# Enhancing death of HIV-infected macrophages using BH3 mimetics.

DR. MORGANE BRUNTON-O'SULLIVAN

INFECTION, INFLAMMATION & INNATE IMMUNITY

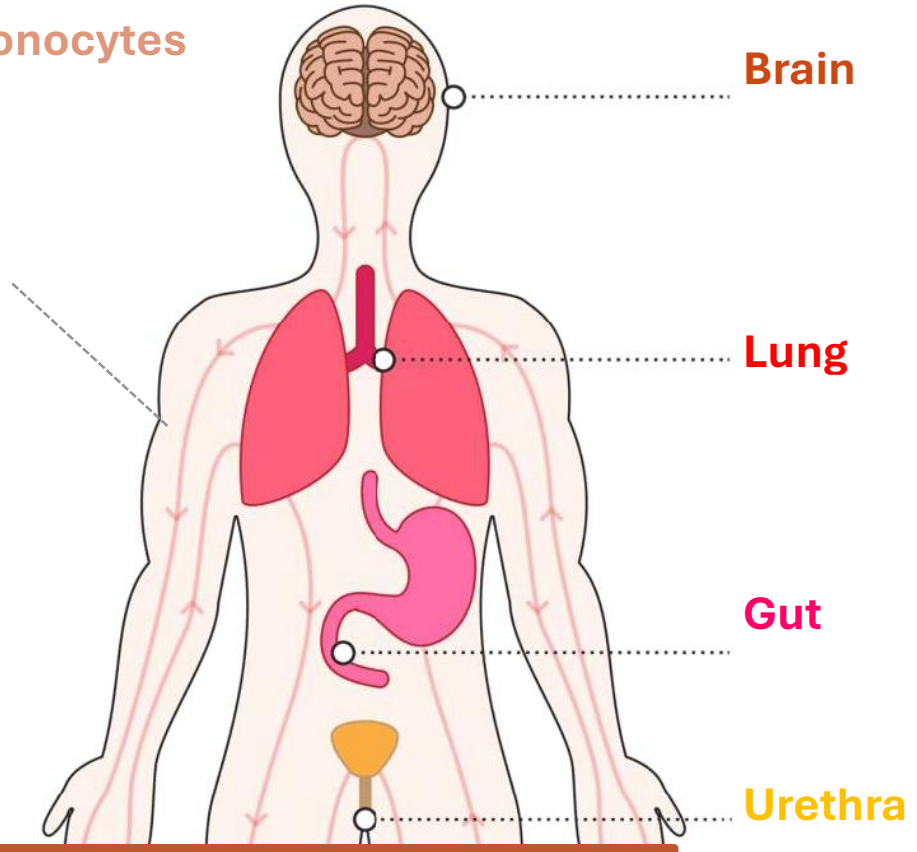
Burnet

reach for the many

# We have no disclosures to declare.

# Macrophage **persist** as an **HIV reservoir** in tissue.

- Macrophages are heterogenous immune cells found across multiple tissue regions of the body.
- Latent HIV-infected macrophages persist in the tissues of people living with HIV despite effective treatment with antiretroviral therapy (ART).
- Replication competent HIV can persist for years in macrophages[1]
- Myeloid-derived HIV can contribute to viral rebound[1,2]




Developing strategies to kill HIV-infected macrophages remains a research priority.

