Spring 5-22-2012

Chimpanzee ad vector technology platform for prophylactic and therapeutic genetic vaccine applications

Stefano Colloca
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from genes to vaccines

Chimpanzee Adenovirus Vectors

Stefano Colloca

Vaccine Technology IV

May 20-25, 2012

Albufeira, Portugal

Contents

- Chimpanzee Adenovirus platform technology
  - The ChAd story
  - Genetic Adjuvant
  - Better cell line
- Immunization modalities
  - Combination of different Adeno vectors
  - Combination of Adeno and Pox vectors
  - Does the order matter?
  - Can the same vector be re-administered?
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Okairos' Technology platform

Pillars of Okairos' platform

Chimp adeno vectors

- Potent inducer of CD8 T cell response
- No pre-existing immunity
- Safe and well-tolerated in > 500 subjects

Genetic adjuvant

- Further boosts CD8 T cell response
- Increases rate, magnitude, breadth and duration
- Breaks tolerance against self-antigens

Procell 92 cell line

- New highly productive proprietary cell line
- Compatible with any gene insert
- FIM of first clinical material 3Q2012

Why adeno?

- For some infections, as well as cancer, protection or clearance can be boosted and they are correlated to a strong T-cell response

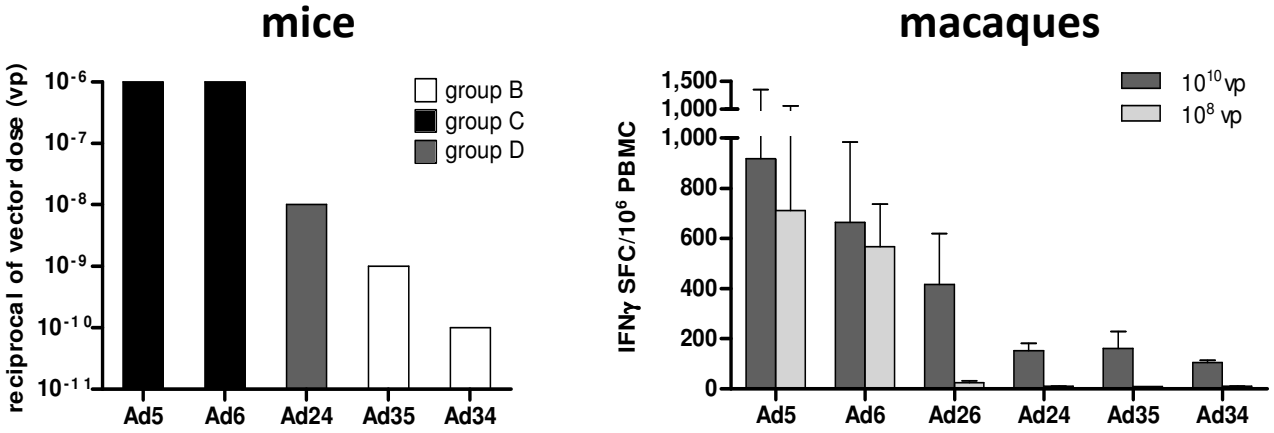
- An efficient way to generate a combined antibody and T-cell response is through an immune-stimulating vector

*Pre-clinical and clinical studies with **non replicating** human adenovirus 5 have shown it to be **safe and highly immunogenic***

- Pre-existing neutralizing antibodies to hAd5 present in humans reduce frequency and magnitude of response

Adenovirus vectors from **rare human serotypes are weak immunogens** in mice and NHP*

Human Ad



* *Colloca et al. Science Translational Medicine, in press*

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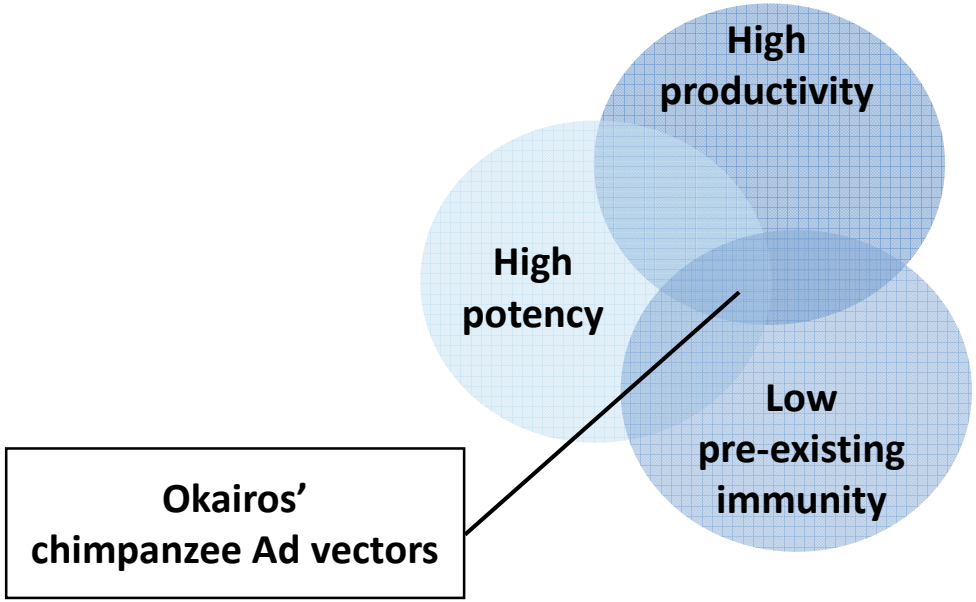
Okairos solution: vectors from chimpanzee adenoviruses

- Chimpanzee adenoviruses are closely related to human Ads
- hAd/ChAd cross-neutralization is very limited
- Human cell lines are permissive for ChAds replication

Okairos' Ad vectors derived from chimpanzees

- Okairos screened samples and isolated virus from over 700 chimpanzees
  - All primates were healthy and well cared for in US and EU facilities
  - Identified more than 100 strains grouped into 25 distinct serotypes



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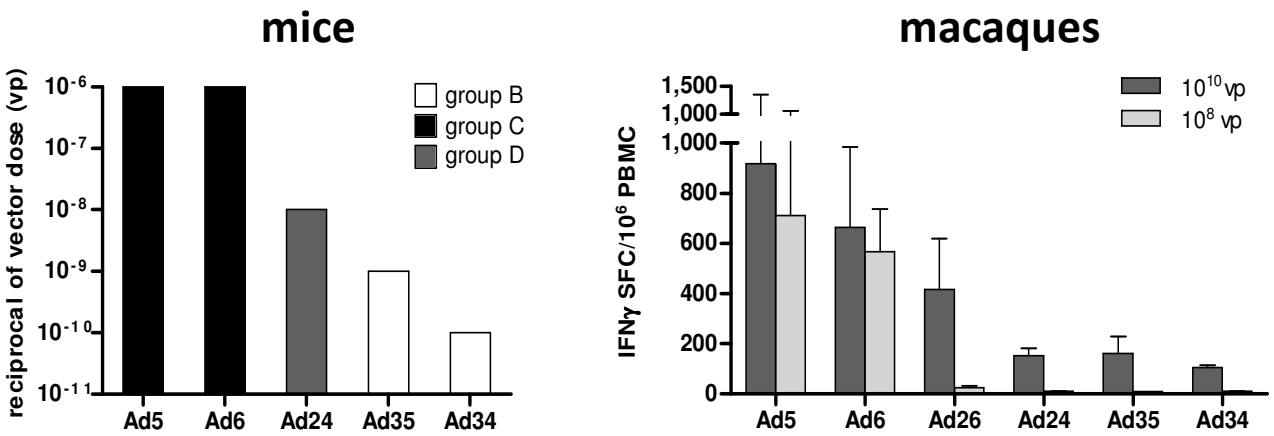
ChAd vectors **are potent immunogens** in mice and NHP*

Human Ad



* *Colloca et al. Science Translational Medicine, 2012*

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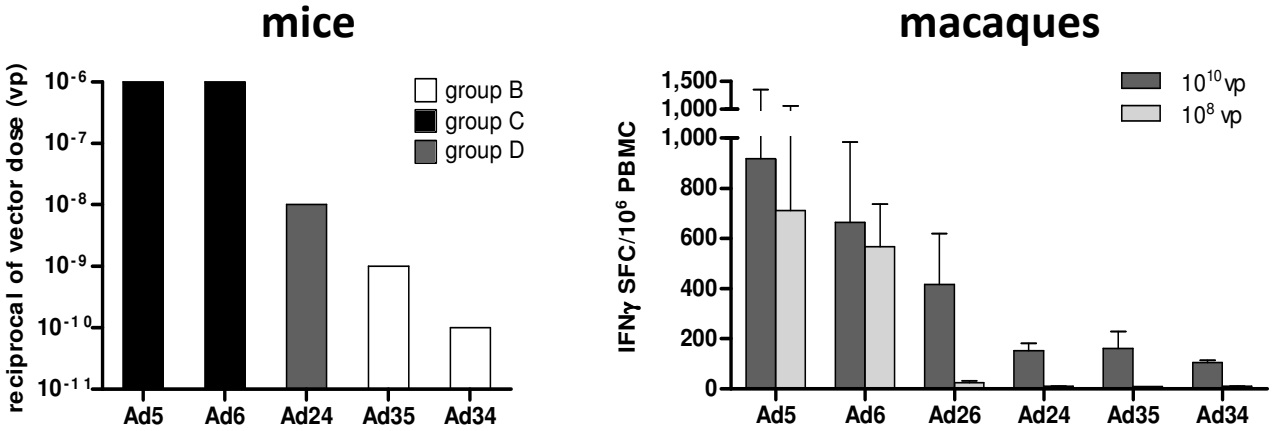
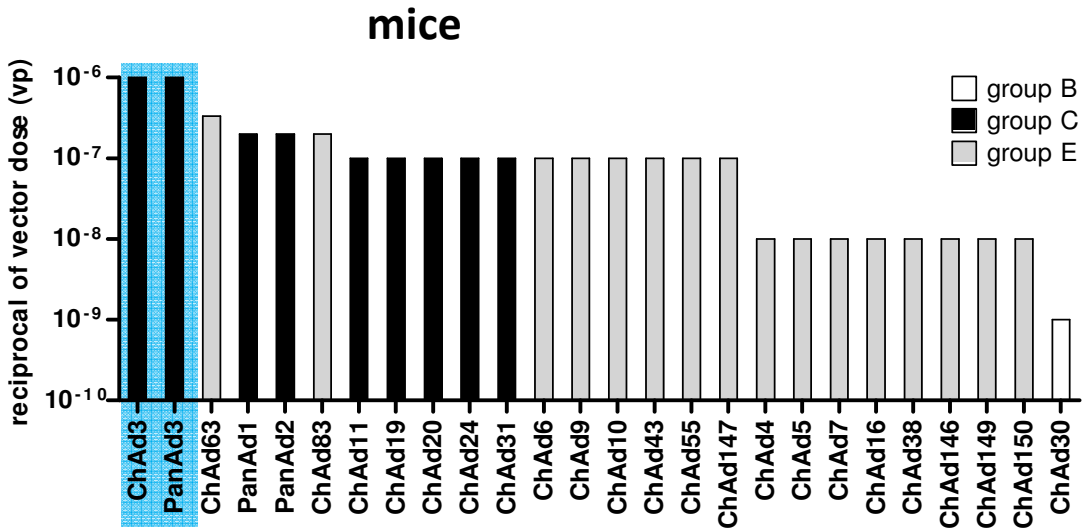


Okairos Chimp Ad



* *Colloca et al. Science Translational Medicine, 2012*

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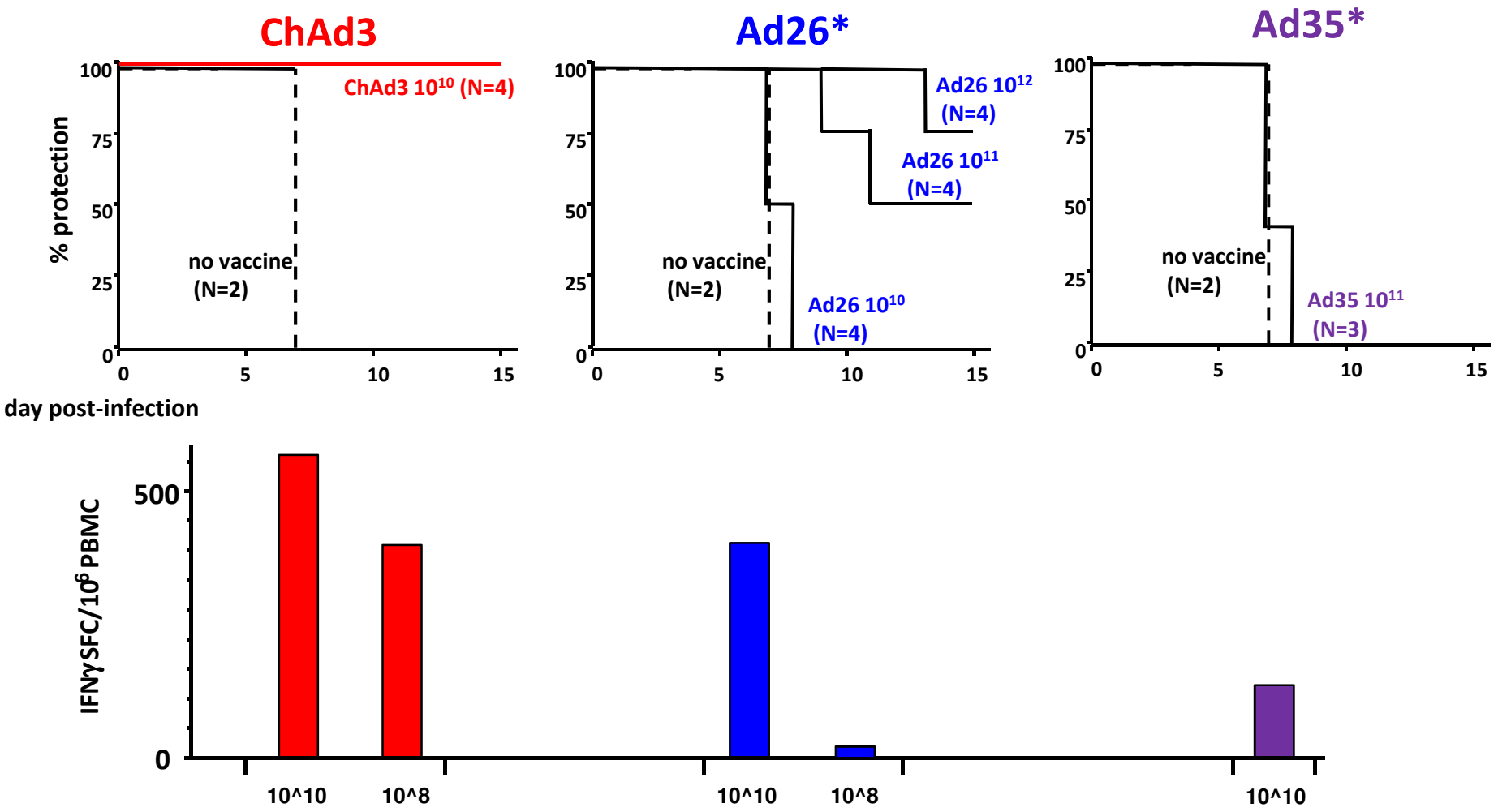
ChAd vectors are the **most effective Ad vaccine carriers**: single injection of ChAd3 provides full protection against EBOV challenge

- ***Mechanism of protection from Ebola challenge is based on CD8 T cells*****



 ** Giesbert TW; J Virol, 2011; collaboration with N.Sullivan & G.Nabel, VRC – NIH - ** Sullivan NJ, Nat Med. 2011 Aug 21;17(9):1128-31*

