

# *Effects of rhIL-7 administration in humans on in vivo expansion of naïve, memory and effector subsets of CD4<sup>+</sup> & CD8<sup>+</sup> T-cells*

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*Cytheris Inc., Vanves, France*



This study was performed as a collaboration between

- National Cancer Institute, NIH, DHSS
- Cytheris, Inc. (Rockville, MD)

Under a Cooperative Research and Development Agreement  
(CRADA # 01649)

- Some of the co-investigators have financial interest in and / or are employees on Cytheris Inc.
- The other co-investigators (including the presenter / Principal Investigator) are federal employees and have no conflict on interest



- IL-7 is a non redundant cytokine
- IL-7 is critical in lymphoid development
- IL-7 is critical in post development lymphocyte homeostasis
- IL-7 multitude of immune properties may have important clinical applications

## *Possible IL-7 use in cancer vaccine / immuno-therapy*

### ■ ↑ Lymphocyte count

- Expansion of naïve / memory T-cell pools
- Anti-apoptotic effect during immune reconstitution following lympho-depleting therapies
- ↑ T-cell proliferation upon engagement of the TCR

### ■ Widening the immune response

- Expansion of naïve T-cell pool increasing the repertoire of T-cell specificities
- ↓ Threshold of immune response
- Recruitment of sub-dominant immune responses

### ■ Generation of better effectors

- ↑ Cytotoxicity of sensitized lymphocytes
- ↓ T-cell apoptosis following antigenic exposure
- ↑ DC function (?)
- *In vitro* or *in vivo*; in autologous or allogeneic settings

# *Phase I study of IL-7 (1)*

NCI protocol 03-C-0152

## Inter-subject dose escalation study

- Recombinant (E. Coli) human IL-7, “CYT 99 007”
- Provided by Cytheris Inc. (Rockville, MD)
- 4 cohorts of 3-6 subjects
- Doses: 3, 10, 30, 60  $\mu\text{g}$  /Kg/ dose
- Given sub-cutaneously every other day for 2 weeks (8 doses)



# *Phase I study of IL-7 (2)*

NCI protocol 03-C-0152

## ■ Primary end points

- Dose Limiting Toxicity (DLT)
- Maximum Tolerated Dose (MTD)

## ■ Secondary end points

- Determine a range of biologically active doses
- Pharmacokinetics and Pharmacodynamics
- Possible anti-tumor activity

# *Phase I study of IL-7 (3)*

NCI protocol 03-C-0152

## Inclusion Criteria

- Diagnosis of incurable malignancy
- Measurable or evaluable disease
- Stable peripheral CD3+ count  $> 300/\text{mm}^3$ 
  - 4 determinations over 2 weeks prior to entry
  - No systemic steroids 2 weeks prior to CD3 determinations
- No therapy in previous 4 weeks with:
  - chemotherapy, cytokine immunotherapy,
  - anti-tumor vaccines or MoAb

# *Phase I study of IL-7 (4)*

NCI protocol 03-C-0152

## Exclusion Criteria

- Hematopoietic malignancies
- Primary carcinoma of the lung
- Life expectancy < 3 months
- HIV, hepatitis B, or hepatitis C
- Need for full anticoagulation or systemic steroids
- Hypertension uncontrolled with standard Rx



# *Phase I study of IL-7*

## *Preliminary results (1)*

- 11 men, 3 women,
- Age from 20 to 71 years (median: 48.5)
- With the following metastatic diseases:
  - renal cell carcinoma (2)
  - malignant hemangiopericytoma (1)
  - melanoma (4)
  - Adenocarcinoma: colon (1), duodenal (1), unknown primary (1)
  - Sarcomas: osteogenic (1), alveolar rhabdomyosarcoma (1), synovial cell (1)
  - Pheochromocytoma (1)

# *Phase I study of IL-7*

## *Preliminary results (2)*

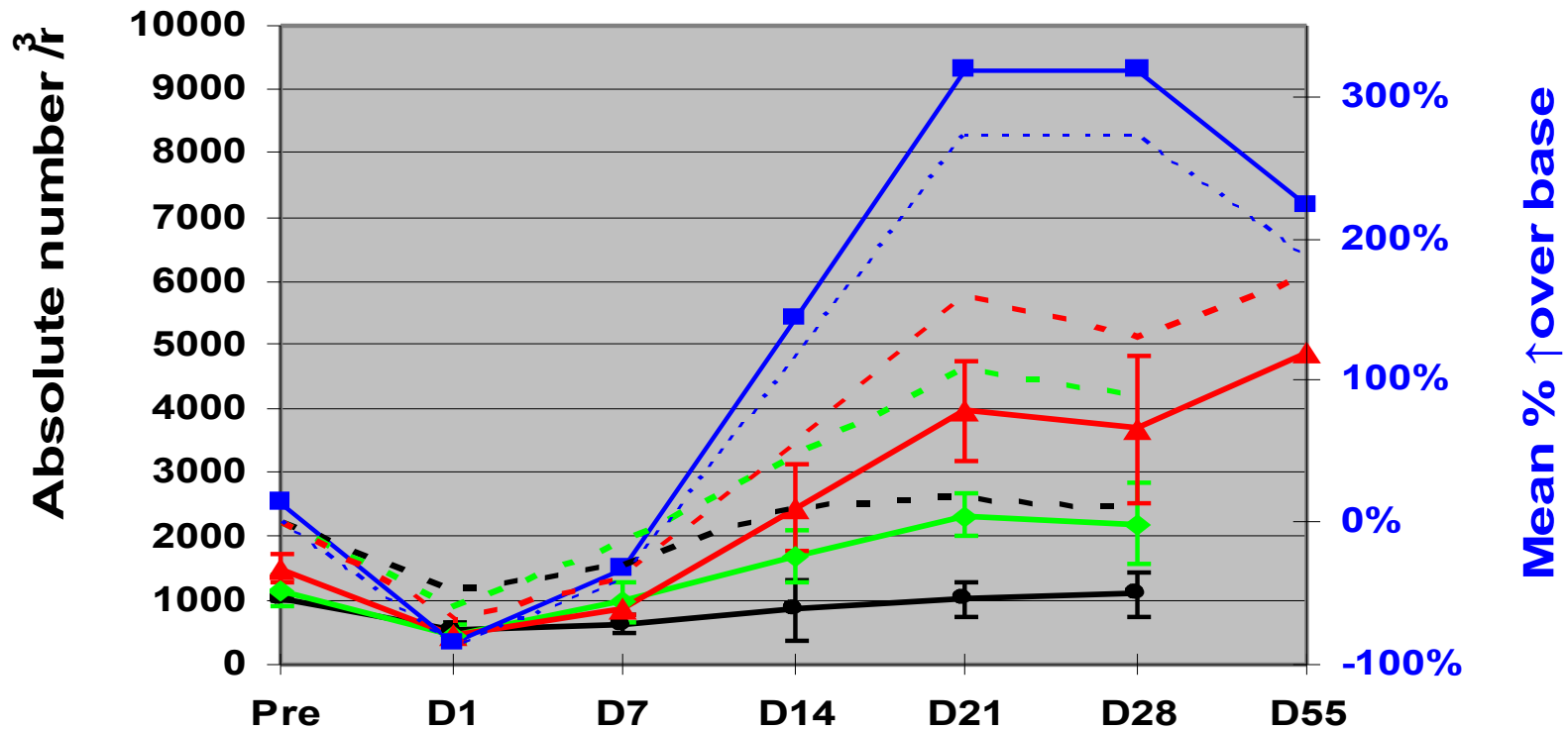
### ■ Toxicity

- Grade 1-2 constitutional symptoms & local reaction
  - Chills, fever, malaise
  - 6-8 hours following injections
  - After most injections, in most subjects receiving  $>3 \mu\text{g} / \text{Kg} / \text{dose}$
- Grade 3 LFT elevation following first injection (DLT)
  - In 1 subject (Rx stopped, normalized within 5 days; possibly related)
- Grade 3 chest pain, hypertension with mild Troponin elevation
  - Patient with Pheochromocytoma
  - After 3 doses (Rx stopped, normalized within 1 day; probably related)

### ■ Immunogenicity

- Non neutralizing anti-IL-7 antibodies (low titers) in 3 subjects
- No neutralizing antibodies (DLT)

# Total circulating lymphocytes

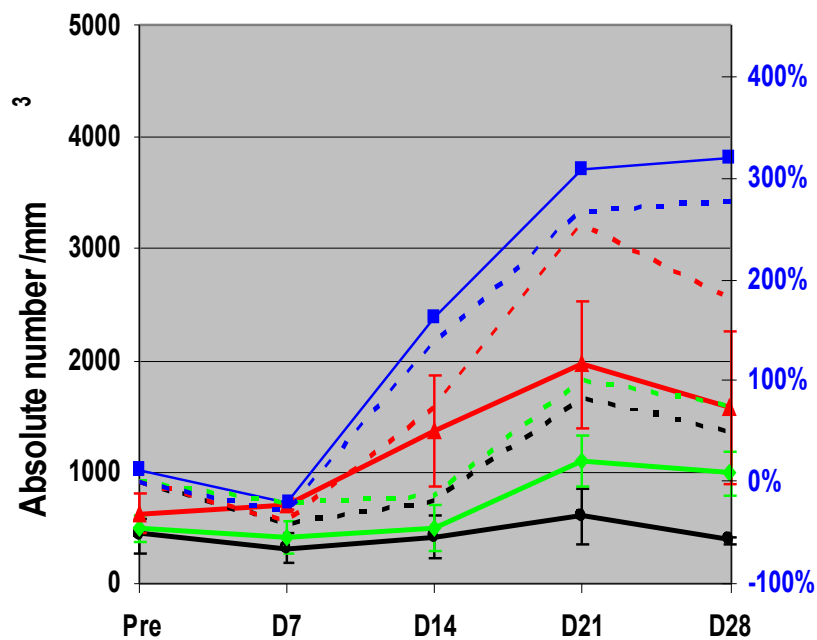


Day 14 = End of treatment

— cohort 1 (3µg) — cohort 2 (10µg) — cohort 3 (30µg) — cohort 4 (60 µg)  
 ---- cohort 1 (%) ---- cohort 2 (%) ---- cohort 3 (%) ---- cohort 4 (%)



