

UNIVERSITY OF PENNSYLVANIA HEALTH SYSTEM
PATHOLOGY & LABORATORY
medicine

Estimating primate effector T cell responses to DNA vaccination

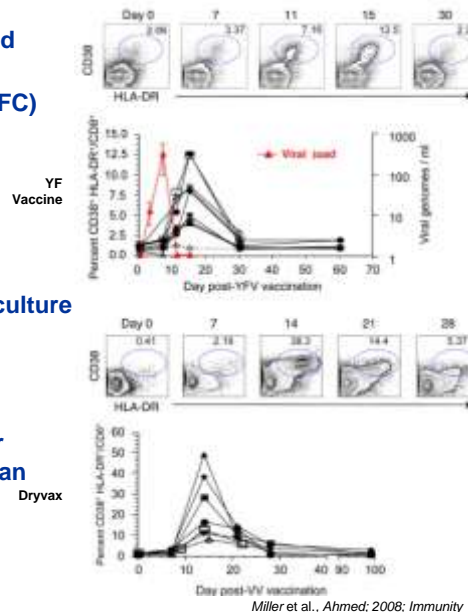
Oct 22nd, 2010

Devon J. Shedlock, PhD

4th Vaccine Renaissance Conference

Estimating vaccine-induced effector T cell responses

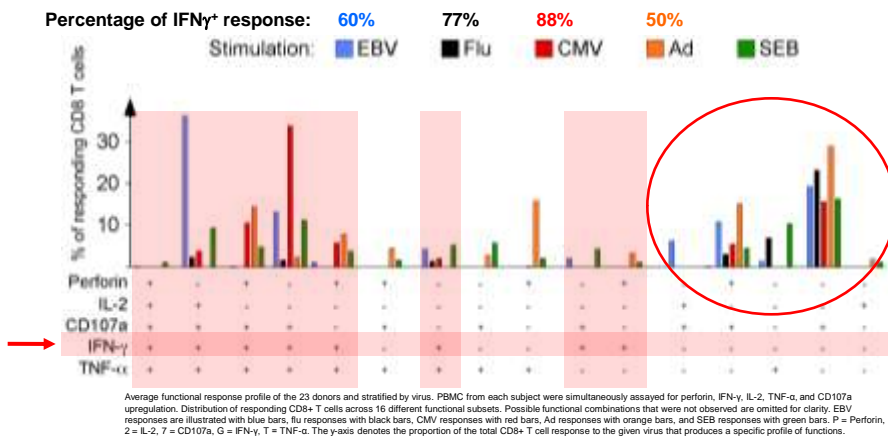
- Vaccine-induced immunity is evaluated using standard immunological assays
 - Polychromatic flow cytometry (PFC)
 - ELISpot assay
 - Proliferation assay
 - Etc...
- These assays require antigenic re-stimulation during *ex vivo* primary cell culture
 - May introduce bias
 - May underestimate
- Simple phenotypic analysis may offer additional information when examining an effector response
 - Tetramer staining
 - Activation markers



Potential bias from standard, ex vivo immunological assays

Assay Type	Ag Restimulation?	Chemicals required	Potential Chemical Bias	Ex vivo Culture	Potential Culture Bias	Effector Readout	Potential Readout Bias
Proliferation Assay		CFSE	Cytotoxicity	96 - 144 h (4-6 days)	Long-term culture/division affects phenotype/function	CFSE dilution	NL
IFN γ ELISpot	Y	-	-	16 - 20 h	NL	IFN γ secretion	Not all effectors make IFN γ
Polychromatic Flow Cytometry		Golgi poisons	Cytotoxicity	4 - 6 h		Multiple cytokine production	limited by which cytokines are assessed

IFN γ responses alone underestimate effector response



Makedonas et al., *Beit*; 2010; *PLoS Path*

- During some human infections, up to 50% of effector may not make IFN γ
- Standard ELISpot assay measuring IFN γ alone may underestimate

