Clinical Studies with Immunotherapies: The Case of Immune Checkpoint Blockers

Thomas A Rasmussen
Hot Topics in HIV
Barcelona, 24 October 2024

AARHUS UNIVERSITY

Central Denmark Region
Aarhus University Hospital

The role of immunotherapies in cure strategies

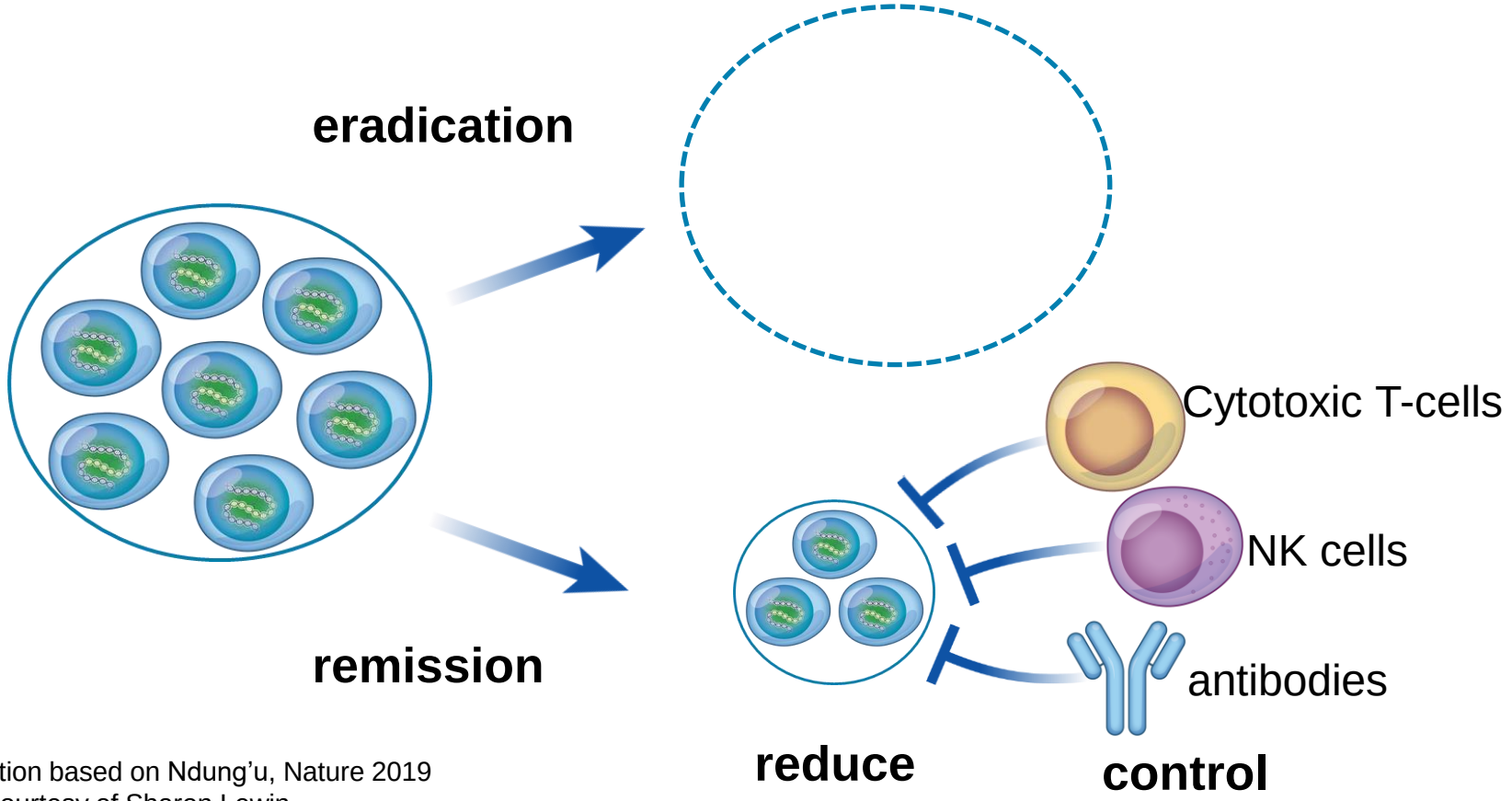
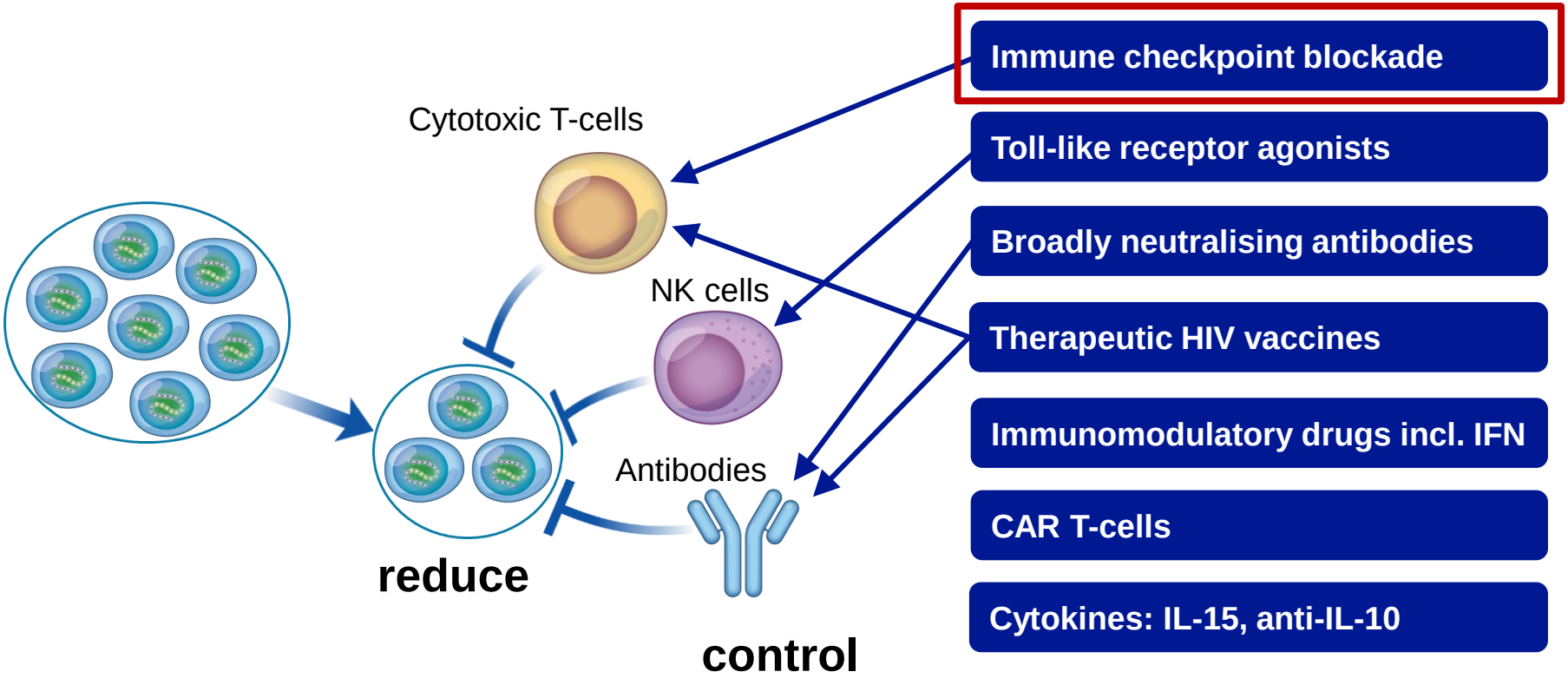
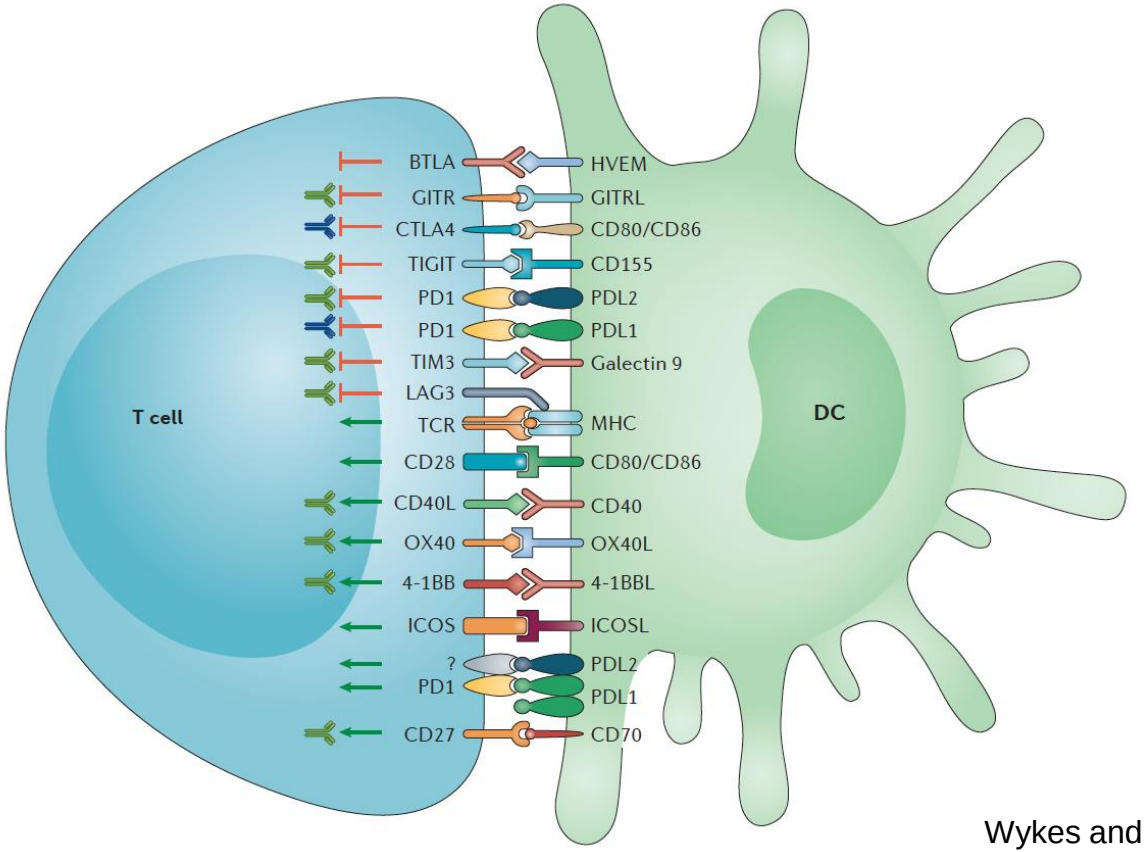