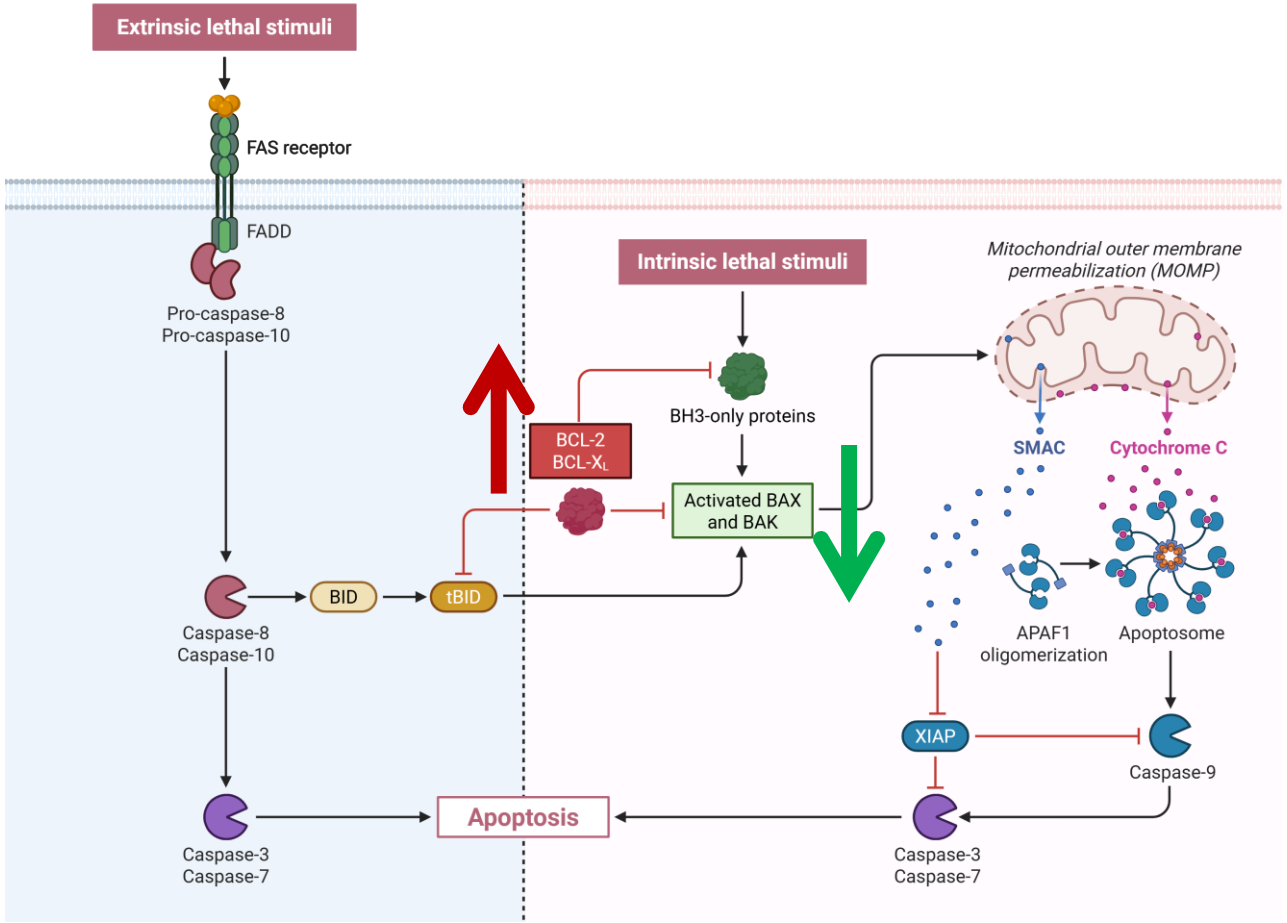
1. Veenhuis et al, Nat Microbiol. 2023;8:833-44 2. Andrade et al, Proc Natl Acad Sci U S A. 2020;117(18):9981-9990

Image created by Hans Kek

# HIV infection **modulates** apoptosis in macrophage

Evidence suggests that HIV infected macrophages are resistant to apoptosis[3]

- HIV infection modulates expression of apoptosis proteins[3,4,5]
  - Downregulation BAX and BAK
  - Upregulation BCL-2 and BCL-XL




3. Campbell et al, mBio. 2019:12;10(6):e02638-19 4. Campbell et al, Cell death and Disease. 2020:11:590 5. Guillemard et al, Virology. 2004 :329:371-380

