Regulating Tregs with IL-2

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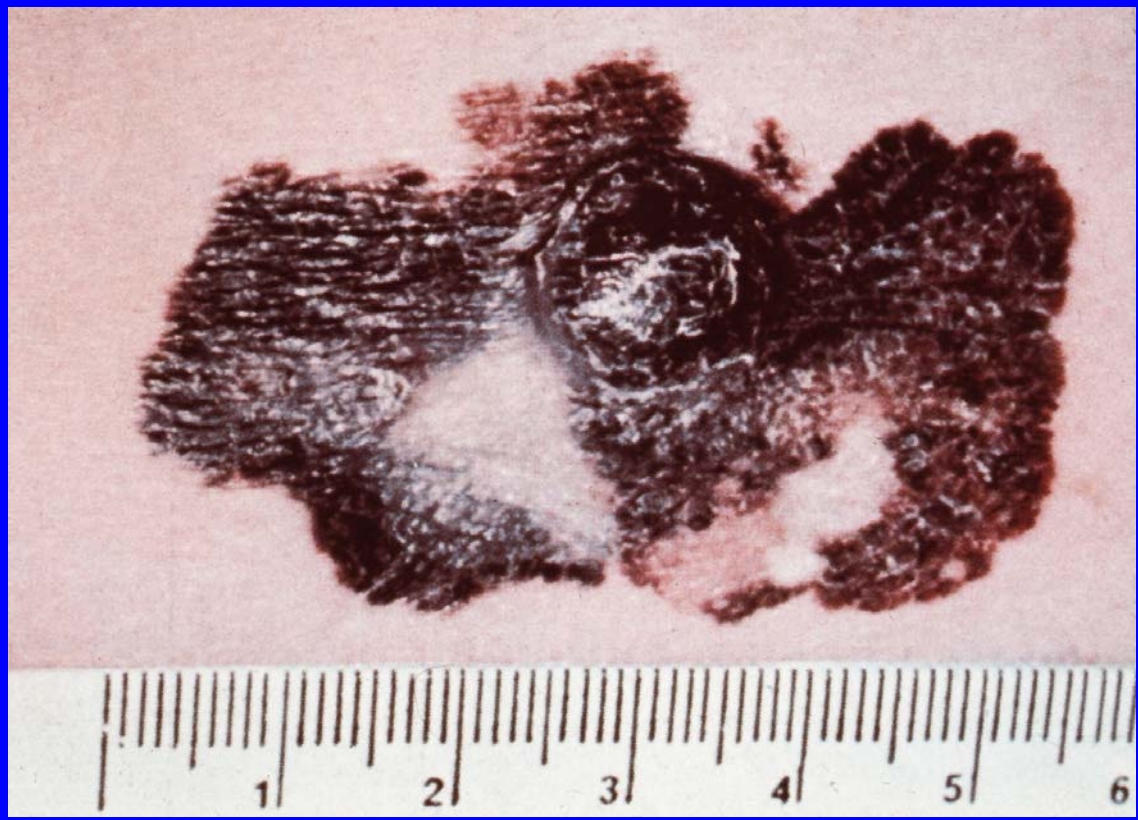
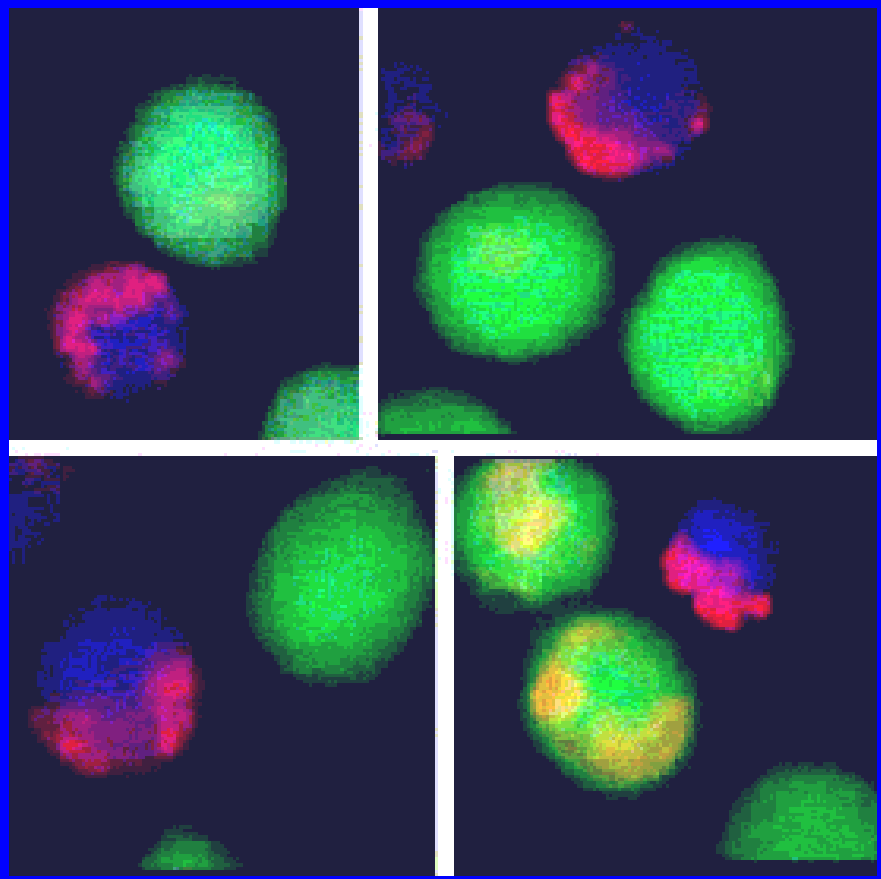
The immune system can induce tumor regression

