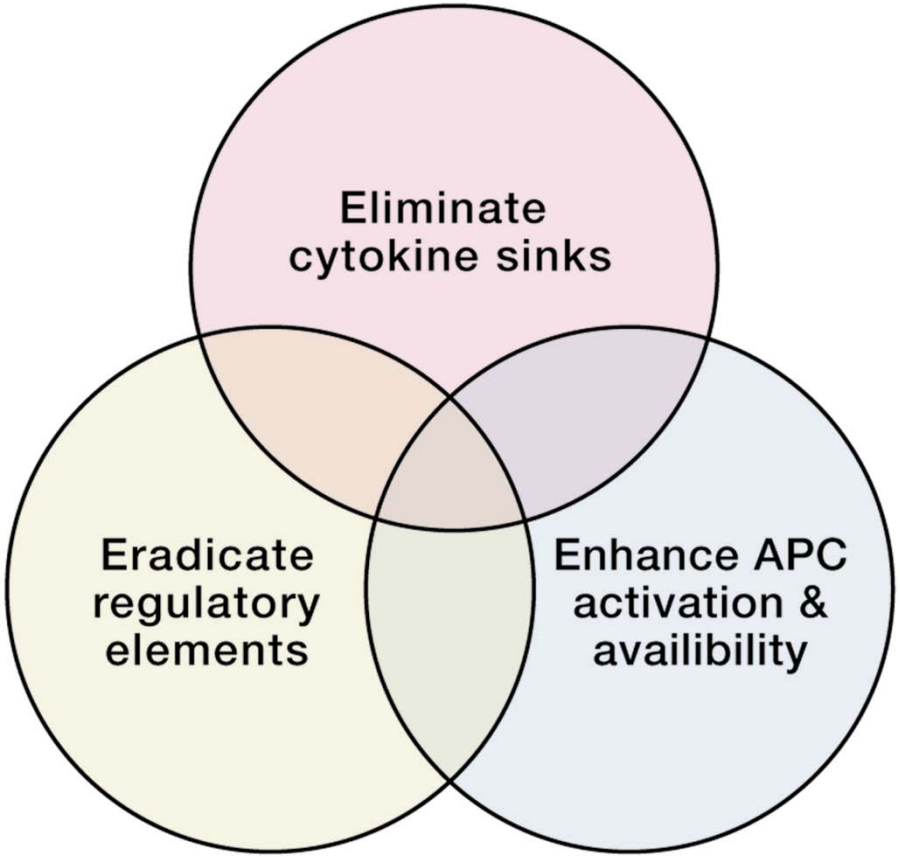
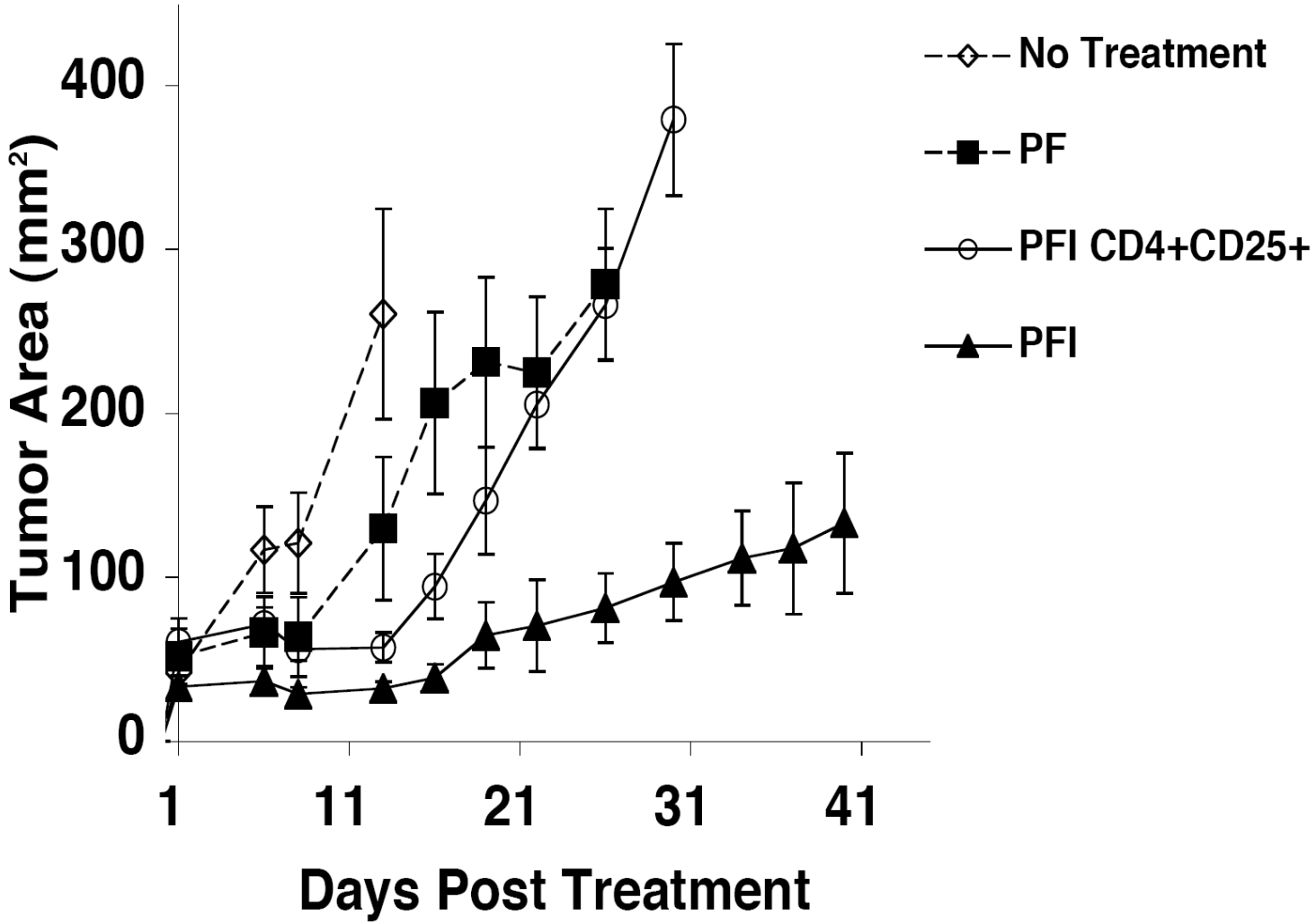
Mechanisms Underlying the Impact of Lymphodepletion on Adoptively Transferred T Cells



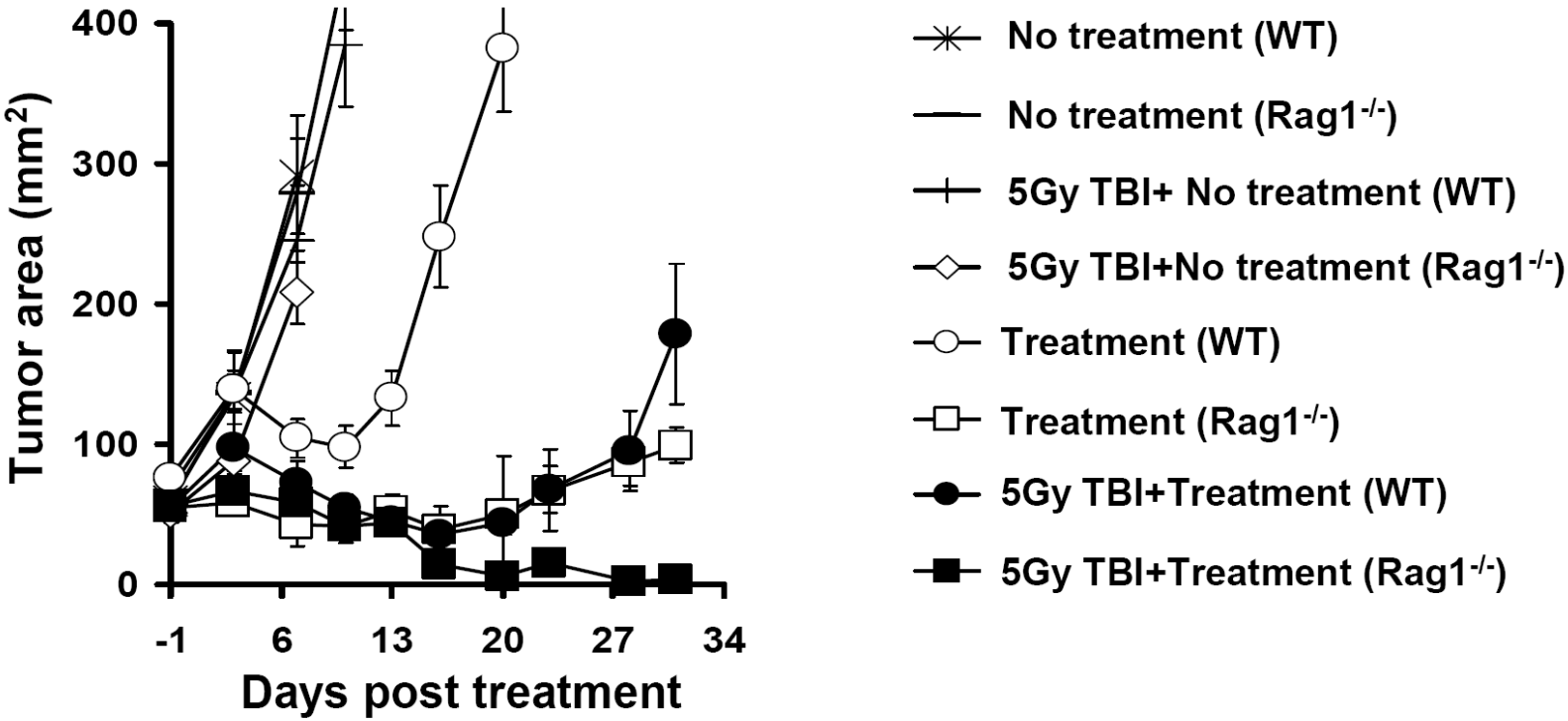
HOST LYMPHODEPLETION PROFOUNDLY ENHANCES THE ANTI-TUMOR EFFECT OF ADOPTIVELY TRANSFERRED CELLS IN MOUSE MODELS

1. **Effect is on the host – not the tumor**
2. **Elimination of T regulatory cells**
3. **Elimination of lymphocytes that are cytokine “sinks” (T cells and NK cells)**
4. **Host IL-7 and IL-15 are essential**
5. **Activation of host antigen presenting cells are critical (antibiotics partially impair effect of TBI)**

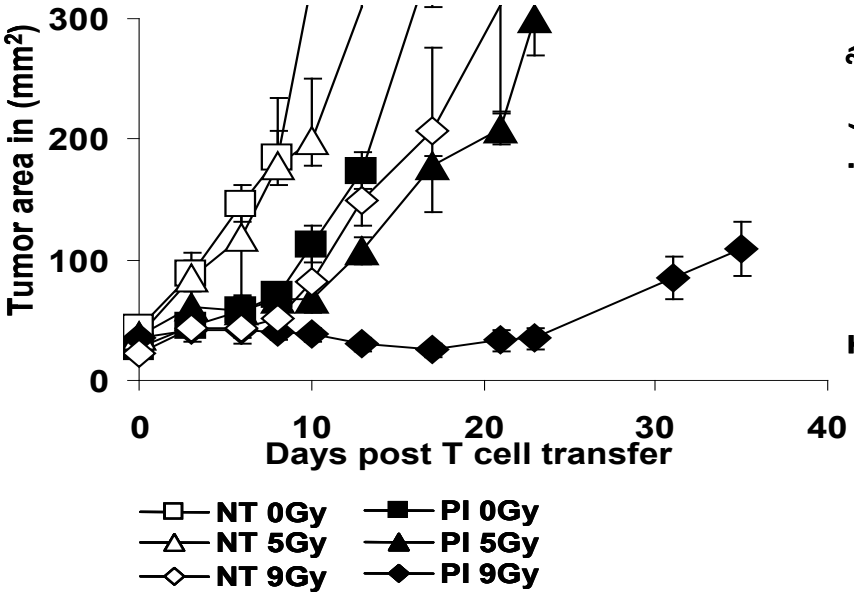
Addition of T Regulatory Cells Damages the Treatment Impact of Adoptively Transferred Cells

