A Cell-Based Vaccine for Neuroblastoma Induces VLA-2 (CD49b) on T Effector-Memory Cells via CD137L

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A member of Children's Hospital and Health System.

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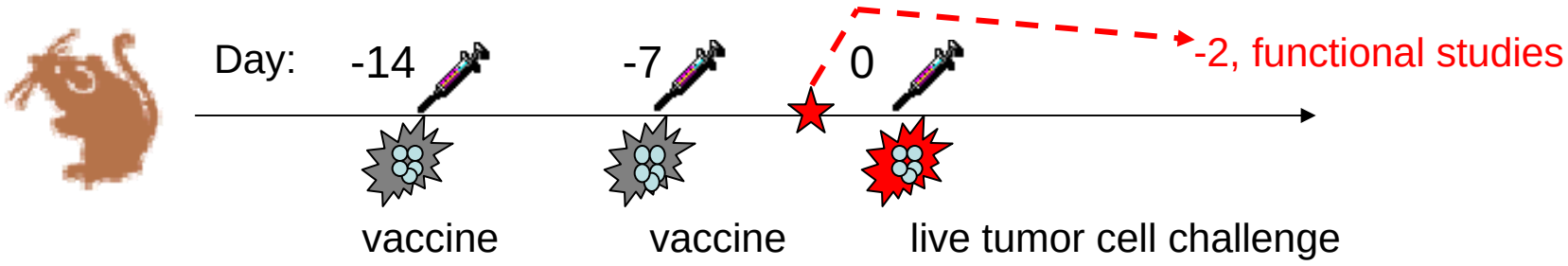
Teaming Up to Cure Childhood Cancer through Research

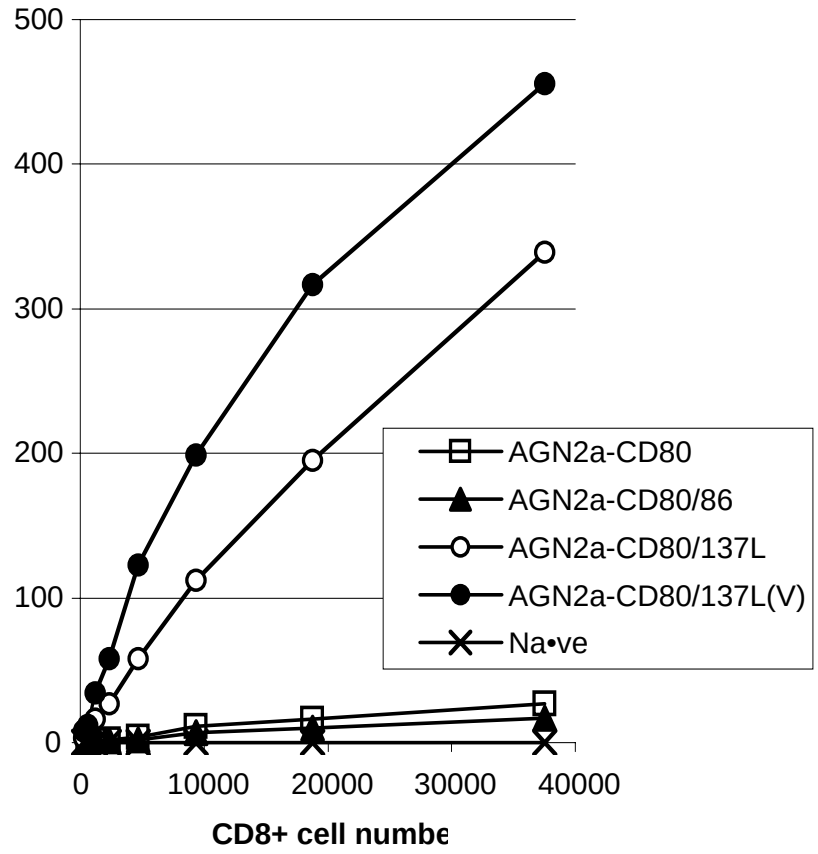
Goal: development of a cell based vaccine with translational impact for patients with advanced neuroblastoma

-survival in advanced disease remains **very** poor
-incremental improvement is seen with HSCT
-model: strain A/J mice immunized with engineered AGN2a
+/- Treg blockade (PC61)
-question: what accounts for the strong anti-tumor effect
mediated by the presence of CD137L
on the surface of the AGN2a-vaccine?

