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.

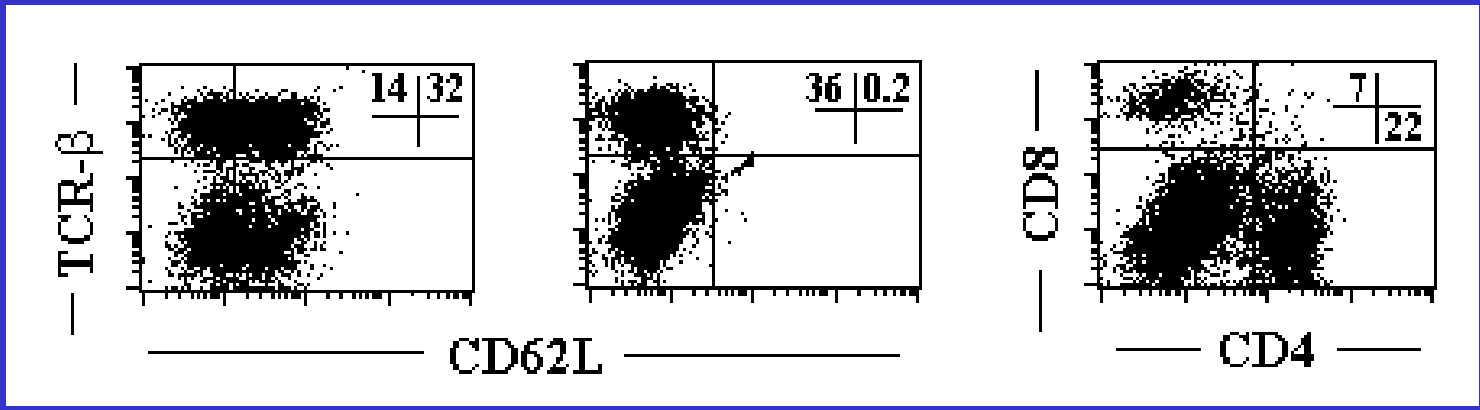
Features of the Preclinical Model
In vivo Source of Sensitized T cells

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.

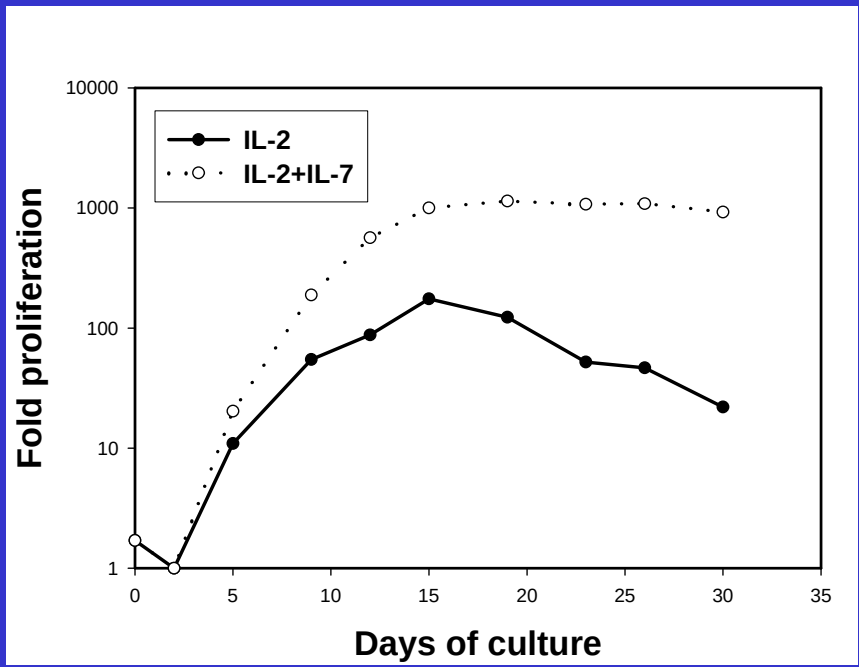
Hypothesis

- Tumor sensitized T cells in draining LN with phenotype $CD62L^{low}$ can be purified by MACS, additional isolation of $CD4^{+}$ and $CD8^{+}$ 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.