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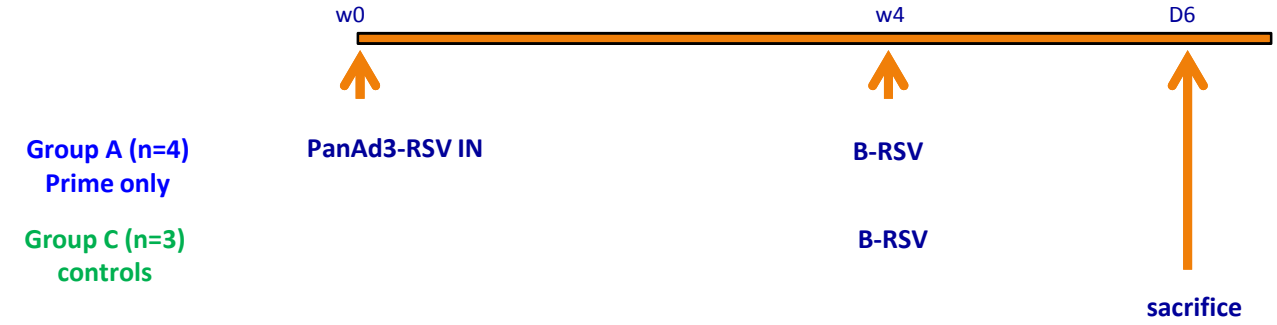
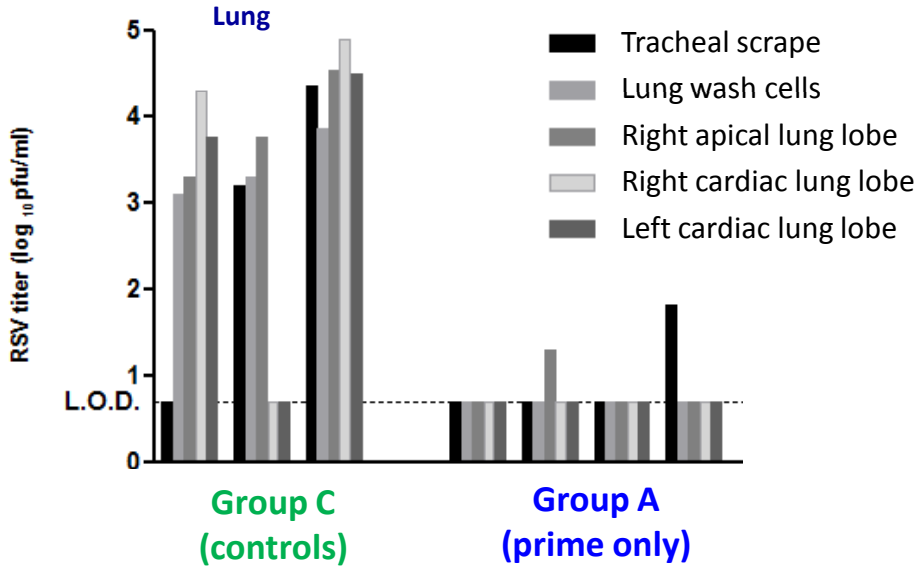
Single administration of **PanAd3 vector expressing RSV F- N/M2-1** and challenge experiment with B–RSV in calves

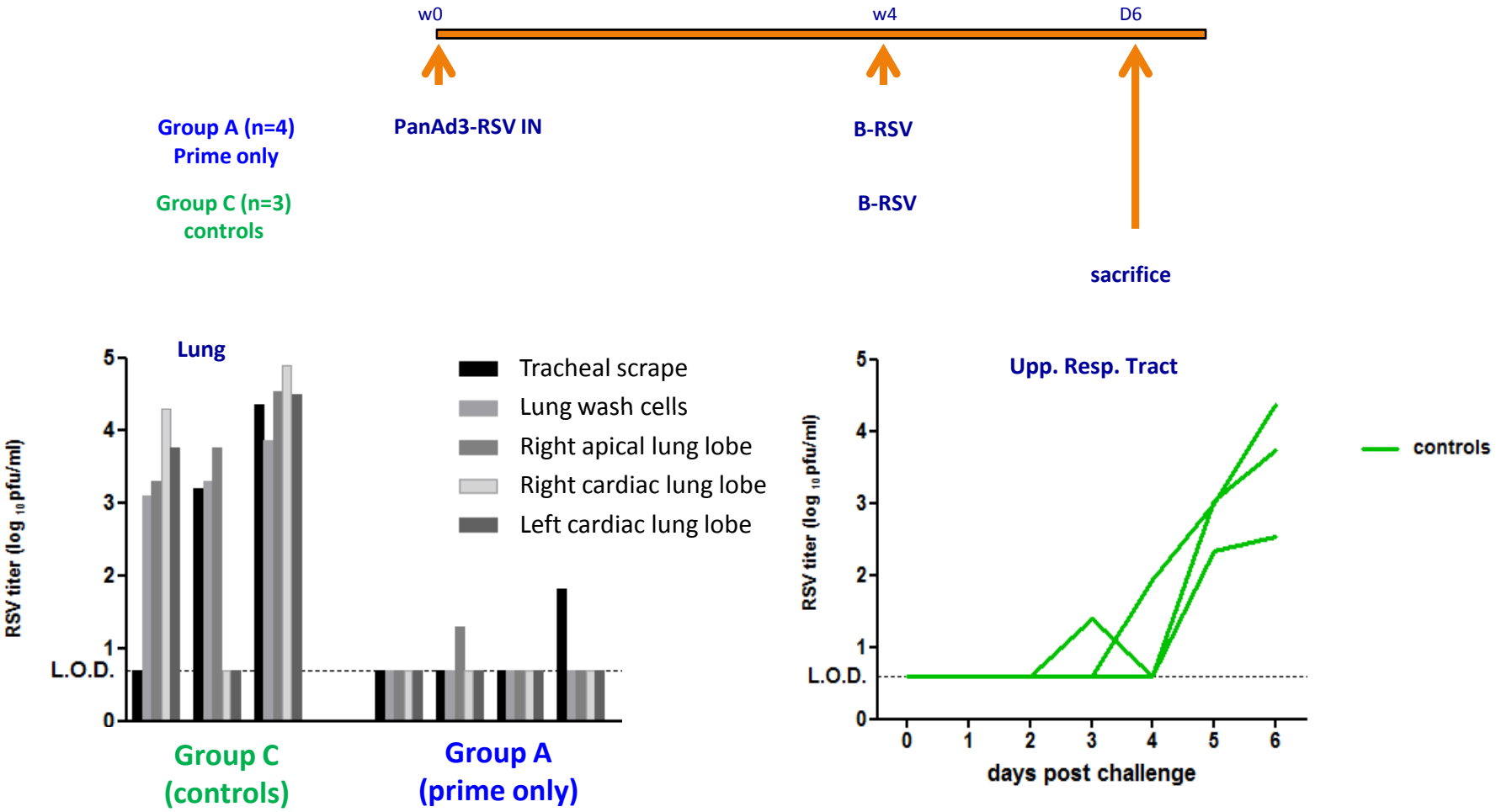




- PanAd3-RSV single administration prevents viral replication in lower respiratory tract and lung

** Collaboration with G. Taylor IAH, UK*

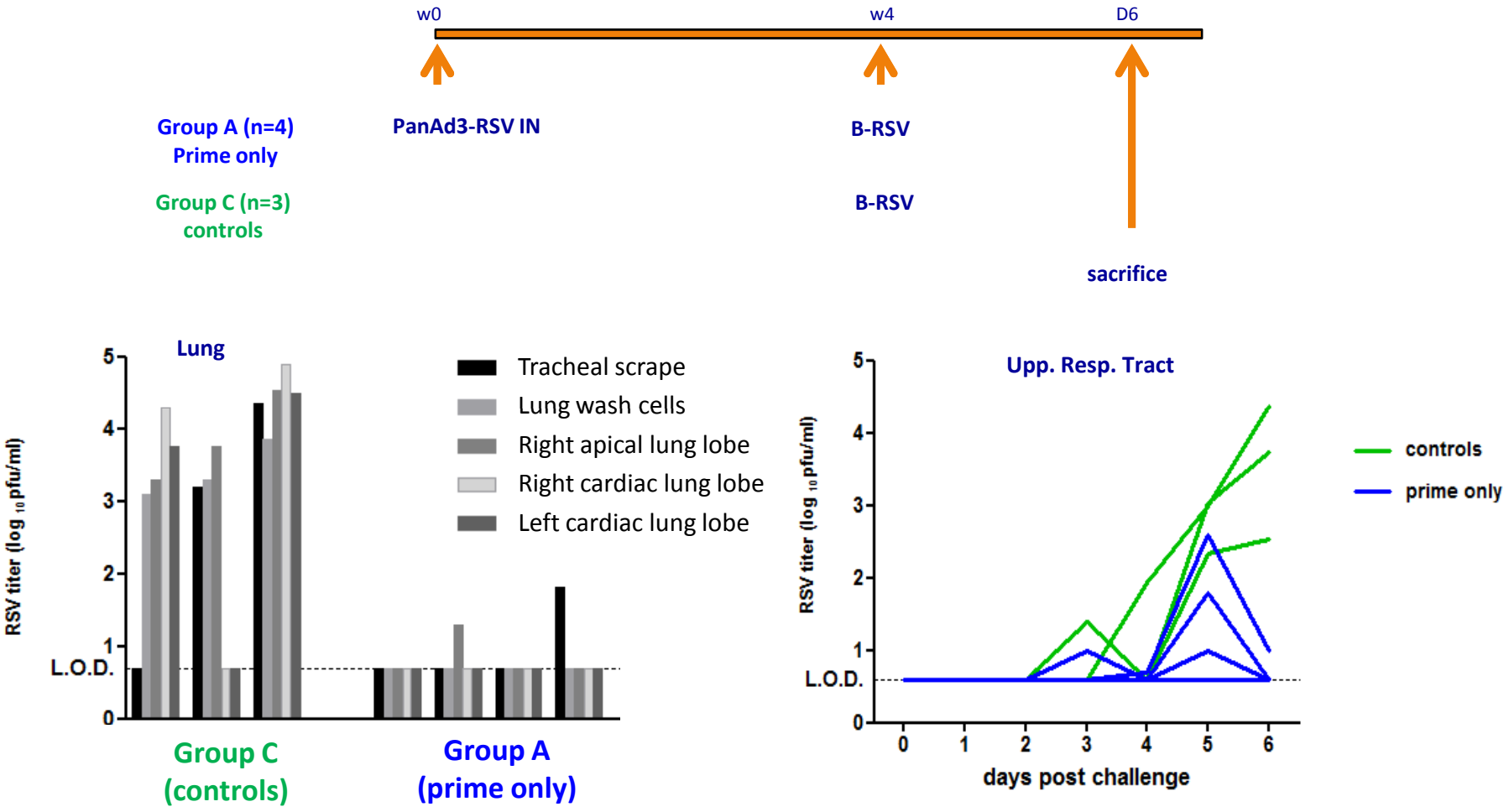
Single administration of **PanAd3 vector expressing RSV F- N/M2-1** and challenge experiment with B–RSV in calves



- Control animals shed RSV in nasal secretions by day 2-3, peaking at day 6 post challenge

** Collaboration with G. Taylor IAH, UK*

Single administration of **PanAd3 vector expressing RSV F- N/M2-1** and challenge experiment with B–RSV in calves

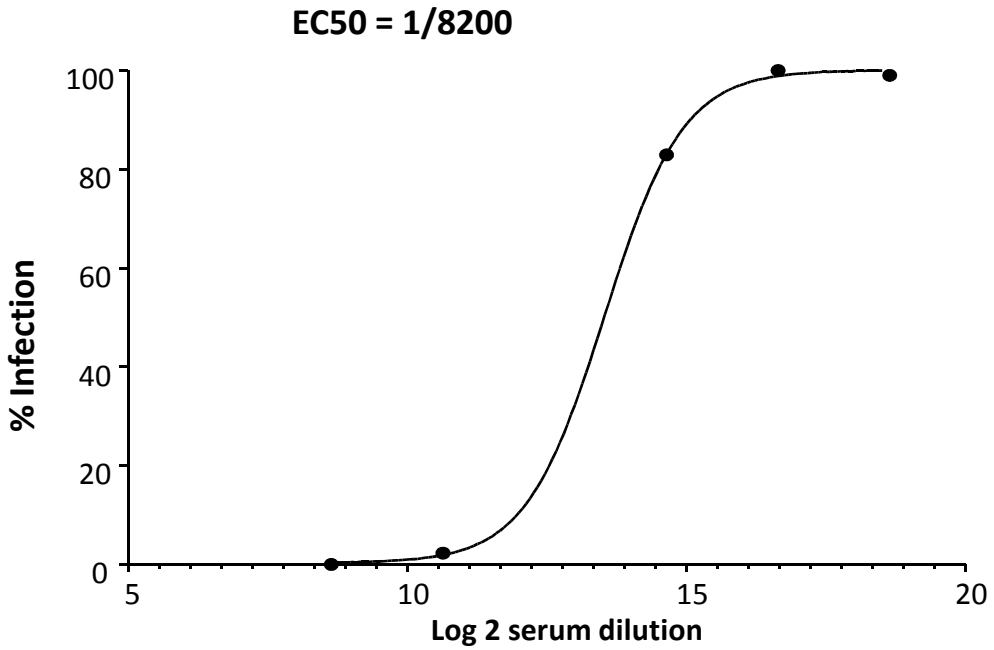


- A single administration of PanAd3 blunts viral replication in the upper respiratory tract as well

** Collaboration with G. Taylor IAH, UK*

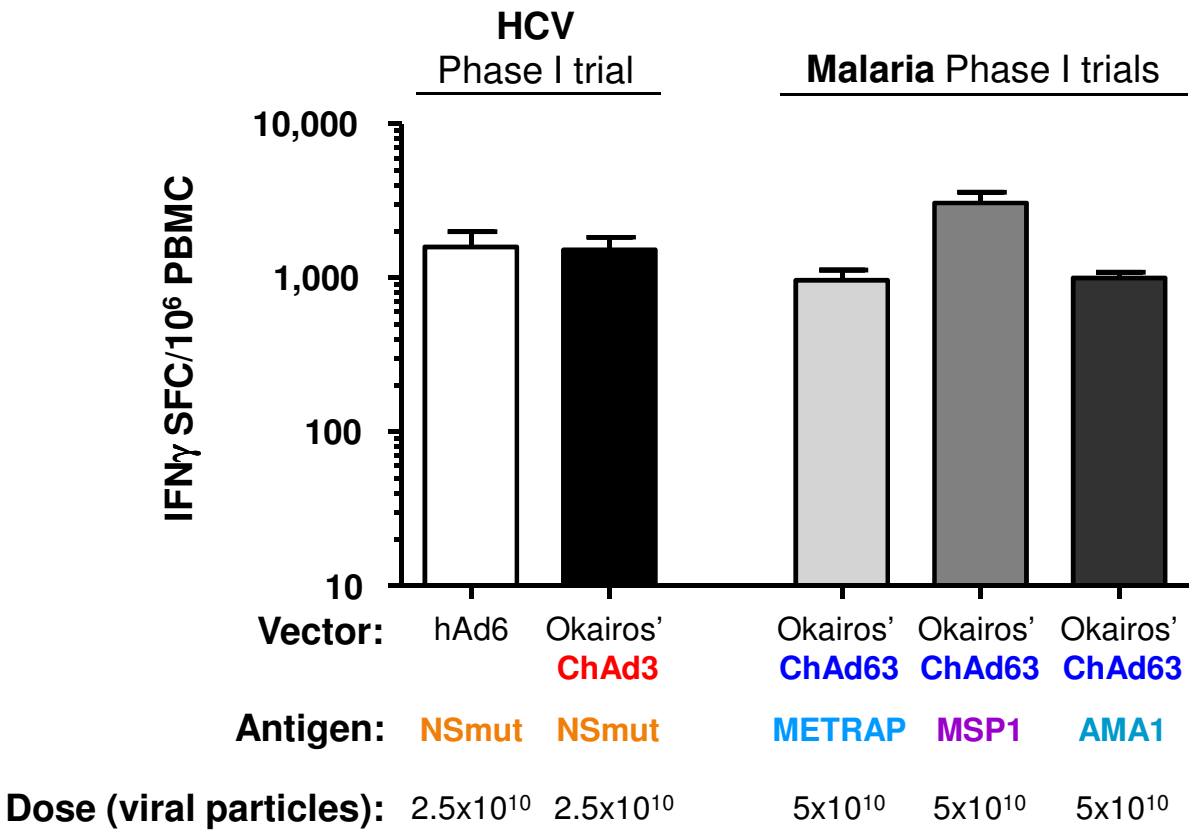
Robust neutralising antibody response generated by RSV vaccine candidate (data from **RSV vaccine program**)