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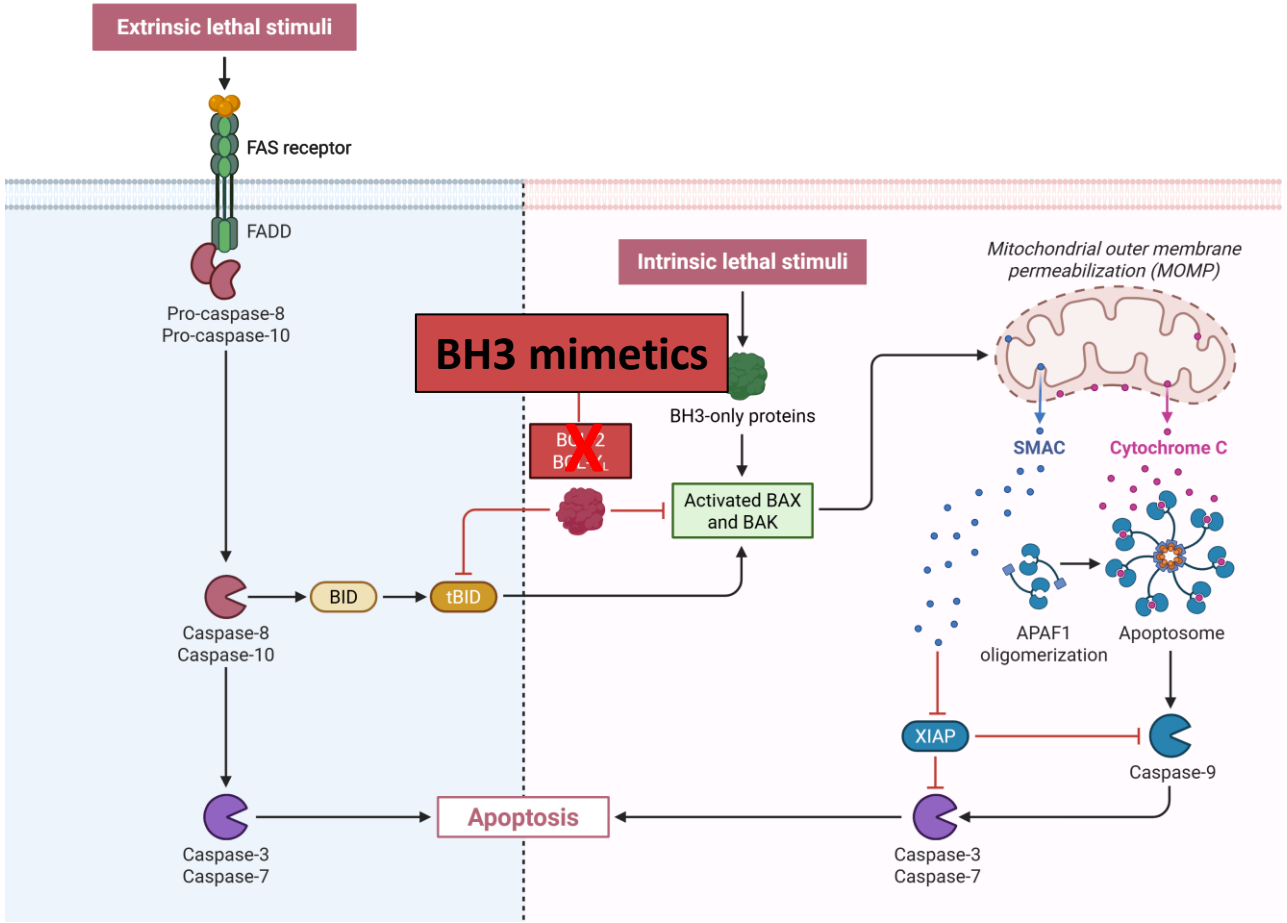
# BH3 mimetics can **enhance** apoptosis

- Evidence suggests that HIV infected macrophages are resistant to apoptosis[3]
- HIV infection modulates expression of apoptosis proteins[3,4,5]
  - Downregulation BAX and BAK
  - Upregulation BCL-2 and BCL-XL
- BH3 mimetic drugs can enhance apoptosis by binding anti-apoptotic proteins and show promise in killing HIV-infected T cells[6].
- Efficacy remains unknown in macrophages.





