

Rotavirus VP6-specific T-cell responses and cellular immunophenotypes in Zambian infants following two and three doses of oral Rotarix vaccine

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Presentation Outline

Background

- Rotavirus vaccines impact
- Outstanding questions and underexplored research areas
- Study Objectives

Methods

- Study Design
- T-cell assay

Results & Discussion

- T-cell responder frequency
- T-cell response and seroconversion
- Unconventional T-cell phenotypes and seroconversion

Conclusion

Rotavirus diarrhoea burden significantly reduced by LORV introduction

- ❑ Rotaviruses are major causes of diarrhoea mortality in children aged <5 years; an estimated 128,515 children in this population continue to die from rotavirus diarrhoea every year globally.
- ❑ Rotavirus has the highest annual diarrhoea mortality compared to other leading etiologies.
- ❑ Live, attenuated, oral rotavirus vaccines (LORV) have been critical in tackling this significant public health problem since introduction.
- ❑ Between 2006-2019, LORV contributed to ~ median 59% global reduction in rotavirus associated diarrhoea hospitalisations in this population.

Troeger C. *et al.* Lancet Infect Dis, 2018; Kyu, H. *et al* Lancet Infect Dis, 2021; Burnett E. *et al.* J Infect Dis, 2020

Rotavirus Vaccine Impact in Zambia

- Introduction of Rotarix vaccine in 2012 was effective (VE 56%) against severe rotavirus diarrhoea
- Annual rotavirus positivity in children with diarrhoea reduced by 51% in <1 year age group
 - Zambia switched to Rotavac vaccine in 2022

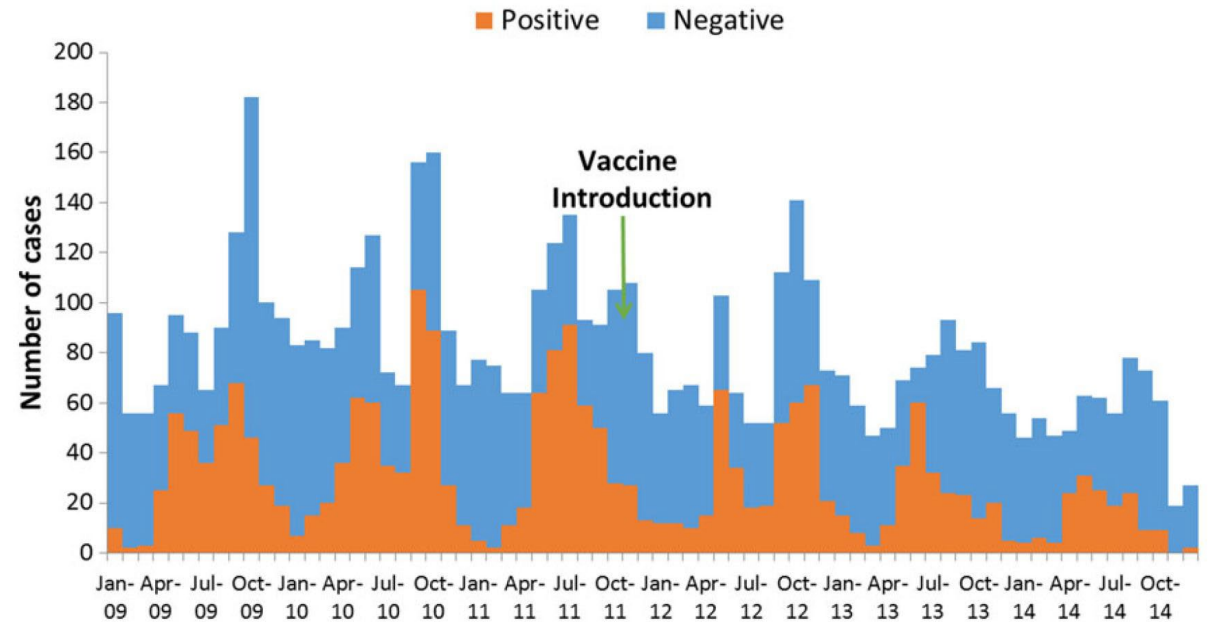


Image from Mpabalwani *et al.* Clin Infect Dis, 2016

Beres LK *et al.* Clin Infect Dis, 2016; Mpabalwani E. *et al.* Clin Infect Dis, 2016; Mpabalwani E. *et al.* Vaccine, 2025

Underexplored Research Area: Rotavirus T-cells

- ❑ Performance of LORV remain suboptimal in low- and middle-income (LMIC) countries and reasons remain incompletely understood.