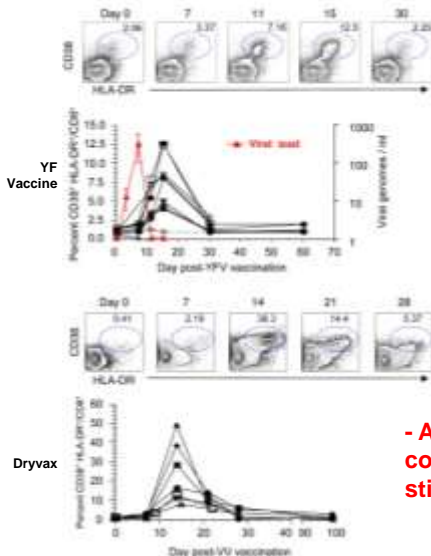
Reduction of potential bias by simple phenotypic analysis

	Assay Type	Ag Restimulation?	Chemicals required	Potential Chemical Bias	Ex vivo Culture	Potential Culture Bias	Effector Readout	Potential Readout Bias
Proliferation Assay			CFSE	Cytotoxicity	96 - 144 h (4-6 days)	Long-term culture may bias phenotype/function	CFSE dilution	NL
IFN γ ELISpot	Functional	Y	-	-	16 - 20 h	NL	IFN γ secretion	Not all effectors make IFN γ
Polychromatic Flow Cytometry			Golgi poisons	Cytotoxicity	4 - 6 h		Multiple cytokine production	limited by which cytokines are assessed
Tetramer staining	Physical	N	-	-	-	-	Direct staining	Reagent limited
Activation marker staining							Activation marker detection	Specificity and background (longitudinal)

- Activation marker staining:

- is not Ag-specific (no evidence detected of bystander activation (Miller et al., Ahmed: 2008; Immunity))
- is affected by non-vaccine-induced responses (longitudinal assessment required)

Underestimation of vaccine-induced effector T cell responses



<u>Stim conditions</u>	<u>Estimated % Response</u>
- Peptides	0.1 – 1.5%
- Infected cells	0.5 – 3.0%
- Activation markers	4 – 13%

- Activation marker staining may be a more comprehensive assessment than ex vivo re-stimulation assays alone

Miller et al. Ahmed: 2008; Immunity

Reagents required for evaluating immunity

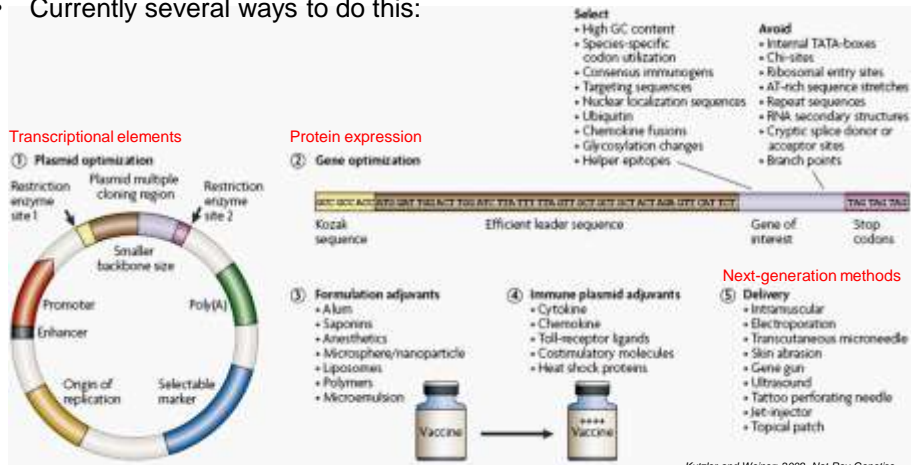
Assay Type	Typical form	Cost	Antigenic processing required?	Epitopic Display	Potential Functional Bias
Peptides	Overlapping xmers	\$\$\$	N (fluid phase exchange)	Limited (xmer length)	[Peptide] must be saturating (functional avidity)
Proteins/pathogen	Whole/ Full length/ Truncated	\$\$	Y (Primary APCs)	Full/Ag	Requires time and APCs for processing and display
Infected cells	Whole/ Irradiated/ Lysed	\$		Full (HLA-restricted)	
Tetramer staining	un/labeled mono/tetramers	\$\$	-	Reagent limited	-
Activation marker staining	-	-		-	

- Activation marker staining:



- is simple, cheap, and not likely affected by potential biases associated w/antigenic re-stimulation

DNA vaccine development and evaluation

- Our lab has helped develop and study DNA vaccines
- Ongoing efforts to increase expression and vaccine immunogenicity
- Currently several ways to do this:

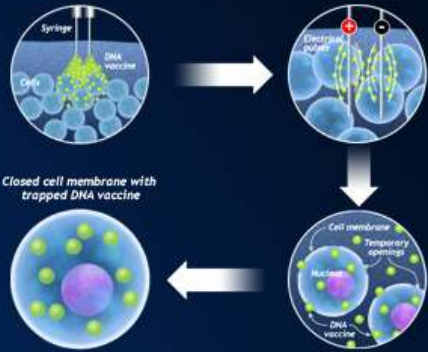



Optimized delivery of DNA vaccines using *In vivo* EP

How Electroporation Delivers DNA Vaccines


- 1 Syringe and needle electrodes are inserted into selected muscle tissue, and the DNA vaccine is injected.
- 2 Controlled, milli-second electrical pulses are applied to the needle electrodes, which then form an electric field.
- 3 The electrical field creates temporary openings in the cell membrane, allowing significantly greater amounts of the DNA vaccine to enter cells.
- 4 The trapped DNA enables cells to produce antigen designed to control cancer & chronic infectious diseases such as HIV. The antigen can also trigger antibody production to prevent diseases.






EP enhancement of DNA vaccine expression


DNA alone




Light + UV



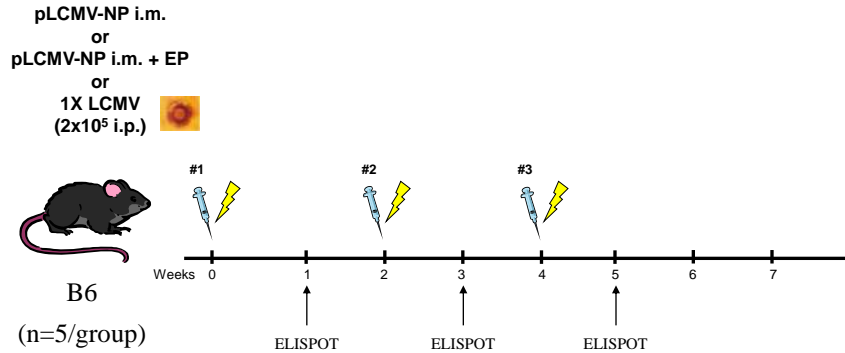
UV



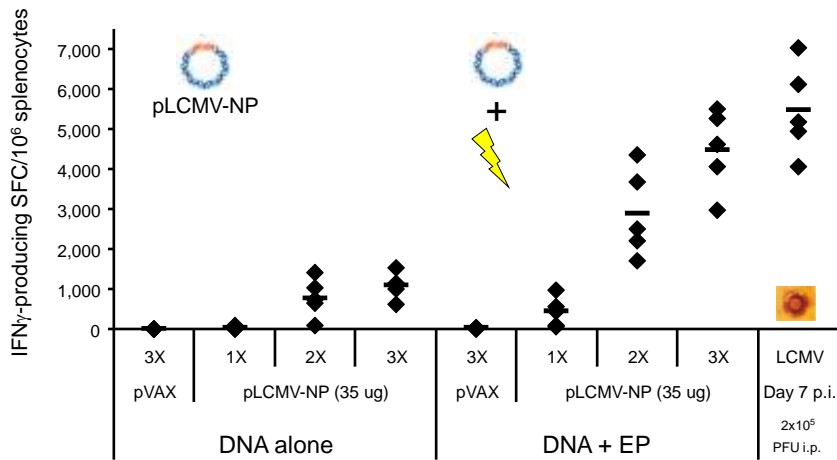
DNA + EP



Can level of T cell immunity from LCMV infection be replicated by DNA vaccination w/EP?