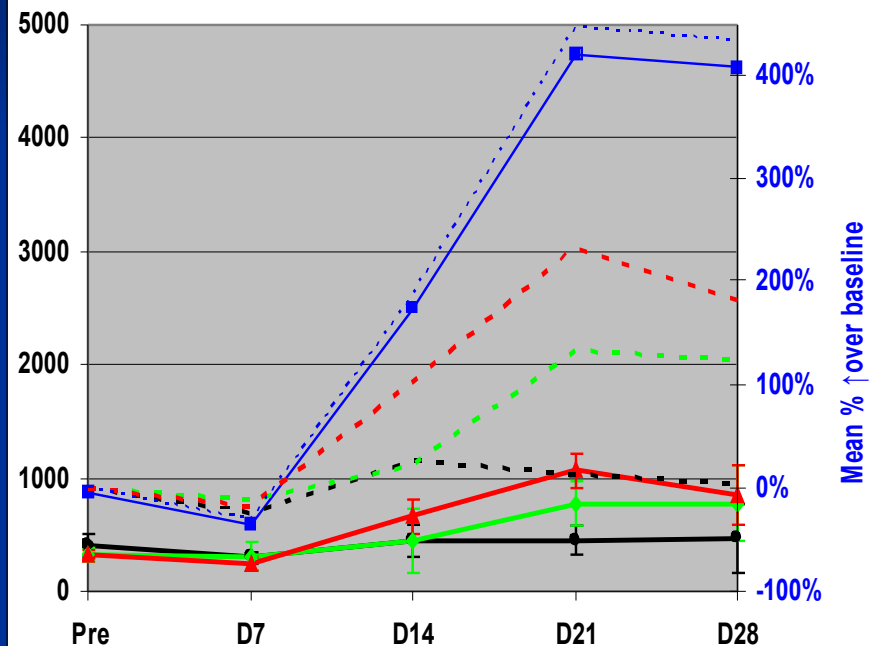
## CD3<sup>+</sup> / CD4<sup>+</sup>



Day 14 = End of treatment

— cohort 1 (3µg) — cohort 2 (10µg) — cohort 3 (30µg) — cohort 4 (60 µg)  
 ---- cohort 1 (%) ---- cohort 2 (%) ---- cohort 3 (%) ---- cohort 4 (%)

## CD3<sup>+</sup> / CD8<sup>+</sup>



## ■ T-cell subsets were defined and analyzed by multicolor Flow Cytometry

- after cell sorting of peripheral blood CD4<sup>+</sup> & CD8<sup>+</sup> cells
- at several time-points before, during and after IL-7 administration

CD4<sup>+</sup> / CD45RA<sup>+</sup> / CD27<sup>+</sup> (\_\_\_\_\_)

CD4<sup>+</sup> / CD45RA<sup>+</sup> / CD31<sup>+</sup> (\_\_\_\_\_ *Recent Thymic Emigrants*)

CD4<sup>+</sup> / CD45RA<sup>-</sup> / CD27<sup>+</sup> \_\_\_\_\_

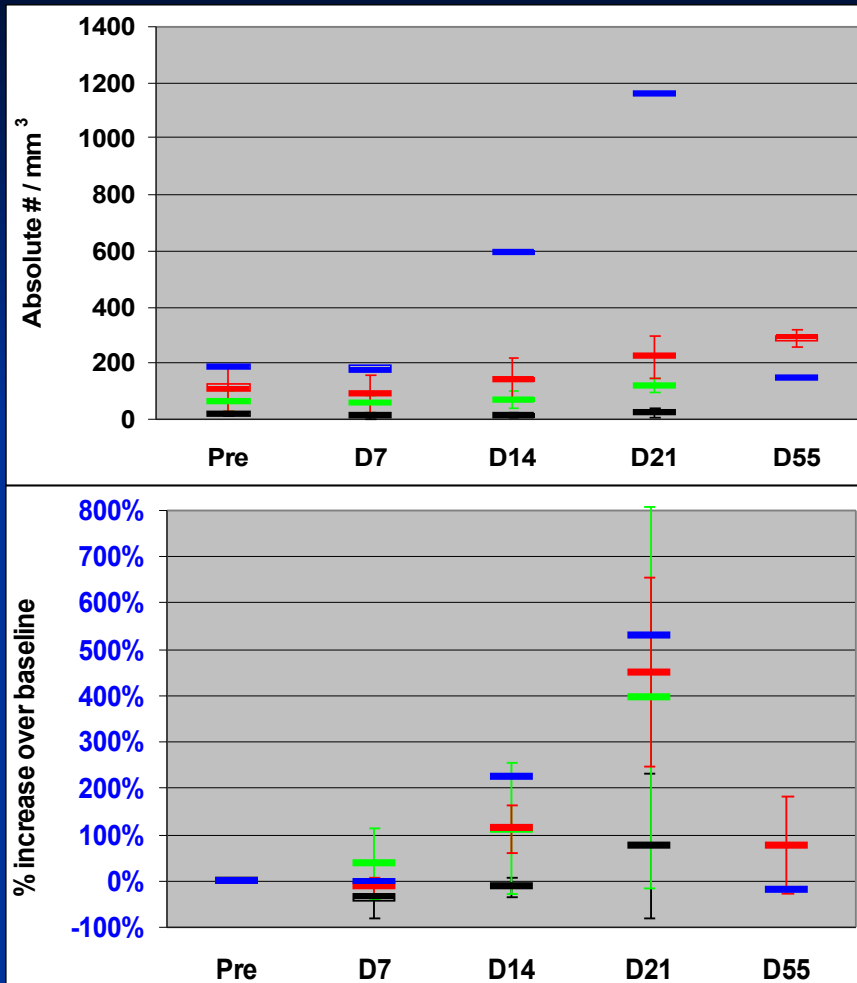
CD4<sup>+</sup> / CD45RA<sup>-</sup> / CD27<sup>-</sup> (\_\_\_\_\_)

CD8<sup>+</sup> / CD45RA<sup>+</sup> / CD27<sup>+</sup> (\_\_\_\_\_)

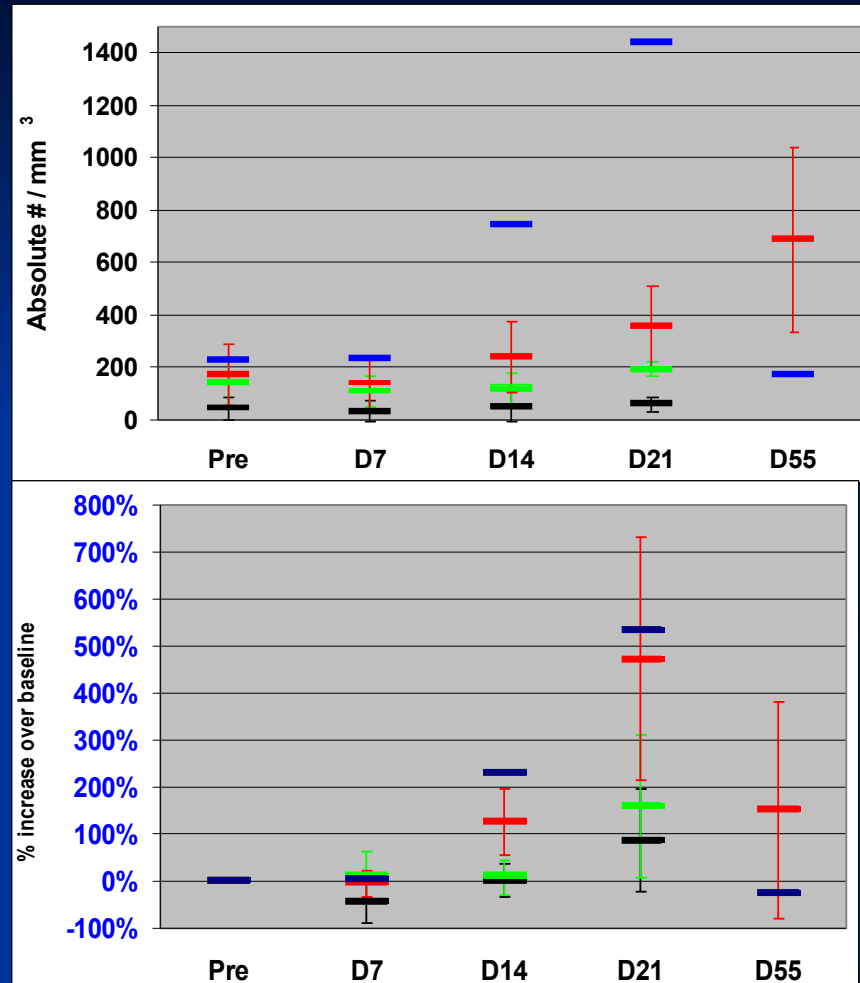
CD8<sup>+</sup> / CD45RA<sup>-</sup> / CD27<sup>+</sup> \_\_\_\_\_

