

Adoptive transfer of T cells genetically modified using the *Sleeping Beauty* system

Laurence J.N. Cooper

Adoptive Transfer session Saturday, October 31st 9:45 AM to 10:15 AM 24th iSBTc

Immunotherapy options for B-lineage (CD19⁺) ALL and lymphoma

- T-cell therapy
- NK-cell therapy.
- Antibody therapy
- Immunocytokines
- Vaccination -

Tumor-specific T cells

- T cells that recognize tumor-associated antigen (TAA) through introduced chimeric antigen receptor (CAR) independent of MHC
- T cells that recognize TAA though endogenous $\alpha\beta$ T-cell receptor (TCR) in context of MHC

Clinical studies using CAR⁺ T cells

| Target | CAR | Major | References |
|----------------------|-----------------------------|----------|---|
| | specificity | toxicity | |
| HIV | CD4 | None | Walker RE, Bechtel CM, Natarajan V, et al. Long-term in vivo survival of receptor-modified syngeneic T cells in patients with human immunodeficiency virus infection. Blood. 2000;96:467-474. |
| HIV | CD4 | None | Mitsuyasu RT, Anton PA, Deeks SG, et al. Prolonged survival and tissue trafficking following adoptive transfer of CD4zeta gene-modified autologous CD4(+) and CD8(+) T cells in human immunodeficiency virus-infected subjects. Blood. 2000;96:785-793. |
| Renal cell cancer | Carbonic anhydrase IX | Liver | Lamers CH, Sleijfer S, Vulto AG, et al. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. J Clin Oncol. 2006;24:220 222 |
| Ovarian cancer | α-folate receptor | None | Kershaw MH, Westwood JA, Parker LL, et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res. 2006;12:6106-6115. |
| Neuroblastoma | CE7R | None | Park JR, DiGiusto DL, Slovak M, et al. Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. Mol Ther. 2007;15:825-833. |
| Lymphoma | CD19 | None | Jensen MC, Popplewell L, DiGiusto DL, et al. A First-in-Human Clinical Trial of Adoptive Therapy Using CD19-Specific Chimeric Antigen Receptor Re-Directed T Cells for Recurrent/Refractory Follicular Lymphoma ASGT Seattle 2007. [abstract] |
| CLL | CD19 | None | Brentjens RJ, Hollyman D, Weiss M, et al. A phase I trial for the treatment of purine analog-refractory chronic lymphocytic leukemia using autologous T cells genetically targeted to the B cell specific antigen CD19. 2008 ASCO Annual Meeting Proceedings. [abstract] |
| Lymphoma | CD20 | None | Till BG, Jensen MC, Wang J, et al. Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. Blood. 2008;112:2261-22671. |
| Neuroblastoma | GD ₂ | None | Pule MA, Savoldo B, Myers GD, et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nat Med. 2008;14:1264-1270. |
| Glioblastoma | IL-13 receptor | None | Yaghoubi SS, Jensen MC, Satyamurthy N, et al. Noninvasive detection of therapeutic cytolytic T cells with (18)F-FHBG PET in a patient with glioma. Nat Clin Pract Oncol. 2008 Nov 18. [Epub ahead of print] |

Rationale

Targeting CD19 determinant on B cells

- CD19 antigen is a 95 kDa B lineage-specific membrane glycoprotein, found on >95% of B-cell COOH lymphomas and B-ALL cells;
- CD19 is rarely lost during the process of neoplastic transformation, but disappears upon differentiation to mature plasma cells;
- CD19 is not expressed on hematopoietic stem cells, nor on normal tissues outside the B lineage;
- CD19 is not shed into the circulation.