Illustration based on Ndung'u, Nature 2019
Slide courtesy of Sharon Lewin

Immunotherapies under investigation for HIV cure



Immune checkpoints modify T cell function



- Multiple interactions between APCs and T cells following the initial MHC/antigen to TCR binding
- Co-stimulatory signals may be inhibitory or stimulatory
- Modify the quality and duration of effector response

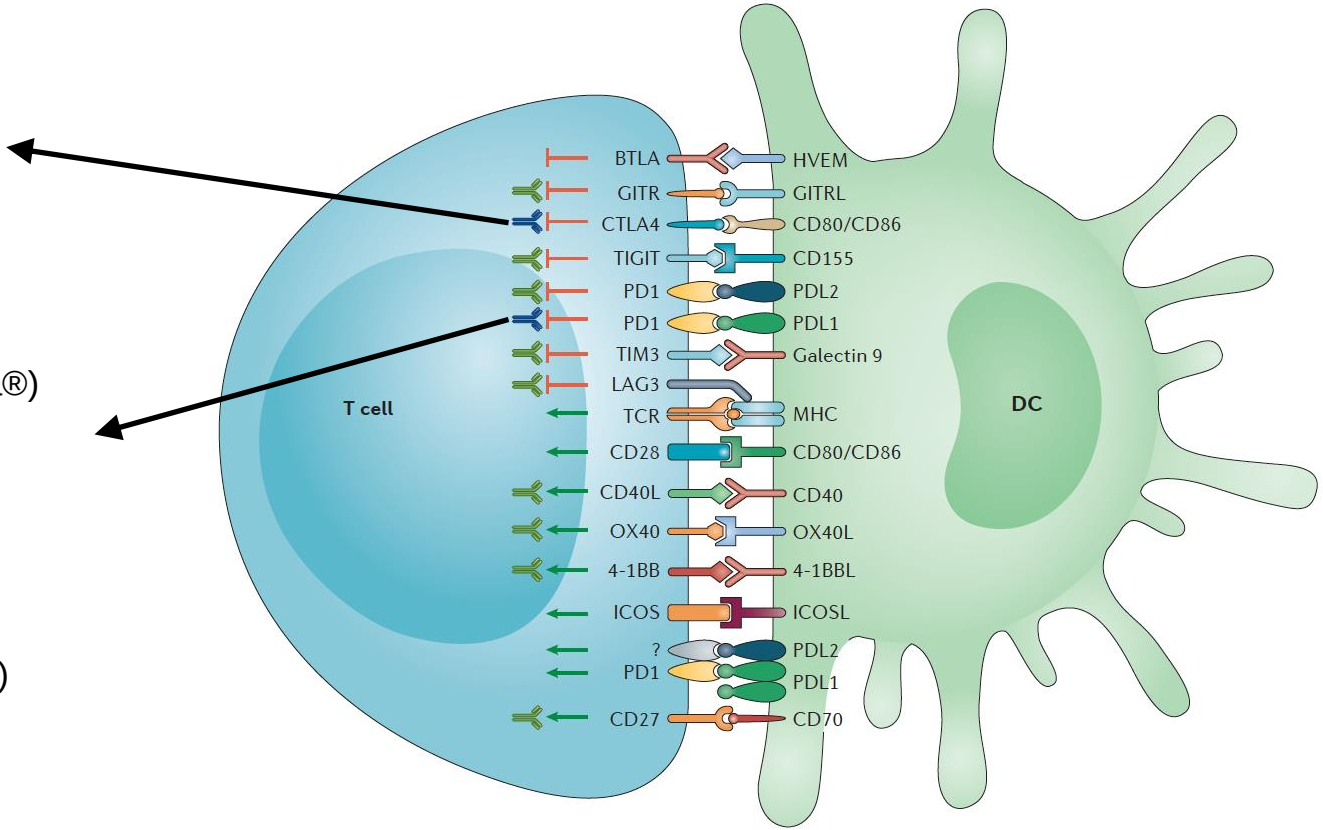
Wykes and Lewin, Nature Reviews Immunology 2018

Licensed immune checkpoints inhibitors include antibodies that block CTLA4 and the PD1/PD-L1 axis

Anti-CTLA4
Ipilimumab (Yervoy®)
Tremelimumab (Imjudo®)

Anti-PD1
Nivolumab (Opdivo®)
Pembrolizumab (Keytruda®)
Cemiplimab (Libtayo®)
Retifanlimab (Zynyz)
Dostarlimab (Jemperli®)
Toripalimab (Loqtorzi®)

Anti-PDL1
Atezolizumab (Tecentriq®)
Avelumab (Bavencio®)
Durvalumab (Imfinzi®)



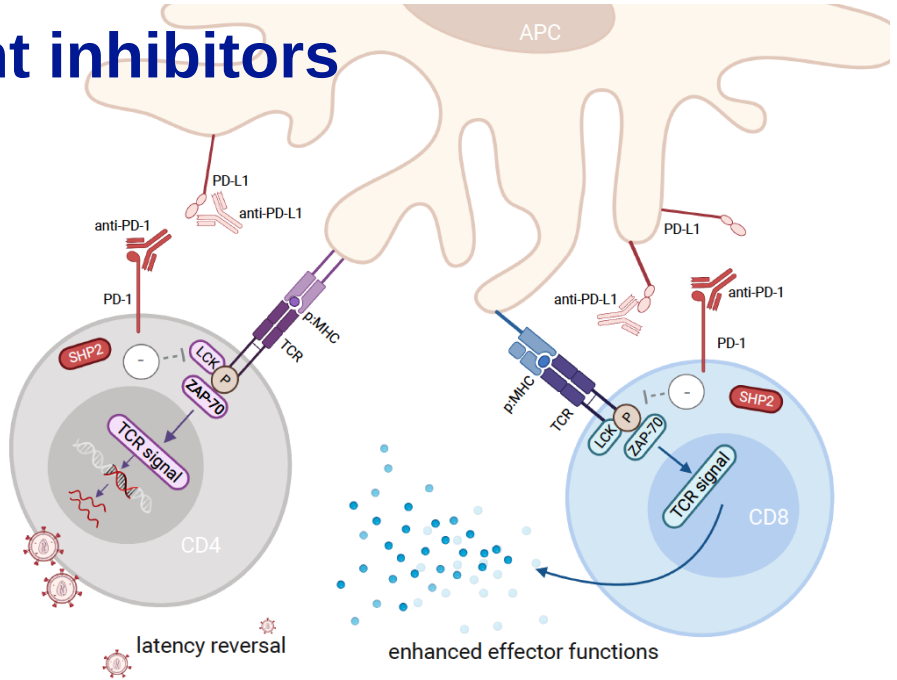
Wykes and Lewin, Nature Reviews Immunology 2018

Dual role of immune checkpoints in HIV persistence

- Inhibitory signalling leads to exhaustion of HIV-specific T-cells
- CD4+ T cells expressing immune checkpoints enriched for HIV