CD8<sup>+</sup> / CD45RA<sup>-</sup> / CD27<sup>-</sup> (\_\_\_\_\_)

## CD4<sup>+</sup> / CD45RA<sup>+</sup> / CD31<sup>+</sup> (*Most Naïve*)



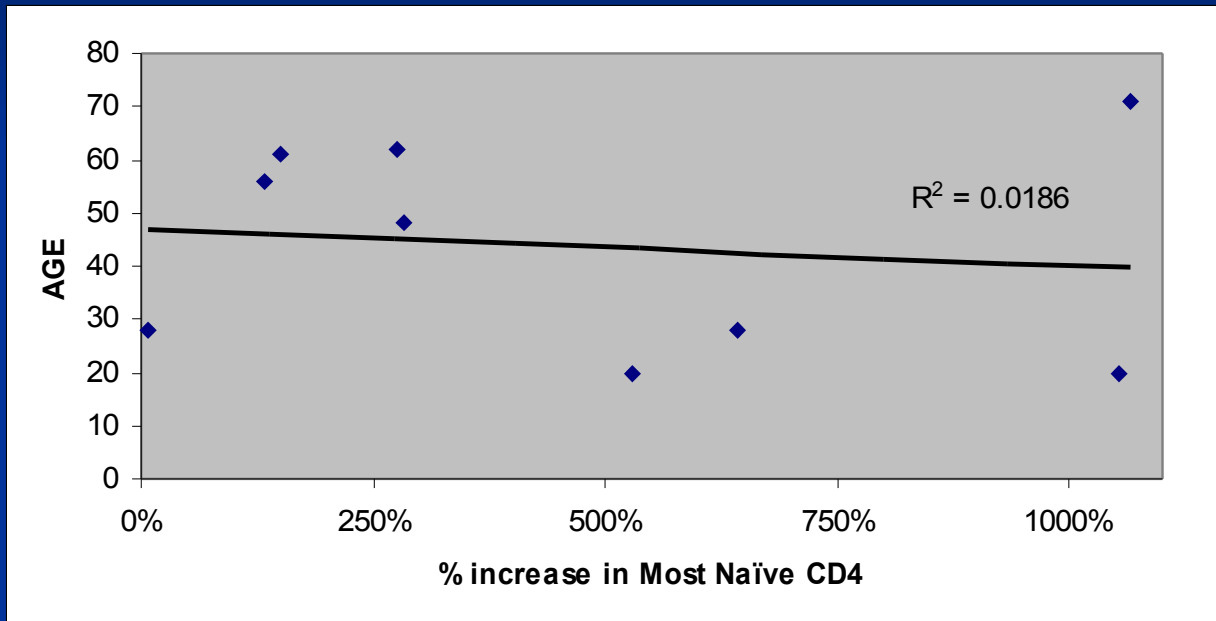
## CD4<sup>+</sup> / CD45RA<sup>+</sup> / CD27<sup>+</sup> (*Naïve*)



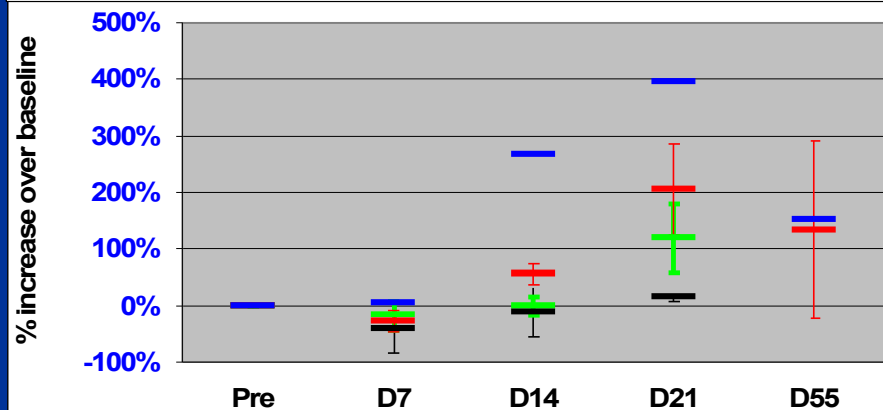
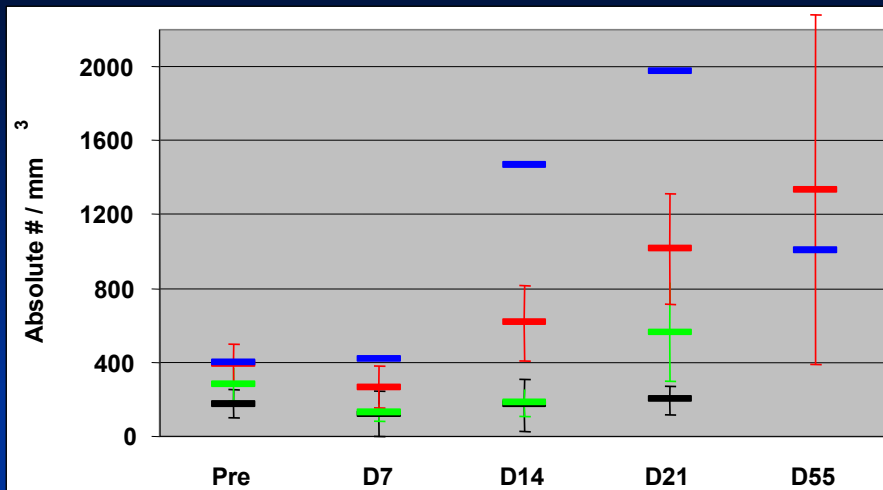
Day 14 = End of treatment

— Mean "1" — Mean "2" — Mean "3" — Mean "4"

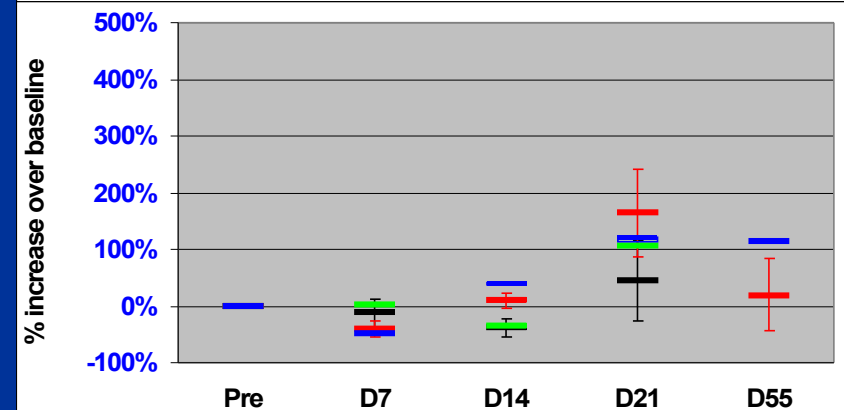
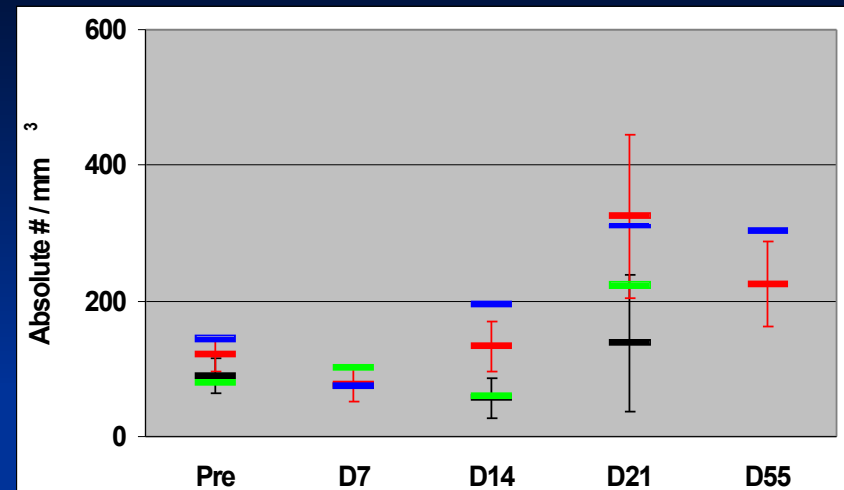
## *Correlation of age with Most Naïve CD4*



## CD4<sup>+</sup> / CD45RA<sup>-</sup> / CD27<sup>+</sup> (*Memory*)



## CD4<sup>+</sup> / CD45RA<sup>-</sup> / CD27<sup>-</sup> (*Effector*)



Day 14 = End of treatment

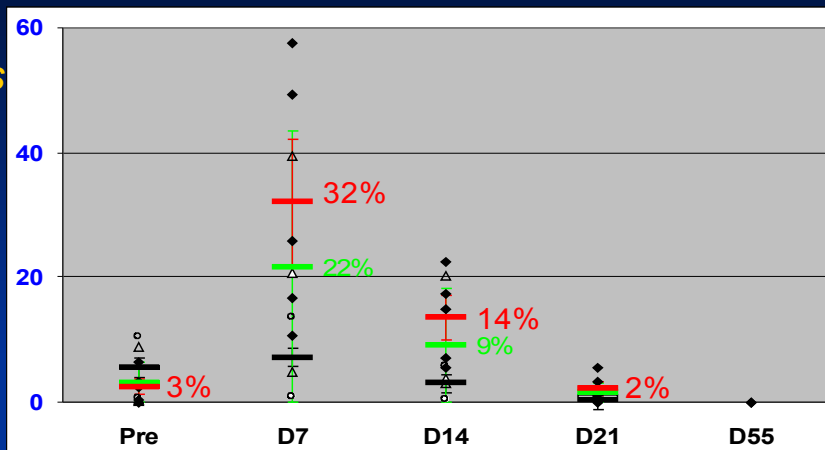
— Mean "1" — Mean "2" — Mean "3" — Mean "4"