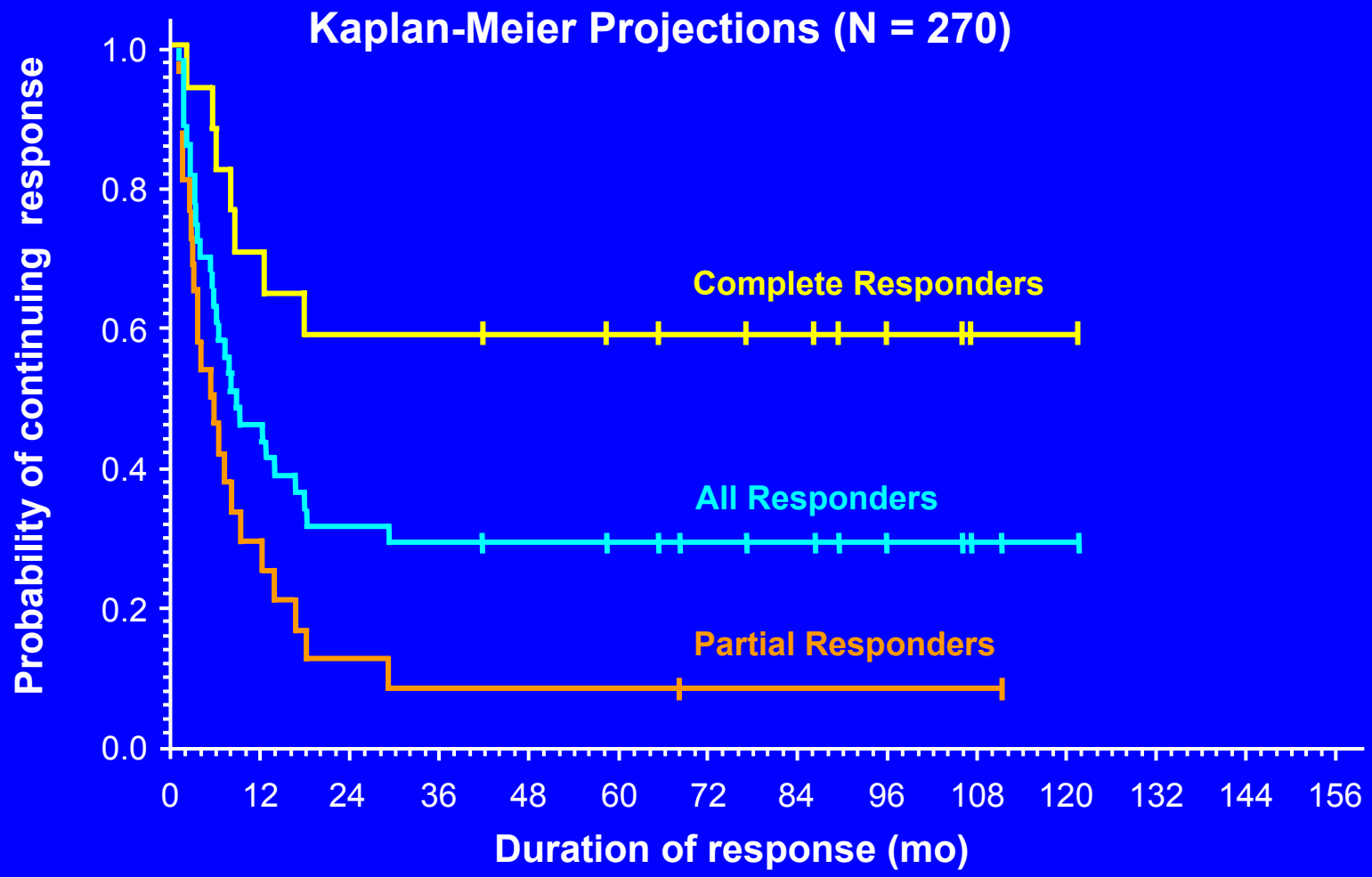


Melanoma regression is mediated by T cells



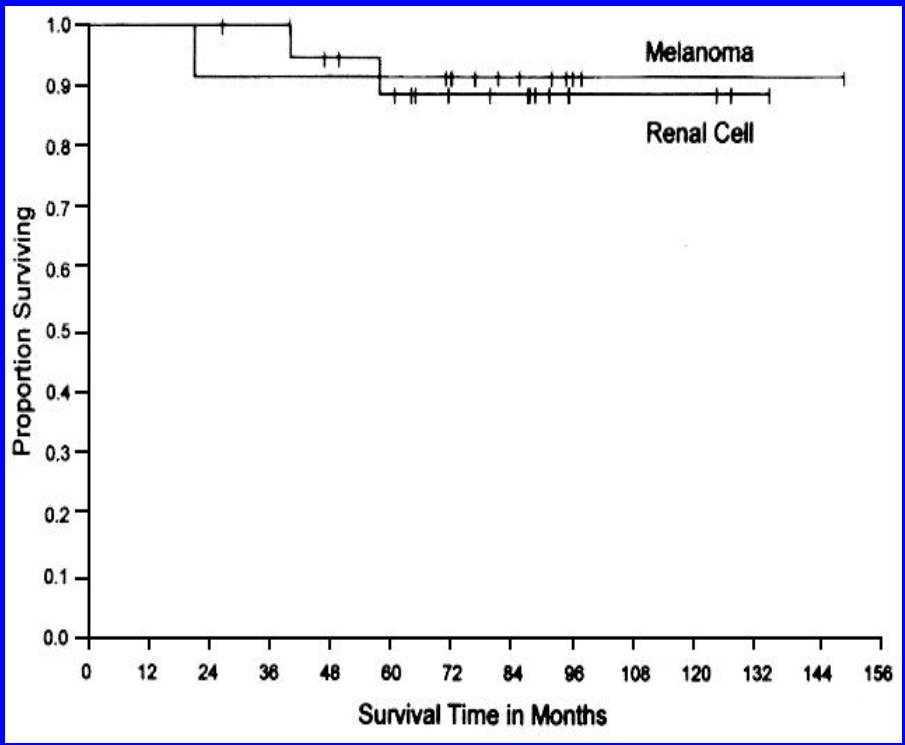
Zweifach 2005

High-dose IL-2 induces durable objective clinical responses in 15-20%



Data on file. Chiron Corporation.

High-dose IL-2 promotes durable disease free survival in responders

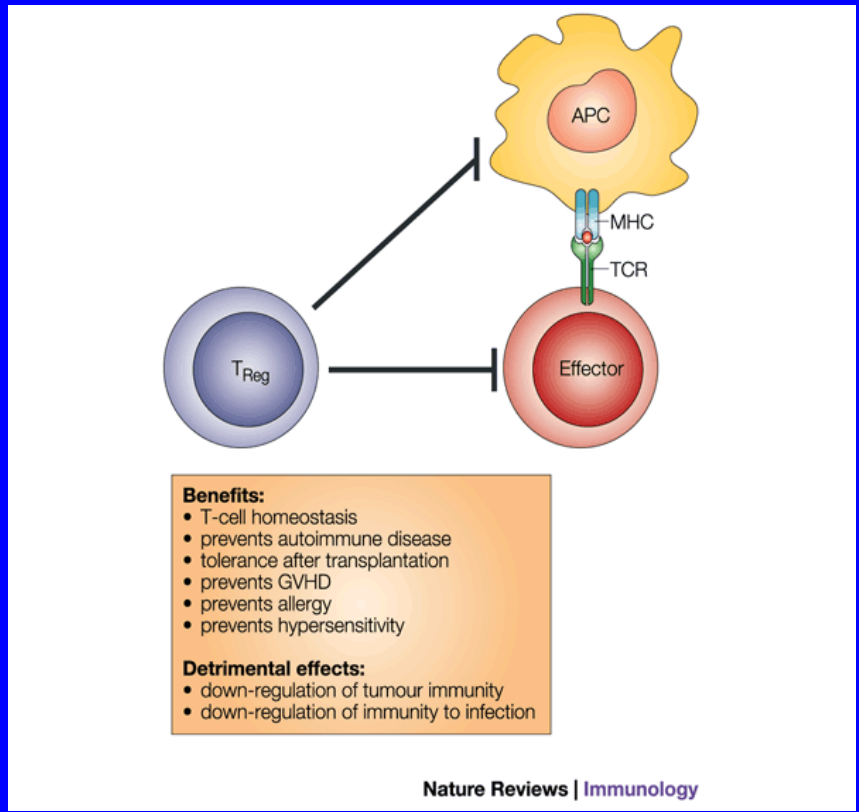


- CR is durable
- 27/33 CR (81%) without recurrence at 39-148 months

Rosenberg et al. Ann Surg 1998

IL-2: The New Paradigm

- Mice deficient in IL-2 or IL-2R demonstrate lethal autoimmunity
- This suggested that the function of IL-2 was more complex
- IL-2 as a regulatory cytokine mediating tolerance?

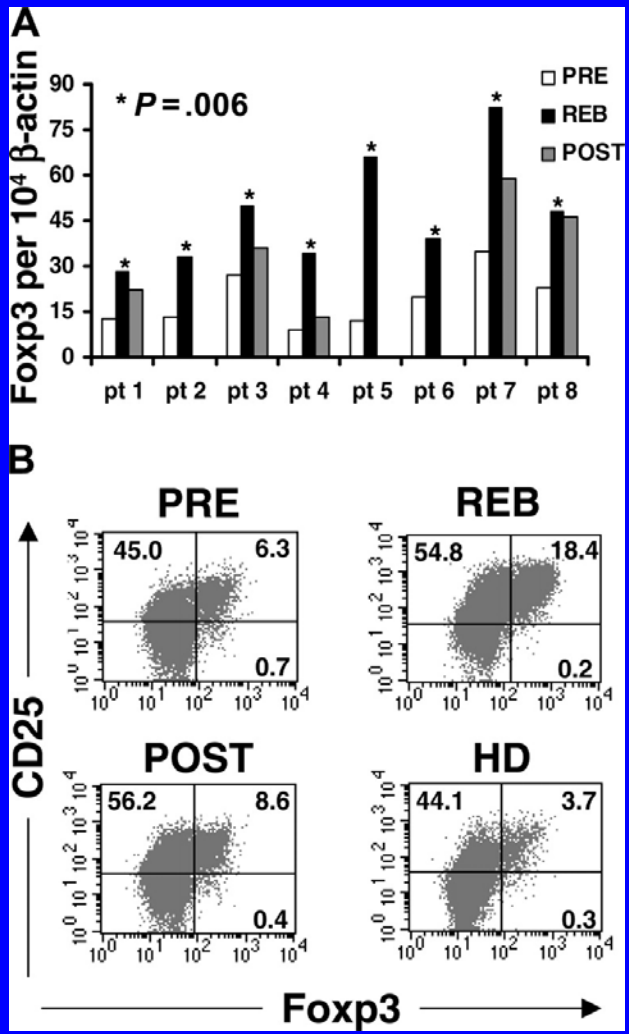


Questions

- What is the effect of ***high-dose IL-2*** on Tregs *in vivo*?

- If Tregs are activated by IL-2 how do we explain the small but defined durable clinical responses observed following high-dose IL-2 therapy?

