

***In situ* targeting of antigen presenting cells within
secondary lymphoid organs as a means to control
immune responses**

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MannKind's Active Immunotherapy Approach

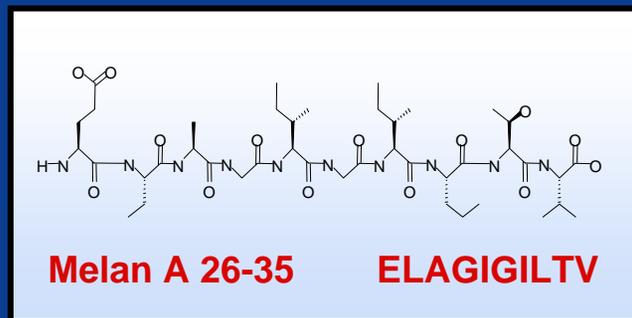
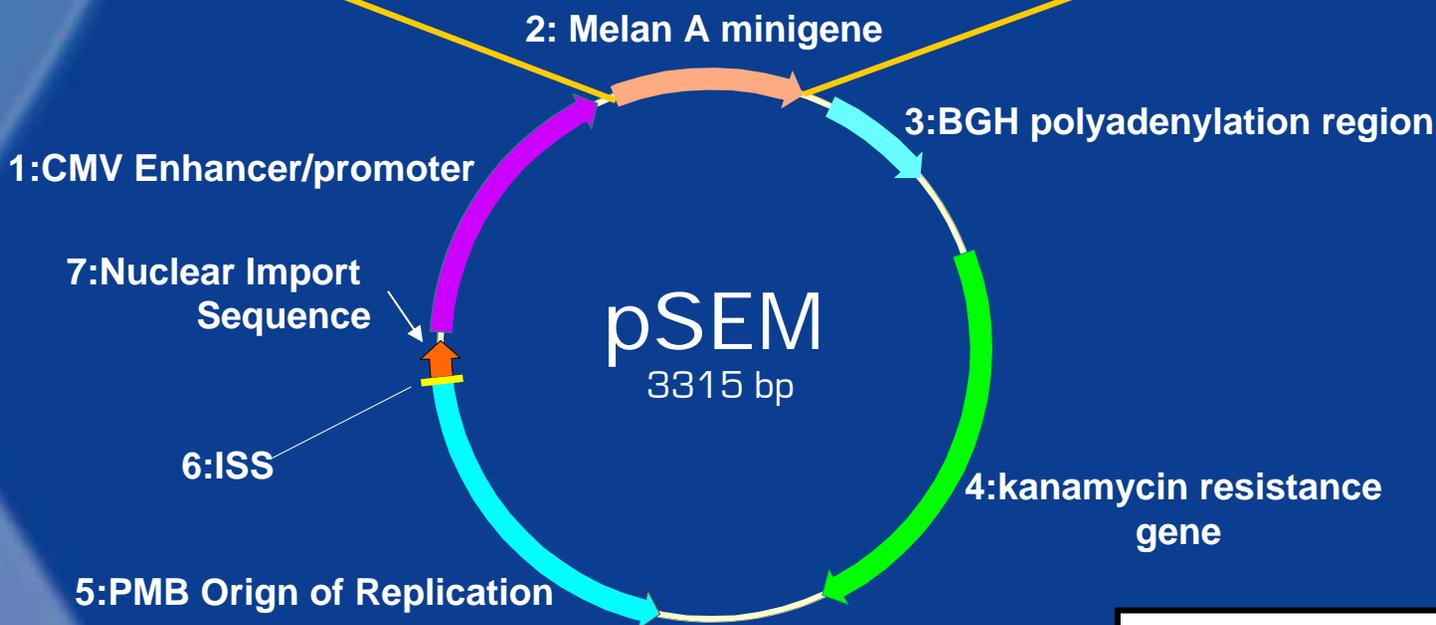
- Method of immunization
 - ✦ Plasmid-prime, peptide-boost

- Route of administration
 - ✦ Intralymphatic targeted delivery of actives
 - Independent control of signal 1 and 2



Model Actives: pSEM and Melan A₂₆₋₃₅ (ELAGIGILTV)

MLLAVLYCL **ELAGIGILTV** **YMDGTMSQV** ³¹**GILT.....CEPV⁹⁶**
Tyr 1-9 Melan A 26-35A27L Tyr 369-377 Melan A 31-96





Preclinical model: HHD-1 mice (Human HLA-A*0201 transgenic H-2D^b^{-/-} β2m^{-/-} double knockout mice)

HHD construct designed:
Hβ₂μ-Hα₁-Hα₂-mα₃-mTM-mcyt

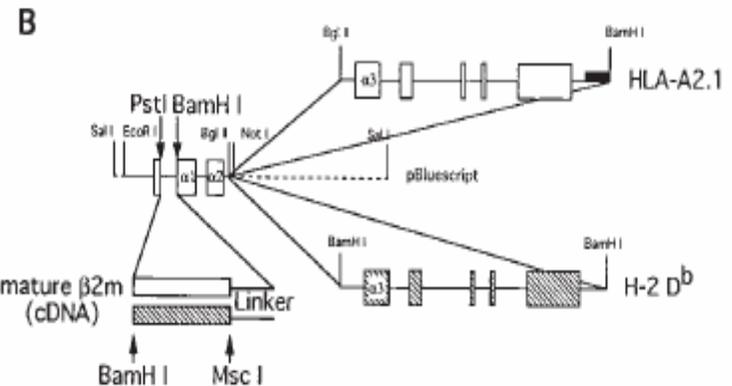
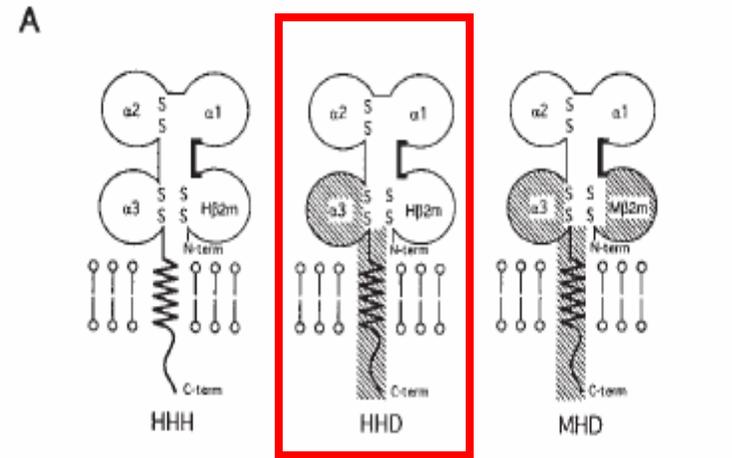
Construct injected into C57Bl/6 x SJL oocytes