# Kinetics of CD4+ Cell Cycling: Most naive

CD4<sup>+</sup> / CD45RA<sup>+</sup> / CD31<sup>+</sup> & Ki67<sup>+</sup>

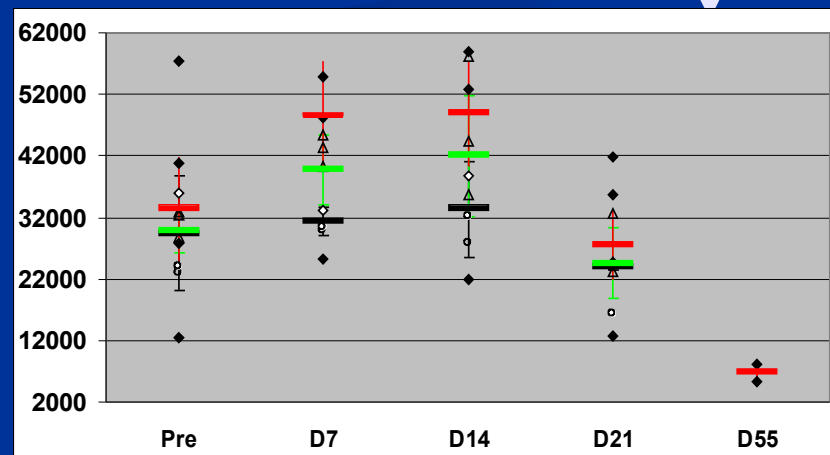
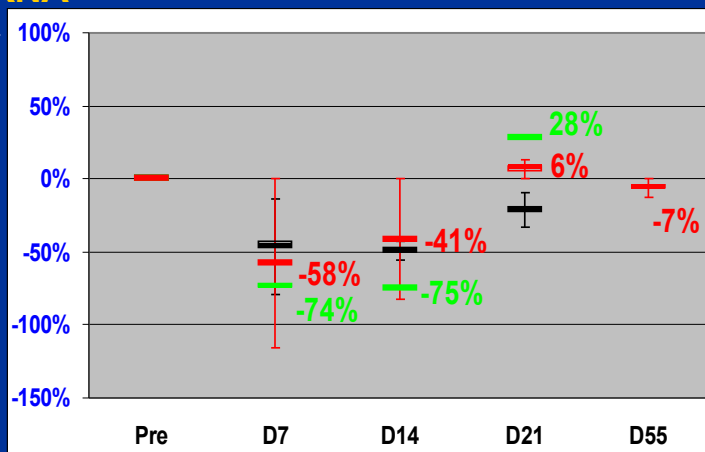
Percent of  
**Most Naive cells**  
in cycle



**Bcl-2:** Mean  
Fluorescence  
Intensity



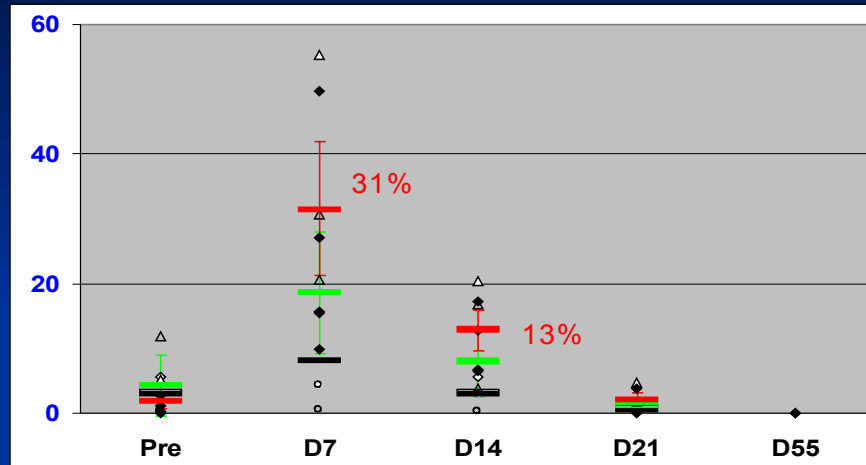
**IL7-R mRNA**  
/ 10<sup>4</sup>  
**Actin mRNA**



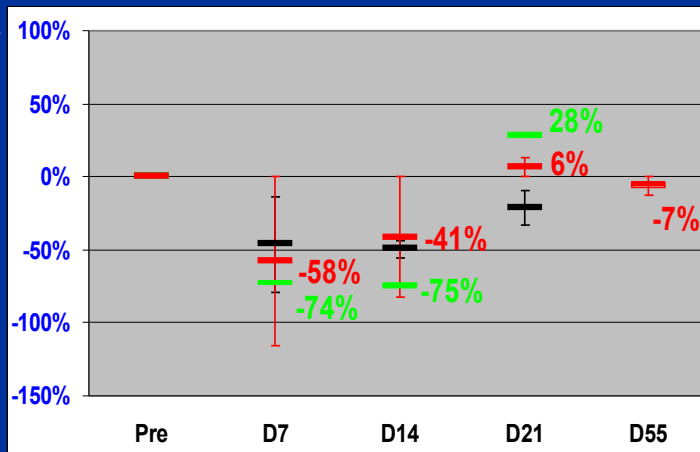
# Kinetics of CD4<sup>+</sup> Cell Cycling: Naive

