Developing Next-Generation Engineered TCR-T Cells with CRISPR

Keystone Symposium: Engineering the Genome

Birgit Schultes, Ph.D. | February 10, 2020

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Intellia Therapeutics is a Full-Spectrum Genome Editing Company

CRISPR creates the therapy

Immuno-oncology
Autoimmune diseases

CRISPR is the therapy

Genetic diseases

In Vivo
Ex Vivo

LNP: Lipid Nanoparticle
Intellia is Employing TCR Adoptive T Cell Therapy for a Broad Range of Cancers

- Recognizes both surface and intracellular tumor antigens
- Physiological activation prevents premature exhaustion
- Natural TCR selection prevents reactivity against healthy tissues
- Preliminary clinical data suggest that TCR T cells show less cytokine release syndrome than CAR-T cells
Our Goal: Engineer T Cell Therapies While Preserving Normal Cell Physiology

CRISPR-Based Engineering

• Enables precise gene KO and insertion

• Replaces endogenous with therapeutic TCR\(^1,2\):
  - Enhances TCR expression and function
  - Reduces mispairing and GvHD risk
  - Improves cell drug quality and potency

Improving Safety and Efficacy Over Current Engineered Cell Therapy Approaches

PRECISE • POTENT • PERSISTENT

KO: Knockout
GvHD: Graft-Vs-Host Disease

\(^1\)Provasi, Genovese et al., Nature Medicine 2012
\(^2\)Mastaglio et al., Blood 2017
Acute Myeloid Leukemia (AML) is Intellia’s Initial Cell Therapy Indication

Cancer of the blood and bone marrow that is *rapidly fatal without immediate treatment*, and is the most common type of acute leukemia in adults.\(^1\)

\(~20K\)  
New cases in the U.S. in 2018\(^1\)

\(>40K\)  
New cases in the 7MM\(^2\) in 2018\(^1\)

\(<30\%\)  
5-year overall survival\(^1\)

**NTLA-5001 in development for AML**

Engineer WT1-specific T cells capable of specifically killing AML blasts

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\(^1\) NIH SEER Cancer Stat Facts: Leukemia – Acute Myeloid Leukemia (AML)

\(^2\) GlobalData EpiCast Report: Acute Myeloid Leukemia July 2017, 7MM: Seven Major Markets (includes U.S.)
Wilms’ Tumor 1 (WT1) is Highly Over-Expressed in AML, Making it an Attractive TCR Antigen

- Identified as top cancer antigen by NCI\(^1\)
- Plays key role in oncogenicity and is expressed in AML progenitor cells
- Intracellular protein expressed across different AML subtypes, independent of driver mutation, and maintained in relapsing disease
- Clinical activity and safety demonstrated with WT1 vaccines and T cell therapy in AML\(^2,3\)

\(^1\)Cheever et al. Clin Cancer Res, 2009
\(^2\)DeStasi et al. Front Immunol. 2015
\(^3\)Chapuis et al. Nature Med. 2019
Path to NTLA-5001, Our WT1-TCR T Cell Development Candidate

1. Identify High-Affinity WT1-TCR
   - Natural TCR from healthy volunteers
   - Specific and potent for WT1 tumor antigen
   - HLA-A*02:01 restricted

2. Engineer T Cell Product with CRISPR/Cas9
   - Eliminate endogenous TCR to prevent mispairing
   - In locus insertion of therapeutic WT1-TCR

3. Characterize T Cell Product
   - TCR avidity
   - Polyfunctionality and anti-tumor reactivity
   - TCR specificity and safety
High Potency WT1-Specific TCRs Identified from Healthy Donor T Cells

Pool of 141 peptides
Length: 15-mers*

**1** In vitro stimulation with WT1 peptide pool

**2** Evaluation of the Expansion of WT1-specific T lymphocytes

**3** Identification of the immunogenic epitope

**4** Determination of HLA restriction

**5** Assessment of target cell recognition

**6** Evaluation of the clonality of the TCR repertoire

**7** High-throughput sequencing of the TCR repertoire

* Peptides containing the immunodominant and well-researched RMFPNAPYL epitope were excluded.
OSR Collaborators Identified Potent and Specific WT1-TCRs