Mice breed on H-2D^b^{-/-} X β₂μ^{-/-} background

Express A*0201 on somatic and BM cells

CTL repertoire selection on A*0201

Mount A*0201-restricted CTL responses

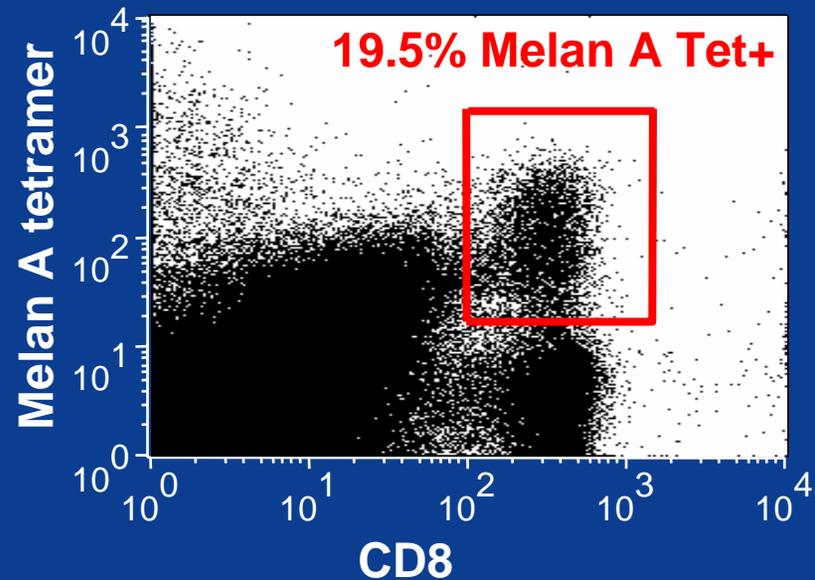
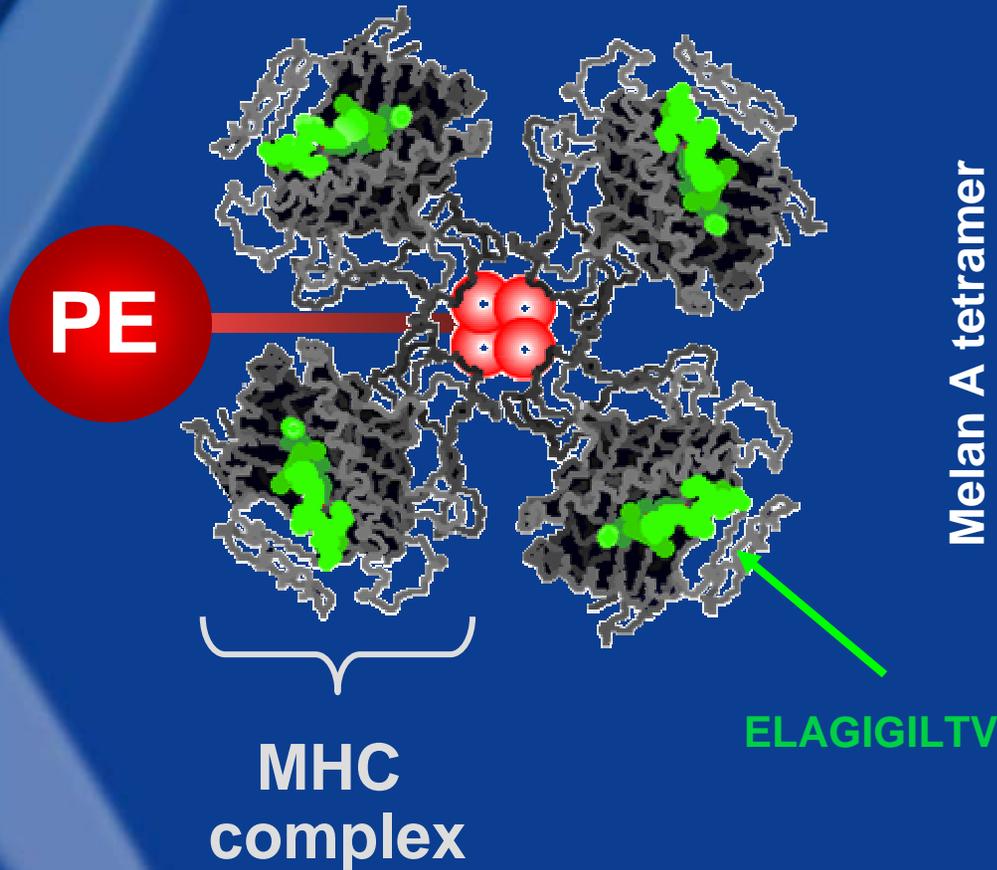


Pascolo et al, J Exp Med 185:2043 1997

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MHC Tetramers for the *ex vivo* quantification of cellular immune response

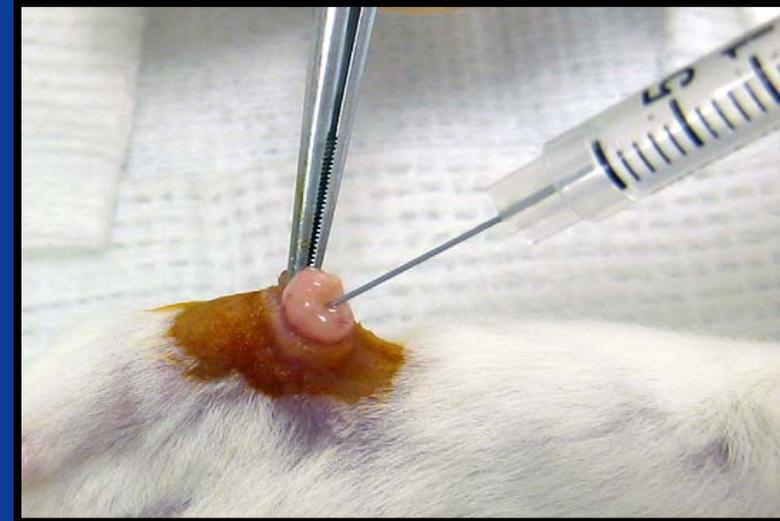
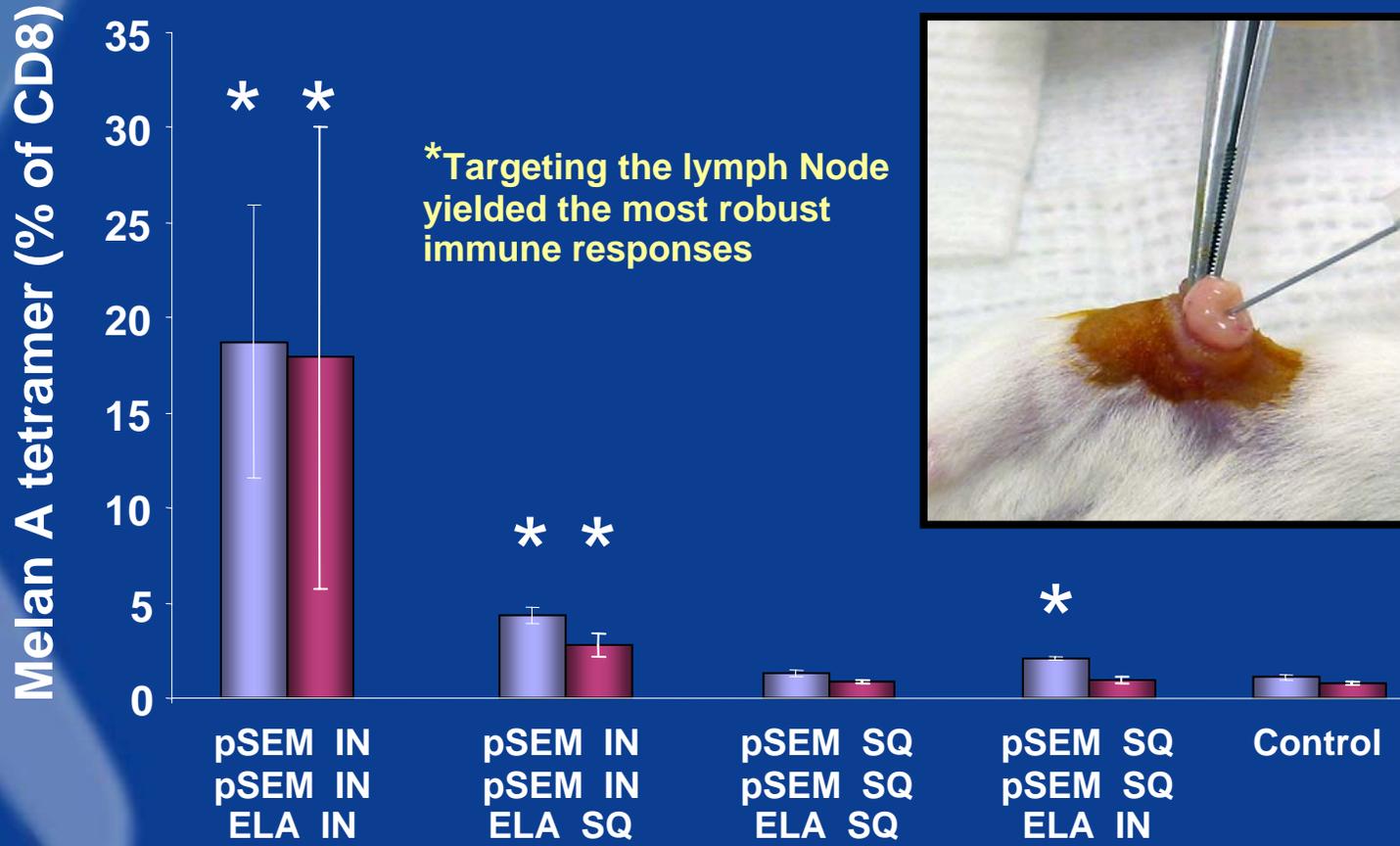


