PD-1 Occupancy Following Low Dose Nivolumab Treatment: The NIVO-LD Trial

Wallace L[1], Lau JSY[1,2,3], Kaiser K[2], Chang JJ[1], Solomon A[1], King H[1], Scher B[1], Beech P[4], Moore M[4], Tang M[2], Le Couteur J[2], Price DJ[1], Meagher N[1], Rasmussen TA[1,5], McMahon JH[2] & Lewin SR[1,2,3]

1 Department of Infectious Diseases, University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Australia
2 Department of Infectious Diseases, Alfred Health and School of Translational Medicine, Monash University, Melbourne, Australia
3 Victorian Infectious Disease Service, Royal Melbourne Hospital at the Peter Doherty Institute for Infection and Immunity, Melbourne, Australia
4 Department of Radiology, Alfred Health, Melbourne, Australia
5 Aarhus University Hospital, Aarhus, Denmark.

For further information, contact james.mcmahon@monash.edu or sharon.lewin@unimelb.edu.au

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

We thank all study participants for generously donating these samples & their time to make this research possible.

Background

Effective antiretroviral therapy (ART) can achieve long-lasting viral suppression in people living with HIV (PLWH), but the persistence of latently infected cells acts as a barrier to achieving cure, necessitating lifelong treatment.

T cell exhaustion

Chronic exposure to HIV antigens can contribute to T cell exhaustion, characterised by reduced effector function and upregulation of immune checkpoint molecules, such as programmed death-1 (PD-1).

PD-1 acts to inhibit T cell activation, proliferation and cytokine production through interactions with its ligands (PD-L1/PD-L2).

PD-1 in HIV

Higher PD-1 expression in PLWH has been associated with increased viral loads and lower CD4 counts.(1,2) In PLWH on ART, PD-1+ memory T cells have higher levels of replication-competent and intact HIV than PD-1- memory T cells.(3)

Nivolumab

Nivolumab is an antibody which binds to PD-1 with high affinity and can reduce PD-1-mediated inhibition of T cell responses. Nivolumab treatment in PLWH may help reverse T-cell exhaustion and **promote anti-HIV immunity.**

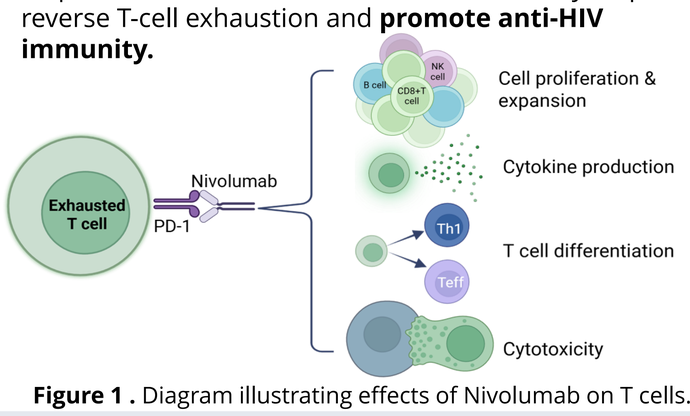


Figure 1 . Diagram illustrating effects of Nivolumab on T cells.

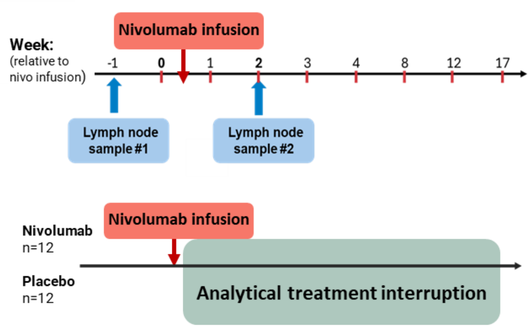
Study Design & Outcomes

Cohort A:

Nivolumab Dosage:
- **0.1mg/kg** (n=6)
- **0.3mg/kg** (n=6)
- **1mg/kg** (n=6)

Cohort B:

Nivolumab Dosage:
One dose selected based on safety & occupancy data from Cohort A



Primary endpoints:
- Safety
- PD-1 Occupancy

| = blood collection

Recruitment completed

Secondary endpoints:
- Safety
- Immunogenicity

Commencing late-2025

Figure 2 . NIVO-LD study design.

Methods

Lymph node occupancy: Fine needle aspirates were collected from a groin lymph node **at baseline and 2 weeks** post-nivolumab infusion**.** Single cell suspensions of fresh cells were then examined for PD1 occupancy using flow cytometry.
= Occupancy in lymph node and whole blood 2 weeks following Nivolumab infusion.