- Seven HLA-A*02:01 restricted TCRs identified to two distinct immunogenic epitopes (outside of well-described RMF epitope)
- Two lead TCRs selected based on avidity and potency assays
- Binding epitopes identified and characterized by alanine scanning
- Lead TCRs recognize VLDFAPPGA epitope
  - Epitope is presented by tumor- and immuno-proteasome
- TCR-binding motif not found in human proteome outside of WT1
Specific Recognition and Killing of Primary AML Blasts Presenting WT1 Epitope

**HLA-A*02:01 Positive**

- **pAML Patient 1**
  - Cytogenetics: intermediate
  - Mutations: FLT3
  - WT1: 26427*

- **pAML Patient 2**
  - Cytogenetics: intermediate
  - Mutations: FLT3, NPMA, DNMT3A
  - WT1: 7185*

- **pAML Patient 3**
  - WT1: 46363*

**HLA-A*02:01 Negative (Control)**

- **pAML Patient 4**

Mean + SD for cytotoxicity of T cell preparations from 3 healthy donors, 6 h apoptosis assay

*WT1 measured by qPCR

**Dose-dependent and HLA-specific cytotoxicity of edited T cells against primary AML blasts overexpressing WT1 across three patient samples**
Lead WT1-TCR T Cells Kill Target Cells with High Avidity

TCR 1 shows low nM avidity
TCR 1 selected as lead candidate

Cytotoxicity of edited T cells against T2 cells pulsed with WT1-derived VLD peptide

tgTCR: transgenic therapeutic TCR
1. Identify High-Affinity WT1-TCR
   - Natural TCR from healthy volunteers
   - Specific and potent for WT1 tumor antigen
   - HLA-A*02:01 restricted

2. Engineer T Cell Product with CRISPR/Cas9
   - Eliminate endogenous TCR to prevent mispairing
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3. Characterize T Cell Product
   - TCR avidity
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   - TCR specificity and safety
CRISPR Engineering Overcomes Key Challenges of Traditional TCR Approaches

**Key Challenges**

- Random insertion of tgTCRs creates mutagenesis risk
- Mixed expression of endogenous and tgTCR
- Mispaired TCRs have unpredictable specificities and pose GvHD risk
- Lower tgTCR expression per T cell leads to reduced efficacy

**Our Solution**

- Precise replacement of endogenous TCR with tgTCR
- No insertional mutagenesis risk
- Reduces TCR mispairing and GvHD risk
- High tgTCR expression per T cell leads to a more efficacious cell product
CRISPR/Cas9 Engineering of tgTCR T Cell Therapies

**Insertion of tgTCR αβ chains at the TRAC locus**

- **Insertion Template**
  - AAV
  - TCRb
  - TCRa
  - TRAC
  - Exon 2
  - In locus insertion

- **CRISPR/Cas9 to TRAC**
  - TCRb
  - TCRa
  - TRAC
  - Exon 2

**CRISPR/Cas9 site-specific KO of TRBC**

- **CRISPR/Cas9 to TRBC**
  - TRBC
  - Exon 2

- **Gene sequence interrupted**

- **No expression of endogenous TCR**
  - (no mispairing)

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**Additional Notes**

- EF1a
- EF1a
Potent and Specific sgRNAs Identified for TRAC and TRBC

sgRNAs show potent TRAC and TRBC KO with >98% KO of endogenous TCR

Off-target analyses demonstrate specificity of sgRNAs
Eliminating Endogenous TCR Results in Homogeneous T Cell Product with >95% of tgTCR Chain Pairs

**TRAC Insertion Only**
Endogenous TCR β chain still present

- Non-edited T cells
- T cells with inserted TCR
  - T cells with varying levels of endogenous TCRβ chains
  - T cells with tgTCRβ only (~20%)

**TRAC Insertion + TRBC KO**
Endogenous TCR α and β chains removed

- Non-edited T cells
- T cells with inserted TCR
  - "Mispaired" TCRs
  - Properly paired TCRs (>95%)

Comparative insertion rates of tgTCR (65-80% CD3+) with or without TRBC KO
Expression of the properly paired tgTCR is greatly increased with TRBC KO

Bind to MHC-Tetramer
TCR α and β Chain Cysteine Modifications Do Not Prevent Mispairing

- TCR α and β Chain Cysteine Modifications Do Not Prevent Mispairing
- TCR with Cysteine Modification
- Natural TCR Sequence
- TRAC Insertion only
- TRAC Insertion + TRBC KO
- Properly paired TCRs (>95%)
- Properly paired TCRs (10-20%)
- Cysteine modification does not solve the mispairing problem and lowers TCR expression per cell
- Inserted tgTCR (Vβ-specific Ab)