IL-2 promotes Treg activity



Ahmadzadeh, M. et al. Blood 2006

Patient Characteristics

Feature	*Melanoma*	*RCC*	*Total*
Tumor	45	12	57
Evaluable	36	12	48
Age (mean)	50	58	52
Sex (M/F)	27/18	8/4	35/22
No. doses			
Cycle 1	8.81	8.6	8.76
Cycle 2	6.64	5.13	6.34
Clinical Response			
CR+PR	9	3	12
PD	32	9	41
NE	4	0	4

High-dose Interleukin-2 Schedule

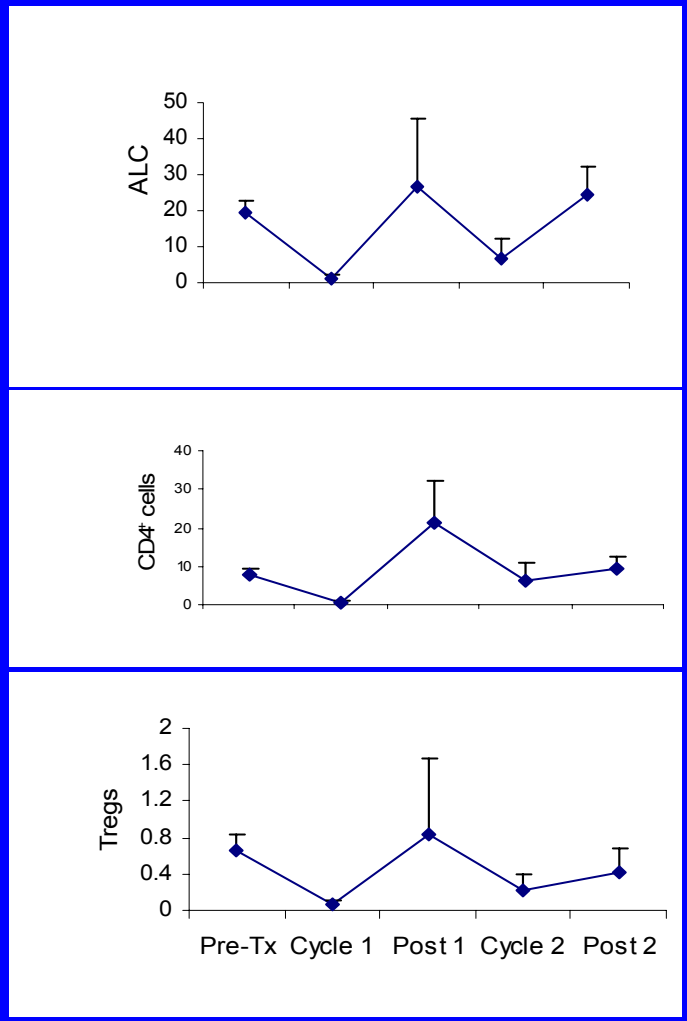
- Cycle 1: rIL-2 600,000 IU/kg I.V. over 15 minutes every 8 hours to a maximum of 15 doses
- 10-14 day rest period
- Cycle 2: rIL-2 600,000 IU/kg I.V. over 15 minutes every 8 hours to a maximum of 15 doses

PBMC Collection

- Prior to treatment (Pre-Tx)
- After the fourth dose of cycle 1 (Cycle 1)
- Two weeks after the first cycle (Post 1)
- After the fourth dose of cycle 2 (Cycle 2)
- Four weeks after the second cycle (Post 2)

- Every 6 months (responders)

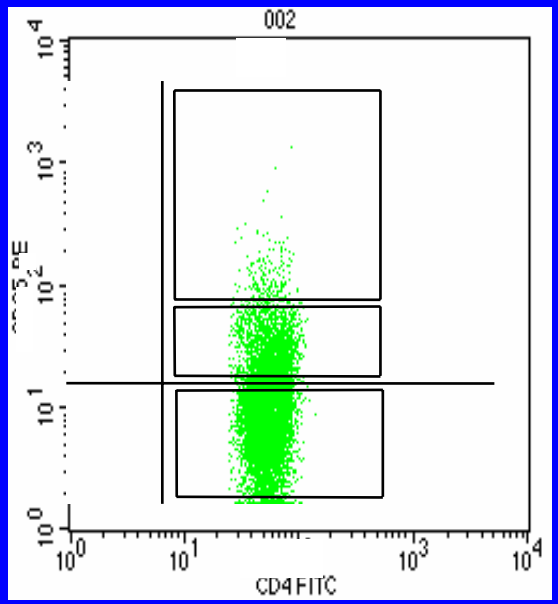
IL-2 induces lymphopenia and rebound in ALC, CD4+ T cells, and Tregs