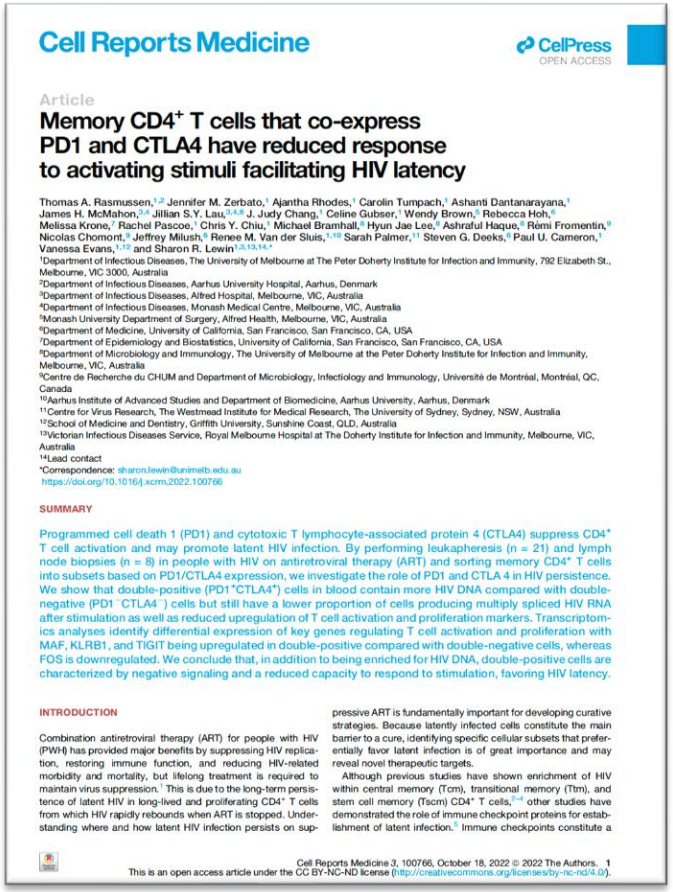
Dual role of immune checkpoint inhibitors in targeting HIV persistence

- Augment HIV-specific T cell function
- Activate HIV from latency



Trautmann Nat Med 2006, Kaufman Nat Immun 2009, Chomont Nat Med 2009, Banga Nat Med 2015, Fromentin Plos Path 2016, Chew Plos Path 2016, Fromentin Nat Comm 2019, McGary Immunity 2017, Harper Nat Med 2020, Rasmussen CID 2020, Uldrick Science TM 2022, Rasmussen CRM 2022

The role of PD-1 and CTLA-4 for HIV Persistence in LN and blood



Cell Reports Medicine

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Article

Memory CD4⁺ T cells that co-express PD1 and CTLA4 have reduced response to activating stimuli facilitating HIV latency

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https://doi.org/10.1016/j.xcrm.2022.100766

SUMMARY

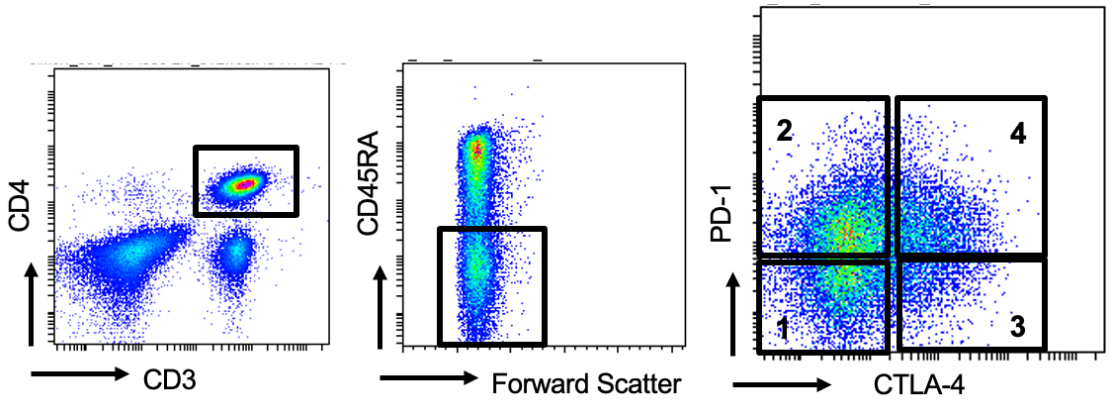
Programmed cell death 1 (PD1) and cytotoxic T lymphocyte-associated protein 4 (CTLA4) suppress CD4⁺ T cell activation and may promote latent HIV infection. By performing leukapheresis (n = 21) and lymph node biopsies (n = 8) in people with HIV on antiretroviral therapy (ART) and sorting memory CD4⁺ T cells into subsets based on PD1/CTLA4 expression, we investigate the role of PD1 and CTLA 4 in HIV persistence. We show that double-positive (PD1⁺CTLA4⁺) cells in blood contain more HIV DNA compared with double-negative (PD1⁻CTLA4⁻) cells but still have a lower proportion of cells producing multiply spliced HIV RNA after stimulation as well as reduced upregulation of T cell activation and proliferation markers. Transcriptomics analyses identify differential expression of key genes regulating T cell activation and proliferation with MAF, KLRB1, and TIGIT being upregulated in double-positive compared with double-negative cells, whereas FOS is downregulated. We conclude that, in addition to being enriched for HIV DNA, double-positive cells are characterized by negative signaling and a reduced capacity to respond to stimulation, favoring HIV latency.

INTRODUCTION

Combination antiretroviral therapy (ART) for people with HIV (PWH) has provided major benefits by suppressing HIV replication, restoring immune function, and reducing HIV-related morbidity and mortality, but lifelong treatment is required to maintain virus suppression.[1] This is due to the long-term persistence of latent HIV in long-lived and proliferating CD4⁺ T cells from which HIV rapidly rebounds when ART is stopped. Understanding where and how latent HIV infection persists on suppressive ART is fundamentally important for developing curative strategies. Because latently infected cells constitute the main barrier to a cure, identifying specific cellular subsets that preferentially favor latent infection is of great importance and may reveal novel therapeutic targets.

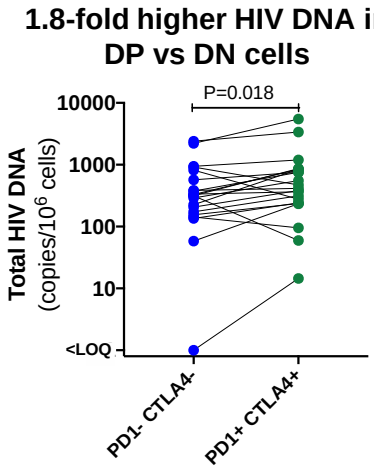
Although previous studies have shown enrichment of HIV within central memory (Tcm), transitional memory (Ttm), and stem cell memory (Tscm) CD4⁺ T cells,[2–4] other studies have demonstrated the role of immune checkpoint proteins for establishment of latent infection.[5] Immune checkpoints constitute a

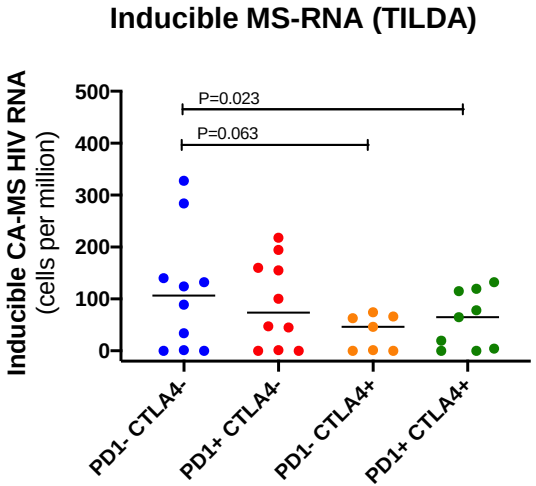
- Leukapheresis (n=21) and LN biopsies (n=8) in PWH on ART
- Memory (CD45RA-) CD4⁺ T cells sorted based on expression of PD1 and/or CTLA4

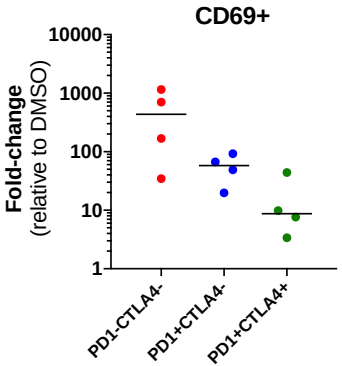


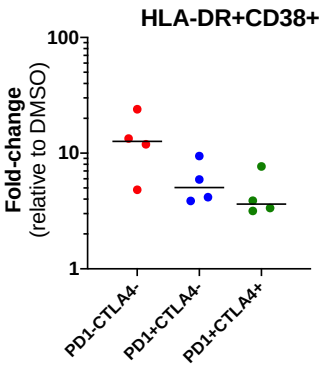
- Within cell subsets we quantified measures of HIV persistence
  - Cell-associated HIV-DNA
  - Unspliced and multiply-spliced HIV RNA
  - Tat/rev Induced Limiting Dilution Assay (TILDA)

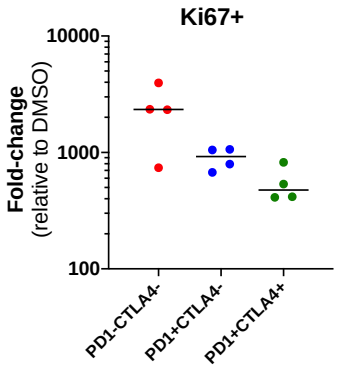
Despite being enriched for HIV DNA, fewer double-positive cells produced MS RNA upon PMA/ionomycin stimulation