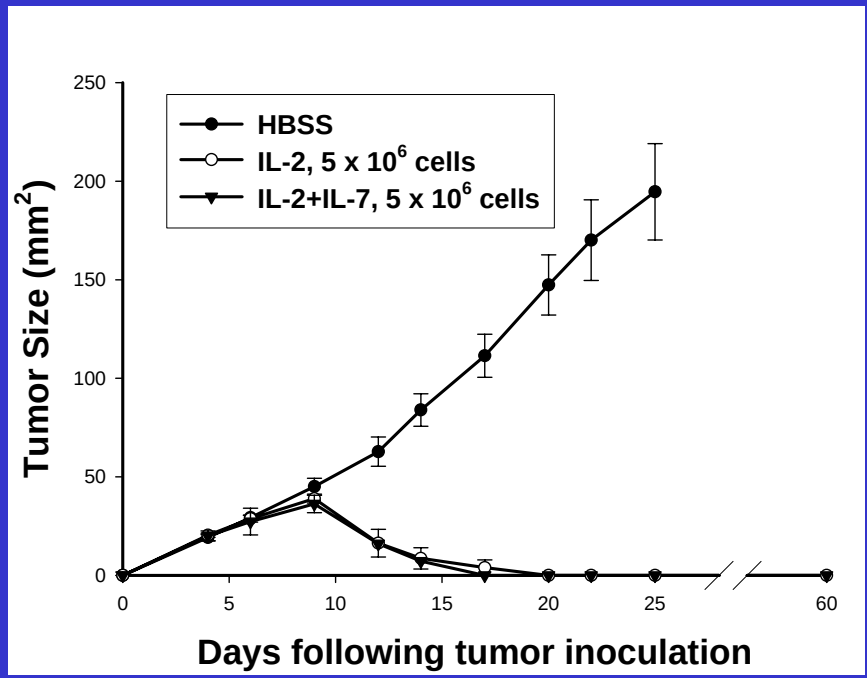
A Day 0 TDLN | Day 0 CD62L^{low} | Day 0 CD62L^{low}