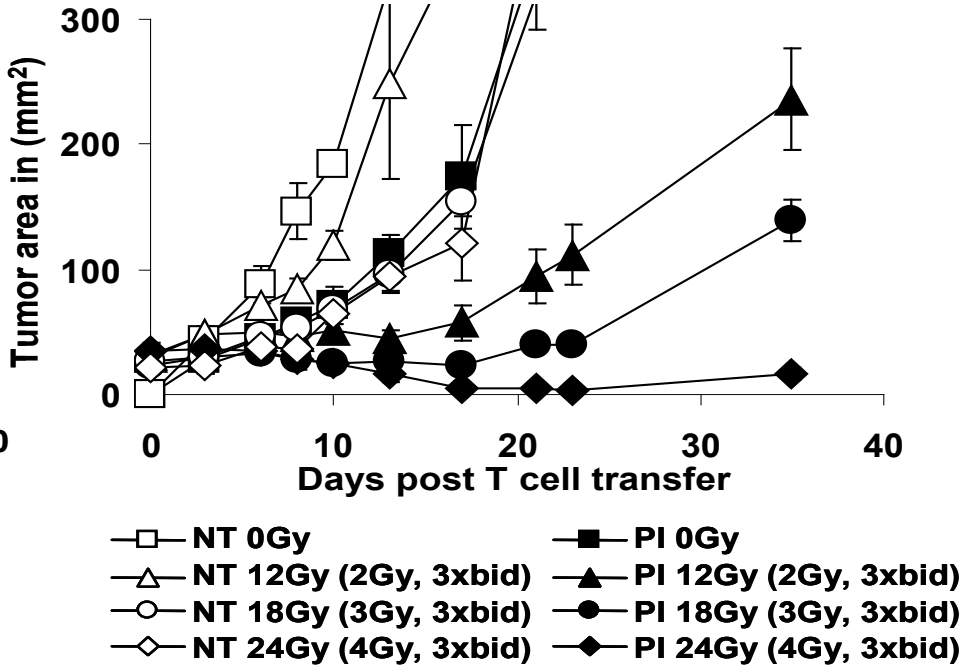


Total body irradiation augments T cell-based immunotherapy even in RAG1-/- mice

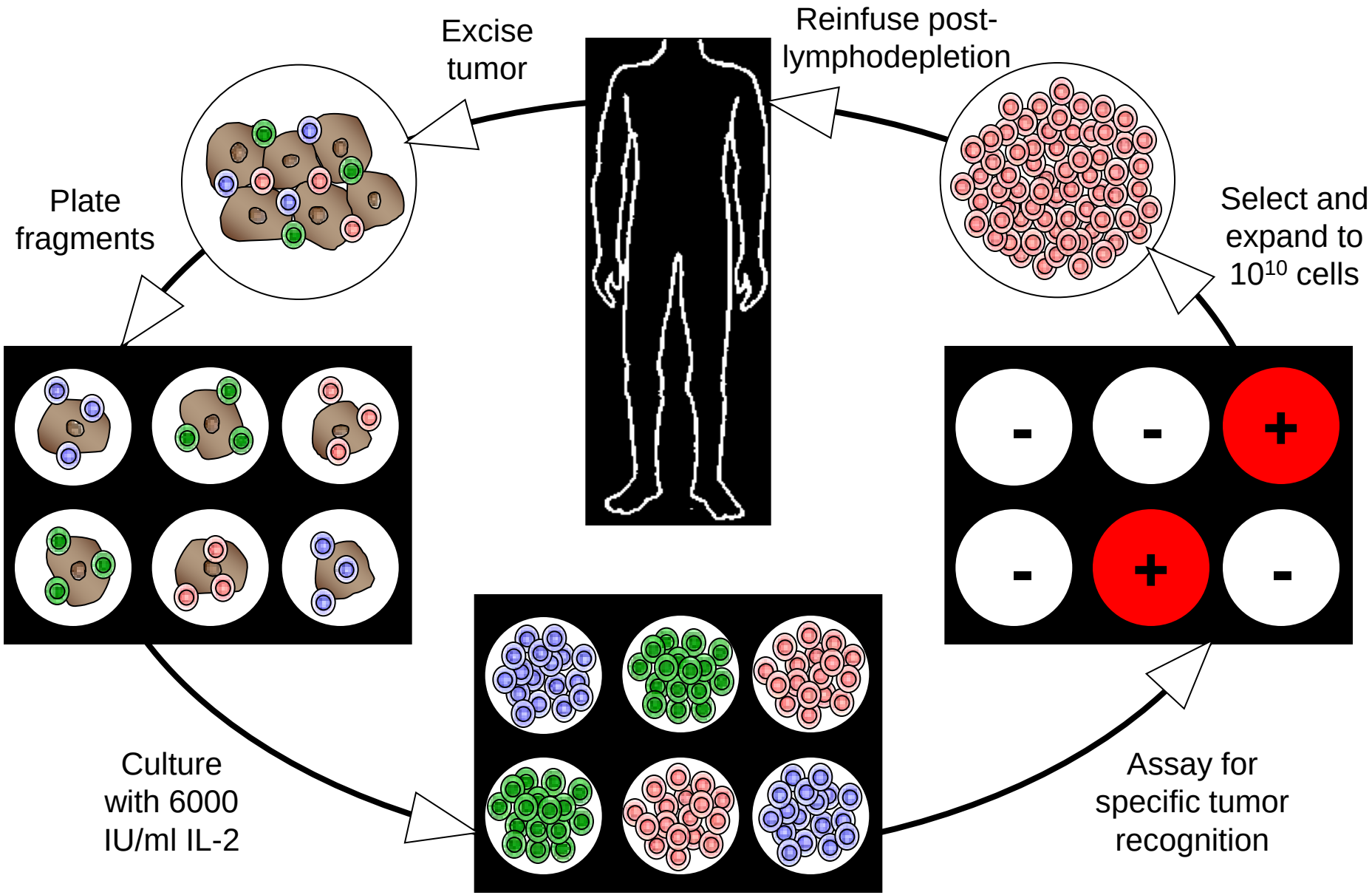


Direct Correlation Between the Amount of Irradiation and the Anti- tumor Effect





Adoptive transfer of tumor infiltrating lymphocytes (TIL)

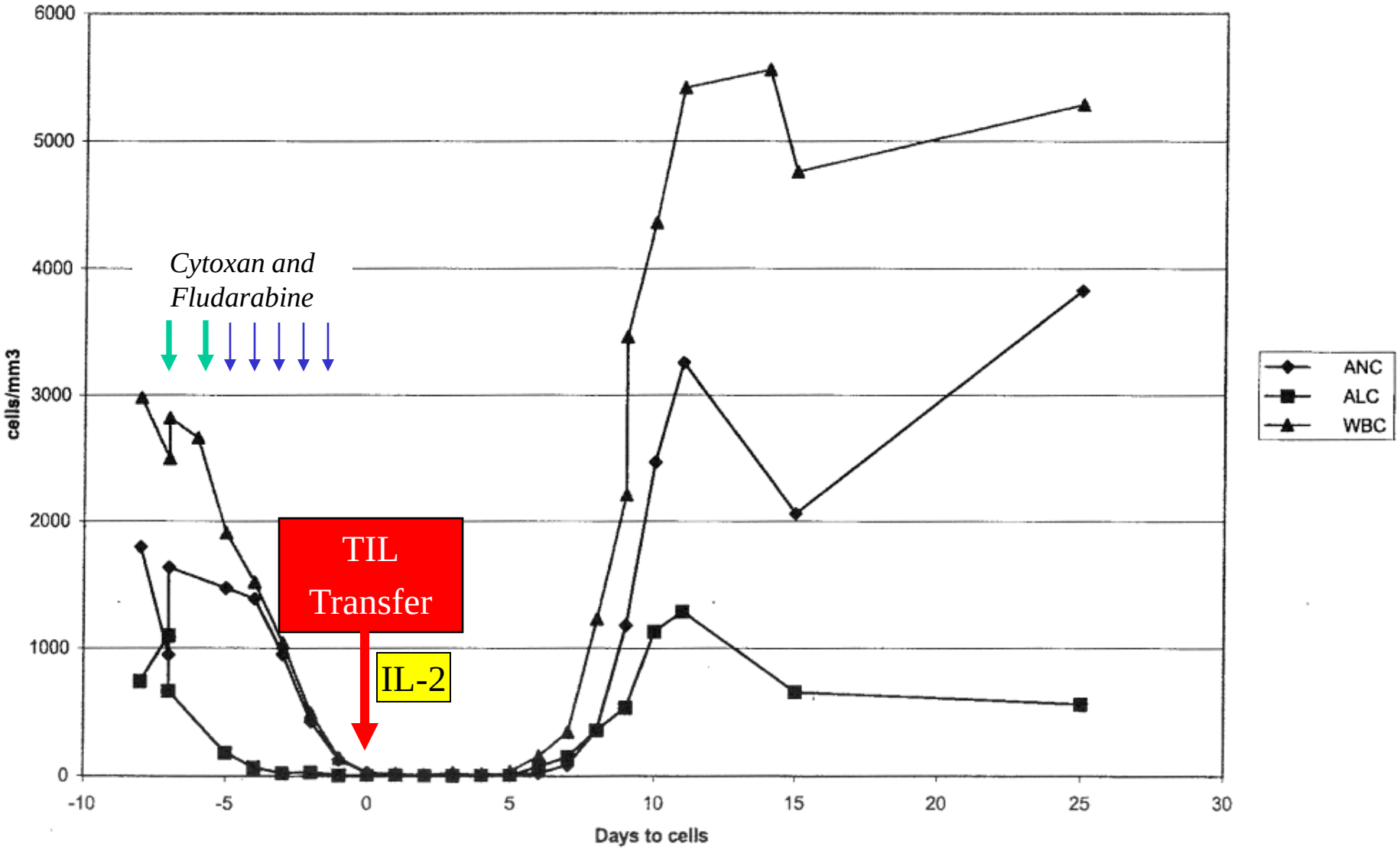


Preparative Regimens for Cell Transfer

	Days										
	-7	-6	-5	-4	-3	-2	-1	0	1	2	3
Non-myeloablative	Cy	Cy	Flu	Flu	Flu	Flu	Flu				
								Cells			
									IL-2	IL-2	IL-2

Cy: Cyclophosphamide 60 µg/kg
Flu: Fludarabine 25 mg/m^2
IL-2: 720,000 IU/kg q8h
Cells: Autologous TIL (1-5 x 10^{10})
CD34$^+$: $\geq$2 x 10^6/kg
TBI: 200 cGy total body irradiation

Integrating Preparative Nonmyeloablative Chemotherapy With Adoptive T-Cell Transfer



Cell Transfer Therapy

(5/1/10)