Vaccine and Challenge Studies (1 x10^6 cells ea. injection)



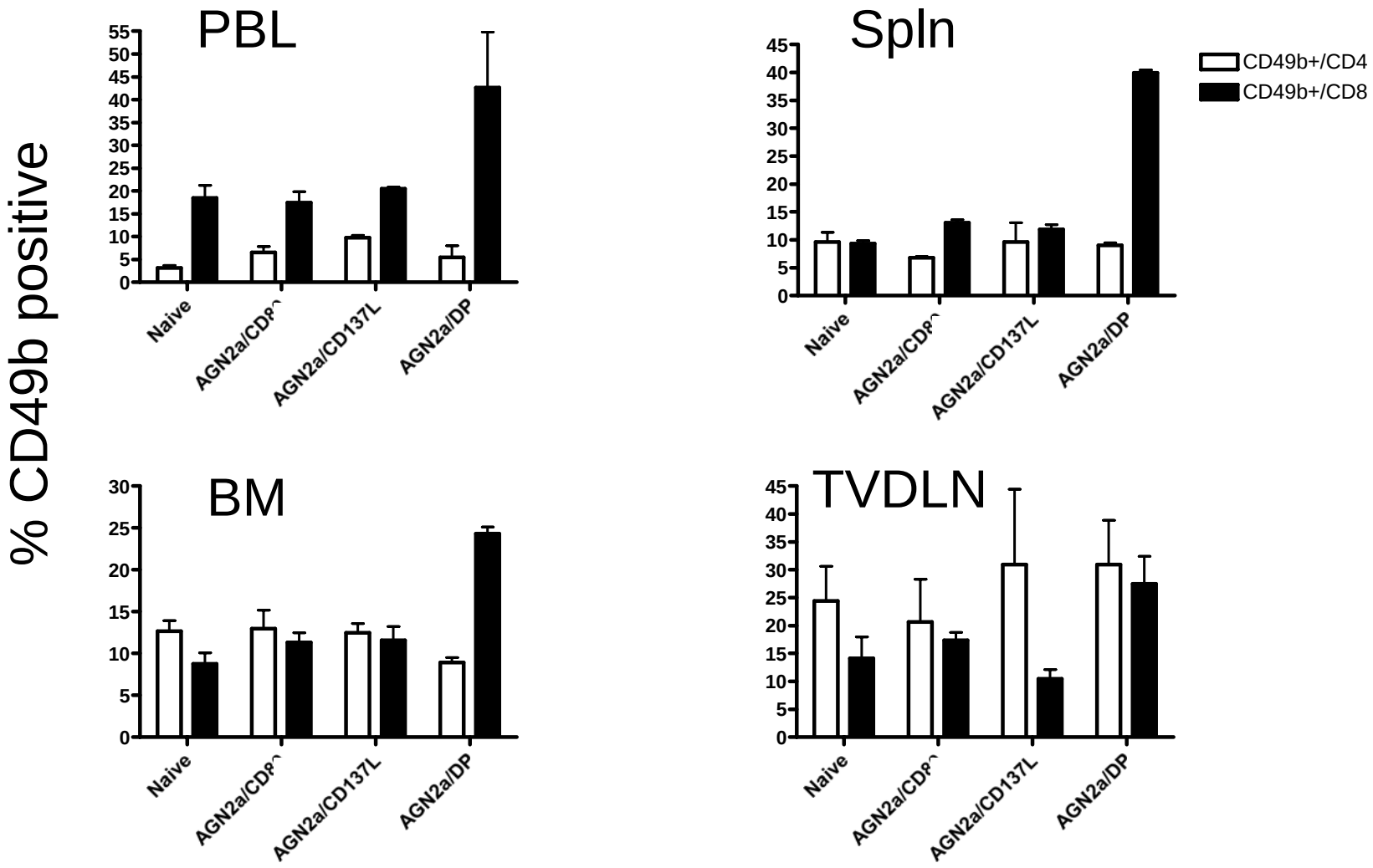
Vaccines: Unmodified AGN2a --> non-protective
AGN2a-CD80 --> non-protective
AGN2a-CD80/86 --> **protective**
AGN2a-CD80/CD137L --> **protective**



