

# BCG AS AN HIV VACCINE VECTOR



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# Mycobacterium bovis **BCG** (bacillus Calmette-Guerin)

## ADVANTAGES

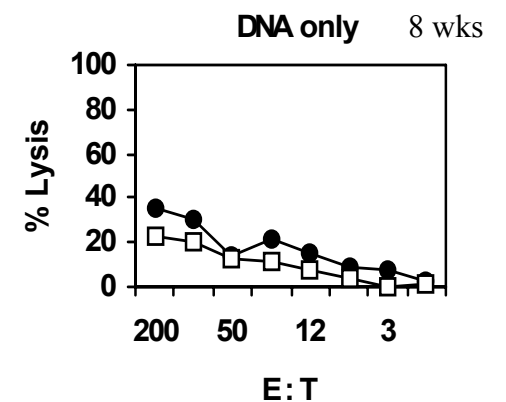
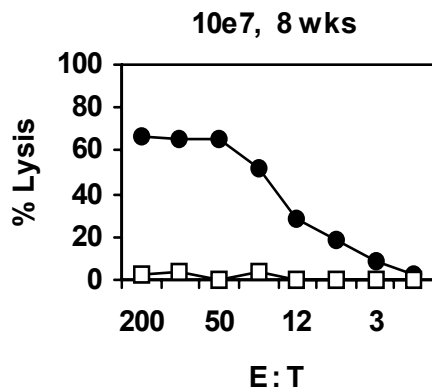
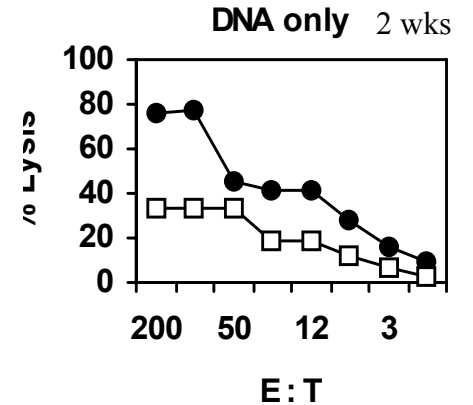
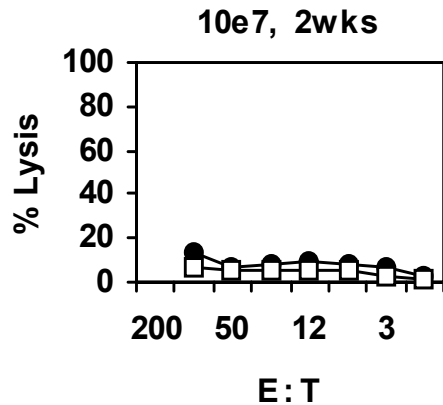
- BCG widely used as a TB vaccine – **3 billion doses** -well established safety profile in babies
- Generate long lived immune responses
- Unaffected by **anti-vector** immunity
- **Oral administration** to generate a mucosal response which may be boosted by non-mucosal vaccines.
- Low cost , low technology production, heat stable

# Mycobacterium bovis **BCG** (bacillus Calmette-Guerin)

## CHALLENGES

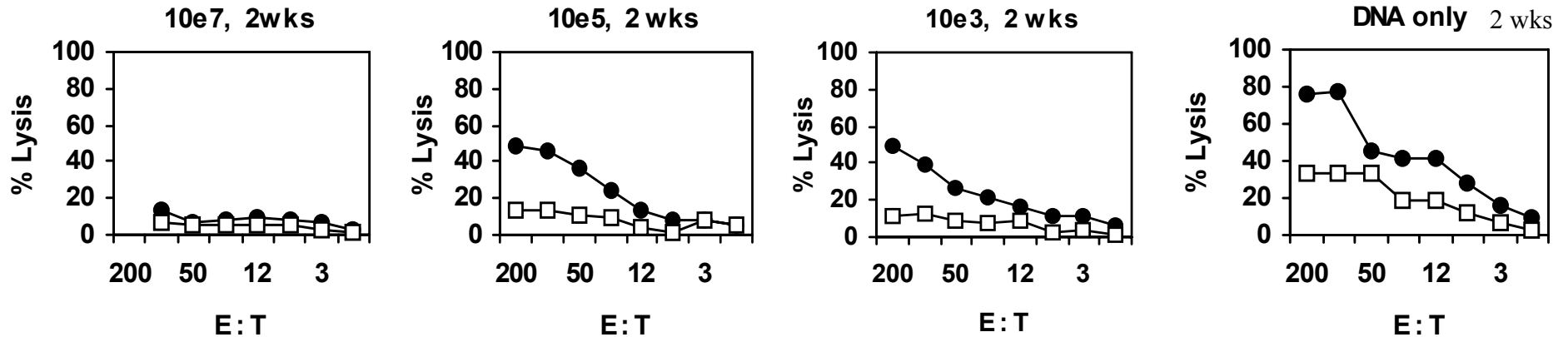
- Slow growing
- Genetic instability- high production of heterologous antigen associated with even slower growth, smaller colonies and genetic rearrangements
- Viral antigens not well expressed compared to bacterial antigens
- Problems with mouse model for immunogenicity

# THE EFFECT OF AN rBCG BOOST ON A pTHGAG PRIMARY RESPONSE AS MEASURED BY CTL ASSAY



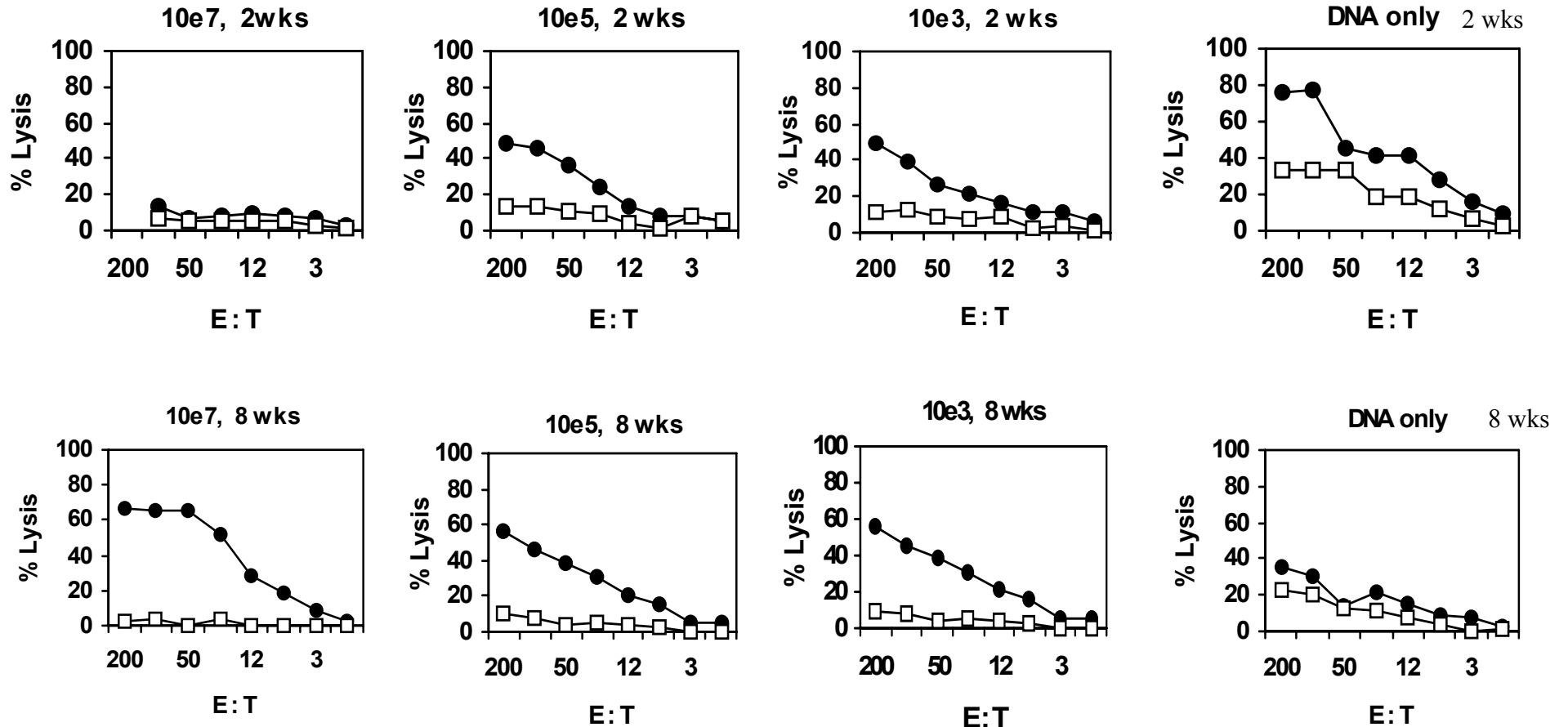
Mice were inoculated with pTHgag (100 $\mu$ g, i.m.), 4 weeks later given an i.p. inoculation of rBCG[pEH+NOTG] (10e3, 10e5 or 10e7 CFU)

