Engineering T-cells for Cancer Therapeutics

Immuno-Oncology Summit
Gary Lee, PhD
August 2017
Current Success Stories in T-cell Immunotherapy

F.D.A. Approves First Gene-Altering Leukemia Treatment

By DENISE GRADY  AUG. 30, 2017

Kite Highlights Durable Complete Remissions Up to 56+ Months in Patients with Chemorefractory Aggressive Non-Hodgkin Lymphoma (NHL) after Anti-CD19 CAR T-Cell Therapy at the National Cancer Institute

- Durable CRs Continued After Recovery of Normal B Cells
- Anti-CD19 CAR T-cell Therapy Can Be Curative for Chemorefractory Aggressive NHL

bluebird bio Announces Interim Phase 1 Dose Escalation Data for its Anti-BCMA CAR T Product Candidate in Patients with Relapsed/Refractory Multiple Myeloma
Achieving T-cell Immunotherapy's Promise

Current challenges facing autologous T-cell immuno-therapies:

• "One Patient, One Product" difficult to scale to meet needs of large populations

• Chemo washout and delayed treatment

• Immuno-modulatory tumor microenvironments in solid tumors

• Limited speed of development for novel, exploratory CARs/TCRs due to the need for viral vector development
Sangamo Universal T-cell Program

• Builds on T-cell engineering process from previous HIV trials; T-cell engineering / expansion process maintains TSCM subpopulation that led to long-term (3+ year) engraftment in all patients

• Highly efficiency (>90%) simultaneous KO and TI; possible due to extreme design densities of new ZFN architecture

• TI for physiological regulation of CAR/TCR expression and increased potency

• Eliminates TCR repertoire, HLA Class I on T-cells; exploring additional novel functionalities

• Efficient editing and expansion process enables QC steps in manufacturing that improve product quality
Clinical Scale Zinc Finger Nuclease-mediated Gene Editing of PD-1 in Tumor Infiltrating Lymphocytes for the Treatment of Metastatic Melanoma

Joal D Beane¹,², Gary Lee³, Zhili Zheng¹, Matthew Mendel³, Daniel Abate-Daga¹,⁴, Mini Bharathan¹, Mary Black¹, Nimisha Gandhi³, Zhiya Yu¹, Smita Chandran¹, Martin Giedlin³, Dale Ando³, Jeff Miller¹, David Paschon³, Dmitry Guschin³, Edward J Rebar¹, Andreas Reik³, Michael C Holmes³, Philip D Gregory³, Nicholas P Restifo¹, Steven A Rosenberg¹, Richard A Morgan¹,⁵ and Steven A Feldman¹

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Received 28 October 2014; accepted 15 April 2015; advance online publication 2 June 2015. doi:10.1038/mt.2015.71
“Off-the-shelf” T-cell Therapy: Eliminating TCR and/or HLA on CAR⁺ T cells

Healthy donor T cells

Patient

TCRαβ

T cells

HLAs

Unwanted response

Rejection

CD19

B cell leukemia/lymphoma

HLAs

Normal cells

Unwanted response

GVHD

Recognize “non-self” (Patient → Donor)

Recognize “non-self” (Donor → Patient)

Intended response

Blood. 2013 Aug 22;122(8):1341-9
Gene editing of TCR alpha constant or TCR beta constant alone is sufficient to eliminate formation of TCR complex.

KO of endogenous TCR eliminated GvHD in mouse models.

ZFNs used in this study was built ~2010.
New ZFN Platform Enables Optimal Targeting Capability

**Modules:** 1- and 2-finger units that recognize base sequence

- >8000 hexamer / module combinations

**Intermodule linkers:**

- Connect adjacent modules
- 6 alternatives for skipping 0, 1, or 2 bp

**ZFP-Fok linker:**

- Links the Fok and ZFP domains
- 5 alternatives for skipping 5-9 bp
- 4 alternatives for reversing Fok-ZFP polarity
Yields Highly Potent, Specific Nucleases from Unparalleled Design Density

Example: TCRα constant region

- 3 exons / 374 bp
- 374 potential locations for cleavage

→ 218 TRAC locations (1 per 1.7 base pairs, or 58%) immediately targetable with current ZFN library
  → Able to construct new ZFNs to target virtually any base pair

- HiFi CRISPR has a theoretical design density of 2-3%, or ~10 targetable locations in TRAC

- High design density (20X relative to HiFi CRISPR) enables selection of most potent and specific nucleases to target TRAC and B2M
>90% TCR KO by FACS

No ZFN controls

TCR KO

1%

92% CD3 neg

ZFN treated
Concordant TRAC Genomic Modification Observed

Gene Modification

% NHEJ (MISEQ)

Untransfected  Transfected

% Gene Modified

% CD3 Neg.
Targeting $\beta_2$-microglobulin (B2M) to Knockout HLA Class I

- Secretion of MHC Class I proteins requires intracellular B2M (Ploegh et. al. PNAS 1979)
- B2M is a relatively small protein (119 aa), located on Chr 15
- Bi-allelic KO likely required to eliminate MHC Class I expression
>90% HLA Class I KO by FACS

No ZFN controls

ZFN treated

94% HLA neg
Concordant B2M Genomic Modification Observed

Gene Modification

%NHEJ (MISEQ)

95.0

0.3

Untransfected Transfected

% HLA Class 1 Neg.