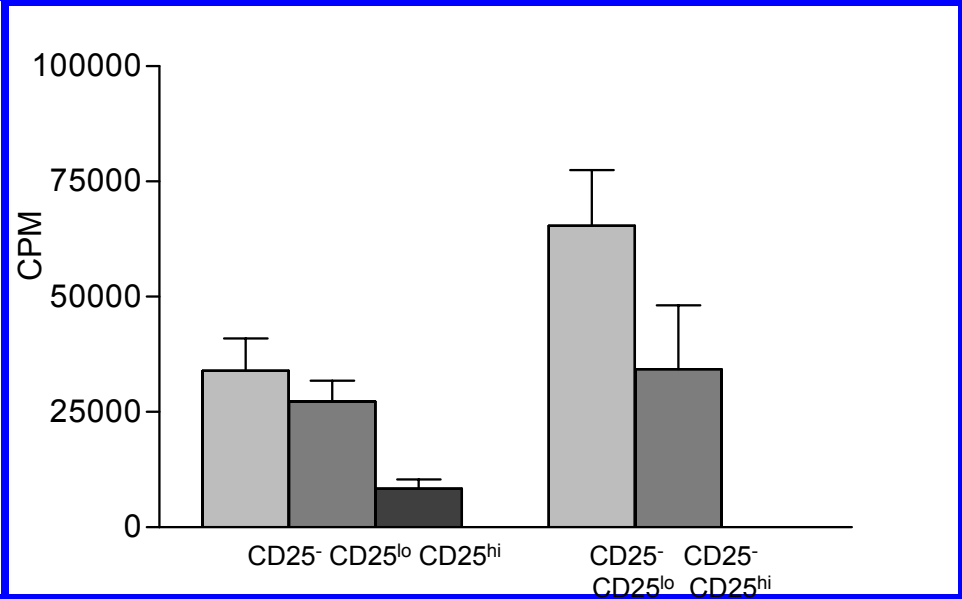


Cesana et al. JCO 2006

$CD4^{+}CD25^{hi}$ are Tregs in humans

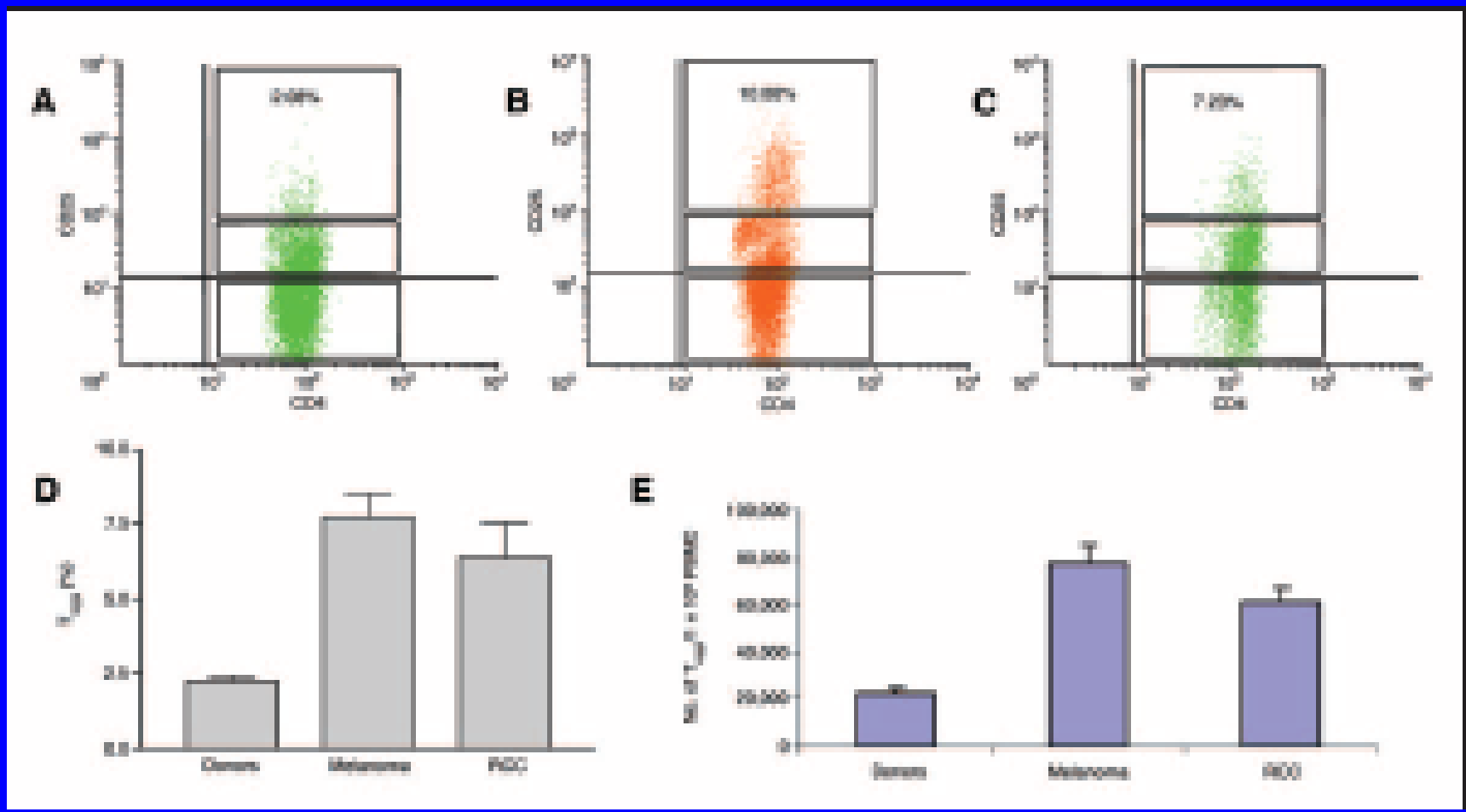
CD25





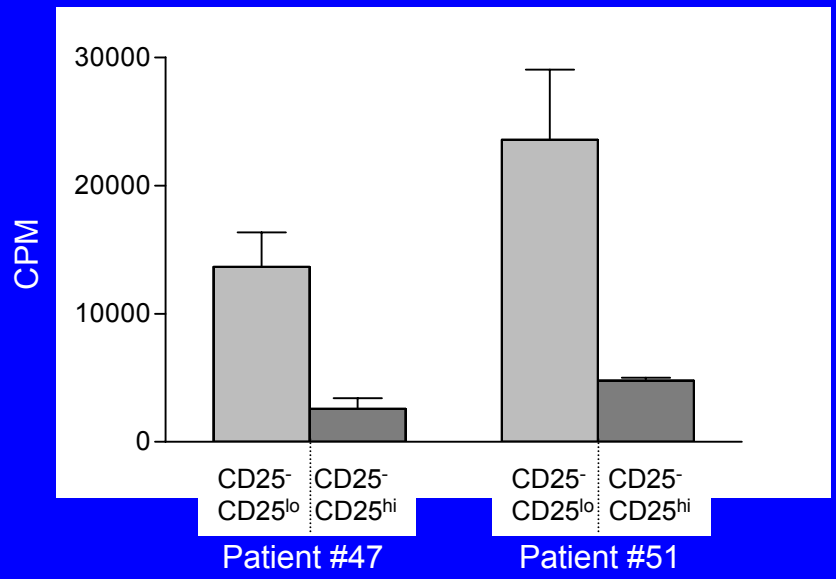
Cesana et al. JCO 2006

$CD4^{+}CD25^{hi}$ T cells are elevated in patients with metastatic MM and RCCA

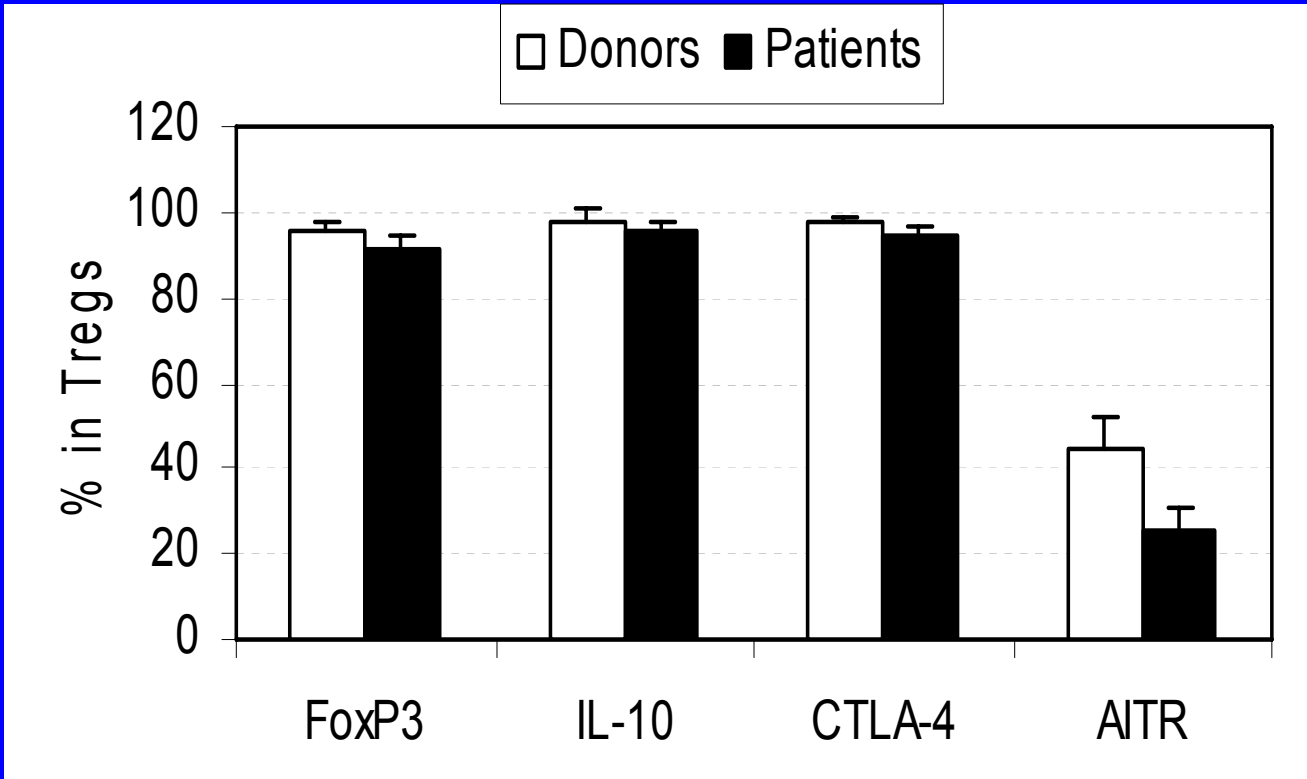


Cesana et al. JCO 2006

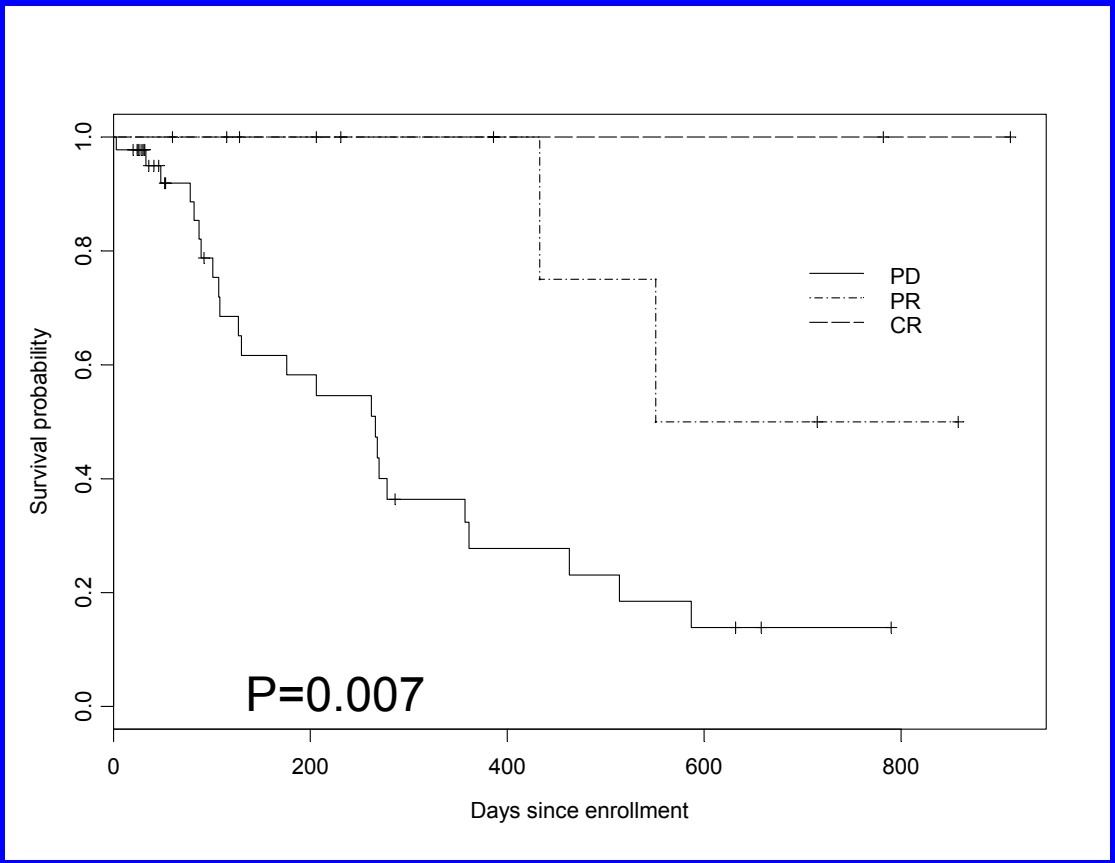
$CD4^{+}CD25^{hi}$ T cells are suppressive in cancer patients