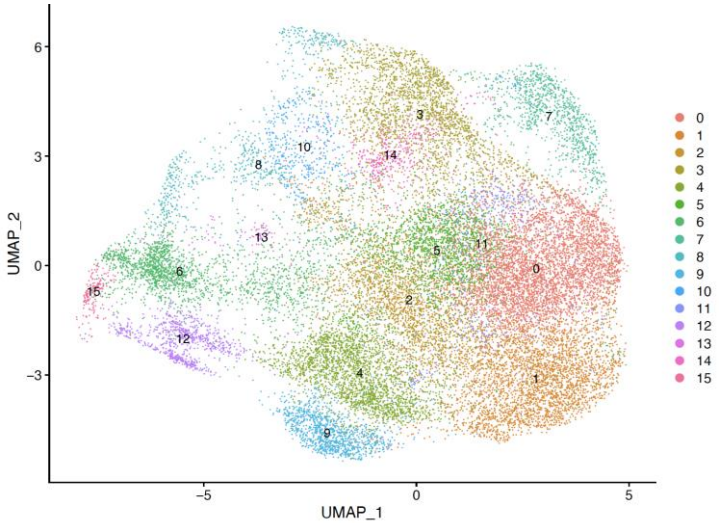


- Cells co-expressing PD1 and CTLA4 are less inducible due to their negative signaling?
- Reduced rates of T cell activation and proliferation in PD1+CTLA4+ cells following PMA/ionomycin for 72h

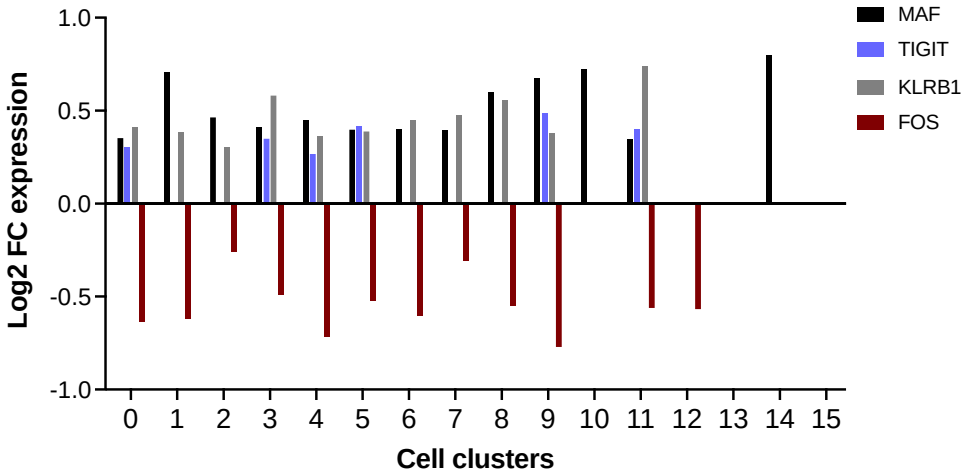
Rasmussen, Cell Reports Medicine 2022

Single-cell RNA seq of double-positive versus double-negative cells

16 distinct cell clusters identified



Top genes with differential expression involved in regulating T cell activation, exhaustion and apoptosis[1-6]



Study conclusion:
In addition to being enriched for HIV-DNA, double-positive cells are characterised by reduced capacity to respond to stimulation, thereby favouring latent HIV infection

1) Yukawa JEM 2020; 2) Peng Eur J Immunol 2007; 3) Huen Int J Cancer 2013; 4) Truong Nat Comm 2019; 5) Chew Plos Path 2016; 6) Fromentin Plos Path 2016

Given the role of PD-1 and CTLA-4 for HIV persistence and T cell exhaustion, can therapeutic blockade of PD-1 and/or CTLA-4

1. **Reverse HIV latency?**
2. **Enhance HIV-specific T cell function?**

Immune checkpoint blockade in people with HIV and cancer

Clinical Infectious Diseases

MAJOR ARTICLE

Impact of A
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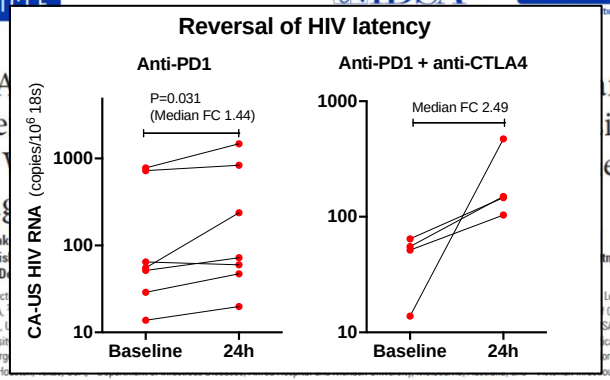

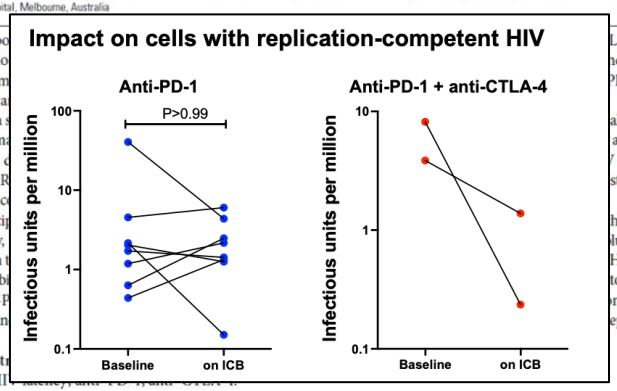

Background. Antibo
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Methods. This was a s
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Conclusions. Anti–P
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Clinical Trials Regist

Keywords. HIV; HI

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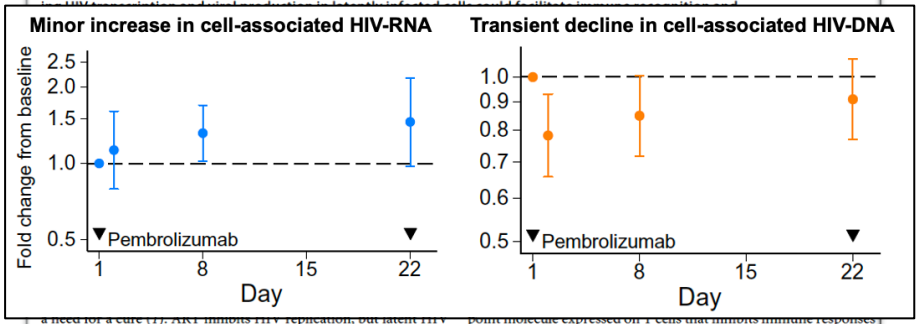
SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

HIV

Pembrolizumab induces HIV latency reversal in people living with HIV and cancer on antiretroviral therapy

Thomas S. Uldrick[1,2,3]*, Scott V. Adams[1], Remi Fromentin[4], Michael Roche[5,6], Steven P. Fling[1], Priscila H. Gonçalves[3], Kathryn Lurain[3], Ramya Ramaswami[3], Chia-ching Jackie Wang[7], Robert J. Gorelick[8], Jorden L. Welker[8], Liz O'Donoghue[1], Harleen Choudhary[1], Jeffrey D. Lifson[8], Thomas A. Rasmussen[6,9], Ajantha Rhodes[6], Carolin Tumpach[6], Robert Yarchoan[3], Frank Maldarelli[3], Martin A. Cheever[1]†, Rafick Sékaly[10], Nicolas Chomont[4], Steven G. Deeks[7], Sharon R. Lewin[6,11,12]*

In people living with HIV (PLWH) on antiretroviral therapy (ART), virus persists in a latent form where there is minimal transcription or protein expression. Latently infected cells are a major barrier to curing HIV. Increas-



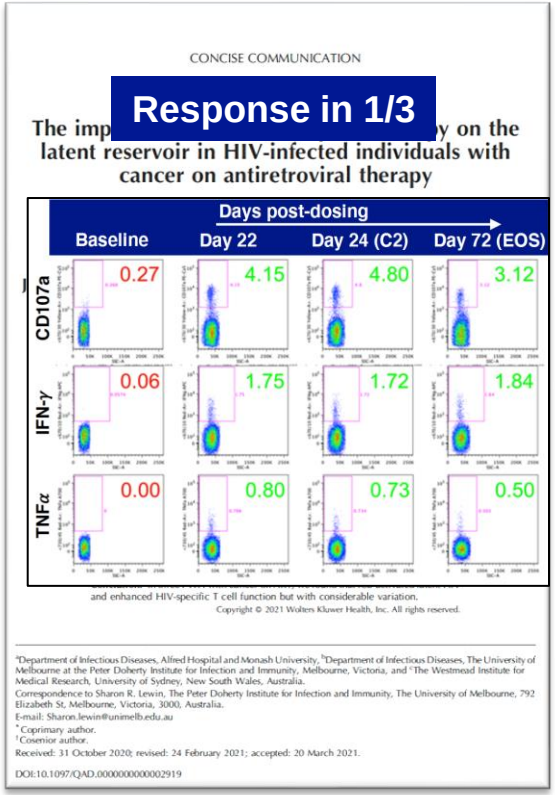
persists as an integrated genome in long-lived CD4$^+$ T cells capable of proliferation with minimal or no antigen expression (*2*). Latency reversal is a proposed approach to induce expression of HIV antigens or virions to allow for immune recognition and elimination of infected cells (reviewed by Zerbato *et al.*) (*3*). Together with enhanced immune clearance, this approach could potentially eliminate cells that contain replication-competent HIV (*4*). Latency-reversing agents evaluated to date in PLWH on ART, including histone deacetylase inhibitors and toll-like receptor agonists, have not consistently demonstrated latency reversal, and no intervention other than allogeneic stem cell transplant has yet shown a sustained

point molecule expressed on T cells that inhibits immune responses against cancers and viral infections. Monoclonal antibodies targeting PD-1 or its ligand, PD-L1, are approved to treat a growing number of cancers, including several HIV-associated malignancies (*6*). PD-1 is up-regulated on CD4$^+$ and CD8$^+$ T cells in PLWH on and off ART (*7*) and, along with other immune checkpoints, is preferentially expressed on latently infected cells in blood and tissue (*8*–*12*). Ex vivo, engagement of PD-1 by its ligand PD-L1 inhibits T cell receptor–mediated activation, allowing for the establishment of HIV latency (*12*). In vitro, anti–PD-1 antibodies enhanced viral production from infected cells when used in combination with a submaximal activating stimulus (*10*, *13*).

