DEVELOPMENT OF NOVEL FULLY HUMAN BISPECIFIC ANTIBODIES FOR ONCOLOGY

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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

![Diagram showing bispecific antibody structure with human IgG and IgG*](image)
REGENERON CLINICAL STAGE BISPECIFIC PROGRAMS FOR ONCOLOGY:

REGN1979: CD20xCD3
REGN5458: BCMAxCD3
REGN4018: MUC16xCD3
REGN1979: A FULLY HUMAN ANTI-CD20 X ANTI-CD3 BISPECIFIC ANTIBODY FOR B CELL MALIGNANCIES

“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.
Best Overall Responses

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

<table>
<thead>
<tr>
<th>REGN1979 dose groups</th>
<th>ORR, n (%)</th>
<th>CR, n (%)</th>
<th>PR, n (%)</th>
<th>Responding patients who did not progress during study treatment, n/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5 mg (n=7)</td>
<td>1 (14.3)</td>
<td>1 (14.3)</td>
<td>0</td>
<td>1 (100.0)</td>
</tr>
<tr>
<td>≥5–≤12 mg (n=5)</td>
<td>5 (100.0)</td>
<td>4 (80.0)</td>
<td>1 (20.0)</td>
<td>4 (80.0)</td>
</tr>
<tr>
<td>≥18–≤40 mg (n=5)</td>
<td>5 (100.0)</td>
<td></td>
<td></td>
<td>5 (100.0)</td>
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**Relapsed/Refractory Diffuse Large B Cell Lymphoma**

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<tbody>
<tr>
<td>&lt;5 mg (n=15)</td>
<td>3 (20.0)</td>
<td>0</td>
<td>3 (20.0)</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>≥5–≤12 mg (n=11)</td>
<td>2 (18.2)</td>
<td>1 (9.1)</td>
<td>1 (9.1)</td>
<td>1 (50.0)</td>
</tr>
<tr>
<td>≥18–≤40 mg (n=10)</td>
<td>6 (60.0)</td>
<td>2 (20.0)</td>
<td>4 (40.0)</td>
<td>3 (50.0)</td>
</tr>
</tbody>
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**CLINICAL SUMMARY FROM ASH 2018: REGN1979 (CD20XCD3) DISPLAYS EFFICACY AND AN ACCEPTABLE SAFETY PROFILE IN PATIENTS WITH R/R B-NHL**

- 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.

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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 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
- BCMA copies
- FMO copies

DiLillo and Olson – In Preparation
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

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

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

Human Bone Marrow Plasma Cells

Human B Cells

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

DiLillo and Olson – In Preparation
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 transfec 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

Implant U266-Luc tumor cells i.v.  
Tumor Establishes  
Day 0

Implant T cells i.p.  
Day 18

Day 31

Days 31-84
Continue dosing animals i.p. twice weekly for 6 total doses

1st Dose Ab i.p.

Dosing was at 0.4 mg/kg

CD3-binding Control bsAb

BCMAxCD3 bsAb

1st Dose

0 of 5 tumor-free

1st Dose

3 of 5 tumor-free

Radiance (Photons/sec)

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

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Subcutaneous MC38/hBCMA Model

- CD3-binding Control bsAb
- BCMAxCD3 (0.4 mg/kg)
- BCMAxCD3 (0.04 mg/kg)

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

- CD3-binding Control bsAb
- BCMAxCD3 bsAb

DiLillo and Olson – In Preparation
BCMAxCD3 BISPECIFIC ANTIBODY CAN RAPIDLY CLEAR ESTABLISHED SYSTEMIC BCMA+ OPM-2 TUMORS IN VIVO

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.

XENOGENIC MODEL: BENCHMARKING BISPECIFIC ANTIBODY TO CAR T IN VIVO

BCMAxCD3 dosed at 0.4 mg/kg

DiLillo and Olson – In Preparation
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:

- Bone marrow Plasma Cells
- Bone marrow B Cells
- Bone marrow T Cells
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
**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
THE OBSERVED IN VITRO ACTIVITY CORRELATES WITH T-CELL ACTIVATION AND CYTOKINE RELEASE

T Cell Activation (CD69)

INFγ  TNFα  IL-10  IL-6  IL-4  IL-2

OVCAR-3

PEO1

Regeneron

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

A. Tumor burden (Avg Radiance) vs Days post implant

- Non-binding control
- REGN4018 0.5mg/kg
- REGN4018 0.1mg/kg
- REGN4018 0.01mg/kg
- CD3-binding control

B. Tumor burden read-out = BLI = Bioluminescence

DAY 6
DAY 14
DAY 20

Crawford et al 2018 Submitted
**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

A. WT + anti MUC16  Hu + anti MUC16  Hu + REGN4018

B. Hu + ID8-VEGF_huMUC16 + REGN4018

Spleen and LN

ID8-VEGF_huMUC16
REGN4018 shows efficacy in both immediate treatment and established syngeneic tumor models.

**ID8-VEGF_HuMUC16 SC model**

A. Tumor Volume (mm$^3$)

- Days post implant:
  - 0
  - 10
  - 20
  - 30
  - 40
  - 50

B. Percent survival

- Days post implant:
  - 0
  - 10
  - 20
  - 30
  - 40
  - 50
  - 60
  - 70
  - 80

C. Pre-Implant vs CD3-binding control vs REGN4018 5mg/kg

- MUC16

Crawford et al 2018 Submitted
REGN4018 SHOWS LINEAR PHARMACOKINETICS AND TRANSIENT DOSE-PROPORTIONAL CRP ELEVATION IN A CYNOMOLGUS MONKEY TOXICITY STUDY

A. Concentration (µg/mL) vs Time (day)

B. C-Reactive Protein (mg/dL) vs Group, Study Day

C. IL-6 (pg/mL) vs Group, Study Day

D. Absolute T cells (per 10^6) vs Study Day

Crawford et al 2018 Submitted
- 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
T cell activation requires presentation of antigen ("SIGNAL 1") via MHC/TCR.

Checkpoint inhibitors can be further combined to (e.g. \(\alpha PD1, \alpha CTLA-4\)) 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

![Flow cytometry plots showing PD-L1 and PD-1 expression on ID8-VEGF_huMUC16Δ cells and FMO controls.](image)

![Survival analysis graph showing different treatment groups with survival curves.](image)

- 5mg/kg CD3-binding control + isotype
- 5mg/kg REGN4018 + isotype
- 5mg/kg CD3-binding control + aPD-1
- 5mg/kg REGN4018 + aPD-1

* and ** indicate statistical significance.
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.
We did not observe any activity when the 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
THE TSAxCD28 BISPECIFIC INDUCED POTENTIATION EXTENDS TO T CELL ACTIVATION, PROLIFERATION, AND CYTOKINE RELEASE

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 serial dilution of Signal 1
COSTIMULATORY BISPECIFIC ANTIBODIES ALSO SHOW SYNERGY WITH xCD3 BISPECIFICS IN IN VIVO TUMOR MODELS

Tumor Burden

Control xCD3
TSA1xCD3
TSA1xCD28
TSA1xCD3 + TSA1xCD28

Dosing d5 and d8 post implantation

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

xCD3 bispecifics dosed at 2.5ug/ms; xCD28 dosed at 100ug/ms
We thank and acknowledge all the patients and their families as well as all medical personnel involved in the REGN1979, REGN4018, and REGN5458 clinical studies.
Thank You!