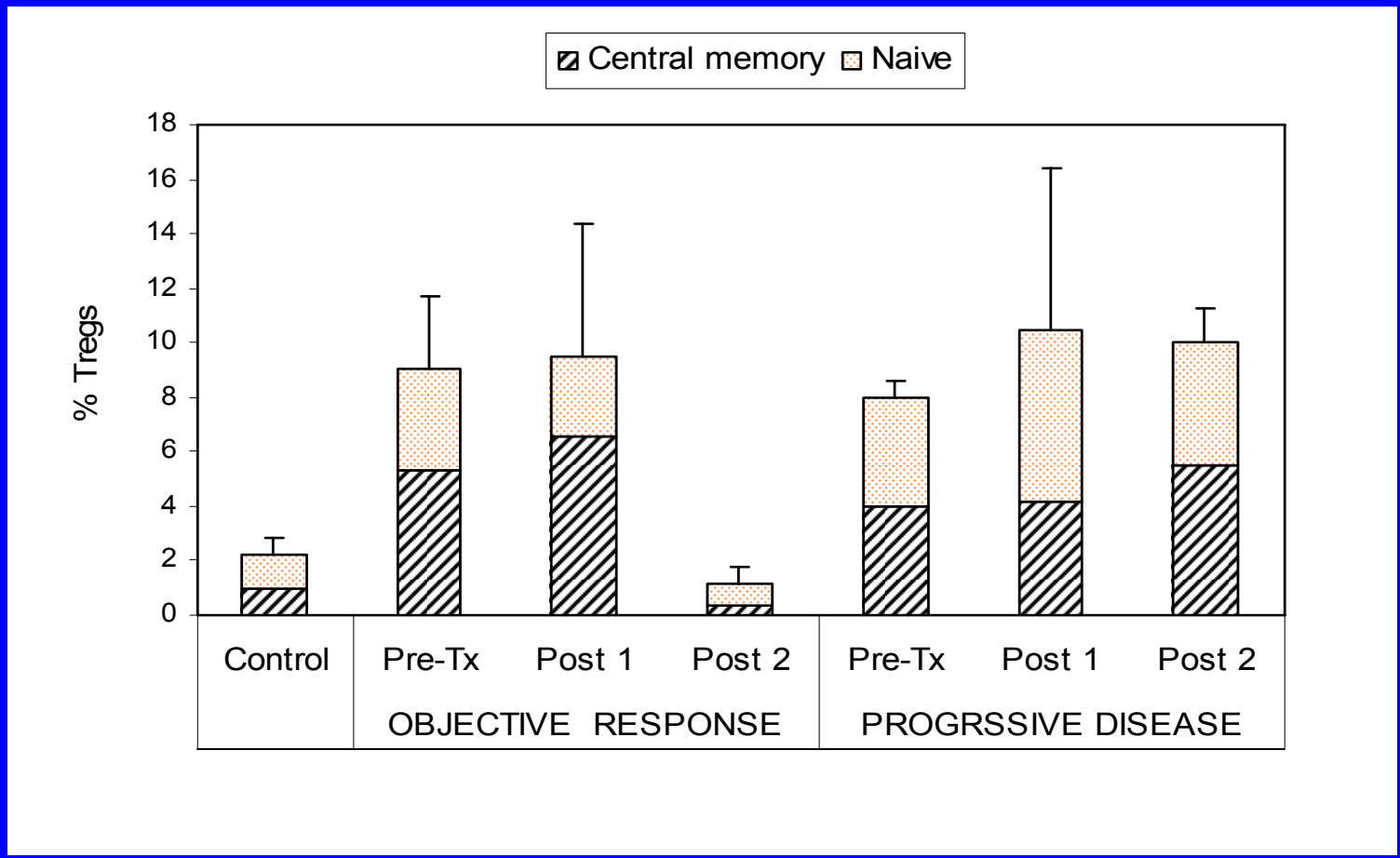
Tregs have a similar phenotype in normal donors and cancer patients



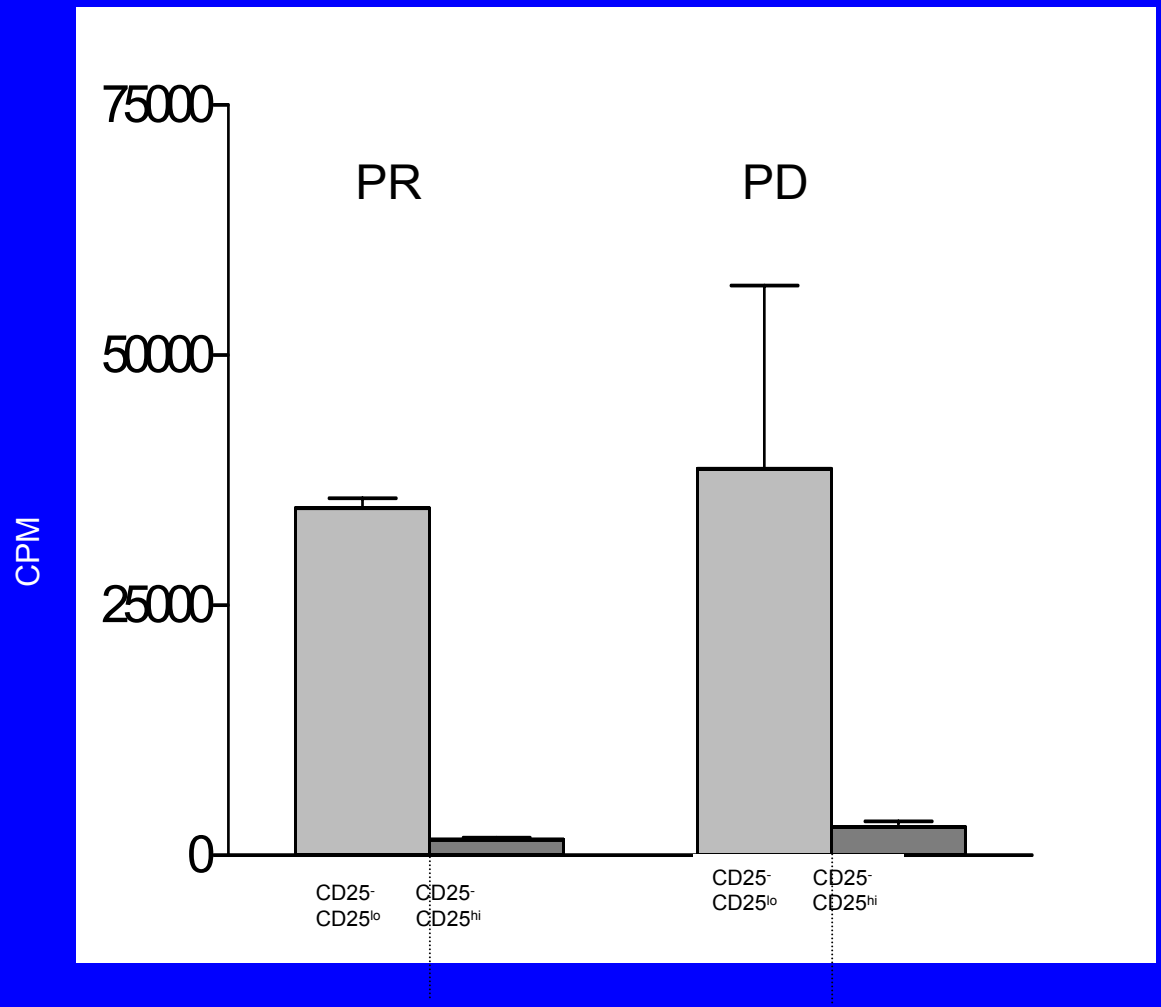
Clinical outcome for high-dose IL-2 patients



