in *in vitro* assays

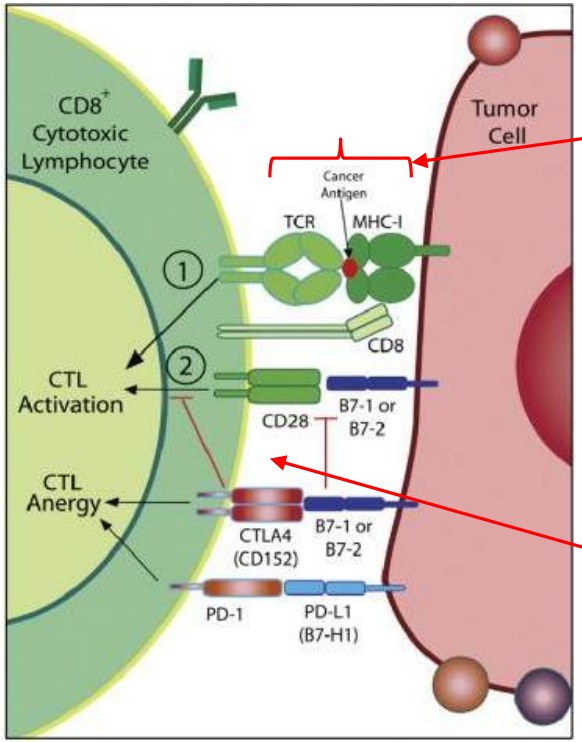
- REGN4018 demonstrated efficacy in multiple *in vivo* ovarian tumor models

- REGN4018 was generally well tolerated in GLP toxicology studies (Crawford A, et al. Cancer Res 2018;78:1777)

- Phase 1 trial initiated for REGN4018 in Ovarian Cancer in 2018 and dose escalation is ongoing

STRATEGIES FOR ENHANCING BISPECIFIC EFFICACY: CAN COMBINATION OF xCD3 BISPECIFICS WITH CHECKPOINT INHIBITORS ENHANCE ANTI-TUMOR EFFICACY?

T cell activation requires presentation of antigen ("SIGNAL 1") via MHC/TCR.

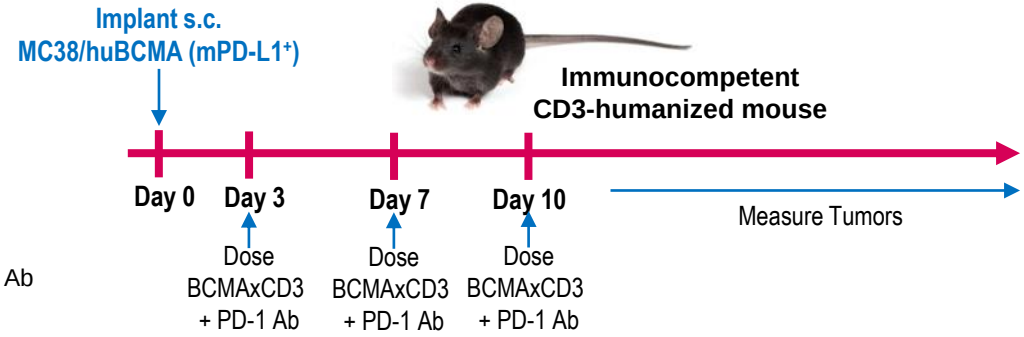


SIGNAL 1

Signal 1 can be mirrored through the use of a TAAxCD3 bispecific antibody.

Checkpoint inhibitors can be further combined to (e.g. αPD1, αCTLA-4) block inhibitory signals from tumor and enhance cytotoxic CD8 T cell activity

