Dual-Switch CAR-T Cells: Inducible Cell Activation and Elimination to Manage Persistence and Toxicity

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Houston, TX
Disclosure Statement

I am an employee and stockholder of Bellicum Pharmaceuticals, Inc.
Challenges in Cell Therapy

Most cell therapies can only be controlled **before** infusion, but **not** after infusion.

**BEFORE INFUSION**
- Collect white blood cells
- Genetic modification
- Patient infusion

**AFTER INFUSION**
- On Target/ Off Organ
- GvHD
- CRS
- Neurotoxicity
- Anergy
- Insufficient expansion
- Lack of persistence
Controllable Cell Therapy

Bellicum’s molecular switches allow control after infusion

BEFORE INFUSION CONTROLLED

Collect white blood cells

Genetic modification

Patient infusion

AFTER INFUSION CONTROLLED

GO switch to activate immune cells

SAFETY switch to eliminate toxic cells

Goal

Improved Benefit / Risk
Dual-Switch Technology
Controlling the activity of CAR-T cells with built-in safety

- **iMC inducible costimulation**
  - Increased persistence and survival of CAR-T cells

- **iRC9 inducible cell elimination**
  - Rapid removal of activated CAR-T cells and reduction of inflammatory cytokines

- **Dual control of CAR-T cells allow for**
  - Treatment of aggressive tumors that need additional CAR-T potency
  - Target or indication toxicity concerns

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**Rimiducid**

- Increased persistence and survival of CAR-T cells

**Temsirolimus**

- Rapid removal of activated CAR-T cells and reduction of inflammatory cytokines

**iMC iRC9**

- Dual control of CAR-T cells allow for
  - Treatment of aggressive tumors that need additional CAR-T potency
  - Target or indication toxicity concerns
DS HER2 CAR-T cells demonstrate iMC-dependent tumor elimination and T cell expansion.

Rim-dependent elimination of OE19 tumors

Rim-dependent CAR-T cell expansion

OE19 tumor IVIS
Radiance scale: Min 5x10^5; Max 1x10^7

T cell IVIS
Radiance scale: Min 1x10^5; Max 1x10^7

- Mock + Rim
- DS CAR + Veh
- DS CAR + Rim

x = euthanized due to high tumor burden
Multiple t test; * p < 0.05; ** p < 0.005; *** p < 0.0005
How do DS CAR-T cells compare to conventional 1\textsuperscript{st} and 2\textsuperscript{nd} generation CAR-T cells?
iMC activation enhances antitumor activity and expansion of DS HER2 CAR-T cells.

<table>
<thead>
<tr>
<th>E:T</th>
<th>1:20</th>
<th>1:10</th>
<th>1:5</th>
<th>1:2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>OE19-GFP (GCU x μm²/well)</td>
<td><img src="image1" alt="Graph" /></td>
<td><img src="image2" alt="Graph" /></td>
<td><img src="image3" alt="Graph" /></td>
<td><img src="image4" alt="Graph" /></td>
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<tr>
<td>Tcell-ONL (RCU x μm²/well)</td>
<td><img src="image5" alt="Graph" /></td>
<td><img src="image6" alt="Graph" /></td>
<td><img src="image7" alt="Graph" /></td>
<td><img src="image8" alt="Graph" /></td>
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</tbody>
</table>

- **Mock**
- **HER2.z**
- **HER2.BB.z**
- **HER2.28.z**
- **DS HER2.z**
- **DS HER2.z+Rim**
iMC activation enhances antitumor activity and expansion of DS HER2 CAR-T cells.

OE19-eGFP  Tcells-ONL  1:20, day 7
iMC activation enhances proinflammatory cytokine secretion of DS HER2 CAR-T cells.

Target = OE19
1:2.5 E:T

One-way ANOVA with Tukey’s multiple comparison
* p < 0.05; ** p < 0.005; *** p < 0.0005
Do HER2 CAR-T cells become exhausted upon repeated antigen exposure?