Treatment	Total	PR	CR	OR (%)
No TBI	43	16 (84, 36, 29, 28, 14, 13, 11, 8 8, 7, 4, 3, 3, 2, 2, 2)	5 (82+, 78+, 76+, 75+, 61+)	21 (49%)

Preparative Regimens for Cell Transfer

	Days										
	-7	-6	-5	-4	-3	-2	-1	0	1	2	3
Non-myeloablative	Cy	Cy	Flu	Flu	Flu	Flu	Flu				
								Cells			
									IL-2	IL-2	IL-2
Ablative		Cy	Cy								
		Flu	Flu	Flu	Flu	Flu					
							TBI				
								Cells			
									IL-2	IL-2	IL-2
									CD34+		

Cy: Cyclophosphamide 60 µg/kg
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Cell Transfer Therapy

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No TBI	43	16 (84, 36, 29, 28, 14, 13, 11, 8 8, 7, 4, 3, 3, 2, 2, 2)	5 (82+, 78+, 76+, 75+, 61+)	21 (49%)
200 TBI	25	10 (57+, 51+, 14, 9 6, 6, 5, 4 3, 3)	3 (65+, 61+, 54+)	13 (52%)

Preparative Regimens for Cell Transfer

	Days										
	-7	-6	-5	-4	-3	-2	-1	0	1	2	3
Non-myeloablative	Cy	Cy	Flu	Flu	Flu	Flu	Flu				
								Cells			
								IL-2	IL-2	IL-2	
Ablative		Cy	Cy								
(200cGy)		Flu	Flu	Flu	Flu	Flu					
							TBI				
								Cells			
								IL-2	IL-2	IL-2	
									CD34+		
Ablative	Cy	Cy									
(1200cGy)	Flu	Flu	Flu	Flu	Flu						
					TBI	TBI	TBI				
								Cells			
								IL-2	IL-2	IL-2	IL-2
									CD34+		

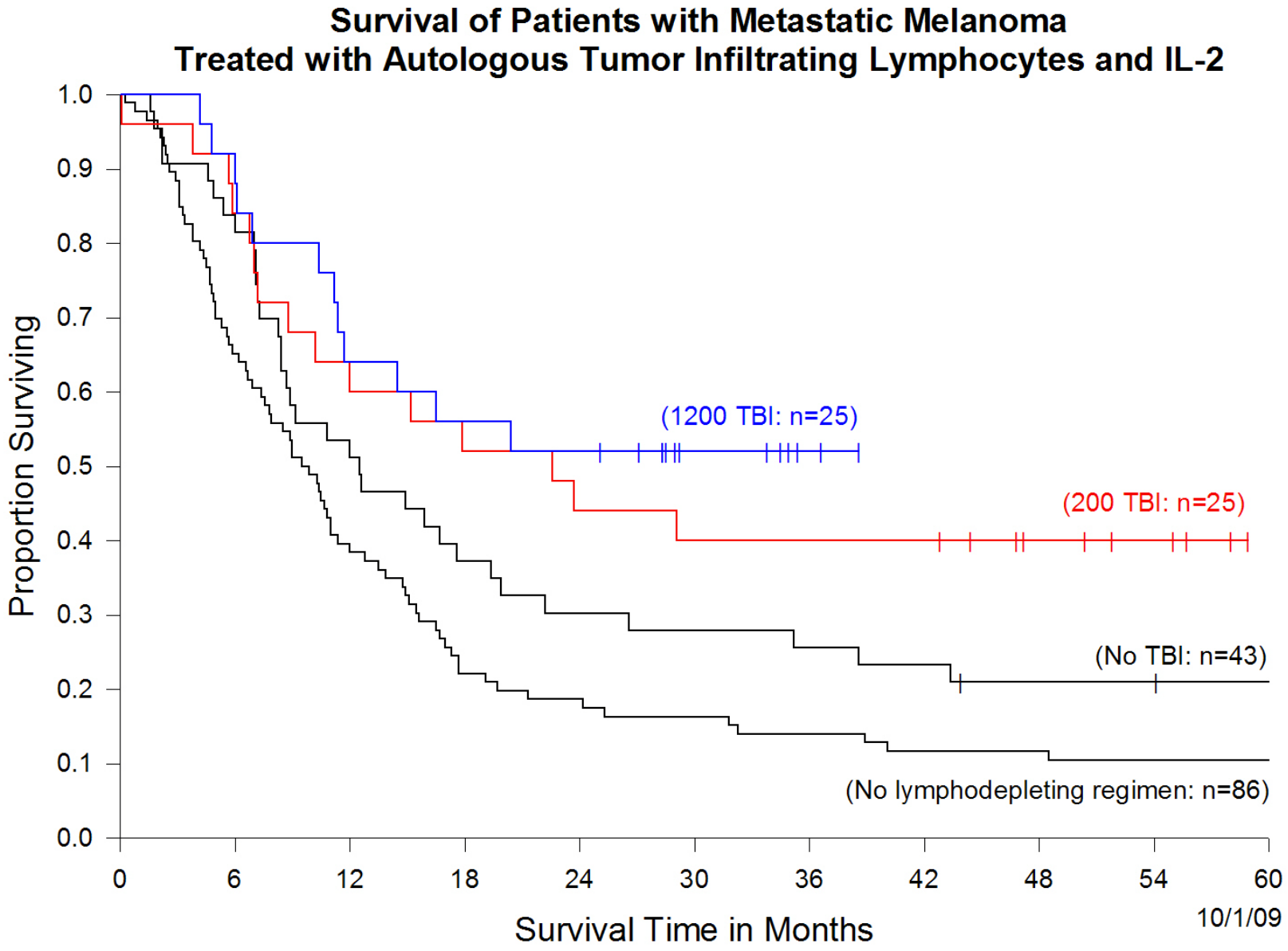
Cell Transfer Therapy

(5/1/10)

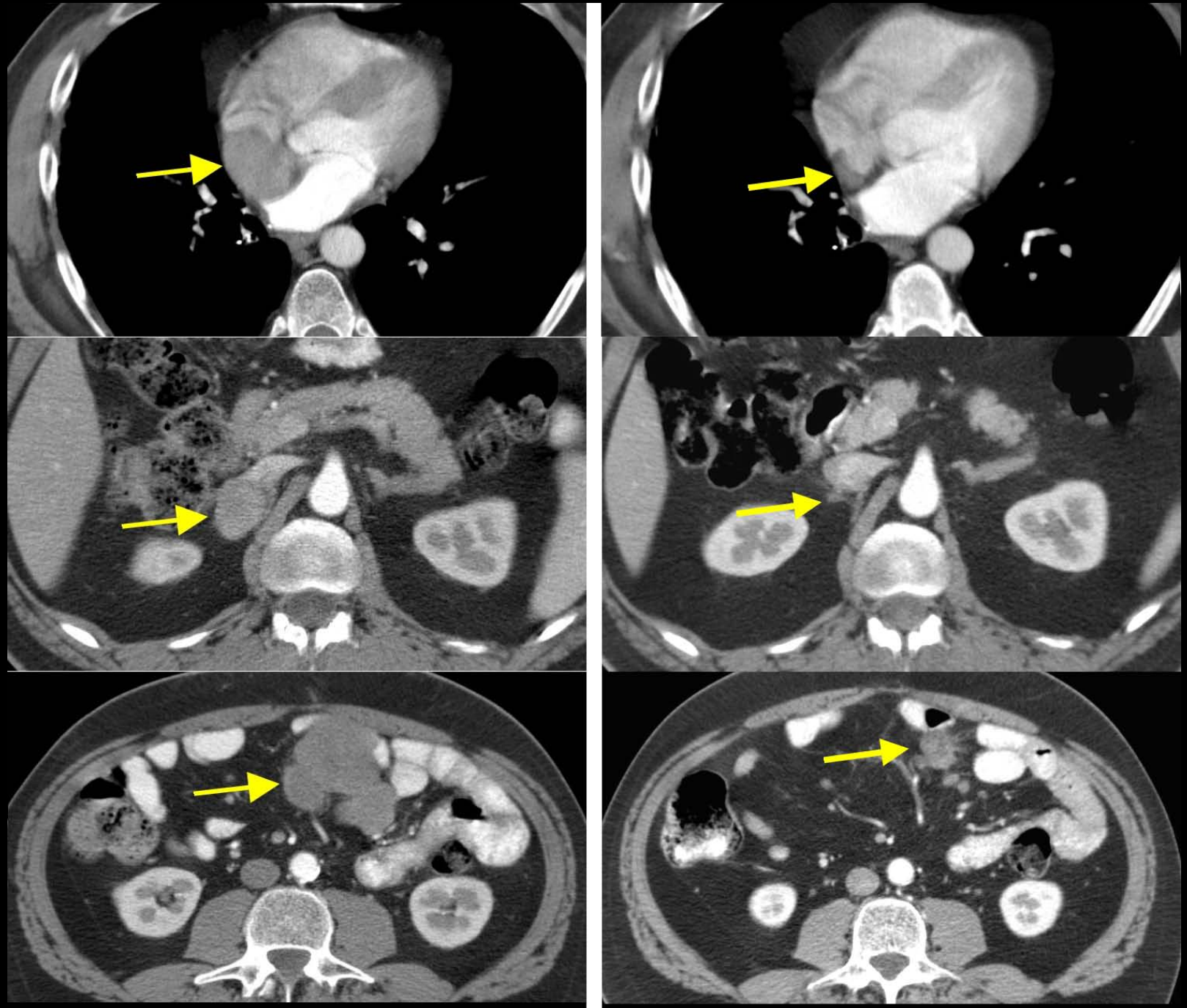
Treatment	Total	PR	CR	OR (%)
No TBI	43	16 (84, 36, 29, 28, 14, 13, 11, 8 8, 7, 4, 3, 3, 2, 2, 2)	5 (82+, 78+, 76+, 75+, 61+)	21 (49%)
200 TBI	25	10 (57+, 51+, 14, 9 6, 6, 5, 4 3, 3)	3 (65+, 61+, 54+)	13 (52%)
1200TBI	25	10 (42+, 35+, 21, 13, 7, 6, 6 5 3, 2)	8 (45+, 41+, 41+, 36+, 35+, 35+, 34+, 19)	18(72%)

(52 responding patients: 42 had prior IL-2, 21 had prior IL-2 + chemotherapy)

(16 complete responses: 15 ongoing at 34 to 82 months)


Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
(1200 TBI: n=25)
(200 TBI: n=25)
(No TBI: n=43)
(No lymphodepleting regimen: n=86)
0
6
12
18
24
30
36
42
48
54
60
Survival Time in Months
10/1/09

R.K.
(NMA)