CD4<sup>+</sup> / CD45RA<sup>+</sup> / CD27<sup>+</sup> & Ki67<sup>+</sup>

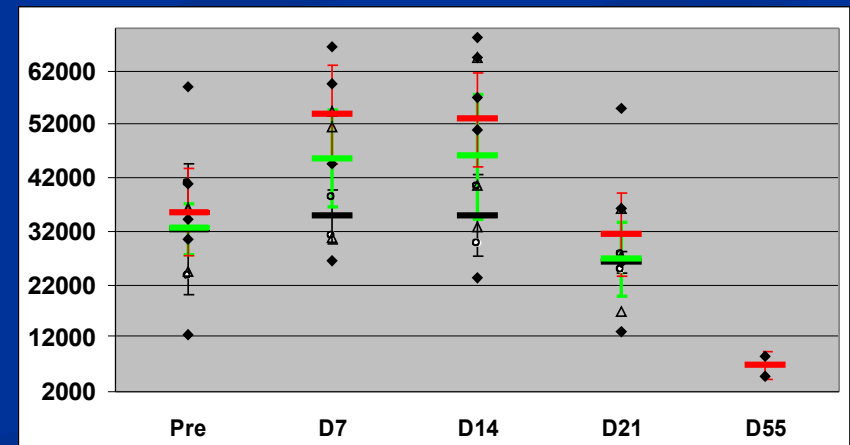
Percent of  
Naive cells  
in cycle



IL7-R mRNA  
/ 10<sup>4</sup>  
Actin mRNA



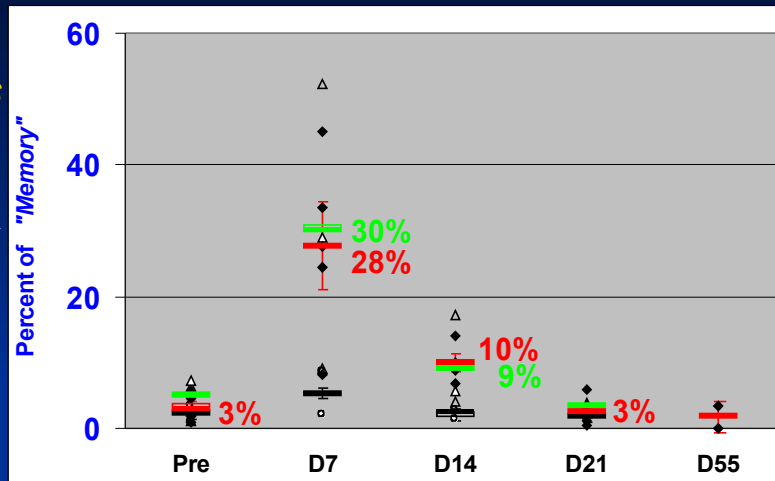
Bcl-2: Mean  
Fluorescence  
Intensity



# Kinetics of CD4<sup>+</sup> Cell Cycling: Memory

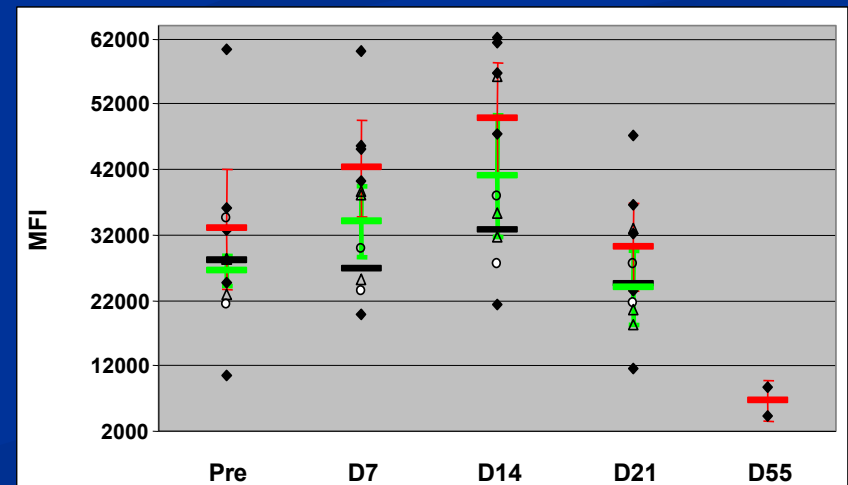
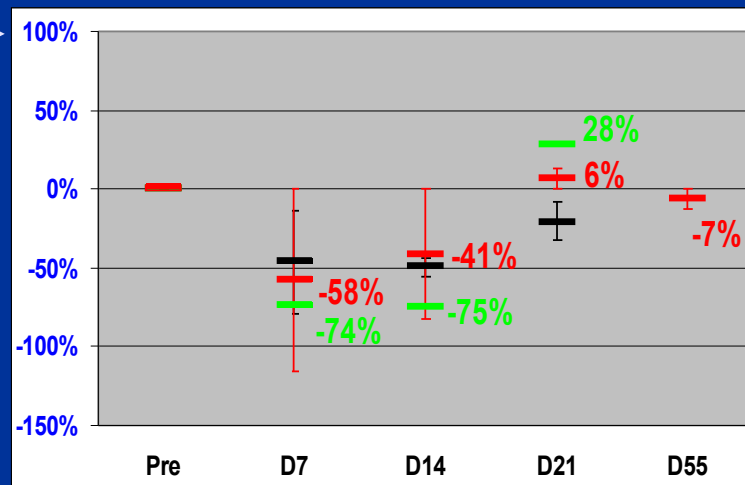
CD4<sup>+</sup> / CD45RA<sup>-</sup> / CD27<sup>+</sup> & Ki67<sup>+</sup>

Percent of  
**Memory** cells  
in cycle



**Bcl-2**: Mean  
Fluorescence  
Intensity

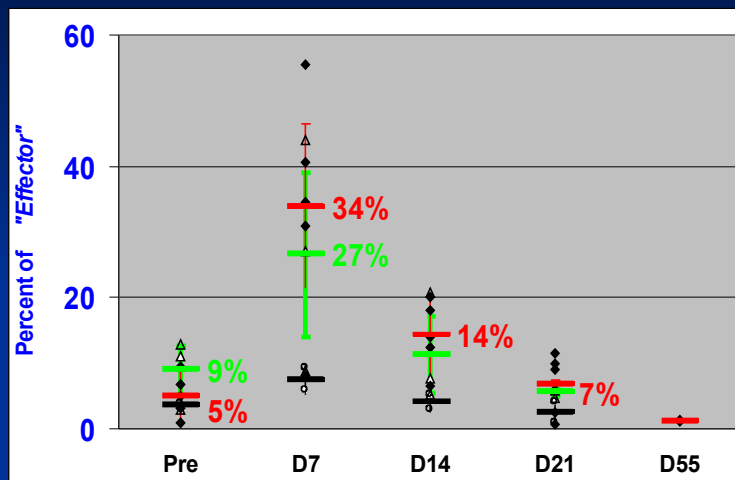
**IL7-R** mRNA  
/ 10<sup>4</sup>  
Actin mRNA



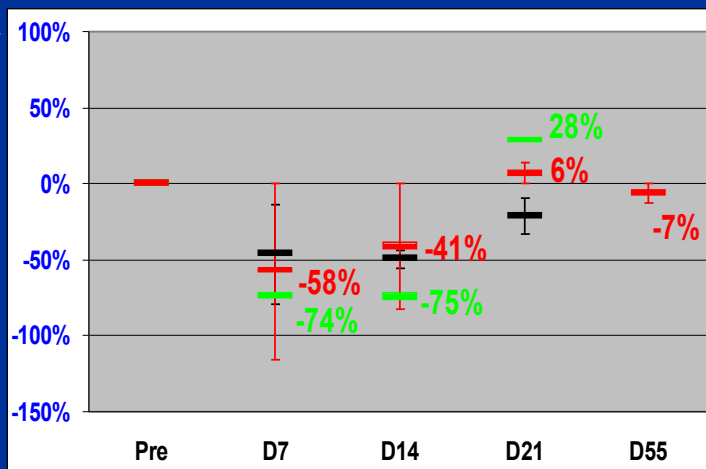
# Kinetics of CD4<sup>+</sup> Cell Cycling: Effectors

CD4<sup>+</sup> / CD45RA<sup>-</sup> / CD27<sup>-</sup> & Ki67<sup>+</sup>

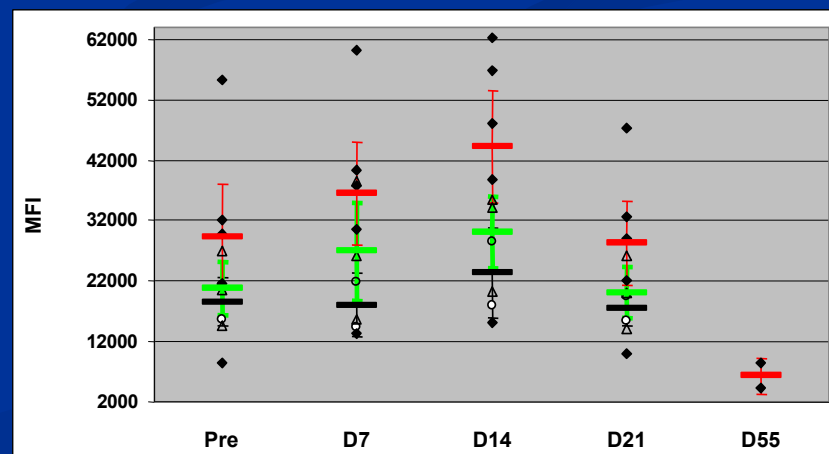
Percent of  
*Effectors* in cycle



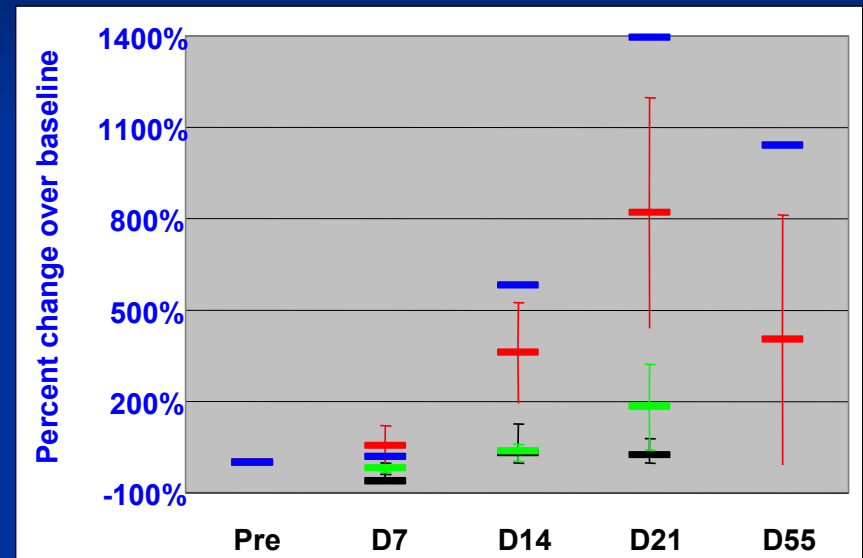
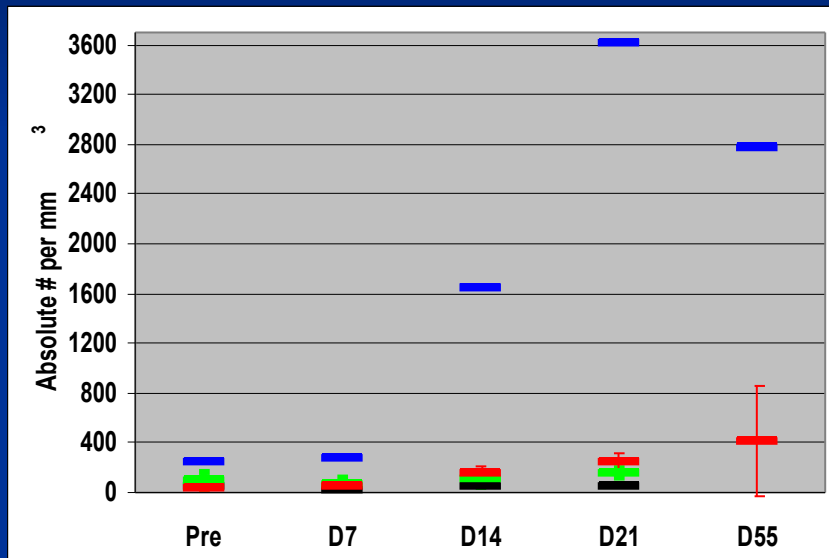
*IL7-R* mRNA  
/ 10<sup>4</sup>  
Actin mRNA