[Lee B. Hum Vaccin Immunother, 2021](#)

- ❑ Current immunogenicity measures are largely based on rotavirus specific immunoglobulin A (RV-IgA) that explains only a fraction of protective mechanisms.

[Lee B et al. Clin Infect Dis, 2018](#)

- ❑ T-cell immunity induced by LORV is understudied and inconsistency between RV-IgA and clinical efficacy signals investigation of additional immune parameters to better understand protective mechanisms

[Laban N et al. Viruses, 2022;](#)

[Angel J, et al. Hum Vaccin Immunother. 2014](#)



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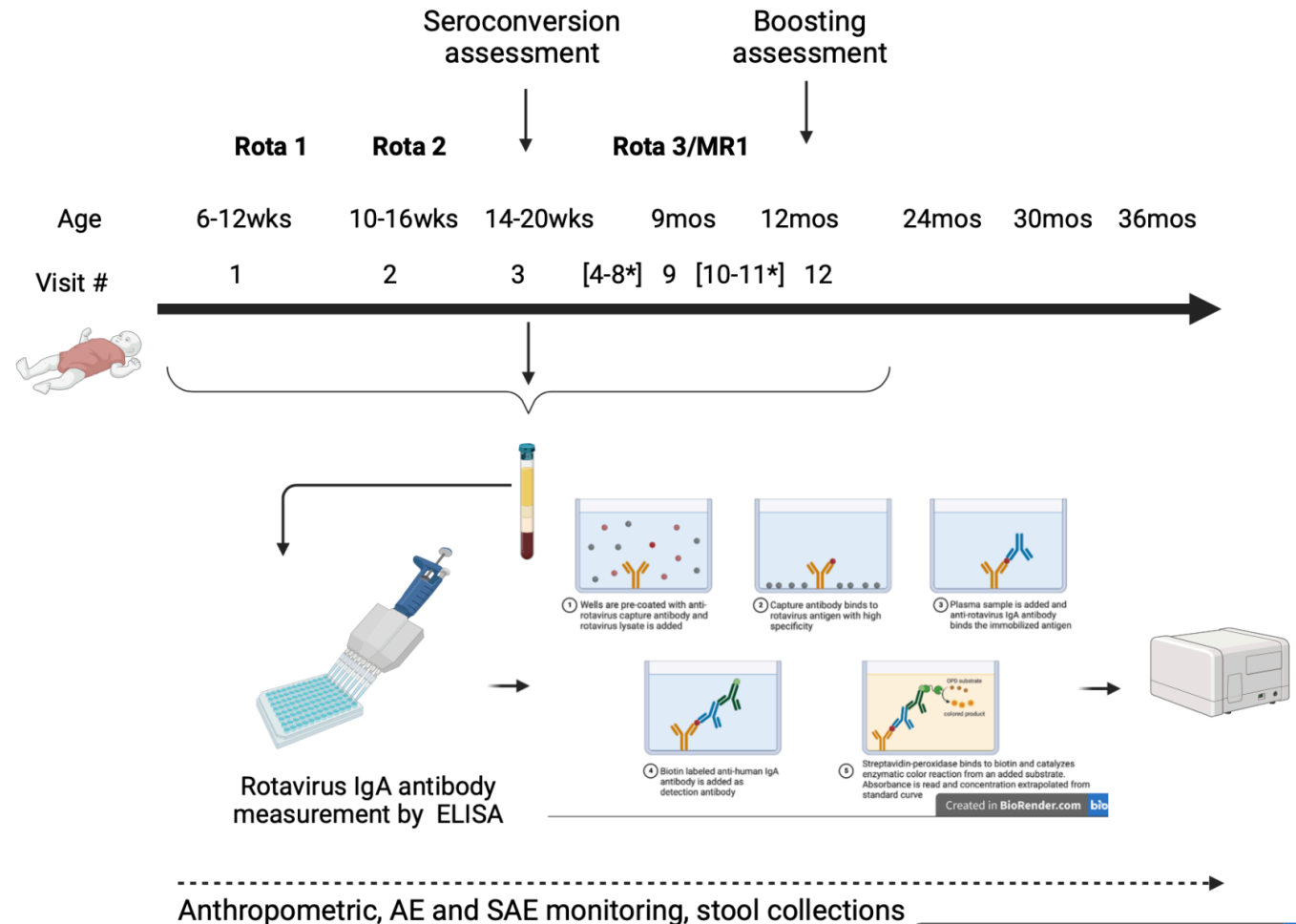
Study Objectives

