EXECUTIVE SUMMARY

- Chimeric Antigen Receptor (CAR) T cells = T cells genetically modified to express a CAR: target specific scFv + signaling components of CD3z + co-stimulatory domain

- Potential liabilities to be considered:
  - On-target on-cancer toxicity
  - On-target off-cancer toxicity
  - Off-target toxicity
  - Vector-related toxicity such as genotoxicity

- Key = good understanding of target expression and specificity
- In vivo toxicity studies are challenging
- Nonclinical safety assessment is mostly qualitative: little impact on human doses
CAR (CHIMERIC ANTIGEN RECEPTOR) T CELLS

First generation:
CD3ζ

Second generation:
CD3ζ + CD28 (or 41BB)

Third generation:
CD3ζ + CD28 +41BB or X,Y, Z, Kill switches, cytokines, chemokine receptors...

Fourth generation and Beyond:
Allogenic ‘off the shelf’ CART
OVERVIEW OF CAR-T THERAPY PROCESS
Nonclinical Safety Considerations for CART

**Potential liabilities to be considered**
- On-target on-cancer toxicity (cytokine release syndrome, CRS; neurotoxicity)
  - Expected and may be impacted by disease burden
  - Severity not necessarily predicted by nonclinical studies
- On-target off-cancer toxicity (driven by target distribution)
- Off-target toxicity (driven by specificity)
- Vector genomic integration (genotoxicity)

**Key elements of nonclinical safety assessment**
- Target expression (cancer cells and normal cells)
- scFv / CAR-T target specificity
- Impact on normal tissues/cells: in vitro cytotoxicity assays complemented by in vivo studies when appropriate
- Vector attributes (modifications to mitigate putative liabilities)
ON-TARGET ON-CANCER TOXICITY: CRS

- Maximum fold change in IL-6:
  - No CRS, grade 1 or 2
  - Grade 3 or 4
  - p = 0.0002

- Maximum fold change in IFNγ:
  - No CRS, grade 1 or 2
  - Grade 3 or 4
  - p = 0.0002

- Spearman:
  - r = 0.81
  - p = 0.0001

- Maximum IL-6 (pg/mL):
  - vs. Maximum CRP (mg/L)
  - p = 0.0001

- Maximum CRP (mg/L):
  - No CRS, grade 1 or 2
  - Grade 3 or 4
  - p = 0.0015

Sources:
- AMGEN
CRS: IMPACT OF DISEASE BURDEN

![Graph showing the impact of disease burden on marrow blasts at enrollment.](image)

- **Y-axis**: Marrow blasts at enrollment (% of mononuclear cells)
- **X-axis**: No CRS, grade 1 or 2 vs. Grade 3 or 4
- **p-value**: 0.039

The graph illustrates the relationship between disease burden and marrow blasts at enrollment, indicating a statistically significant difference between the two groups.
NEUROTOXICITY

- CD19 CARTs: tumor burden, high CART dose, CRS, and preexisting neurologic comorbidities = increased risk of neurologic AEs
ON-TARGET OFF-TUMOR EFFECTS: DEPLETION OF TARGET EXPRESSING NORMAL CELLS

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use YESCARTA safely and effectively. See full prescribing information for YESCARTA.

5.7 Hypogammaglobulinemia

B-cell aplasia and hypogammaglobulinemia can occur in patients receiving treatment with YESCARTA. In Study 1, hypogammaglobulinemia occurred in 15% of patients. Monitor immunoglobulin levels after treatment with YESCARTA and manage using infection precautions, antibiotic prophylaxis and immunoglobulin replacement.
## GENE AND TARGET EXPRESSION ANALYSIS

### Gene model analysis
- Orthologs
- Splice variants
- Annotation accuracy
- Protein variant alleles
- Oncogenic mutations

### Target expression analysis
- RNASEq
- qPCR
- ISH
- IHC
- WB
- MS
**Example of Body Map Expression**

All tissues with FPKQ >= 5 in at least one sample (sorted by median human expression)

- Very high expression in certain immune cells and bone marrow cells.
- Some low expression signals in other tissues.
- Some data available in other species (not as complete data set)

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<th>Tissue Type</th>
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<th>Rat</th>
<th>Mouse</th>
<th>Cyto</th>
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- Heatmap color scale: 0 to 512+
IMMUNOHISTOCHEMISTRY

Cyno cerebellum

Human cerebellum

Punctate cytoplasmic staining
IN VIVO STUDIES – NONHUMAN PRIMATE MODEL(S)

Safety of Targeting ROR1 in Primates with Chimeric Antigen Receptor-Modified T Cells
Carolina Berger12, Daniel Sommermeyer1, Michael Hudecek3, Michael Berger1, Ashwini Balakrishnan1, Paulina J. Paszkiewicz4,5, Paula L. Kosasih1, Christoph Rader6,7, and Stanley R. Riddell1,2,5
Cancer Immunol Res; 3(2) February 2015

Creation of the First Non-Human Primate Model That Faithfully Recapitulates Chimeric Antigen Receptor (CAR) T Cell–Mediated Cytokine Release Syndrome (CRS) and Neurologic Toxicity Following B Cell–Directed CAR–T Cell Therapy
Agne Taraseviciute, Leslie Kean, and Michael C Jensen
Blood 2016 128:651;
Absolute numbers of GFP T cell (n=1) and CD20 CAR T cell (n=3) expansion and persistence in rhesus macaques (top graph).