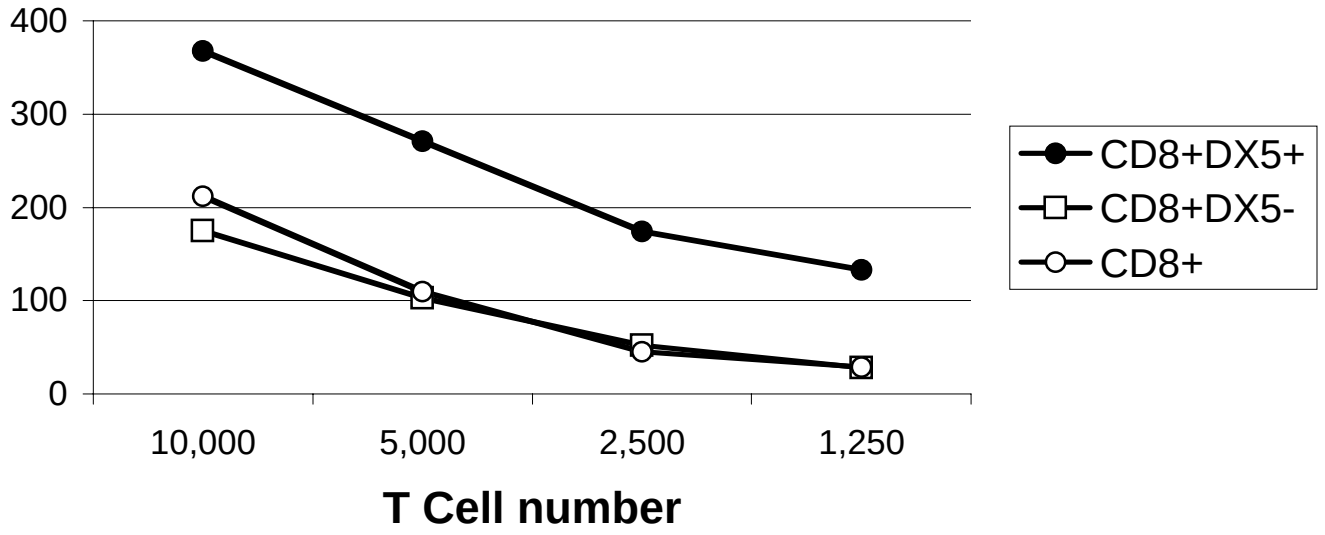
To analyze vaccine-induced effector cells, CD8 splenocytes were collected 5 days after secondary vaccination and analyzed (no-restim) by IFN-γ ELISPOT using wt AGN2a targets.

What cell type accounts for the effects induced by CD137L?

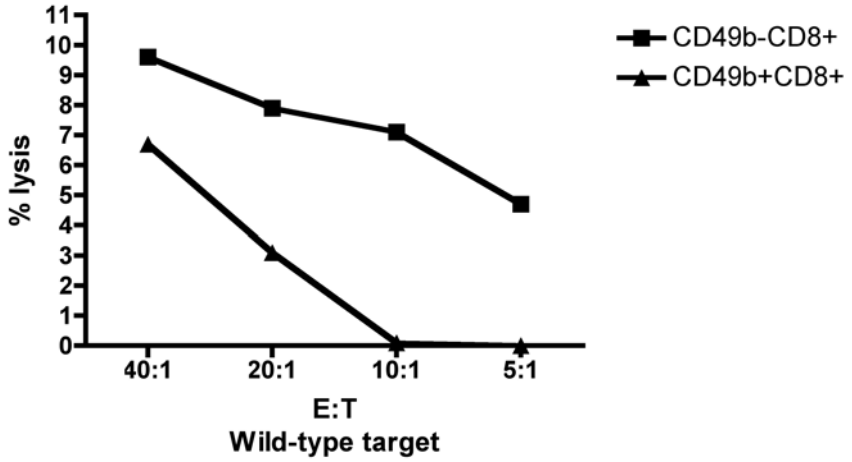
Increased Expression of VLA-2 (CD49b/DX5) on AGN2a-CD80/CD137L (DP)-induced T cells



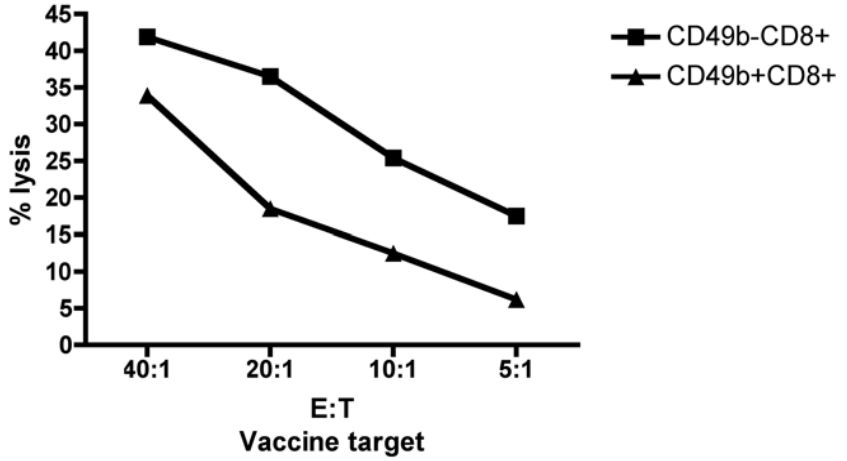
ELISPOT Reactivity, VLA-2+ CD8 Effector T Cells



AGN2a target



AGN2a-DP target



What is CD49b/VLA-2/DX-5?