*Bcl-2*: Mean  
Fluorescence  
Intensity



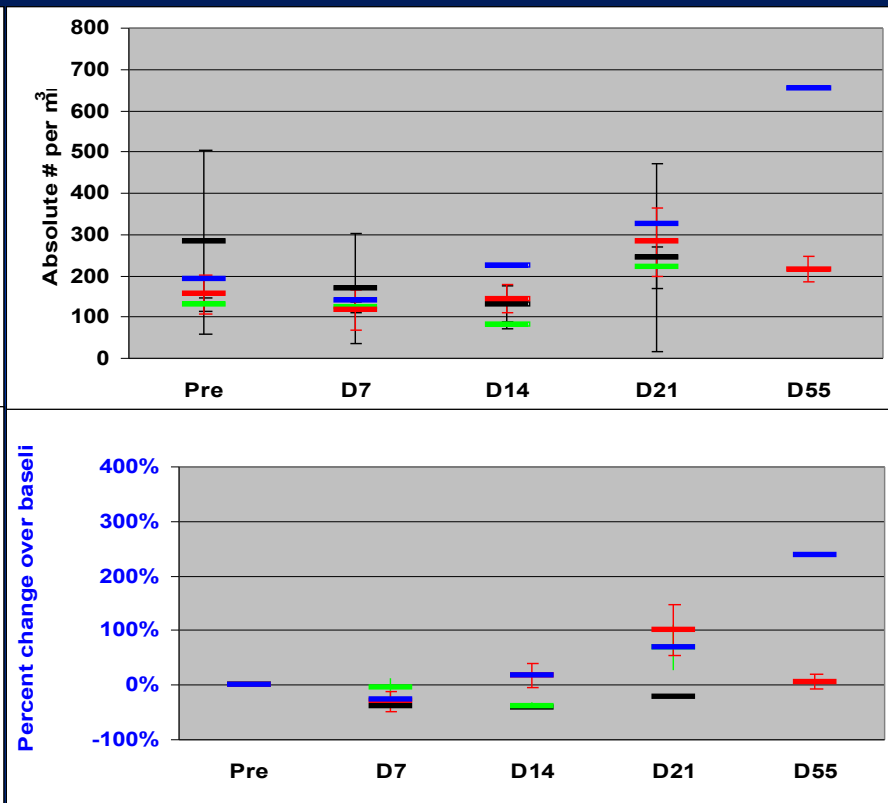
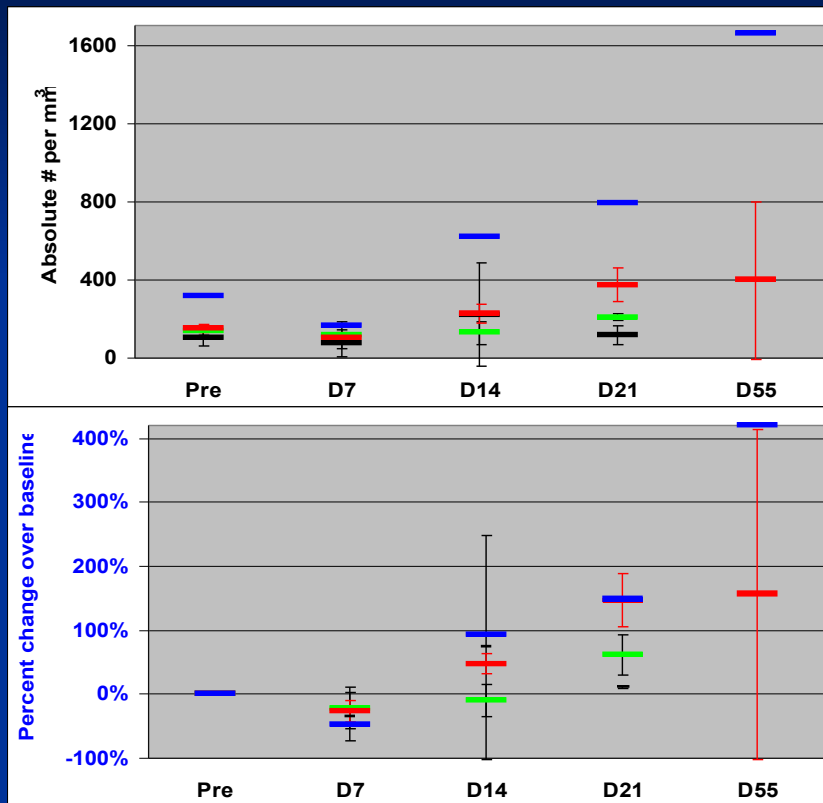
## CD8<sup>+</sup> / CD45RA<sup>+</sup> / CD27<sup>+</sup> (*Naïve*)



Day 14 = End of treatment  
 — Mean "1" — Mean "2" — Mean "3" — Mean "4"

## CD8<sup>+</sup> / CD45RA<sup>-</sup> / CD27<sup>+</sup> (Memory)

## CD8<sup>+</sup> / CD45RA<sup>-</sup> / CD27<sup>-</sup> (Effector)

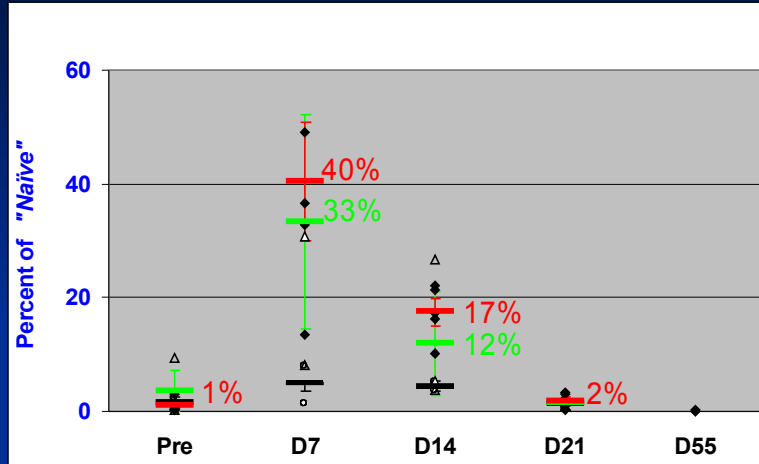


Day 14 = End of treatment  
 — Mean "1" — Mean "2" — Mean "3" — Mean "4"

# Kinetics of CD8<sup>+</sup> Cell Cycling: Naive

CD8<sup>+</sup> / CD45RA<sup>+</sup> / CD27<sup>+</sup> & Ki67<sup>+</sup>

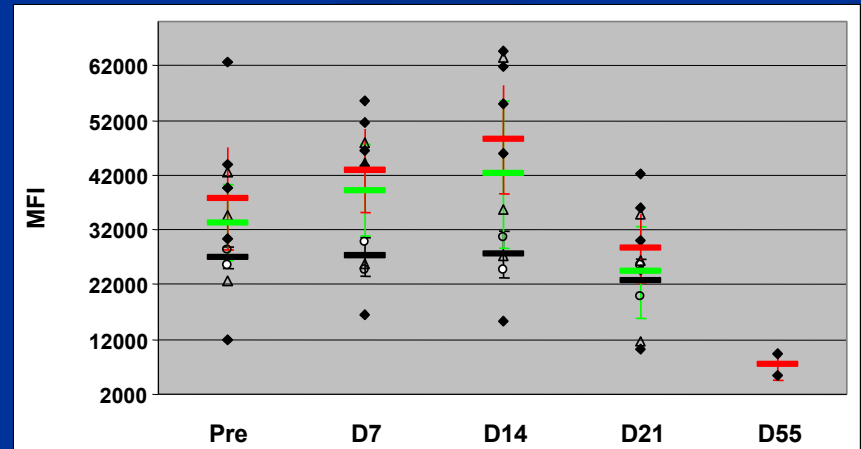
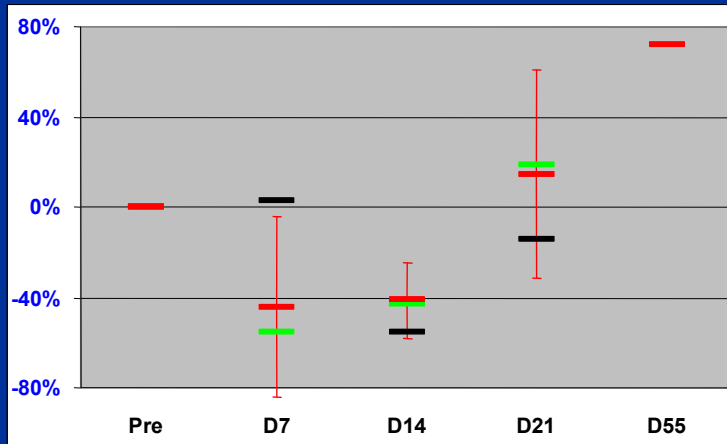
Percent of  
**Naive** cells  
in cycle



