Adoptive Immunotherapy of Cancer with Polyclonal Hyperexpanded CD4^+ and CD8^+ Tumor-sensitized T cells.

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Theoretical Advantages of Adoptive Immunotherapy *versus* Active Immunotherapy

1. Sequester T cells from immunosuppressive factors produced by the tumor (TGF-β, IL-10).
2. Isolate T cell subsets based on phenotype.
3. Optimize T cell proliferation to amplify the immune response.
4. Modify T cells (gene transfer, cytokine exposure)
5. Condition the host/tumor without detrimental effects on T cells.
Features of the Preclinical Model

*In vivo* Source of Sensitized T cells

1. Naïve hosts are inoculated with weakly immunogenic MCA205 which grows progressively.
2. Draining LNs are highly enriched for sensitized T cells.
3. Upon antigen sensitization, T cells downregulate CD62L.
4. Freshly isolated CD62L$^{low}$ cells are functionally defective.
5. Antigen-independent *in vitro* stimulation with anti—CD3 confers effector function. (CD28 stimulation not required)
6. T cells respond to tumor-specific antigens, traffic to tumor in all anatomic sites and establish a memory response.
Hypothesis

• Tumor sensitized T cells in draining LN with phenotype CD62L\textsuperscript{low} can be purified by MACS, additional isolation of CD4\textsuperscript{+} and CD8\textsuperscript{+} subsets.
• Repetitive anti-CD3 stimulation can lead to extensive polyclonal proliferation with retention of effector function.
• Optimal conditions for proliferation differ between CD4 and CD8 T cells.
A Day 0 TDLN            Day 0 CD62L\text{low}            Day 0 CD62L\text{low}

Days of culture
0 5 10 15 20 25 30 35
Fold proliferation
1
10
100
1000
10000
IL-2
IL-2+IL-7

Days following tumor inoculation
0 5 10 15 20 25 60
Tumor Size (mm²)
0
50
100
150
200
250
HBSS
IL-2, 5 \times 10^6 cells
IL-2+IL-7, 5 \times 10^6 cells

B Proliferation following anti-CD3 stimulation

C Therapy of 3-day s.c.tumor

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**A**

Day 0 TDLN

Day 0 CD62L\text{low}

Day 0 CD62L\text{low}

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**B**

Proliferation following anti-CD3 stimulation

**C**

Therapy of 3-day s.c.tumor
Can we restimulate with anti-CD3 and retain effector function?
A  Anti-CD3 restimulation day 14

B  Day 23 FACS

C  Therapy of 10-day pulmonary tumors

D  Therapy of 3-day s.c. tumors
Does hyperexpansion of T cells through repetitive anti-CD3 abrogate effector function?
A  CD8 T cell proliferation

B  Therapy of 3-day pulmonary tumors

C  Therapy of 3-day s.c. tumors

D  Therapy of 3-day i.c. tumors
Is it possible to hyperexpand CD4 and retain effector function?
A  CD4 T-cell Proliferation

B  Therapy of 3-day s.c. tumor

C  Therapy of 3-day i.c. tumor

D  Therapy of 3-day s.c. tumor with CD4 or CD8
TCR V$\beta$ phenotype of CD62$^{\text{low}}$ subset is similar to total LN T cells.

TCR V$\beta$ phenotype of $10^8$-fold hyperexpanded T cells is similar to starting population.

TCR spectratype analysis does not demonstrate oligoclonality.

*In vitro* functional analysis by intracellular IFN-$\gamma$ assay shows persistence of function in hyperexpanded cells.
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