MART-1 (Beckman Coulter)



More effective induction of immune responses by intra-lymphatic immunization

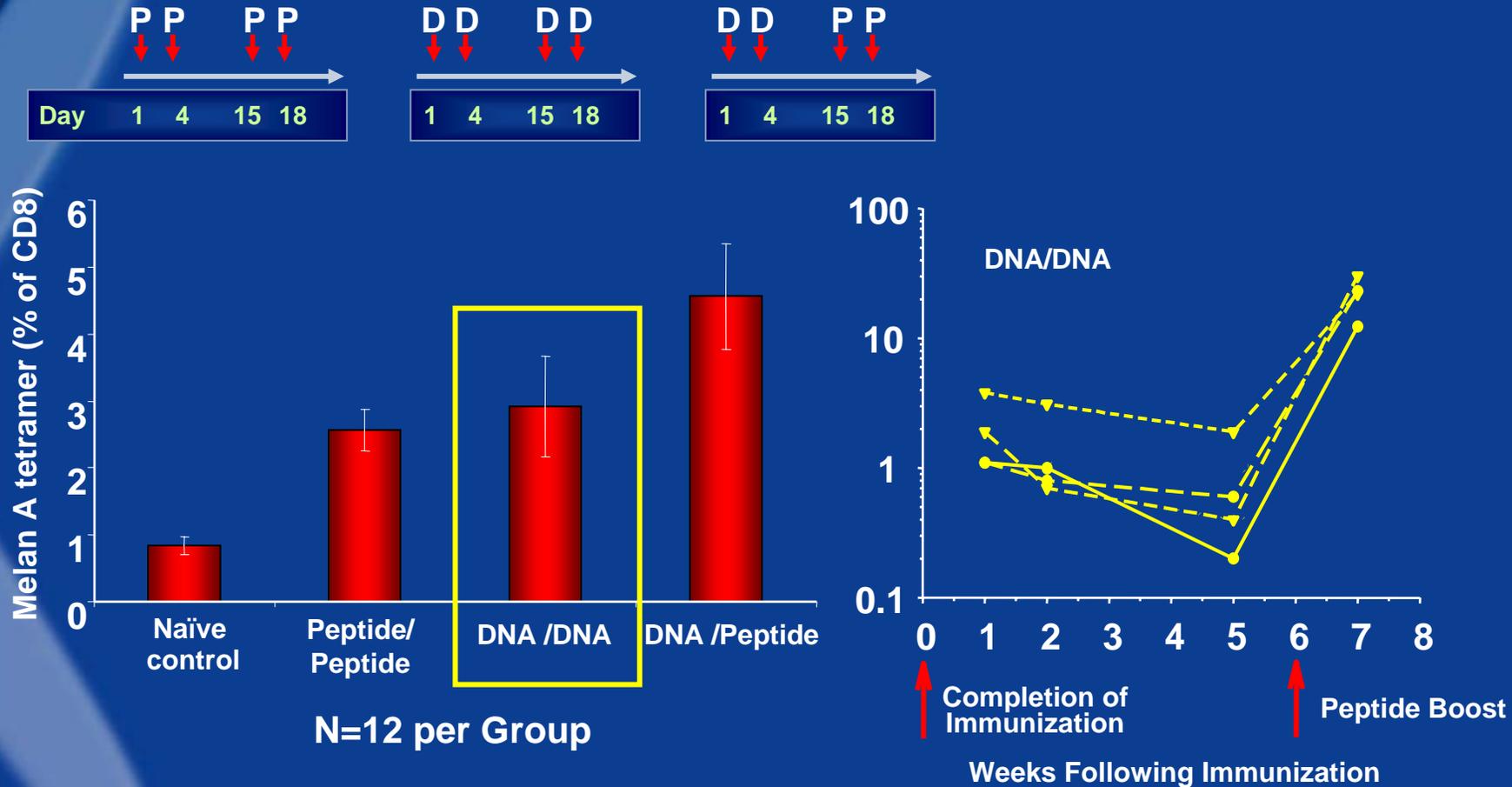
15 days post boost 43 days post boost



N=12 per Group

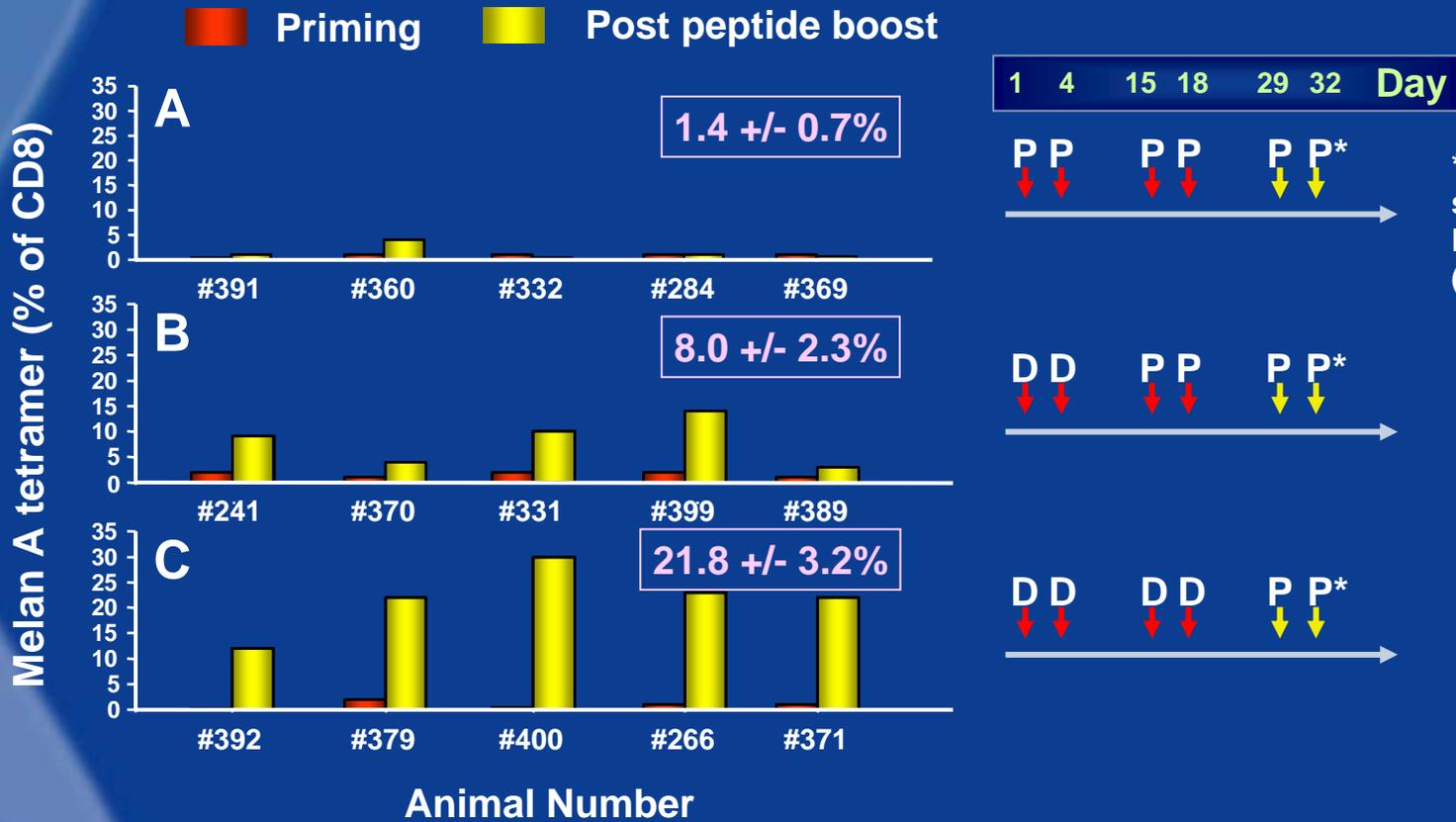