Can immune checkpoint blockade enhance HIV-specific T cell function?

Anti-PD-1/anti-CTLA-4

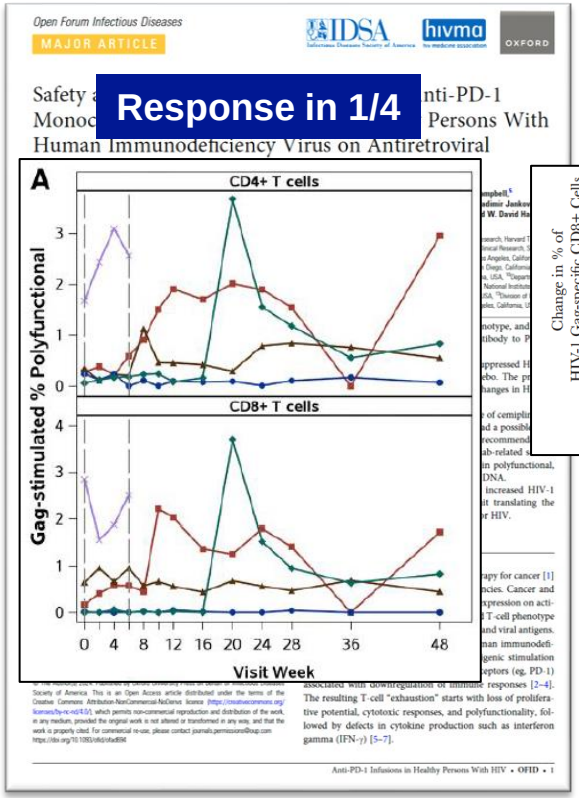
Response in 1/3



Lau, AIDS 2021

Anti-PD-1 (0.3 mg/kg)

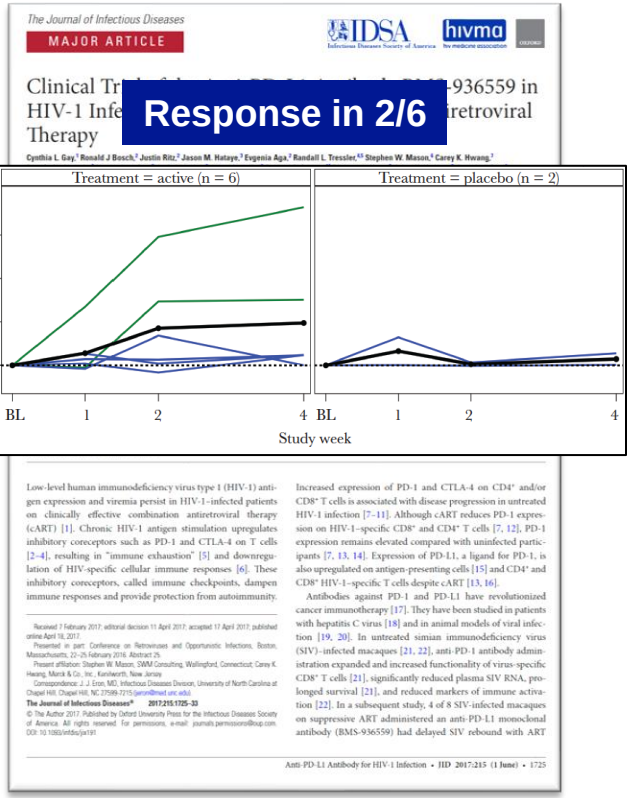
Response in 1/4



Gay, OFID 2024

Anti-PD-L1 (0.3 mg/kg)

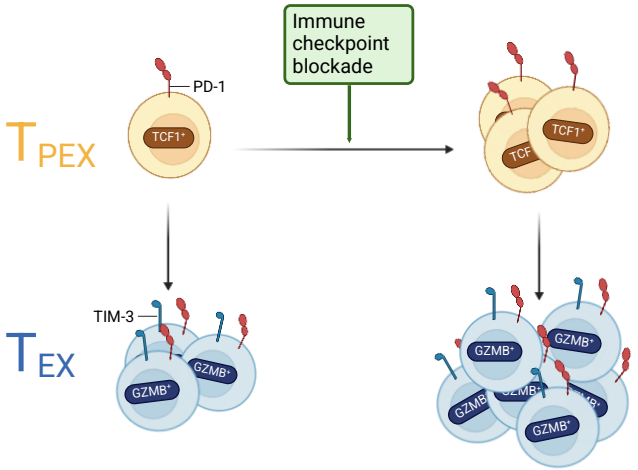
Response in 2/6



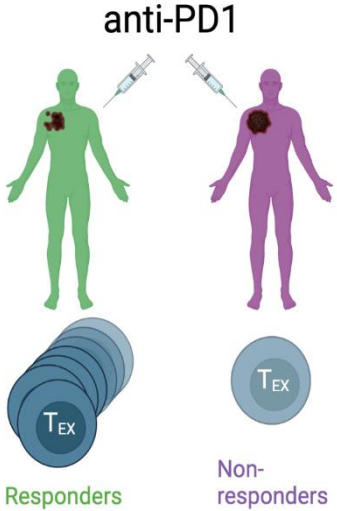
Gay, JID 2017

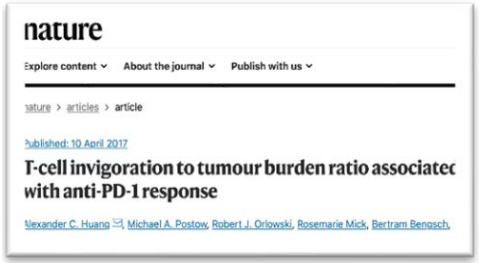
Immune predictors of a successful response to anti-PD-1

TCF-1 expressing precursor exhausted T cells (T_{PEX}) are responsible for the reinvigorated CD8 T cell response in cancer



T_{PEX} display self-renewing capacity and give rise to expansion of granzymeB-expressing exhausted CD8 T cells (T_{EX}) following PD-1 blockade

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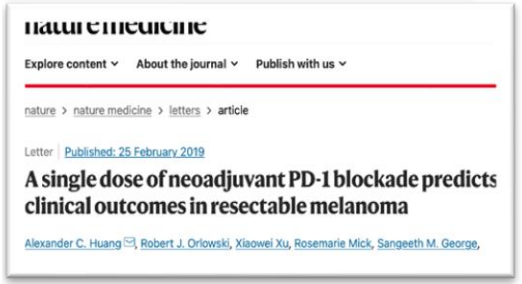
Published: 10 April 2017

T-cell invigoration to tumour burden ratio associated with anti-PD-1 response

Alexander C. Huang, Michael A. Postow, Robert J. Orlowski, Rosemarie Mick, Bertram Bengsch,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Letter | Published: 25 February 2019

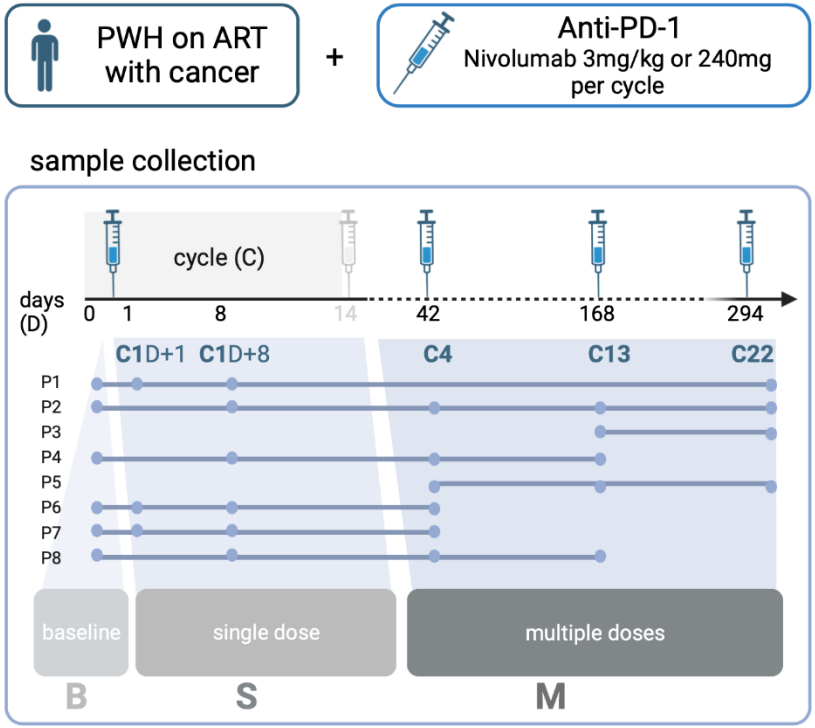
A single dose of neoadjuvant PD-1 blockade predicts clinical outcomes in resectable melanoma