- ❑ To detect rotavirus specific T-cells in vaccinated children
- ❑ To profile fluctuations of diverse circulating conventional, gut homing and unconventional T-cell phenotypes post-vaccination
- ❑ To explore the relationship between these T-cell responses and RV-IgA seroresponse



Parent Randomised Control Trial

- ❑ Infants enrolled aged 6 to 12 weeks (N=214)
- ❑ RCT aim: to study the RV-IgA boosting by a third dose of Rotarix LORV
- ❑ RCT was conducted in a peri-urban community in Lusaka, Zambia 2018-2023



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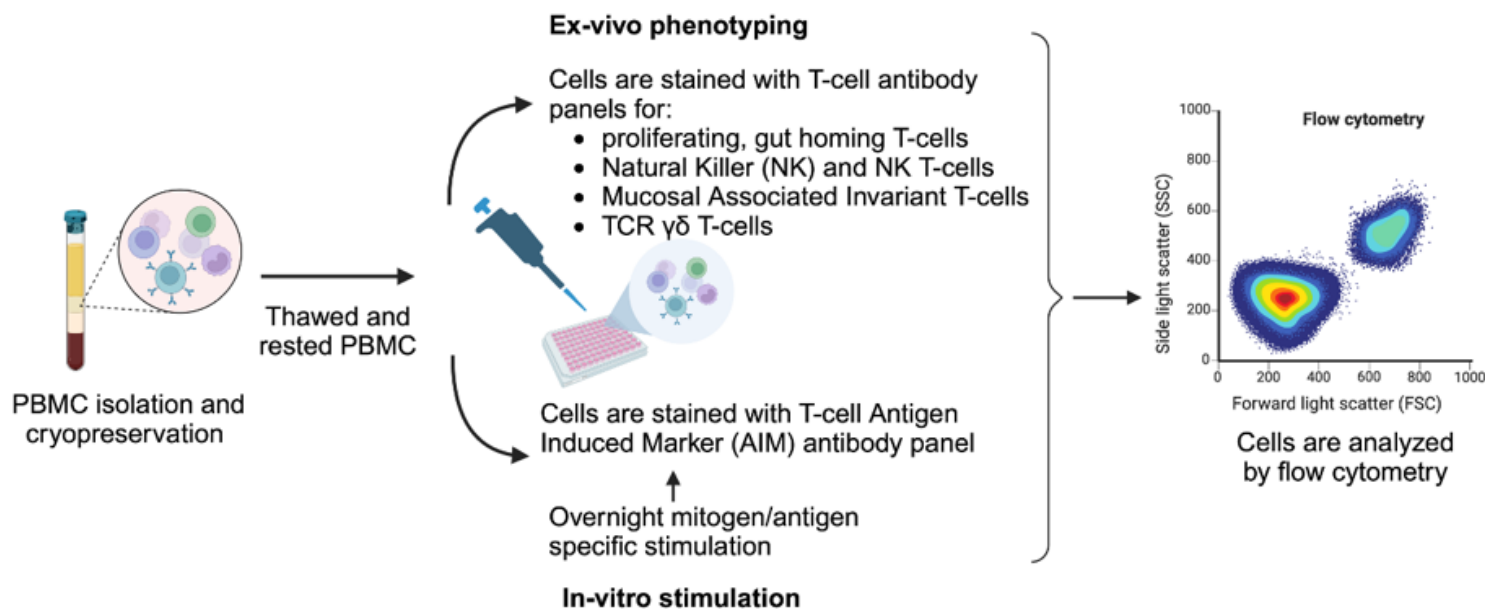


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Nested T-cell study

- PBMC collection and testing nested under the parent RCT (n=36)

T1	T2	T3	T4	T5	T6	T7	T8
Pre-vaccine	7days post dose 1	Pre-dose 2	7days post dose 2	1 month post-dose 2	Pre-dose 3	7days post dose 3	3 months post dose 3
Ex-vivo phenotyping	Ex-vivo phenotyping	Ex-vivo phenotyping	Ex-vivo phenotyping	Ex-vivo phenotyping	Ex-vivo phenotyping	Ex-vivo phenotyping	Ex-vivo phenotyping
In-vitro stimulation				In-vitro stimulation	In-vitro stimulation		In-vitro stimulation