More effective induction of immune responses by alternating plasmid (priming) with peptide (boost)



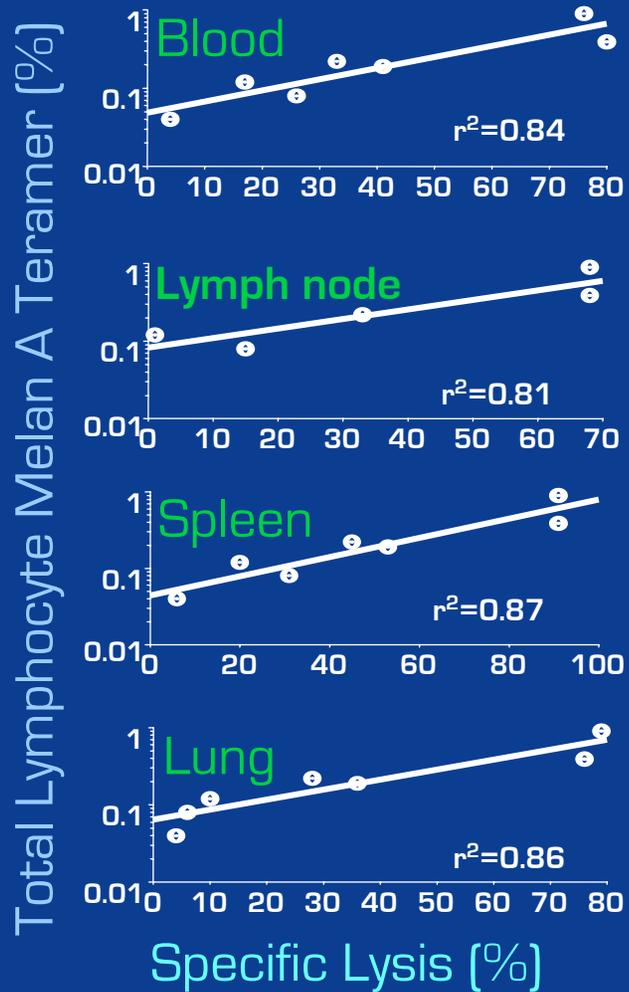
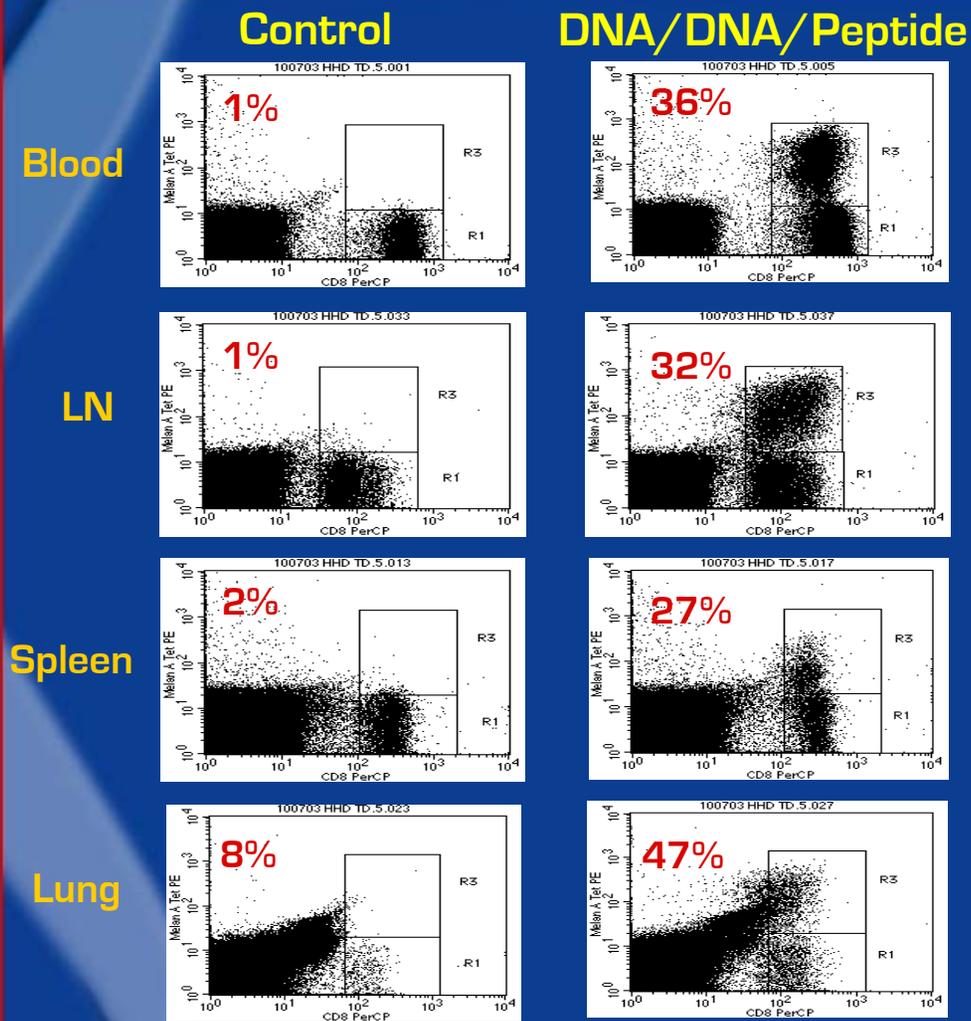


Refinement of the prime-boost regimen to maximize the expansion of antigen specific T cells