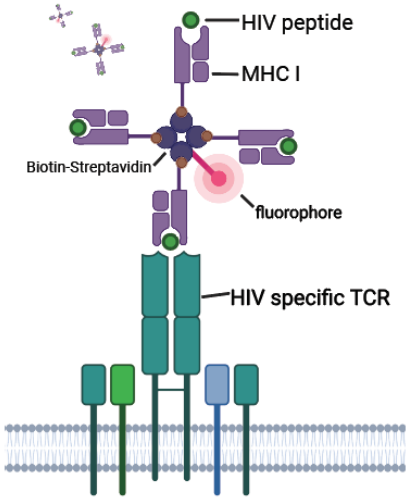
Alexander C. Huang, Robert J. Orlowski, Xiaowei Xu, Rosemarie Mick, Sangeeth M. George,

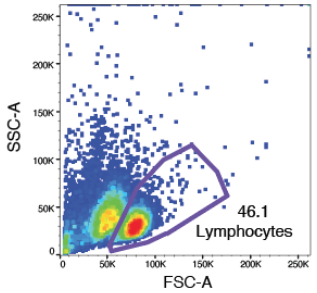
Kallies A. et al. Nat Rev Immunol 2019, Huang A C. et al. Nature 2017, Huang A C. Nat Med. 2019.

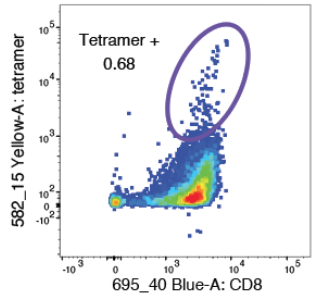
Understanding the HIV-specific response to anti-PD-1

Celine Gubser





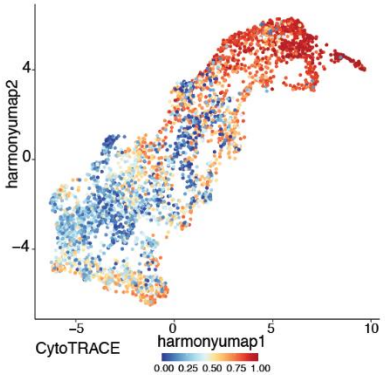


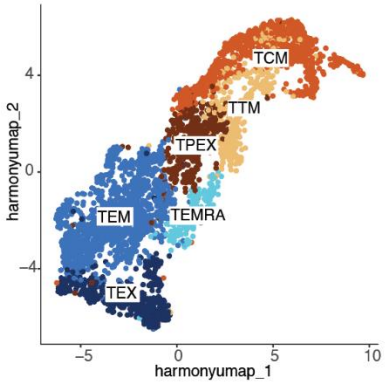


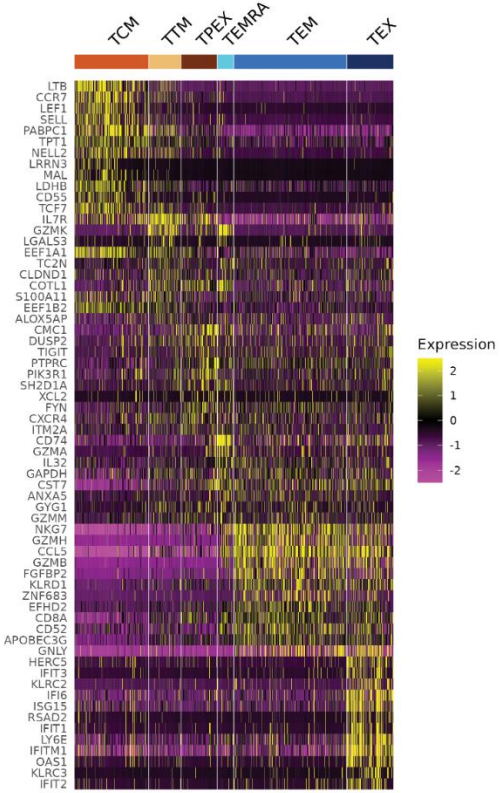
Gubser, submitted

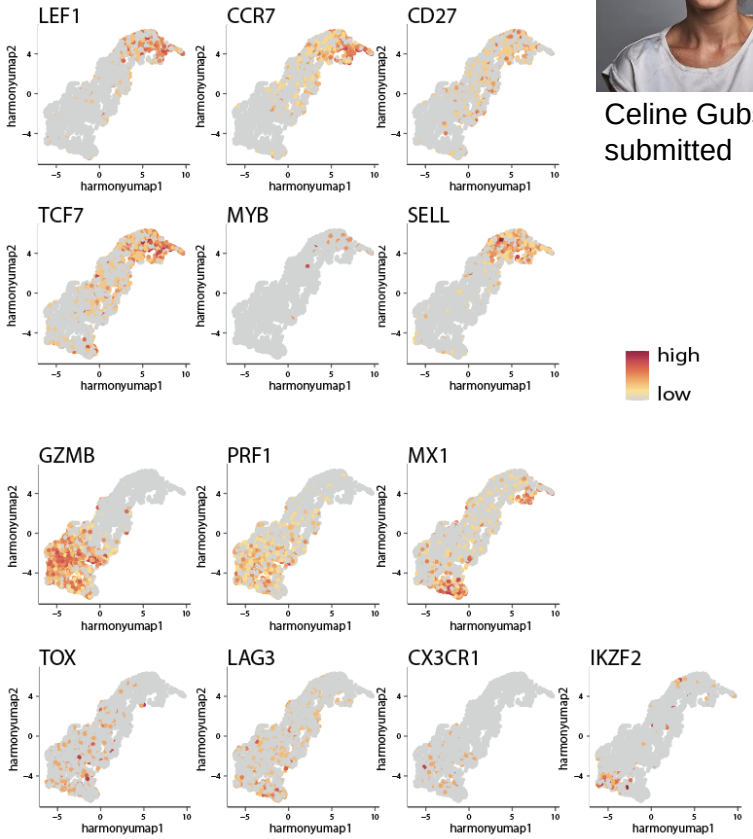
HIV tetramer+ CD8 subpopulations and cluster annotation

n=8 participants, all study timepoints represented







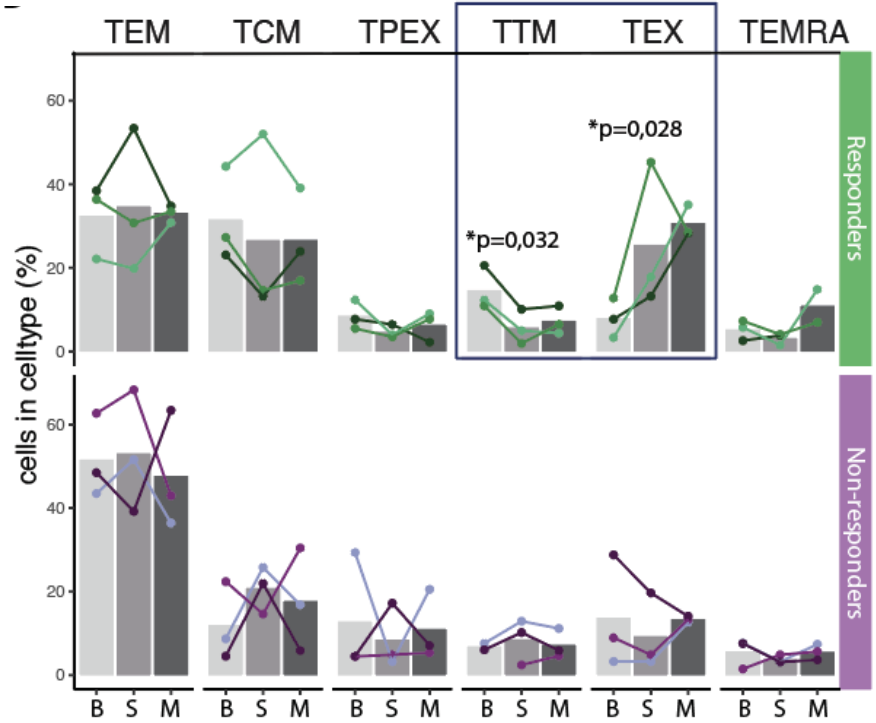


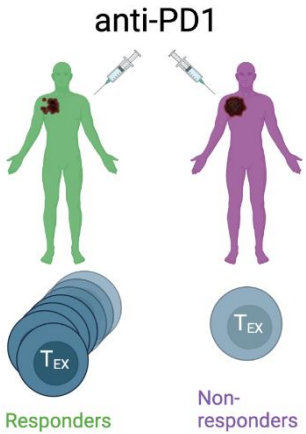
Celine Gubser, submitted

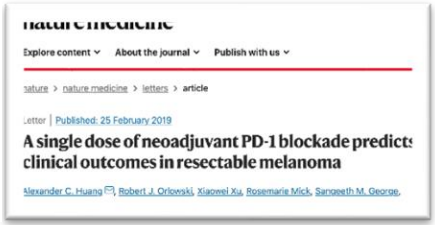
Hu et al., 2022; Tsui et al., 2022; Wang et al., 2020; Wang et al., 2022