Neutralising titers induced in mice by Okairos RSV vaccine



- Neutralizing antibody titer EC_{50} = 1/8200 (both A and B RSV types) is ***100 fold higher*** than:
  - trough levels present in **sera of infants treated with Synagis**
  - sera from adults and **infants protected from hospitalization**

Multiple clinical studies have **validated the safety and strength of ChAd vectors in humans***



- Peak responses shown from U.Oxford/Okairos' HCV and malaria vaccine clinical trials

* *Barnes et al. Science Translational Medicine, 2012; O'Hara J.Infect. Disease, 2012; Sheehy et al. Molecular Therapy, 2011*

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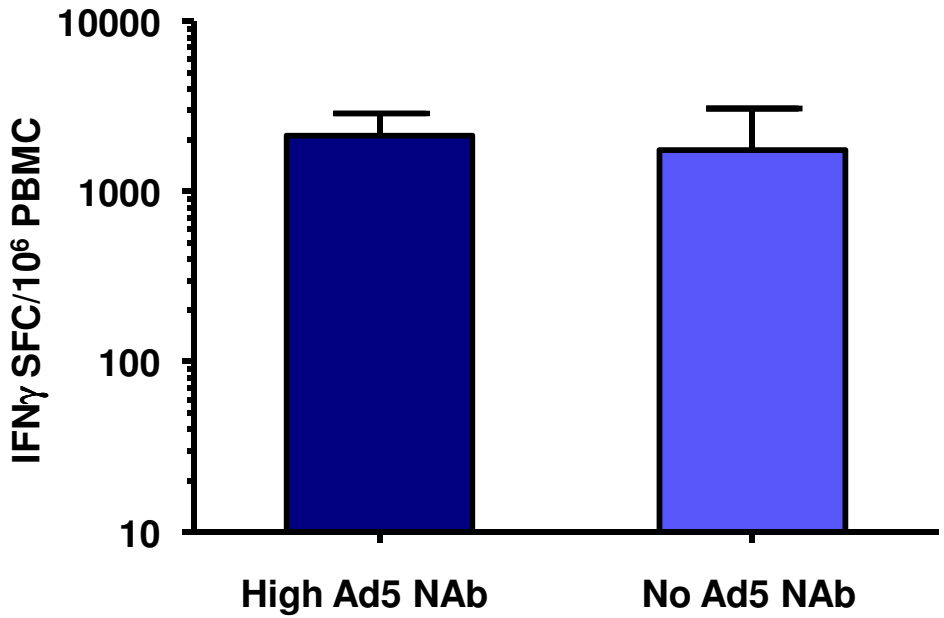
Chimp Adeno are insensitive to pre-existing anti-Adeno immunity

present in in humans (data from **HCV vaccine program***)



* *Barnes et al. Science Translational Medicine, 2012*

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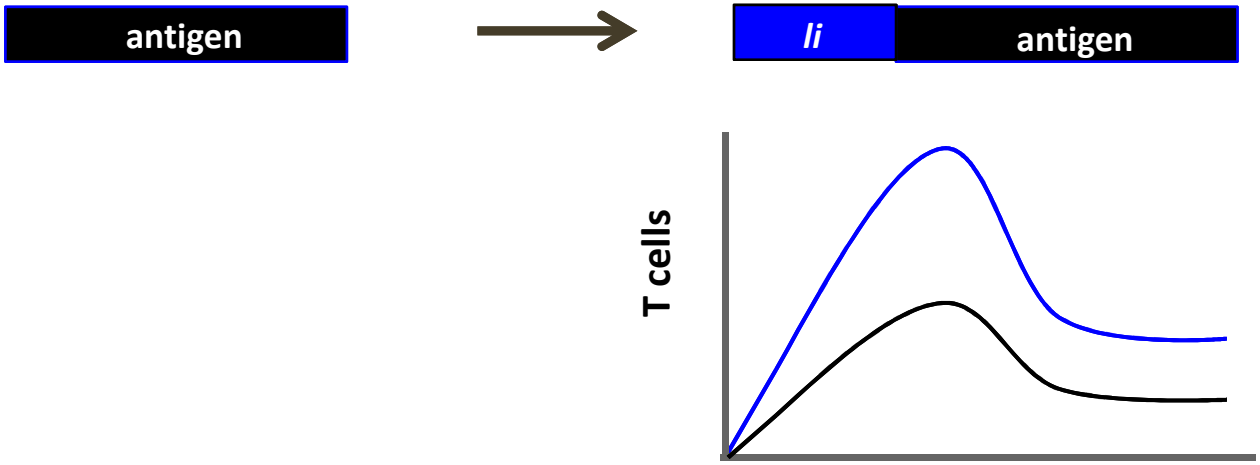
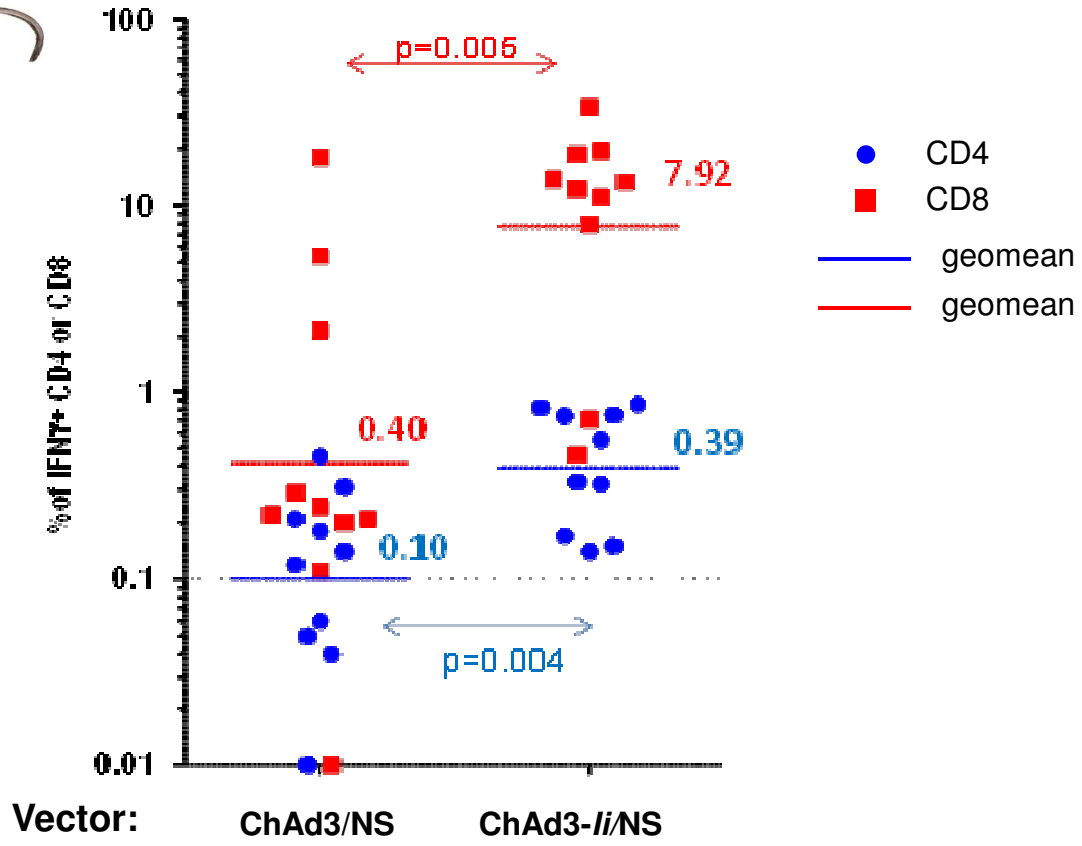
MHC-II Invariant chain (*Ii*) is a genetic adjuvant

- Fusion of mouse Ii to Adeno encoded antigen increases presentation on MHC-I (Holst, J of Immunology 2008)
- Invariant chain fusion increases intra-cellular concentration of antigen – ER accumulation (P. Holst, unpublished)

➢ Hypothesis: re-location and intracellular storage of antigen, favourable for antigen presentation, leads to **increased T cell response**



MHC-II Invariant chain *(li)* increases strength of CD4 and CD8 T-cell response in mice



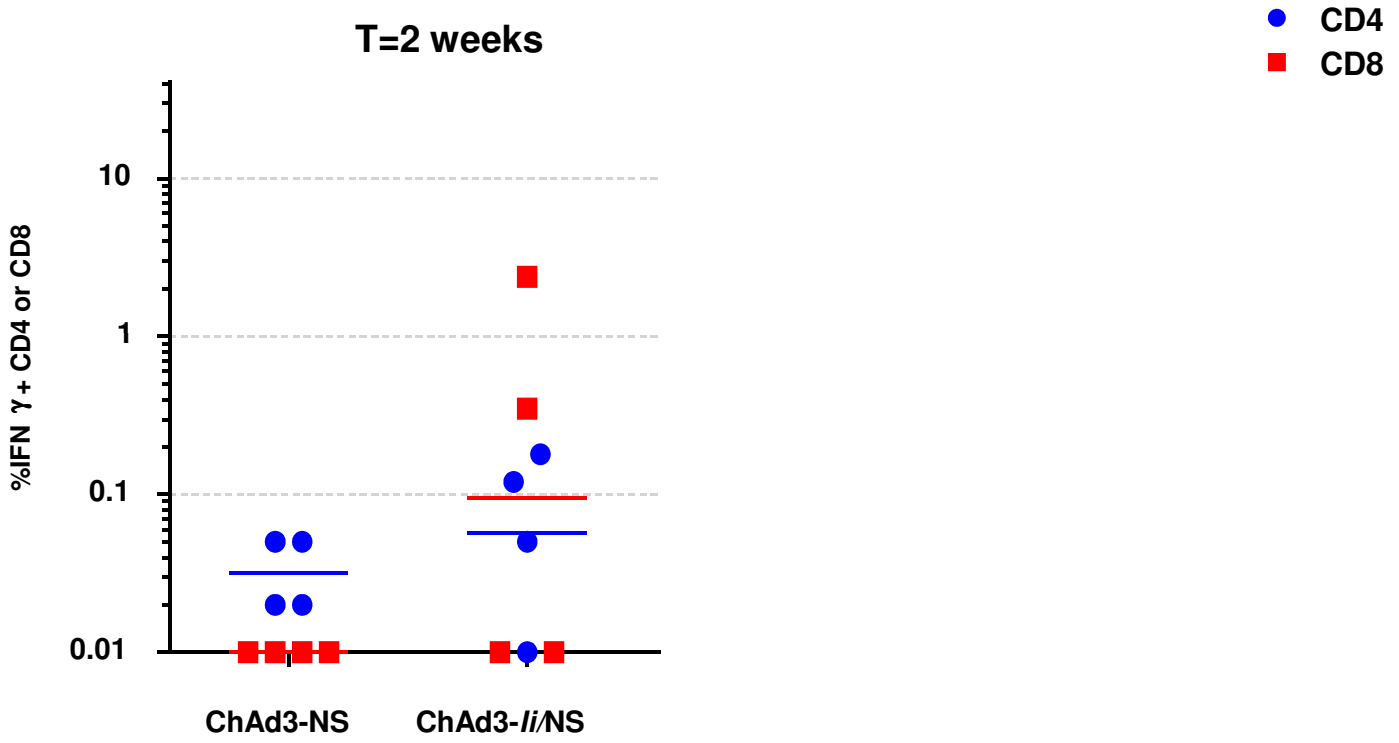
- Similar data with different Ad vectors (ChAd3, ChAd63, Ad5) and different antigens:

1. Ebola GP
2. Malaria METRAP
3. LCMV GP and NP
4. MHV-68 M2 and M3
5. VSV GP

- 10 outbred mice vaccinated per group
- Test on splenocytes by IFNγ ICS at week 2
- Bar represents geometric mean per group

li improves levels and onset of CD4 and CD8 T-cell response in non-human primates

- 4 NHPs immunized per group
- IFNγ ICS data collected at 2 and 4 weeks post vaccination
- Line represents geometric mean



- First in man planned in early 2013

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Procell-92: a novel manufacturing cell line for an unmet technological need

Need for a new proprietary cell line with:

- Full history documentation and clean records
- Ability to support production of Ad vectors expressing toxic/interfering transgenes
- Better productivity

Advantages of Procell-92 expressing TetR:

- Successful production of genetically stable Ad vectors expressing toxic/interfering transgenes:
  - *Ad6-HCV E1E2p7*
  - *ChAd3-HIV GP155*
  - *ChAd63-Malaria CS*
- Increased yield of ChAd vectors in comparison to 293 or PER.C6 was observed for all vectors tested so far

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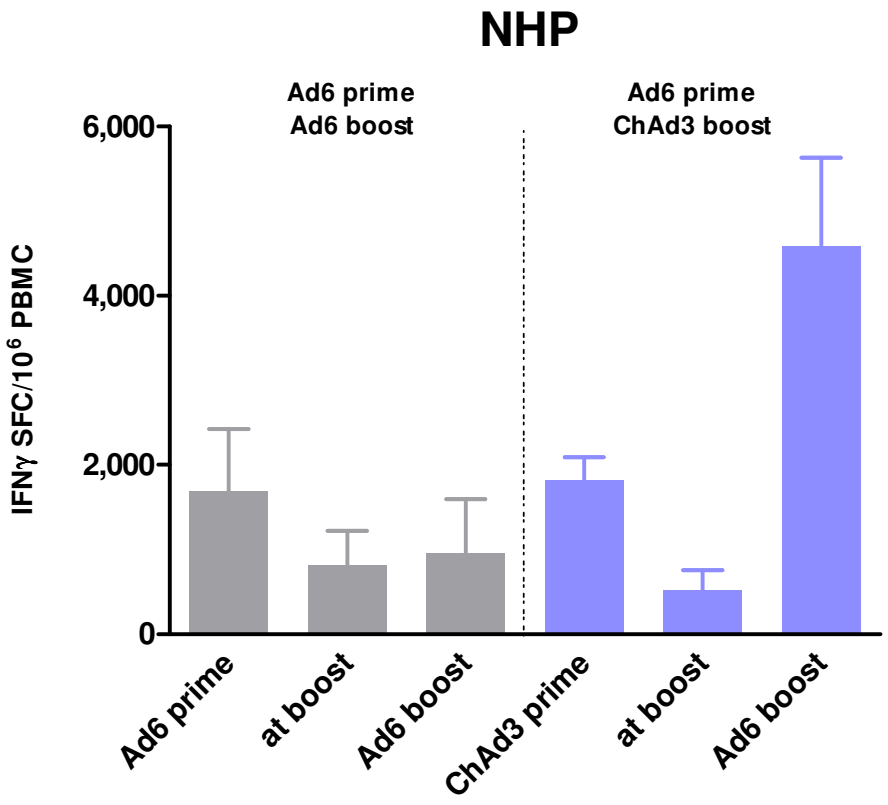
Heterologous prime/boost with different group C Ad vectors is more efficient than homologous prime/boost in NHP