Tregs decrease to normal levels after the cycle 2 in objective responders

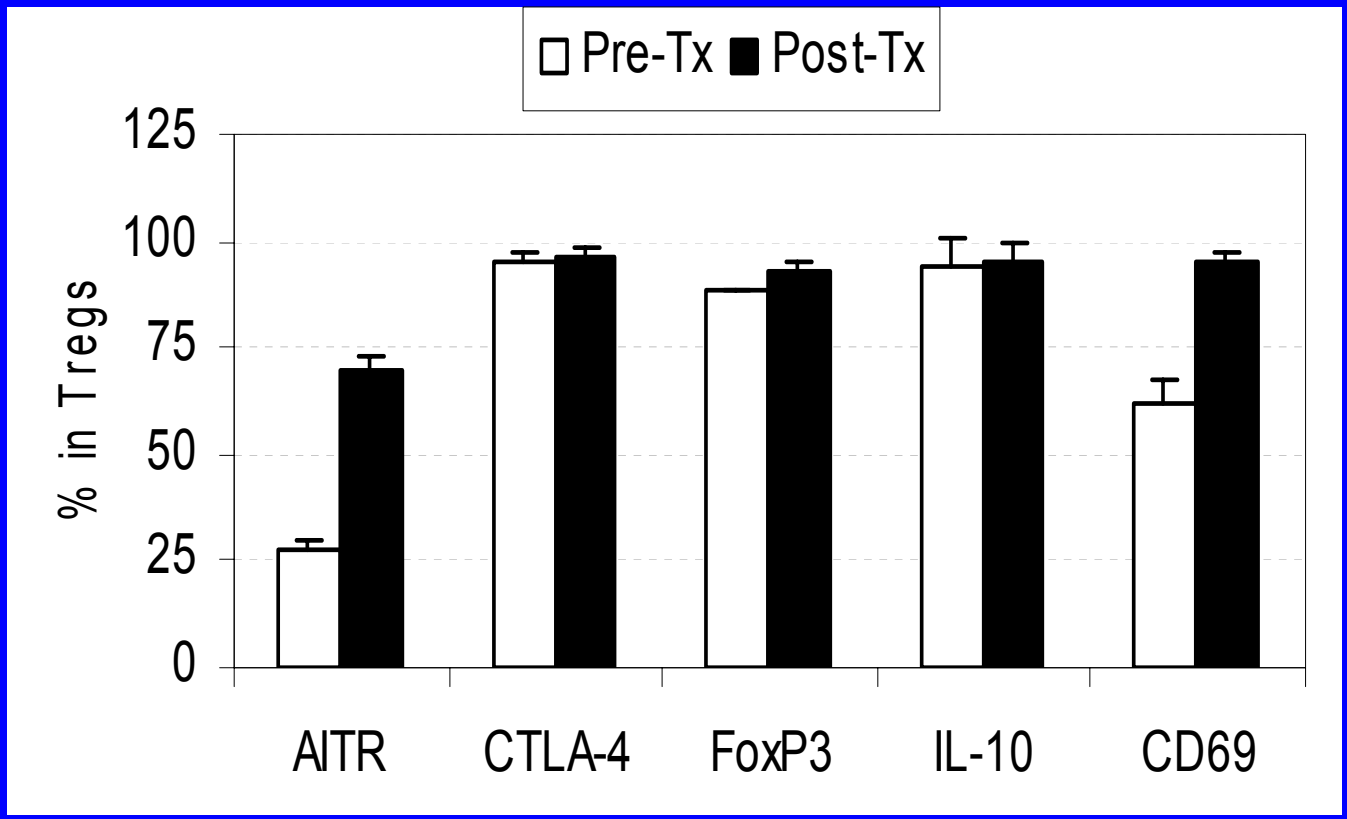


Tregs are suppressive in responding and non-responding patients



Treg phenotype is similar before and after high-dose IL-2

c.



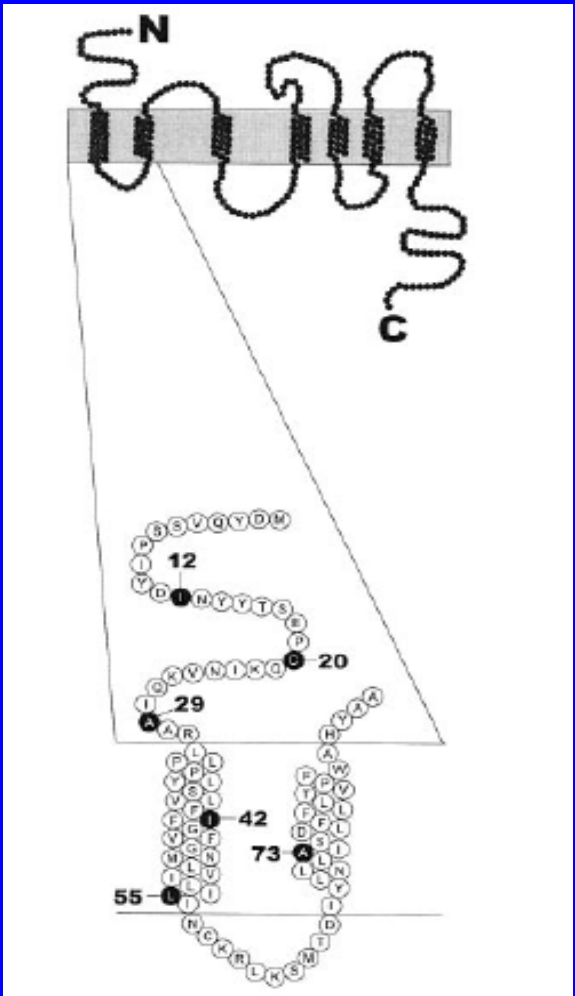
The change in Treg frequency is associated with clinical response

	Mean change in Treg frequency			
Time	***PD***	***PR***	***CR***	***P-value****
Pre-Tx – Post 1	2.05%	1.52%	0.19%	0.826
Pre-Tx – Post 2	5.09%	2.37%	-7.85%	0.004

Why do patients exhibit differential Treg responses to IL-2?

- Drug effect
  - Dose
  - Schedule
  - Route of Administration
- Patient effect
  - Genetic polymorphism of immune responsive genes
  - Phenotypic characteristics

CCR5

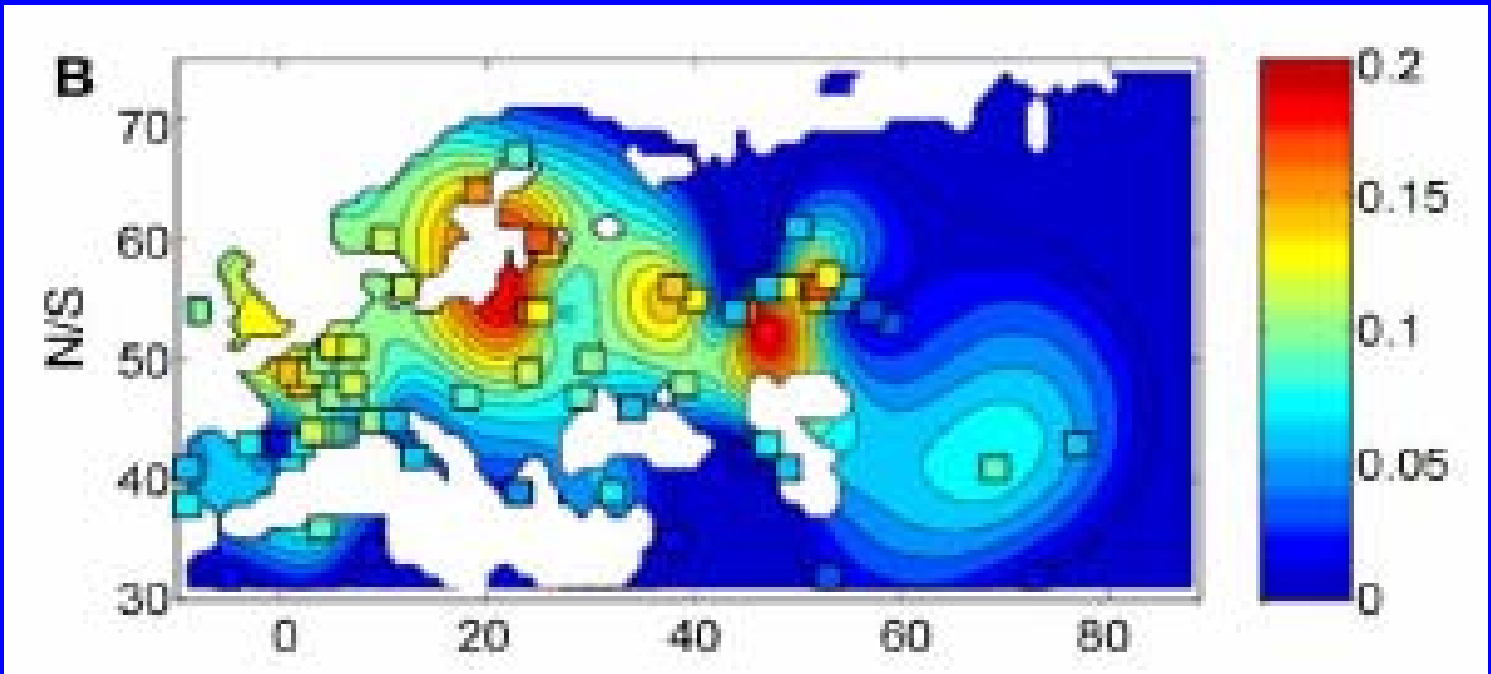


- G protein coupled receptor
- Binds CCL3, 4, 5, 8, 11, 14α and 16
- Expressed on T cells, NK cells, immature DCs, macrophages and basophils
- Functions to distribute effector cells in infection
- Role in priming innate and adaptive immunity