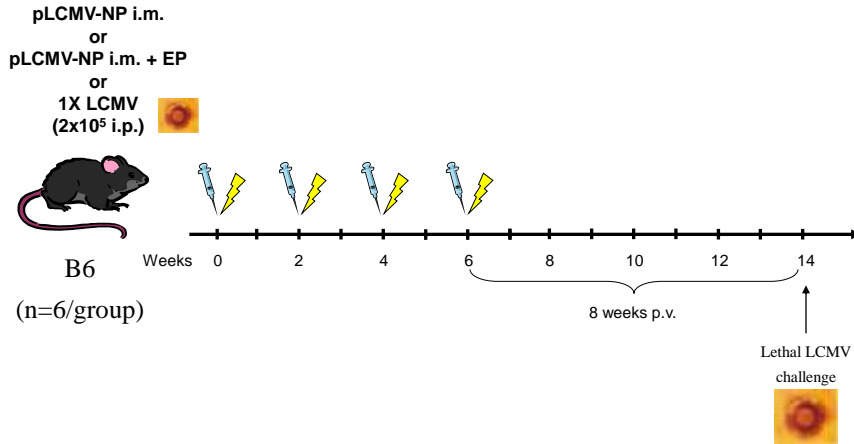
EP enhancement of DNA vaccine immunogenicity



Shedlock et al., Weiner, 2010; Submitted

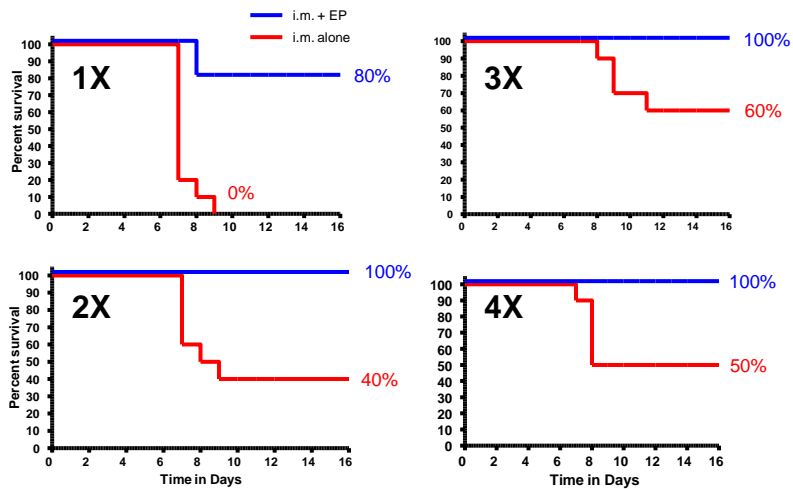
- T cell responses from 3X DNA vaccination w/EP are ~80% of acute LCMV response

Are immune responses from DNA vaccination w/EP protective against lethal LCMV challenge?



Shedlock et al., Weiner, 2010; Submitted

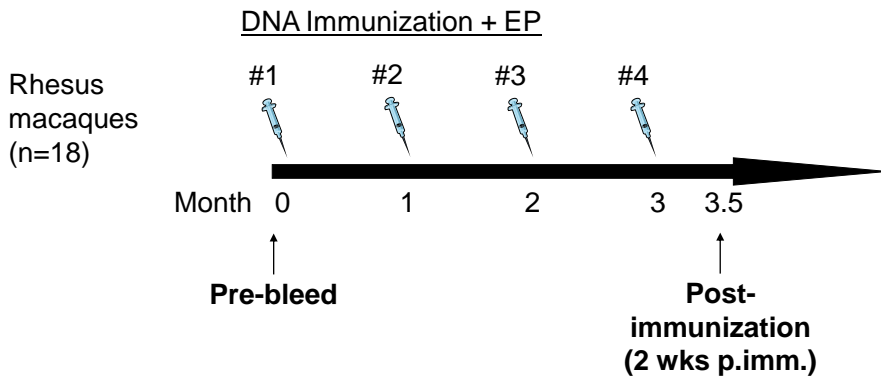
Can DNA vaccination protect against lethal LCMV challenge?