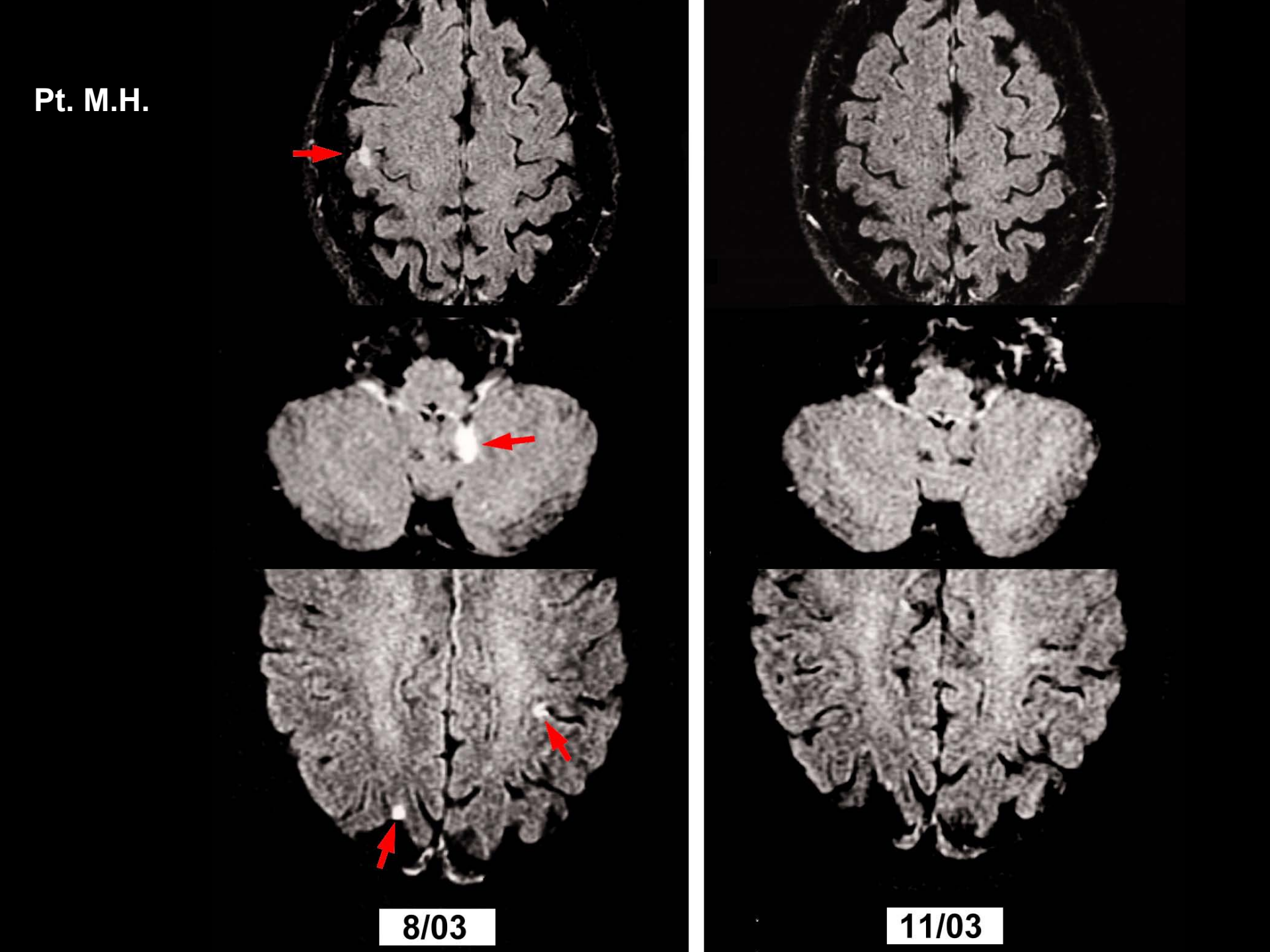
Pre-Treatment

14 Months

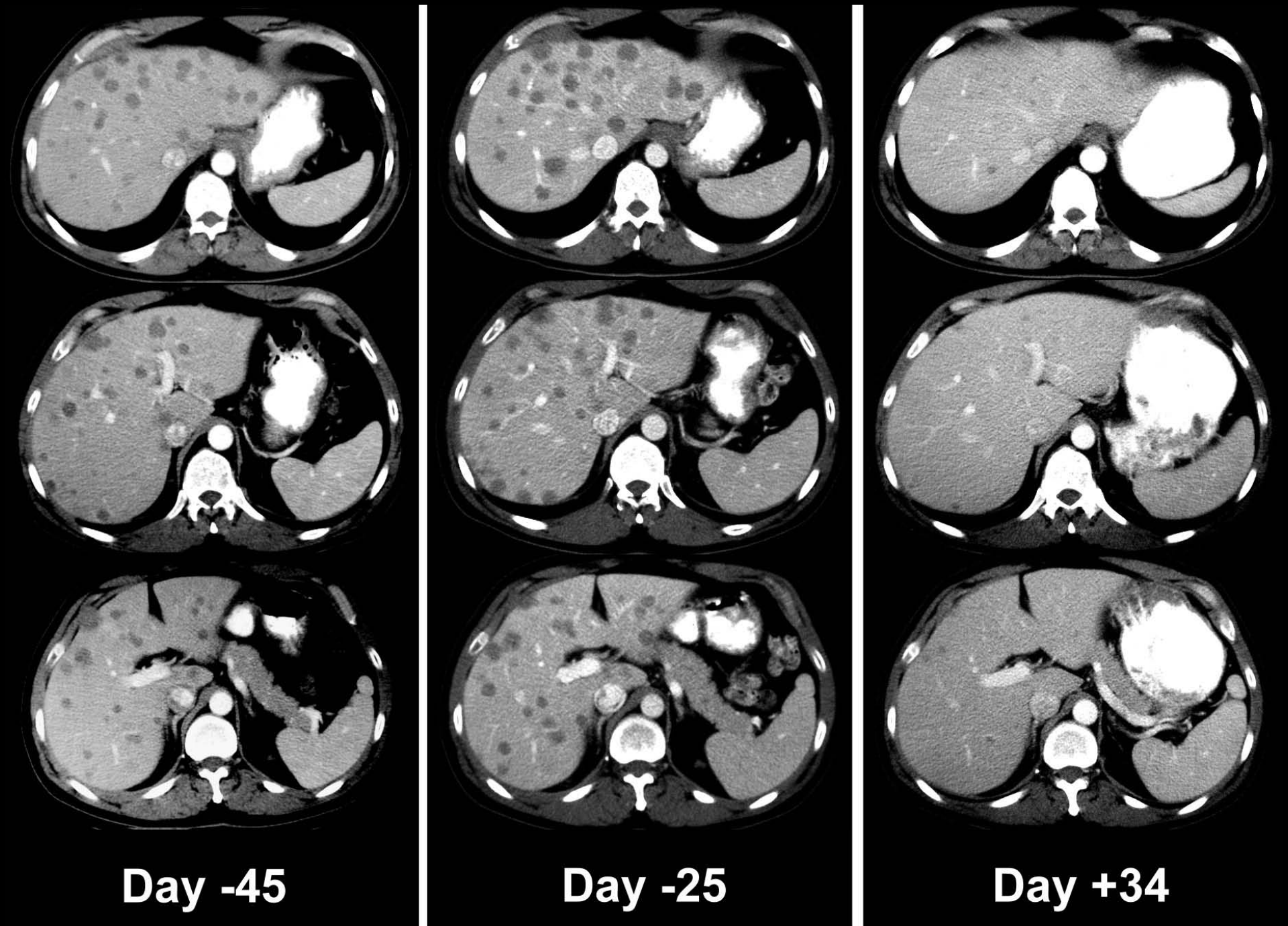
C.K. (200cGy) Pre 12 days

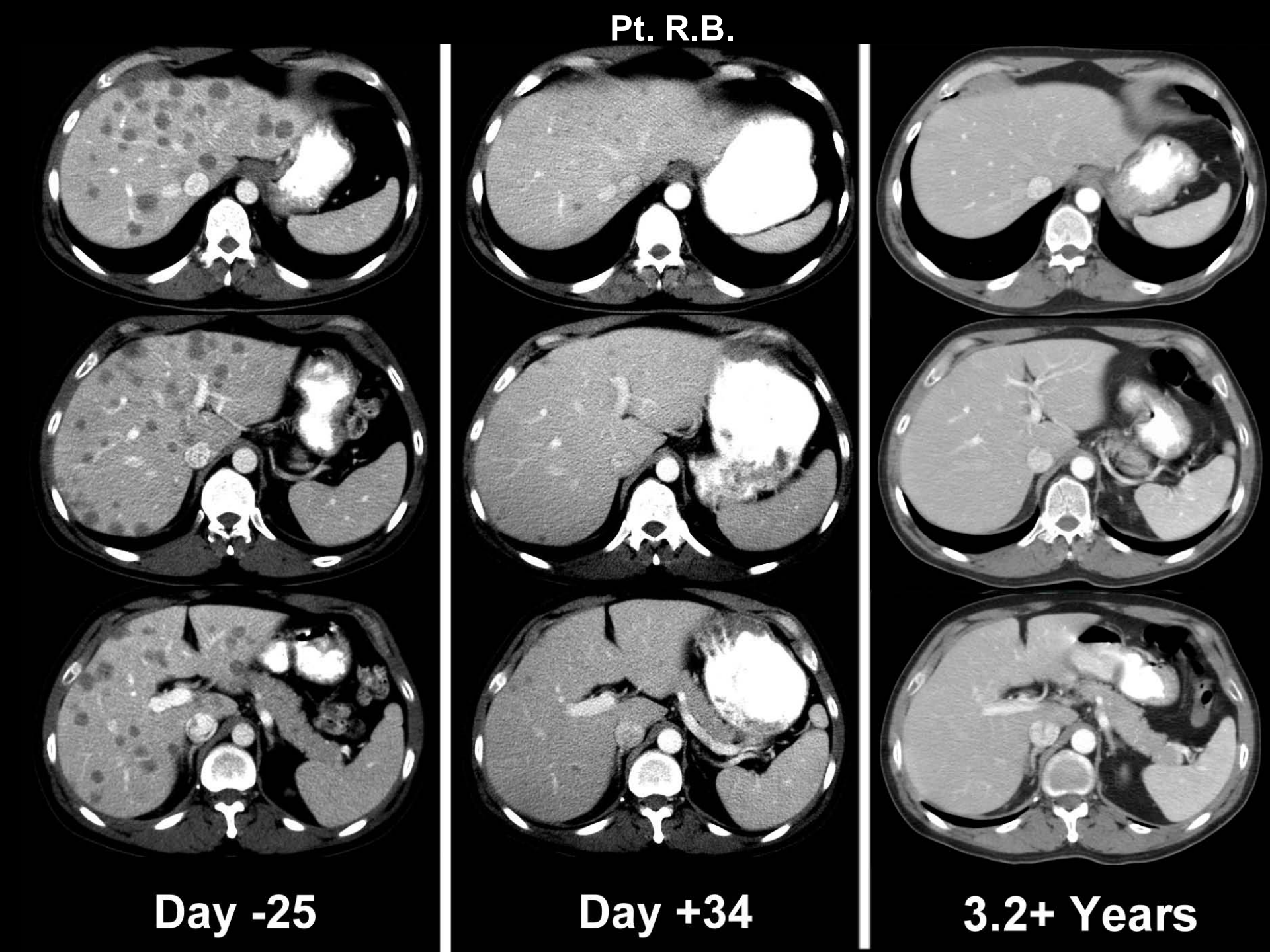
Pt. M.H.

Pt. M.H.