**Bcl-2**: Mean  
Fluorescence  
Intensity



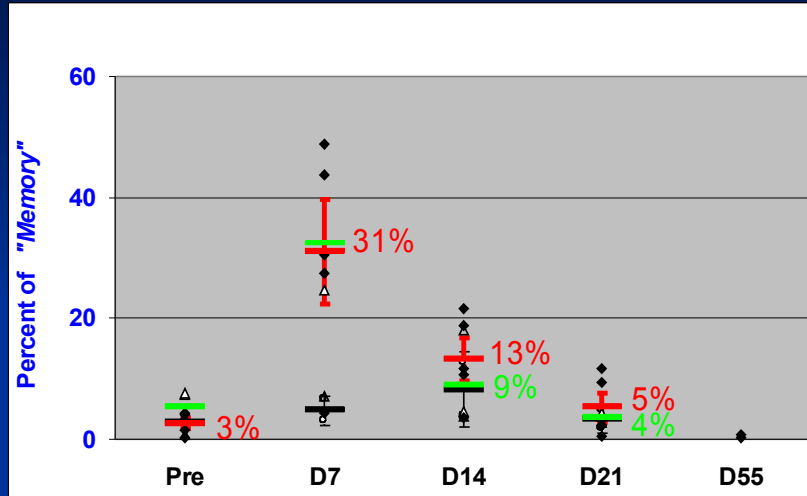
**IL7-R** mRNA  
/ 10<sup>4</sup>  
Actin mRNA



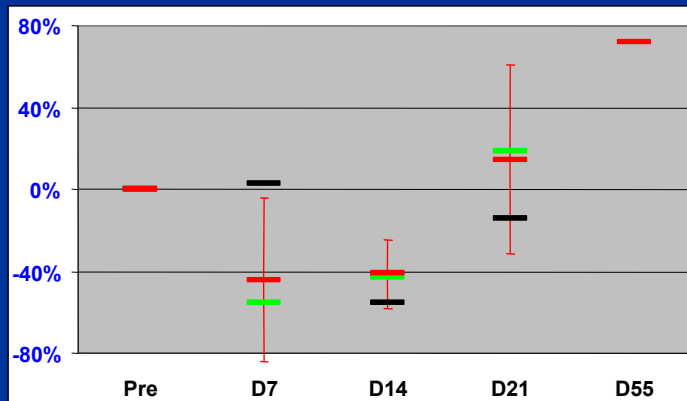
# Kinetics of CD8<sup>+</sup> Cell Cycling: Memory

CD8<sup>+</sup> / CD45RA<sup>-</sup> / CD27<sup>+</sup> & Ki67<sup>+</sup>

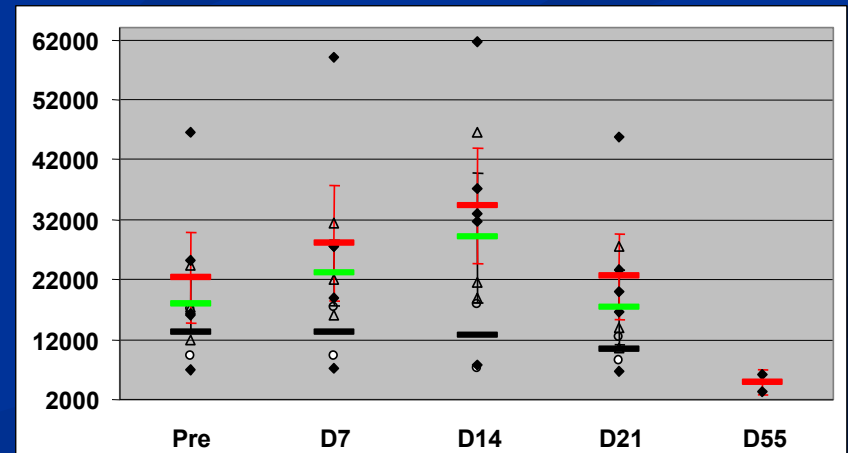
Percent of  
Memory cells  
in cycle



IL7-R mRNA  
/ 10<sup>4</sup>  
Actin mRNA



Bcl-2: Mean  
Fluorescence  
Intensity

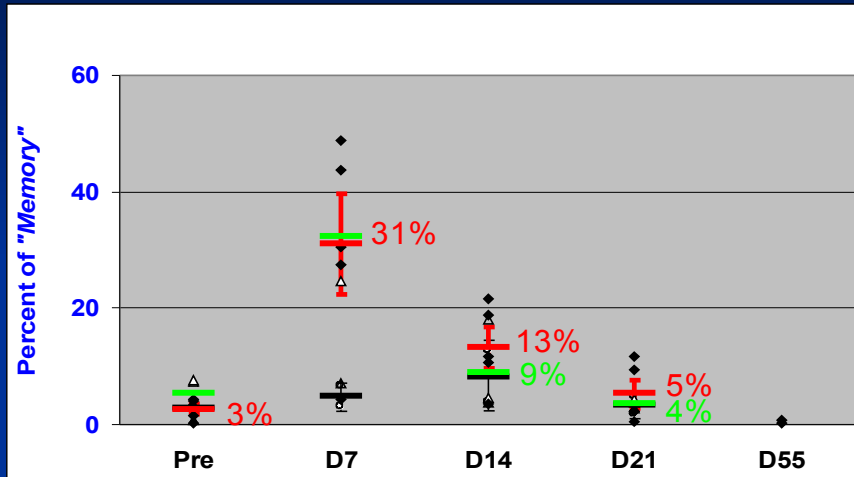




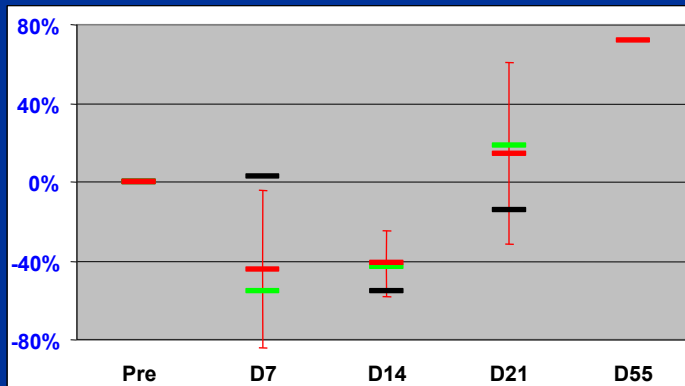
# Kinetics of CD8<sup>+</sup> Cell Cycling: Effectors

CD8<sup>+</sup> / CD45RA<sup>-</sup> / CD27<sup>-</sup> & Ki67<sup>+</sup>

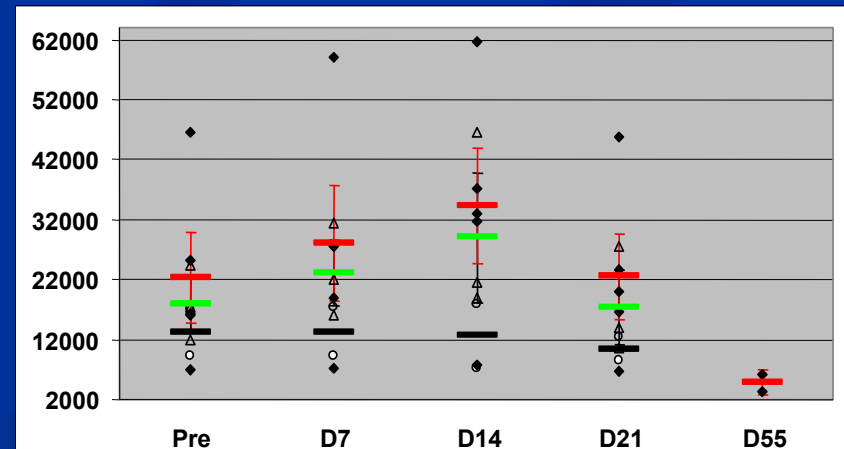
Percent of  
**Memory** cells  
in cycle



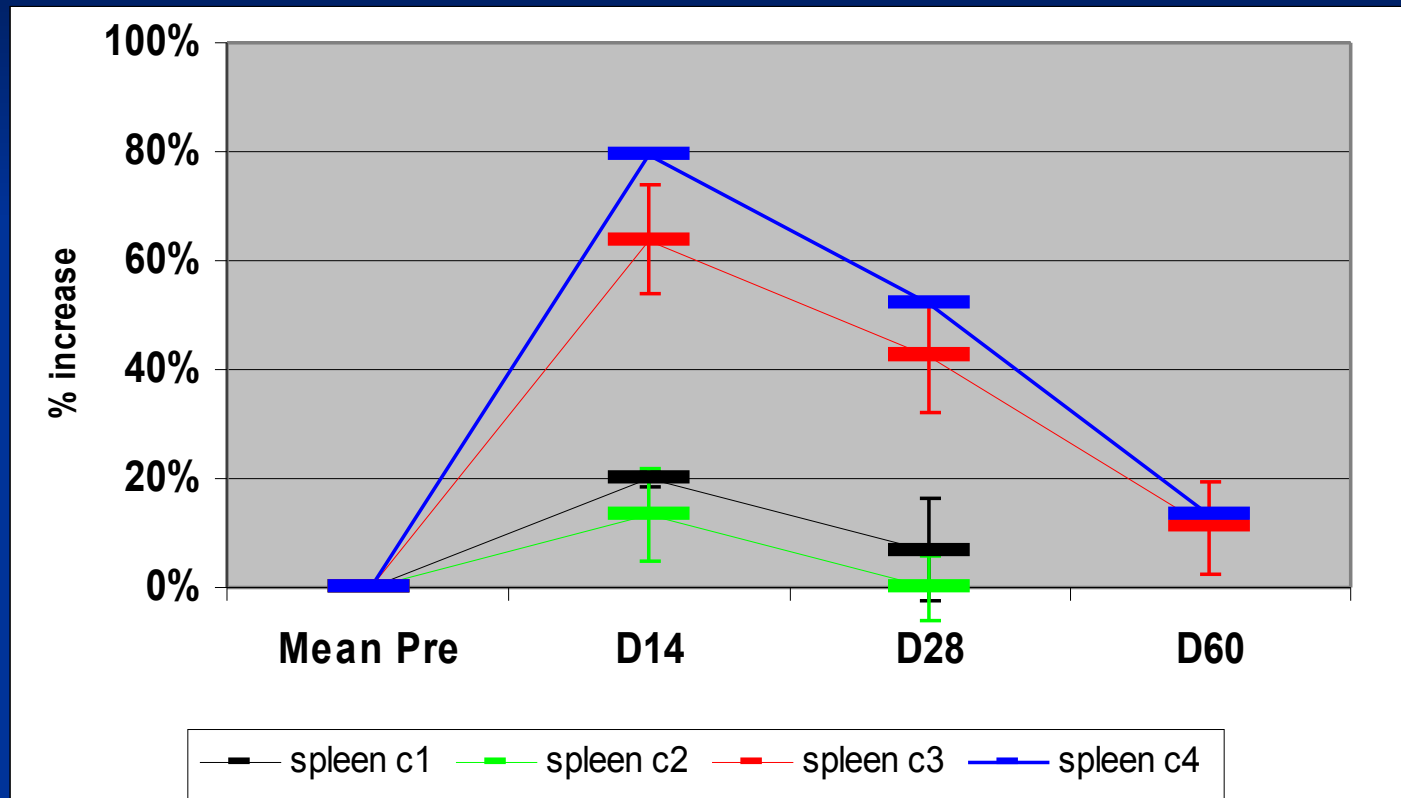
**IL7-R** mRNA  
/ 10<sup>4</sup>  
Actin mRNA



