Precise Targeting of AML With OR/NOT Logic-Gated Gene Circuits in CAR-NK Cells

Brian S. Garrison, Han Deng, Gozde Yucel, Nicholas W. Frankel, Marcela Ayala Guzman, Russell M. Gordley, Michelle Hung, Derrick Lee, Marcus Gainer, Kathryn Loving, Jenny Chien, Tiffany Pan, Wesley Gorman, Nelia Leemans, Alice Lam, Travis Wood, Wilson Wong, Philip Lee, Tim Lu, Gary Lee

ASGCT – Abstract #77
6:00pm ET, May 12, 2021
Disclosure

- Employee of Senti Biosciences, and receive salary and benefit from the company
- This presentation included verbal remarks by the presenter that are not included here
Senti’s Gene Circuit “Software” Platform Technologies Embed Logic Into Cell & Gene Therapies, Which May Address Certain Challenges Facing Existing Medicines

About Gene Circuits

Senti creates novel and proprietary combinations of DNA sequences as gene circuits that implement biological logic.

Senti’s gene circuits may power “smart” cell and gene therapies with enhanced therapeutic properties.

Source: Adapted from Science, 359:6376 (2018)
Senti’s NOT GATE Technology is Intended to Solve a Fundamental Problem in Cancer Therapy

Question: How do we prevent on-target/off-tumor toxicity?

Answer: NOT Logic Gate

How Does the NOT GATE Work?
Senti’s NOT GATE Technology is Intended to Solve a Fundamental Problem in Cancer Therapy

Tumor Antigen Engagement Triggers Cancer Cell Killing

Engineered Cell

Cytokines

Cytotoxic granules

Activation

Cancer Cell

Apoptosis

Safety Antigen Engagement Enables Protection of Healthy HSCs

Engineered Cell

Healthy Cell

NOT GATE (iCAR)
For Acute Myeloid Leukemia (AML), We Believe that Different Therapies Designed to Target Multiple Antigens are Needed

Challenges for Current Acute Myeloid Leukemia (AML) Therapies

**Single-target Toxicity:** Challenging to comprehensively target all AML subsets, including blasts and AML leukemic stem cells (LSCs), with single antigens → decreased efficacy and increased relapses

**Indiscriminate Toxicity:** Challenging to target AML cells without also killing healthy hematopoietic stem cells (HSCs) with single antigens → toxicity/safety issues

Bone marrow transplant carries morbidity/mortality risks and limitations due to donor availability

Senti’s Approach to the Challenges of AML

**Key Takeaways**

We believe that protection of 10-20% of healthy HSCs would be sufficient to enable hematopoietic recovery and provide clinical benefit to patients
OR/NOT GATE: TARGETING BOTH AML BLAST AND AML LSCs WHILE PRESERVING HEALTHY HSCs

Deep Clearance of AML Blasts and LSCs While Sparing Healthy HSCs

<table>
<thead>
<tr>
<th>Sense Inputs</th>
<th>Compute Decision</th>
<th>Respond with Outputs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD33</td>
<td>No Logic</td>
<td>AML Blasts: X</td>
</tr>
<tr>
<td>FLT3, CD33</td>
<td>OR GATE Only</td>
<td>AML LSCs: X</td>
</tr>
<tr>
<td></td>
<td>(FLT3 OR CD33)</td>
<td>Healthy HSCs/PCs: X</td>
</tr>
</tbody>
</table>

Antigen: CD33, FLT3, EMCN

CD33 is over-represented on AML blast cells; FLT3 is a marker for AML LSCs; HSCs=hematopoietic stem cells; PCs = progenitor cells
OR Logic Gating

Targeting the Tumor-Associated Antigens FLT3 and CD33
Senti Identified FLT3/CD33 as an Ideal OR GATE Target Antigen Pair for AML

**OR/NOT GATE: TARGETING BOTH AML BLAST AND AML LSCs WHILE PRESERVING HEALTHY HSCs**

1. **Membrane Protein**
   - 7,860 genes
2. **High in AML**
   - 2,611 genes
3. **Low Non-Hematopoietic Tox**
   - 2,152 genes
4. **Confirmation: High in AML**
   - 112 genes
5. **Stricter Tox Filtering**
   - 40 genes
6. **Manual Curation**
   - 7 genes

