THERAPEUTIC IMPLICATIONS OF PREPARING AND ADMINISTERING INNATE IMMUNE CELLS

9:40 am to 10:10 pm
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Forward-looking statements

This presentation contains certain **forward-looking information about ZIOPHARM Oncology, Inc.** that is intended to be covered by the safe harbor for "forward-looking statements" provided by the Private Securities Litigation Reform Act of 1995, as amended. Forward-looking statements are statements that are not historical facts, and in some cases can be identified by terms such as "may," "will," "could," "expects," "plans," "anticipates," and "believes." These statements include, but are not limited to, statements regarding the progress, timing and results of preclinical and clinical trials involving the Company’s drug candidates, and the progress of the Company’s research and development programs. All of such statements are subject to certain risks and uncertainties, many of which are difficult to predict and generally beyond the control of the Company, that could cause actual results to differ materially from those expressed in, or implied by, the forward-looking statements. These risks and uncertainties include, but are not limited to: whether chimeric antigen receptor T cell (CAR T) approaches, Ad-RTS-IL-12, TCR and NK cell-based therapies, or any of our other therapeutic candidates will advance further in the pre-clinical or clinical trials process and whether and when, if at all, they will receive final approval from the U.S. Food and Drug Administration or equivalent foreign regulatory agencies and for which indications; whether chimeric antigen receptor T cell (CAR T) approaches, Ad-RTS-IL-12, TCR and NK cell-based therapies, and our other therapeutic products will be successfully marketed if approved; the strength and enforceability of our intellectual property rights; competition from other pharmaceutical and biotechnology companies; and the other risk factors contained in our periodic and interim SEC reports filed from time to time with the Securities and Exchange Commission, including but not limited to, our Annual Report on Form 10-K for the fiscal year ended December 31, 2015, and our Quarterly Report on Form 10-Q for the quarter ended September 30, 2015. Readers are cautioned not to place undue reliance on these forward-looking statements that speak only as of the date hereof, and we do not undertake any obligation to revise and disseminate forward-looking statements to reflect events or circumstances after the date hereof, or to reflect the occurrence of or non-occurrence of any events.
Some of technology described was advanced through research conducted at the MD Anderson Cancer Center by Laurence J.N. Cooper, M.D., Ph.D. On May 7, 2015, Dr. Cooper was appointed as the Chief Executive Officer at ZIOPHARM. Dr. Cooper is now a Visiting Scientist at MD Anderson.
Premise & Promise: 3\textsuperscript{rd} party (allogeneic) innate immune cells can be infused as an off-the-shelf therapy

• This lends itself to centralized manufacturing for distribution to multiple points-of-care
• Immune cells can be pre-prepared in advance of need
• Immune cells can be shipped to multiple centers
• Immune cells can be infused on demand rather than when available
• Undertake Phase IIb multi-center trials to establish efficacy
• Establish maximal tolerated dose from a well-described immune-cell product
• Undertake combination (immunotherapy) trials
Discuss two approaches for off-the-shelf immunotherapy

• T cells expressing $\gamma^\delta$TCR which do not lead allo-reactivity, yet maintain specificity through TCR and also introduced chimeric antigen receptor (CAR)

• NK cells do not express TCR, yet maintain specificity through endogenous inhibitory/activating receptors
Cellular templates propagated on activating and propagating cells (AaPC)

- NK cells
- \( \gamma \delta \) T cells
- K-562 cells (genetically modified)
- NKT cells
- \( T_{\text{reg}} \) cells
- \( T_{H17} \) cells
- \( \alpha \beta \) T cells
Genetically edited off-the-shelf T cells

- 3rd party T cells expressing $\alpha\beta$ TCR may cause intolerable graft-versus-host-disease (GvHD)
Eliminating TCR on CAR+ T cells

Sleeping Beauty
Transposon/transposase system

Intended response

CD19
HLAs
B-cell leukemia/lymphoma

Unwanted response
GVHD

TCRαβ
Zinc finger nuclease

Patient

Normal cells
HLAs

Blood. 2013 Aug 22;122(8):1341-9
Off-the-shelf $\text{TCR}^{\text{neg}}\text{CAR}^{\text{+}} \ \alpha\beta \ T \ cells$