3. Campbell et al, mBio. 2019:12;10(6):e02638-19 4. Campbell et al, Cell death and Disease. 2020:11:590 5. Guillemard et al, Virology. 2004 :329:371-380 6. Arandjelovic & Kim et al, Cell Rep Med. 20234(9):101178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# Aims

**Investigate macrophage heterogeneity in HIV infection and BH3 mimetic response.**

**Aim 1.**

- Quantify the impact of HIV infection on macrophage viability.

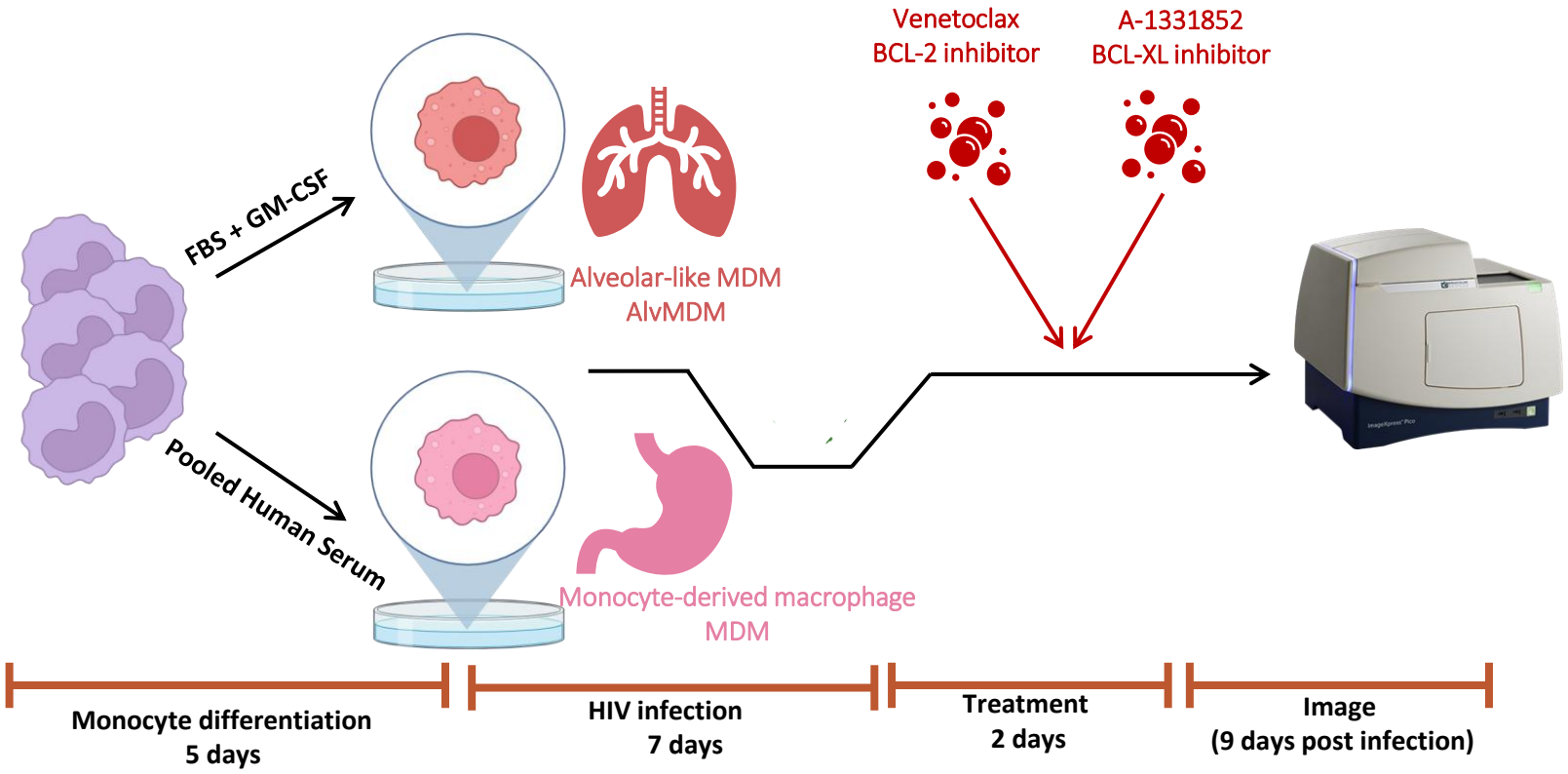
**Aim 2.**

- Examine if BH3 mimetics can sensitise HIV-infected macrophages to virus-mediated death.

**Aim 3.**

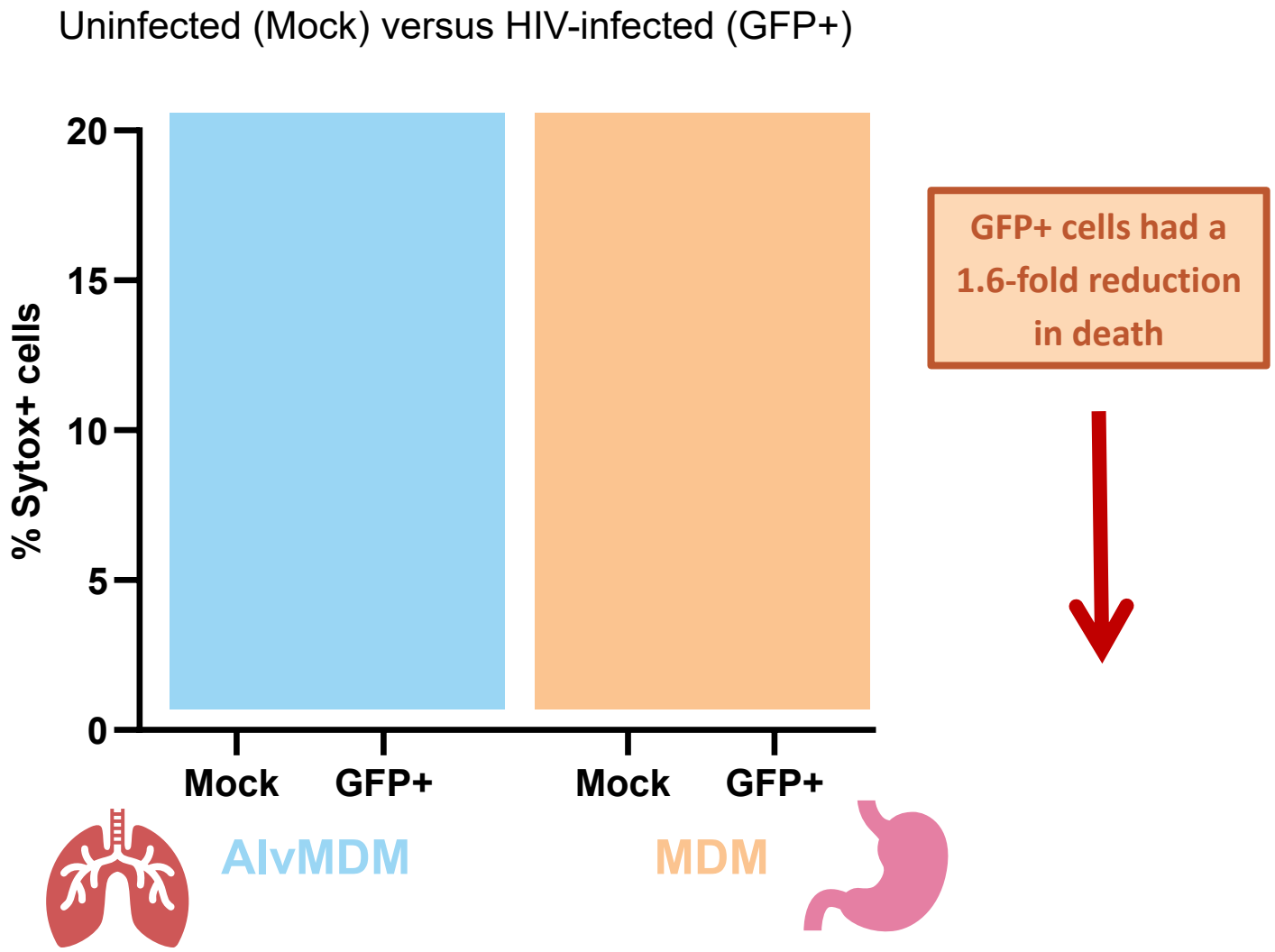
- Identify novel targets to enhance death in HIV-infected macrophages using single-cell RNA sequencing analysis (scRNA-seq).