1985: "**VLA-1: a T cell surface antigen which defines a novel late stage of human T cell activation**," M.E. Hemler, J.G. Jacobson, M.B. Brenner, D. Mann, J.L. Strominger. *Eur J Immunol* 15:502

2000: "**NK markers are expressed on a high percentage of virus-specific CD8+ and CD4+ T cells**," M.K. Slifka, R.R. Pagarigan, J.L. Whitton. *J Immunol* 164:2009

2000: "**Integrin $\alpha 2\beta 1$ (VLA-2) is a principal receptor used by neutrophils for locomotion in extravascular tissue**," J. Werr, J. Johansson, E.E. Eriksson, P. Hedqvist, E. Ruoslahti, L. Lindbom. *Blood* 95:1804

1997: "**Amplification of tumor immunity by gene transfer of the co-stimulatory 4-1BB ligand: synergy with the CD28 co-stimulation pathway**," I. Melero, N. Bach, K. Hellstrom, A. Aruffo, R.S. Mittler, L. Chen. *Eur J Immunol* 28:1116.

>When T cell responses arise in the context of infectious disease, a clear pattern of differentiation can be proposed

>We do not know which T cell population are correlated with protective immune responses in vaccinated/tumor-bearing hosts, in which case exposure to antigen may be chronic. *Where are the VLA-2+ cells?*

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

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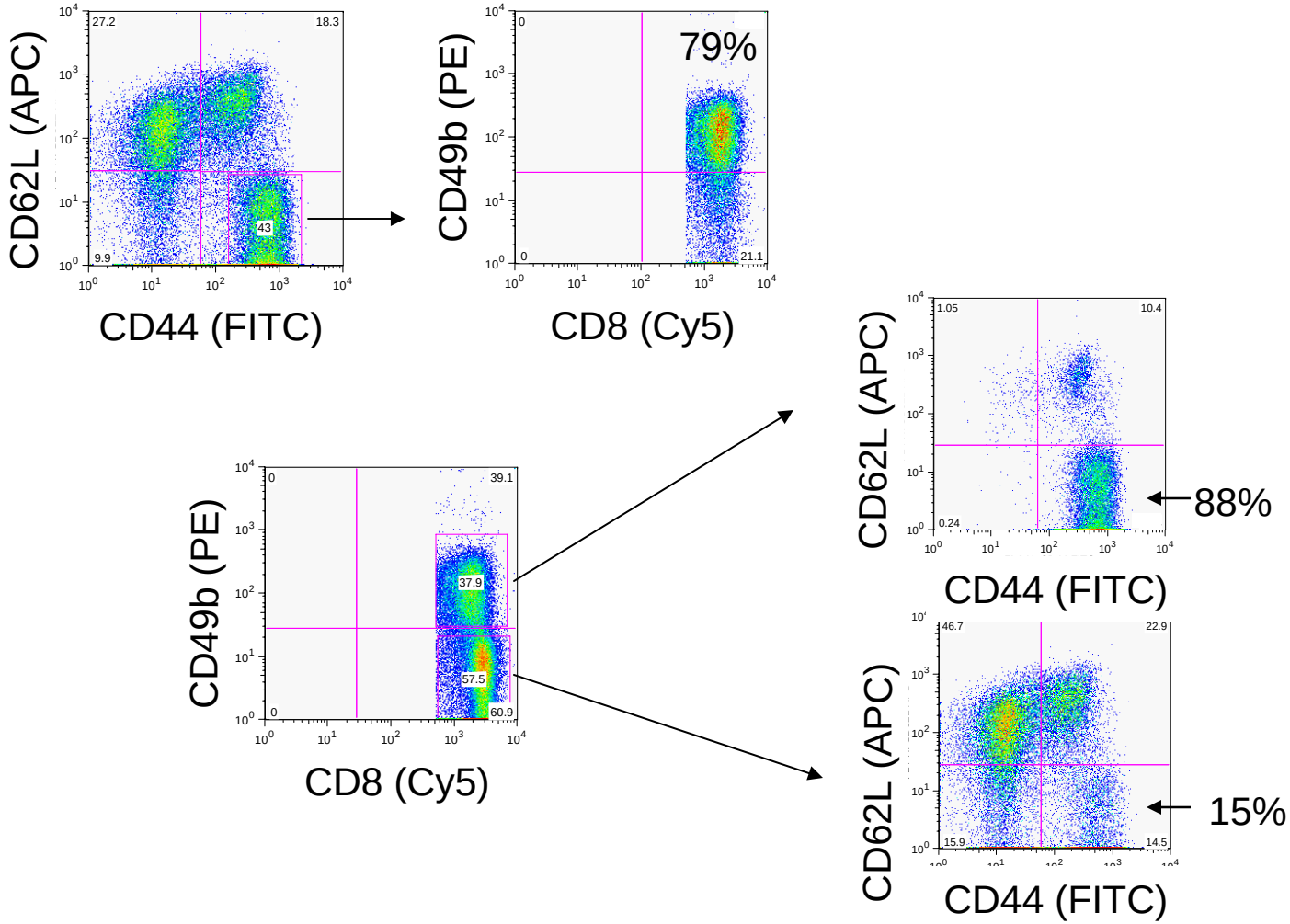
Badanovic and Harty, 2003, Nat Immunol 4:212