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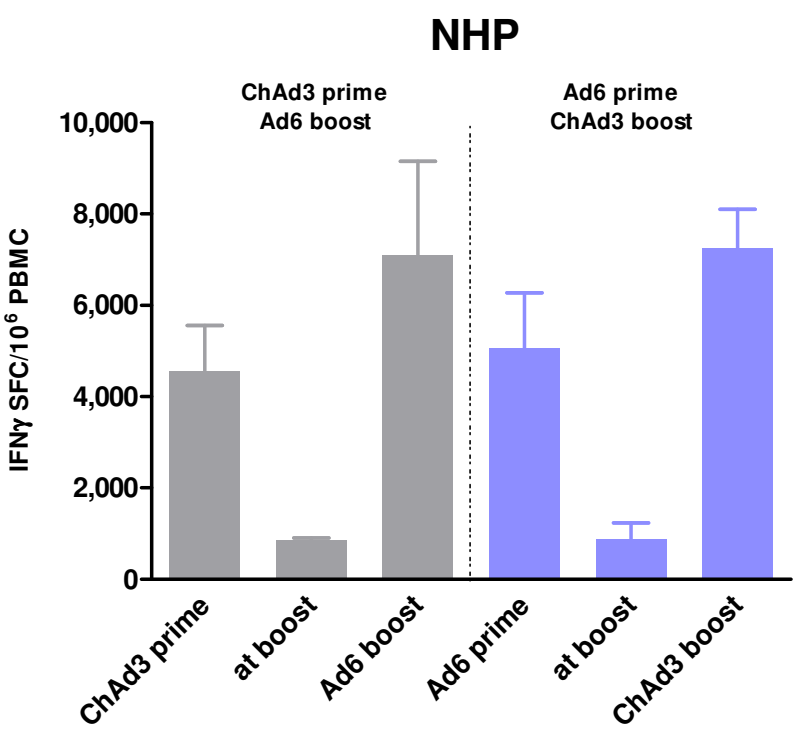
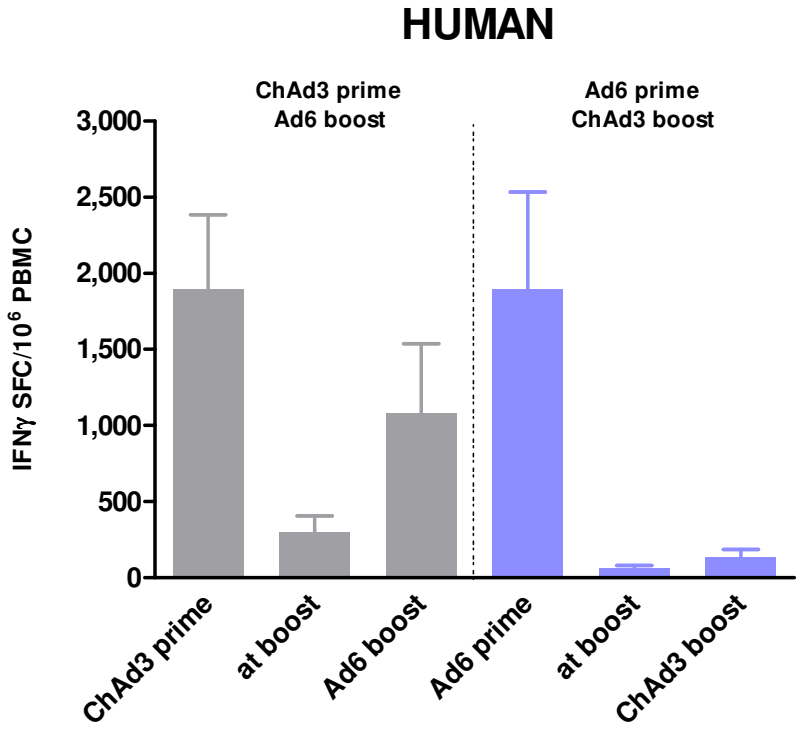
Heterologous prime/boost with different group C Ad vectors in NHP and in Humans*





- In humans the heterologous prime/boost with different group C Ad vectors **is not as efficient as in NHP**, and ChAd3 is a better primer
- No correlation with anti-vector T cell response
- Some correlation with anti-vector nAb

* *Barnes et al. Science Translational Medicine, 2012*

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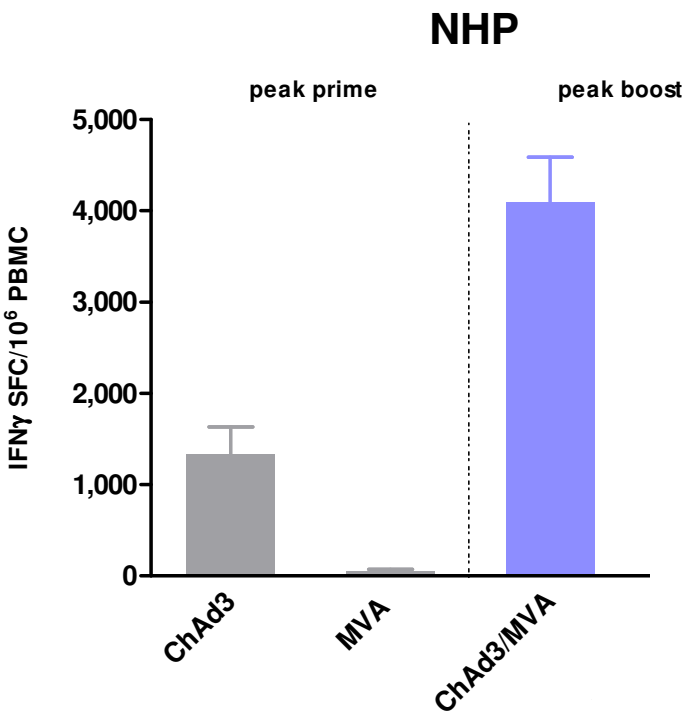
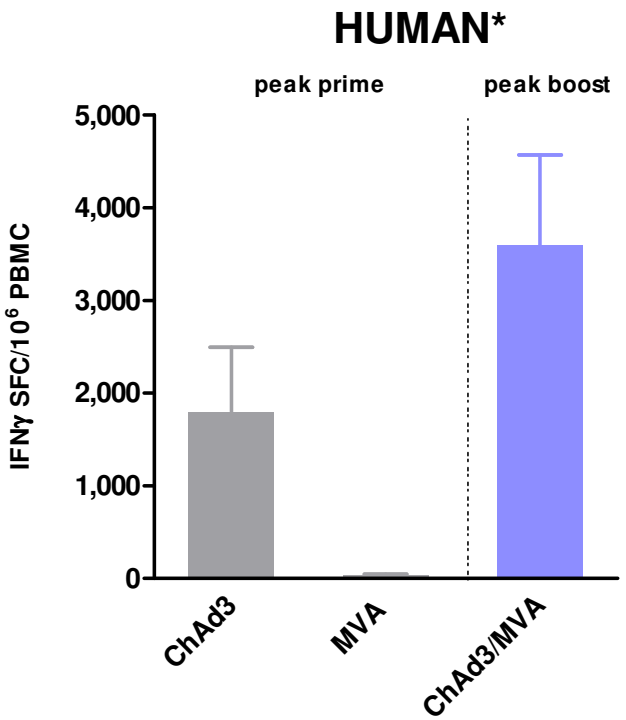
ChAd/MVA is the best combination





* *Collaboration with P.Klenerman & E.Barnes, U.Oxford*

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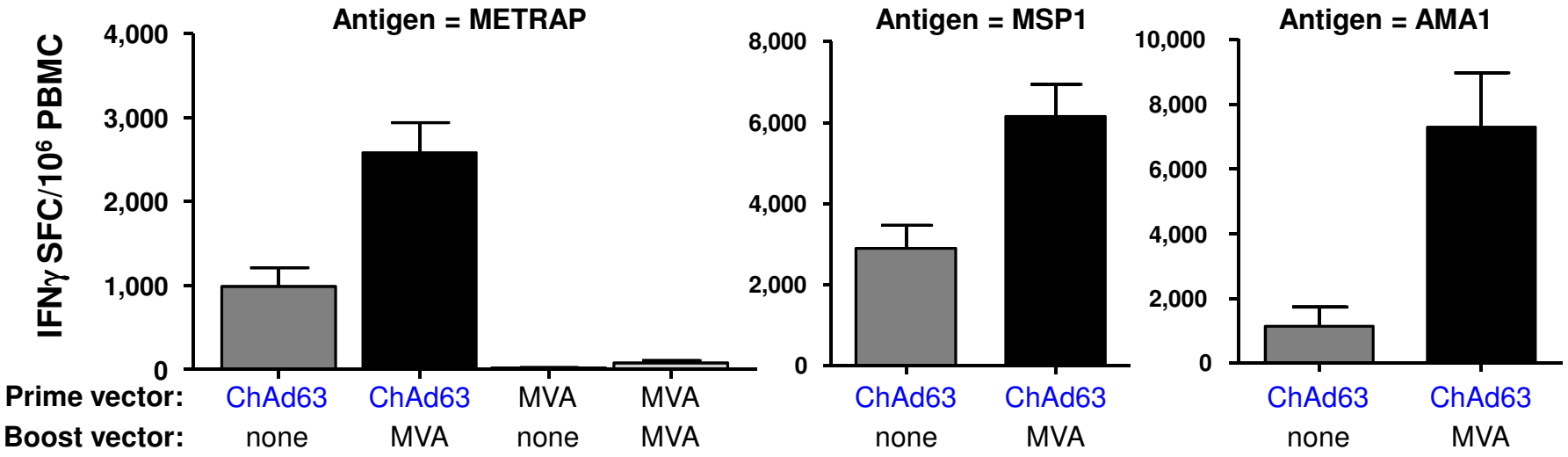
The high immunological potency of **ChAd/MVA** Heterologous prime/boost is **independent from the ChAd serotype or the antigen** (data from Malaria vaccine program)

- Data from different Phase I trials with vectors encoding different Malaria antigens*



* Peak responses shown from U.Oxford/Okairos' malaria vaccine clinical trials

* *O'Hara J.Infect. Disease, 2012; Sheehy et al. Molecular Therapy, 2011*

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Overview of ChAd/MVA clinical trials completed/ongoing to date

- Over 500 subjects vaccinated
- No safety issue
- 100% response rate
- 1000 – 18000 SFC/million PBMC
- Balanced CD4/CD8

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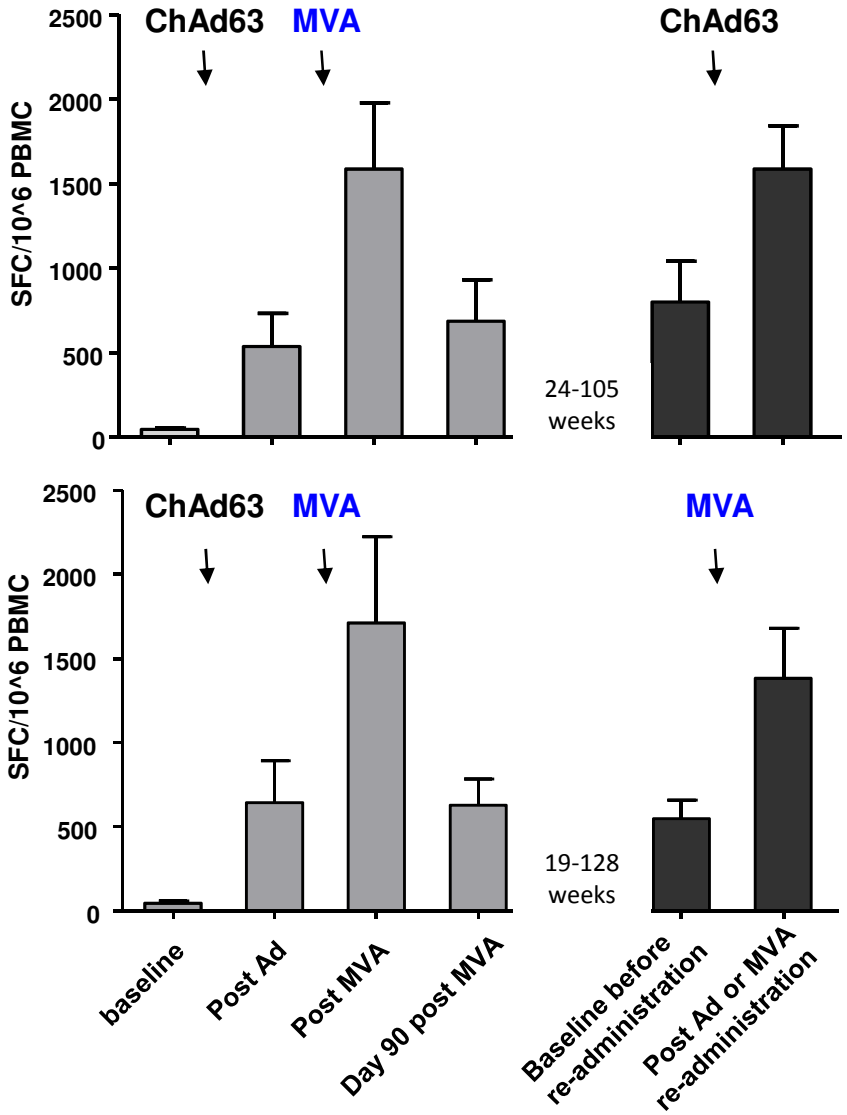
ChAd and MVA vectors can be re-administered in humans

(data from Malaria vaccine program - vectors encoding Malaria METRAP*)

IFNγ ELISpot



* *Collaboration with A.Hill, U. Oxford*

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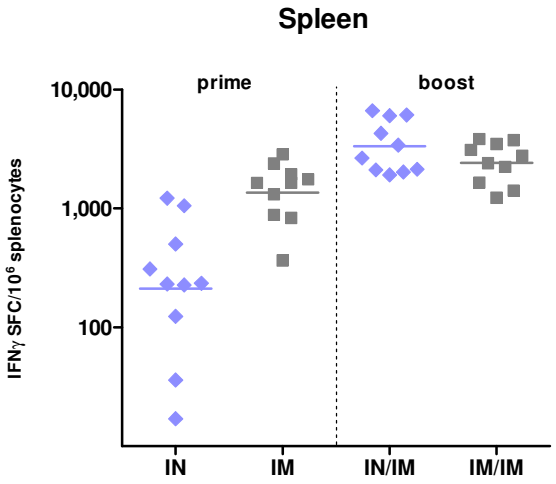
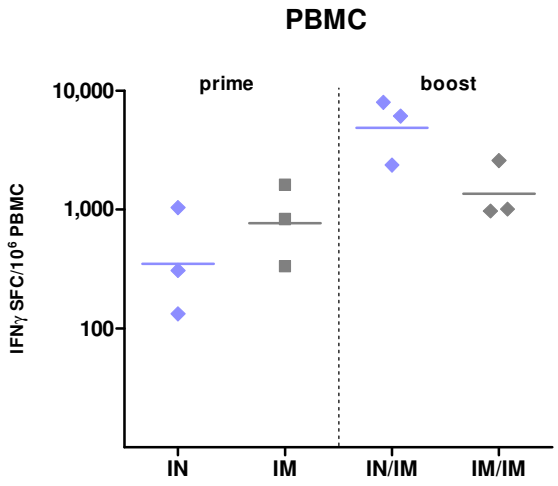
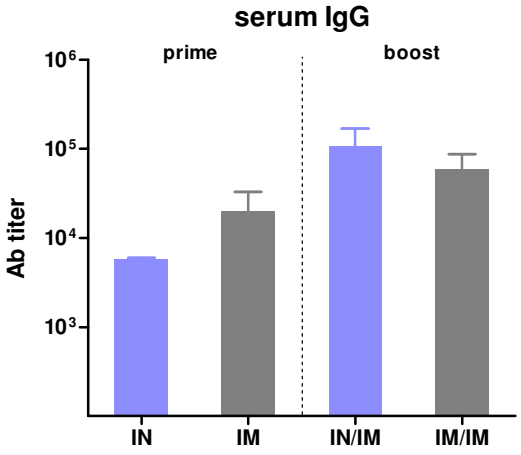
Intranasal ChAd priming can be efficiently combined with **intramuscular MVA boosting**: the **'Mixed route'**