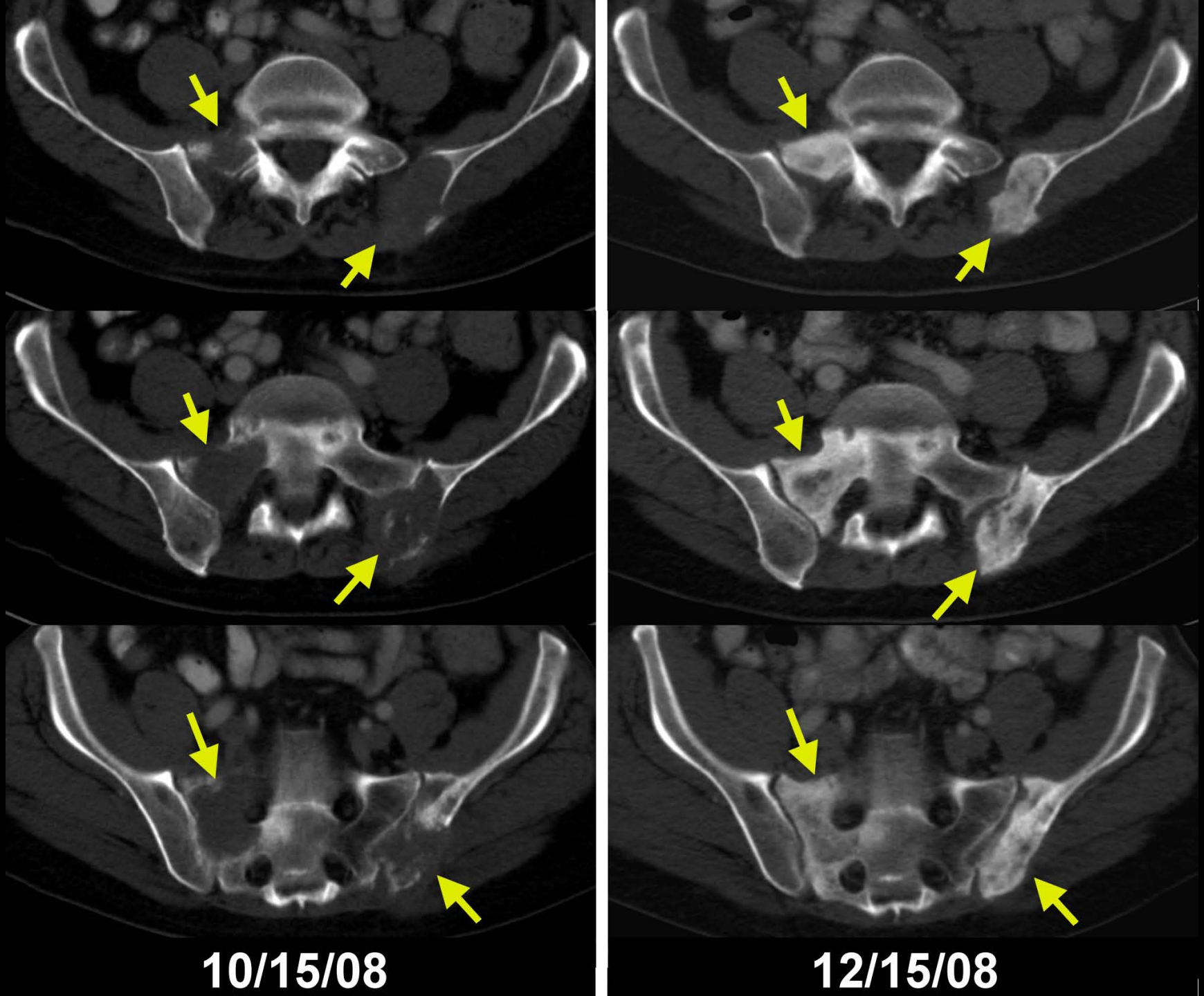
Pt.R.B.




Pt. R.B.
Day -25
Day +34
3.2+ Years

E.H.

Young TIL; CD8



A.H.: N-M cell transfer

March, 2005

April, 2008

Hypothesis of Mechanism of Cancer Regression Following Cell Transfer

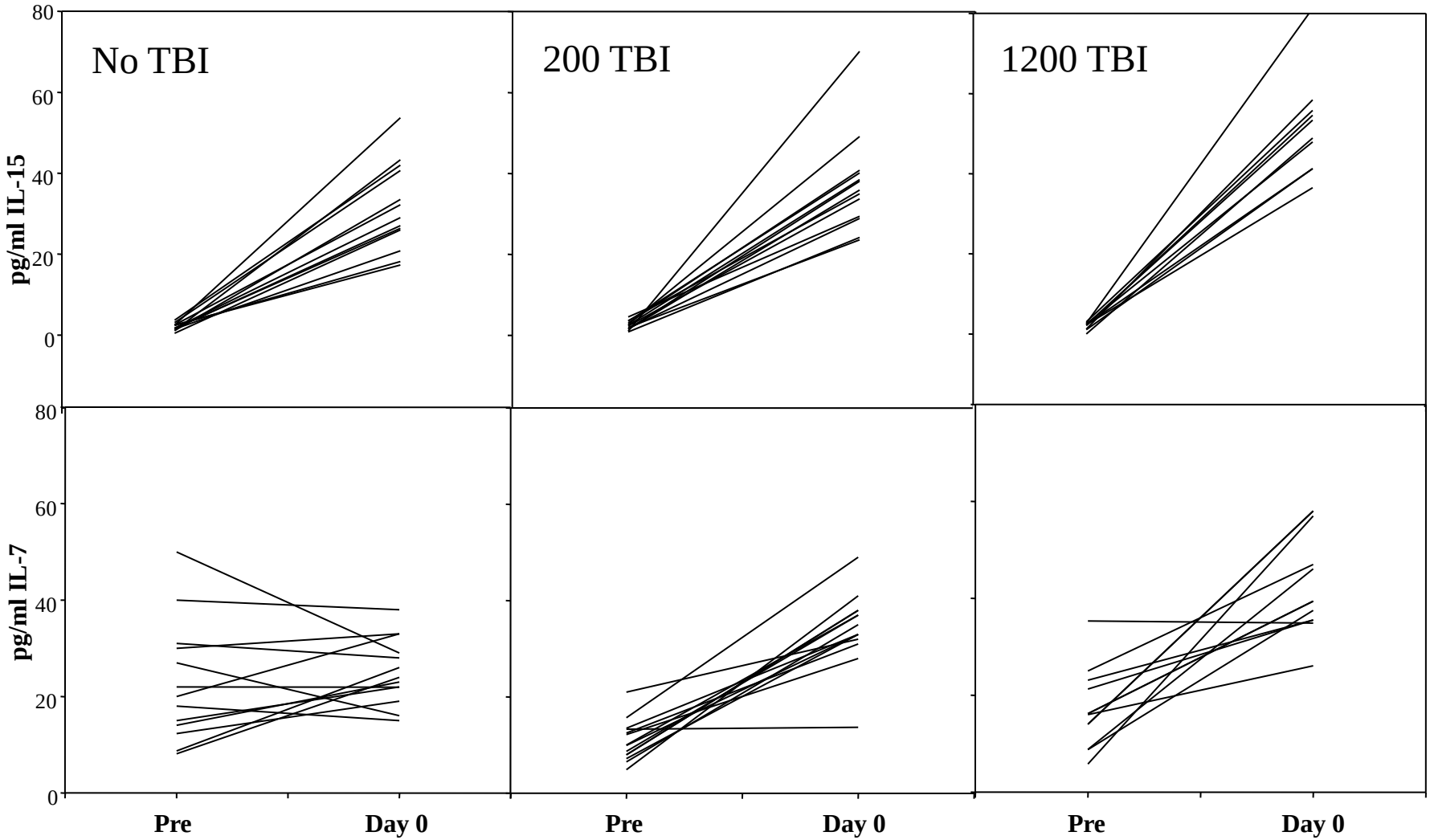
The lymphophenic environment

1) eliminates T regulatory (suppressor) cells

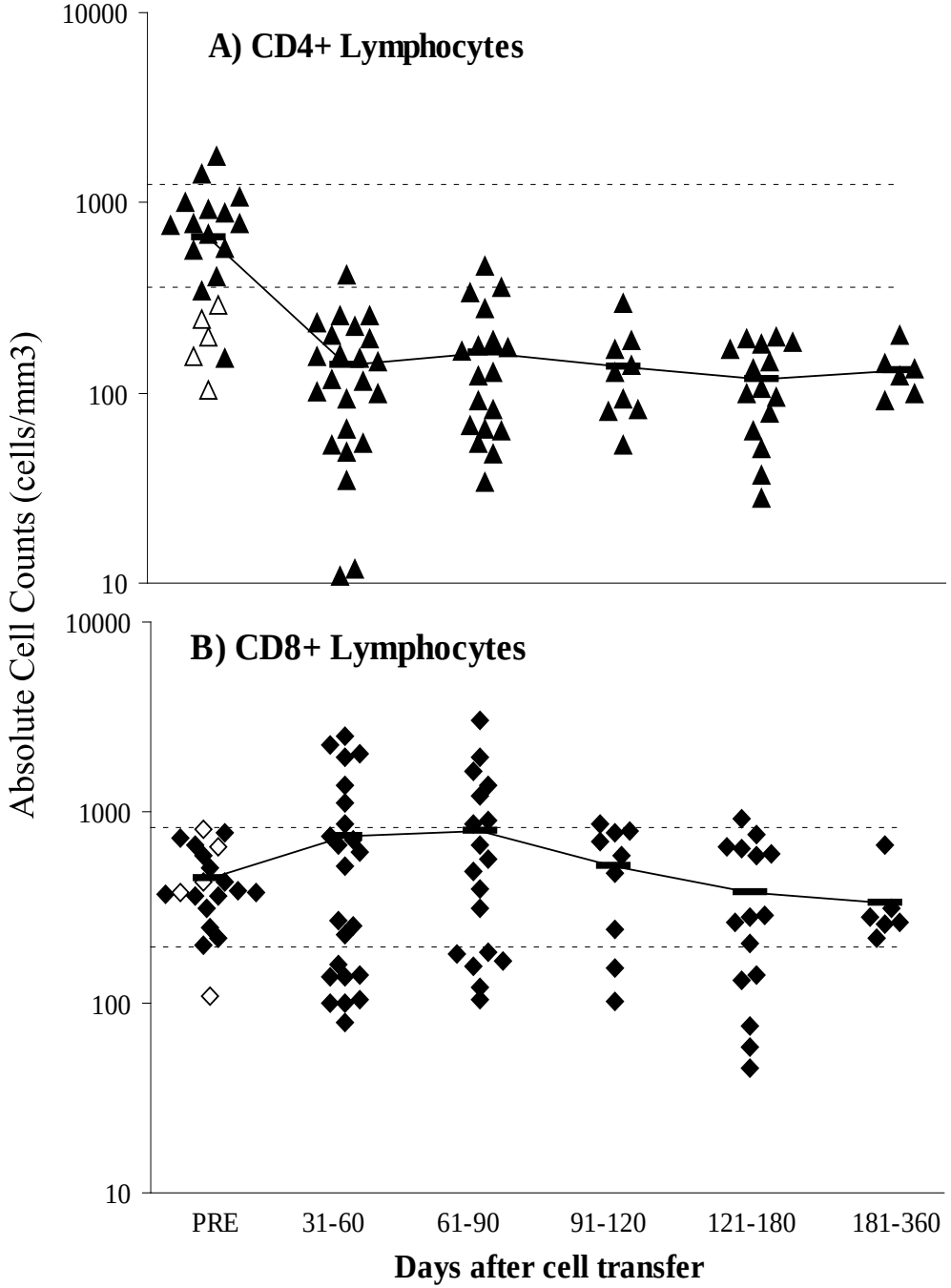
2) eliminates competition for homeostatic cytokines (IL-7, IL-15) vital for T cell survival

In the lymphopenic host, anti-tumor T cells proliferate, persist, infiltrate organs, recognize cancer antigens and destroy cancer cells.