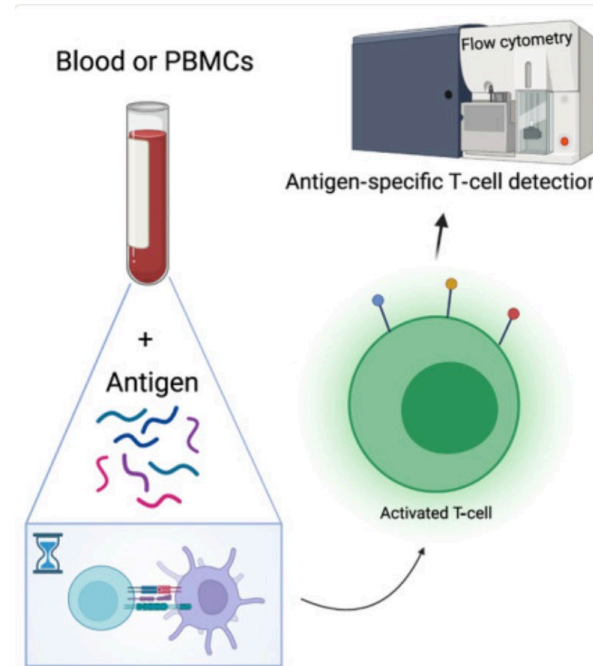


Rotavirus VP6-specific T-cell AIM assay

6-color AIM panel	
CD14	Monocytes
CD19	B-cells
Live/dead	Viability
TCR $\alpha\beta$ +	T-cell receptor
CD4	CD4 subset
CD69	T-cell activation
CD134	
CD137	

Antigen: Rotavirus
VP6 peptide pool

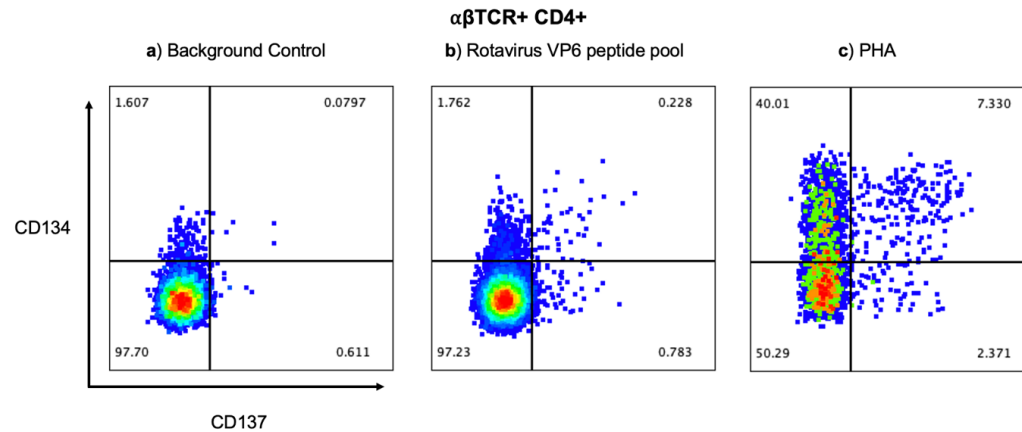
37°C, 5% CO₂
for 20 hours



- **Activated CD8 T-cells:**
TCR $\alpha\beta$ +CD4-(CD8+)
CD69+CD137
- **Activated CD4 T-cells:**
TCR $\alpha\beta$ +CD134+CD137+

- Antigen-specific activated CD4 and CD8 T-cell frequencies were determined for each infant sample after subtraction of the corresponding DMSO background frequency at the specific timepoint.