Rapid T_{EX} expansion and concomitant T_{TM} contraction in certain donors after the first infusion of anti-PD1

Celine Gubser, submitted

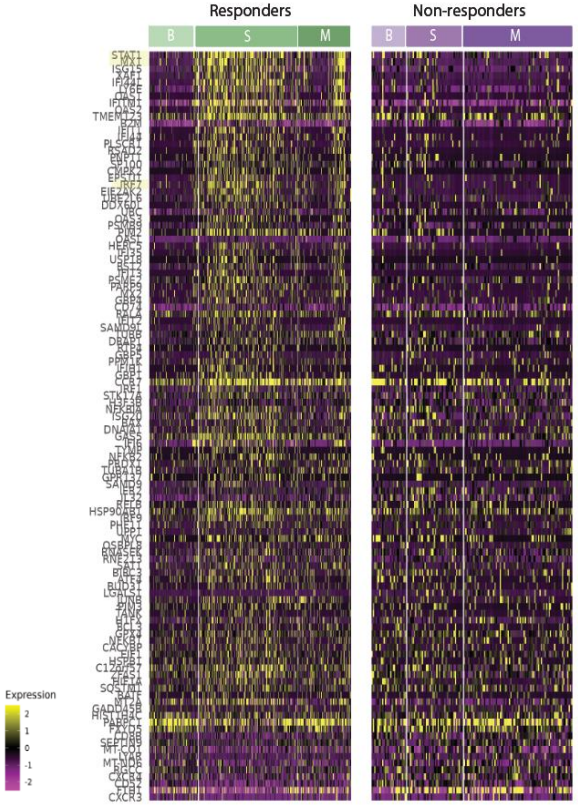


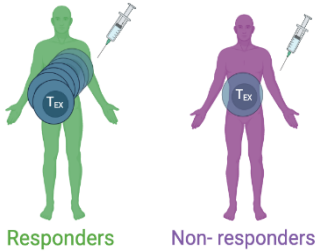


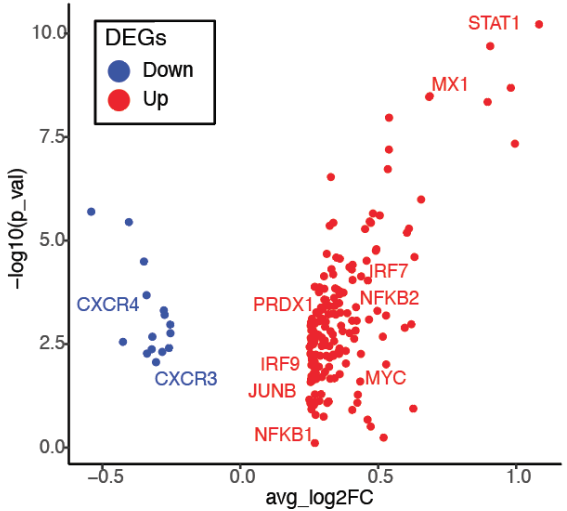


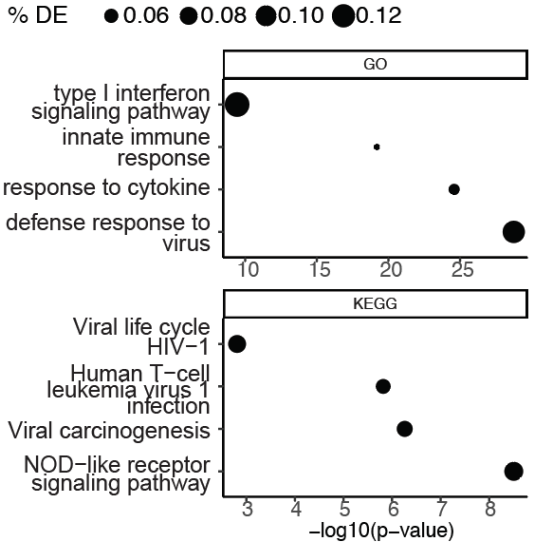
Interferon signaling gene transcriptional signature in T_{CM} of responders after a single dose of anti-PD1

Celine Gubser, submitted







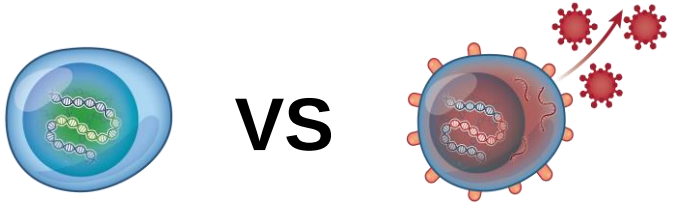


Study summary

Following a single dose of anti-PD-1, a subset of participants displayed:

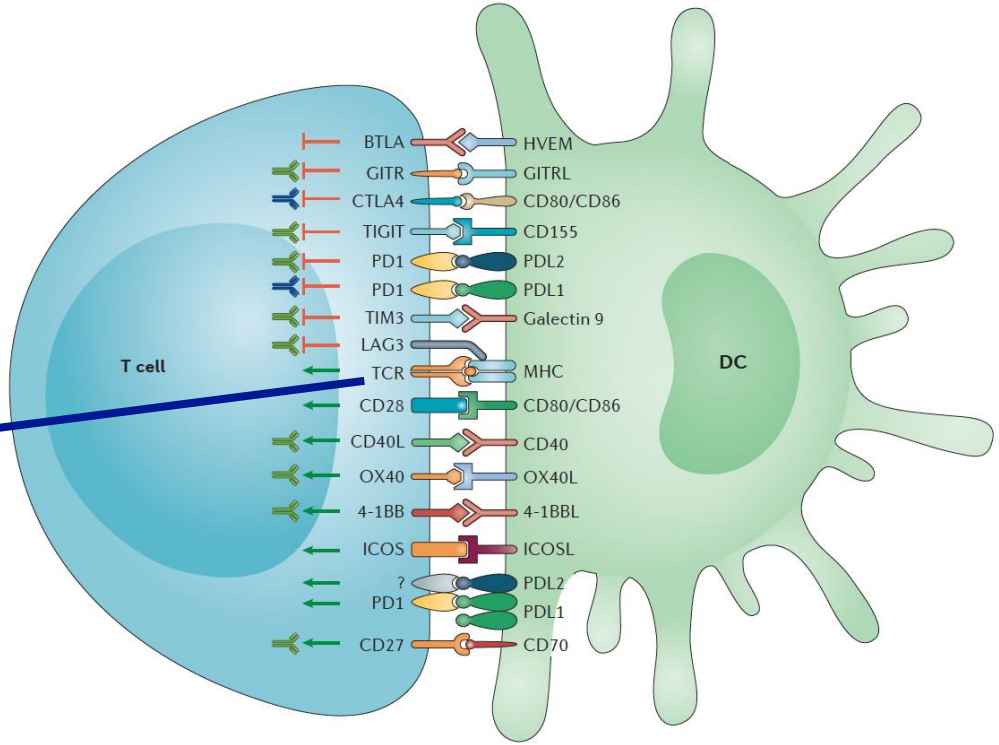
- Rapid expansion of T_{EX} cells
- Distinct transcriptomic signature in T_{CM} related to type I interferon signalling, cytokine response and the HIV-1 viral cycle
- Range of ‘new’ TCR clonotypes in the HIV specific T_{EX} cell compartment
- Together this indicates a favourable effect of PD-1 blockade on HIV-specific CD8 T-cells at the transcriptomic level consistent with reversal of T-cell exhaustion in some but not all recipients

Enhanced effect in the setting of HIV expression?



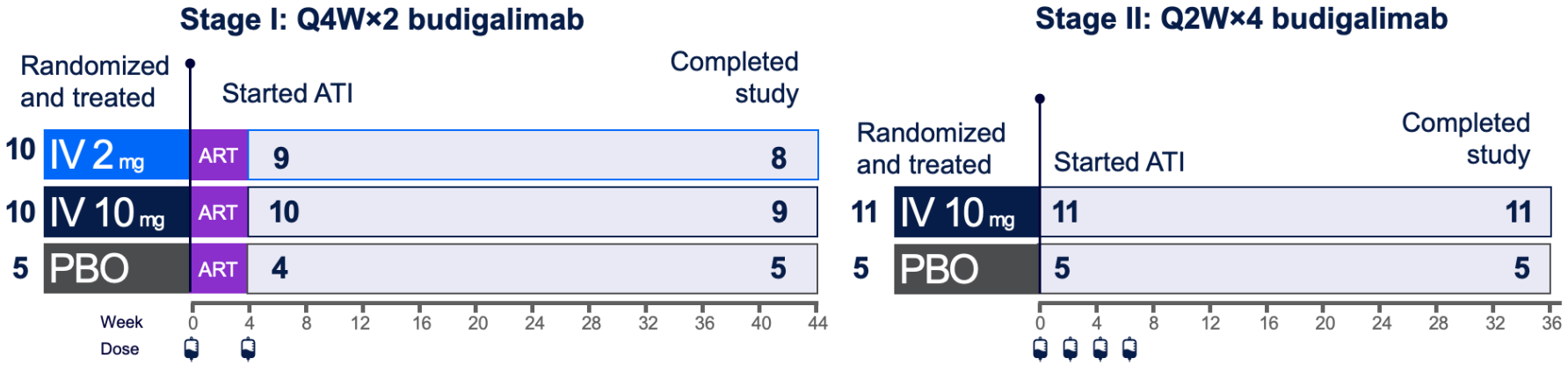
VS

Immune checkpoints modulate T-cell activity in the setting of antigen recognition