3rd party (allogeneic) 
$\alpha\beta \ T \ cells$

Recipient

$\text{HLA} \ 1$

$\text{HLA} \ 2$

$\text{HLA} \ 3$

$\text{TCR}^{\text{neg}}\text{CAR}^{\text{+}} \ T \ cells$

pre-prepared

$\text{HLA} \ 4$
Eliminating HLA and TCR on CAR$^+$ T cells

Healthy donor T cells

Patient

TCR$\alpha$$\beta$

T cells

CD19

B cell leukemia/lymphoma

Normal cells

Recognize "non-self" (Patient $\rightarrow$ Donor)

Unwanted response

Rejection

Intended response

Recognize "non-self" (Donor $\rightarrow$ Patient)

Unwanted response

GVHD
OTS HLA A\textsuperscript{neg} TCR \textsuperscript{neg} CAR \textsuperscript{+} T cells

Blood. 2013 Aug 22;122(8):1341-9
Off-the-shelf $\text{HLA}^{\text{neg}}\text{TCR}^{\text{neg}}\text{CAR}^+$ $\alpha\beta$ T cells

3\textsuperscript{rd} party (allogeneic) $\alpha\beta$ T cells

HLA 1

$\text{HLA}^{\text{neg}}\text{TCR}^{\text{neg}}\text{CAR}^+$ T cells pre-prepared

Recipient

HLA 4
Cellular templates propagated on AaPC

- NK cells
- NKT cells
- TH17 cells
- T_reg cells
- aβ T cells
- γδ T cells

K-562 cells genetically modified
T cells differ in TCR heterodimer
Antigen recognition by TCRγδ

• Repertoire: 3 Vδ and 14 Vγ (humans)

➢ THEREFORE:

➢ Can target multiple ligands on the tumor surface
➢ Protect against infection in immunocompromised patients
➢ Could be used for viral or bacterial therapy

• Vδ1:
  • MICA/B (γ1)
  • Phycoerythrin (γ1)
  • Heat shock proteins (γ4)
  • CD1d (γ4)
  • EBV and CMV
  • Dendritic epidermal T cells (γ5)

• Vδ2:
  • Bacterial alkylamines and Listeria monocytogenes (γ2)
  • Phospho-antigens (γ9)
  • F1-ATPase and Apo-A1 (γ9)
  • Mycobacterium tuberculosisi (γ9)

• Vδ3:
  • CMV and HIV (γ3)
Clinical application of $\gamma\delta$ T cells

- Limited frequencies impede direct infusion of peripheral $\gamma\delta$ T cells
- Standard expansion protocols used for $\alpha\beta$ T cells are not applicable for $\gamma\delta$ T cells
- Bisphosphonates discovered to expand $V\delta 2$ cells
- No clinically relevant method to expand $V\delta 1$ or $V\delta 3$ cells
- $V\delta 1$
  - Correlated with durable CR following $\alpha\beta$ T cell-depleted hematopoietic stem-cell transplantation
  - Not directly infused into patients to date
- $V\delta 2$
  - Bisphosphonates, e.g., Zoledronic acid (Zometa), used to expand $V\gamma 9V\delta 2$ cells
    - ex vivo and/or in vivo
  - Objective clinical responses achieved in solid and hematological tumors
  - Durable CR were not achieved
- $V\delta 3$
  - Nothing known to date about their anti-tumor immunity
  - Not directly infused into patients to date
γδ T cells numerically expand on activating and propagating cells

Flow plots are representative of 10 normal donors
Data are mean ± SD (n = 4) pooled from two independent experiments

Dr. Drew Deniger
Propagated $\gamma\delta$ T cells maintain polyclonal TCR$\gamma\delta$ repertoire
$V\delta$ subsets differ in surface phenotype

Flow plots are representative of four normal donors; Data are mean ± SD (n = 4) Pooled from 2 independent experiments.
Vδ subsets lyse broad range tumor cells


Data are mean ± SD (n = 4)
Pooled from 2 independent experiments
Polyclonal $\gamma\delta$ T cells eliminate tumors \textit{in vivo}.