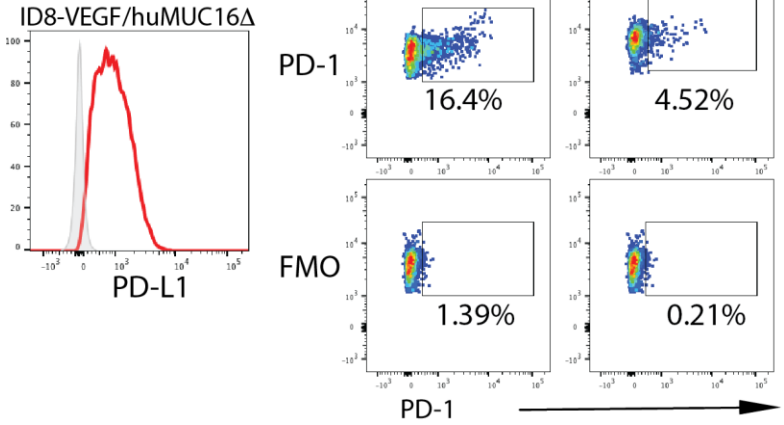
REGN5458 (BCMAXCD3) DEMONSTRATES COMBINATORIAL EFFICACY WITH PD-1 BLOCKADE

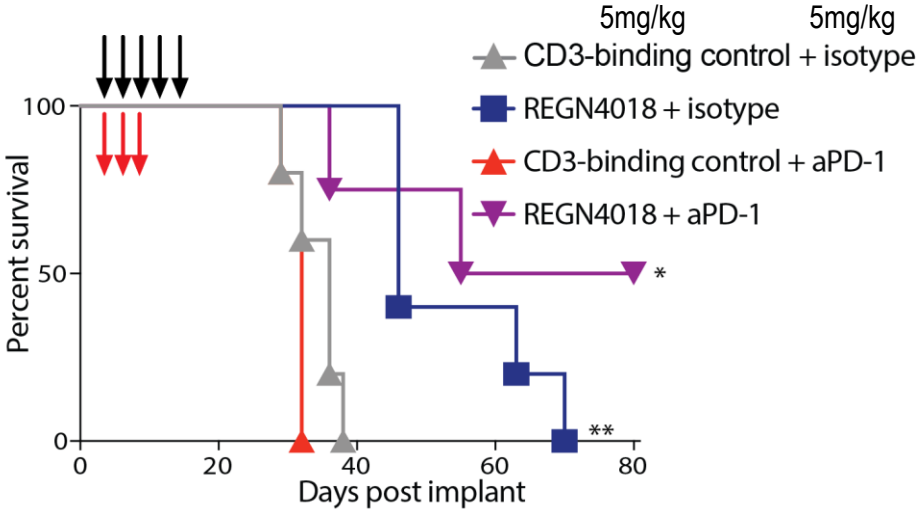


PD-1 BLOCKADE ENHANCES ANTI-TUMOR EFFICACY OVER REGN4018 ALONE IN A SYNGENEIC MODEL

ID8-VEGF_huMUC16 IP Ascites model:

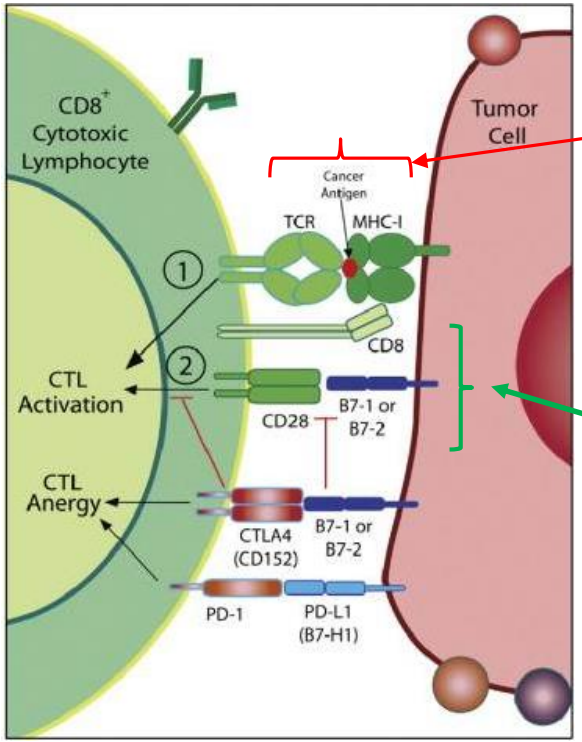
- PD-L1 is expressed on ID8 cells ex vivo
- PD-1 is expressed on a subset of T cells in the ascites





STRATEGIES FOR ENHANCING BISPECIFIC EFFICACY: CAN COMBINATION OF xCD3 BISPECIFICS WITH COSTIMULATORY AGONISM ENHANCE BISPECIFIC EFFICACY?

Optimal T cell activation requires presentation of antigen ("SIGNAL 1") via MHC/TCR and signaling through costimulatory pathways ("SIGNAL 2").