**Absolute # GFP+ or CD20+ Cells**

- Recipient 1 GFP
- Recipient 1 CD20 CAR
- Recipient 2 CD20 CAR
- Recipient 3 CD20 CAR

**Absolute # B cells**

Agne Taraseviciute et al. Blood 2016;128:651
AMGEN’S EXPERIENCE: OVERVIEW OF STUDY STEPS

1: Collection of blood (9.5 mL/kg)
   Isolation and freezing of PBMCs (50-90 million cells)

2: Activation / transduction / expansion

3: PBMCs characterization (flow)
   Functional testing
   Formulation, sterility testing and freezing

4: non-myeloablative lymphodepletion

5: CAR-T IV infusion

6: Monitoring (safety, PD, cell persistence) followed by necropsy

1: Collection of blood (9.5 mL/kg)
   Isolation and freezing of PBMCs (50-90 million cells)
IN VIVO LYMPHODEPLETING CY/FLU REGIMEN: EFFECTS SIMILAR TO THOSE OBSERVED IN HUMANS

<table>
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<tr>
<th></th>
<th>IL-15 pg/mL (fold change)</th>
<th>MCP-1 pg/mL (fold change)</th>
<th>Perforin pg/mL (fold change)</th>
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<td>Animal #1 baseline</td>
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<td>CART day (predose)</td>
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<td>CART day (8 hrs)</td>
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<tr>
<td>CART day (8 hrs)</td>
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</tbody>
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GOOD TRANSDUCTION OF CYNOMOLGUS MONKEY T CELLS WITH RETROVIRAL VECTOR

- 53.25 to 73.90% CAR positive cells
- Similar transduction efficiency as compared to human cells transduced with a lentiviral vector
The NHP CAR-T cells generated in this study had specific cytotoxicity.
TEST ARTICLE – DOSE LEVELS

<table>
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<th>Animal #</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Dose CAR+/kg</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Dose total cells/kg</td>
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<td></td>
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</tr>
<tr>
<td>Transduction %</td>
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</tr>
</tbody>
</table>

- Formulation: Frozen suspension of T cells in 5% DMSO and 2.5% human albumin
- IV Dosing volume: 21 mL administered over 30 minutes
- No adjustment for a specific CD4:CD8 ratio
- Reference: Approved CD19 CART clinical dose (YESCARTA) = 2x10^6 CAR+/kg; max 2x10^8 CARTs
OUTCOME OF THE STUDY AND LESSONS LEARNED

- No evidence of expansion/persistence of the CARTs
- No toxicity
  - Transduction, formulation, dosing are technically feasible in the cynomolgus model
  - Preconditioning dosing regimen was optimized
  - Critical components that may impact value of a nonhuman primate model
    - CAR-T functional status
    - Level of target expression in healthy animals
  - Relying on nonhuman primate studies is challenging and other approaches are necessary
A POSSIBLE ALTERNATIVE DE-RISKING STRATEGY: USING CD3-BISPECIFIC MOLECULES
ADVANTAGES AND CAVEATS OF CD3 BISPECIFIC AS SURROGATES

• PROS
  – Different modality but similar MOA: leveraging T-cell mediated cytotoxicity
  – Exposure can be controlled and maintained for a period of time

• CONS
  – Potency may differ
  – Biodistribution may differ
IN VITRO ASSAYS CAN BE CONDUCTED TO FURTHER EVALUATE RISK OF CYTOTOXICITY AGAINST NORMAL HUMAN CELLS

- This assessment can be performed using primary human cells, induced pluripotent stem cell-derived models, or established cell lines.
- Should include cells where a target expression signal has been detected.
- Can include cells with no known expression of the target of interest as a way to document specificity.
- Can also include cells overexpressing related proteins to further ascertain specificity.
IN VITRO CYTOTOXICITY - EXAMPLE

Edited
CAVEATS OF IN VITRO CYTOTOXICITY ASSAYS

- Procurement of certain cell types can be challenging, in particular if a very specific regional organ/tissue origin is desired
- Perfect replication of target expression in intact tissue can be a challenge
- A prioritization of cell types to be tested is necessary
CONCLUSIONS

• The nonclinical safety assessment of CARTs is presenting unique challenges

• The understanding of target expression in normal tissues is pivotal

• The combination of various in vivo and in vitro studies, when feasible, contributes to the assessment despite known limitations
ACKNOWLEDGEMENTS