

*Effects of rhIL-7 administration in humans
on in vivo expansion of naïve, memory and
effector subsets of CD4⁺ & CD8⁺ 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 μg /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)

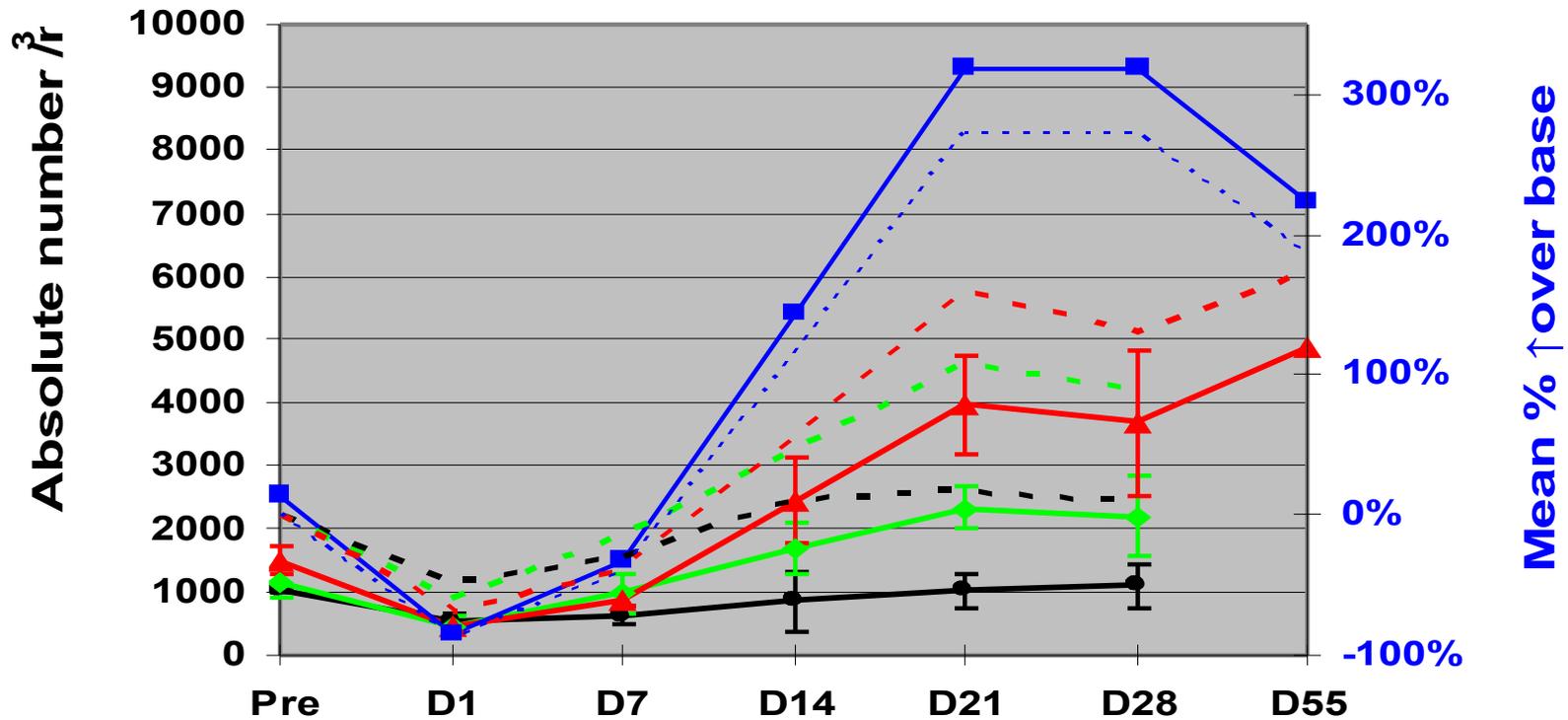
■ Toxicity

- 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



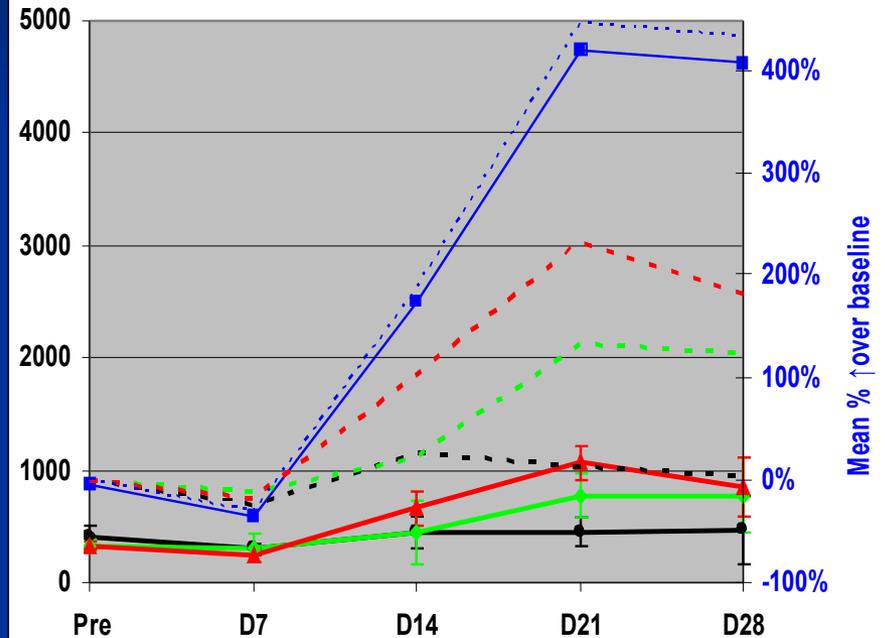
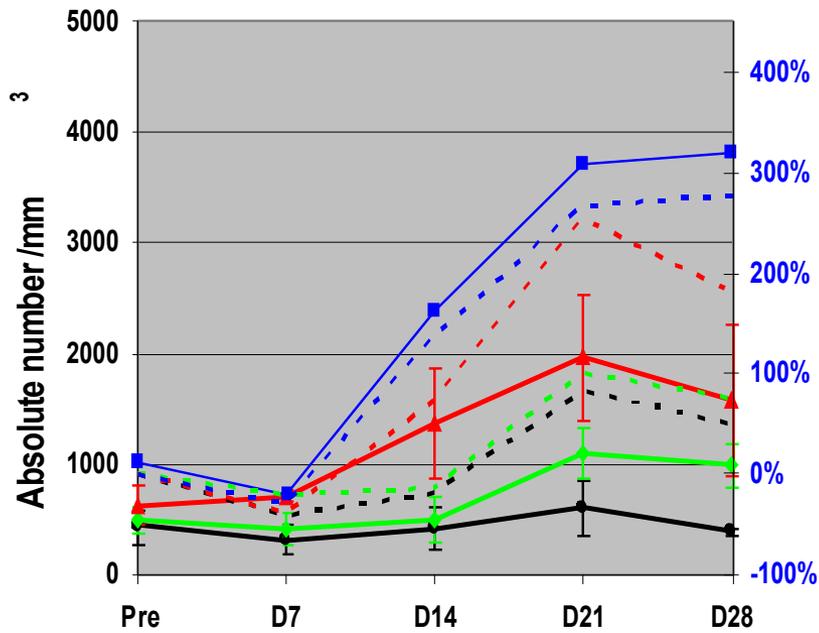
Day 14 = End of treatment

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



CD3⁺ / CD4⁺

CD3⁺ / CD8⁺



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 (%)



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

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

CD4+ / CD45RA+ / CD27+ (_____)

CD4+ / CD45RA+ / CD31+ (_____ *Recent Thymic Emigrants*)

CD4+ / CD45RA- / CD27+ _____

CD4+ / CD45RA- / CD27- (_____)