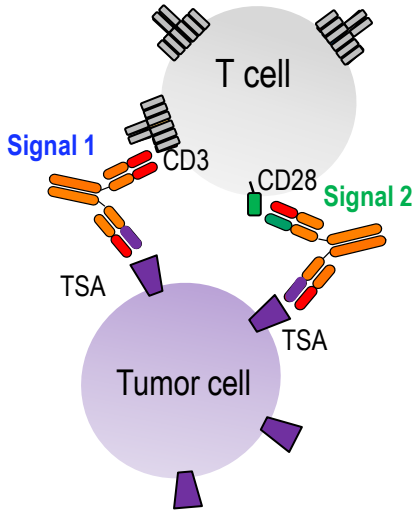
SIGNAL 1

Signal 1 can be mirrored through the use of a TAAxCD3 bispecific antibody.

SIGNAL 2

Can we activate the co-stimulatory pathway by using agonist antibodies? i.e. activate signal 2.

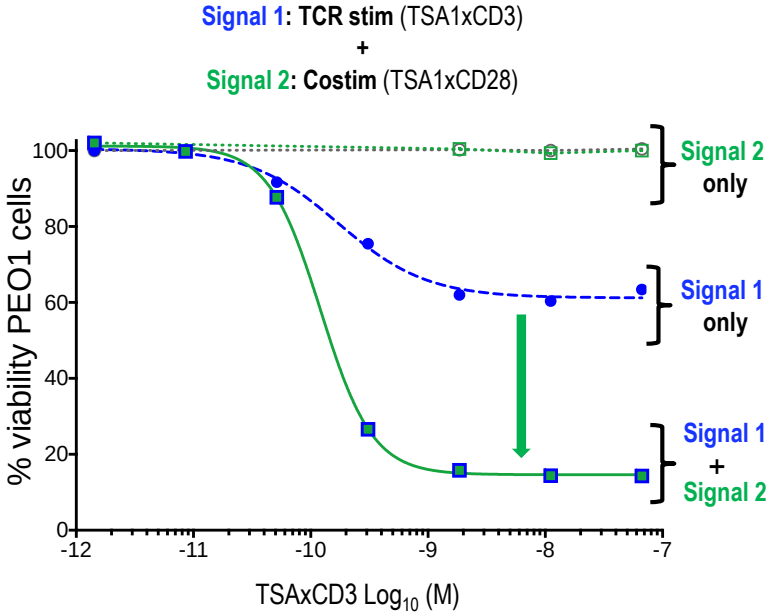
THE ADDITION OF TUMOR-TARGETED CO-STIMULATORY BISPECIFIC POTENTIATES KILLING OF OVARIAN CANCER CELLS



TSAxCD3 Bispecific Antibody

TSAxCD28 Costimulatory Bispecific Antibody

Cytotoxicity with Combination Treatment



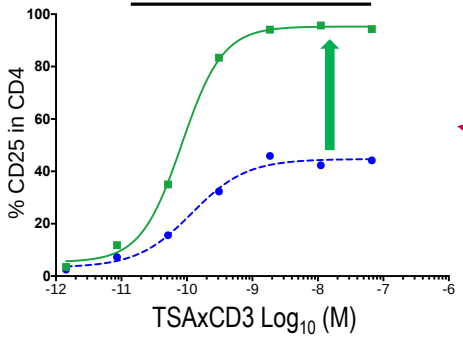
- We selected a non-competing co-stim binding arm to enhance the in-vitro potency of the xCD3 bispecific
- We did not observe any activity when the cells were only treated with a TSAxCD28 alone
- The addition of a TSAxCD28 to a TSAxCD3 bispecific resulted in a dramatic increase in cytotoxicity

Skokos et al, 2019 – Manuscript submitted

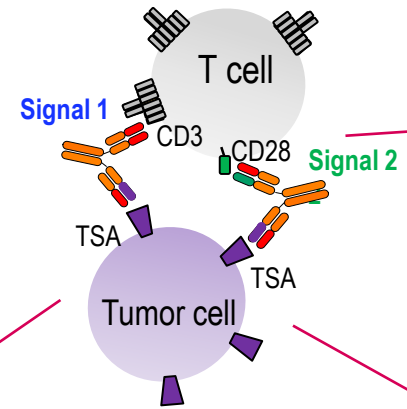
In-vitro 96 hour cytotoxicity assay with unstimulated human PBMC; 4:1 Effector: Target ratio
For Combo treatment, a fixed amount of Signal 2 (2.5ug/ml) was added to the titration of Signal 1