Peripheral blood mononuclear cell (PBMC) occupancy: PBMCs were isolated from whole blood collected at study intervals between **baseline and 17 weeks post-infusion**. Samples for all visits for each participant were frozen and then thawed at the same time and examined for PD1 occupancy using flow cytometry.
= Duration of occupancy in PBMCs

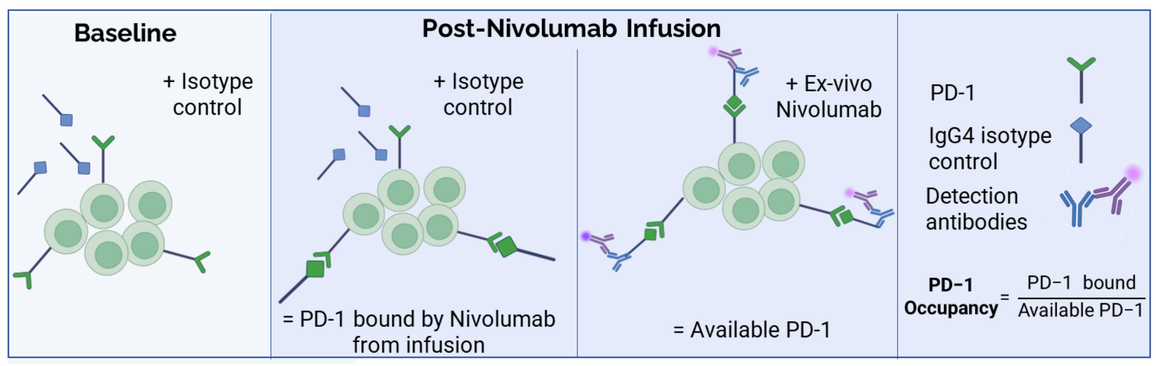


Figure 3. Schematic of PD-1 occupancy assay design.

Participant Characteristics

Recruitment criteria: virologically suppressed PLWH on ART

Table 1. Characteristics of study participants. IQR= interquartile range.

	0.1mg/kg n=6		0.3mg/kg n=6		1mg/kg n=6		Total n=18	
Characteristic	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Gender								
Male	**5**	83%	**6**	100%	6	100%	17	94%
Female	1	17%	0	0%	0	0%	1	6%
HIV subtype								
B	2	33%	2	33%	4	67%	8	44%
C	1	17%	1	17%	0	0%	2	11%
AE	0	0%	0	0%	2	33%	2	11%
AG	0	0%	1	17%	0	0%	1	6%
Unknown	3	50%	2	33%	0	0%	5	28%
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**
Age	50	35-55	50	32-55	38	38-60	49	36-55
CD4 cells/µl	734	(637–956)	797	(762–946)	614	(566–658)	716	(622-879)
Years since HIV diagnosis	13	(12–20)	18	(10–22)	17	(13–31)	14	(12-22)

References

1. Trautmann 2006., Nat. Med 2.Fromentin 2019, Nat Comms 3.D'Souza 2007., J Immunol

Results 1. Nivolumab treatment increases PD-1 occupancy in the lymph node

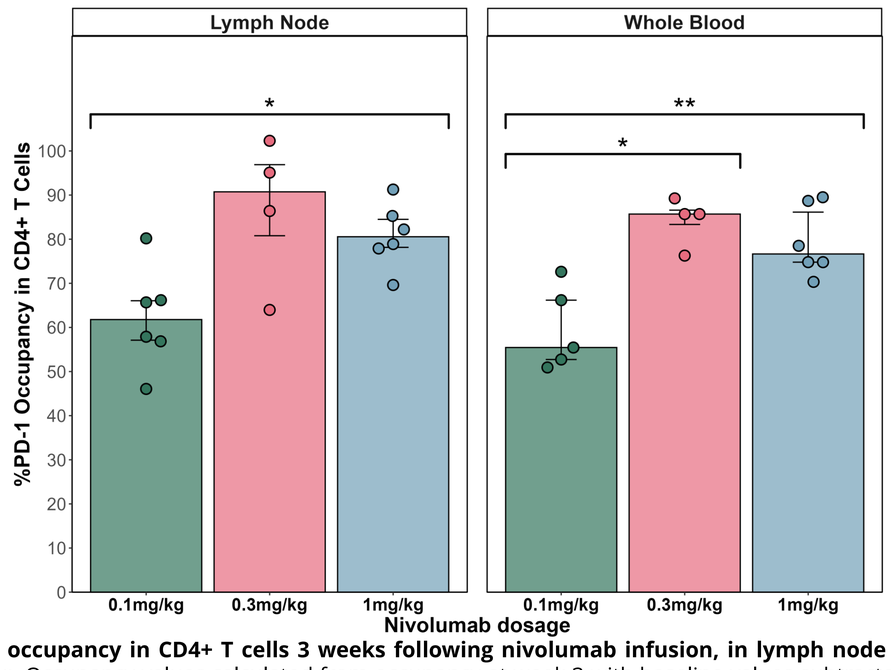


Figure 4.PD-1 occupancy in CD4+ T cells 3 weeks following nivolumab infusion, in lymph node and whole blood samples. Occupancy values calculated from occupancy at week 3 with baseline values subtracted. Columns represent median for each dosage group with error bars representing interquartile range. **=p<0.01 and *=p<0.05 from Wilcoxon rank-sum test.