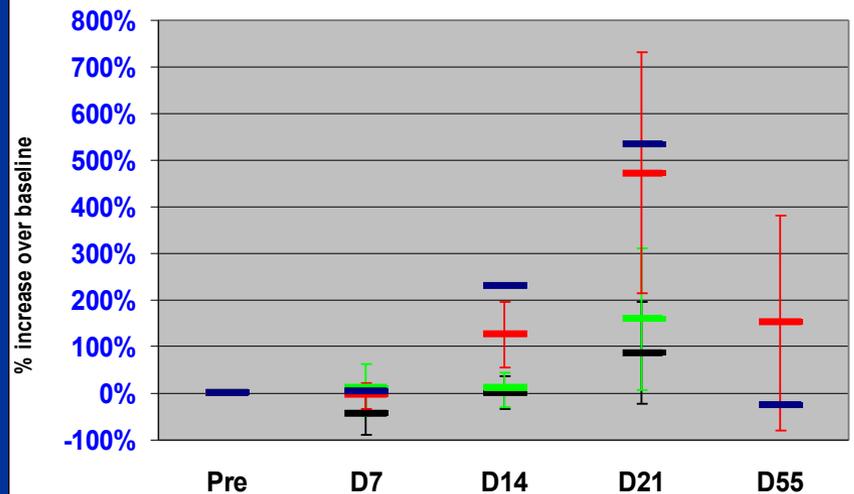
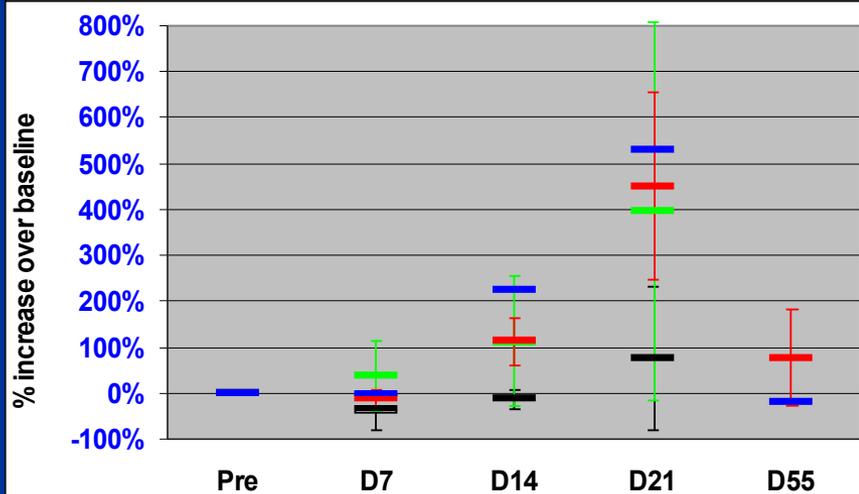
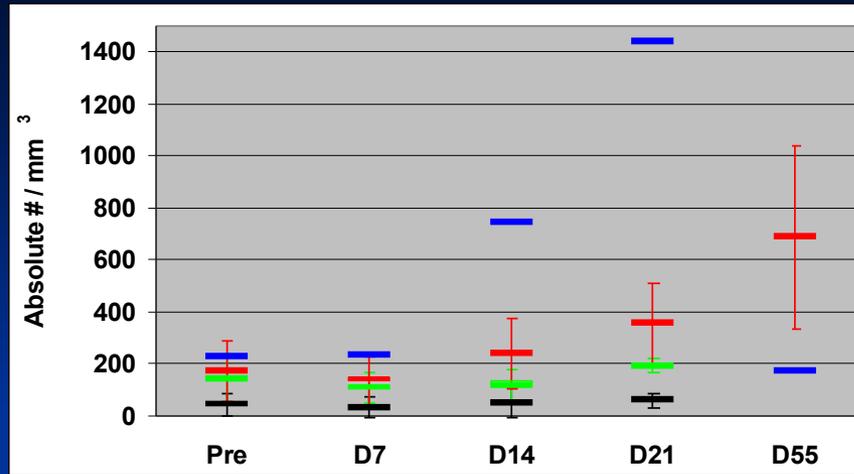
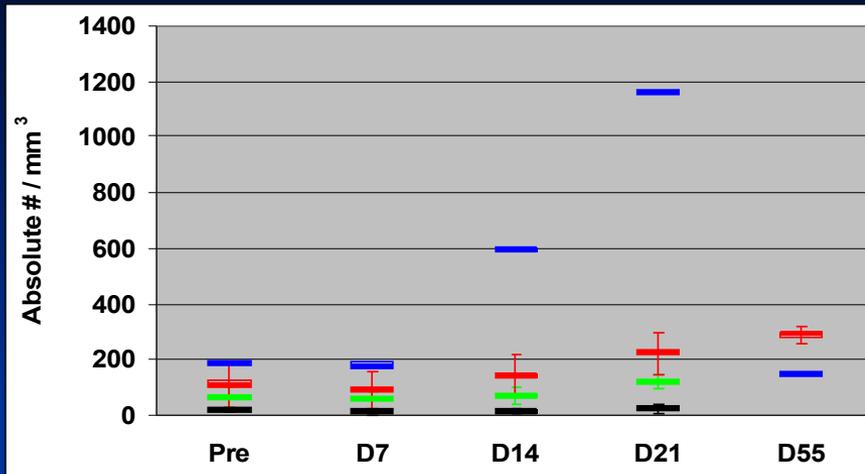
CD8+ / CD45RA+ / CD27+ (_____)

CD8+ / CD45RA- / CD27+ _____

CD8+ / CD45RA- / CD27- (_____)

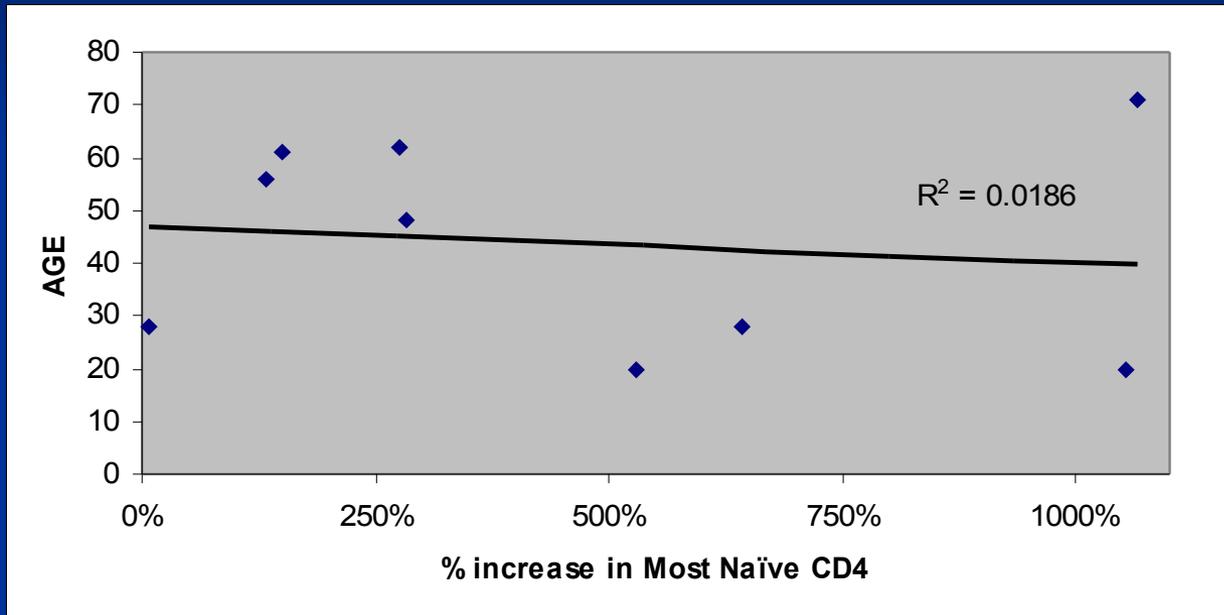
CD4⁺ / CD45RA⁺ / CD31⁺ (Most Naïve)

CD4⁺ / CD45RA⁺ / CD27⁺ (Naïve)