Yu and Anasetti, 2005, Nat Med 11:1282

Flow Cytometric Gating for CD49b+ T_{EM} from Vaccinated A/J mice, splenocytes

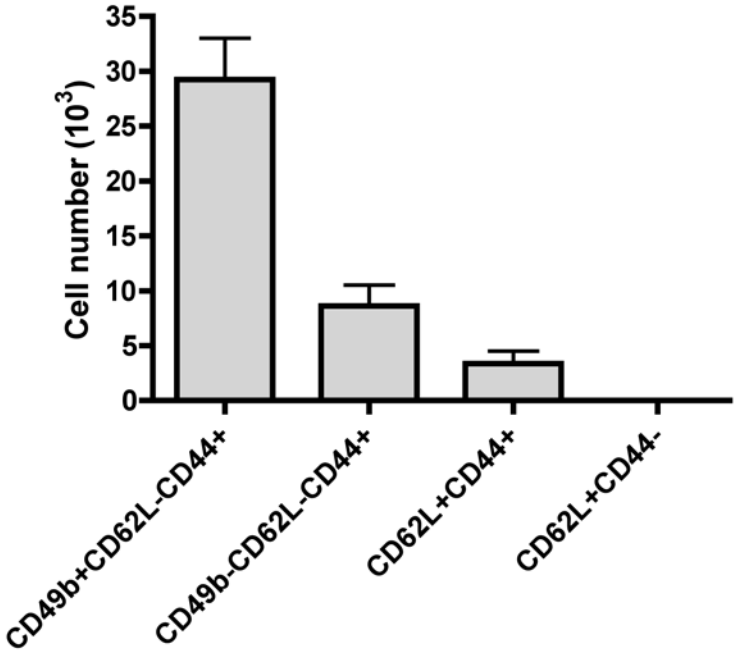


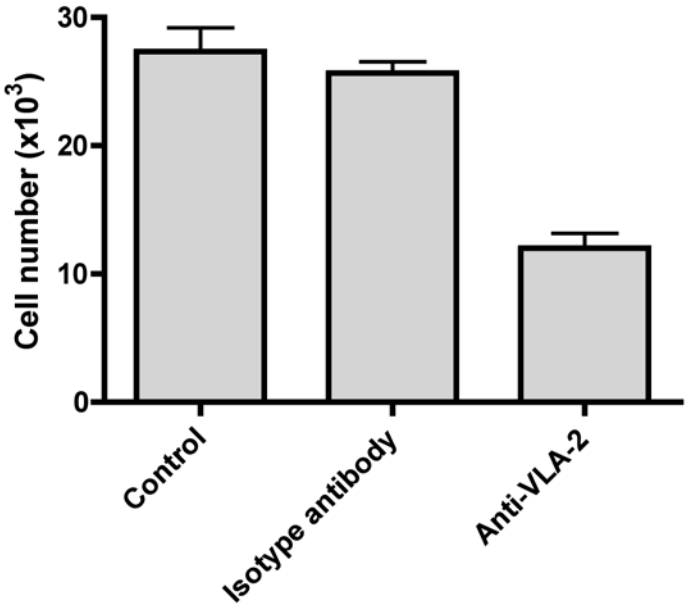
VLA-2 functions as an adhesion receptor: Matrigel invasion

2x AGN2a-CD80/CD137L vaccinations

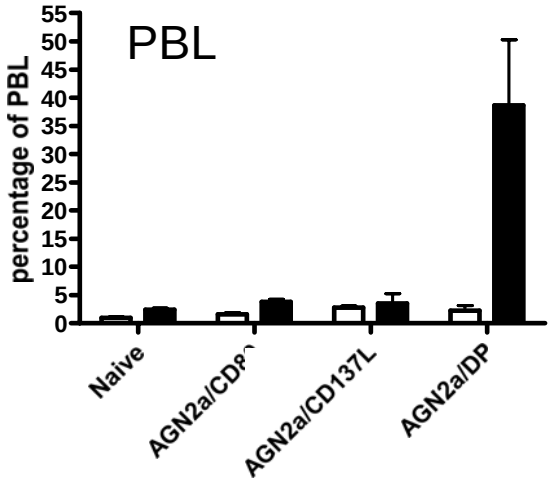
CD8 select (autoMACS) splenocytes, flow sort for CD44, CD62L, CD49b