B Proliferation following anti-CD3 stimulation

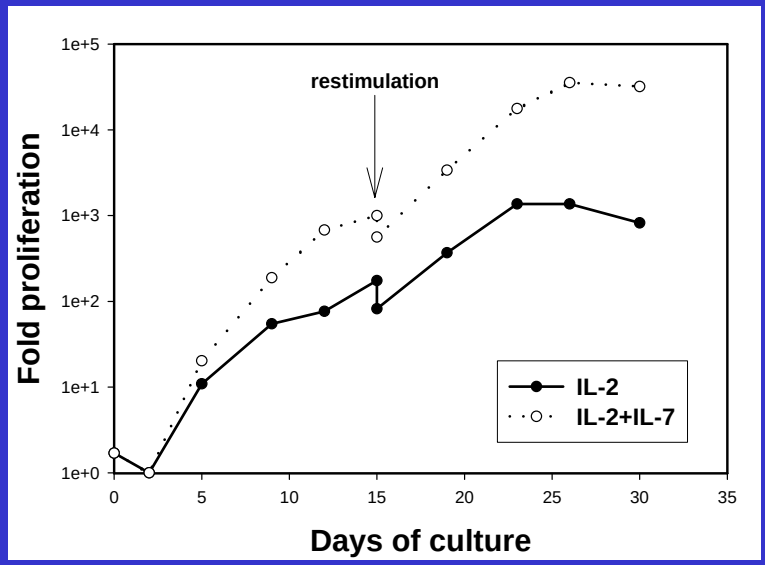


C Therapy of 3-day s.c.tumor

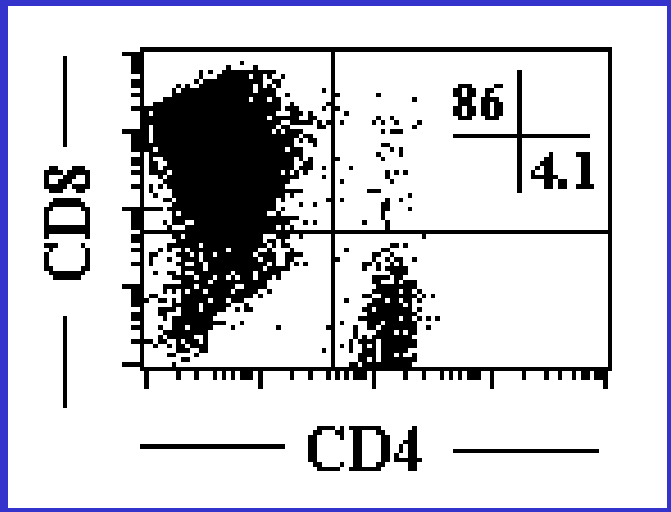


Can we restimulate with anti-CD3 and retain effector function?