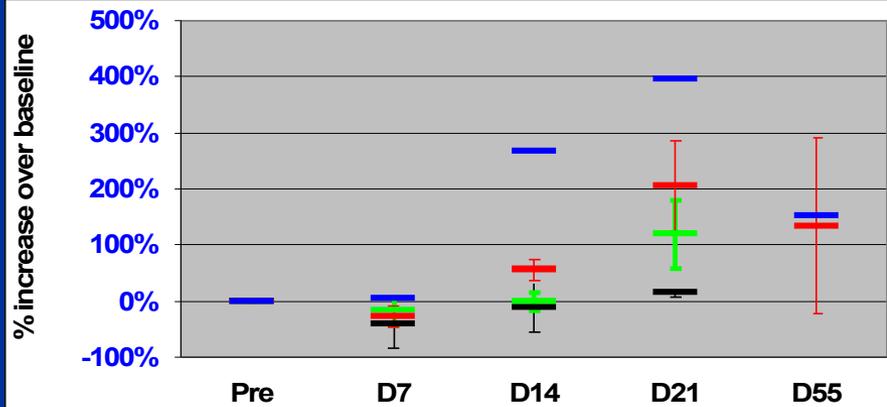
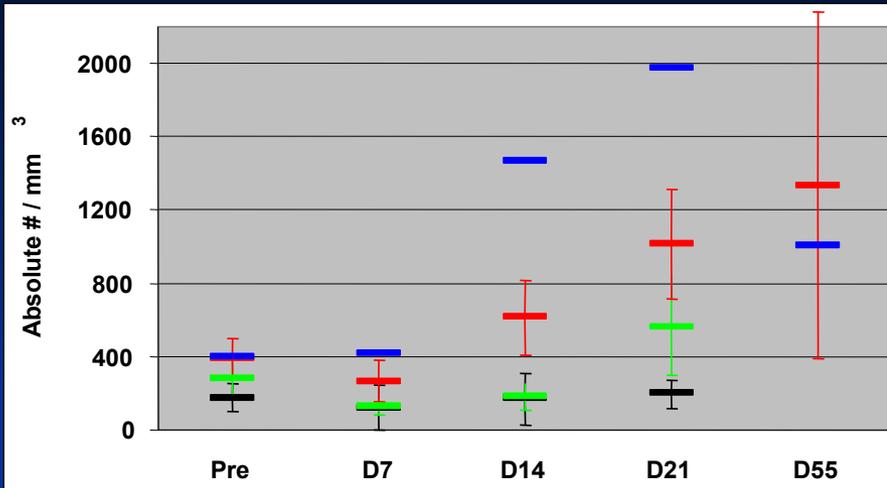


Day 14 = End of treatment
 — Mean "1" — Mean "2" — Mean "3" — Mean "4"

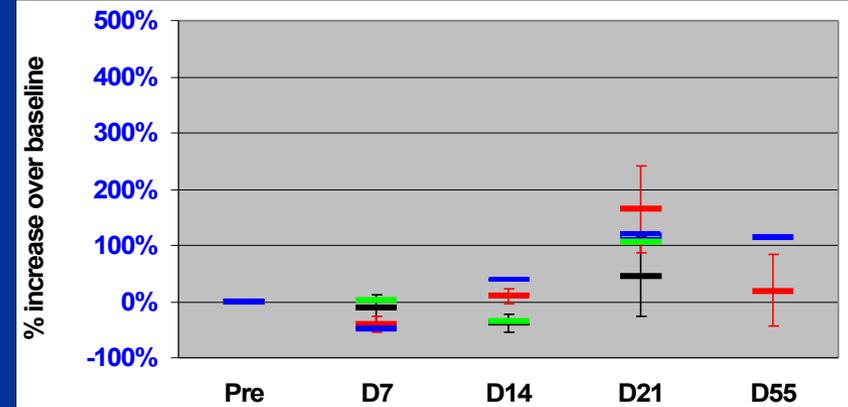
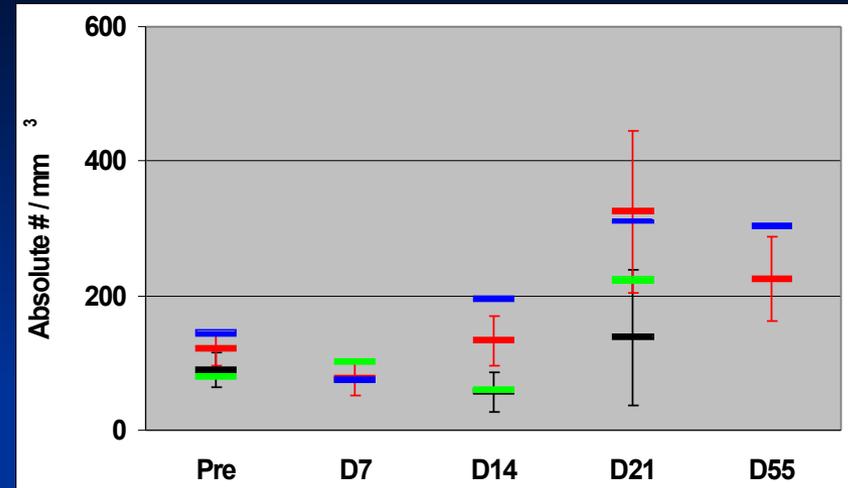
Correlation of age with Most Naïve CD4



CD4⁺ / CD45RA⁻ / CD27⁺ (*Memory*)



CD4⁺ / CD45RA⁻ / CD27⁻ (*Effector*)



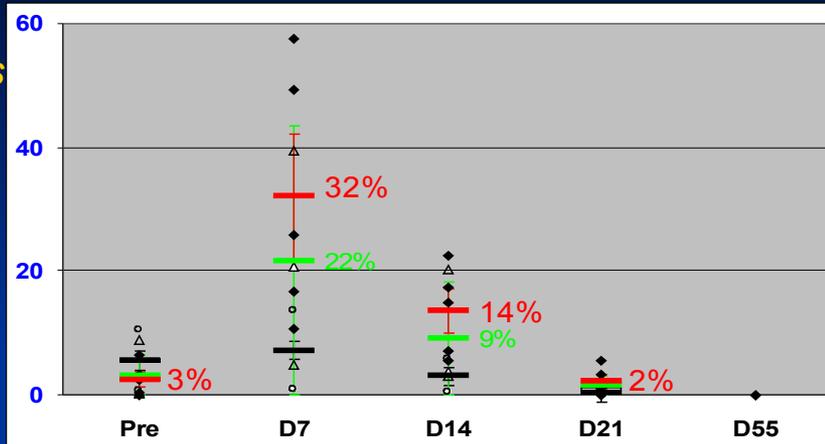
Day 14 = End of treatment

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

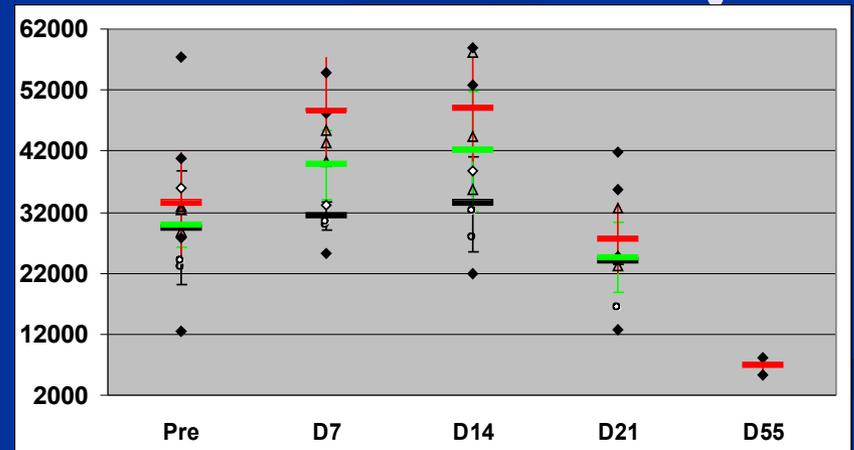
Kinetics of CD4+ Cell Cycling: Most naive