THE TSAxCD28 BISPECIFIC INDUCED POTENTIATION EXTENDS TO T CELL ACTIVATION, PROLIFERATION, AND CYTOKINE RELEASE

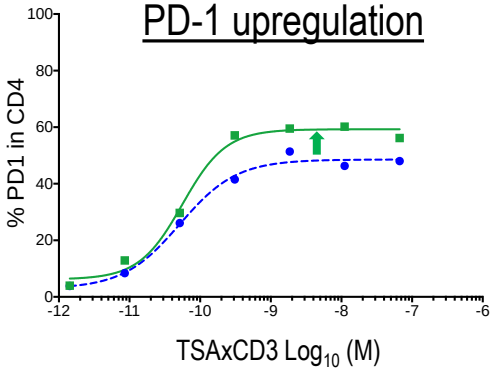
T cell activation



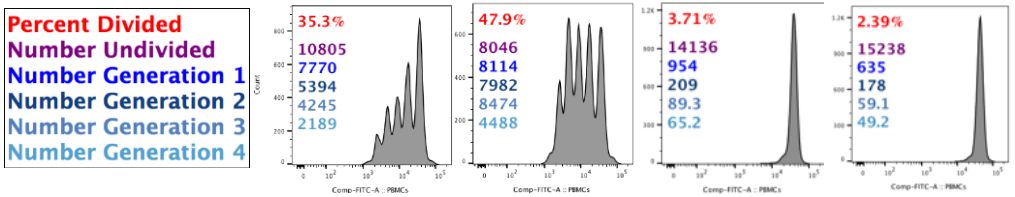
Signal 1 + Signal 2



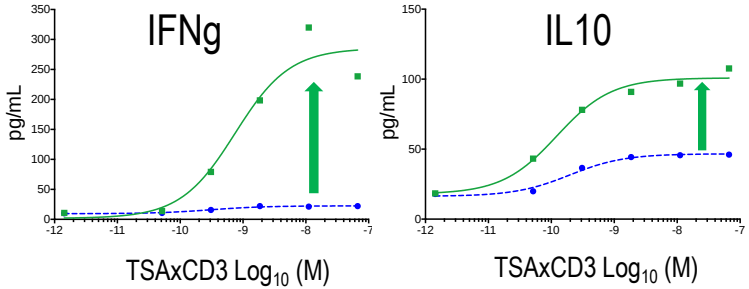
PD-1 upregulation



T cell proliferation



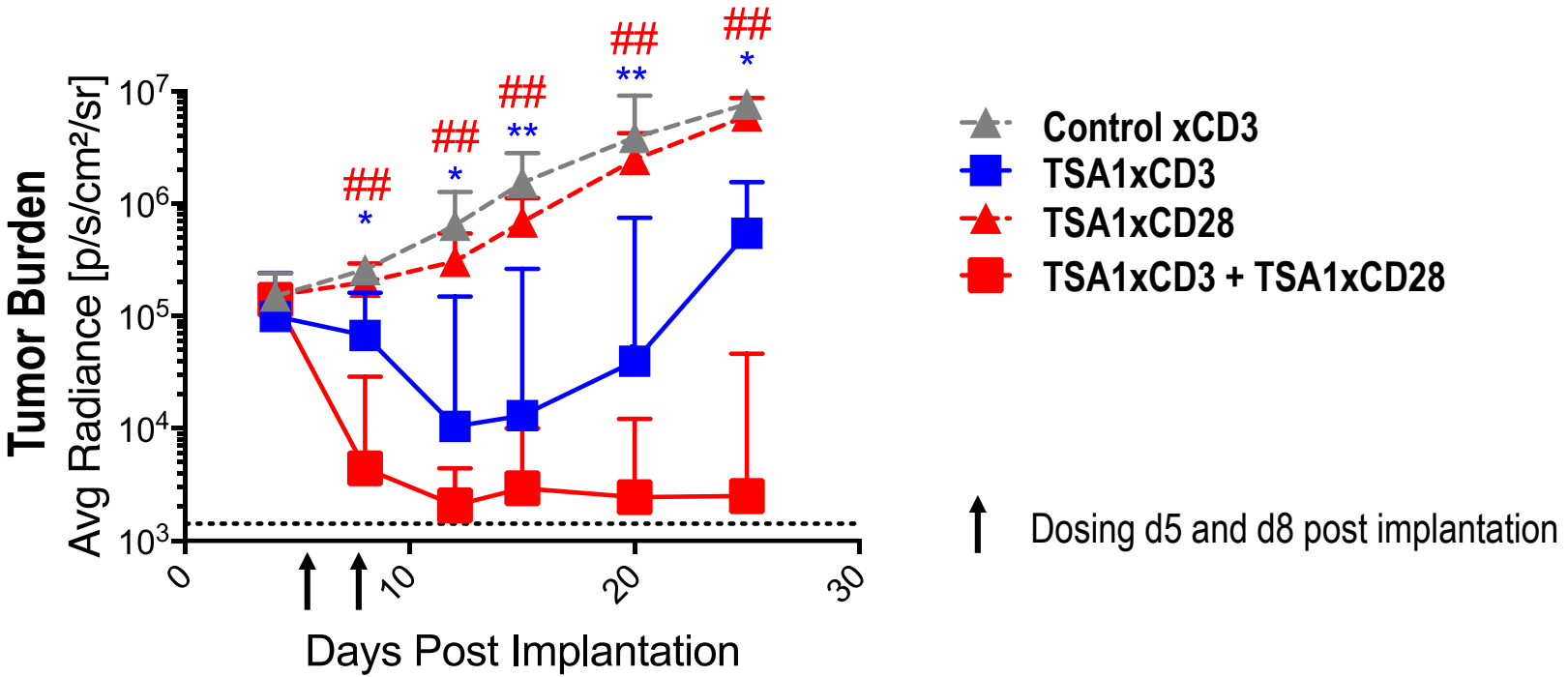
Cytokine release



REGENERON

In-vitro 96hour cytotoxicity assay with unstimulated human PBMC; 4:1 Effector:Target ratio