- **High on AML**
- **Low on healthy tissues**
  - FLT3 (CD135)
  - CD33 (Siglec3)
  - Target 3
  - Target 4
  - Target 5
  - Target 6
  - Target 7

**Source:** Internal data

20,216 Protein coding genes (NCBI)
Focusing Only on CD33 Addresses AML Blasts but Largely Misses AML LSCs

Key Takeaways

- Based on our preclinical study, when only CD33+ cells are targeted, a substantial number of AML LSCs may not be targeted.
- We believe this may lead to suboptimal efficacy and relapses.

Source: Internal data
By Targeting either FLT3 or CD33 Antigens With an OR GATE, We Believe that Comprehensive Killing of Cancer Cells Is Possible

Key Takeaways

- Based on our preclinical studies, targeting either FLT3 or CD33 antigens enables more comprehensive targeting of cancer cells.

- We believe improved efficacy may be achieved if both major populations of AML Blasts and AML LSCs are comprehensively targeted.

Source: Internal data
OR GATE: CAR-MEDIATED NK CELL KILLING

OR GATE Facilitated Improved Killing of Cancer Cells Expressing Either Antigen Target Based on Preclinical Data

Key Takeaways

- Only targeting the FLT3 antigen had modest FLT3++ cell killing but little CD33++ cell killing
- Only targeting the CD33++ antigen poorly addressed FLT3++ cells
- Being able to target either antigen may result in more robust CAR-mediated cell killing

Source: Internal data

* p < 0.05, ** p < 0.01
OR GATE: CAR-MEDIATED NK CELL KILLING

OR GATE Facilitated Improved Killing of Cancer Cells Expressing Either Antigen Target Based on Preclinical Data

Key Takeaways

- Only targeting the FLT3 antigen had modest FLT3++ cell killing but little CD33++ cell killing
- Only targeting the CD33++ antigen poorly addressed FLT3++ cells
- Being able to target either antigen may result in more robust CAR-mediated cell killing

Percent of CAR-Mediated Killing

<table>
<thead>
<tr>
<th>Antigen(s) Targeted</th>
<th>FLT3 (Only) CAR-NK</th>
<th>CD33 (Only) CAR-NK</th>
<th>FLT3 OR CD33 CAR-NK</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLT3 (Only) CAR-NK</td>
<td>40</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>CD33 (Only) CAR-NK</td>
<td>20</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>FLT3 OR CD33 CAR-NK</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
</tbody>
</table>

1:2 (E:T) ratio; 4hrs

Leukemia Cell Line

- FLT3++
- CD33++

* p < 0.05, ** p < 0.01

Source: Internal data
OR Gated CAR-NK Cells Demonstrated Significant Cytotoxicity Against Primary AML Samples

Primary AML Samples With Expanded Blast Populations

Healthy BMMC  
AML #857 (M2)  
AML #847 (M3)

CD45  
SSC

AML blasts

FLT3 or CD33 CAR-NK Cells Killed Primary AML Cells

AML #857 (M2)  
AML #847 (M3)

Percent killing

Unengineered NK Cells  
Engineered CAR-NK Cells

1:1 (E:T) ratio

** P ≤ 0.01; *** P ≤ 0.001

Source: Internal data
FLT3 OR CD33 CAR-NK Cells Significantly Suppressed Tumor Growth in Preclinical AML Xenotransplantation Study

Key Takeaways

FLT3 OR CD33 CAR-NK cells achieved statistically significantly greater anti-tumor activity compared to untreated control mice (p < 0.01) and mice treated with unengineered NK cells (p < 0.05)

Source: Internal data
FLT3 OR CD33 CAR-NK Cells Significantly Increased Mouse Survival in Preclinical AML Xenotransplantation Study

Key Takeaways

FLT3 OR CD33 CAR-NK cells significantly suppressed tumor growth and increased animal survival in an MV4-11-based AML xenotransplantation model.