Impact of Lymphodepletion on Serum Levels Of IL-15 and IL-7



Recovery of lymphocytes in patients receiving N-M chemotherapy



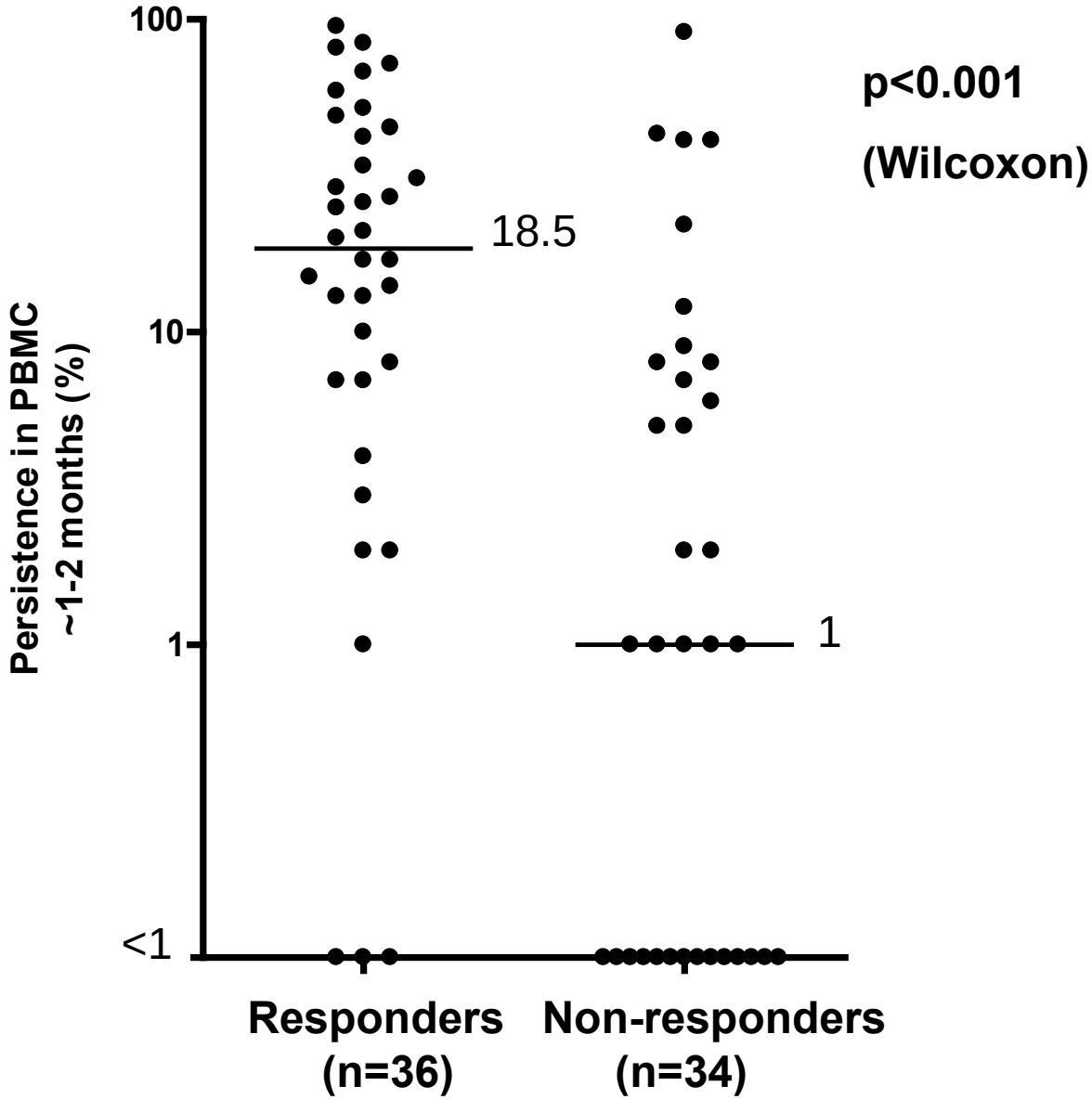
Three Factors that Correlate with Cancer Regression Following Cell Transfer Therapy

1. Persistence of the transferred cells

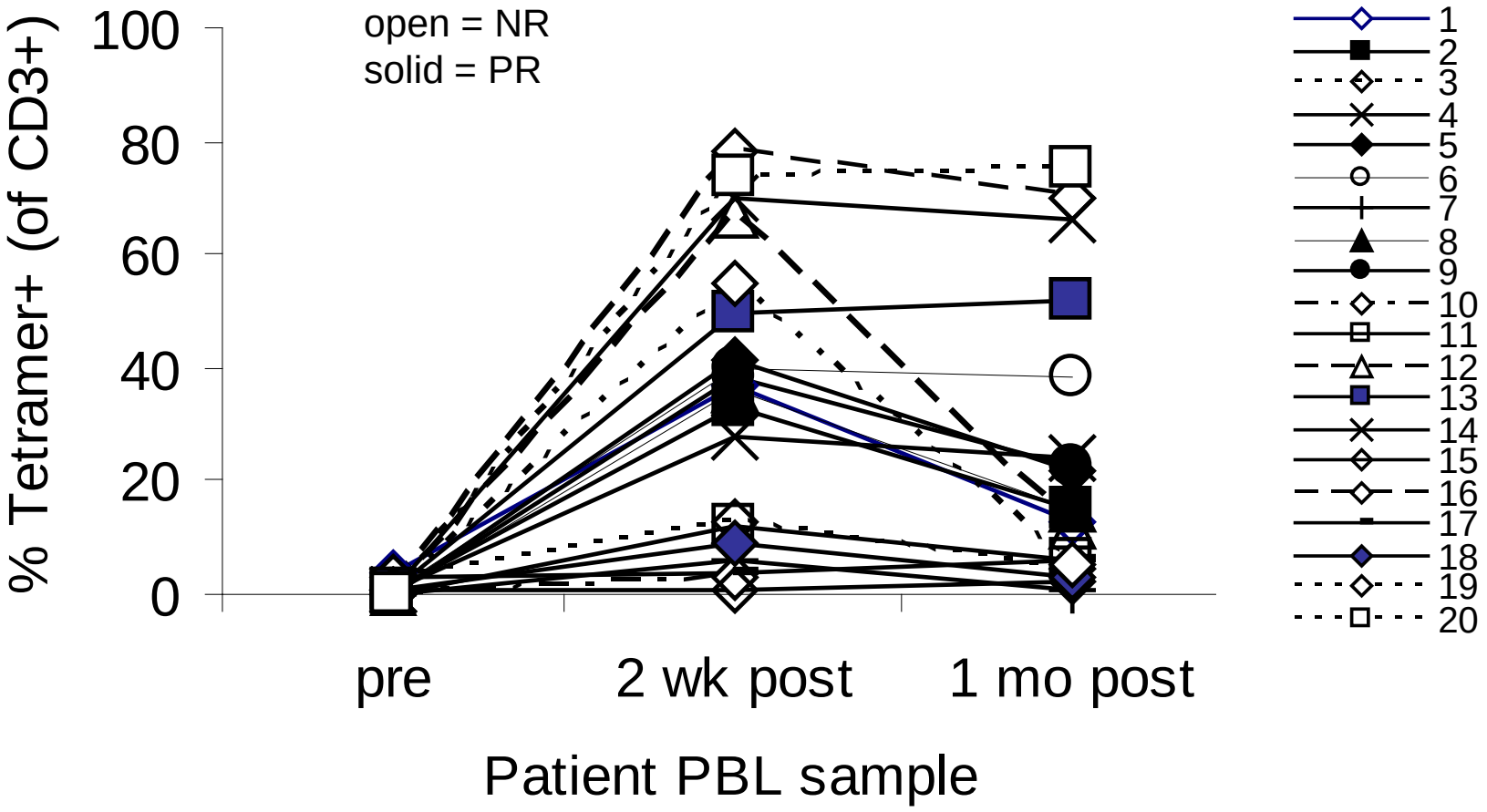
2. Telomere length

3. Expression of CD27 (following IL-2 withdrawal)

T cell persistence: NMA, 200 and 1200 TBI trials



Persistence of infused cells in PBL at one month: Tetramer analysis



Persistence of infused cells in PBL at one month: Elispot analysis (IFNg)

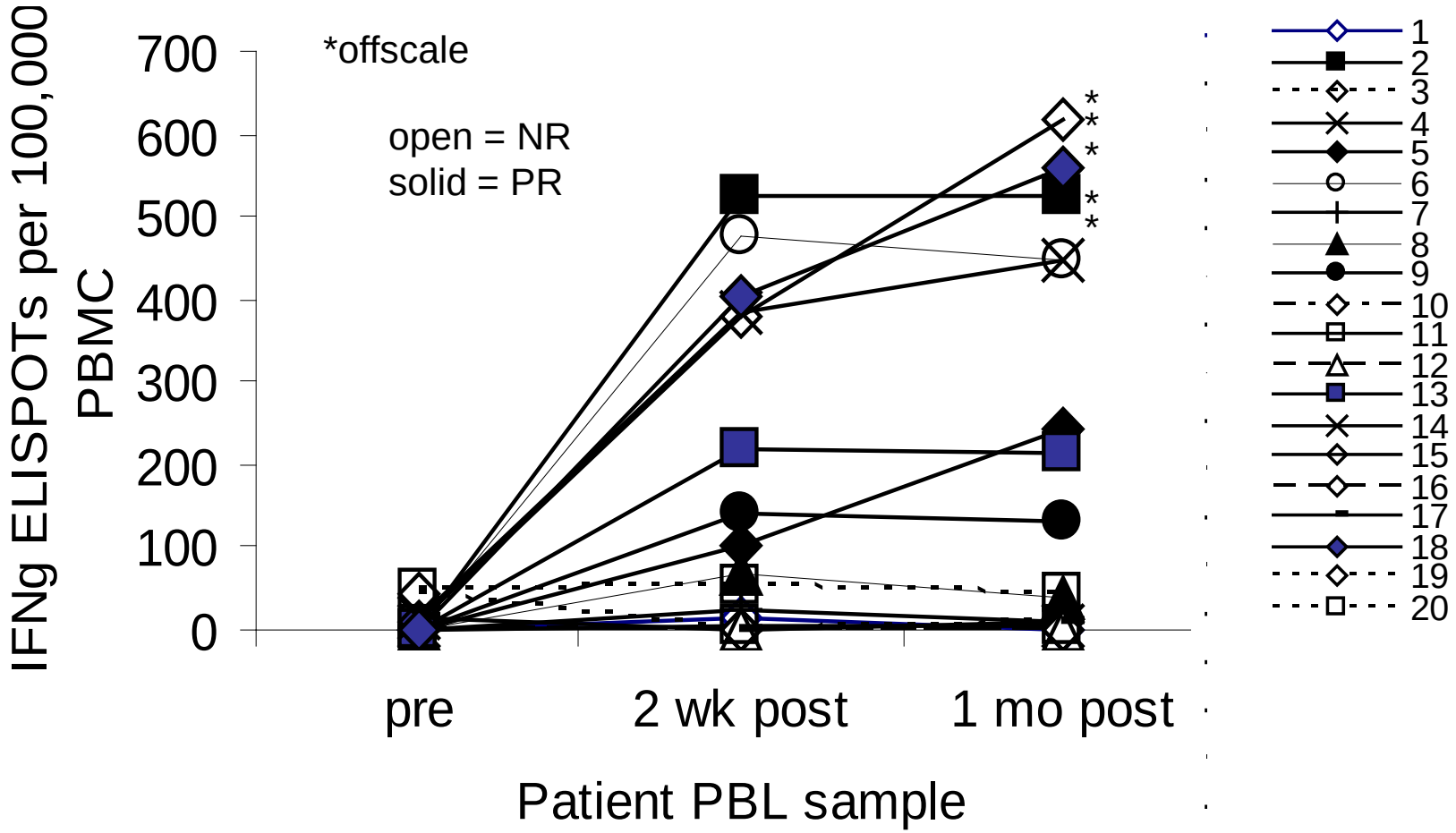


Figure 2D(L)

