Adoptive transfer strategies: impacting Tregs and vaccines

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Overview: Adoptive T Cell Therapy

- **Effector T cells**: schedule nad combination dependent effects
- **Adoptive transfer of Tregs**
General Approaches for Adoptive T Cell Therapy

A.

Harvest PBMC by apheresis

T cell in vitro activation and expansion

B.

TIL cell isolation

Cancer patient

Lymphodepleted cancer patient

± HSC

Host condition chemotherapy ± radiotherapy

TIL cell in vitro activation and expansion

PB T cell transfer

TIL cell transfer

J Clin Invest 2007 117:1466-76
Cell Culture Approaches for Adoptive T Cell Therapy

Starting T cell repertoire

TILs or PBMCs

Antigen-specific stimulation

Antigen and APC

Antigen and APC

Polyclonal stimulation

Cyclic stimulation with CD3- and CD28-specific antibodies

7-10 d

Functional development
Treg cell depletion
Genetic modification
T cell selection and/or expansion in the host

Time

6 w
T Cell Expansion in Lymphopenic Hosts
Enhanced CD8 Effector Function

Potential mechanisms:
- Role of lymphopenia
- Depletion of Tregs, NKT, B cells?
- Removal of cytokine sinks?
  IL-2 vs IL-7/-15/-21 regulation

Day 12 p HSC

Day 2 p HSC

Wrzesinski et al, J Clin Invest 2007;117:492
Multiple Myeloma

- Plasma cell neoplasm characterized by serum monoclonal Ab, osteolytic lesions, pathological fractures, anemia, hypercalcemia
- 15% of hematologic malignancies
- Autologous transplants are highly effective for tumor reduction (first line therapy), but cures are infrequent.
- GVM/GVT: Allogeneic transplants can induce cures, but treatment-related risks are high.
Adoptive transfer of vaccine primed T cells augments immunity in lymphodepleted hosts: Summary of first trial

- First successful randomized multicenter adoptive immunotherapy trial
- Accelerated recovery of CD4 and CD8 counts to normal levels by day 42 (P=0.004)
- Protective antibody levels established by day 30
- Improved proliferative capacity of CD4 T cells to vaccine carrier antigen (P<0.01) and to Staphylococcal enterotoxin B (P=0.004)

=> Adoptive transfer of vaccine primed T cells appears to facilitate establishment of CD4 T central memory cells


But what about tolerance?
Phase I/II Combination Immunotherapy after ASCT for Advanced Myeloma of hTERT/Survivin Vaccination Followed by Adoptive Transfer of Vaccine-Primed Autologous T cells
Phase I/II Combination Immunotherapy after ASCT for Advanced Myeloma of hTERT/Survivin Vaccination Followed by Adoptive Transfer of Vaccine-Primed Autologous T cells

PIs: Aaron Rapoport, U Maryland
    Edward Stadtmauer, U Pennsylvania

INDs:
    Vaccine (Vonderheide)
    T cells (June)

Design: Randomized (biologic) comparison
    1) Autologous T cells day 2 post ASCT
    2) Vaccine + vaccine primed T cells

Status:
    Protocol open to accrual
    18 patients enrolled
Myeloma Trial #2 Protocol Flow

Myeloma → Myeloma Cell Storage → Pre-Transplant Therapy → Biologic "randomization" → Study Enrollment → HLA-A2 STATUS

A2+ → Arm A
A2- → Arm B
Mobilization
Stem Cell Collection
High-dose Melphalan
Stem Cell Transplant

Immune Assessment Studies at Day 60, 100, and 180

T Cell Infusion- Day +2

T Cell In Vitro Activation and Expansion to Infuse $10^{10}$ Cells

TERT, Survivin, CMV, PCV

HLA-A2+ (Arm A)

PCV boosters at Day +14, 42, and 90

TERT, Survivin, CMV + PCV boosters at Day +14, 42, and 90

HLA-A2- (Arm B)

T Cell Collection

Mobilization
Stem Cell Collection
High-dose Melphalan
Stem Cell Transplant

T Cell Infusion- Day +2

PCV boosters at Day +14, 42, and 90

PCV

HLA-A2- (Arm B)

TERT, Survivin, CMV

Study Day

- 100

- 90

- 80

- 70

- 60

- 50

- 40

- 30

- 20

- 10

0

10

20

30

40

50
## T-cell Recovery - Myeloma Trial #2

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T Cell Leukocytosis Post Day 2 Adoptive Transfer

- Schedule dependent effects of costimulated T cell infusion
  - Prolonged T cell leukocytosis in patients after day 2 T cell infusion
  - Rapid normalization of T cell counts with homeostasis after day 12 T cell infusion
**CD4/CD8 T-cell Recovery – Comparison to Previous Adult Myeloma Trial**

**CD4 Recovery**
- Day +2 (RED) – Current Study
- Day +12 (BLUE) – Prior Study
- Day +100 (GREEN) – Prior Study

**CD8 Recovery**
- Day +2 (RED) – Current Study
- Day +12 (BLUE) – Prior Study
- Day +100 (GREEN) – Prior Study
Vaccine + day 2 T cell boost trial: Myeloma Interim Summary

- Safety to date: no HSC engraftment issues
- Clinical responses promising
- Unexpected:
  - Lymphocytosis: sustained in many patients
  - T cell engraftment syndrome in 6 patients (skin rash, fever, diarrhea)
- Above implies major schedule dependent (day 2 vs day 12) difference in T cell engraftment and effector functions
T Cell Engraftment Syndrome and auto-GVHD with day 2 autologous T cells

- T cell engraftment syndrome: onset by day 14 with rash, diarrhea, fever (n=6).
- Steroid responsive (n=3).