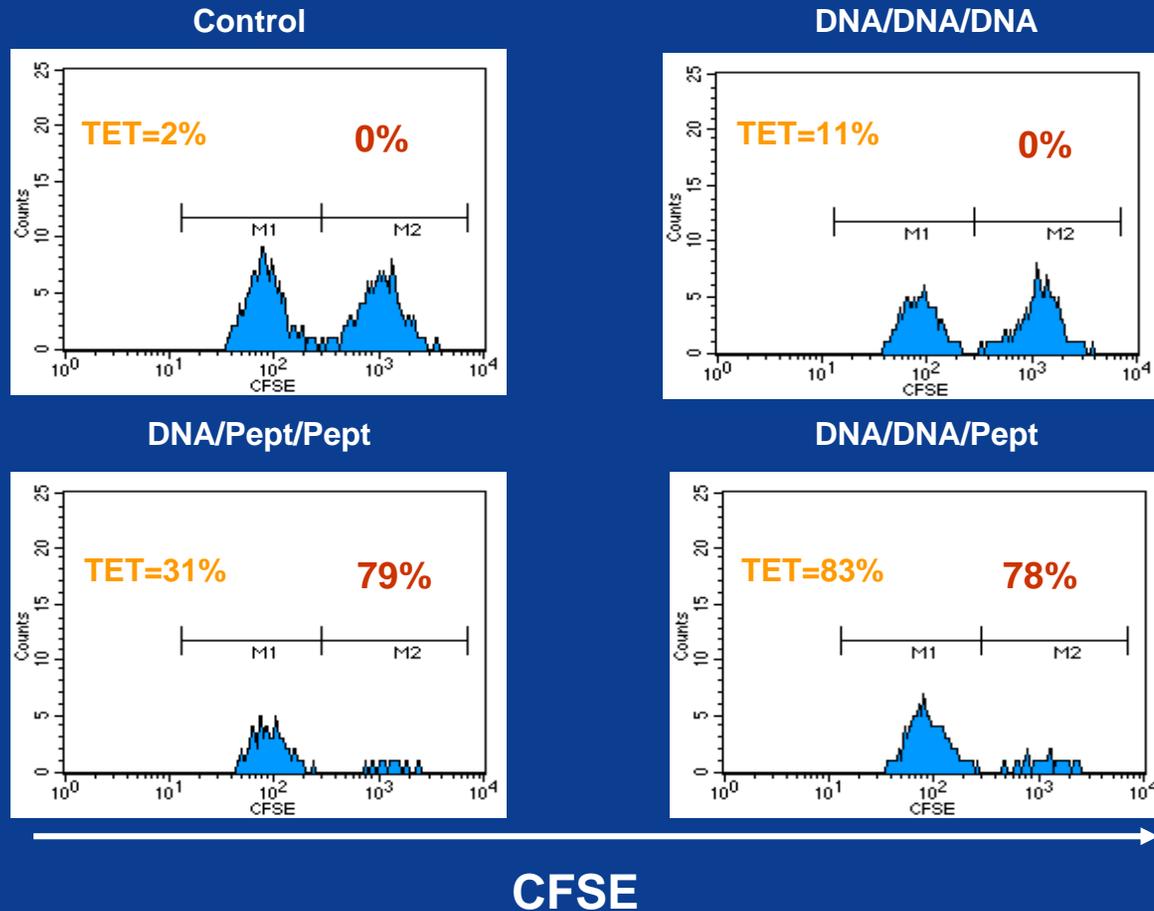
Enhanced response seen in lymphoid and non-lymphoid organs correlates with efficacy





Clearance of human tumor cells in animals immunized by DNA priming – peptide boost

In vivo cytotoxicity

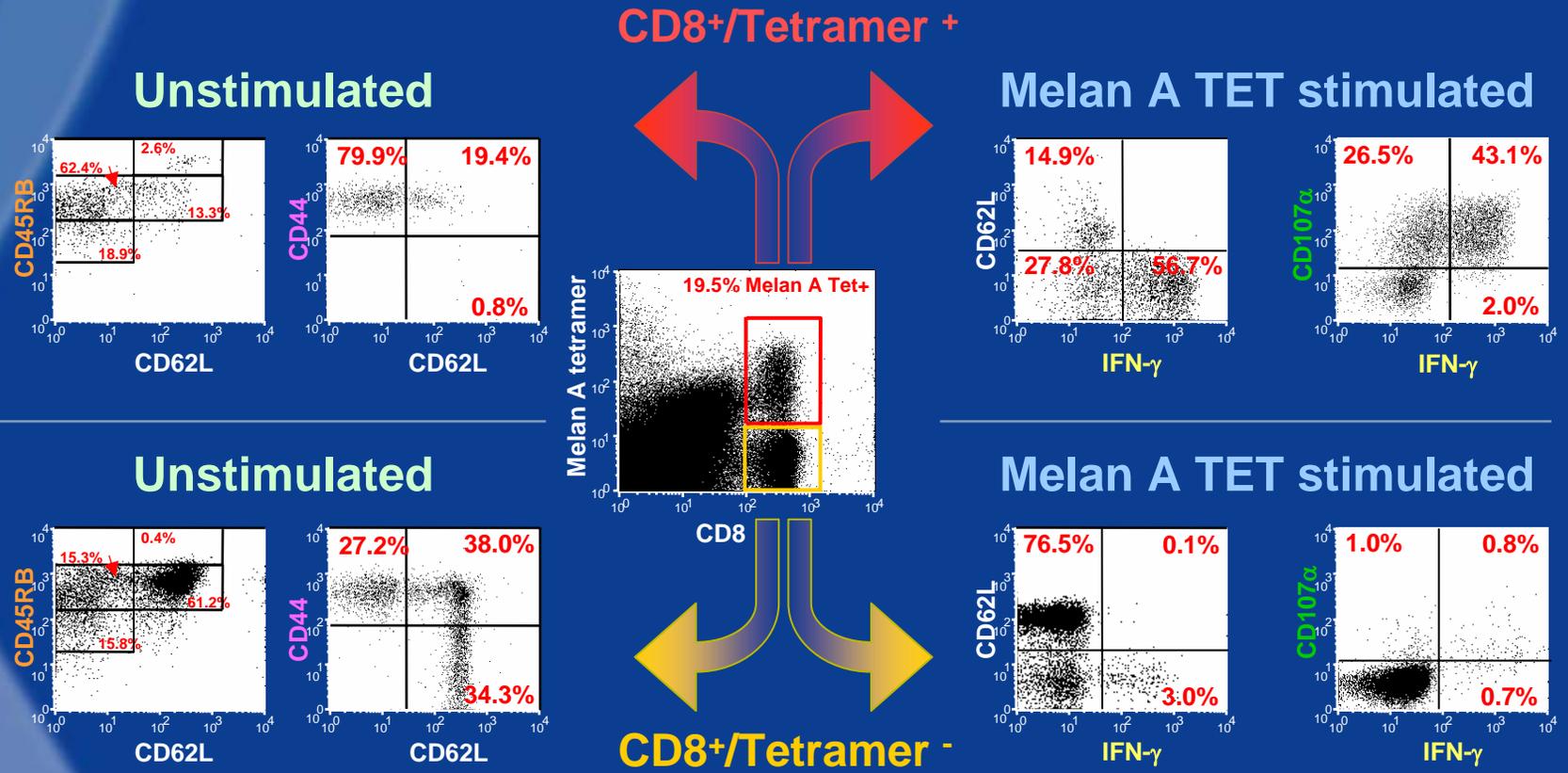


Mechanism of Action Studies

Characterization of the phenotypic profile and functional status of the T cell response induced by plasmid-priming and peptide-boost