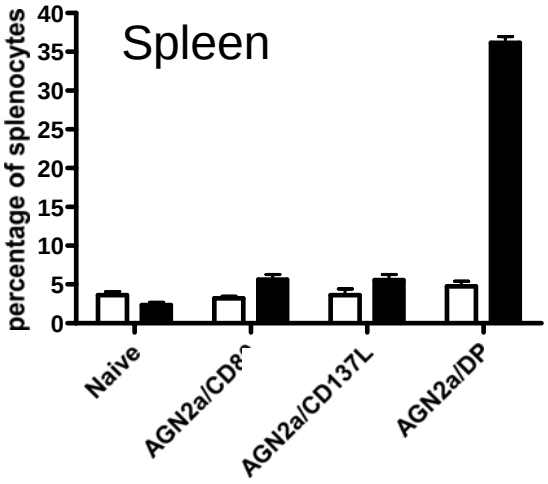
Culture on Matrigel +IL-2 for 48 h, Count infiltrating cells

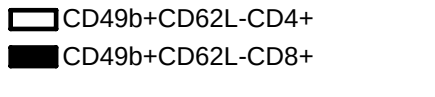


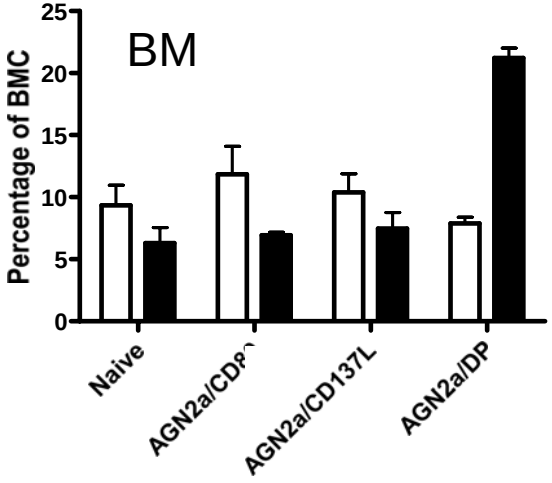


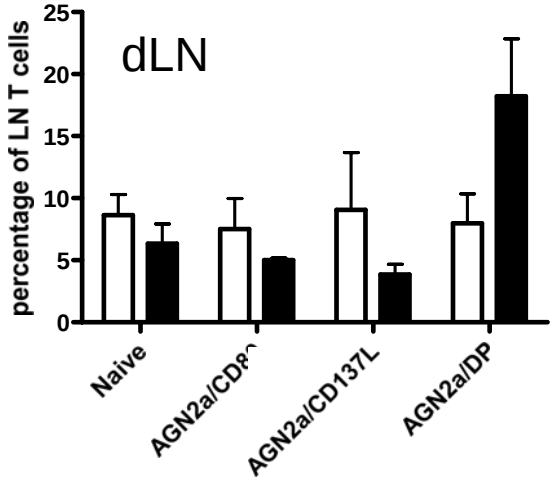
Increased Expression of VLA-2 in all lymphocyte compartments upon gating for T_{EM}