Shedlock et al., Weiner, 2010; Submitted

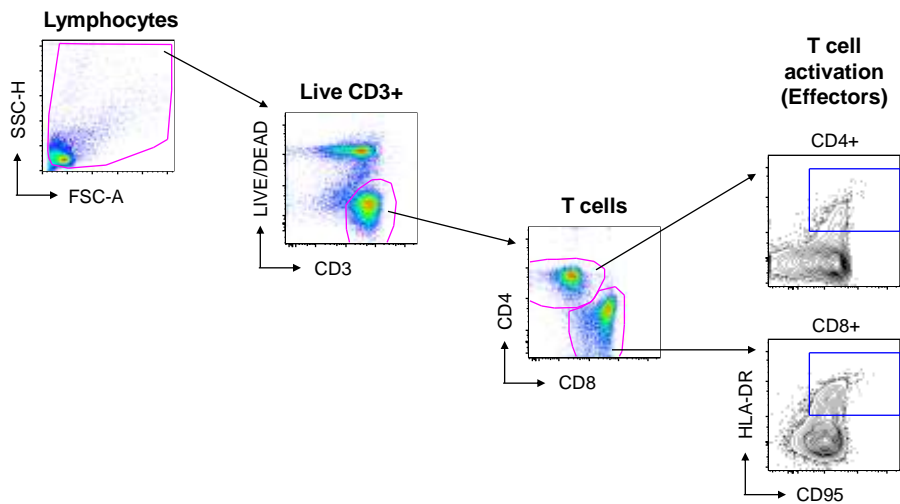
- DNA + EP is 100% protective after 2 immunizations

Gating Strategy for flow-based activation analysis in NHP



Beisik et al., Boyer, 2010; Submitted

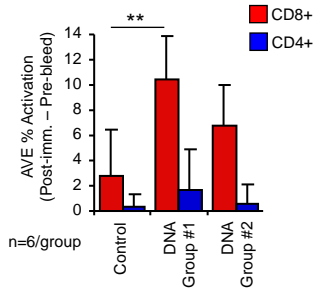
Gating Strategy for flow-based activation analysis in NHP



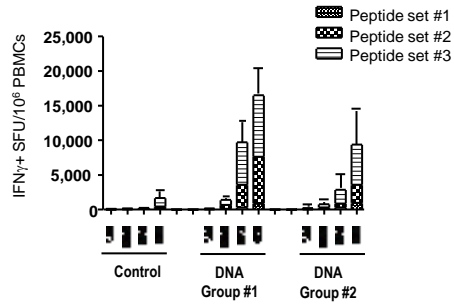
Beisik et al., Boyer, 2010; Submitted

HLA-DR activation correlates with ELISpot data

Activation Staining



ELISpot



Total AVE

~15,000 spots = 1.5% Ag-specific

x2.5 (T cells are ~40% of PBMC)

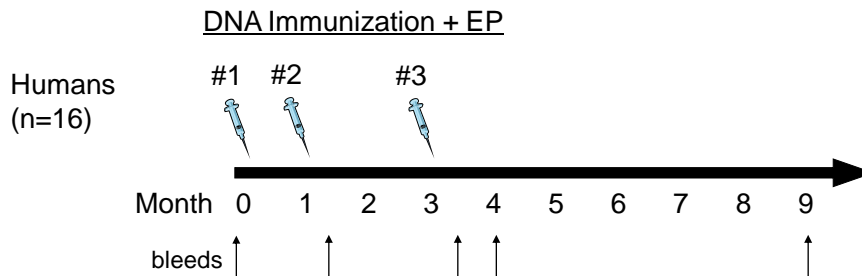
x2 (best assume for IFN γ)