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# THE EFFECT OF AN rBCG BOOST ON A pTHGAG PRIMARY RESPONSE AS MEASURED BY CTL ASSAY



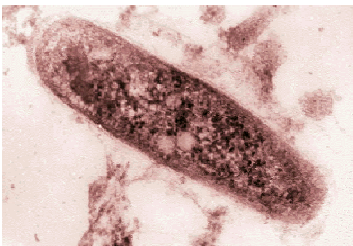
Mice were inoculated with pTHgag (100 $\mu$ g, i.m.), 4 weeks later given an i.p. inoculation of rBCG[pEH+NOTG] (10e3, 10e5 or 10e7 CFU)

PRIME WITH GAG EXPRESSING DNA VACCINE  
BOOST WITH GAG EXPRESSING BCG AT  
DIFFERENT DOSES

- BCG suppresses immune response in mice at early time points
- Suppression is dose related
- Suppression is time related

# PILOT EXPERIMENTS IN BABOONS rBCG PRIME AND VLP BOOST

**Gerald K Chege\* EG Shephard, A Jaffray, R  
Thomas, S Makhubela, JM Maclean, EP  
Rybicki and A-L Williamson**

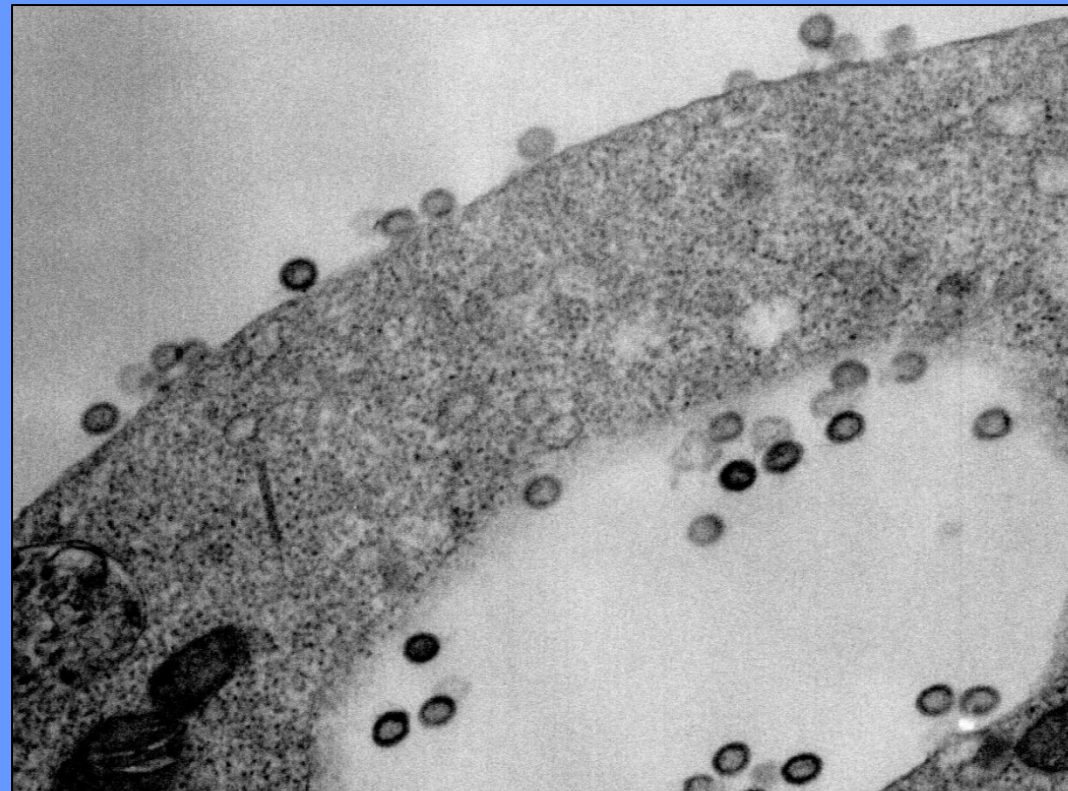
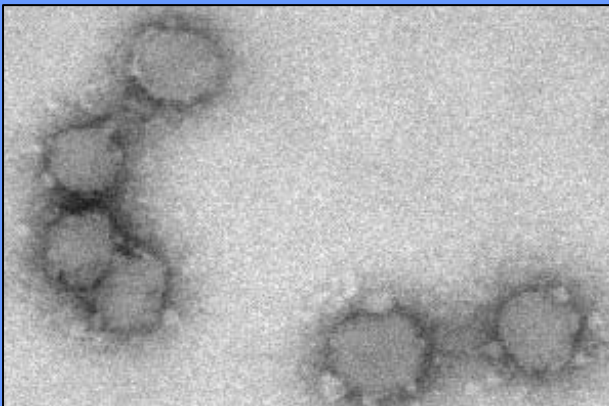


- BCG VACCINATION WAS SUBCUTANEOUS
- 4 DOSES OF BCG GIVEN
  - EXPRESSING GAG OR NOT
  - WEEKS 0, 14, 24, 40
- BOOSTED WITH VIRUS LIKE PARTICLES
  - WEEKS 92, 104

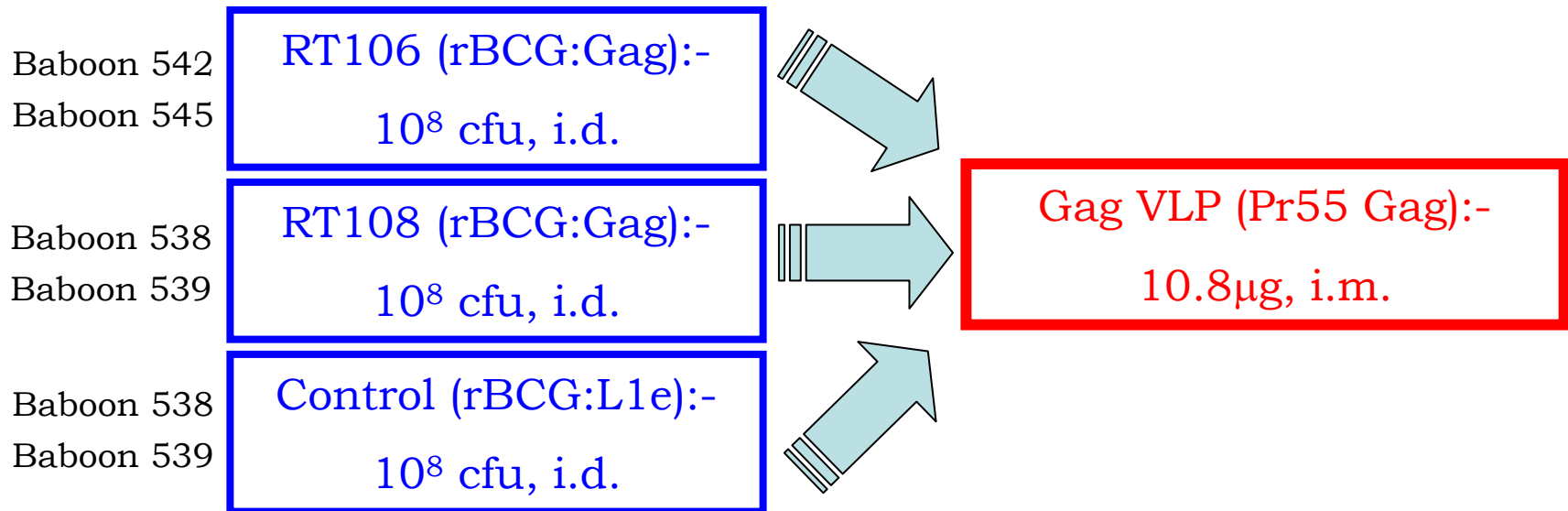
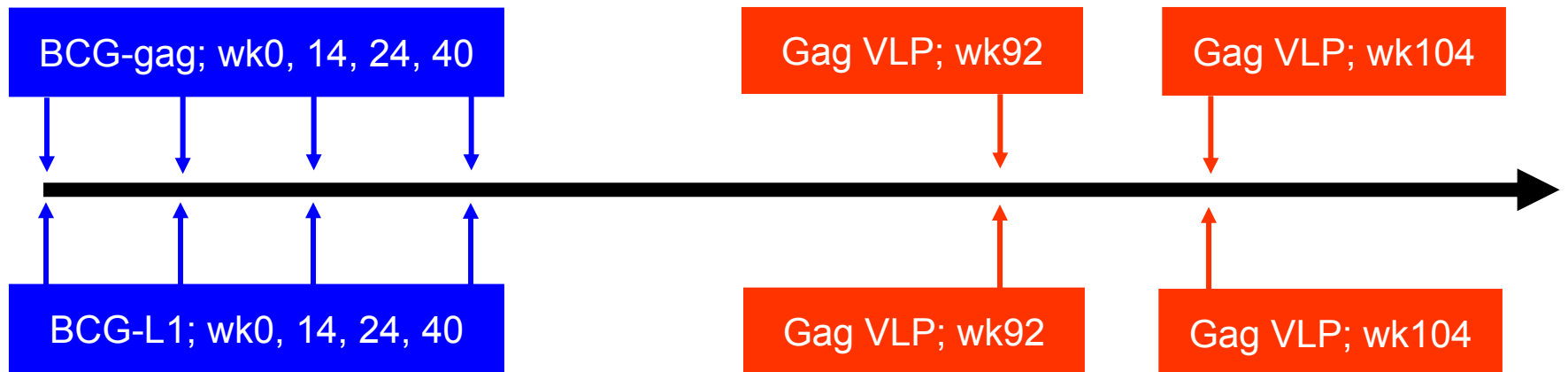