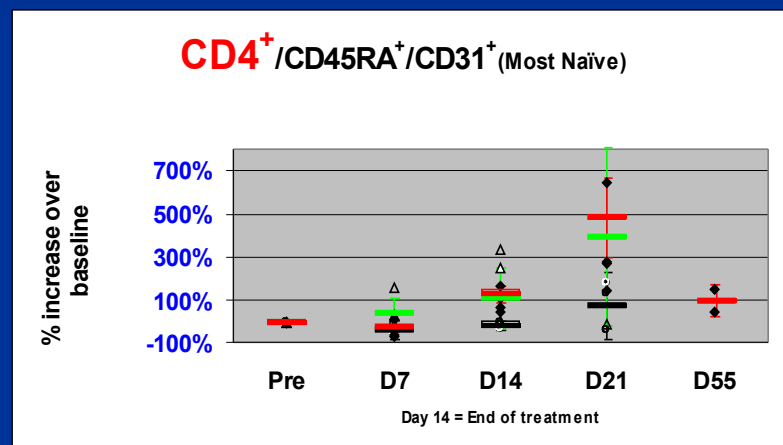
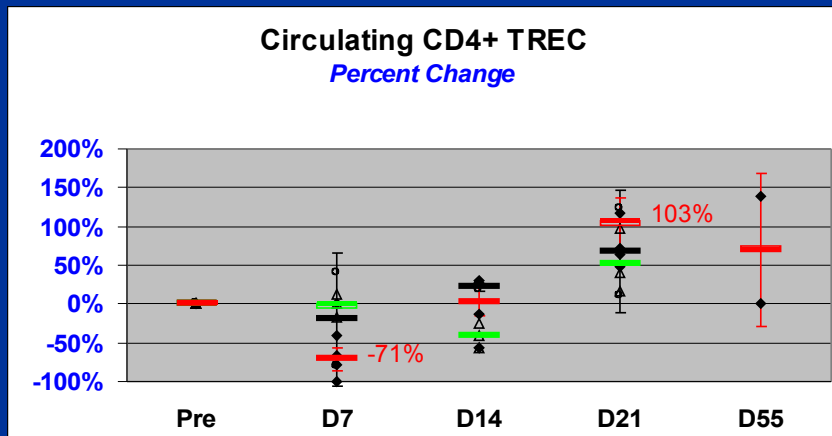
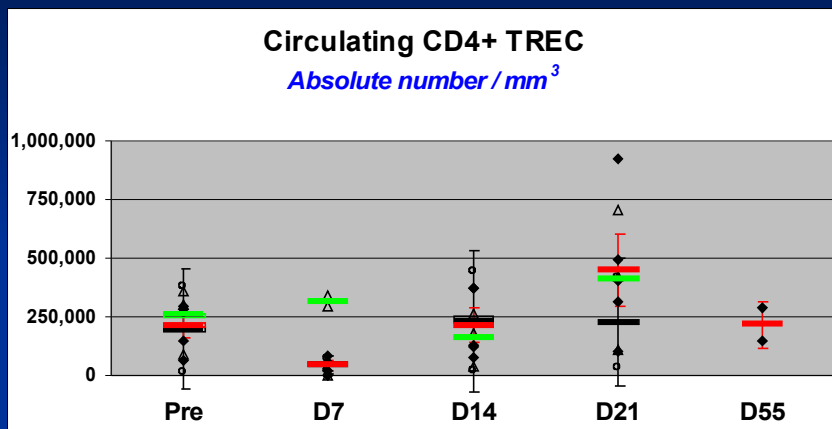
**Bcl-2**: Mean  
Fluorescence  
Intensity



## *Mean % Increase over baseline of spleen size (bi-dimensional product by CT)*

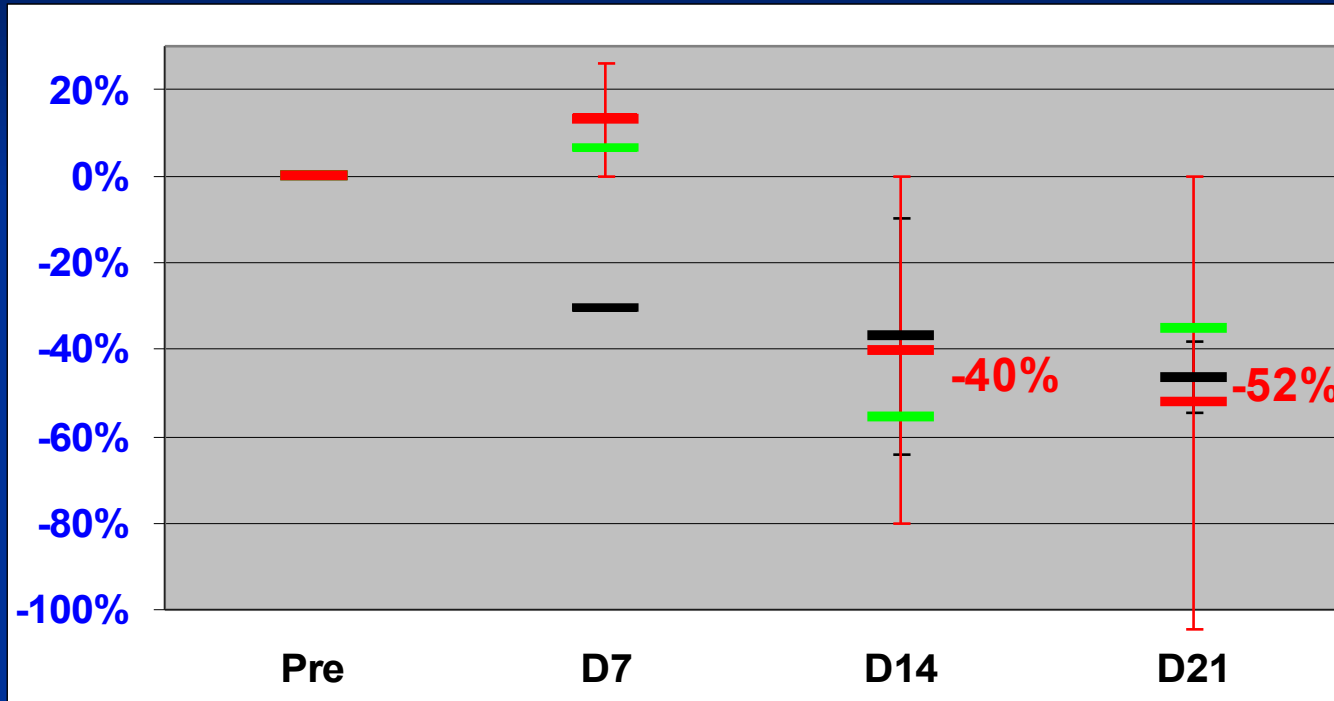


# TREC analysis: CD4<sup>+</sup>



# CD4 T-regs / Fox-P3

*Percent change over baseline in  
FoxP3 mRNA copies per 10<sup>4</sup> Actin copies*



# *Conclusions (1)*

IL-7 appears to have, in humans, the range of biologic activity foreseen from animal data:

- Initial (Day 1) tissue redistribution of circulating T-lymphocytes
- Characteristic down-regulation of the IL-7 R $\alpha$  and up-regulation of Bcl-2
- Reversible lymphoid organ enlargement: spleen, LN
- Induction of massive proliferation and expansion of T-lymphocytes subsets
  - In most naïve CD4 (Recent Thymic Emigrants)
  - In naïve, memory & effector subsets (CD4 & CD8)
  - Regardless of the subjects age
- These effects are:
  - Dose-dependent
  - Maximum within 1<sup>st</sup> week
  - Sustained several weeks after the end of IL-7 exposure
  - More pronounced in the naïve subsets

## *Conclusions (2)*

- These findings set the stage for the design of clinical studies evaluating the possible role of IL-7 in augmenting immune responses in the context of anti-tumor vaccines and immunotherapy

# Acknowledgements

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