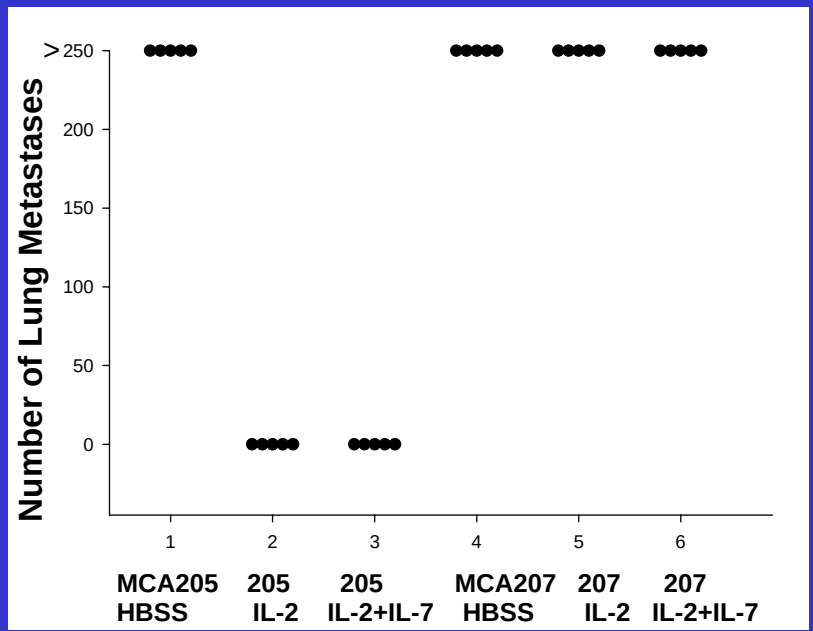
A Anti-CD3 restimulation day 14



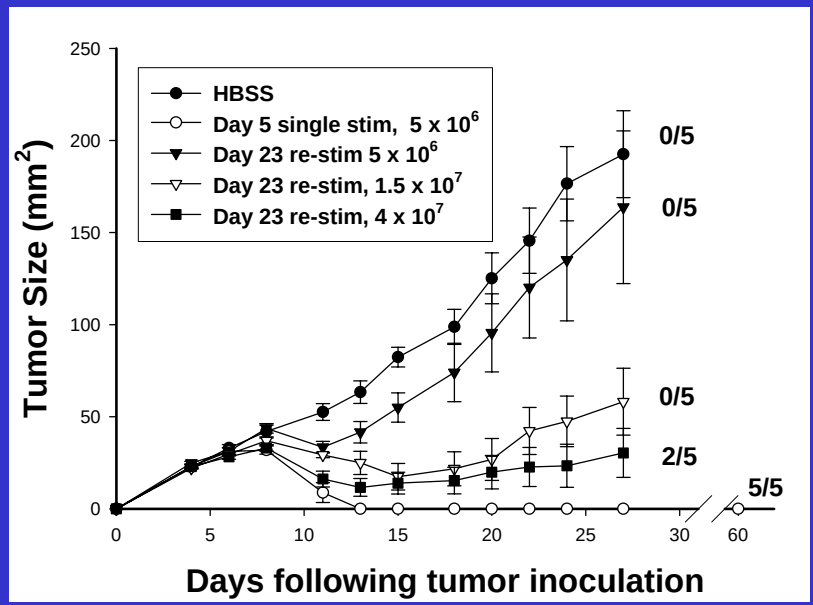
B Day 23 FACS



C Therapy of 10-day pulmonary tumors

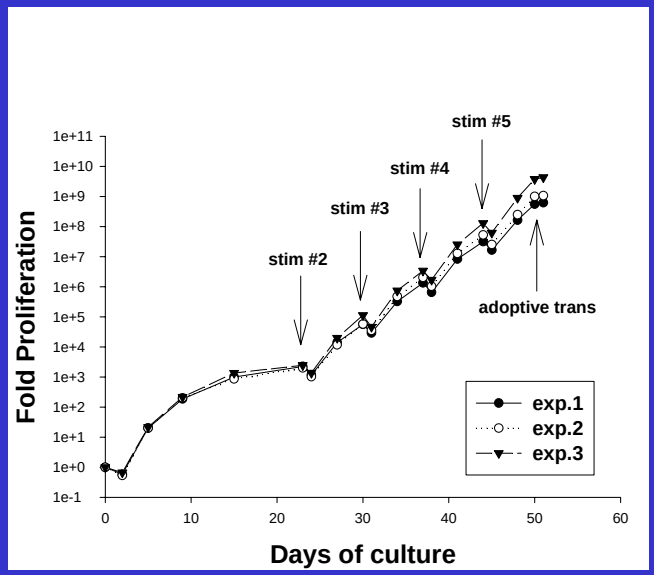


D Therapy of 3-day s.c. tumors

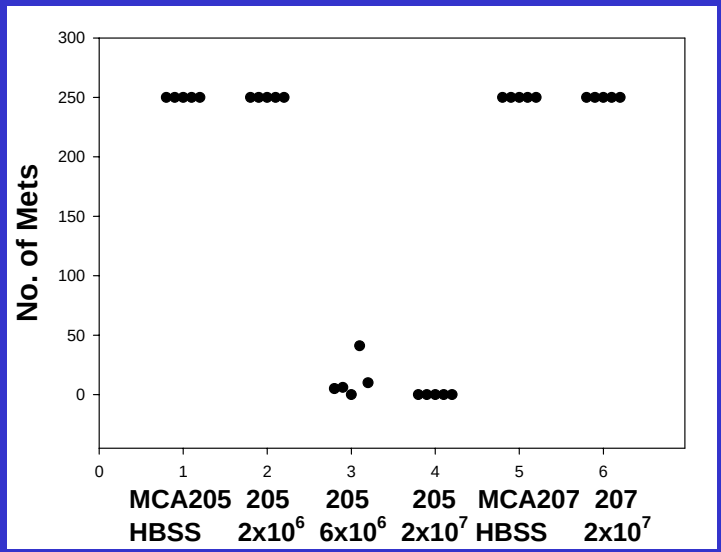


Does hyperexpansion of T cells through repetitive anti-CD3 abrogate effector function?