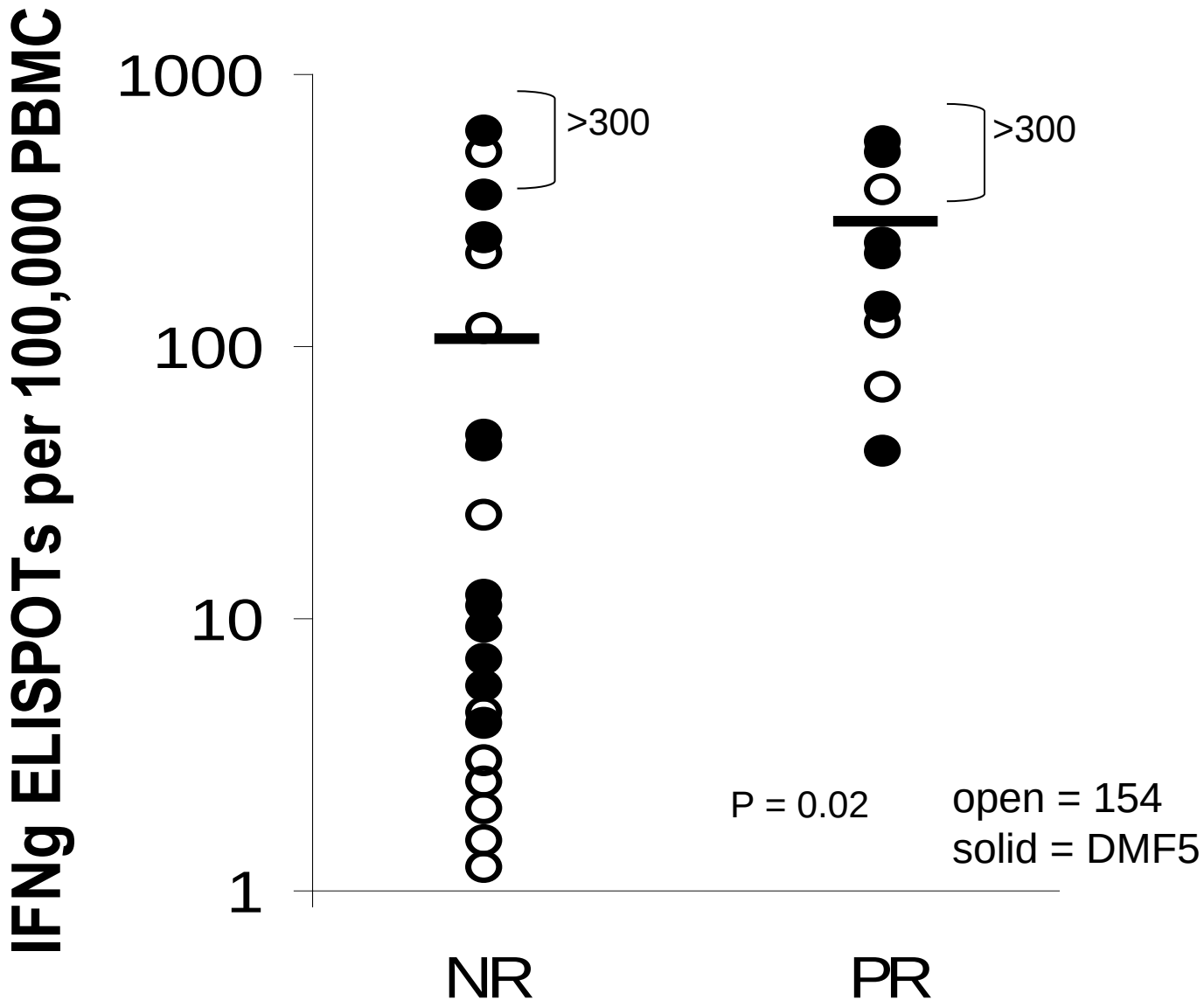
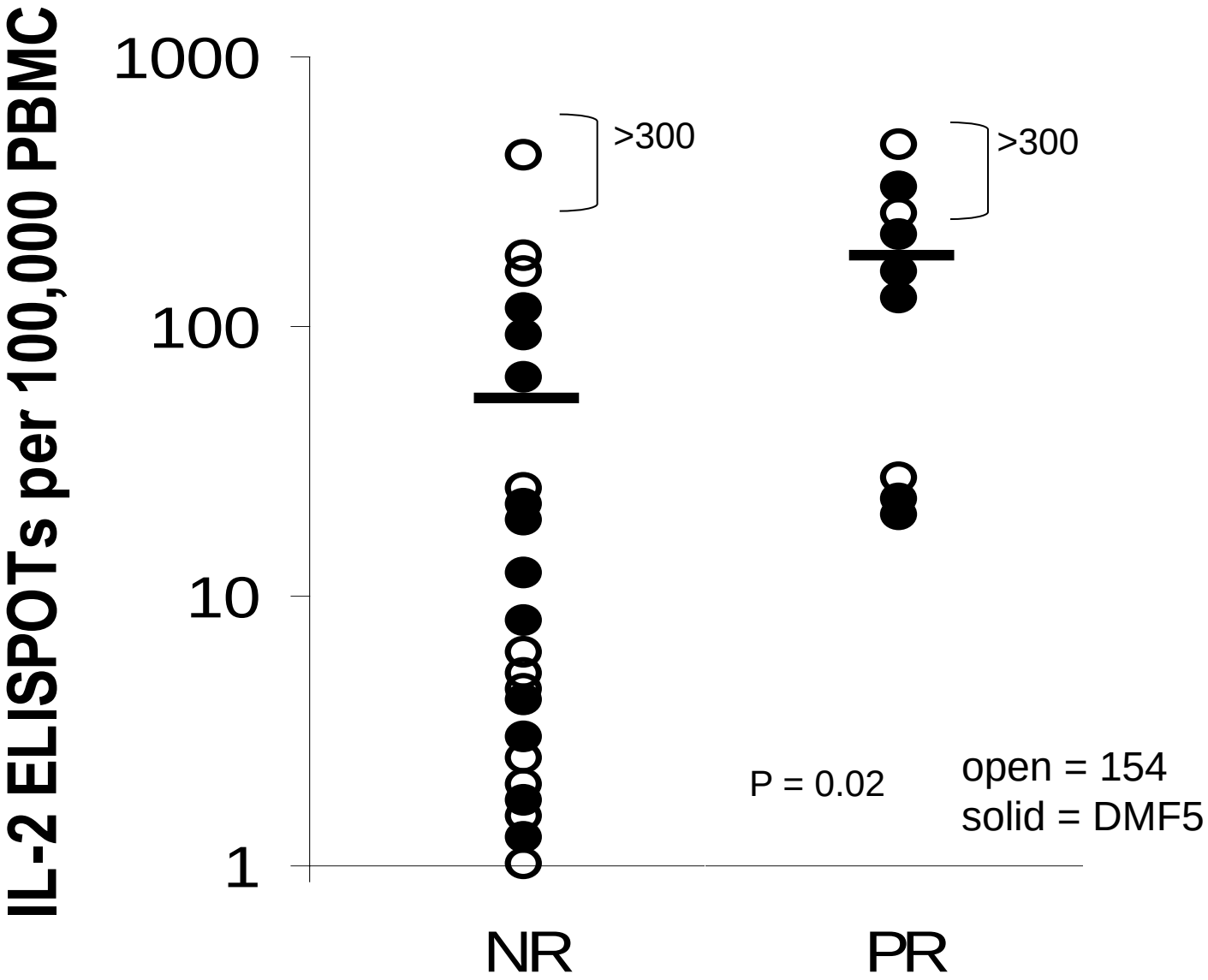
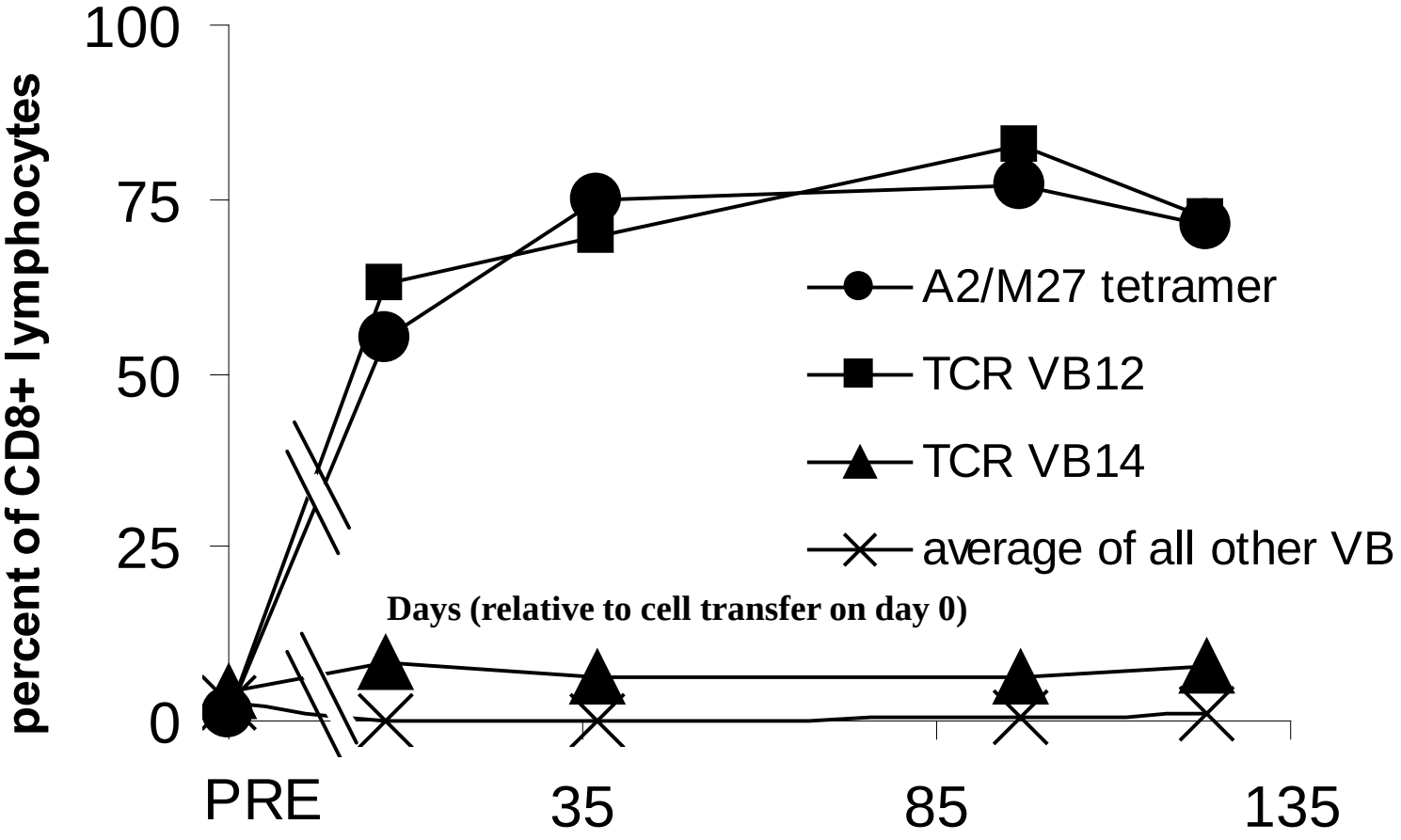
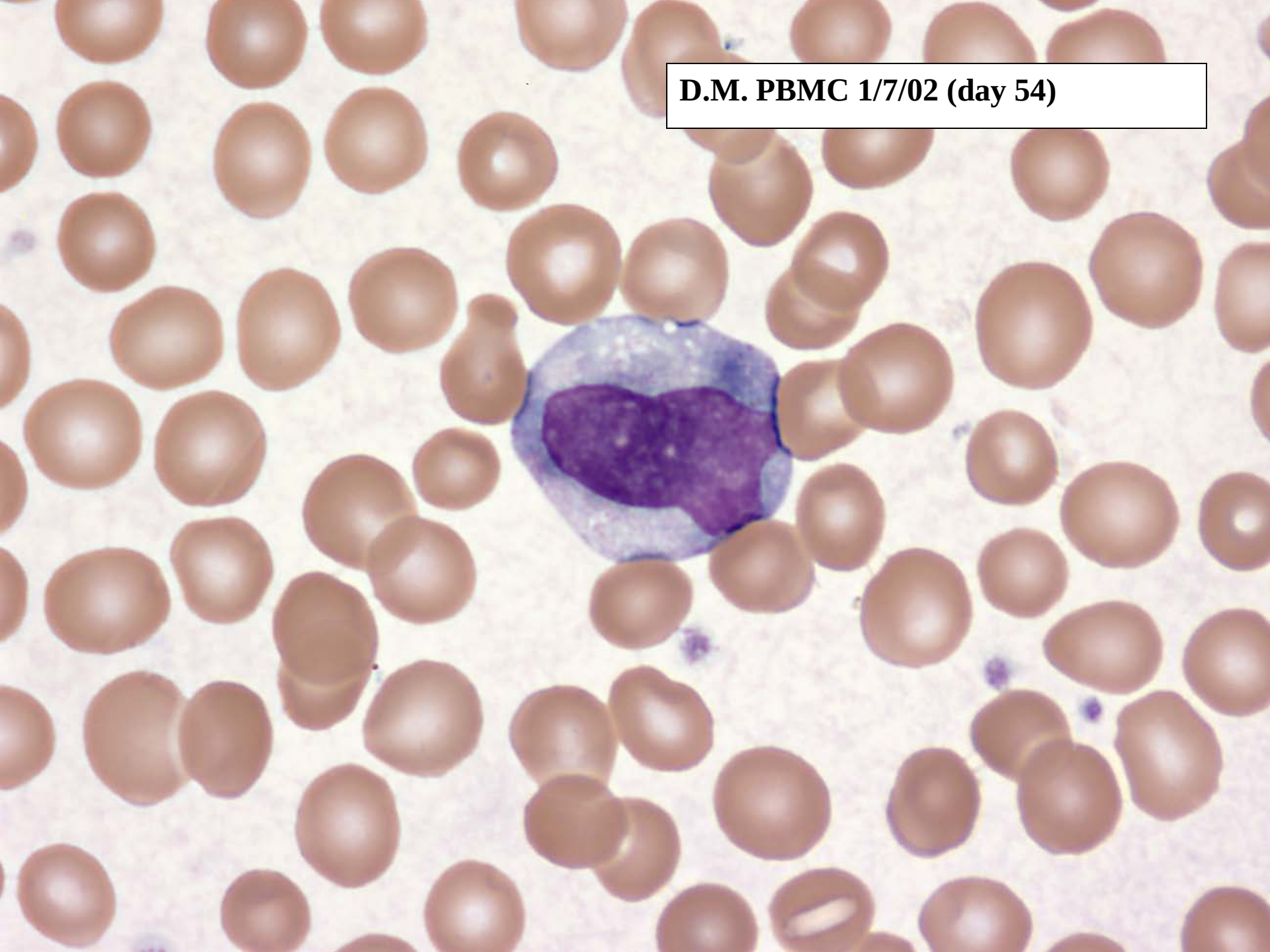

