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

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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 x 10^6 cells ea. injection)

Day: -14 -7 0 -2, functional studies
vaccine vaccine live tumor cell challenge
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

PBL

SpIn

BM

TVDLN

% CD49b positive

CD49b+/CD4

CD49b+/CD8

Naive

AGN2a/CD8

AGN2a/CD137L

AGN2a/DP

Naive

AGN2a/CD8

AGN2a/CD137L

AGN2a/DP

Naive

AGN2a/CD8

AGN2a/CD137L

AGN2a/DP

Naive

AGN2a/CD8

AGN2a/CD137L

AGN2a/DP
ELISPOT Reactivity, VLA-2+ CD8 Effector T Cells

**AGN2a target**
- CD49b-CD8+
- CD49b+CD8+

**AGN2a-DP target**
- CD49b-CD8+
- CD49b+CD8+

**T Cell number**

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Wild-type target

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


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?*

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

![Graph showing cell numbers](image-url)
Increased Expression of VLA-2 in all lymphocyte compartments upon gating for $T_{EM}$

- PBL
- Spleen
- BM
- dLN

Lymphocytes

$T_{EM}$

(49b+/CD62L-)
In vivo kinetics of CD8+, CD49b+ and TEM populations, following primary or recall vaccine challenge with AGN2a-CD80/CD137L

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**Graphs:**

- **PBL:** Percentage of CD8 over time in peripheral blood lymphocytes (PBL).
- **Spleen:** Percentage of CD49b+CD8+ and CD62L-CD44+CD8+ over time in spleen.
- **BM:** Percentage of CD8 over time in bone marrow (BM).
- **dLN:** Percentage of CD8 over time in draining lymph nodes (dLN).

**Legend:**

- CD49b+CD8+
- CD62L-CD44+CD8+

**Key Points:**

- Days after vaccination: 0, 10, 20, 30, 40, 50, 60, 70, 80.
- Re-vaccination with 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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Support: MACC Fund
MCW Cancer Center/ACS
NIH RO1 CA 100030