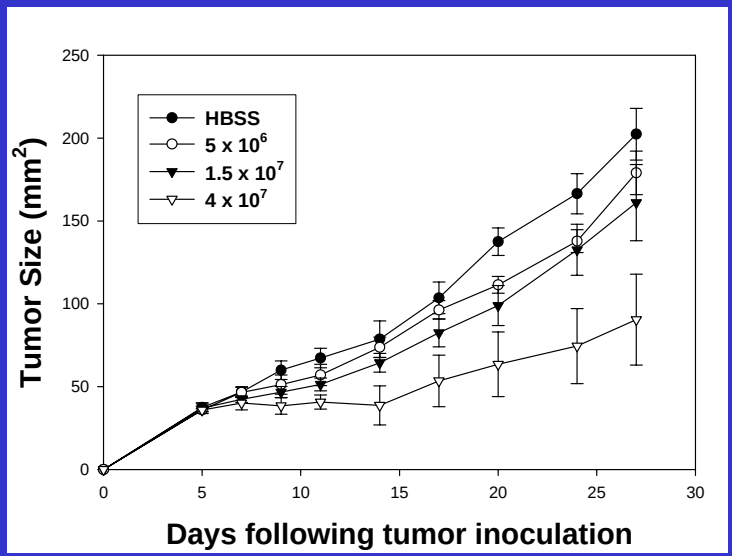
A CD8 T cell proliferation



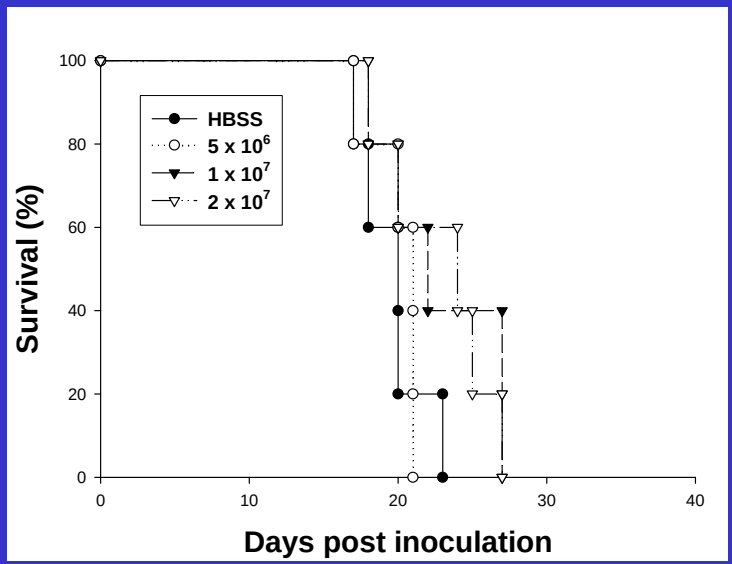
B Therapy of 3-day pulmonary tumors



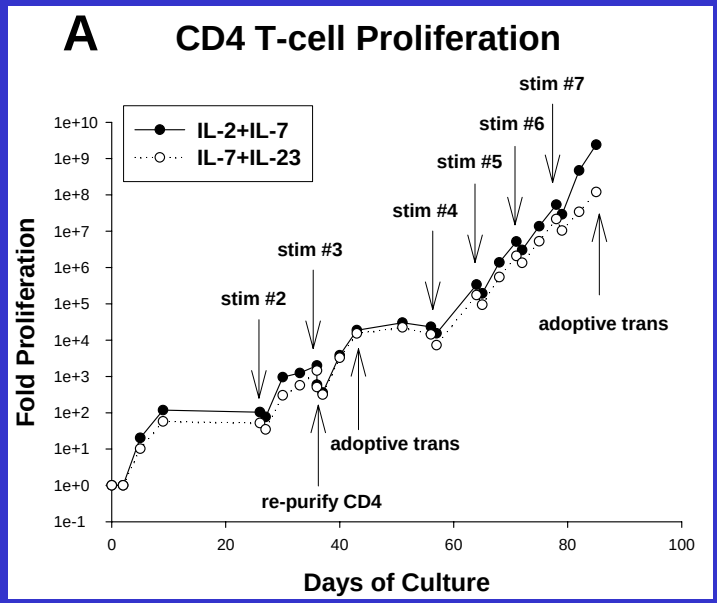
C Therapy of 3-day s.c. tumors