IFNg ELISPOTs per 100,000 PBMC
1000
100
10
1
>300
>300
P = 0.02
open = 154
solid = DMF5
NR
PR

Figure 2D(C)

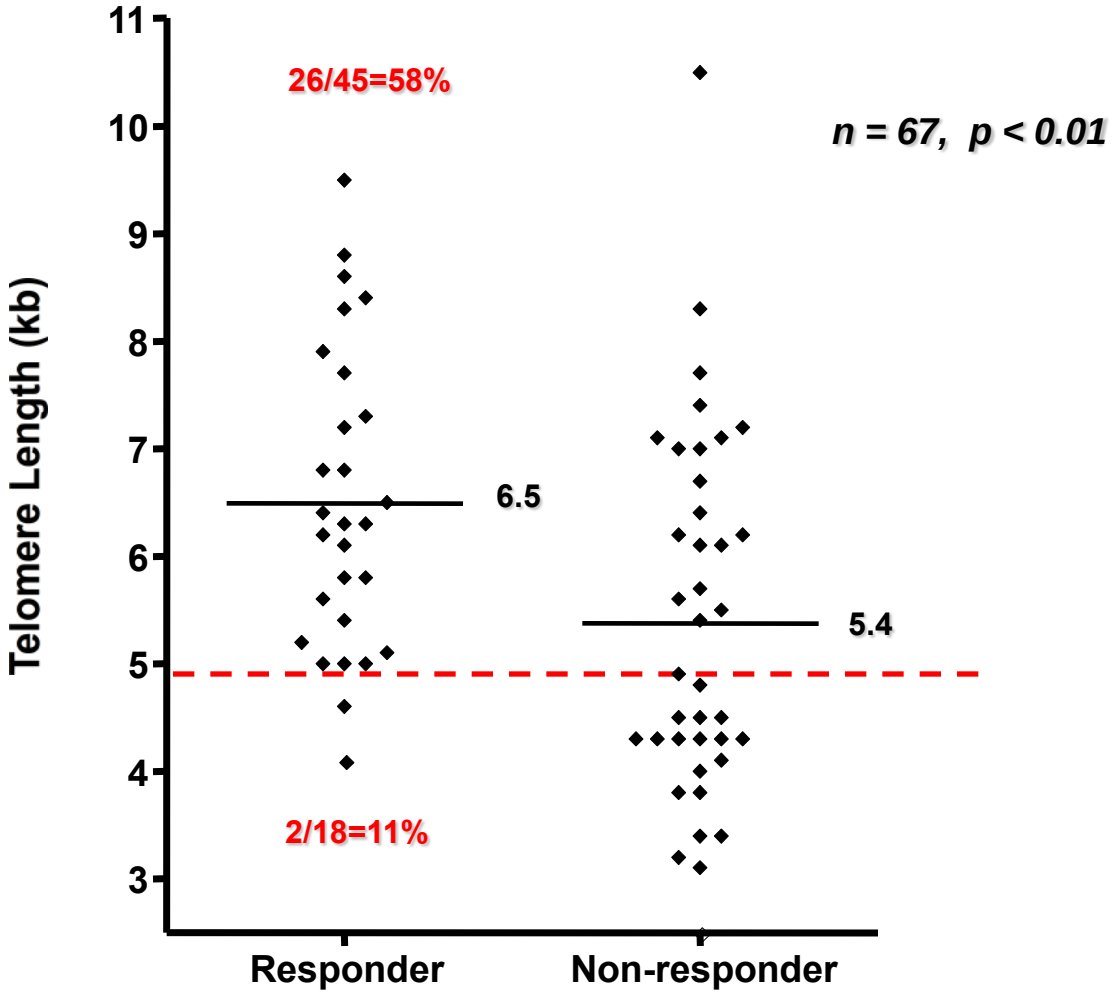

IL-2 ELISPOTs per 100,000 PBMC
1000
100
10
1
>300
>300
P = 0.02
open = 154
solid = DMF5
NR
PR

Persistence of transferred cells – Patient DM

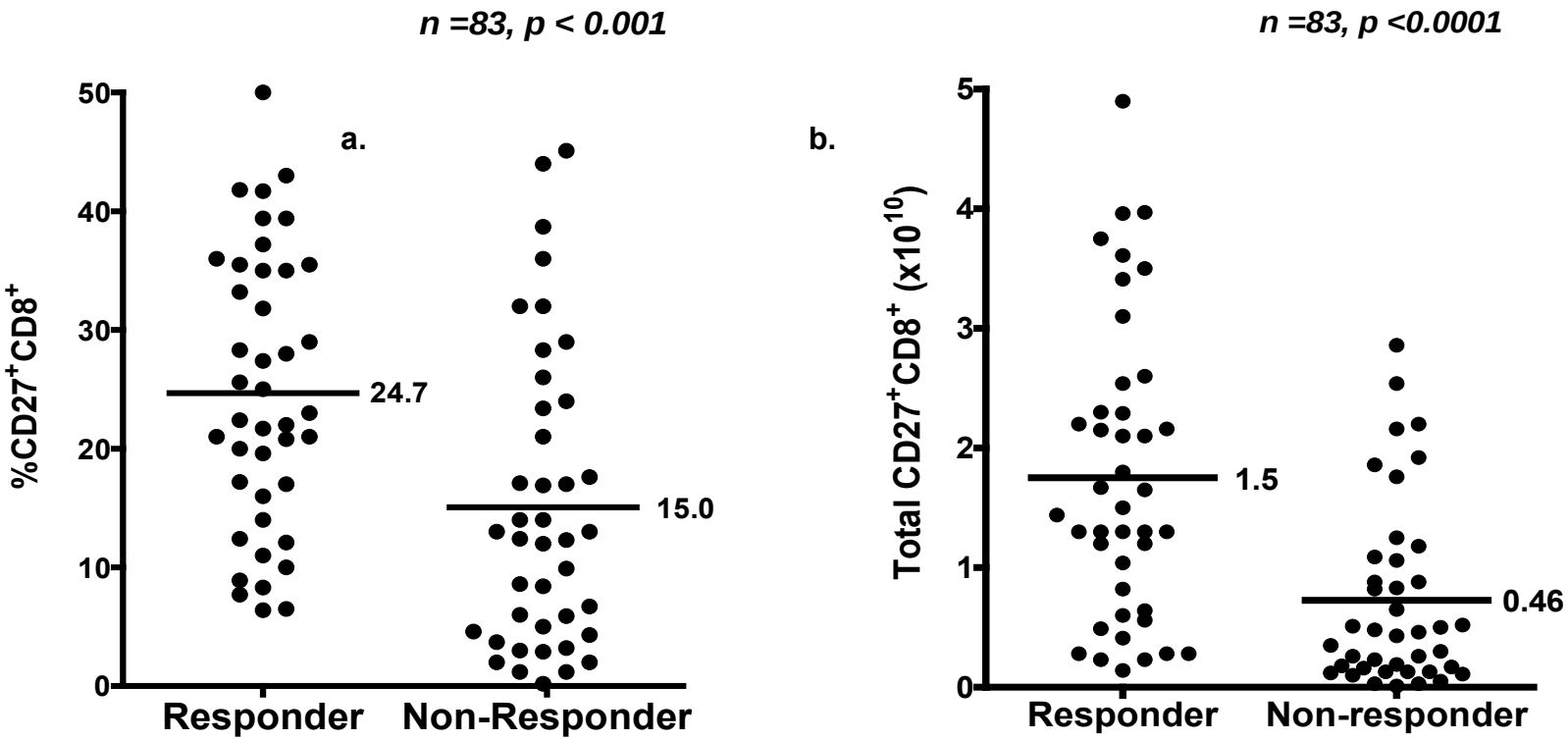



D.M. PBMC 1/7/02 (day 54)

Telomere Length of the infusion TIL significantly associated with response



Cellular differentiation associated with clinical response



CONCLUSION

In the presence of host lymphodepletion adoptive transfer of anti-tumor T cells can mediate regression of established metastatic cancer.