P-Values:
(3) vs (1): p < 0.01
(3) vs (2): p < 0.01
(2) vs (1): p < 0.01

Source: Internal data
NOT Logic Gating

NOT GATE for protection of healthy cells
Based on Preclinical Data, Targeting Only FLT3 Addresses AML LSCs But May Kill Healthy HSCs as Well

**Key Takeaways**
- AML LSCs can be effectively killed by targeting FLT3+ cells
- However, FLT3 expression on AML cells has a large overlap with Healthy HSCs, leading to toxicity challenges

Source: Internal data
Senti’s NOT GATE Technology is Intended to Solve a Fundamental Problem in Cancer Therapy

**Tumor Antigen Engagement Triggers Cancer Cell Killing**

- Engineered Cell
- Cytokines
- Cytotoxic granules

**Cancer Cell**
- Activation
- Apoptosis

**Safety Antigen Engagement Enables Protection of Healthy HSCs**

- Engineered Cell
- Healthy Cell
- NOT GATE (iCAR)

**Diagram:**
- Activating CAR (aCAR)
- Inhibitory CAR (iCAR)
- Tumor-associated Antigen
- Safety Antigen
Senti Leverages Bioinformatics Approaches To Identify Suitable Safety Antigens and Screen NOT GATE Architectures To Build Next-Gen Precision Cell Therapies

Digital Presentation:
Precise Tumor Targeting with NOT GATE Chimeric Antigen Receptor Gene Circuits
Tuesday May 11 from 8:00–10:00am ET (abstract #960)
Senti Identified a NOT GATE Safety Antigen Called EMCN That Distinguishes AML LSCs From Healthy HSCs

Key Takeaways
- The ‘NOT GATE’ uses EMCN as a Safety Antigen input to differentiate between healthy HSCs and AML cells
- This enables targeted killing of cancer cells while sparing Healthy HSCs, thereby improving the therapeutic window

Healthy HSCs (from healthy donor)

EMCN+ = 70.2%

EMCN
Side Scatter (SSC)

AML LSCs (from AML patient)

EMCN+ = 0%

EMCN
Side Scatter (SSC)

Source: Internal data
NOT GATE Enabled Protection of Cells Expressing EMCN While Maintaining On-Target Killing of FLT3+ Cancer Cells

Key Takeaways

- Without an EMCN-specific NOT GATE, FLT3+ cells undergo NK cell-mediated killing.
- With EMCN-specific NOT GATE, presence of EMCN on target cells reduce cytotoxicity by 67% while preserving NK cell-mediated killing of FLT3+ cancer cells.
- We believe that protecting 10-20% of Healthy HSCs is clinically meaningful.

ns = not significant; ** p < 0.01, *** p < 0.001
Source: Internal data
NOT GATE Enabled Protection of Cells Expressing EMCN While Maintaining On-Target Killing of FLT3+ Cancer Cells

Key Takeaways

- Without an EMCN-specific NOT GATE, FLT3+ cells undergo NK cell-mediated killing.
- With EMCN-specific NOT GATE, presence of EMCN on target cells reduce cytotoxicity by 67% while preserving NK cell-mediated killing of FLT3+ cancer cells.
- We believe that protecting 10-20% of Healthy HSCs is clinically meaningful.

Source: Internal data

ns = not significant; ** p < 0.01, *** p < 0.001
Senti’s R&D Headquarters (South San Francisco, CA, USA)

HQ and R&D Center

- Senti’s multi-modal preclinical research labs – South San Francisco, CA
- SSF is a major Biotech hub with high density of facilities and R&D talent in over 150 companies
- Proximity to major biopharma sites, institutions, and SFO
Together, We Can Outsmart Complex Diseases With Intelligent Medicines.

**Oral Presentation:**
Small Molecule-Regulated Gene Circuit for Controlling Cytokine Expression in Cell Therapies
Friday May 14 from 1:45–2:00pm ET (abstract #214)

**Digital Presentation:**
Precise Tumor Targeting with NOT GATE Chimeric Antigen Receptor Gene Circuits
Tuesday May 11 from 8:00–10:00am ET (abstract #960)

Brian.garrison@sentibio.com