mouse



NHP





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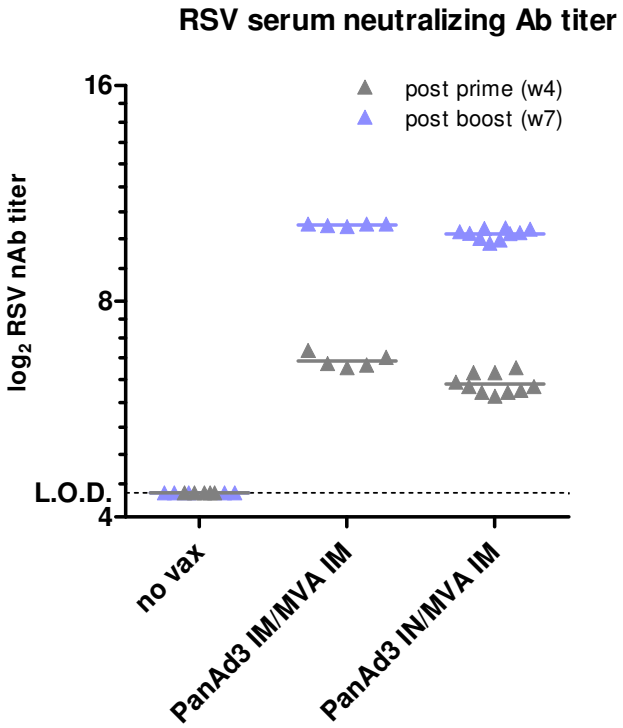
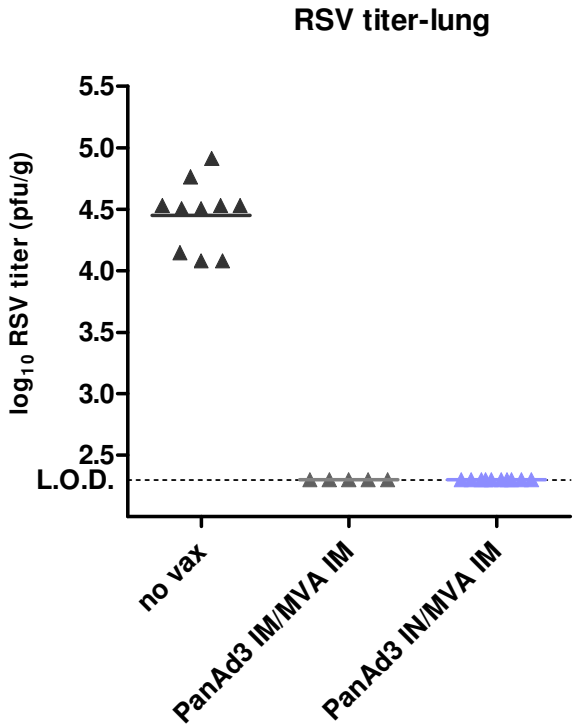
‘Mixed-route’ ChAd/MVA regimen is induces highest protection from RSV challenge in cotton rats





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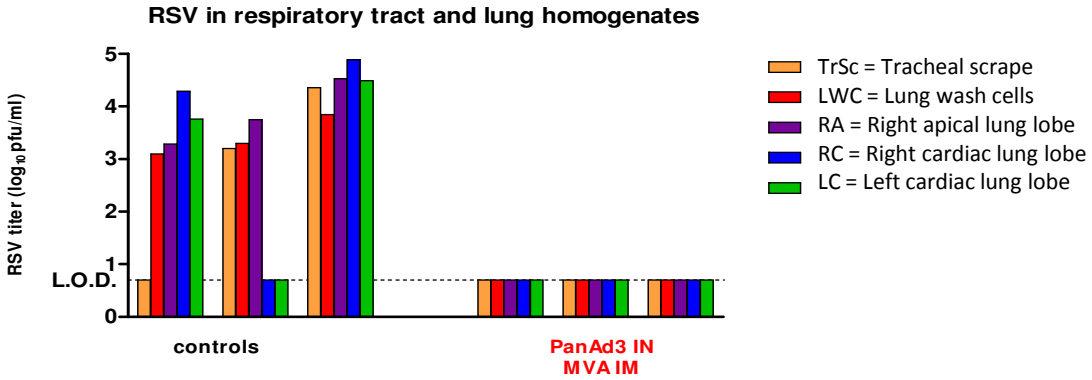
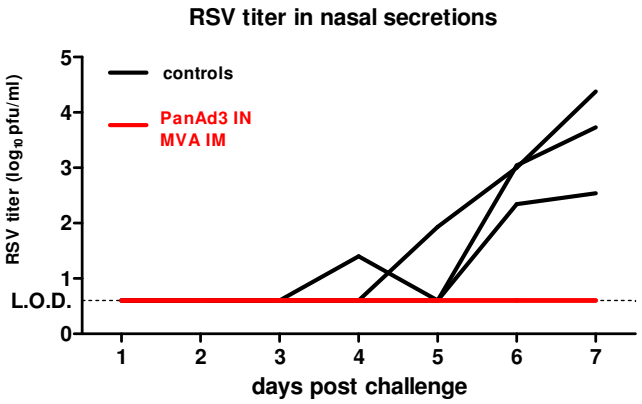
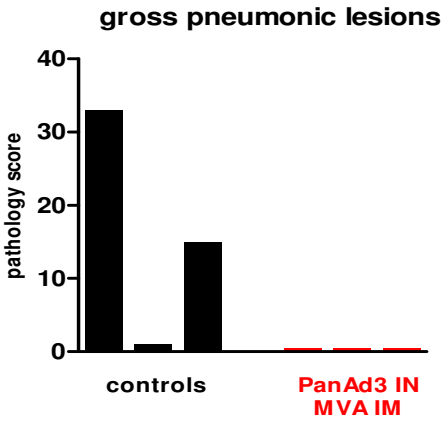
PanAd3/MVA RSV vaccine is **safe and fully protective** against **heterologous B-RSV challenge** in seronegative newborn calves*







- Phase I study starts 3Q 2012

* *Collaboration with G.Taylor, IAH, UK*

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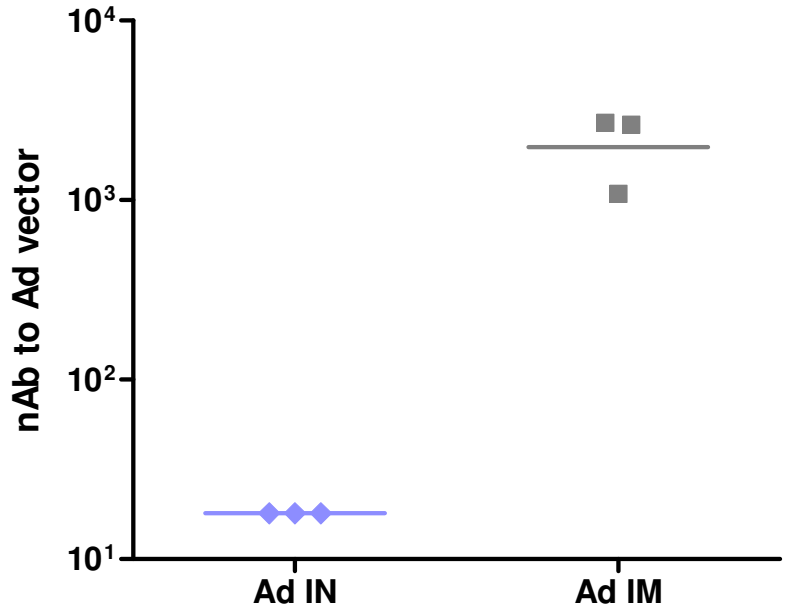
Intranasal Ad prime does not induce systemic neutralizing antibodies to the vector: option for re-administration

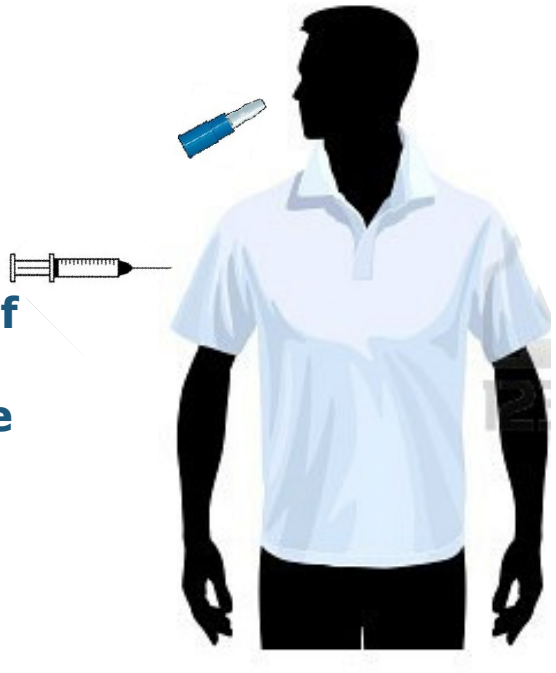
- NHP (N=3) were immunised with 5x10^10 vp PanAd3-RSV by the intranasal (IN) or intramuscular route (IM)
- nAb measured 4 weeks post immunization



Administration of the same Ad vector by the 'Mixed route': A new concept for homologous prime/boost

1° vector administration by intranasal route

2° administration of the same vector by intramuscular route

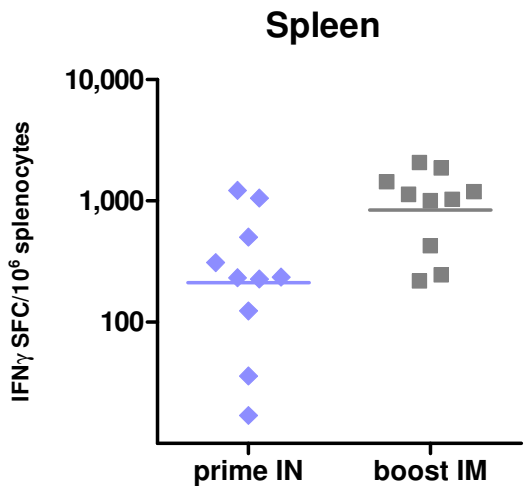


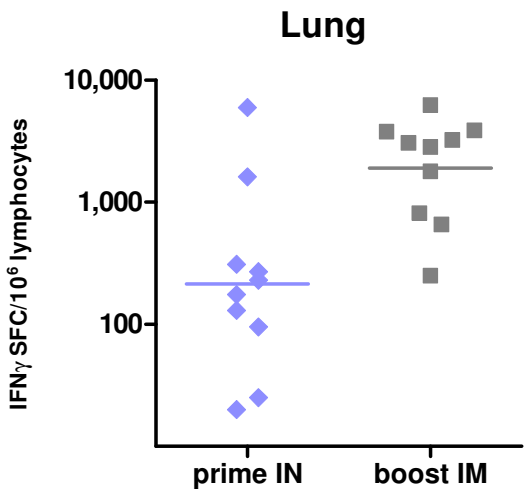
- **Priming of Ag-specific response**
- **No induction of systemic anti-Adeno antibodies**

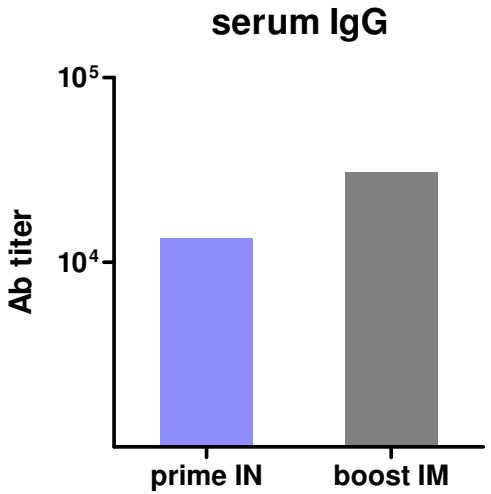
- **Vector is NOT neutralized**
- **Efficient boosting of Ag-specific immunity**

Efficient boosting by re-administration of the same ChAd vector by the 'Mixed route'

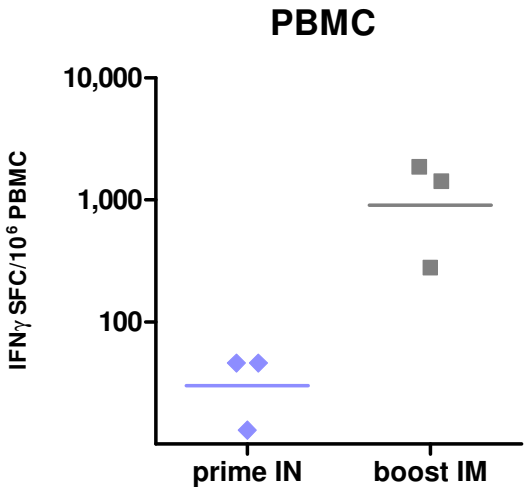
mouse

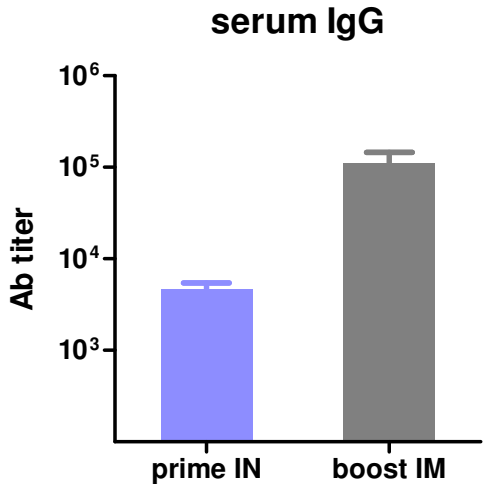






NHP





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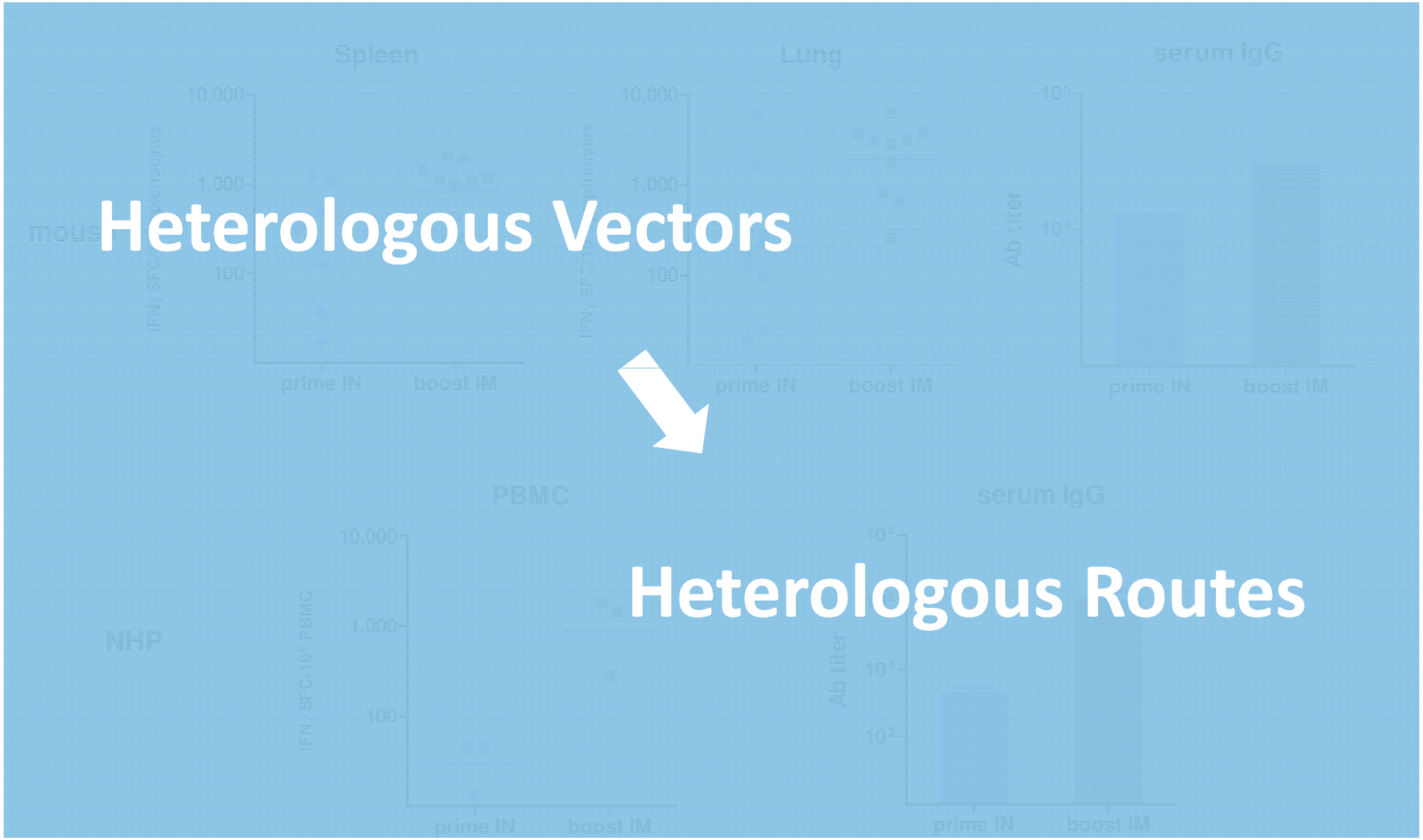
‘Mixed route’: a paradigm shift