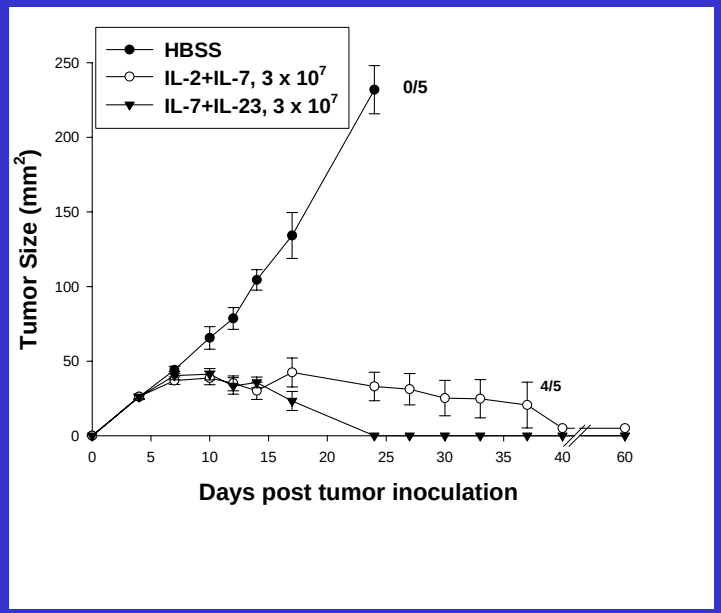


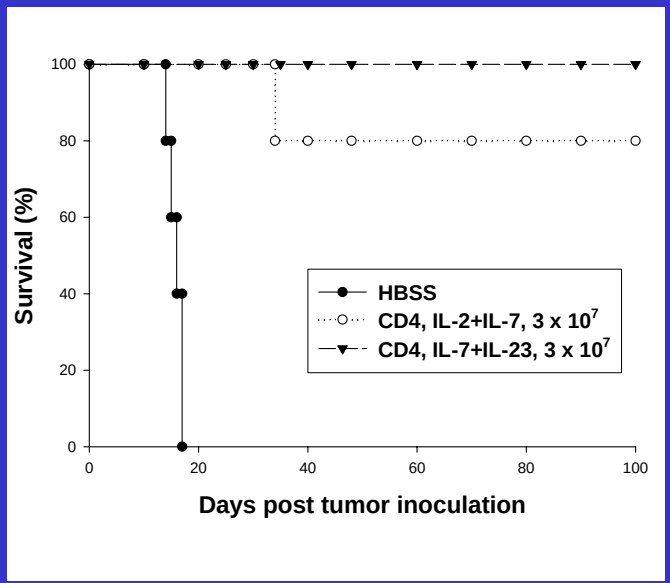
D Therapy of 3-day i.c. tumors

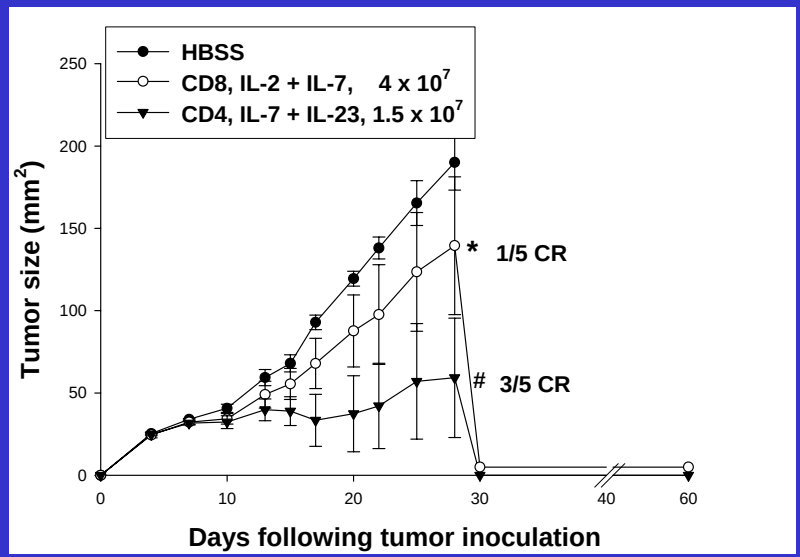


Is it possible to hyperexpand CD4 and retain effector function?