CCR5Δ32 Polymorphism

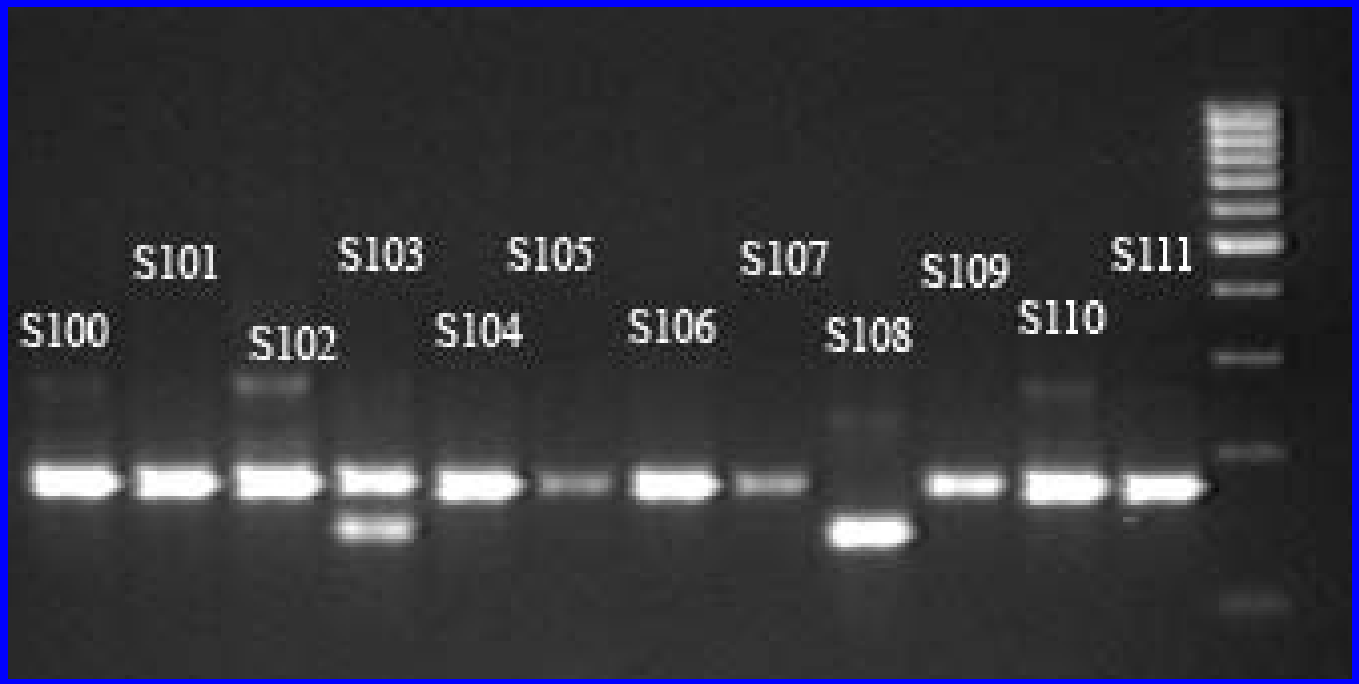
- 32 base pair deletion reported 894 B.C. – 1306 A.D.
- Receptor if dysfunctional and not expressed on the cell surface
- Prevalence in Caucasian population
  - 10-14% heterozygous
  - 1% homozygous
- Role in disease prevention?
- Associated with disease outcome
  - Increased renal allograft survival
  - Increased resistance to HIV infection
  - Increased resolution of Hepatitis B (& C?) infection
  - Increased protection against NHL

Modern frequency of the CCR5Δ32 allele



Galvani and Novembre, Microbes & Infect 2005

CCR5 Polymorphism Δ32 detected by PCR analysis of PBMC



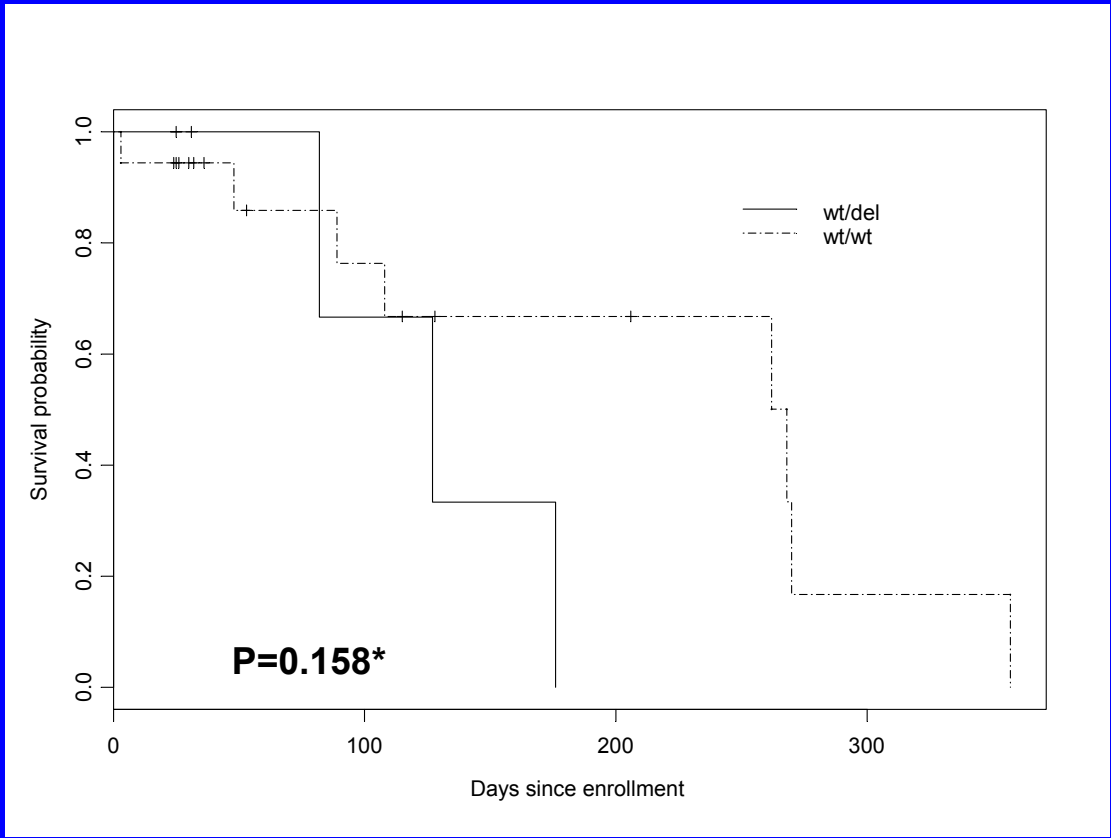
↑↑ ↑

CCR5Δ32 is inversely correlated with overall survival in IL-2 patients

Group	N	Median (d)	95% CI	P
• CR	3	NA	NA	0.007*
• PR	9	705	(433, NA)	
• PD	45	266	(130, 463)	
• wt/wt	18	268	(108, NA)	0.158
• wt/del	5	127	(82, NA)	

*Relative risk of wild type to polymorphism is 0.60 with 95% CI (0.28, 1.26).

CCR5Δ32 is inversely correlated with response to IL-2



*Relative risk of wild type to polymorphism is 0.60 with 95% CI (0.28, 1.26).

CCR5 Validation Set

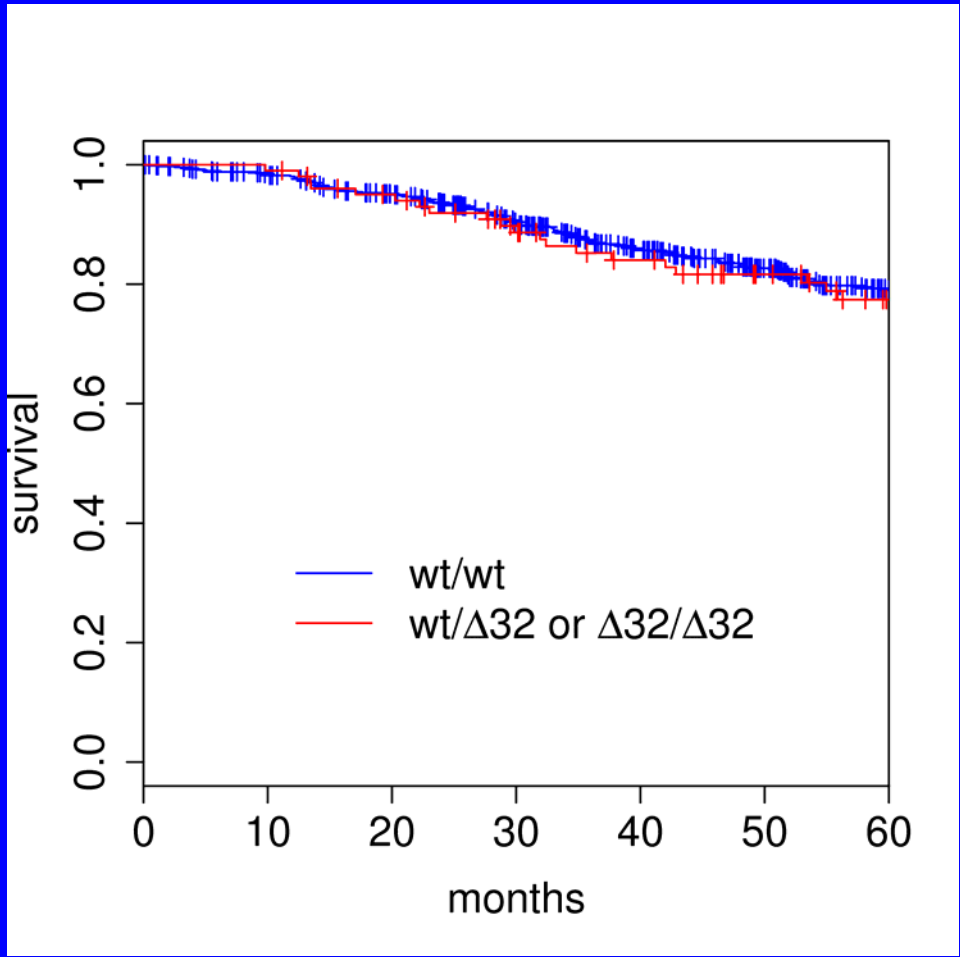
Feature	*Stage III*	*Stage IV*	*Total*
N	302	261	782
Age	56.0	54.2	56.4
Sex (M/F)	172/130	153/100	434/344
CCR5			
wt/wt	259	229	680
wt/Δ32	39	27	90
Δ32/Δ32	4	5	12