# Methodology




Hoechst
GFP (HIV-infected)
Sytox Deep Red (dead cells)

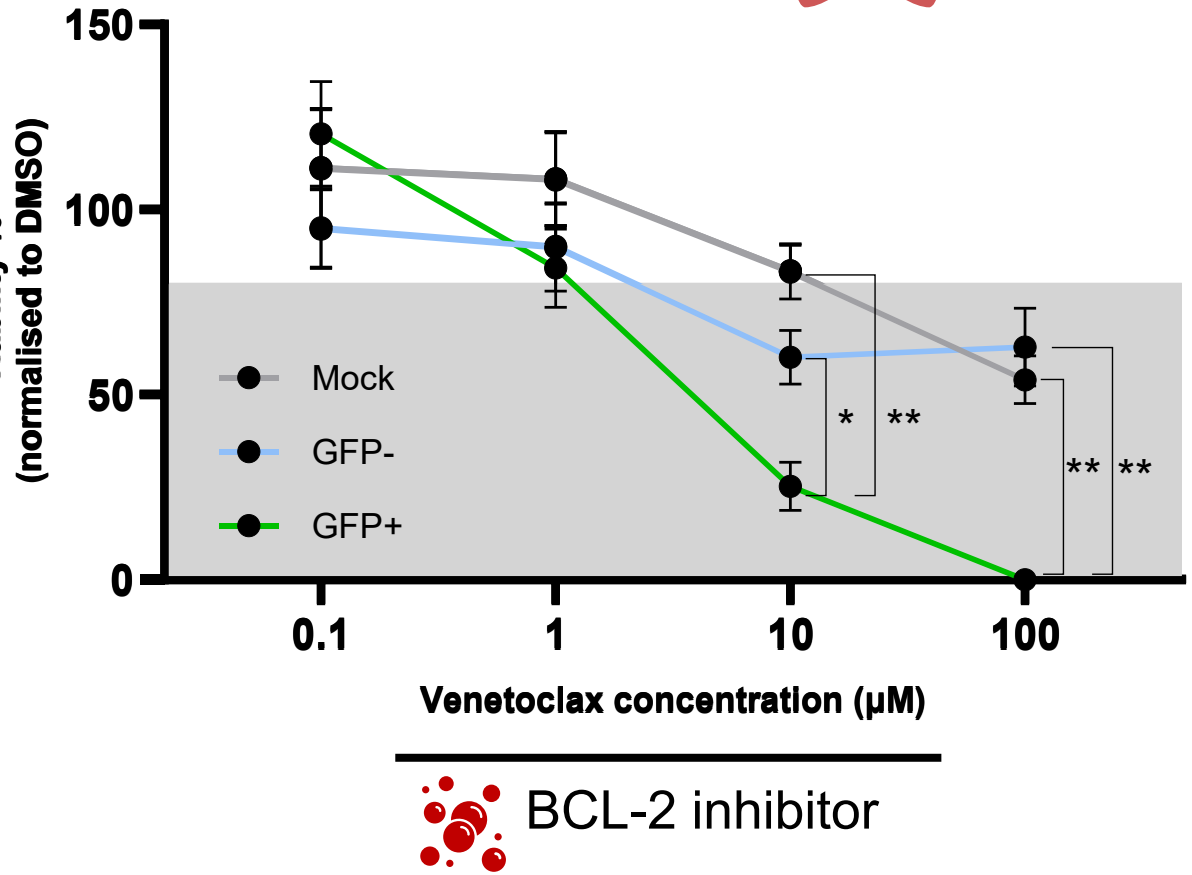
# HIV-infection **differentially modulates death** in macrophages