A
CD4 T-cell Proliferation
IL-2+IL-7
IL-7+IL-23
stim #2
stim #3
stim #4
stim #5
stim #6
stim #7
adoptive trans
re-purify CD4
adoptive trans
Fold Proliferation
Days of Culture


B
Therapy of 3-day s.c. tumor
HBSS
IL-2+IL-7, 3 x 10^7
IL-7+IL-23, 3 x 10^7
0/5
4/5
Tumor Size (mm^2)
Days post tumor inoculation


C
Therapy of 3-day i.c. tumor
HBSS
CD4, IL-2+IL-7, 3 x 10^7
CD4, IL-7+IL-23, 3 x 10^7
Survival (%)
Days post tumor inoculation

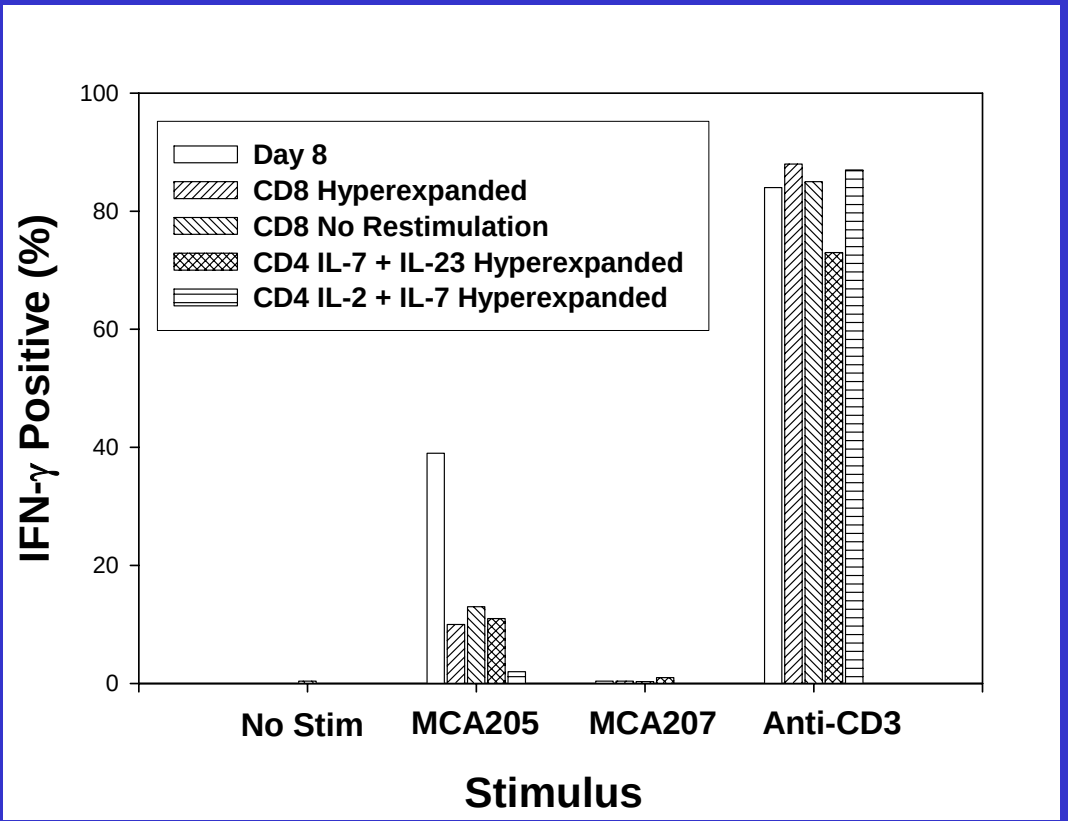

D
Therapy of 3-day s.c. tumor with CD4 or CD8
HBSS
CD8, IL-2 + IL-7, 4 x 10^7
CD4, IL-7 + IL-23, 1.5 x 10^7
* 1/5 CR
# 3/5 CR
Tumor size (mm^2)
Days following tumor inoculation

TCR Vβ phenotype of $CD62^{low}$ subset is similar to total LN T cells.

TCR Vβ phenotype of 10^8-fold hyperexpanded T cells is similar to starting population.