CD4⁺ / CD45RA⁺ / CD31⁺ & Ki67⁺

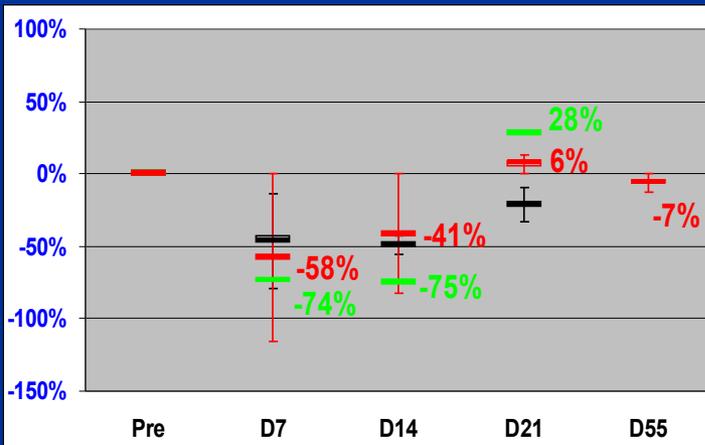
Percent of
Most Naive cells
in cycle



Bcl-2: Mean
Fluorescence
Intensity



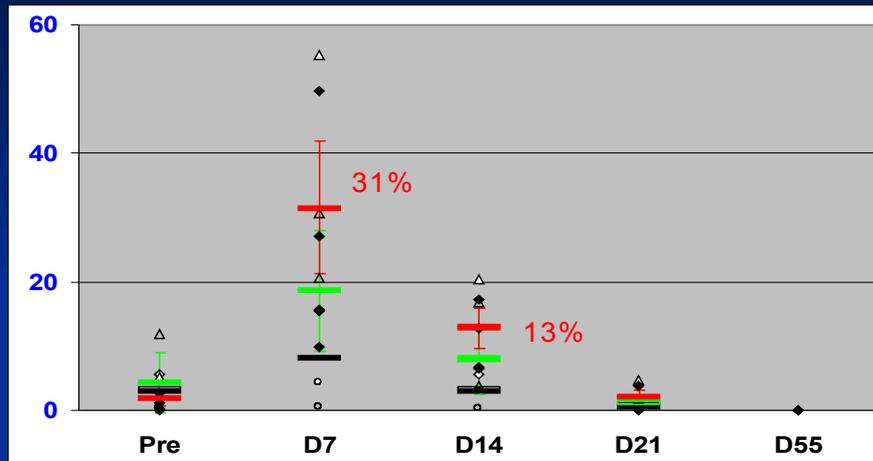
IL7-R mRNA
/ 10⁴
Actin mRNA



Kinetics of CD4⁺ Cell Cycling: Naive

CD4⁺ / CD45RA⁺ / CD27⁺ & Ki67⁺

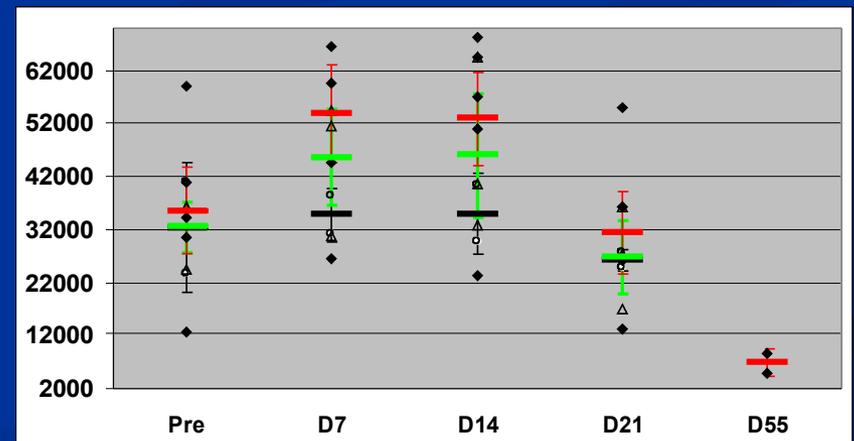
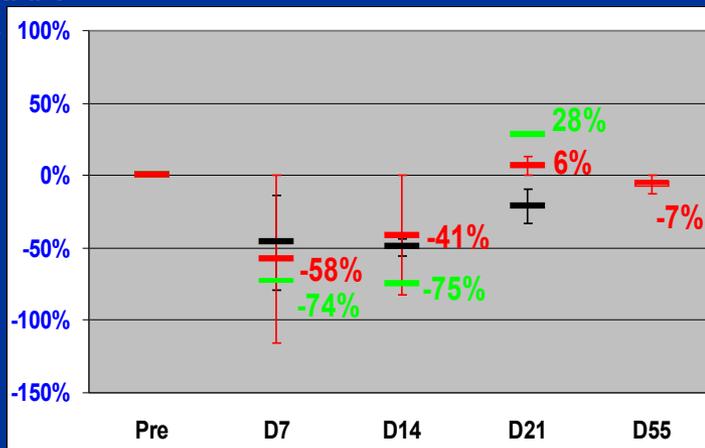
Percent of Naive cells in cycle



Bcl-2: Mean Fluorescence Intensity



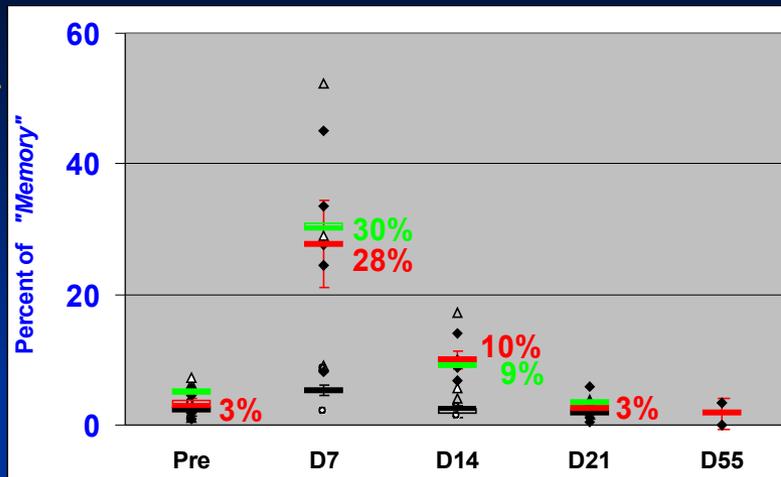
IL7-R mRNA / 10⁴ Actin mRNA



Kinetics of CD4⁺ Cell Cycling: Memory

CD4⁺ / CD45RA⁻ / CD27⁺ & Ki67⁺

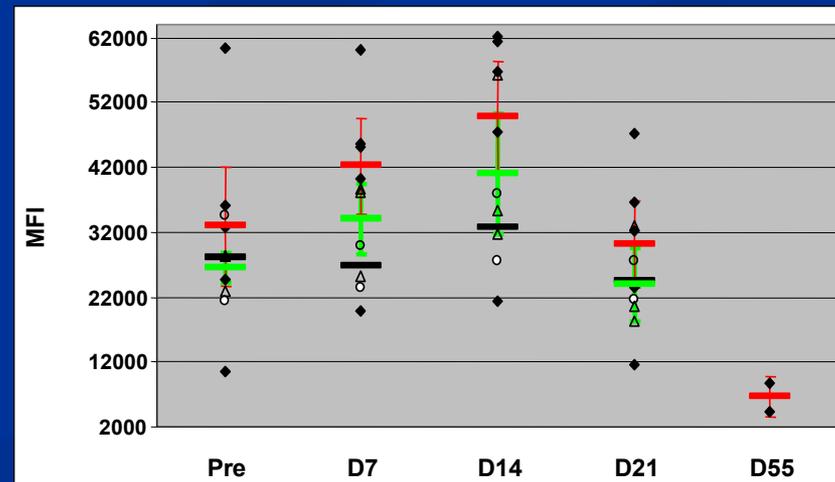
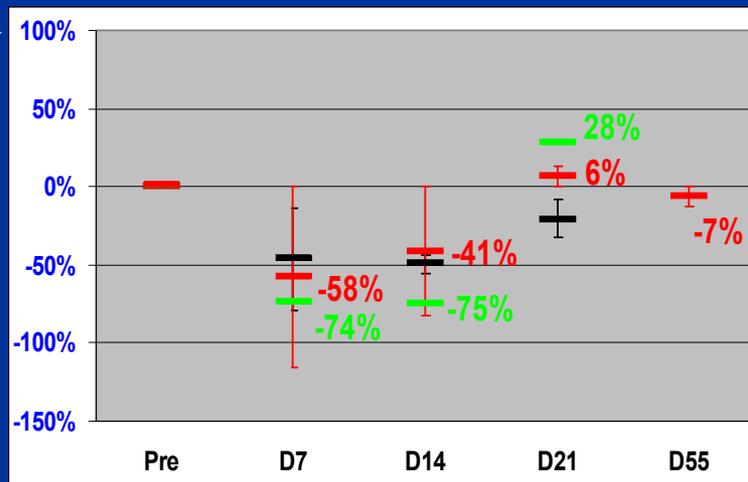
Percent of Memory cells in cycle