## BACULOVIRUS PRODUCTION OF HIV VIRUS LIKE PARTICLES

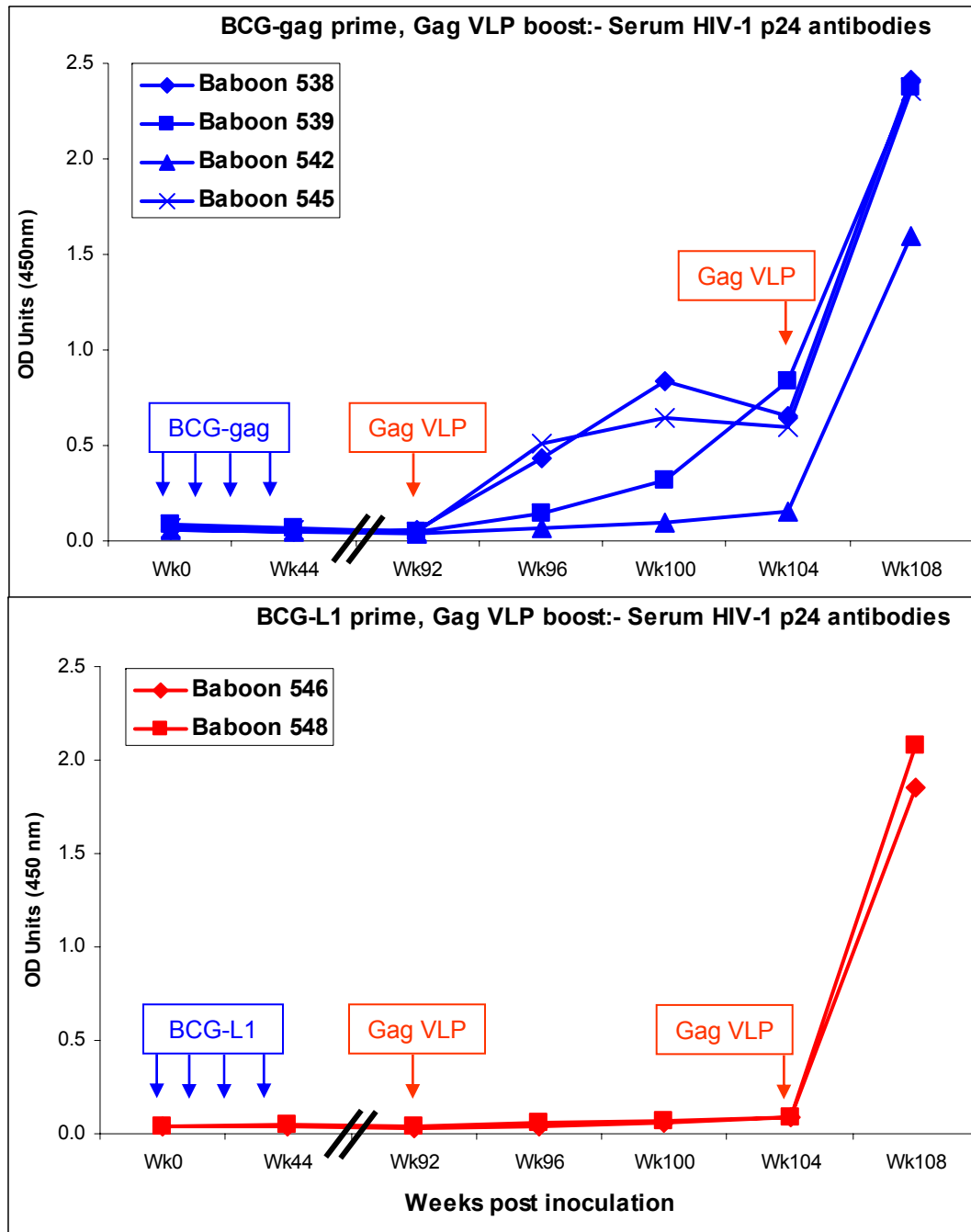
A. Jaffray, E.P. Rybicki.