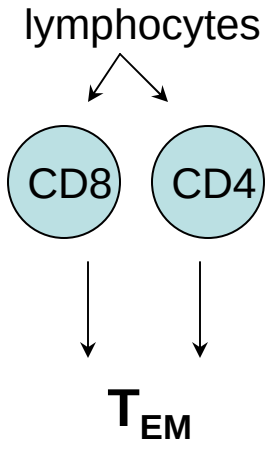








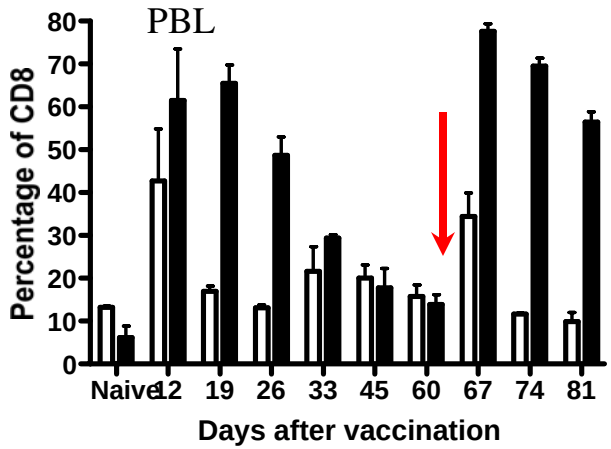


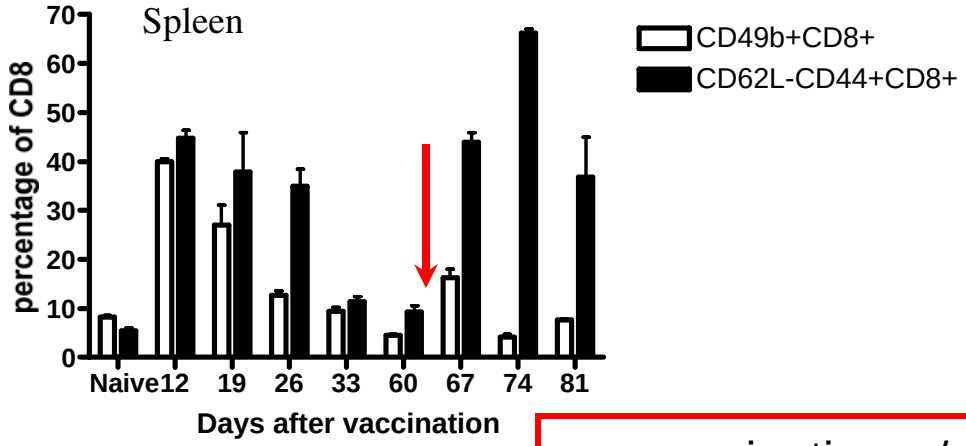


T_{EM}

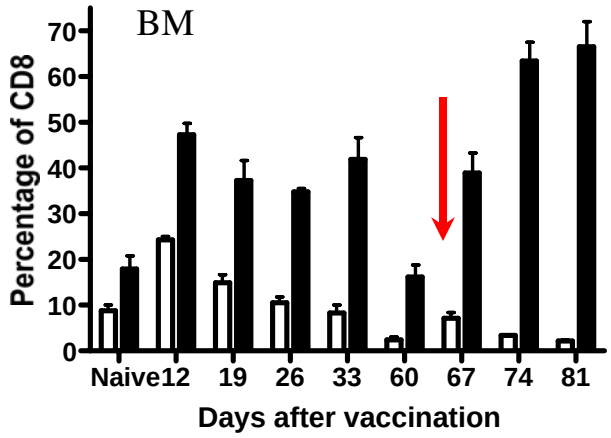
(49b+/CD62L-)

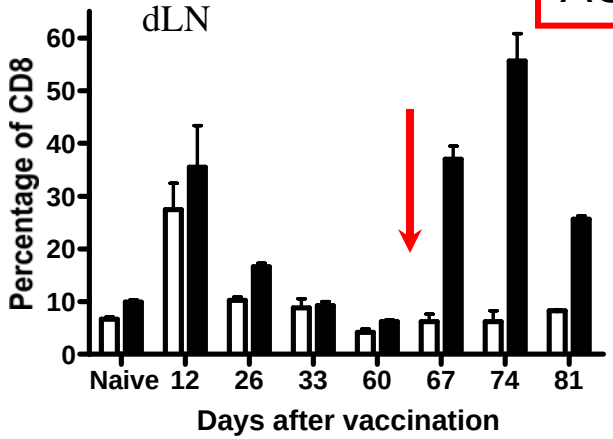
In vivo kinetics of CD8+, CD49b+ and T_{EM} populations, following primary or recall vaccine challenge with AGN2a-CD80/CD137L