Bcl-2: Mean Fluorescence Intensity



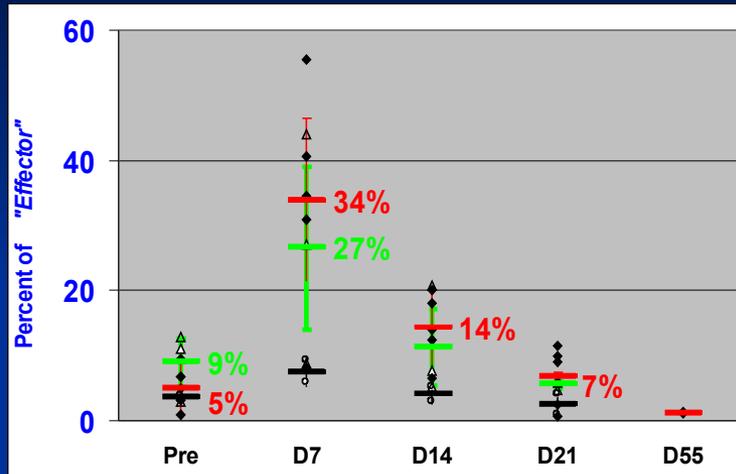
IL7-R mRNA / 10⁴ Actin mRNA



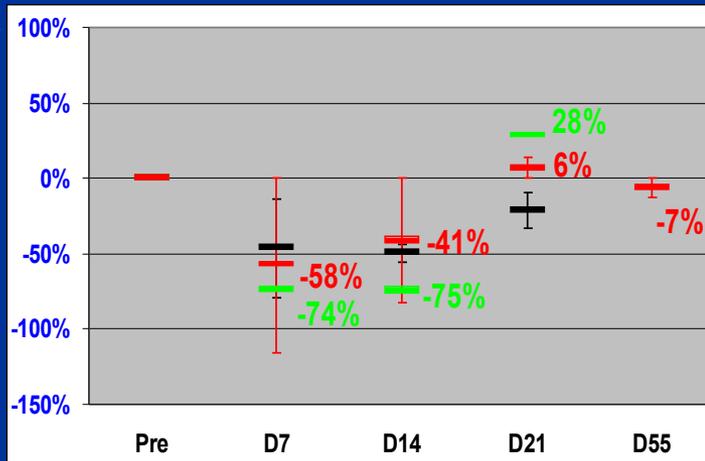
Kinetics of CD4⁺ Cell Cycling: Effectors

CD4⁺ / CD45RA⁻ / CD27⁻ & Ki67⁺

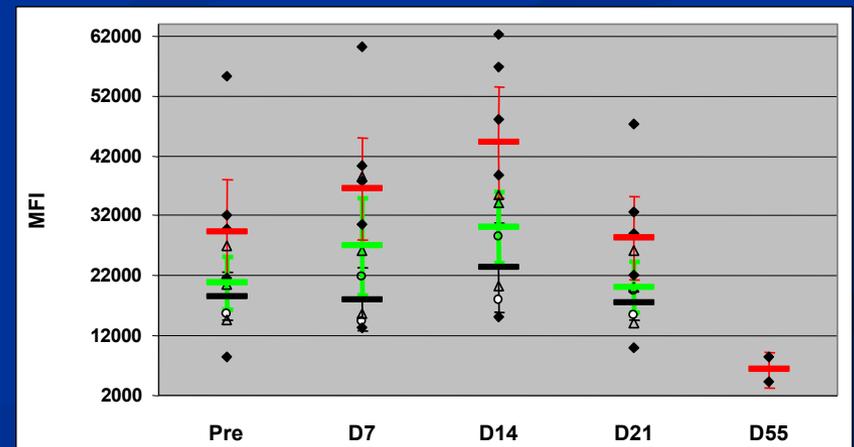
Percent of
Effectors in cycle



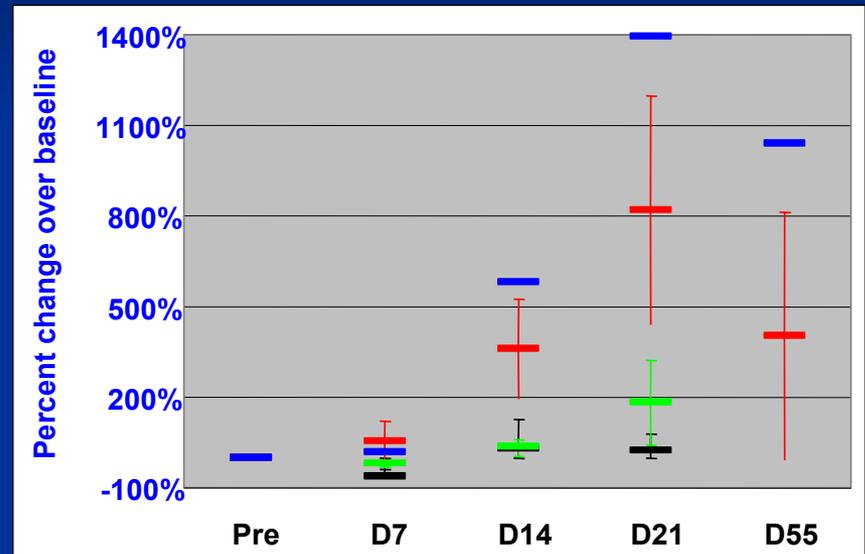
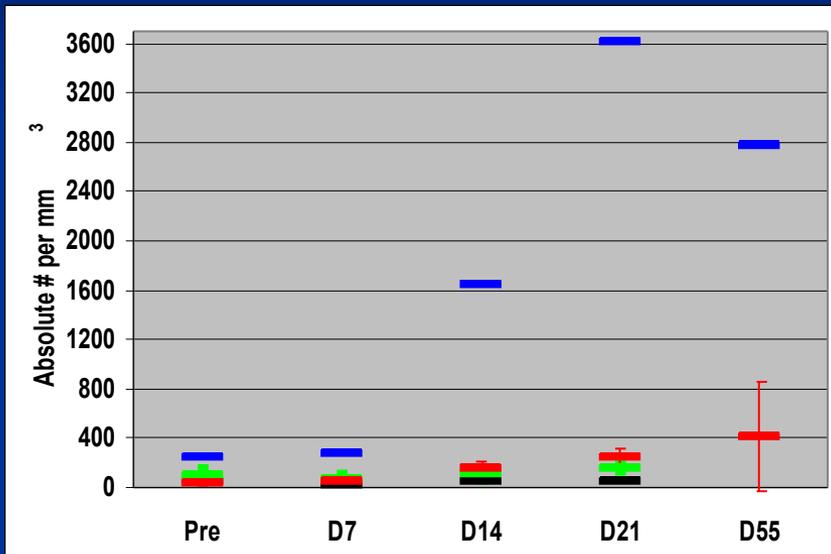
IL7-R mRNA
/ 10⁴
Actin mRNA



Bcl-2: Mean
Fluorescence
Intensity



CD8⁺ / CD45RA⁺ / CD27⁺ (Naïve)