# Immunization Regimen

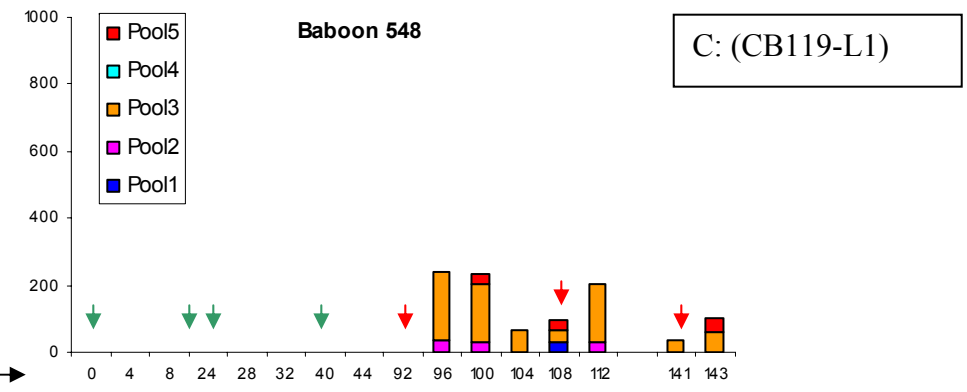
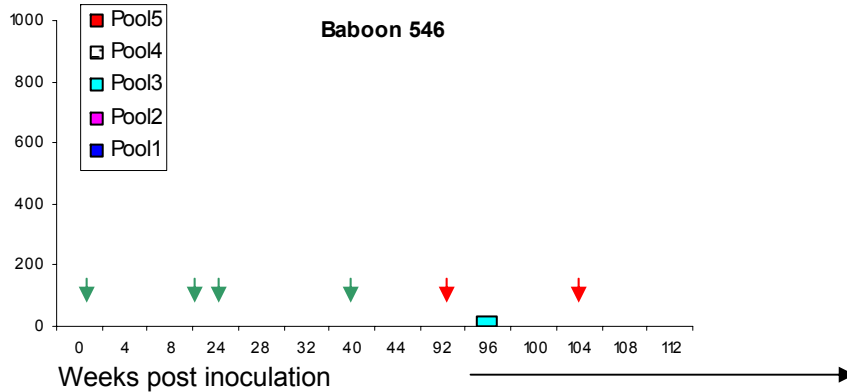
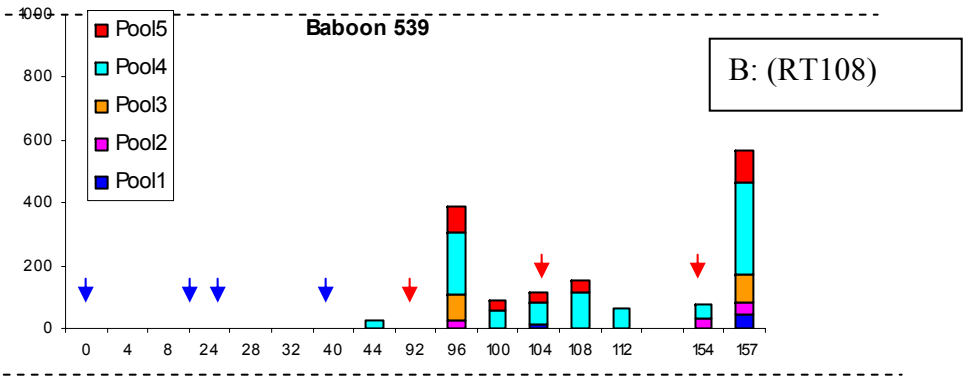
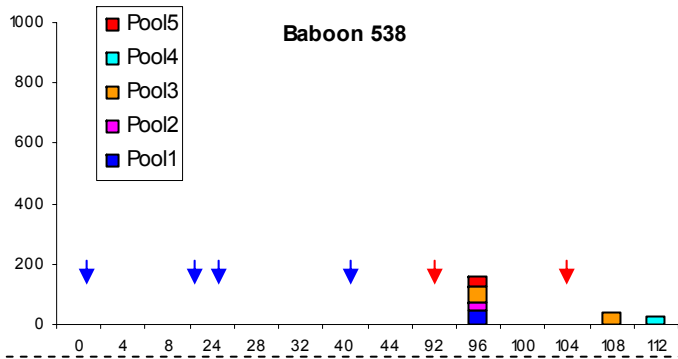
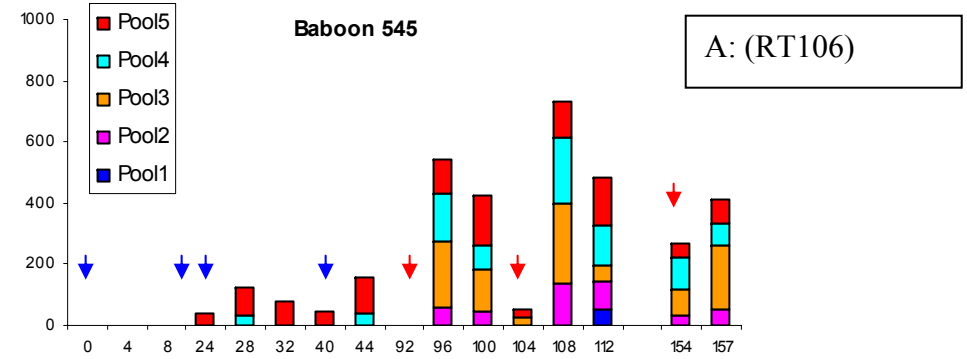
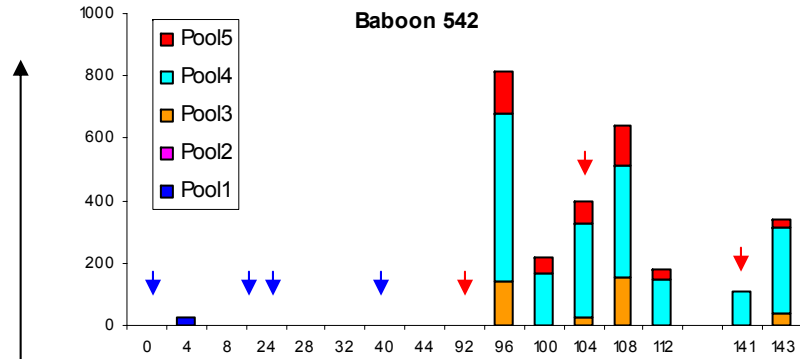


# Serum HIV-1 p24 binding IgG antibodies



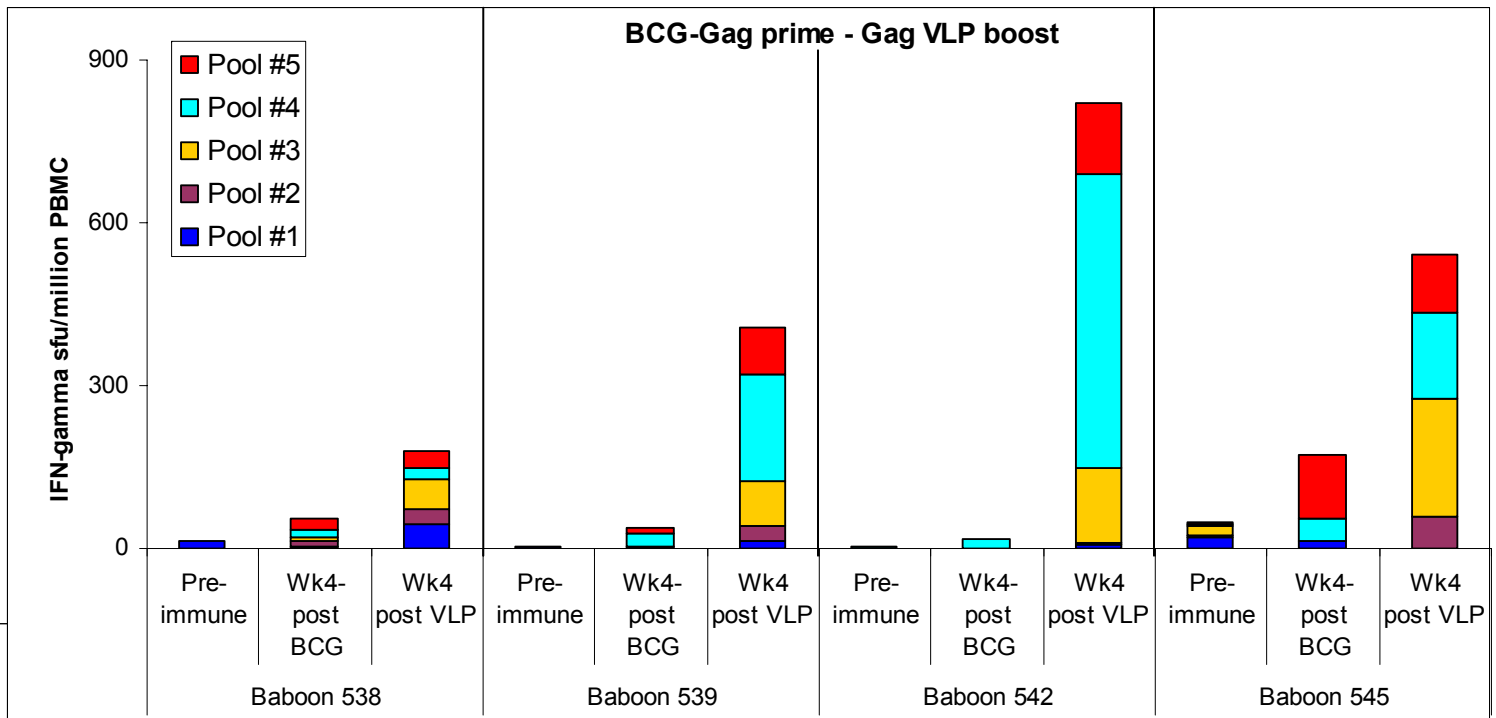
# rBCG(Gag) PRIME/(Gag)VLP BOOST immunogenicity in baboons