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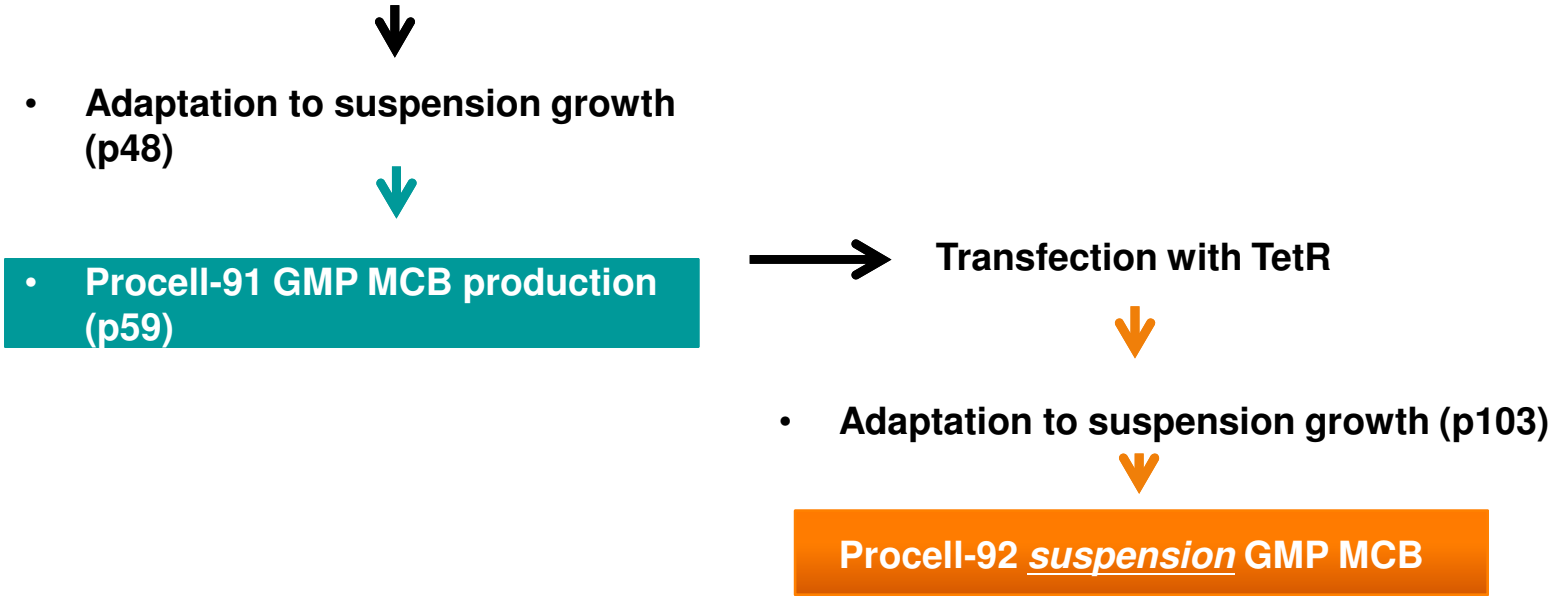
Funding

Back up

Procell-91 and Procell-92 Cell line history

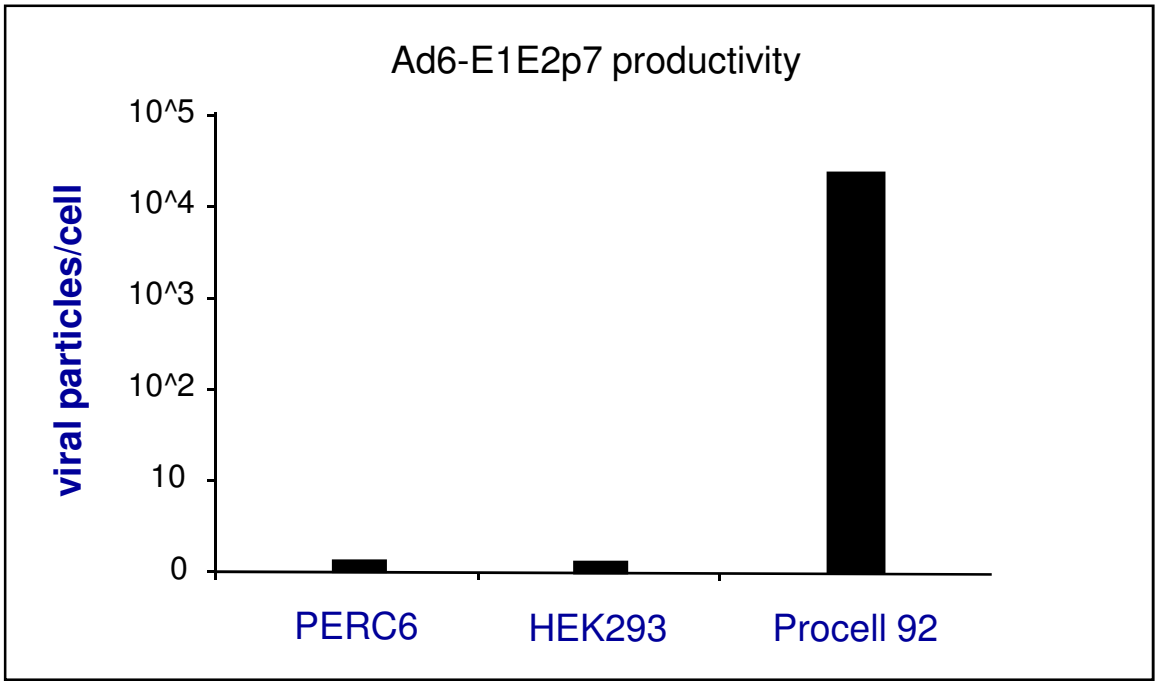
- Procell-91 and Procell-92 are proprietary derivative of HEK293, designed to optimize vector expression independent of insert
  - Obtained 293 cells frozen in 1975 (prior to prion disease onset) from Dr F. Graham (p10)
  - Cells were expanded under defined conditions to generate a research cell bank (p33)



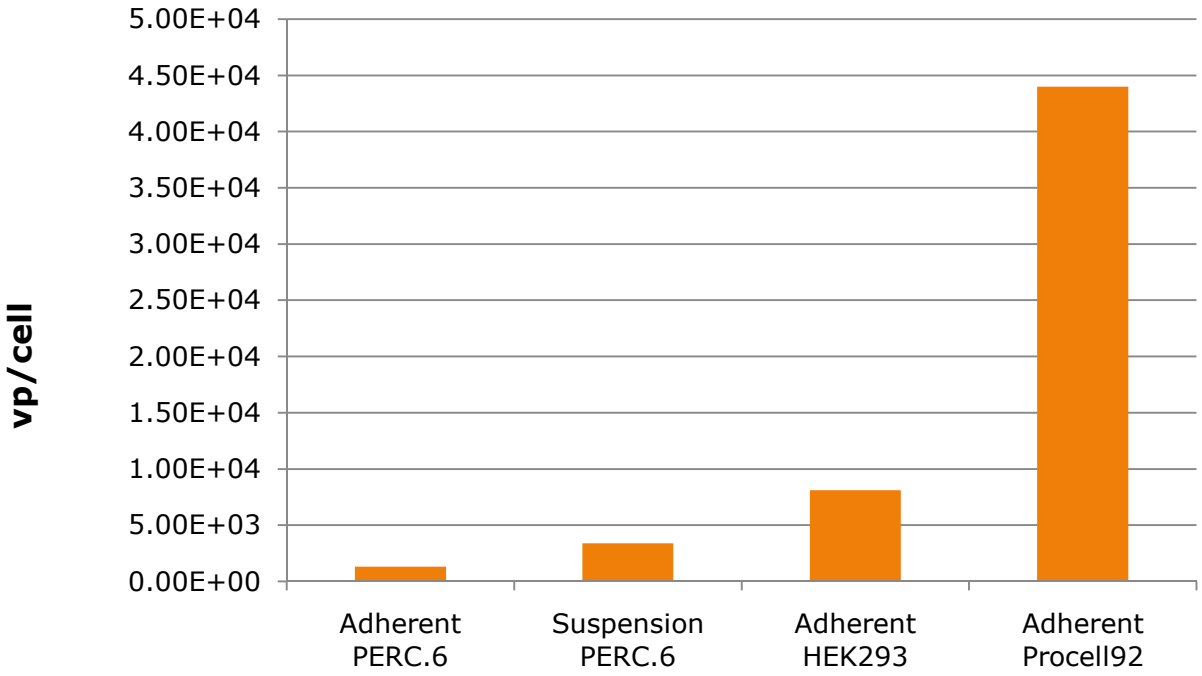
Procell-92 allows growth of Ad vectors encoding toxic genes

Ad6 vector encoding 3 HCV genes:

1. *E1 (transmembrane domain)*
2. *E2 (transmembrane domain)*
3. *p7 (highly toxic ion channel protein)*



Increased productivity of ChAd3NSmut in Procell-92

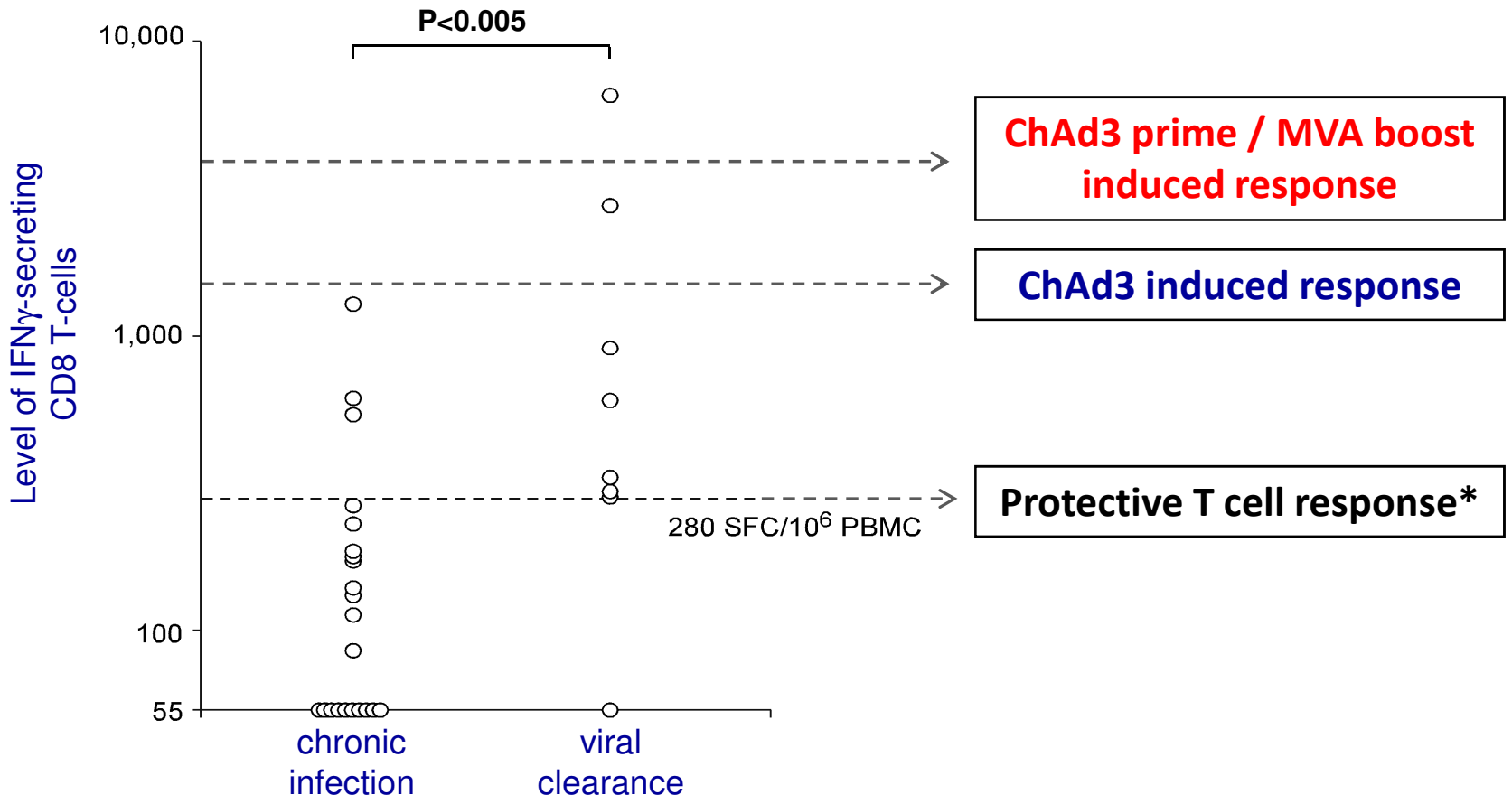


Ad5 is best-in-class vector for CD8 T cell induction in humans

	Immunogenicity in humans	Clinical Safety	Ease of manufacturing	Integration	Pre-existing Immunity
Human Ad5	**very high (>CD8)**	**excellent**	**excellent**	**none**	**high**
Alphavirus	high (>CD4)	excellent	problematic	none	none
Lenti	untested	untested	problematic	yes	none
ALVAC	low	good	adequate	none	none
MVA	low	good	adequate	none	none
DNA	low	excellent	excellent	none	none

- Pre-existing neutralizing antibodies present in humans reduce immunogenicity (hAd5)
- Human adeno vectors based on rare serotypes are less immunogenic (hAd24, hAd26, hAd35)

The ChAd/MVA **HCV candidate vaccine** induced **T cell response is well above the 'protective threshold'**