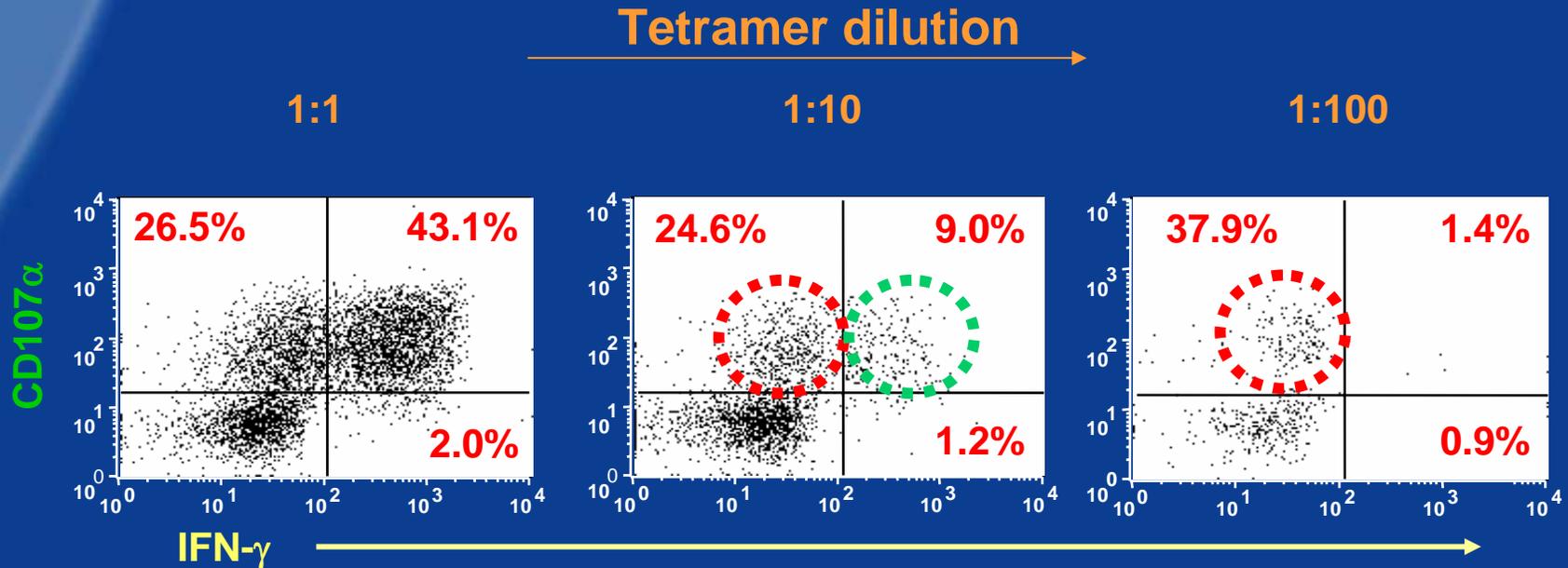


Phenotypic analysis of Melan A₂₆₋₃₅ specific CD8+ T cells before and after *ex vivo* stimulation





Functional specific T cells rapidly acquired the expression of CD107a and IFN- γ following Melan A tetramer stimulation

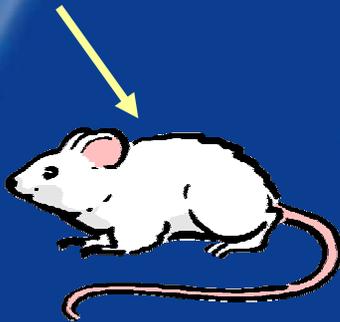


A lower stimulating threshold for CD107a expression was observed following limiting dilution of the Melan A tetramer (right panel).



Control of the immune response by targeted lymph node delivery

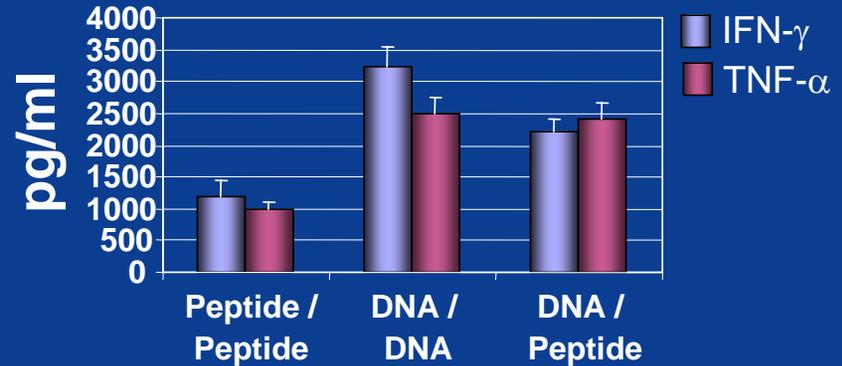
pSEM plasmid and/or Melan A 26-35 peptide (intra lymph node admin)