% Gene Modified
Codelivery Enables >80% Double Knockout

Control  TCRα ZFNs  B2M ZFNs  TCRα + B2M ZFNs

- 96% CD3(-)
- 92% HLA(-)
- 82% double knockout
Specificity Assessed Via End Capture Assay

Cleave genome
Integrate donor

K562 cells
delivery via nucleofection
400 ng RNA / ZFN, 1 uM oligo duplex

donor

Sequence genome
adjacent to donor

Linear amplification,
adaptor ligation, PCR

Sequence reveals candidate cleavage site.

Assess indels at candidate
off-target loci

off-target?

CD4 T cells
delivery via RNA electroporation
TCRα ZFN Exhibit No Evidence of Cleavage at Non-Target Loci in Human Primary T-cells

<table>
<thead>
<tr>
<th>Locus</th>
<th># of sequences recovered</th>
<th>% indels in followup study</th>
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<td>8784</td>
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<td>102757228</td>
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assay background not significant
### B2M ZFN Exhibit No Evidence of Cleavage at Non-Target Loci in Human Primary T-cells

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<thead>
<tr>
<th>Locus</th>
<th>Sequences recovered</th>
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<th>p-value</th>
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Assay background not significant
ZFN-Mediated Targeted Integration With AAV Donors

ZFNs provided as mRNA

Donor provided as rAAV2/6

Integrated Donor

Published online 2 November 2015
Nucleic Acids Research, 2016, Vol. 44, No. 3 e30
doi: 10.1093/nar/gkv1121

Highly efficient homology-driven genome editing in human T cells by combining zinc-finger nuclease mRNA and AAV6 donor delivery

Jianbin Wang*, Joshua J. DeClercq, Samuel B. Hayward, Patrick Wai-Lun Li, David A. Shivak, Philip D. Gregory, Gary Lee and Michael C. Holmes
ZFN-Mediated Targeted Integration of an GFP Expression Cassette

ZFNs provided as mRNA

CD19 CAR Donor provided as rAAV2/6

Integrated Donor
Concerted Knockout + Integration in T-cells

**Gene KO**

TCR locus

- **CD3-**
  - 88%

B2M locus

- **HLA-**
  - 93%

**Targeted Integration**

- 71% TI (CD3- / GFP+)

- 72% TI (HLA- / GFP+)
90% Double KO of TCR and HLA Class I, with 90% Targeted Gene Insertion

Sham

ZFNs only

ZFNs + B2M Donor

ZFNs + TRAC Donor

HLA-/CD3-

HLA-/CD3-/GFP+
ZFN-Mediated Targeted Integration of CD19 CAR

- ZFNs provided as mRNA
- CD19 CAR Donor provided as rAAV2/6
- Integrated Donor
ZFN-Mediated Editing Enable Efficient Targeted Insertion of CD19 CAR into the TRAC Locus

**TCR**

- 96.5%

**HLA**

- 83.1%

**CD19 CAR**

- 61.2%
T-cell Killing Assay: CD19 Antigen Specific Cytotoxicity

Target cells only

<table>
<thead>
<tr>
<th>No T-cells</th>
<th>Sham Treated T-cells</th>
<th>TCR KO T-cells</th>
</tr>
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<tbody>
<tr>
<td>Controls-Target - Stained</td>
<td>Controls-Target + UT</td>
<td>Controls-TRAC + Target</td>
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<tr>
<td>CD19- K562 54.4%</td>
<td>CD19- K562 54.9%</td>
<td>CD19- K562 52.2%</td>
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<td>CD19+ K562 45.6%</td>
<td>CD19+ K562 45.1%</td>
<td>CD19+ K562 47.8%</td>
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</table>

Target cells + CAR neg T-cells
ZFN Engineering CD19 CAR-T Efficiently Kills Target Cells *In Vitro*

**T-cell: Target cell Ratio**

<table>
<thead>
<tr>
<th>Ratio</th>
<th>2:1</th>
<th>1:1</th>
<th>0.5:1</th>
<th>0.25:1</th>
<th>0.125:1</th>
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</thead>
<tbody>
<tr>
<td>TI into B2M</td>
<td>99.6%</td>
<td>0.4%</td>
<td>99.1%</td>
<td>9.9%</td>
<td>72.6%</td>
</tr>
<tr>
<td>TI into TRAC</td>
<td>99.9%</td>
<td>0.1%</td>
<td>99.9%</td>
<td>0.1%</td>
<td>98.3%</td>
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</table>
CD19 CART-T Dose Response Curve Observed

(T-cell: Target cell Ratio)
Highly Efficient T-cell Engineering Enables One-Step Universal T-cell Manufacturing Process

TCR and B2M Knockouts
mRNA Electroporation

CAR Targeted Insertion
AAV6 Transduction

Expansion & Selection
~150 x 10⁹ T-cells Post Expansion and Selection

Testing & Release

~500 Treatment Doses Per T-cell run

Cell Purification

Apheresis: Cell Procurement

5 x 10⁹ T-cells Post Enrichment

Product Infusion

3 x 10⁸ Dose per Treatment

5 x 10⁹ T-cells Post Expansion and Selection

~150 x 10⁹ T-cells Post Expansion and Selection
Summary

• Highly active and specific ZFN reagents to KO TCR and HLA Class I developed
  • 90+% double KO achieved

• T-cell engineering process optimized to mediate >90% TI using a AAV2/6 GFP donor

• TI of CAR or antigen-specific TCR
  • T-cell effector function against antigen positive target cells demonstrated

• ZFN mediated T-cell engineering may be deployed to engineer and develop next generation ACT products
## Acknowledgements

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