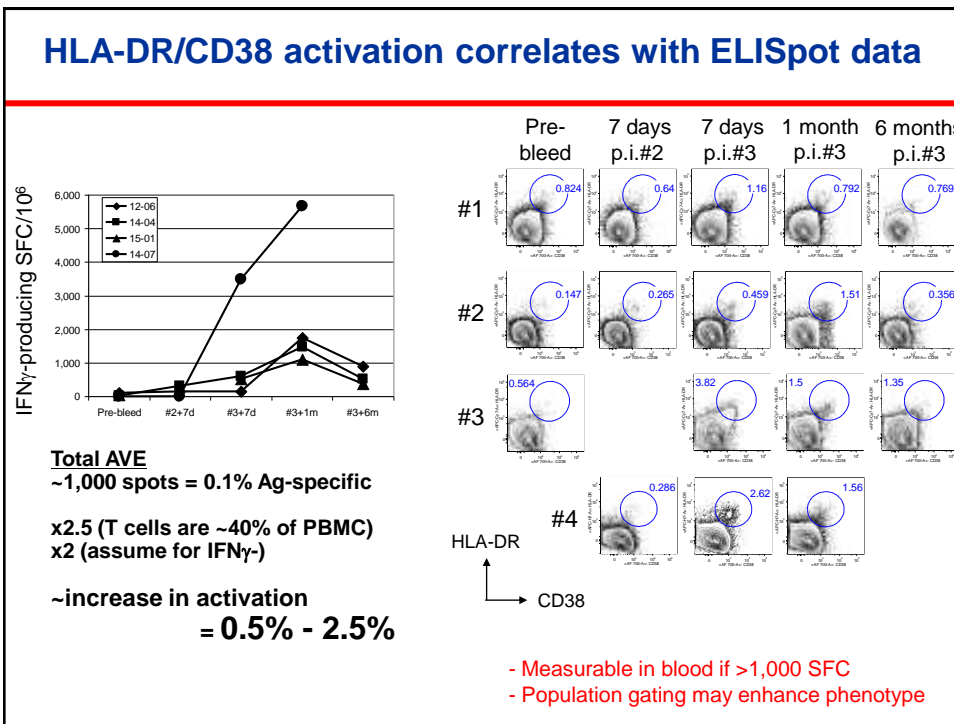
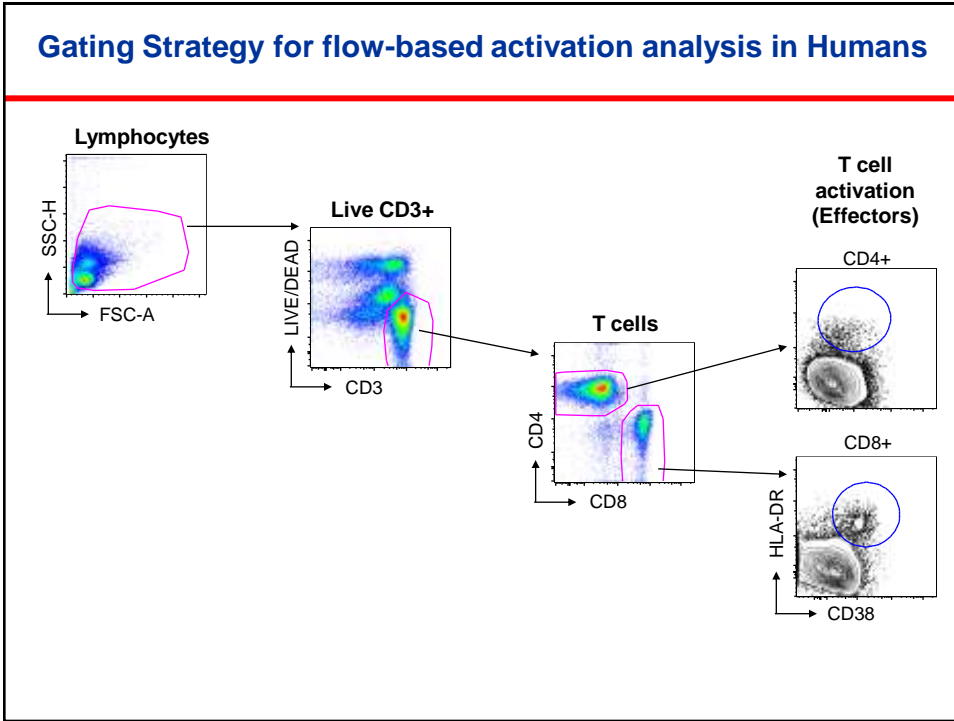
~ increase in T activation = (3.8 - 7.5%)