Representative images of BLI from groups at corresponding times; Data are mean ± SD (n = 6-9). Survival (n = 10); p-value and hazard ratio (H) calculated by Gehan-Breslow-Wilcoxon test.
Off-the-shelf $\gamma\delta$ T cells

3rd party (allogeneic)
$\gamma\delta$ T cells

Recipient

HLA 1

HLA 2

HLA 3

T cells
pre-prepared

HLA 4
Bi-specific CAR⁺ γδ T cells

CD19-specific CAR

Electroporation with Sleeping Beauty DNA plasmids

Transposon/Transposase

From peripheral blood mononuclear cells collected by steady-state apheresis

Mol Ther. 2013 Mar;21(3):638-47
CAR$^+$ γδ T cells Numerically Expand on activating and propagating cells (AaPC)

Flow plots are representative of four normal donors; Data are mean ± SD (n = 4) Student’s paired, 1-way t-test for statistical analysis

Mol Ther. 2013 Mar;21(3):638-47
Re-directed cytolysis of CD19+ tumors
Re-directed cytolysis of CD19+ tumors

Data are mean ± SD (n = 5) and are representative of 2 independent experiments; Flux images are at Day +22 Two-way ANOVA with Bonferroni’s post-test for BLI flux data, Student’s paired, 2-tailed t-tests for post-mortem analysis; **p<0.01, and ***p<0.001

Mol Ther. 2013 Mar;21(3):638-47
Off-the-shelf CAR$^+$ $\gamma\delta$ T cells

3rd party (allogeneic) $\gamma\delta$ T cells

Recipient

HLA 1

HLA 2

HLA 3

HLA 4

CAR$^+$ T cells pre-prepared
Cellular templates propagated on AaPC

- NK cells
- NKT cells
- TH17 cells
- T_{reg} cells
- αβ T cells
- γδ T cells
- K-562 cells

genetically modified
NK cells

- GvHD did not develop when allogeneic haploidentical and 3rd party NK cells were infused
  - Blood. 2007 Jul 1;110(1):433-40
  - Expert Opin Biol Ther. 2014 Jul;14(7):947-54
  - Abstract 929, ASH 57th 2015 meeting
  - Abstract 102, ASH 57th 2015 meeting
Natural Killer Cells: Beyond CAR<sup>+</sup> T cells

- Target tumors such as AML so do not require CAR
  - Killing is independent of a specific (known) target antigen
- Do not have T-cell receptor (TCR), so do not require genetic editing to eliminate TCR
  - May be used as an off-the-shelf therapeutic
- Build on promising proof-of-principle trials, *e.g.*, ongoing at MDACC infusing autologous and allogeneic NK cells
  - Manufactured using engineered AaPC to generate large numbers
Propagate NK cells on AaPC expressing tethered IL-21
Evaluate in a clinical trial

Off-the-shelf NK cells

- 3rd party donor identified as universal donors
- Propagate on AaPC
- Cryopreserve banks
- Infuse into medically-fragile patients with AML

Off-the-shelf NK cells
Off-the-shelf NK cells

Universal Donor #2
Peripheral blood
Co-culture on specialized feeder cells
Bank #2
Infusion product
NK cells
Defined
AML
Recipients (patients)
Infusion #2
NK cells
NK cells
NK cells
Off-the-shelf NK cells

Universal Donor #3
Peripheral blood
Co-culture on specialized feeder cells
Bank #3
Infusion product
NK cells

AML Recipients (patients)
Infusion #2

Defined
Off-the-shelf NK cells

3rd party (allogeneic) NK cells

Recipient

HLA 1

HLA 2

HLA 3

NK cells pre-prepared

CAR+ NK cells

3rd party (allogeneic) NK cells

Recipient

HLA 1

HLA 2

HLA 3

CAR+ NK cells pre-prepared

Recipient

HLA 4
Elimination of HLA-A increase the chances of matching OTS T cells with donor

Limited pool of universal donors matches with large number of recipients

Off-the-shelf HLA-matched NK cells

3rd party (allogeneic)
NK cells

Recipient

HLA A^{neg}
NK cells pre-prepared

3rd party (allogeneic)
NK cells

Recipient

HLA A^{neg}CAR+
NK cells pre-prepared
Summary

• The ability to propagate large numbers of $\gamma\delta$ T cells on AaPC while retaining effector function provides a pathway to infusing as OTS therapy
• The ability to propagate large numbers of NK cells on AaPC while retaining effector function provides a pathway to infusing as OTS therapy
• Expression of CAR to redirect effector function may improve potential as OTS therapeutic
• Sourcing cells from HLA-disparate donors and genetic editing may avoid immune-mediated rejection and improve therapeutic effect
Thank you