## IFN- $\gamma$ ELISPOT responses to Gag peptide pools (1-5)

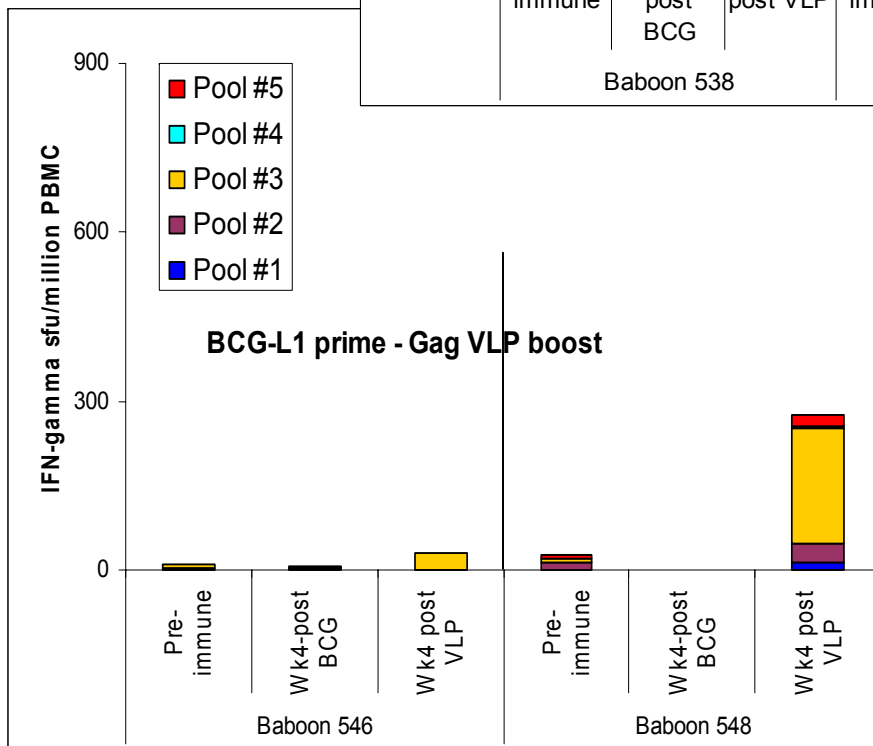


SFU/10<sup>7</sup> PBMC

**BCG-Gag prime - Gag VLP boost**



**BCG-L1 prime - Gag VLP boost**



Cellular immune responses (ELISPOT)

## Identified epitopic regions in immunized baboons: 4wks post BCG-GagC & 1<sup>st</sup> VLP

**Colour Codes**

**HIV-1C p24 region**

**HIV-1C p2p7p1p6 region**

**epitopic region in p24 also identified in subtype C HIV-1 infected humans**

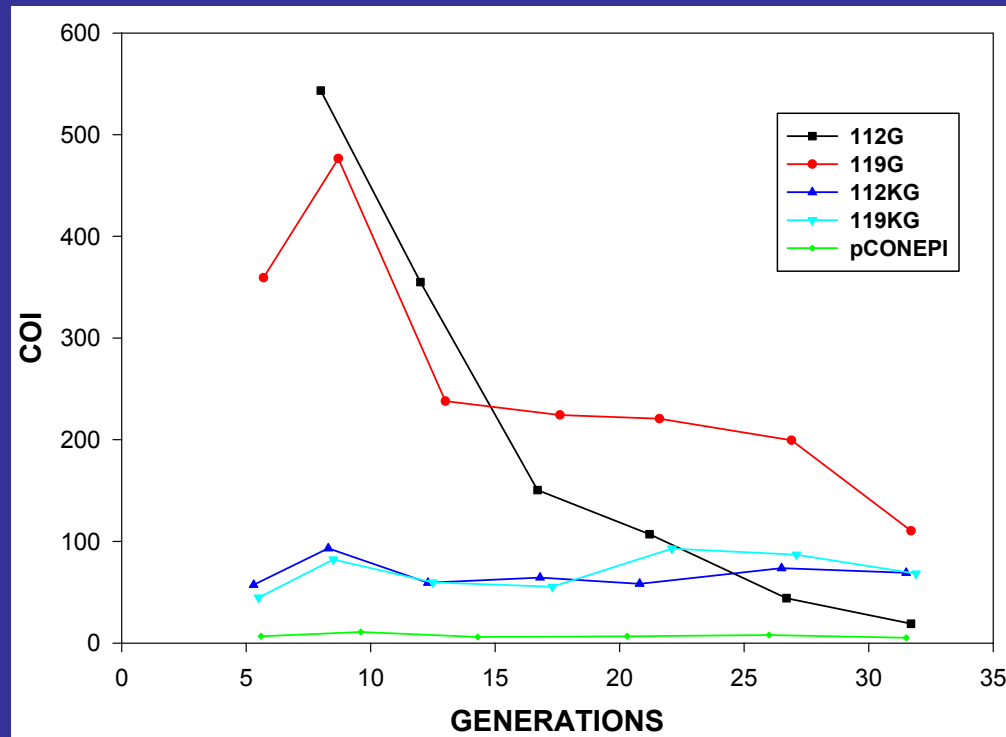
rBCG prime	Baboon Number	Major pool	Identified peptide number (and sequence)	IFN- $\gamma$ sfu/10 <sup>6</sup> of identified peptides	
				4 weeks post BCG prime	4 weeks post VLP boost
RT106	B542 <sup>Gag</sup>	#3 #4 #5	30 <sup>(215LHPVHAGPIAPGQMREPR<sup>232</sup>)</sup>	2	245
			53 <sup>(380KGPKRIVKCFNCGKEGHI<sup>398</sup>)</sup>	2	90
			54 <sup>(388CFNCGKEGHIARNCRAPR<sup>406</sup>)</sup>	8	613
			55 <sup>(397HIARNCRAPRKKGCWK<sup>412</sup>)</sup>	2	255
			56 <sup>(403RAPRKKGCWKCGKEGHQM<sup>420</sup>)</sup>	0	250
			57 <sup>(411WKCGKEGHQMKDCTERQA<sup>428</sup>)</sup>	2	185
			58 <sup>(419QMKDCTERQANFLGKIW<sup>435</sup>)</sup>	0	110
	66 <sup>(474KDREPLTSLKSLFGSDPLSQ<sup>493</sup>)</sup>	0	260		
	B545 <sup>Gag</sup>	#2 #3 #4 #5	23 <sup>(161AFSPEVIPMFTALSEGA<sup>177</sup>)</sup>	0	25
			36 <sup>(255PVGDIYWKRWILGLNKIV<sup>273</sup>)</sup>	6	70
			37 <sup>(264WILGLNKIVRMYSVSI<sup>281</sup>)</sup>	0	55
			38 <sup>(272IVRMYSVSI<sup>289</sup>)</sup>	13	105
			40 <sup>(287GPKEPFRDYVDRFFKTLR<sup>304</sup>)</sup>	0	70
			41 <sup>(298YVDRFFKTLRAEQATQDV<sup>312</sup>)</sup>	6	43
54 <sup>(388CFNCGKEGHIARNCRAPR<sup>406</sup>)</sup>			6	160	
66 <sup>(474KDREPLTSLKSLFGSDPLSQ<sup>493</sup>)</sup>	90	150			
RT108	B538 <sup>Gag</sup>	#3	37 <sup>(264WILGLNKIVRMYSVSI<sup>281</sup>)</sup>	NT	22
			38 <sup>(272IVRMYSVSI<sup>289</sup>)</sup>	NT	42
	B539 <sup>Gag</sup>	#3 #4 #5	36 <sup>(255PVGDIYWKRWILGLNKIV<sup>273</sup>)</sup>	0	27
			54 <sup>(388CFNCGKEGHIARNCRAPR<sup>406</sup>)</sup>	2	342
			56 <sup>(403RAPRKKGCWKCGKEGHQM<sup>420</sup>)</sup>	4	27
			57 <sup>(411WKCGKEGHQMKDCTERQA<sup>428</sup>)</sup>	9	110
66 <sup>(474KDREPLTSLKSLFGSDPLSQ<sup>493</sup>)</sup>	0	155			
CB119-L1e	B546 <sup>Mock</sup> B548 <sup>Mock</sup>	#3 #3	36 <sup>(255PVGDIYWKRWILGLNKIV<sup>273</sup>)</sup>	0	20
			37 <sup>(264WILGLNKIVRMYSVSI<sup>281</sup>)</sup>	0	20
			40 <sup>(287GPKEPFRDYVDRFFKTLR<sup>304</sup>)</sup>	0	222
			41 <sup>(298YVDRFFKTLRAEQATQDV<sup>312</sup>)</sup>	0	287

## **Summary of rBCG prime – Virus Like Particle Boost in Baboons**

- **BCG-GagC primed both antibody and cellular HIV-1 specific immune responses which were boosted and broadened by VLPs after 1 year**
- **Some of the epitopes recognized in HIV infected people were also recognized in vaccinated baboons**
- **CD8 and CD4 responses were detected to specific peptides (FACS data not shown)**

- rBCG expressing Gag used in baboon experiments unstable in culture
- Major problem for cGMP
- Major effort from UCT to solve this problem

Relative levels of p24 produced by recombinants after multiple passages in liquid culture