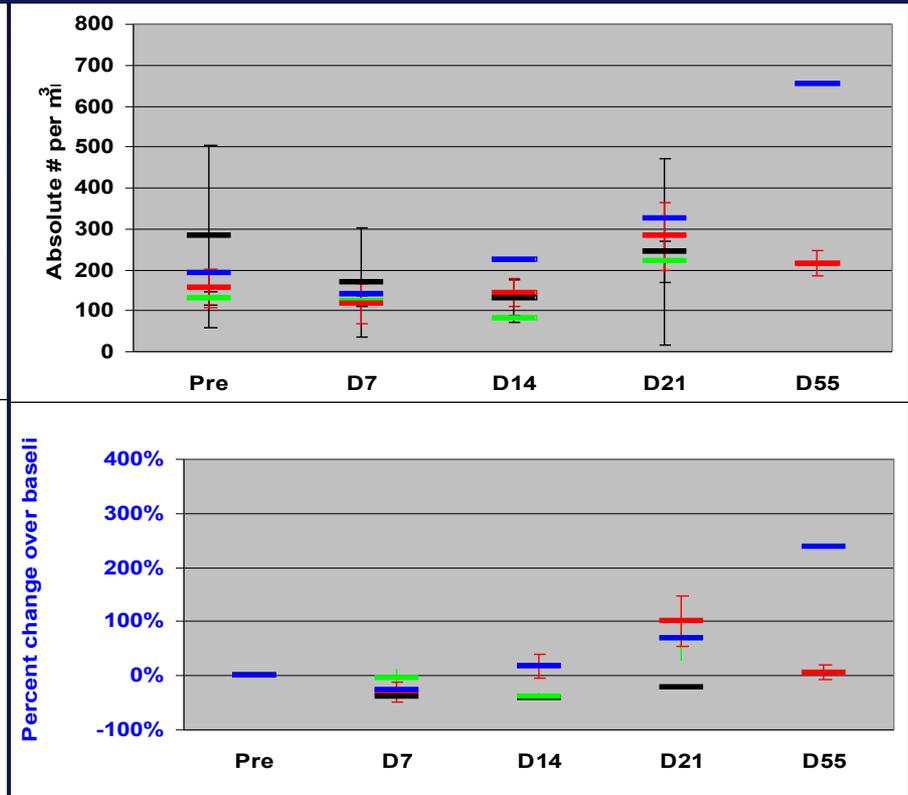
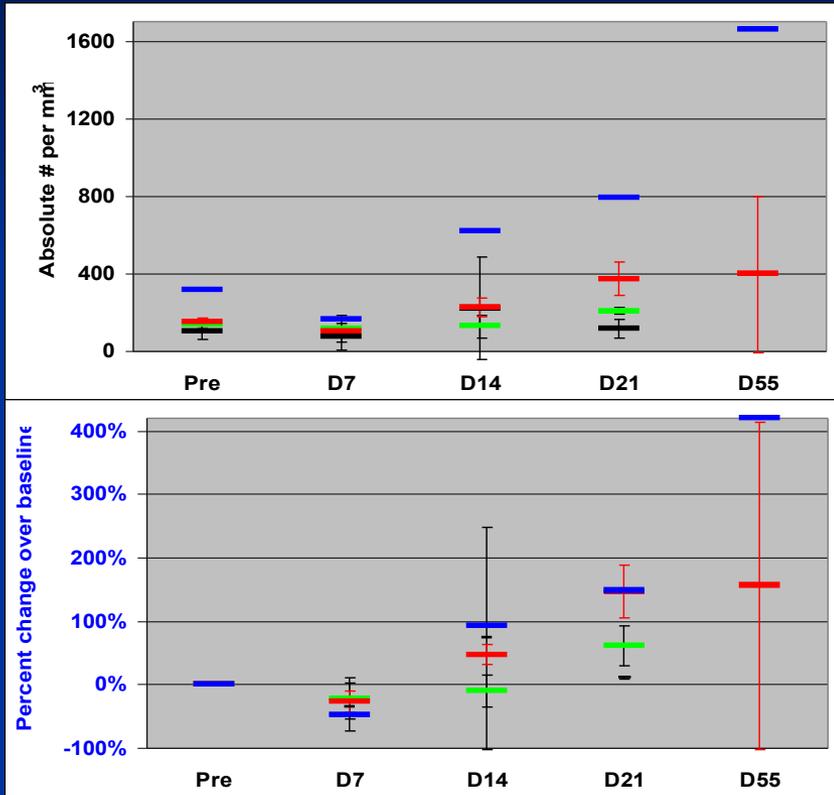


Day 14 = End of treatment
 — Mean "1" — Mean "2" — Mean "3" — Mean "4"



CD8⁺ / CD45RA⁻ / CD27⁺ (Memory)

CD8⁺ / CD45RA⁻ / CD27⁻ (Effector)

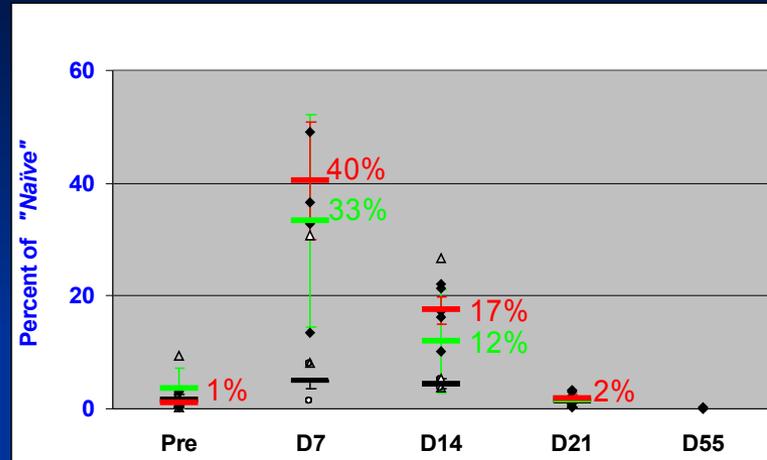


Day 14 = End of treatment
 — Mean "1" — Mean "2" — Mean "3" — Mean "4"

Kinetics of CD8⁺ Cell Cycling: Naive

CD8⁺ / CD45RA⁺ / CD27⁺ & Ki67⁺

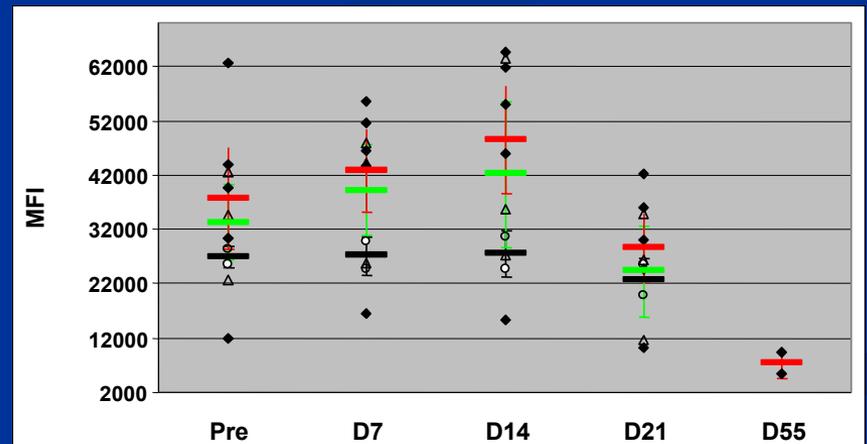
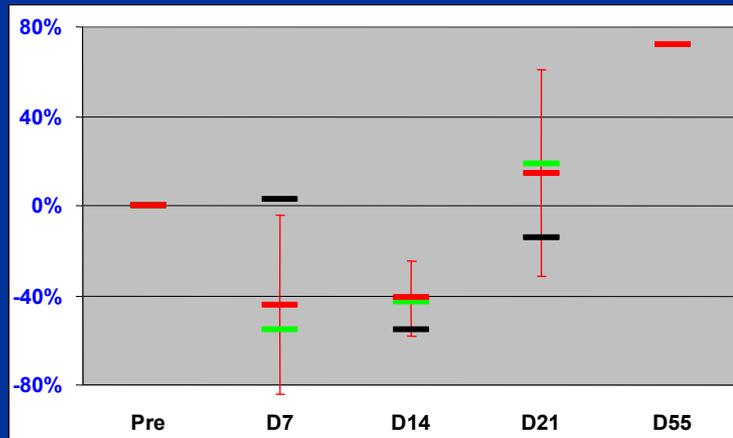
Percent of Naive cells in cycle