CCR5 polymorphism does not predict survival in melanoma

782 melanoma pts

-680 wt/wt CCR5
-102 hetero- or homozygous Δ32 CCR5

Kaplan-Meier plot
p=0.89

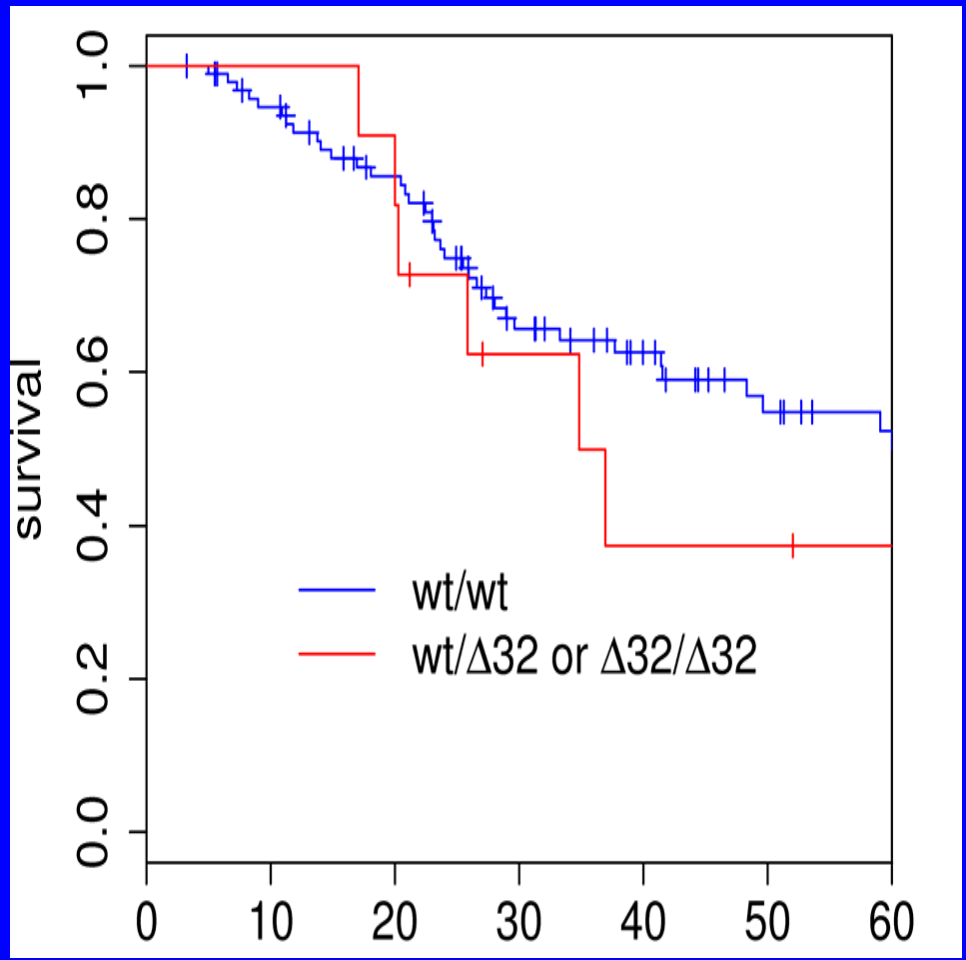


Intact CCR5 is necessary for response to IL-2

261 Stage IV melanoma patients

139 received IL-2
123 wt/wt
16 wt/Δ32 or Δ32/Δ32

Kaplan-Meier plot
p=0.029

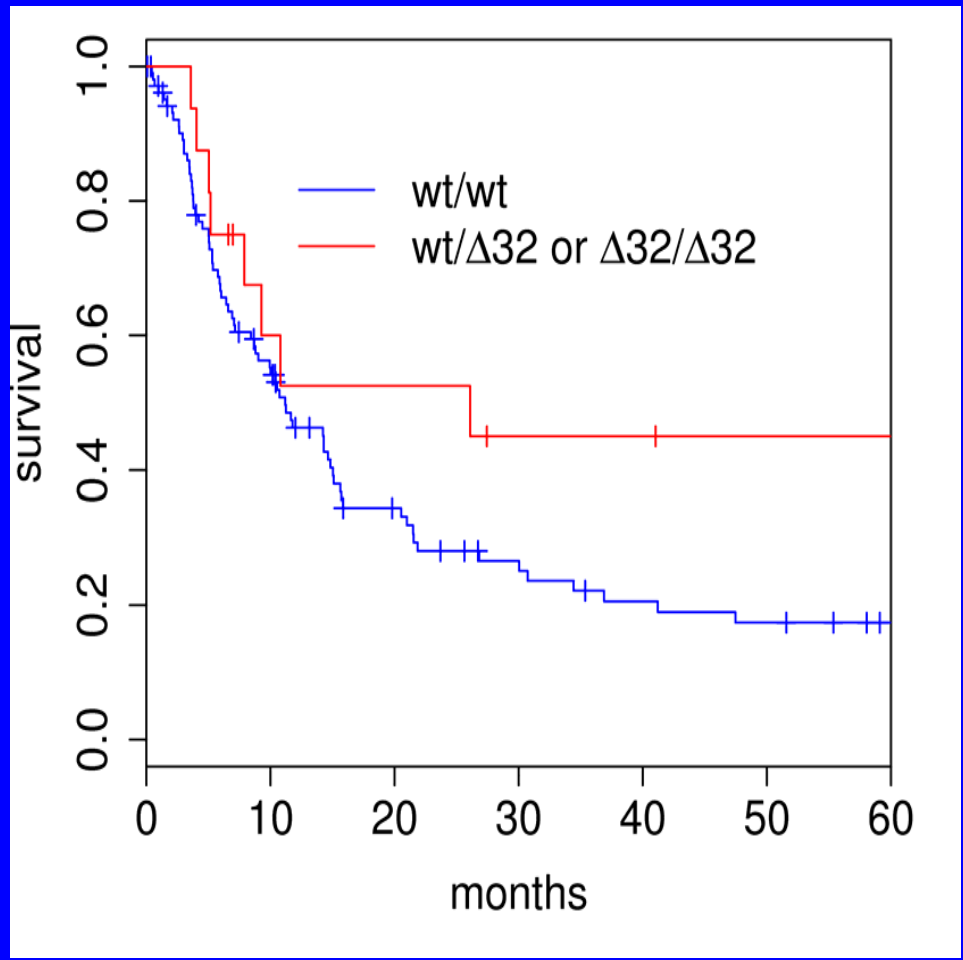


Intact CCR5 may predict worse prognosis in patients who do not receive immunotherapy

261 Stage IV melanoma patients

122 did not receive IL-2
106 wt/wt
16 wt/Δ32 or Δ32/Δ32

Kaplan-Meier plot
p=0.12



Conclusions

1. High-dose IL-2 induces a durable objective response in 15-20% of patients with metastatic melanoma and renal cell carcinoma
2. Tregs are elevated in patients with melanoma and renal cell carcinoma
3. High-dose IL-2 increases the Treg frequency in most patients
4. Patients who achieve an objective clinical response with IL-2 showed a *7-fold decrease* in Tregs
5. The CCR5Δ32 polymorphism is associated with a poor response to immunotherapy
6. Other immune responsive genes may also influence the response to tumor immunotherapy

Acknowledgements

- **THE TUMOR IMMUNOLOGY LABORATORY**
  - Qin Wang, PhD
  - Seunghee Kim-Schulze, PhD
  - Giovanni Cesana, MD
  - Dae Won Kim, MD
  - Dorota Morociewicz
- **COLUMBIA CLINICAL TEAM**
  - Bret Taback, MD
  - Desiree Ratner, MD
  - George Niedt, MD
  - William Sherman, MD
  - Gail DeRaffele, RN
  - Josephine Mitcham

- **JULIUS-MAXIMILIANS UNIVERSITY**
  - Dabid Schrama, PhD
  - Jurgen C. Becker, MD, PhD

- The Dana Foundation
- Chiron Corporation