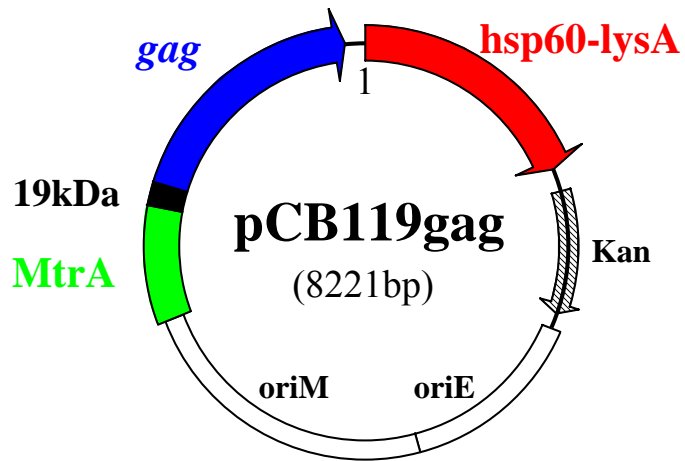
# Multiple Approaches to Improvement of rBCG Immunogenicity

- **Shuttle vector**
  - Mtr promoter – down regulated in culture upregulated in macrophages
  - 19kDa leader – exports/anchors antigen to cell surface
  - BCG codon optimized HIV genes
- Investigation of **BCG as a prime** with a heterologous MVA or VLP boost
- **BCG strain-** harmonize with TB vaccine initiatives

# Optimization of pCB119 export based vaccine construct

## 1<sup>st</sup> GENERATION

Vector provided by William R. Jacobs  
Jr. Albert Einstein College of Medicine

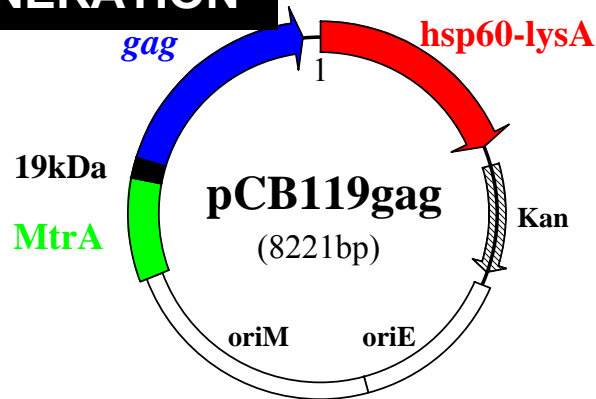


- Unhealthy/poor growth rBCG
- UNSTABLE
- Poor immune responses
- Boosted in baboons by VLPs



# Optimization of pCB119 export based vaccine construct

## 1<sup>st</sup> GENERATION



- Unhealthy/poor growth rBCG
- UNSTABLE
- Poor immune responses in mice
- BCG prime boosted in baboons by VLPs

## 2<sup>ND</sup>

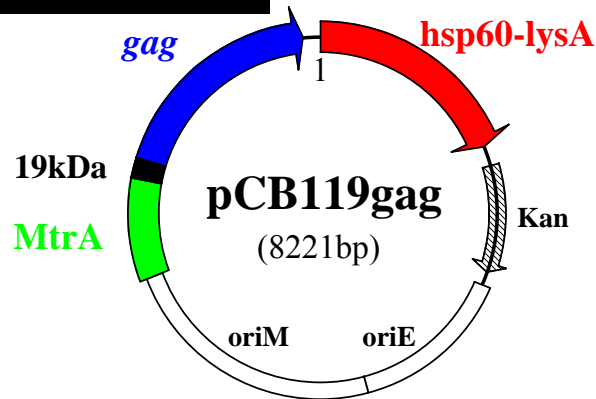
pCB119GO $\Delta$



- Codon optimised gag
- Truncated gag (-p7,p1,p6)
- Healthier/more stable rBCG

# Optimization of pCB119 export based vaccine construct

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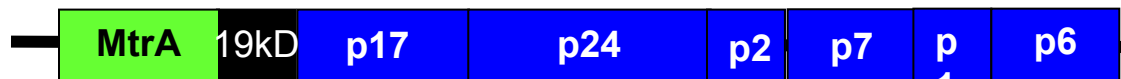
pCB119GO $\Delta$



- Codon optimised *gag*
- Truncated *gag* (-p7,p1,p6)
- Healthier/more stable rBCG

## 3<sup>RD</sup> GENERATION

pEM119GO



wtBCG[pEM19GO] + panBCG[pEM19GO]

- Full length codon opt. *gag*
- Hsp60-lysA deleted
- HEALTHY + STABLE

# PanCD BCG

William R. Jacobs Jr. Albert Einstein College of Medicine

## Could the immunogenicity of rBCG be improved by changing the strain?

- Pantothenate (vitamin B5) is an essential precursor for cellular processes such as energy metabolism and fatty acid metabolism
- Auxotrophic PanCD BCG made by deletion of two genes required for the synthesis of pantothenate (*panCD*).
- *In culture vitamin B 5 is added – limited growth in vivo*

# BCG prime – MVA boost Immunogenicity in BALB/c mice – Comparison of PanCD BCG-gag and WT BCG-gag

**PRIME: rBCG**



[10<sup>7</sup> cfu i.p.]

12 WKS

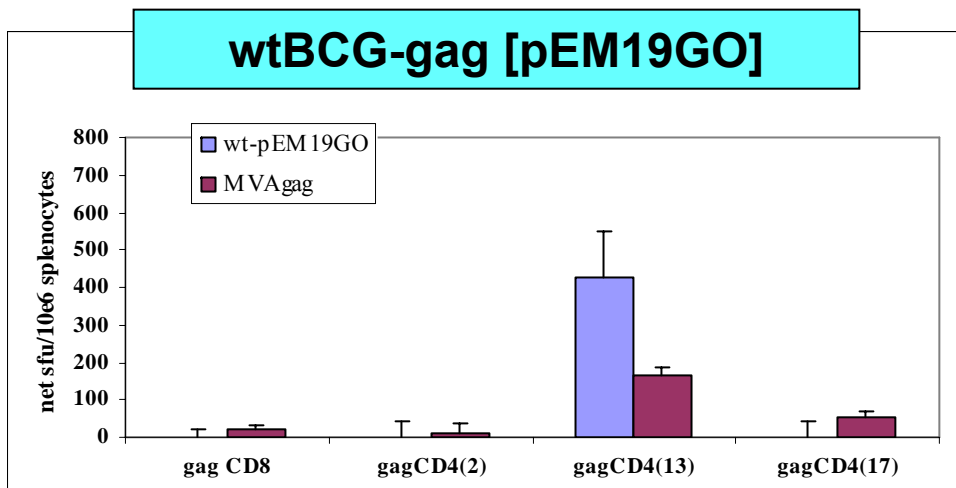
**BOOST MVA-gagC**



[10<sup>7</sup> pfu i.m.]

2 WKS

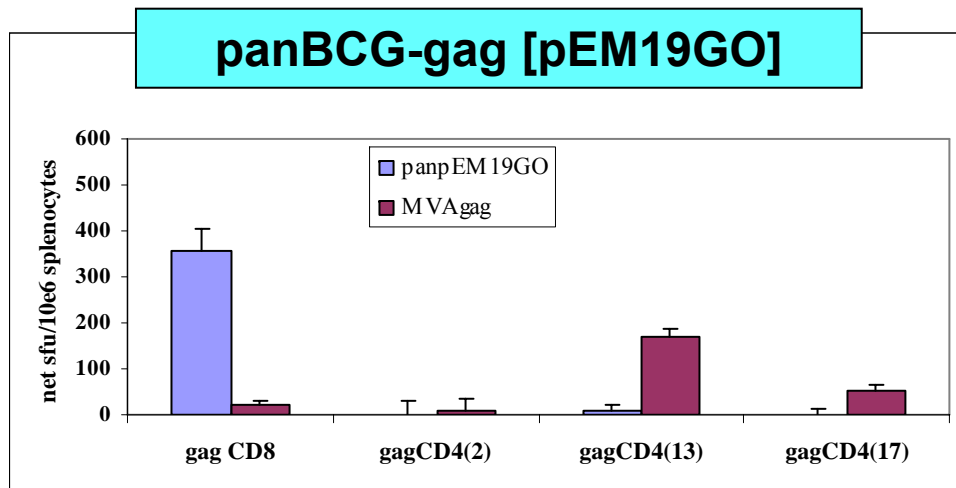
**IFN $\gamma$  ELISPOT Assay on splenocytes**