* *Spada et al, 2004, Gut; Folgori et al, 2006, Gut*

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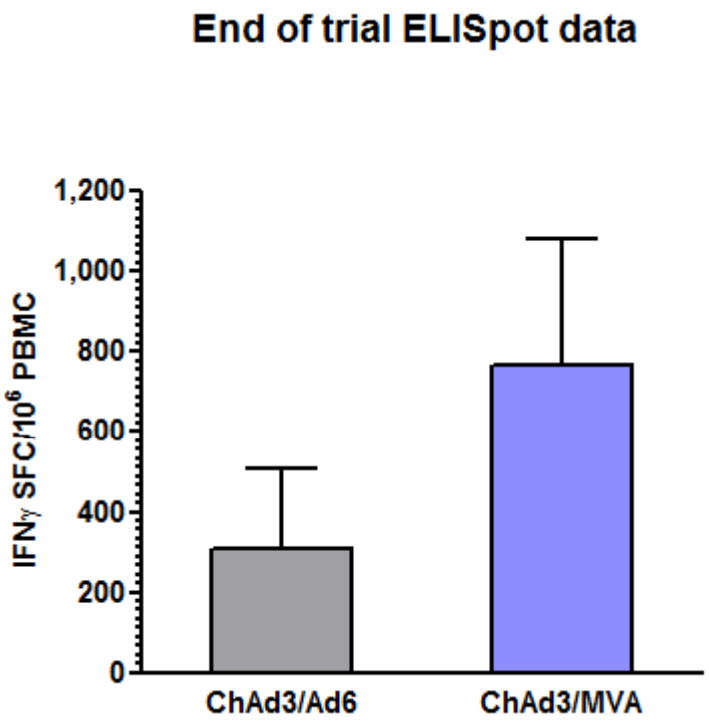
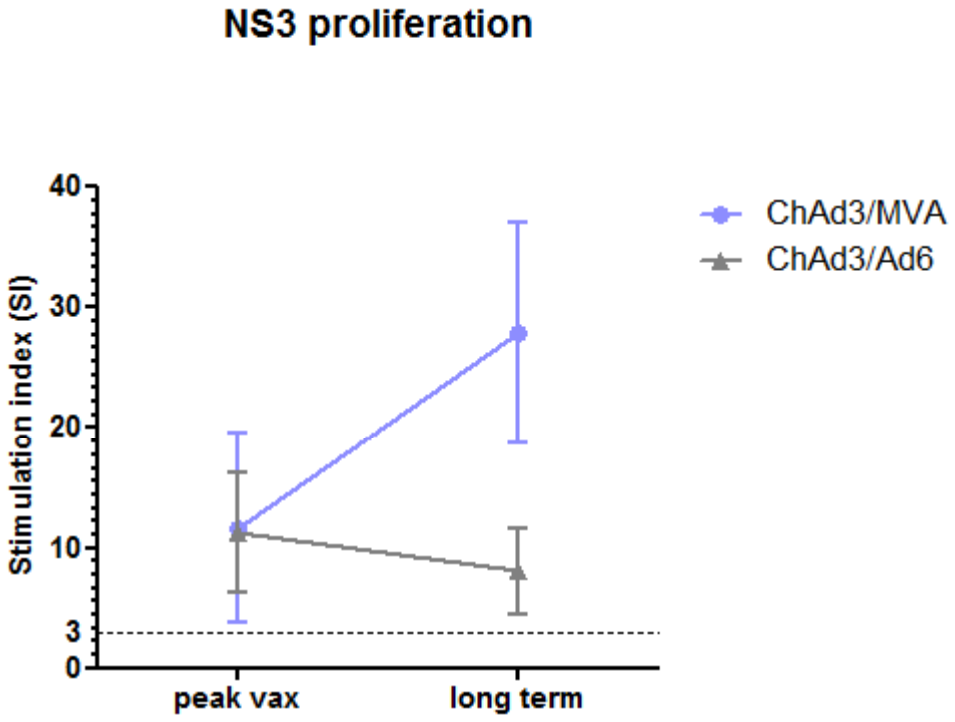
The **ChAd/MVA regimen** induces high levels of **memory response** in humans (data from HCV vaccine program*)





- FDA approved **Phase II efficacy study** in high risk individuals **currently ongoing** in US (collaboration with NIH, J. Hopkins & UCSF)
- First and only prophylactic HCV vaccine in Phase II
- Mechanism based only on T cells

* *Collaboration with P.Klenerman & E.Barnes, U.Oxford*

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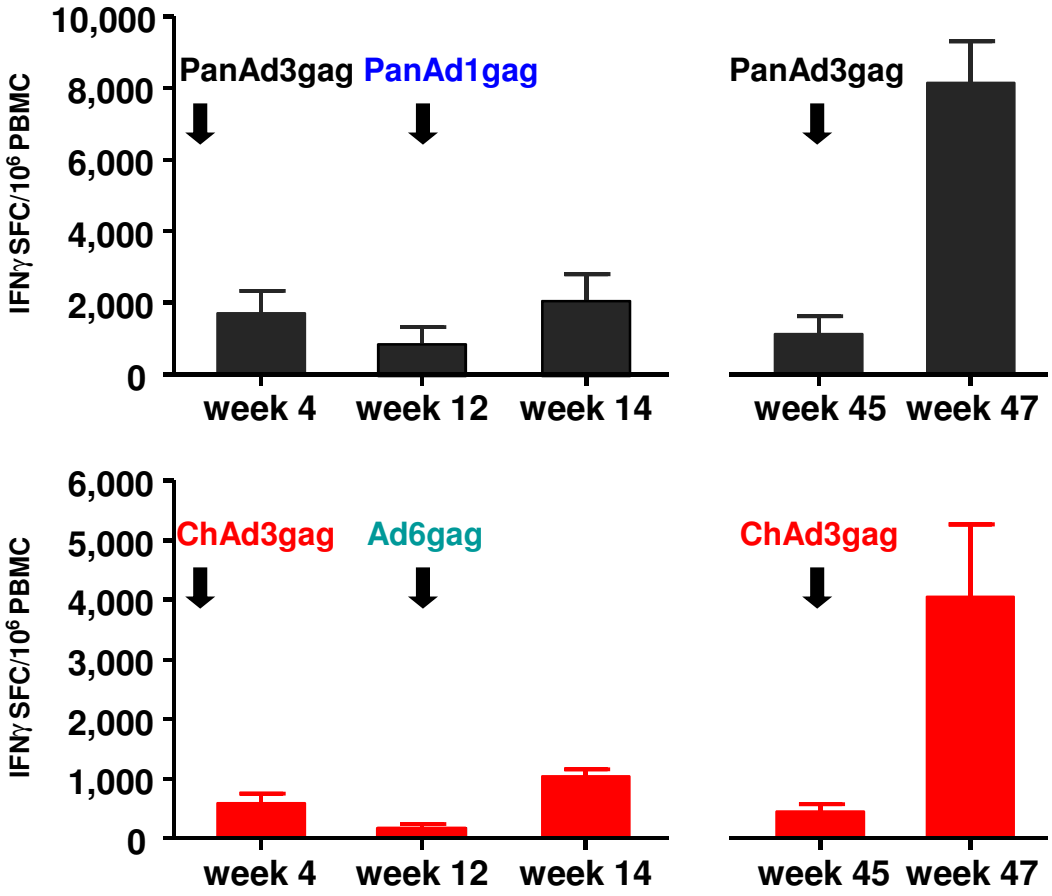
Heterologous prime/boost with different ChAd serotypes improves T-cell response and allows re-administration

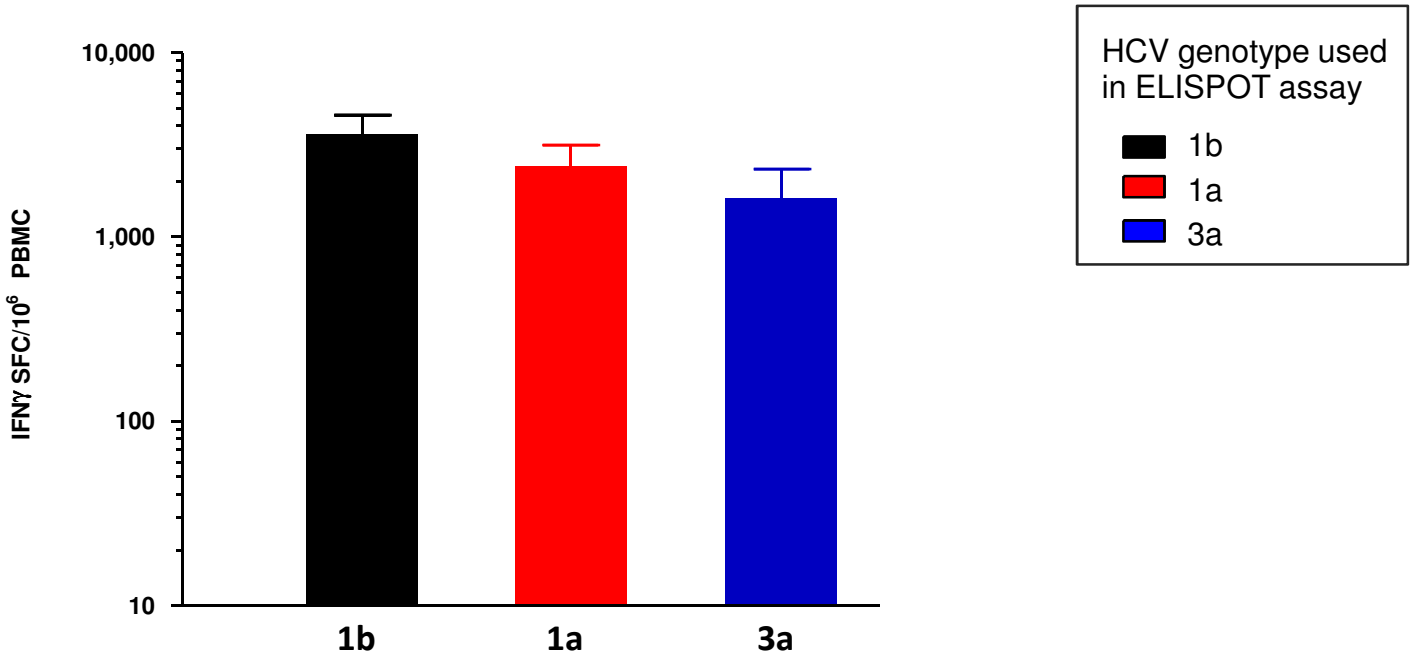
IFNγ ELISpot



- Our data suggest that injection of a different vector after priming (another Adeno or MVA) improves efficacy of re-administration by establishing a larger pool of antigen-specific memory T cells

ChAd/MVA induces a **cross reactive T-cell response** across divergent HCV genotypes (data from HCV vaccine program*)

Prime vector (dose): ChAd3 ($2.5x10^{10}$)
Boost vector (dose): MVA ($2x10^{8}$)
Antigen in vectors: genotype 1b



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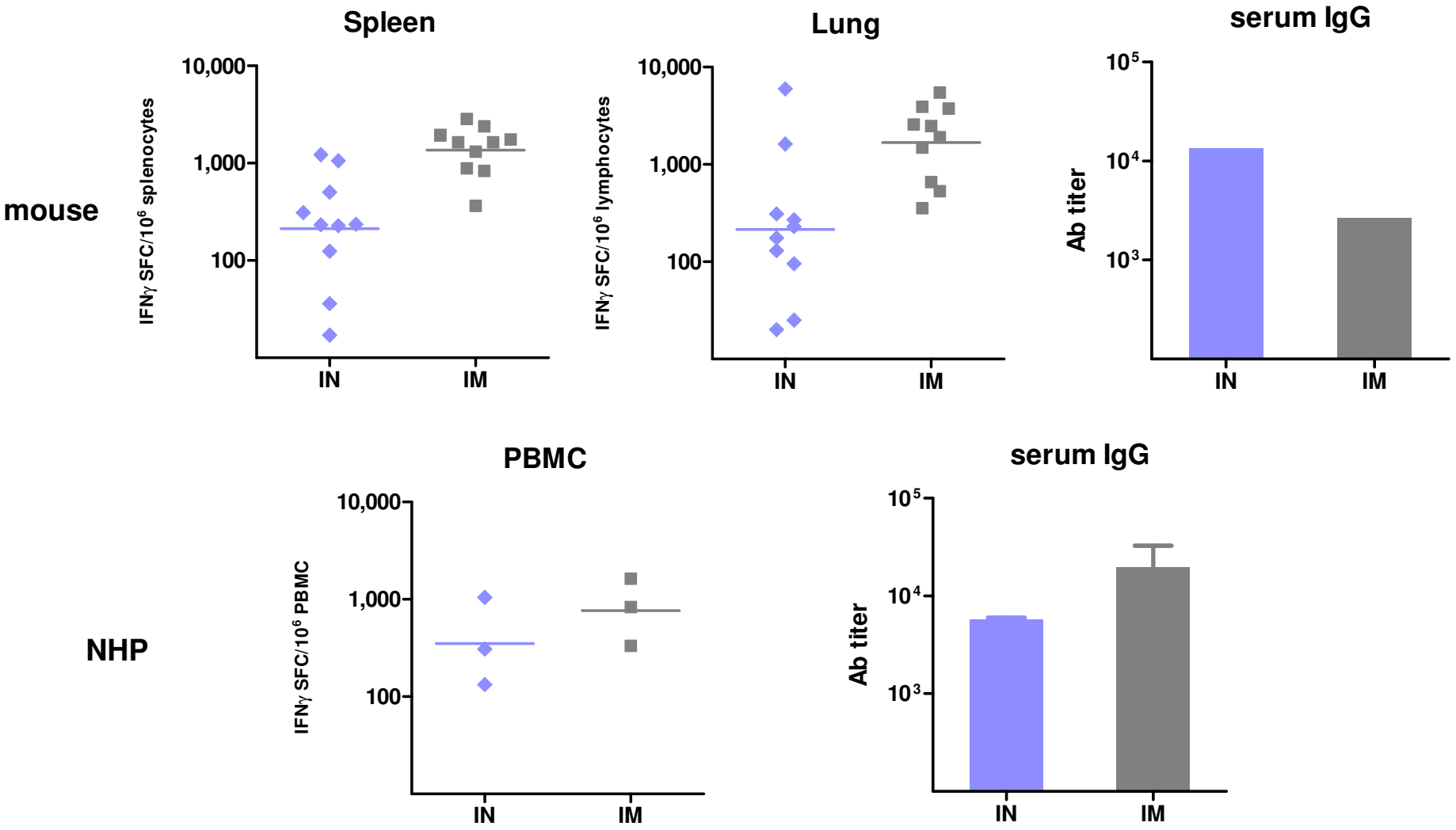
Intranasal (IN) ChAd administration induces both T- and B-cell responses in mice and NHP



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Back up

Vaccination and challenge experiment in calves: **Animals and Challenge virus***

Animals:

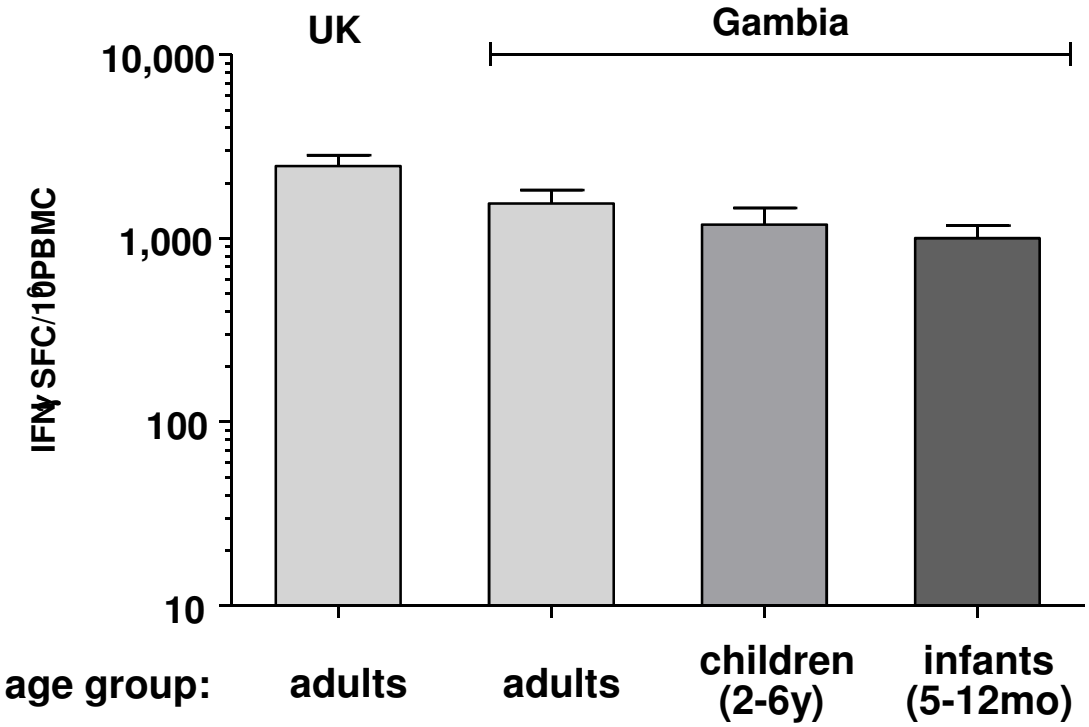
- 4 -6 weeks old calves
- partially colostrum deprived (fed with colostrum only at birth to avoid intestinal infections)
- Absence of B-RSV neutralizing antibodies tested by plaque reduction assay