**Effector:** HER2 CAR-T cells  
**Target:** OE19 (irradiated)  
1:1 E:T

- **Day 0 coculture**
- **Day 2** ELISA
- **Day 7** Count & FACS coculture
- **Day 9** ELISA
- **Day 14** Count & FACS coculture
iMC-activated DS HER2 CAR-T cells are not exhausted upon repeated antigen stimulation.

Cytokine release

Two-way ANOVA with Tukey’s multiple comparison
* p < 0.05; ** p < 0.005; *** p < 0.0005
iMC-activated DS HER2 CAR-T cells are not exhausted upon repeated antigen stimulation.

Two-way ANOVA with Tukey's multiple comparison

* p < 0.05; ** p < 0.005; *** p < 0.0005
iMC activation enhances antitumor activity and expansion of DS CD19 CAR-T cells.

Nalm6-eGFP Tcells-ONL; D236; 1:5, day 7
DS CD19 CAR-T cells demonstrate iMC-dependent tumor elimination and T cell expansion.

Rim-dependent elimination of Nalm6 tumors

Rim-dependent CAR-T cell expansion

**Nalm6 tumor IVIS**
Radiance scale: Min 2x10^6; Max 1x10^9

**T cell IVIS**
Radiance scale: Min 3x10^5; Max 1x10^7

- x = euthanized due to high tumor burden
- Multiple t test; * p < 0.05; ** p < 0.005; *** p < 0.0005
iRC9 activation allowed for titratable T cell elimination and remaining DS CAR-T cells retained the ability to control tumor re-challenge.
iRC9 activation allowed for titratable T cell elimination and remaining DS CAR-T cells retained the ability to control tumor re-challenge.

- **Nalm6-eGFP/Fluc**
  - 5x10^5 i.v.

- **CAR-T**
  - 10x10^6 i.v.

- **Nalm6-eGFP/Fluc**
  - 5x10^6 i.v.

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**Tem-dependent elimination of DS CAR-T cells**

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<tr>
<th>Tem (mg/kg)</th>
<th>Rim (mg/kg)</th>
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<tr>
<td>10</td>
<td>1</td>
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<td>0.1</td>
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**Nalm6-eGFP/Fluc**

- Avg. Radiance (p/s/cm^2/sr)
- T cell injection (days)
- Nalm6 re-challenge

**Tem-dependent reduction of IFN-γ**

- Avg. Radiance
- Normalized cytokine

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iRC9 activation allowed for titratable T cell elimination and remaining DS CAR-T cells retained the ability to control tumor re-challenge.
Summary

Molecular switches designed to control CAR-T dynamics in vivo

- Transient toxicity and efficacy (2nd gen CARs)
- Prompt resolution of toxicity (2nd gen CARs + safety switch)
- Prolonged efficacy (DS CARs)

Toxicity
Efficacy
Highly Differentiated Portfolio

Control switch(es) selected to address situation-specific challenge

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<thead>
<tr>
<th>PRODUCT CANDIDATE</th>
<th>DISCOVERY</th>
<th>CLINICAL PROOF OF CONCEPT</th>
<th>PIVOTAL</th>
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<tbody>
<tr>
<td>Rivo-cel (BPX-501)</td>
<td>E.U.: Pediatric Leukemias, Lymphomas, and Inherited blood disorders (+allo-HSCT)</td>
<td>12+ Years AML + MDS (+allo-HSCT) – THRIVE</td>
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<td>(Allogeneic Polyclonal T-cells)</td>
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<tr>
<td>BPX-601 GoCAR-T PSCA</td>
<td>Pancreatic, Gastric, &amp; Prostate Cancers</td>
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<td>BPX-603 GoCAR-T HER-2</td>
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<td>BPX-802 GoCAR-T Target TBA</td>
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<td>Liquid Tumors</td>
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Acknowledgements

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