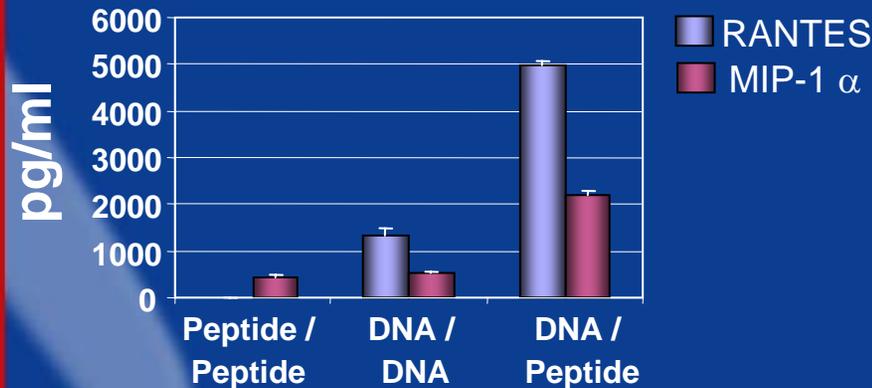
Peptide-stimulated splenocytes

ELISA, Luminex

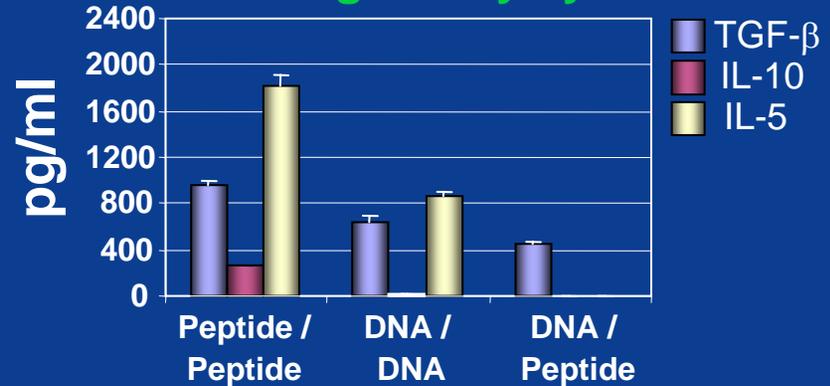
Pro-inflammatory cytokines



Chemokines

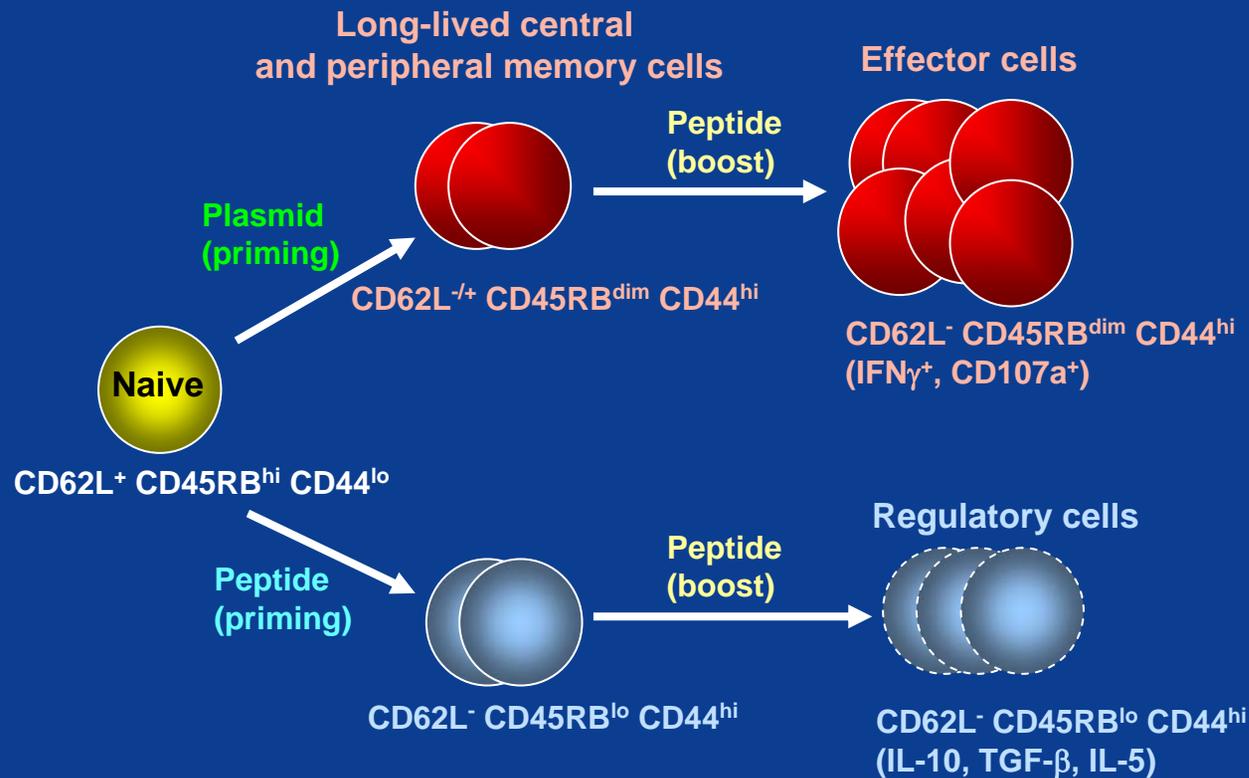


Immune regulatory cytokines



Dichotomy between T cell profile elicited by DNA priming or peptide priming

Proposed mechanism of action of plasmid versus peptide priming



Priming with DNA and boosting with peptide achieves substantially higher immune responses (Th1), and is associated with an increase in the rate of responders and the magnitude of response



Summary

- Targeted delivery of plasmid primes a long lasting population of central and peripheral memory cells, with significant expansion capability
- Effector cells resulting from plasmid prime – peptide boost display a complex functional profile
- Immune signatures of DNA versus peptide are substantially different
 - ✦ Independent control of signal 1 and 2 in context of lymph node targeting may allow effective manipulation of the magnitude and profile of immune response



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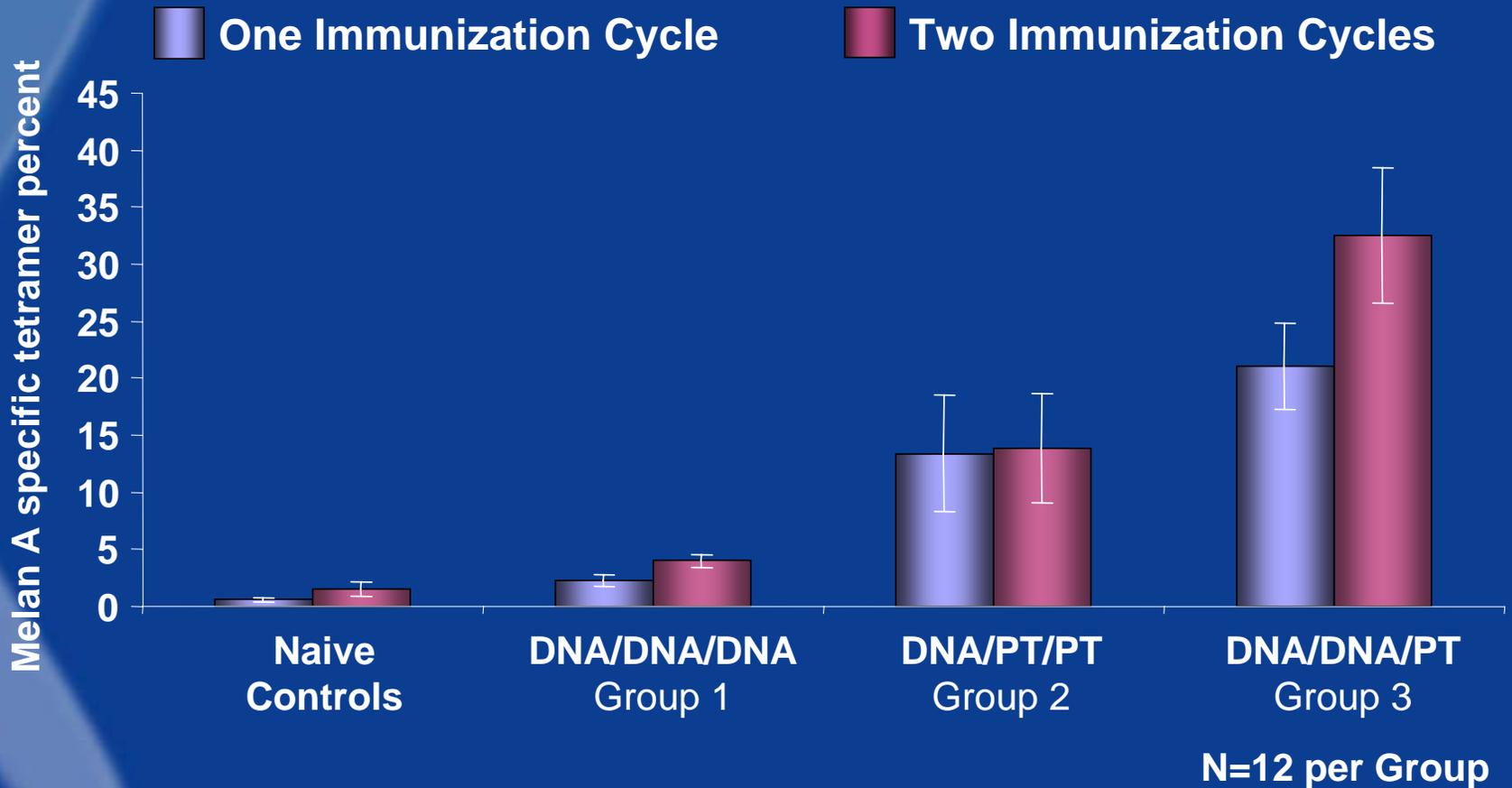
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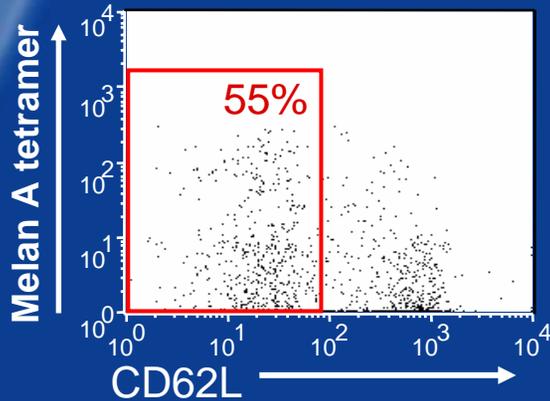
Enhanced immunity following two therapeutic cycles: Rational for clinical protocol