**Induced a Gag-specific:  
CD4+ response (wt BCG)  
CD8+ response (panBCG)**

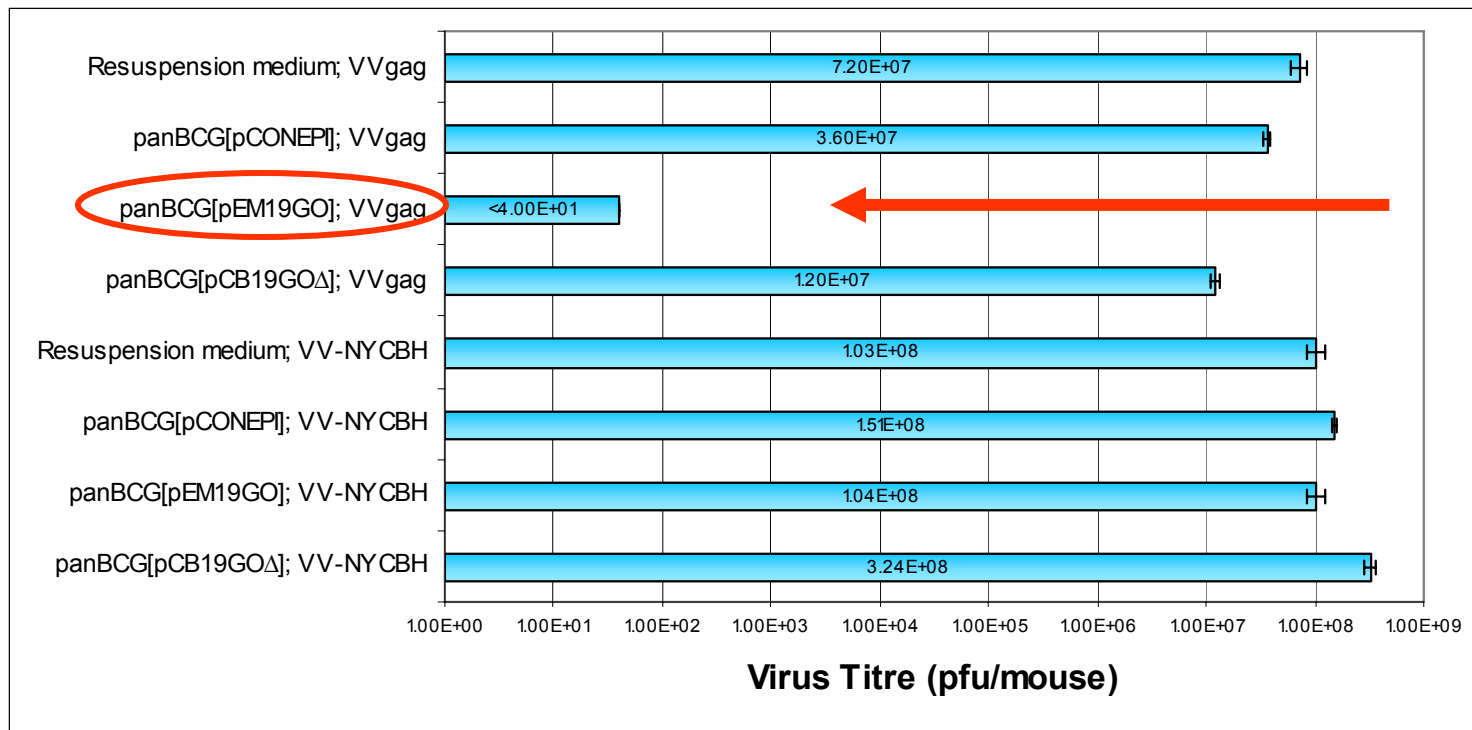
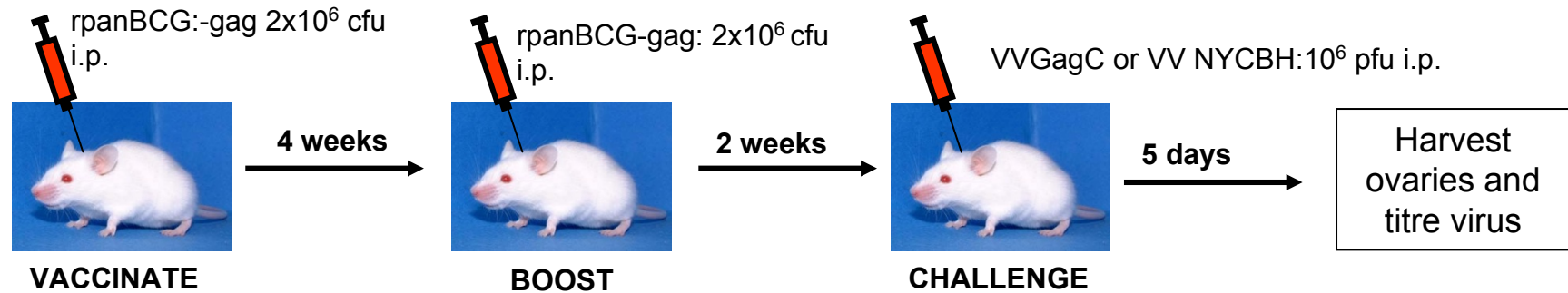


**Good strategy to prime immune system**



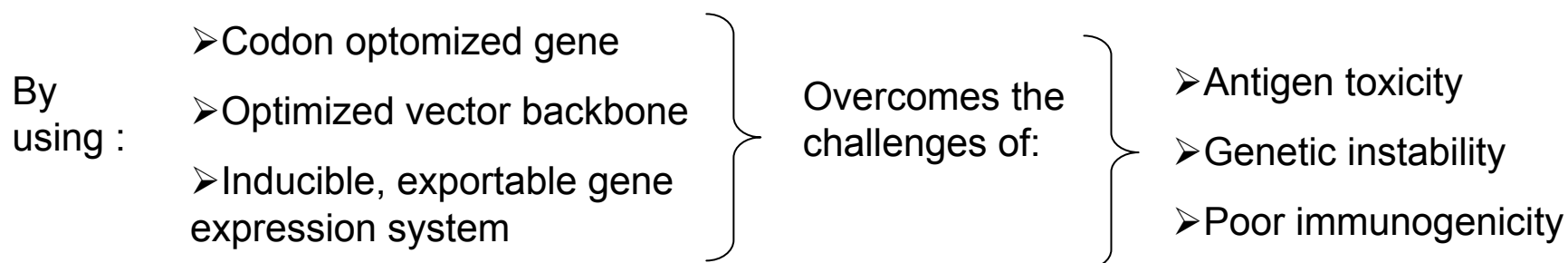
**PAN auxotroph strain suitable for use in immunocompromised individuals**

# Gag-specific CD8+ T cell response induced by panBCG-gag[pEM19GO] protects mice against a challenge of Vaccinia virus expressing Gag (VVGagC)



**Complete protection when vaccinated with panBCG-gag**

# Candidate HIV Vaccine based on recombinant BCG panBCG-gag[pEM19GO] Developed



Good strategy to prime immune system

Attenuated panBCG strain is attenuated -safer alternative for vaccination of immunocompromised individuals

EXPERIMENTS  
IN BABOONS

(By: Gerald Chege)