Bcl-2: Mean Fluorescence Intensity



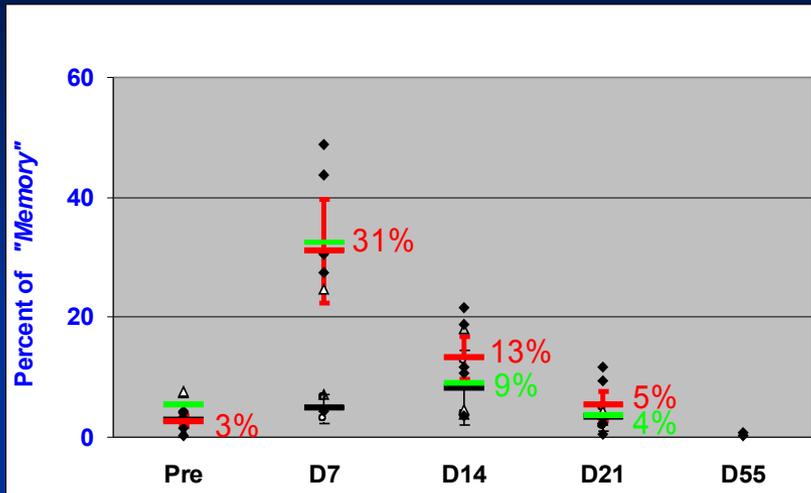
IL7-R mRNA / 10⁴ Actin mRNA



Kinetics of CD8⁺ Cell Cycling: Memory

CD8⁺ / CD45RA⁻ / CD27⁺ & Ki67⁺

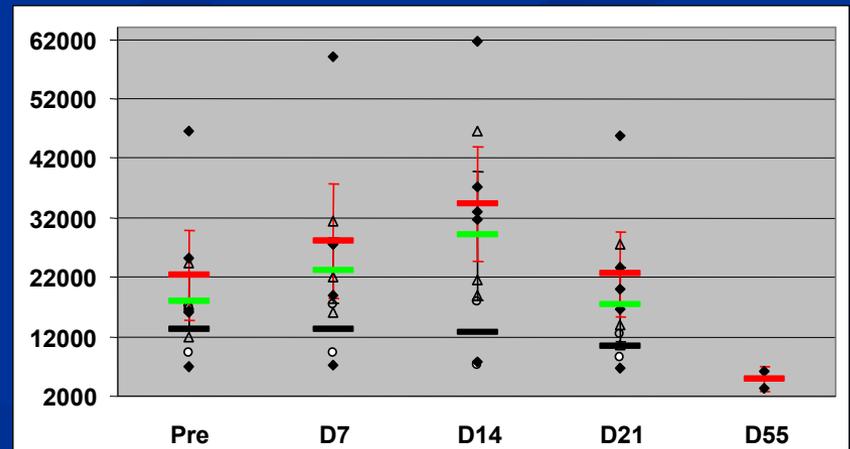
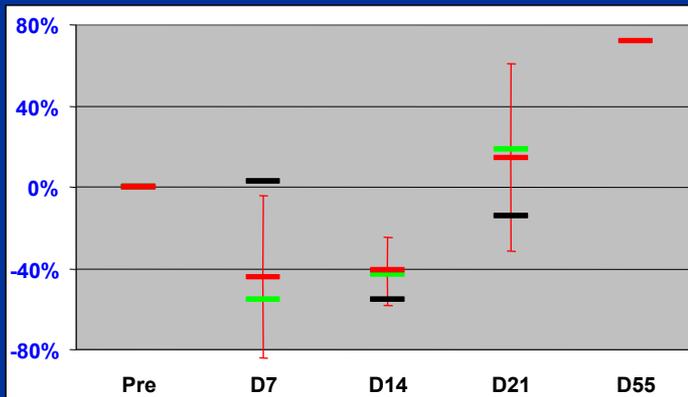
Percent of Memory cells in cycle



Bcl-2: Mean Fluorescence Intensity



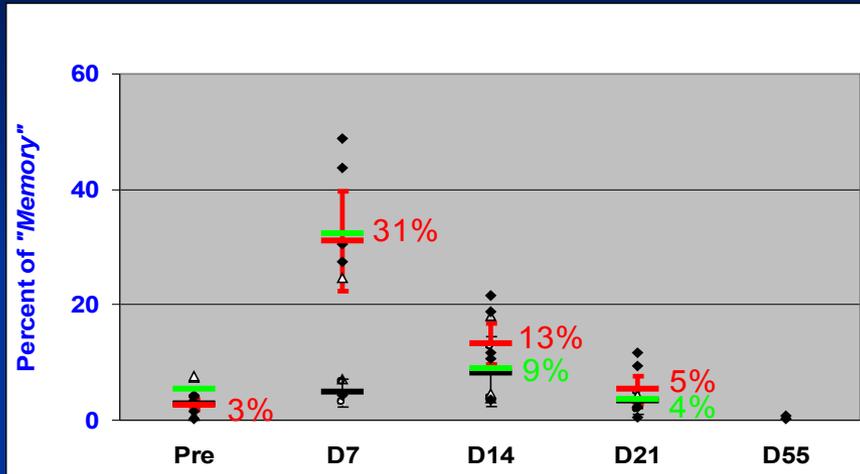
IL7-R mRNA / 10⁴ Actin mRNA



Kinetics of CD8⁺ Cell Cycling: Effectors

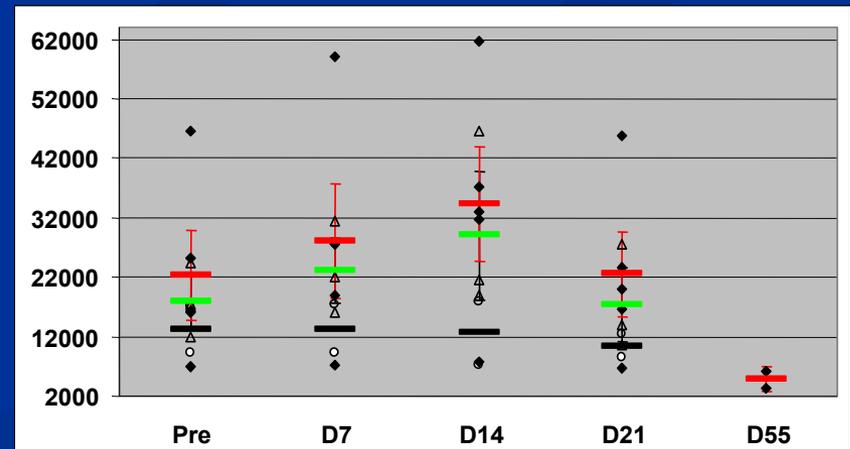
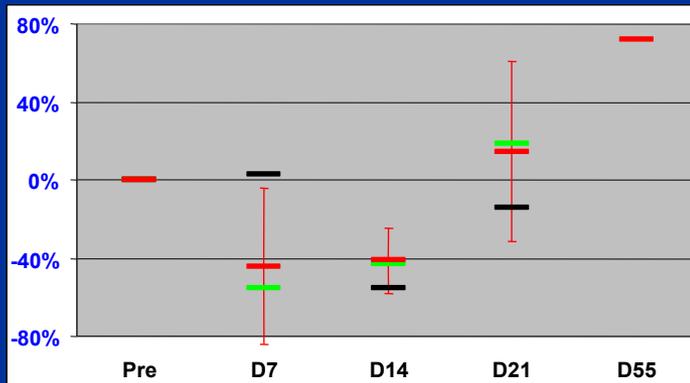
CD8⁺ / CD45RA⁻ / CD27⁻ & Ki67⁺