Challenge:

- Highly virulent BRSV isolate 3761 Snook strain
- Isolated from a nasal swab of a calf with distress respiratory syndrome in 2003. The virus was amplified by 3 passages in newborn calves to generate the BRSV-3761 inoculum
- Animals are infected by Intranasal and intratracheal inoculation of 1 x 10^4 pfu
- All animals exhibit viral replication in the lung and in the nose and develop pulmonary disease

** Collaboration with G. Taylor IAH, UK*

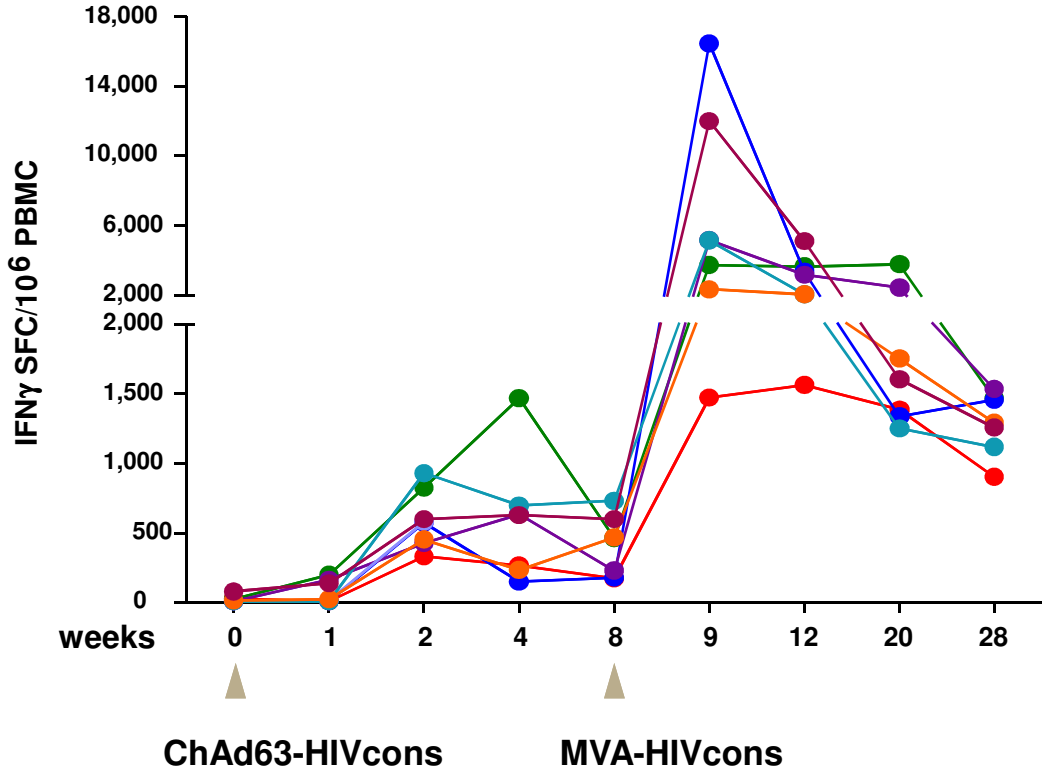
Malaria – ChAd63/MVA METRAP shows equivalent immunogenicity in adults, children and infants



- First evidence of Adeno immunogenicity in infants

ChAd63/MVA HIV-cons induces the highest T cell immunity to date (data from **HIV vaccine program**)

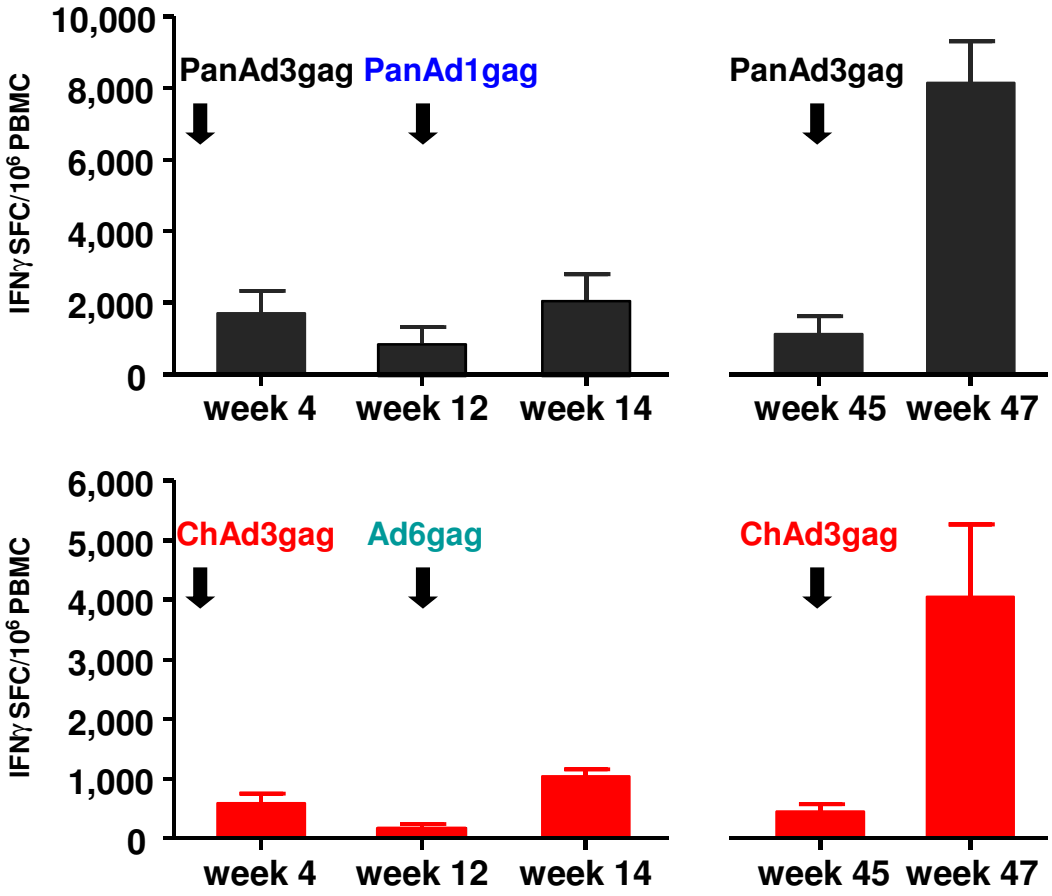
- Data from Phase I trial with vectors encoding the HIV-cons antigen*



* *Collaboration with T.Hanke, U. Oxford*

Heterologous prime/boost with different ChAd serotypes improves T-cell response and allows re-administration

IFNγ ELISpot



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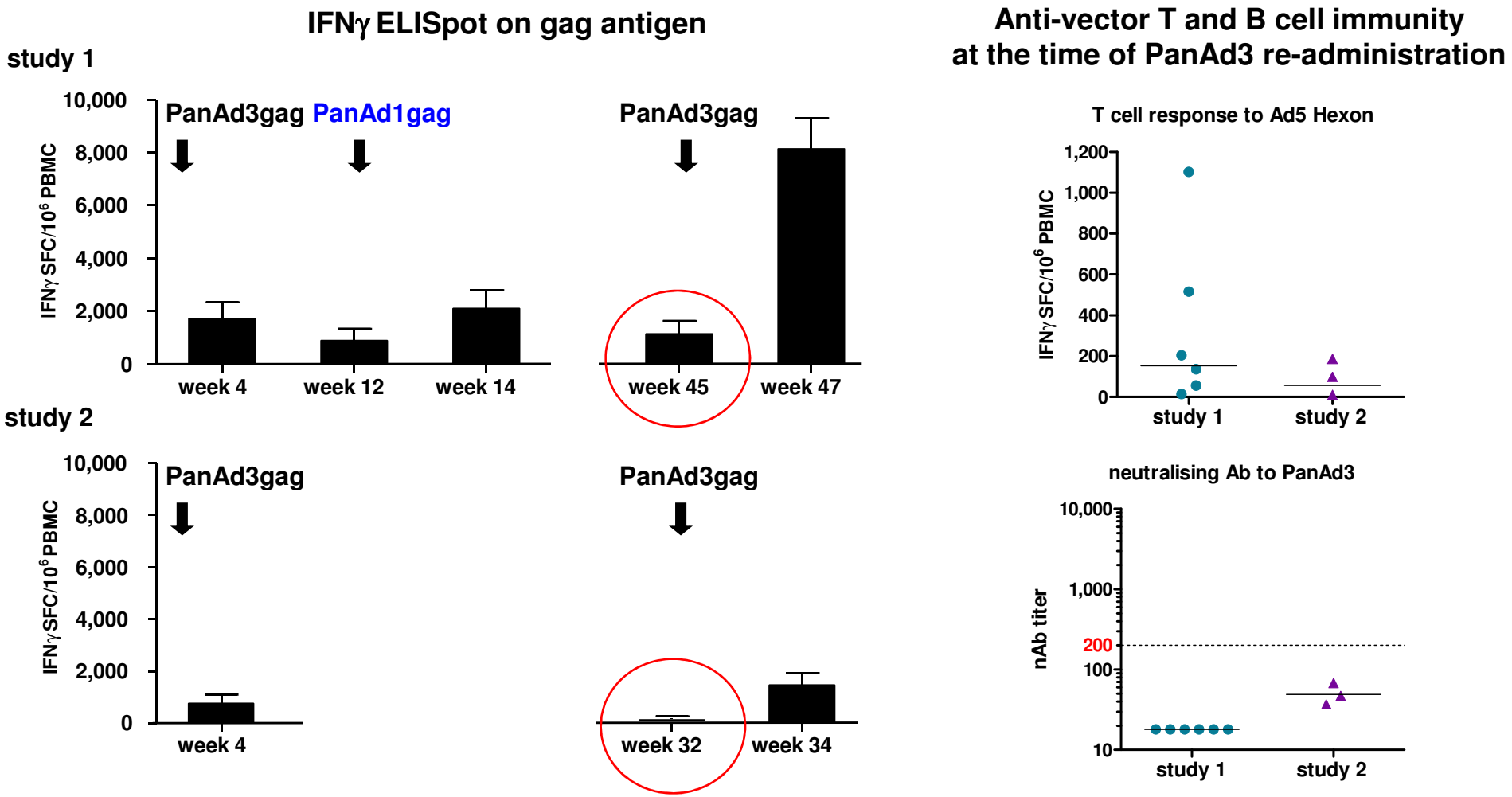

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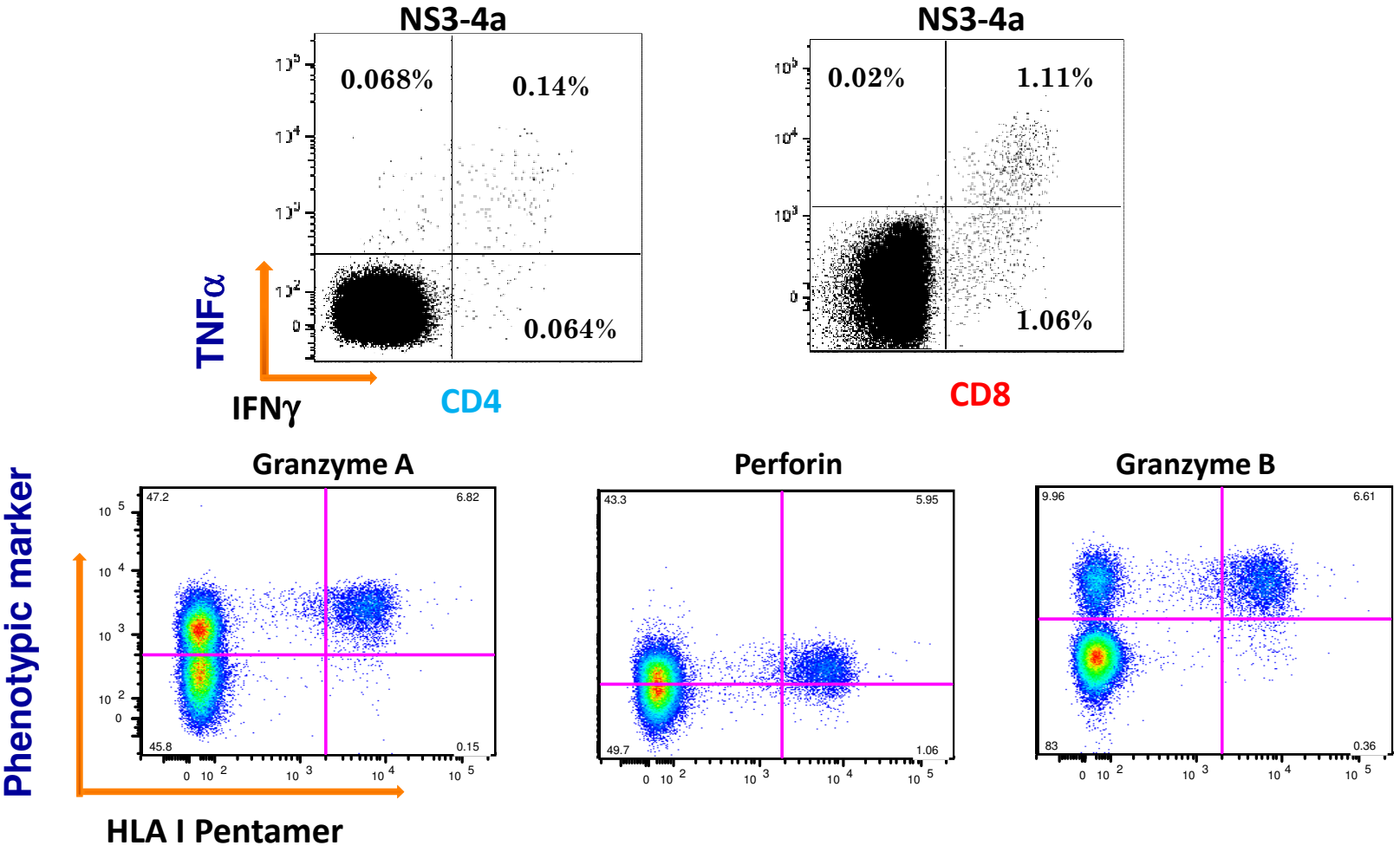
ChAd3 induces **poly-functional T-cells in humans**
(data from **HCV vaccine program**)

- **ICS and Pentamer staining from a representative volunteer 4 weeks post ChAd3 prime***



* *Barnes et al. Science Translational Medicine, 2012*

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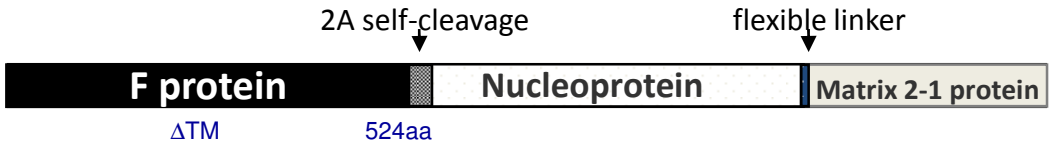
The Okairos RSV vaccine multi-antigen

Vaccine encoded Antigens



Why F, N and M2-1?

- **F** is a known **protective antigen** (The Impact-RSV study group 1998 Pediatrics 102:531)
- It is **highly conserved** between strains from the A and B subgroups
- Contains human **T cell epitopes** (Cherrie AH 1992 J Virol 66:2102)
- **Soluble F** protein induces **higher neutralizing titers** than membrane-bound form (Cseke G 2007 J Virol 81: 698; Kohlmann R 2009 J Virol 83:12601)
- **N and M2-1 are highly conserved** between RSV strains and are known to be a source of **many T-cell epitopes** (Anderson R 2010 Future Microbiol 5:585)
- **Genetic vaccines encoding either F or N or M2-1 each confer some degree of protection in animal models** (reviewed in Graham B 2011 Immunol Reviews 239: 149; Boxus M 2007J Virol 81:6879)