re-vaccination w/ AGN2a-CD80/137L



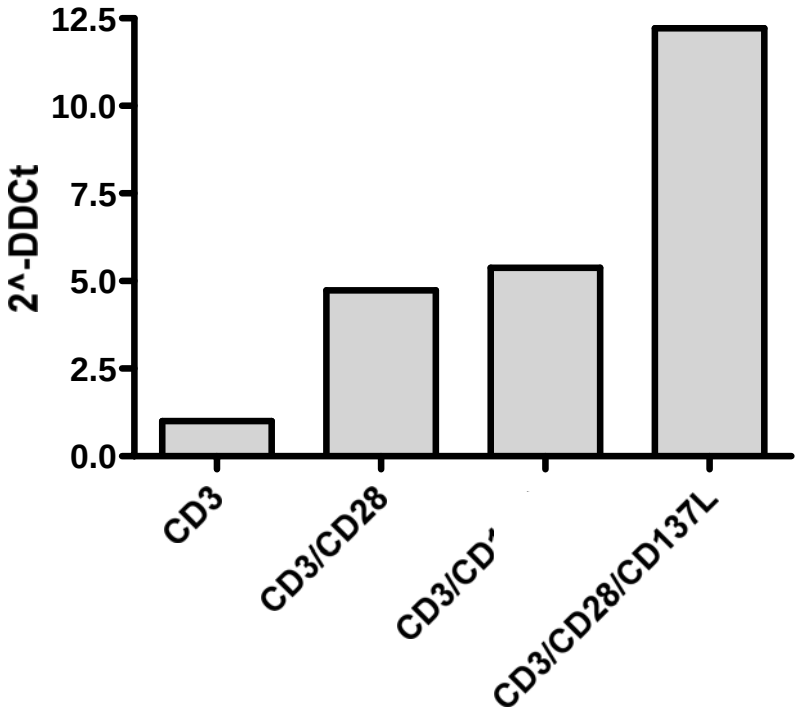


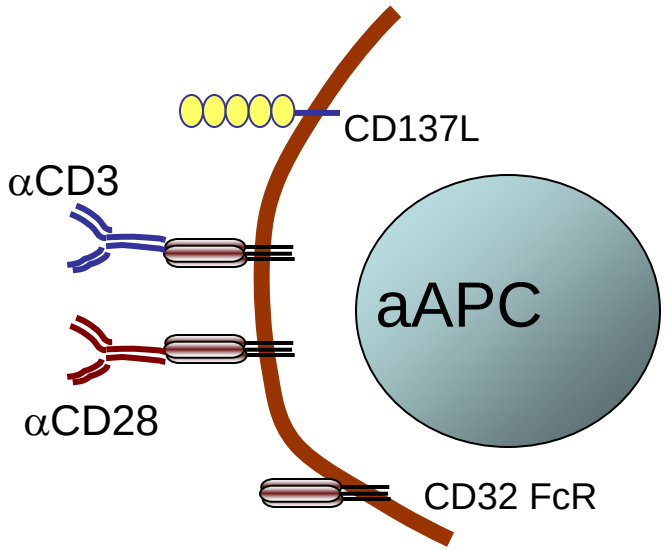
CD137L Increases CD49b Transcription (mRNA)

-incubate naïve splenocytes with aAPC, 20 U/ml IL-2

-on day 3, prepare cDNA, carry-out quantitative real-time PCR using SyberGreen detection

-normalize signal to HPRT, report fold change





Conclusions:

>CD137L induces a VLA-2+ T_{EM} population upon primary vaccination in the context of a cell-based vaccine

>VLA-2+ cells have superior tumor lysis, collagen invasion and IFN-g production

>VLA-2 expression is not as pronounced upon recall

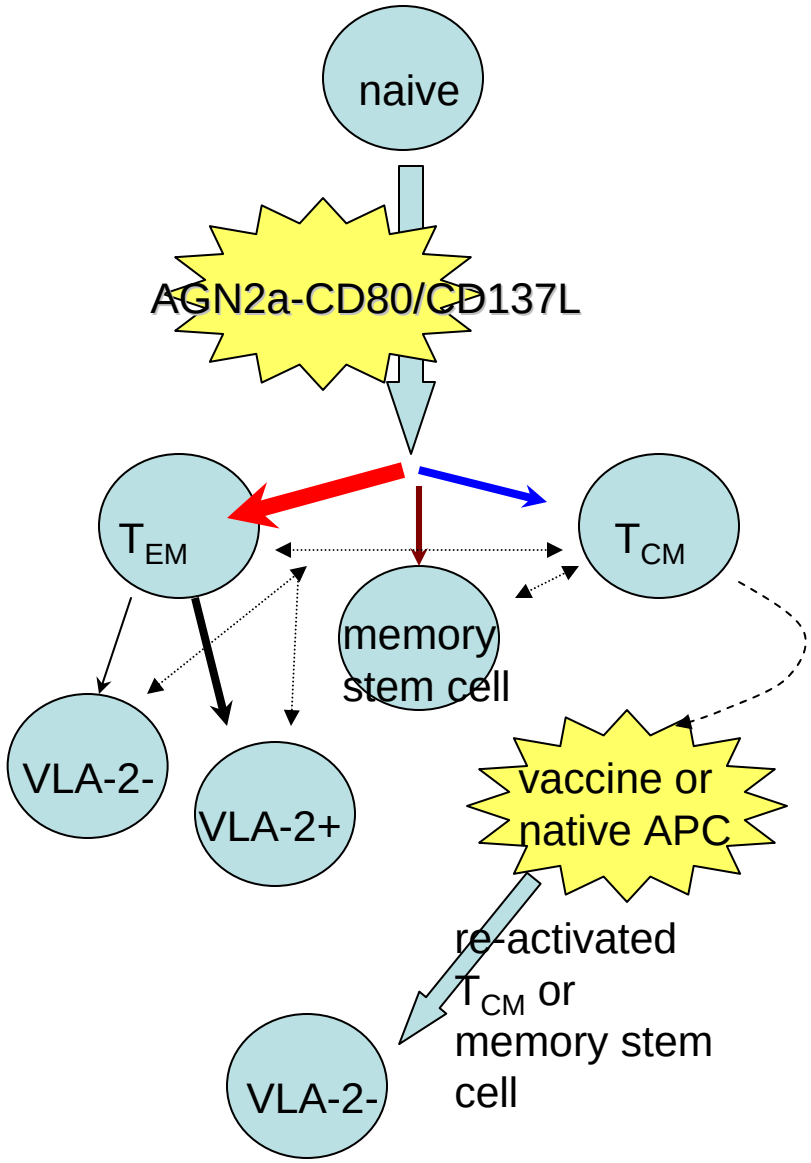
vaccination and may indicate:

*CD137L interactions are not required to activate differentiated effectors

*different APC are driving the reaction

*VLA-2 is restricted to “newly” activated T cells, and these are consumed rapidly during an immune response

>VLA-2 may be required to seed anti-tumor effectors into peripheral tissue where they may reside mediate a response, or differentiate to Tm



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