Percent of Memory cells in cycle

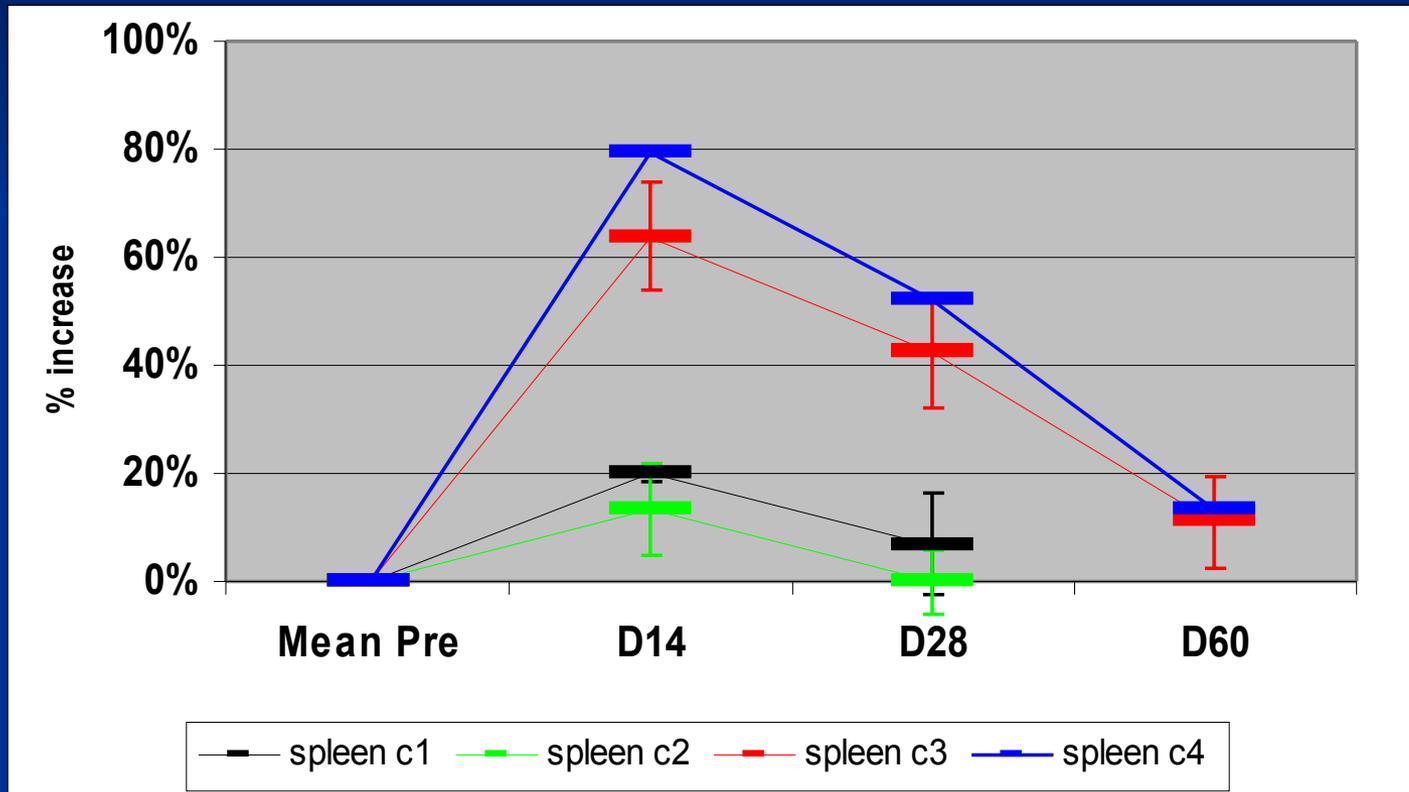


Bcl-2: Mean Fluorescence Intensity

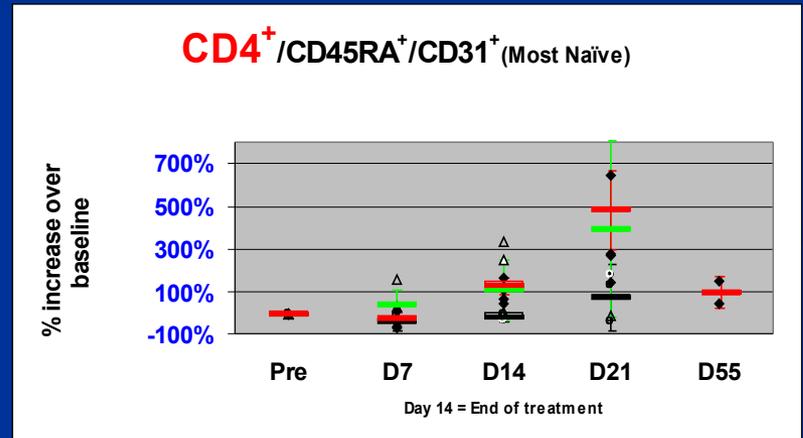
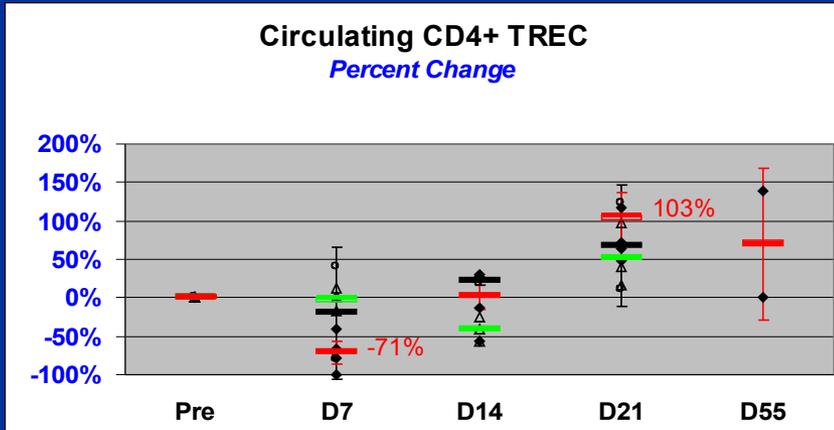
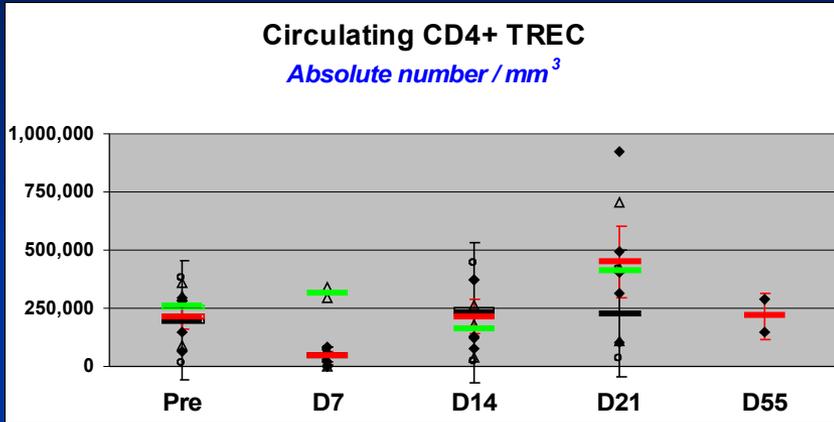
IL7-R mRNA / 10⁴ Actin mRNA



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

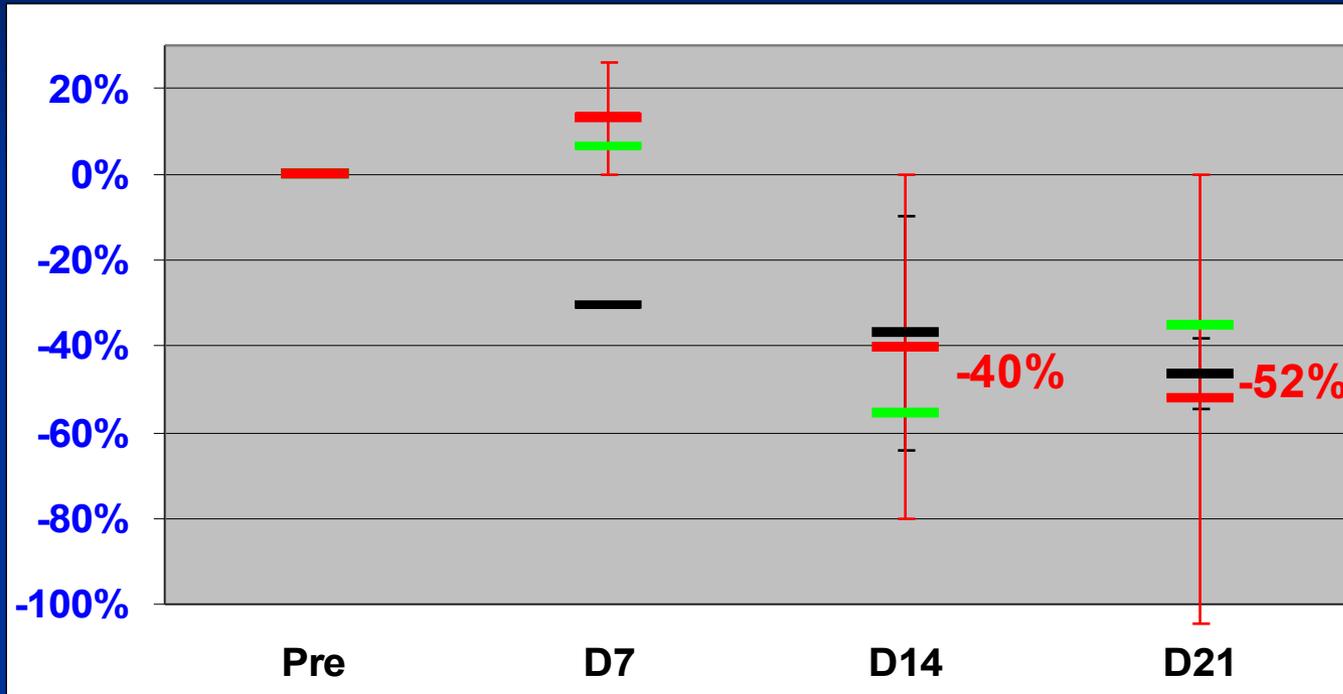


TREC analysis: CD4⁺



CD4 T-regs / Fox-P3

Percent change over baseline in
FoxP3 mRNA copies per 10^4 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 α 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 1st 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

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