Few Rotavirus VP6-specific T-cell responders

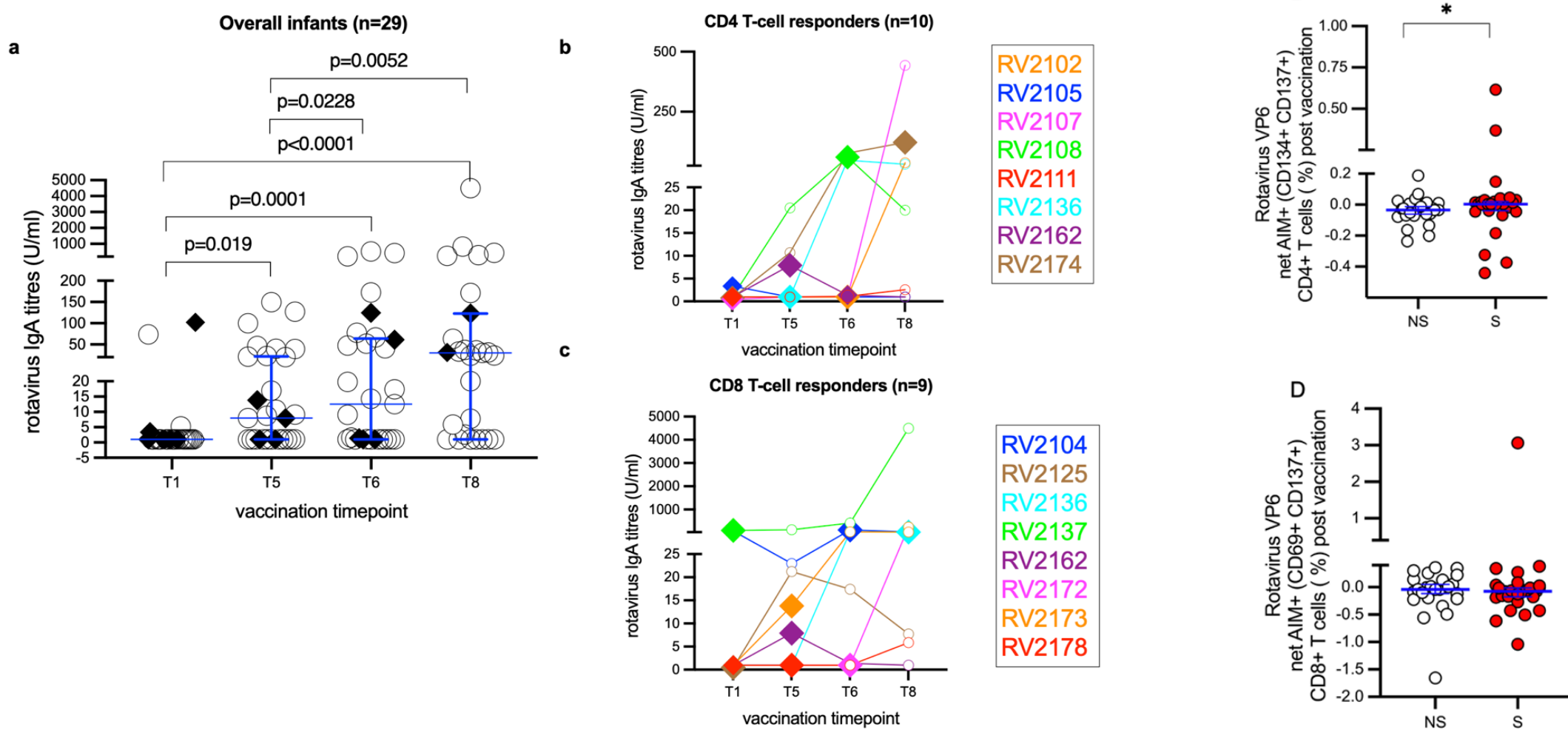


Representative plot for Rotavirus CD4 T-cell responder

Number and proportion of antigen-specific AIM+ CD4+ and CD8+ T-cell responders

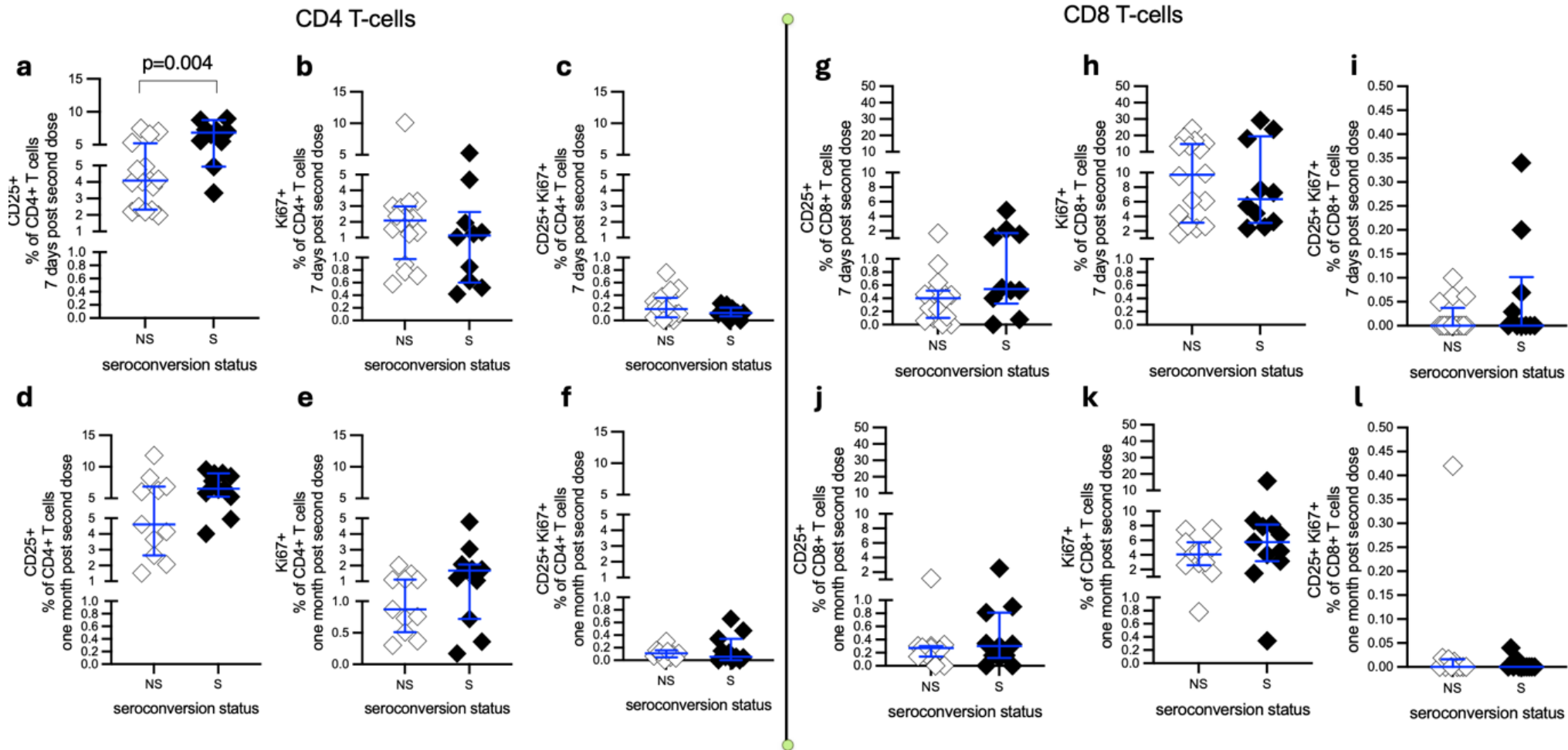
	CD4+CD134+CD137+ T-cell responders n of Total (%)				CD8+CD69+CD137+ T-cell responders n of Total (%)			
	T1	T5	T6	T8	T1	T5	T6	T8
Rotavirus VP6	4/17 (23.5)	2/18 (11.1)	3/23 (13.0)	1/11 (9.1)	3/17 (17.7)	3/18 (16.7)	2/22 (9.1)	1/12 (8.3)
HCMV	1/10 (9.1)	1/16 (6.3)	2/21 (9.5)	0/10 (0)	2/11 (18.2)	2/16 (12.5)	7/20 (35.0)	8/11 (72.7)
PHA	13/14 (92.9)	16/16 (100.0)	16/16 (100.0)	9/9 (100.0)	14/14 (100.0)	16/16 (100.0)	16/16 (100.0)	10/10 (100.0)

Rotavirus VP6-specific T-cells modestly enriched in seroconverters but detected with and without RV-IgA