- Day 13: N/V/Diarrhea, T=38
- Day 14: 800cc stool, T=37.6
- Day 15: 1300cc stool, T=38
- Day 16: 900cc stool, T=38.1
- Day 17: 500cc stool, T=38.1
- Day 18: 300cc stool, T=37.7
- Day 20: no diarrhea/fever
Schedule Dependent Effects of T cell transfer on CD8 count

CD8+ cells/μl

- No T cells
- T cells D+12
- T cells D+2
- SCT
- D+30
- D+60
- D+90

CD8 count varies with time and treatment.
“Engraftment Syndrome”

- GVHD-like features with or without fever
- Not seen in pts receiving d+12 or d+90 T cells
- No delay in hematopoietic recovery after Day +2 transfers of costimulated T-cells
- T cell recovery is accelerated compared to randomized controls and is schedule dependent (day +2 vs day +12)
- T cell recovery shows sustained levels above normal, suggesting that early recovery may not be subject to normal homeostatic mechanisms
Issues To Be Addressed: T Cell Leukocytosis And Engraftment Syndrome

- Schedule dependent immune reconstitution, toxicity and/or anti-self/tumor effects. Is this a good thing?
- Why does it occur with day 2 and not post day 12 infusions?
- Potential mechanisms
  - r/o trivial (microchimerism with allo)
  - Homeostatic cytokine milieu day 2 vs day 12
  - Treg depletion or Th17 generation on day 2?
Treg Tolerance Mechanisms

- Subsets: nTregs and iTregs
- Act to limit effector response to self-antigens by blocking cytokines and proliferation
- FoxP3 required for Treg function
  - Mouse deficiency: Scurfy
  - Human deficiency: IPEX (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked inheritance)
- Cancer: acquired gain of function and number of Tregs

Sakaguchi, Sci American 2006
Use of Adoptive T Cell Immunotherapy To Tip the Balance of Teff and Tregs in vivo

**Treg depletion**
Adoptive transfer of T cells depleted of Tregs might increase effector T cell function in vivo
Potential use for vaccine adjuvant and cancer patients
Safety profile: unknown risk of autoimmunity

**Treg augmentation**
Adoptive transfer of Tregs might induce immunosuppression or tolerance
Potential uses for GVHD, autoimmunity and organ transplantation
Safety profile: unknown risk of immunosuppression
Potential Forms of Adoptive Cellular Immunotherapy with Tregs

- Transferred Cells
  - Polyclonal donor Tregs
  - Cord blood Tregs
  - Alloantigen specific Tregs
  - Autoantigen specific Tregs
  - Gene-modified Tregs

Indications
- Graft versus Host Disease
- Cord blood HSC
- Solid organ transplantation
- Autoimmunity
- Infertility
- Various
Activated and Expanded CD4⁺CD25⁺ Cells Can be Used to Treat Lethally Irradiated Recipients of Full MHC Mismatched Donor Grafts

**Donor T cells expand 50-100 fold by d.5/6 post-BMT**

Ex Vivo Expanded Mouse Tregs for GVHD Treatment

Taylor, et al.
Blood. 2002;99:3493

Hoffman, et al.
Development of Human Treg GMP Compliant Culture Systems

CD3/28 Bead aAPC or KT32/4.1BBL aAPC

* Note difference in scale
Development of an in vivo model to test expanded human Treg cell function

CD4⁺CD25⁺ were transduced with GFP lentiviral vector and expanded by KT86 aAPCs and rapamycin for 21 days.

Analysis:
1. In vitro suppression assay and phenotype
2. Weight and visual inspection for GVDH.
3. Ratio of GFP to non-GFP positive CD4 T cells
4. T cell infiltration into lung and liver
Ex Vivo Expanded Human Treg Prevent Lethal Xeno-GVHD in NOG mice

NOG mice (8 wks) were injected IP with 10 million PBMCs and 2 million expanded nTregs (6 mice per group).
• Human Tregs prevent xeno-GVHD immunopathology in NOD/\(\gamma\)C-/- mice

• GMP compliant cell culture systems permit efficient ex vivo expansion of polyclonal CD4+CD25+FoxP3+ nTregs.

• These cells are currently in phase I clinical trials at the University of Minnesota.
Lessons Learned: Effector T Cell Transfers

• Schedule dependent effects uncovered “engraftment syndrome” with autologous T cells
  – Subset of patients develop a T cell “engraftment syndrome” with features of GVHD
  – Relationship to chemotherapy
  – Host lymphopenia

• Combination dependent effects (neuroblastoma trial)
  – Cluster of Transplant Associated Microangiopathy (TAM)
  – Associated with irradiation, isotretinoin, and T cell infusions

• Pre-clinical models in mice are poorly predictive for the above
Case Studies: Lessons and Issues

• Key Strategic Decisions
  – Gene therapy or not?

• Impact of Regulatory Interactions
  FDA and NIH/RAC very helpful
  Redundancy and poor harmonization of reporting requirements

• Financial Considerations: Projected Costs vs. Reality
  – Academic development:
    • Advantages, can take on longer term projects and are less risk adverse than small biotech
    • Disadvantages: resource constrained. No grant budget can support a cell based therapy trial

• Lessons Learned
  – Teamwork required
  – Environment is critical
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