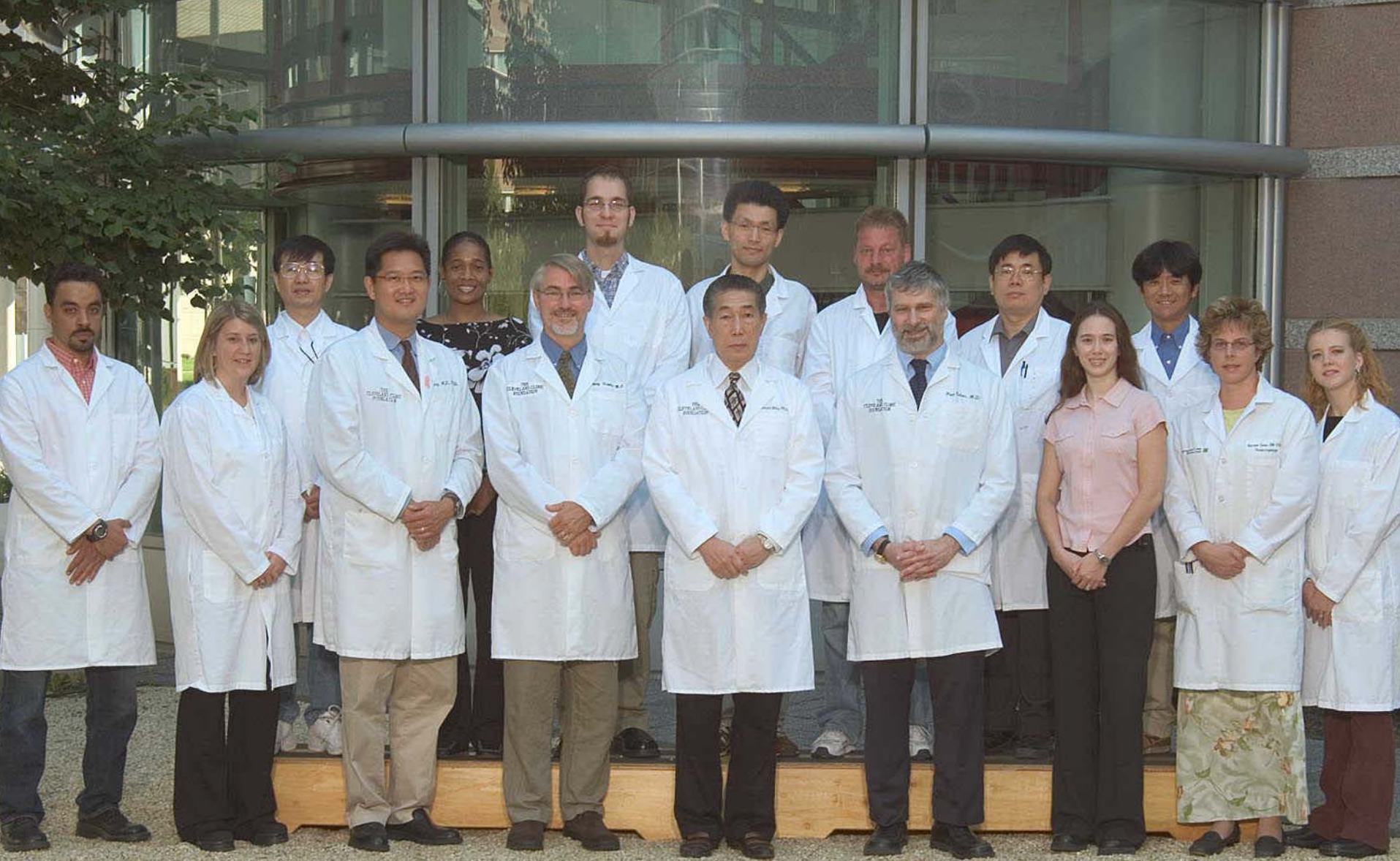
TCR spectratype analysis does not demonstrate oligoclonality

In vitro functional analysis by intracellular IFN-γ assay shows persistence of function in hyperexpanded cells.



Acknowledgements

- Julian Kim
- Hallie Graor



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