Graphs showing:
- WT1 TCR expression level (Vb8+ MFI)
- TRAC Insertion + TRBC KO
- TRAC Insertion only
High and Uniform tgTCR Expression Results from Full KO of Endogenous TCR

Extent of mispairing is TCR-dependent but detectable in all tested TCRs
Elimination of Endogenous TCR Significantly Improves T Cell Product Target Recognition and Activation

**T Cell Degranulation After Peptide Stimulation**

- TRAC Insertion + TRBC KO
- TRAC Insertion only

 EC$_{50}$: 0.01 nM
 EC$_{50}$: 1.35 nM

**T Cell Cytokine Release After Peptide Stimulation**

- TRAC Insertion + TRBC KO
- TRAC Insertion only

 EC$_{50}$: 0.02 nM
 EC$_{50}$: 0.14 nM

Increased cytotoxicity and cytokine secretion both observed following peptide stimulation
Endogenous TRAC and TRBC KO Reduces Off-Target Reactivity


dilution of cell trace Violet in dividing cells

Without both TRAC and TRBC KO, mispaired TCRs lead to strong alloreactivity of T cells
EF1α Promoter Drives Higher and More Uniform Expression of tgTCR than TRAC Promoter

Promoterless (PL) TCR template + TRAC and TRBC edit

EF1α promoter TCR template + TRAC and TRBC edit

EF1α promoter TCR template – No CRISPR edit

No AAV No CRISPR edit

Donor-specific Vβ8 frequency

Endogenous TRAC promoter drives lower expression of TCR α and β chains

EF1α promoter provides near physiological expression of the tgTCR construct

No episomal expression in final product
Superior Cytokine Production and Tumor Cell Killing with EF1α Promoter

Higher TCR expression with EF1α promoter allows for:

- Lower peptide threshold
- Higher cytokine production
- Better tumor cell killing at naturally presented WT1 peptide levels
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   - TCR avidity
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Lead TCR Shows Superior Target Recognition *In Vitro* vs. Other Natural WT1-Specific TCRs, Comparable to Affinity-Enhanced TCRs

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**T Cell Cytokine Release After Peptide Stimulation**

- Natural TCR (WT1-VLD)
- Natural TCR (WT1-C4-RMF)
- Affinity-enhanced TCR A
- Affinity-enhanced TCR B

**T Cell Degranulation After Peptide Stimulation**

- Natural TCR (WT1-VLD)
- Affinity-enhanced TCR A
- Affinity-enhanced TCR B

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Lead TCR Avidity:
EC$_{50}$ = 6.3 nM

Lead TCR Avidity:
EC$_{50}$ = 0.013 nM
Lead TCR T Cells Proliferate in Response to WT1+ Tumor Cells

Engineered T cells proliferate in response to:
- Naturally processed WT1 peptide (K562-A2.1)
- WT1 peptide-pulsed tumor cells (OCI-AML3)
Lead TCR T Cells Kill Primary AML Blasts

Cytogenetics: intermediate
Mutations: FLT3, NPMA, DNMT3A
6 h apoptosis assay
Lead TCR Shows No Cytotoxicity Against Bone Marrow CD34+ Cells Expressing Normal Levels of WT1

Mean ± SD for Incucyte apoptosis assay, CD34+ bone marrow cells (HLA-A*0201 positive and negative) ± TCR 1 T cell preparations from 3 donors

Apoptosis level in HLA-A*02:01-positive HSCs is comparable to that in negative control levels
Multiple Workstreams to Advance Cell Therapy Efficacy in Solid Tumors

Allogeneic Cell Source
• Edit determinants of allo-reactivity to achieve persistence in presence of T and NK cells

Functional Modulation
• Knockout and/or knock-in (insertion) of key receptors to modulate T cell functionality

Solid Tumor Efficacy
• CRISPR screening to unravel targetable key regulators of T cell fitness in different tumor microenvironments

NK: Natural killer
Key Takeaways

• NTLA-5001 nominated as WT1-TCR-directed development candidate

• Consistent high-level editing efficacy
  – >98% KO of endogenous TCRs
  – >70% in locus insertion of tgTCRs

• TCR shows nanomolar avidity

• Specific and potent killing of WT1-positive AML blasts

• No detectable bone marrow cell toxicity

• Expect to submit an Investigational New Drug (IND) application in 1H 2021

• AML program provides foundation for expansion into solid tumors
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