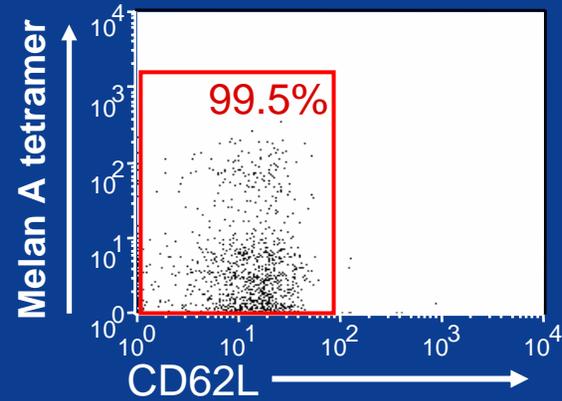


Luminex multiplex cytokine analysis of sorted CD62L negative cells from Melan A₂₆₋₃₅ immunized animals

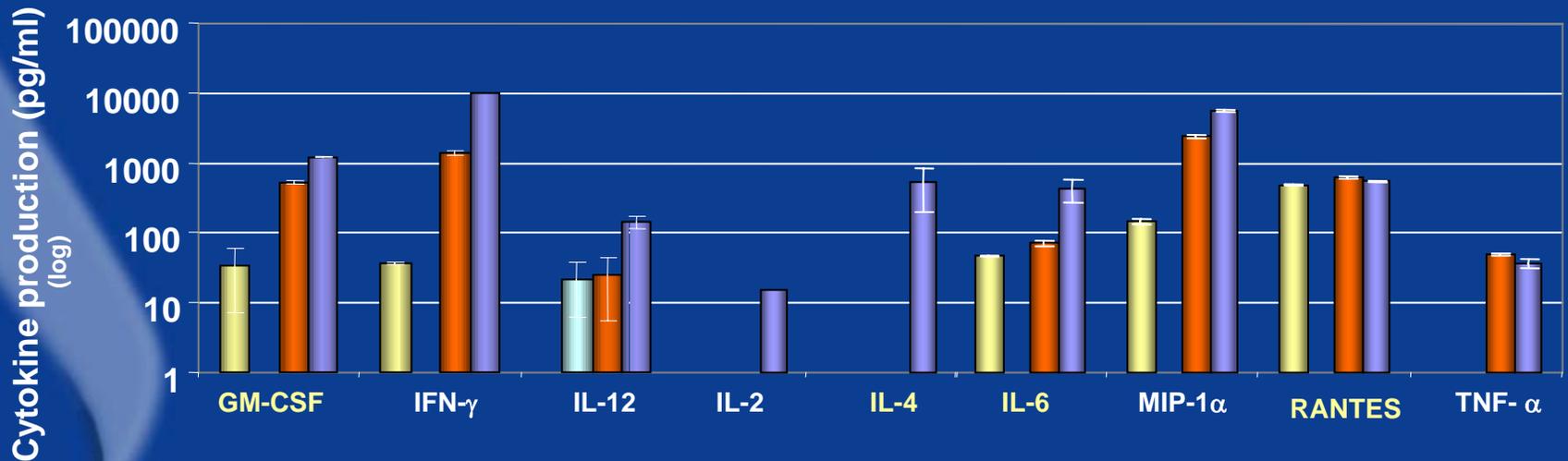
Presort



Post sort

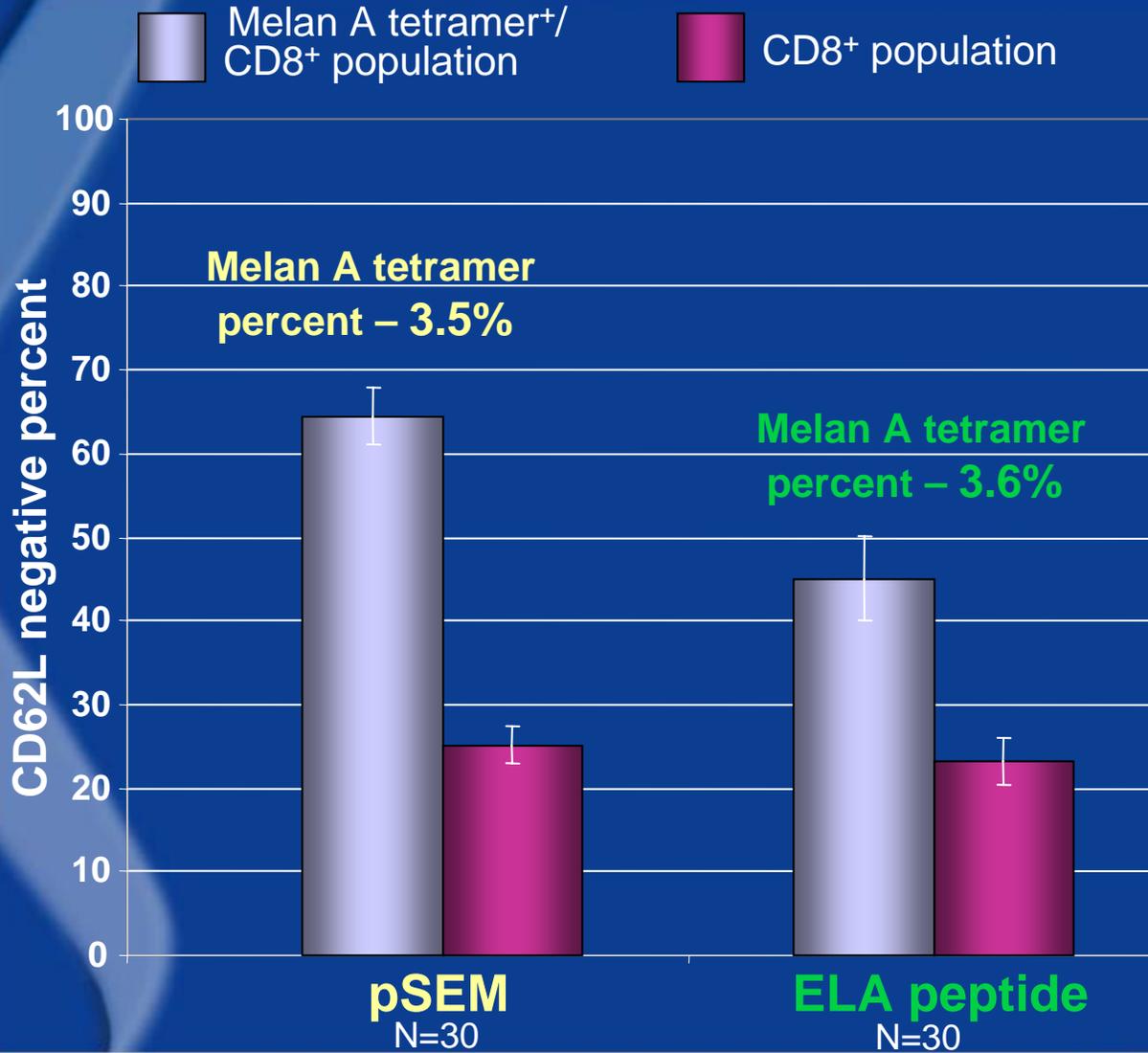


CD62L⁺ Naïve CD62L⁻ Naïve CD62L⁺ immunized CD62L⁻ immunized





CD62L negative population of Melan A immunized animals identified from CD8+ lymphocytes



Animals immunized with 4 injections of pSEM plasmid (1mg/mL) or 4 injections of Melan A (A27L) peptide analogue (1mg/mL). CD62L negative population analysis performed on CD8+ and Melan A+/CD8+ populations.

A student's t-test value of 4.74 was determined from the Melan A tetramer+/CD8+ population comparing the pSEM immunized group and the ELA peptide immunized group



MannKinds Active Immunotherapy Approach

- Route of administration
 - ✦ Intralymphatic vs. subcutaneous injection
- Method of immunization
 - ✦ Optimized protocol in transgenic mice
- Mechanism of Action
 - ✦ Antigen specific effector cells
- Conclusions