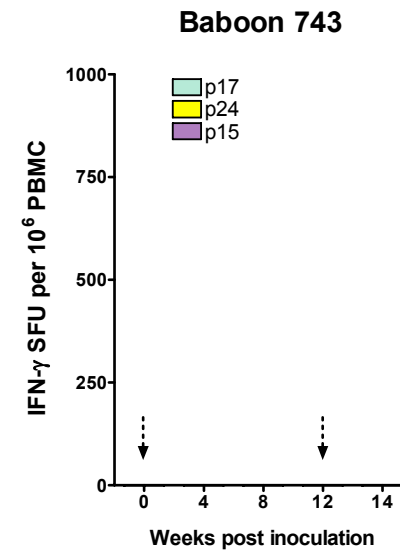
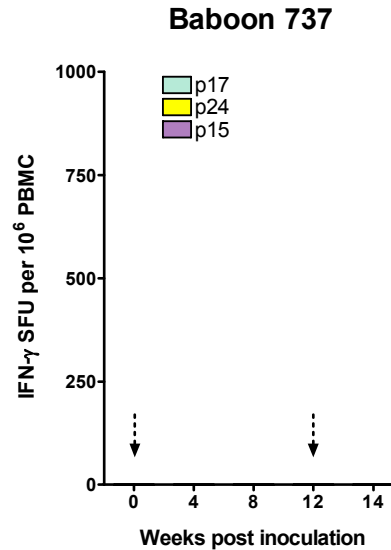
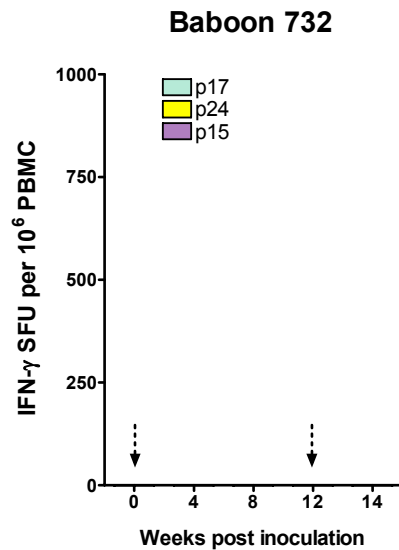
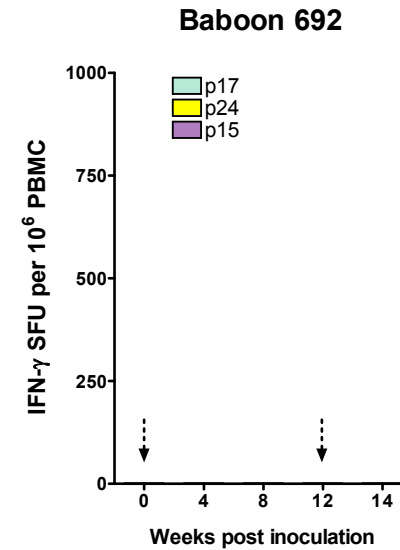
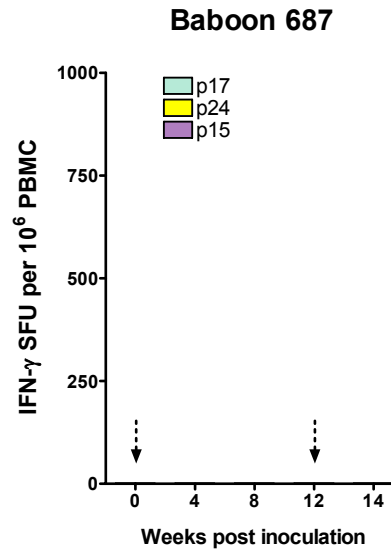
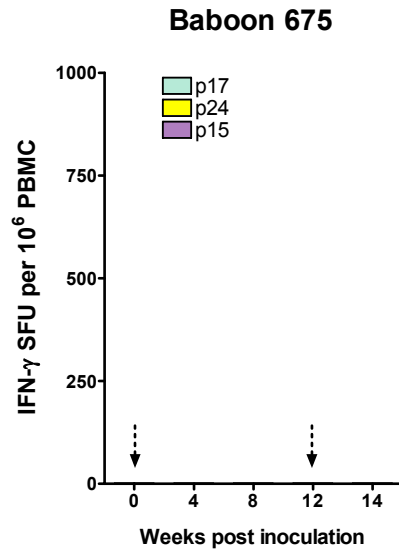


panBCG-gag  
[pEM19GO] prime  
VLP boost



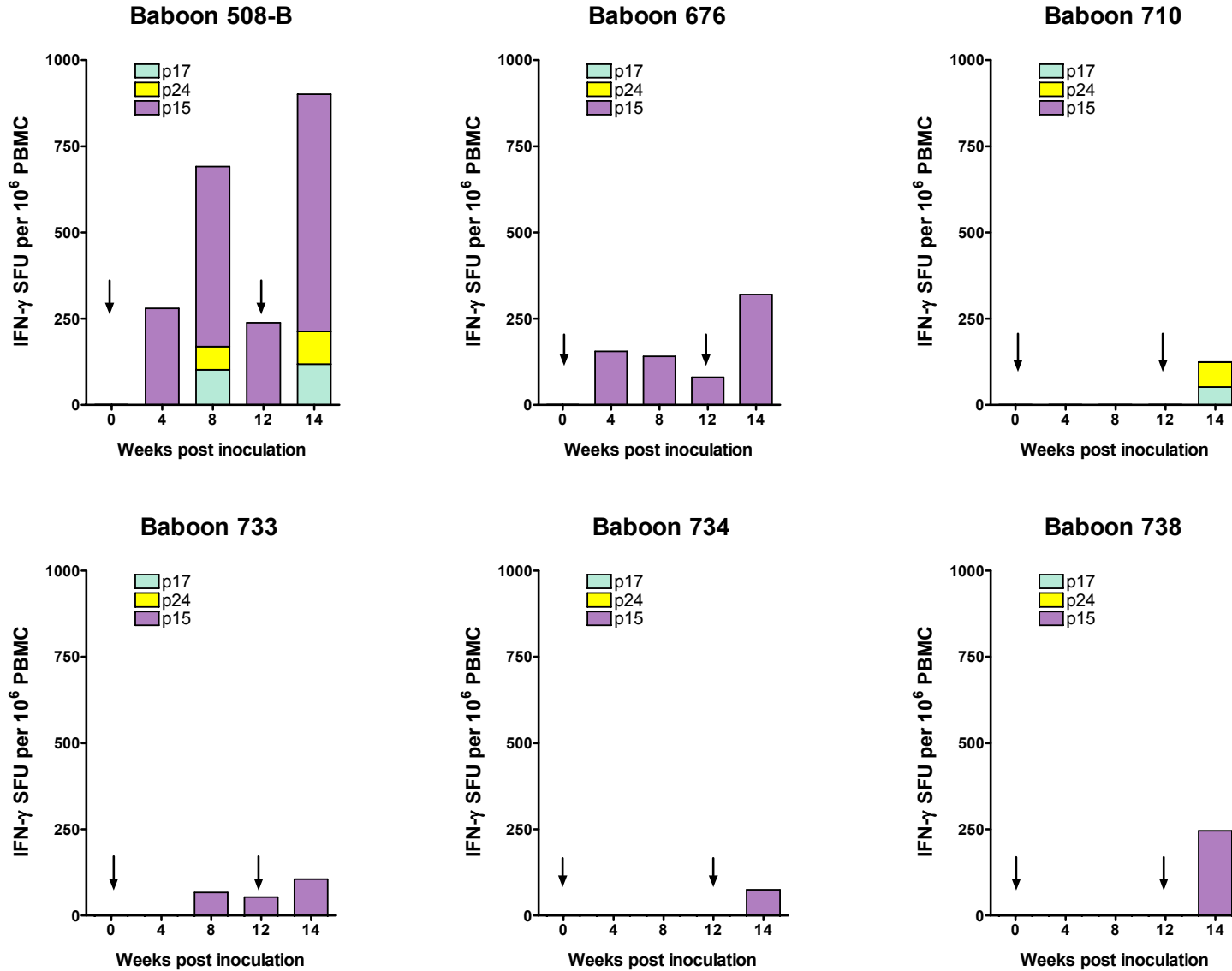
# CONTROL GROUP: immunized with rBCGpan:pCONEPI vaccine

## NO ELISPOT Response to HIV-1 Gag



# EXPERIMENTAL GROUP: immunized with panBCG-gag:pEM19GO vaccine

## ELISPOT Responses to HIV-1 Gag



# rBCG Conclusions

- We now have a much better understanding of how to improve expression and stability in rBCG
- rBCG expressing HIV genes should be further investigated to prime immune responses boosted by other heterologous candidate HIV vaccines for eventual clinical testing in both adults and children
- Oral immunization should be further investigated in non human primate models

# BCG Team at University of Cape Town

## Prof Anna-Lise Williamson (PI)

### BCG

Dr Ros Chapman, Dr Helen Stutz

### Immunology

A/Prof Enid Shephard and team, Dr Gerald Chege (Primate)

### Vaccinia Virus Challenge

Dr Niki Douglas


### Major Collaborators

William Jacobs Jnr, Albert Einstein College of Medicine, New York  
Clive Gray NICD, Johannesburg

### Previously at UCT

Dr Will Bourn, Dr Robin Thomas



A scenic photograph of a beach at sunset. The foreground shows a sandy beach with gentle waves washing onto the shore. In the middle ground, there are dark, jagged rocks protruding from the water. The background features the iconic Table Mountain range under a clear, orange-hued sky. The text 'Acknowledgements SAAVI and NIH for funding' is overlaid in yellow at the bottom of the image.

**Acknowledgements  
SAAVI and NIH for funding**