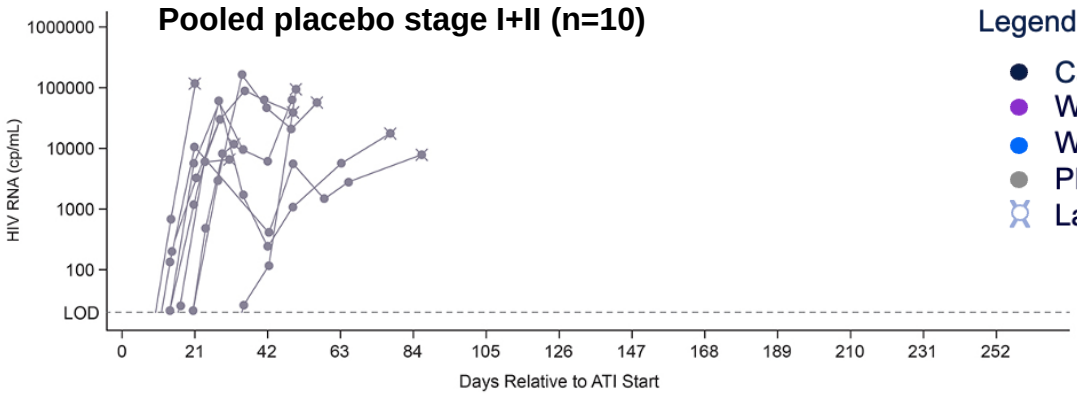
Anti-PD-1 budigalimab in the setting of ART interruption (M19-939)

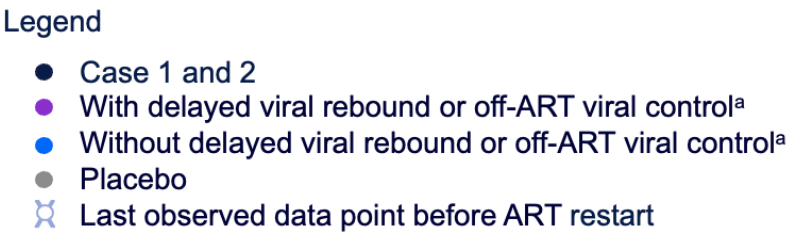
Study design:

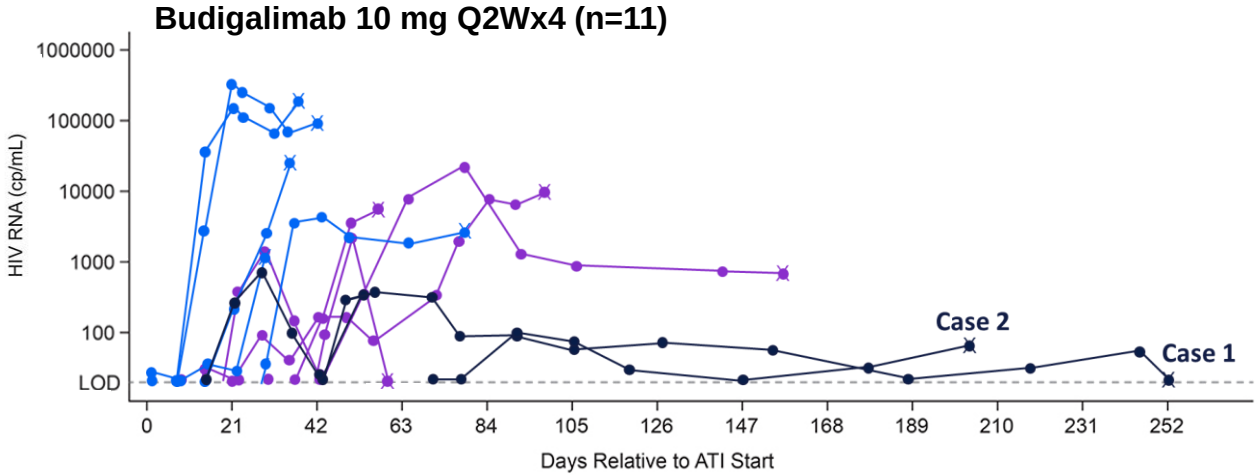


Routy, EACS 2023

Anti-PD-1 budigalimab in the setting of ART interruption (M19-939)

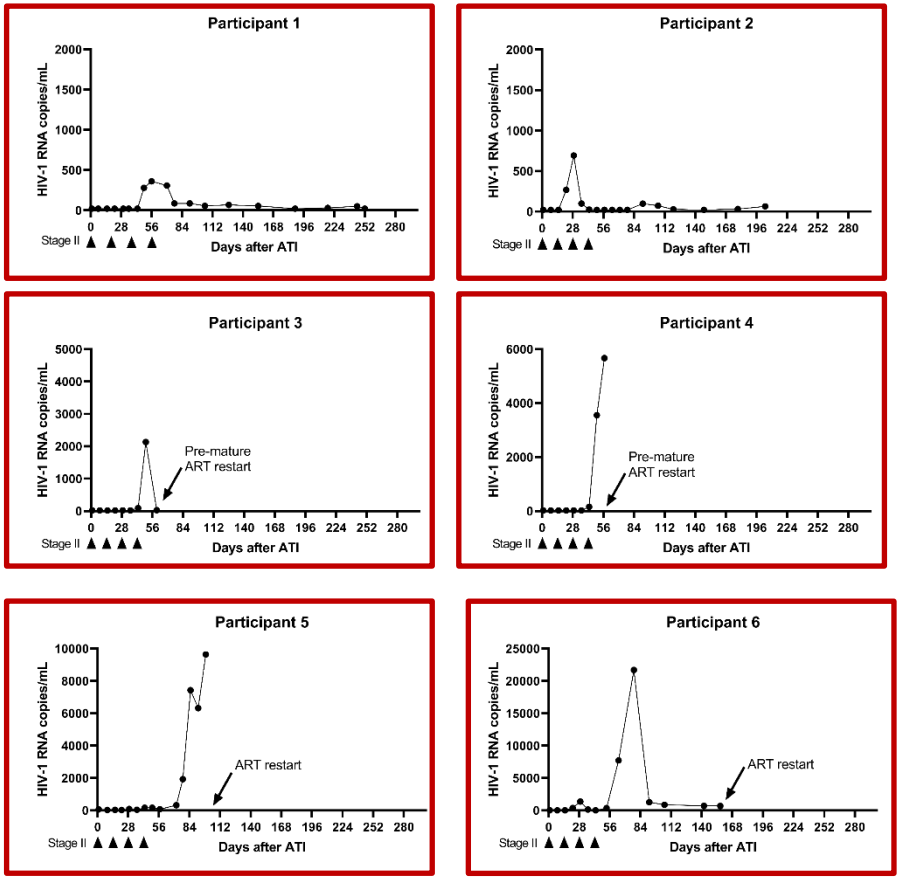






Routy, EACS 2023

Anti-PD-1 budigalimab in the setting of ART interruption (M19-939)



Biomarker exploration:

- Increases in gag-specific T cell responses seen only in a subset of samples and correlating with viral load rather than control
- Bulk RNAseq: 126/130 DEGs increased with high viral load and did not identify “controllers”
- A trend towards expansion of CXCR5+CD8+ T cells, T follicular helper-like (T_{FH}) cells and CCR6+CD4+ T cells in participants with enhanced control

Routy, EACS 2023 and Krishnan, CROI 2024

Conclusions

- Latent HIV is enriched in cells expressing some immune checkpoints (PD-1, CTLA-4, TIGIT, LAG-3) and blocking these can activate HIV from latency, but the effect is modest
- Increased expression of immune checkpoints (PD-1, CTLA-4, TIGIT) mediate T-cell exhaustion during chronic HIV infection and blocking these might reverse immune exhaustion
- Concern and occurrence of immune-related adverse events following PD-1 blockade has limited clinical studies focused on cure/remission
- Emerging data indicate a potential effect on delaying rebound/inducing control when blocking PD-1 in the setting of ART interruption, but numbers are still small and virological control not clearly linked to enhanced T-cell function

Acknowledgements

Department of Infectious Diseases, Aarhus University Hospital
Ole Søgaard
Martin Tolstrup
Lars Østergaard
Jesper Gunst
Rachel Pascoe
Rikke Olesen
Mariane Schleimann
And many more…

Department of Infectious Diseases, Alfred Health and Monash University
James McMahon
Jill Lau
Michelle Hagenauer
Janine Roney

The Doherty Institute of Infection and Immunity, University of Melbourne
Sharon Lewin
Celine Gubser
Vanessa Evans
Judy Chang
Jenn Audsley
Ajantha Rhodes
Barbara Scher
And many more…

UCSF, San Francisco
Steven Deeks
Peter Hunt
Rachel Rutishauser
Jeffrey Milush
Peter Hunt
Rebecca Hoe
Melissa Krone

Westmead Millennium, Sydney
Sarah Palmer

Oregon Health Sciences University
Afam Okoye
Louis Picker

University of Montreal
Nicolas Chomont
Remi Fromentin

Johns Hopkins University
Christine Durand
Danielle Rigau