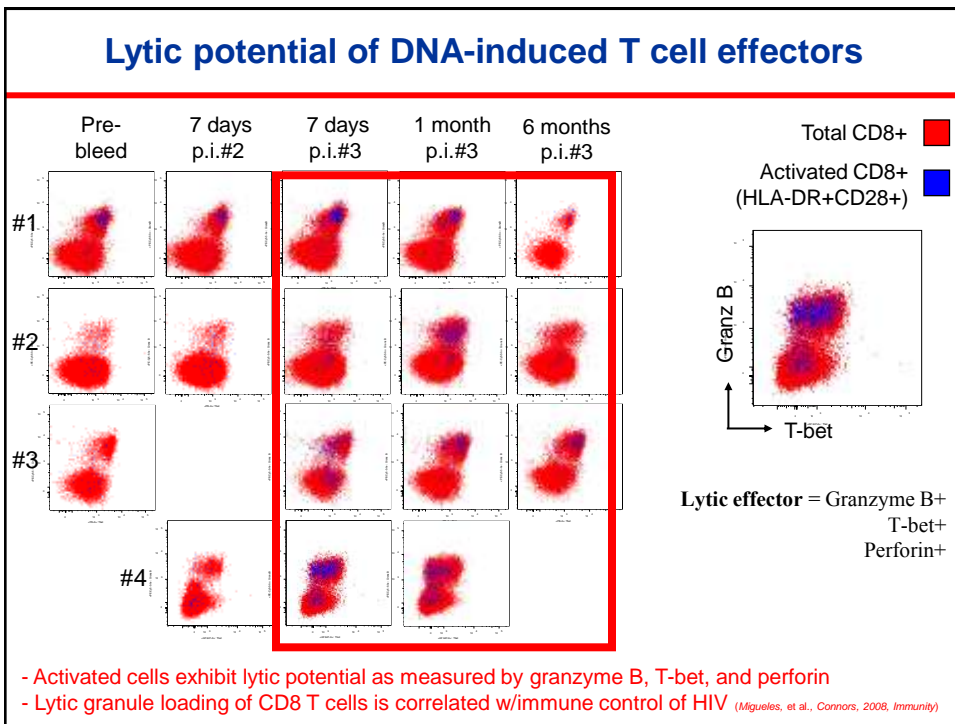
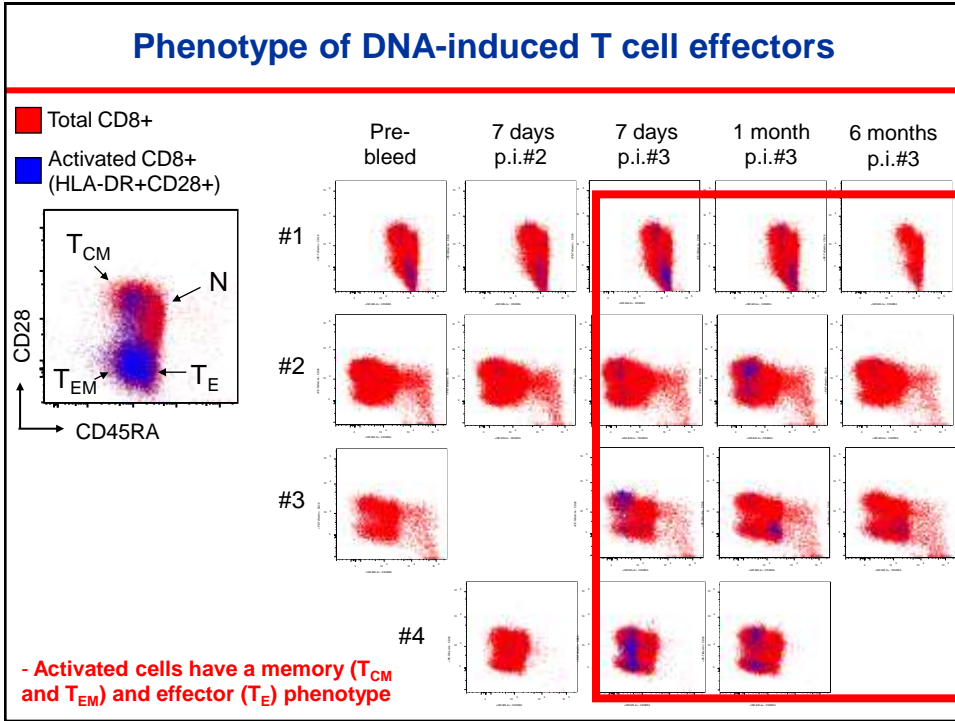
~ increase in CD8 T activation = (7.6 - 18.8%)

Belske et al., Boyer, 2010; Submitted

Gating Strategy for flow-based activation analysis in Humans







Summary

- DNA vaccination w/EP is immunogenic in RMs and humans
- Primate blood-derived, activated T effectors can be estimated by direct HLA-DR staining (if > 1,000 SFC by ELISpot)
 - activation data correlates with standard immunological assays
- Activated T cells have a memory- and effector-like phenotype
- Lytic potential of effectors can be assessed directly
 - re-stim triggers de-granulation
 - this is first study to demonstrate DNA-induced lytic potential
- This technique may provide a less biased assessment of global vaccine-induced activation
 - should be combined w/standard immunological assays



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