2. Higher dosage nivolumab treatment results in prolonged PD-1 occupancy

Following 1mg/kg Nivolumab, **PD-1 occupancy remaining elevated above baseline at 17 weeks** (mean occupancy at 17 W=21% in CD4+ T cells) and returned to baseline by week 23.

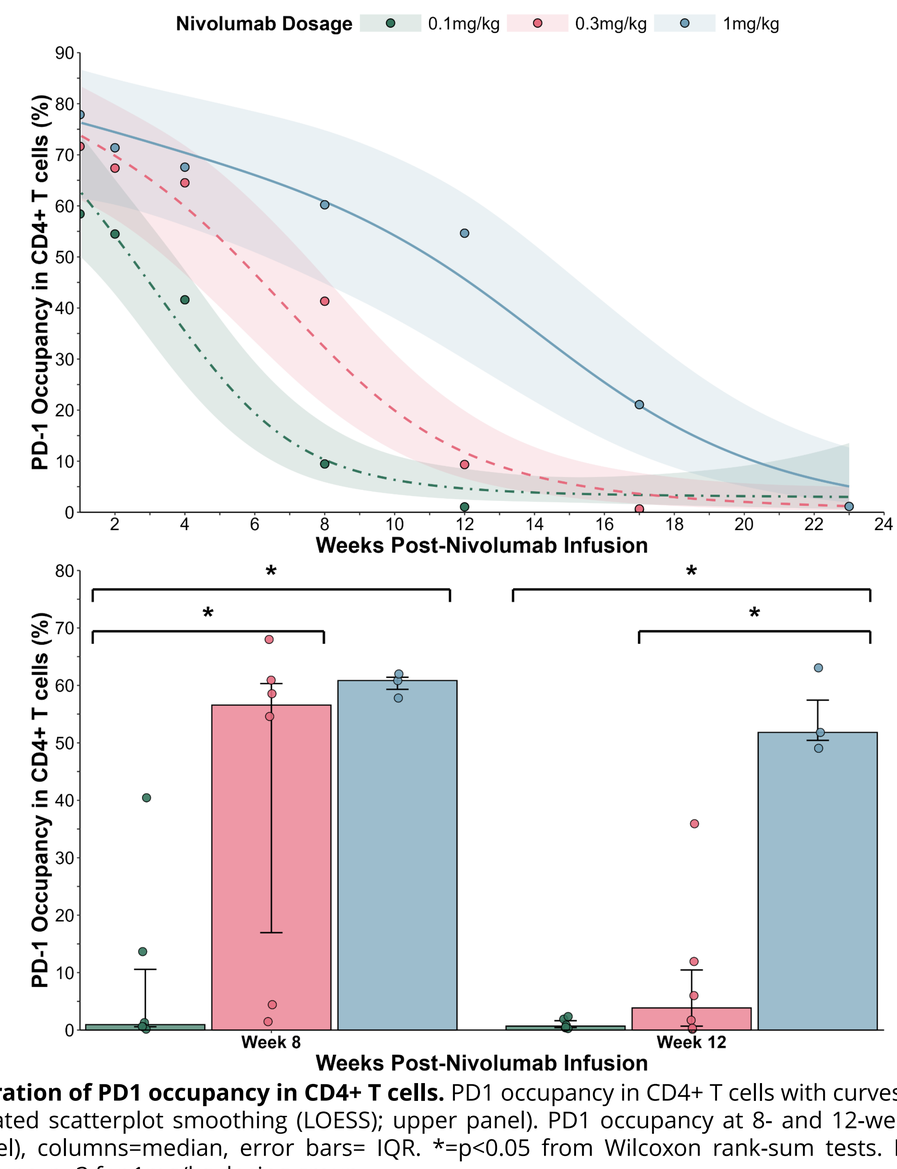


Figure 5. Duration of PD1 occupancy in CD4+ T cells. PD1 occupancy in CD4+ T cells with curves generated using locally estimated scatterplot smoothing (LOESS); upper panel). PD1 occupancy at 8- and 12-weeks post-infusion (bottom panel), columns=median, error bars= IQR. *=p<0.05 from Wilcoxon rank-sum tests. N=6 for 0.1- and 0.3mg/kg groups, n=3 for 1mg/kg dosing group.

Conclusions

All doses of Nivolumab resulted in increased PD-1 occupancy in study participants, with lymph node occupancy comparable to levels in whole blood.

Nivolumab at 0.3- and 1mg/kg resulted in greater occupancy than 0.1mg/kg in lymph node and whole blood. No significant difference was observed between 0.3- and 1mg/kg at this timepoint. In PBMCs, the duration of PD-1 occupancy increased with Nivolumab dosage, with both 0.3- and 1mg/kg resulting in prolonged occupancy compared to 0.1mg/kg dosing.

Implications: A single low-dose nivolumab in PLWH on ART leads to high and prolonged PD-1 occupancy

Future investigations::
- Assess changes in reservoir size, HIV transcriptional activity and HIV-specific T-cell function
- Enrolment in Cohort B which includes an analytical treatment interruption to commence shortly in Melbourne & Singapore.