For Combo treatment, a fixed amount of Signal 2 (2.5ug/ml) was added to the serial dilution of Signal 1

COSTIMULATORY BISPECIFIC ANTIBODIES ALSO SHOW SYNERGY WITH xCD3 BISPECIFICS IN *IN VIVO* TUMOR MODELS



*p<0.05 or **p<0.01 vs TSAxCD3 ##p<0.01 vs combination

xCD3 bispcifics dosed at 2.5ug/ms; xCD28 dosed at 100ug/ms

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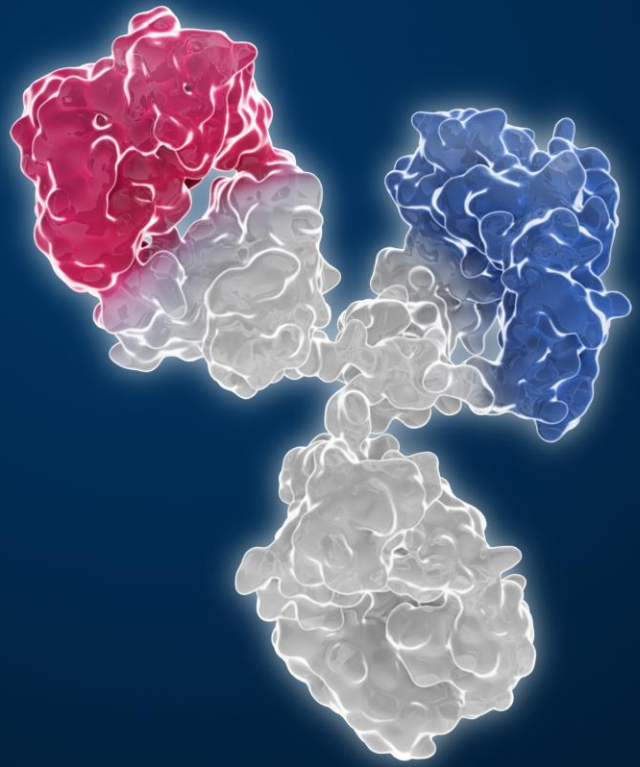

Thank You!

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