 NH_2

Improve T-cell therapeutic potential Improve persistence

- Proliferative potential
- CAR
 - 1st generation
 - 2nd generation
 - 3rd generation
- Improve culturing environment
 - aAPC
 - Cytokines
- Type of T-cell
 - Memory
 - Naïve

Most clinically-effective T-cell therapies includes *ex vivo* antigendependent proliferation

 Therefore we developed culture systems ex vivo that select for T cells that can sustain CAR-dependent proliferation in vivo

Experimental design Programming the µenvironment Numerically expand T cells under polarizing conditions





Sleeping Beauty Transposition

Transposon (Donor) sequences flanked by inverted repeats are integrated into genome



Numeric expansion of CAR⁺ T cells on aAPC



Relative efficiency of stable gene expression by SB system

| DNA | | Day 0 | | Day 28 | | |
|---|----------|----------|------------|----------|----------|------------|
| piasmid | CD4+CAR+ | CD8+CAR+ | Total CAR+ | CD4+CAR+ | CD8+CAR+ | Total CAR⁺ |
| No DNA | 0.63 | 0.18 | 1.61 | | | |
| SB _{ase} | 0.96 | 0.75 | 1.16 | | | |
| SB _{on} | 15.9 | 9.5 | 27.0 | 0.36 | 0.36 (| 0.6 |
| SB _{on} & SB _{ase} | 13.29 | 7.87 | 22.0 | 16.98 | 25.89 | 43.0 |

71 fold

SB T-cell data

CD19-dependent cytotoxicity



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CD19-specific CARs



Relative in vivo T-cell persistence



Relative anti-tumor effect



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Which T-cell sub-population to genetically modify?

Research article

Adoptive transfer of effector CD8⁺ T cells derived from central memory cells establishes persistent T cell memory in primates

Carolina Barger ¹ Michael L. Jersen, ¹ Pater M. Lanetter, ¹ Mice Denge, ¹ Carole Ellerit, ² and Darries B. Holdel²

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Adoptively transferred effector cells derived from naïve rather than central memory CD8⁺ T cells

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Outgrowth of CAR⁺ T cells with memory cell phenotype



Safety of transposition

Translation to clinical trials

Applied Cellular Therapy (ACT)



Translational pipeline



Schematic



Trial Principal Investigator = Dr. Partow Kebriaei design Time Line for IRB # 2007-0635 Infusion of PBSC Enrollment Day 0 Leukapheresis #1 Leukapheresis #2 (Steadystate, to obtain PBMC (To obtain G-CSF mobilized 28 day post-T-cell infusion monitoring to manufacture T cells) PBSC, approximate time point) Week 1 Week 2 Week 3 Week4 Week 5 Week 6 Whek 7 Week 9 Week 11 W Week 10 Interval therapy Indeterminate time interval; Conditioning therapy Day -7 to Day -1 but least 49 days are anticipated to be needed to manufacture and release CD19-specific autologous T cells Window for infusion of T cells Day +2 to +7 Conditioning therapy Daily SQ IL-2, if eligible. Day -7 to Day -1 Ideally, starting day of, but after, T-cell infusion

Day-7

Admit Hydration Day-6

Carmustine

Day-5

Day-4

Etoposide Cytarabine Day-3

Day-2

Day-1

Melphalan

Day 0

Day+1

Rituoimab

Day +2 to Day +16 (assuming T cells infused day +2)

Day+14

Rituoimab

T-cell trials at MDACC

| Trial | Agent | Preclinical | Phase I |
|---------------------|---|---------------|---------|
| Lymphoma | Autologous CD19- specific T cells | \rightarrow | Х |
| ALL and lymphoma | Allogeneic CD19-specific T cells | \rightarrow | Х |
| ALL and lymphoma | Allogeneic CD19-specific UCB-derived T cells | \rightarrow | Х |

NK-cell trials at MDACC

| Trial | Agent | Preclinical | Phase I | Phase II |
|---------------|---|---------------|---------------|----------|
| Neuroblastoma | Haploidentical NK cells | \rightarrow | Х | |
| ALL | Haploidentical NK cells and Epratuzumab | \rightarrow | Х | |
| AML | Haploidentical NK cells | \rightarrow | \rightarrow | Х |

Future developments

- Altering the host environment to facilitate persistence and function of transferred T cells
- Altering intrinsic properties of T cells that are selected or engineered for therapy

Lessons and Take Home Messages

• Key points

- SB system can be used to genetically modify human T cells

• Potential impact on the field

- SB system can be adapted for human application

- Lessons learned
 - Genetically modified T cells can be generated with improved persistence and therapeutic potential

THANKS!