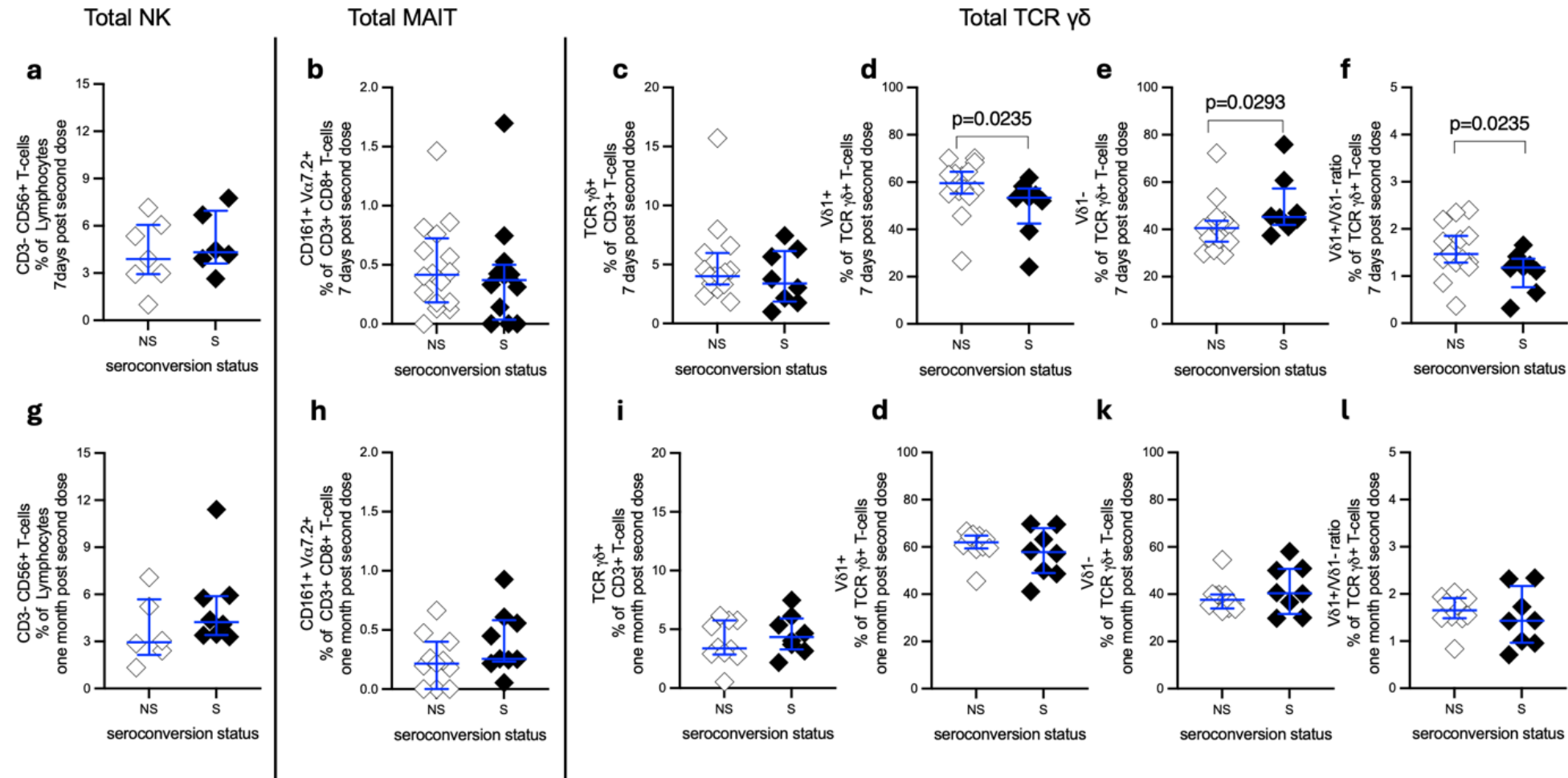
Relationship between rotavirus VP6-specific T-cell responses and rotavirus antibody titres

Higher CD4+CD25+ T-cell frequency in seroconverters



Ex vivo CD4+ and CD8+ T-cell frequencies at one week and one month post two dose vaccination by seroconversion status

Higher V δ 1 $\gamma\delta$ T-cell frequency in non-seroconverters



Ex vivo total NK, MAIT and TCR gamma delta T-cell frequencies one week and one month post two dose vaccination by seroconversion status

Conclusions

- ❑ We demonstrated the utility of the T-cell AIM assay in rotavirus vaccine studies
- ❑ Our findings suggest limited induction of rotavirus VP6-specific T-cell responses following rotavirus vaccination in Zambian children but rarity in circulation may reflect short lived responses or sequestration at local sites.
- ❑ Further characterization of the vaccine induced VP6-specific CD4+ T-cells enriched in seroconverters is warranted.
- ❑ MHC class restriction of the presented VP6 peptides among infants may have contributed to the low number of T-cell responders observed
- ❑ The investigation of rotavirus T-cell responses using a more comprehensive peptide pool covering known immunogenic structural and non-structural viral proteins may have increased the frequency of responding infants

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