n=6, Paired t test, *p<0.05

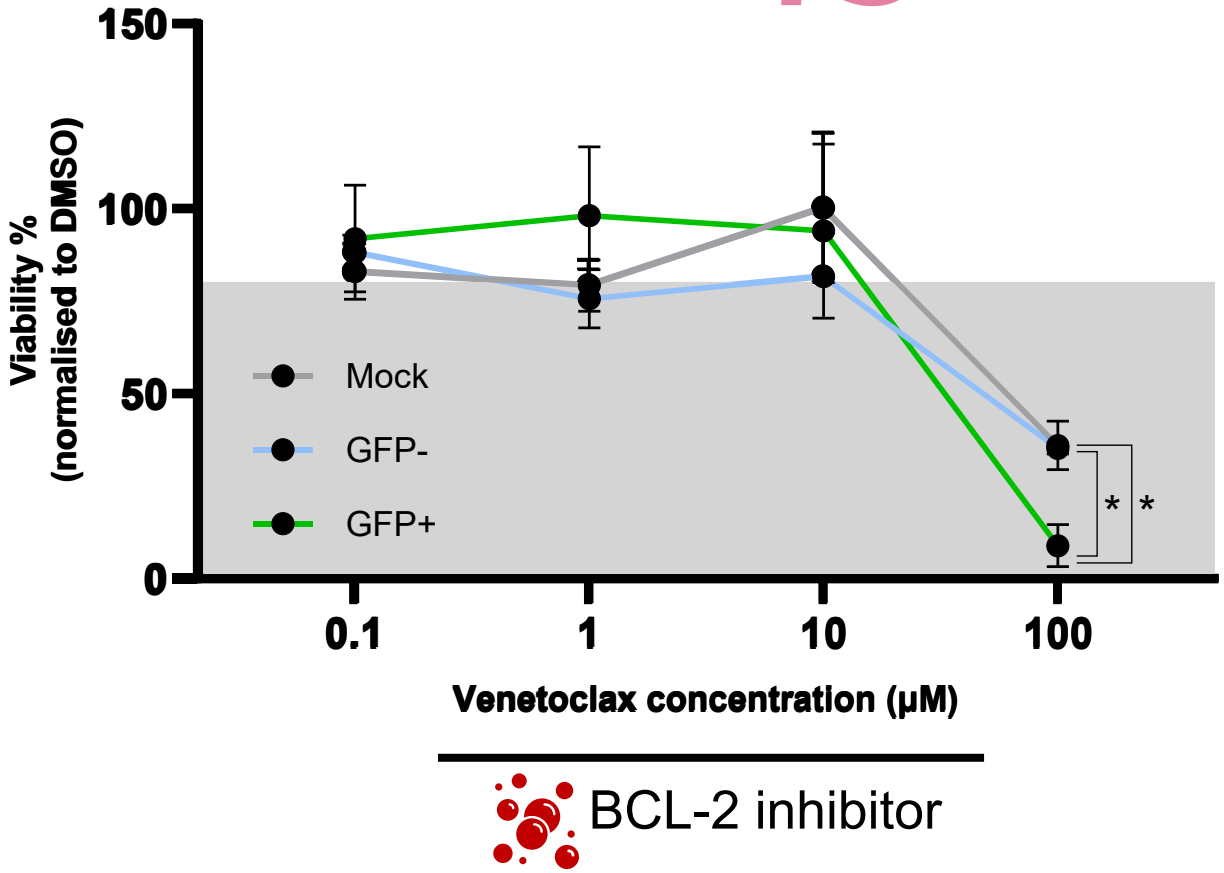
# HIV-infected AlvMDM are **sensitive** to BCL-2 inhibition

AlvMDM




BCL-2 inhibitor

MDM

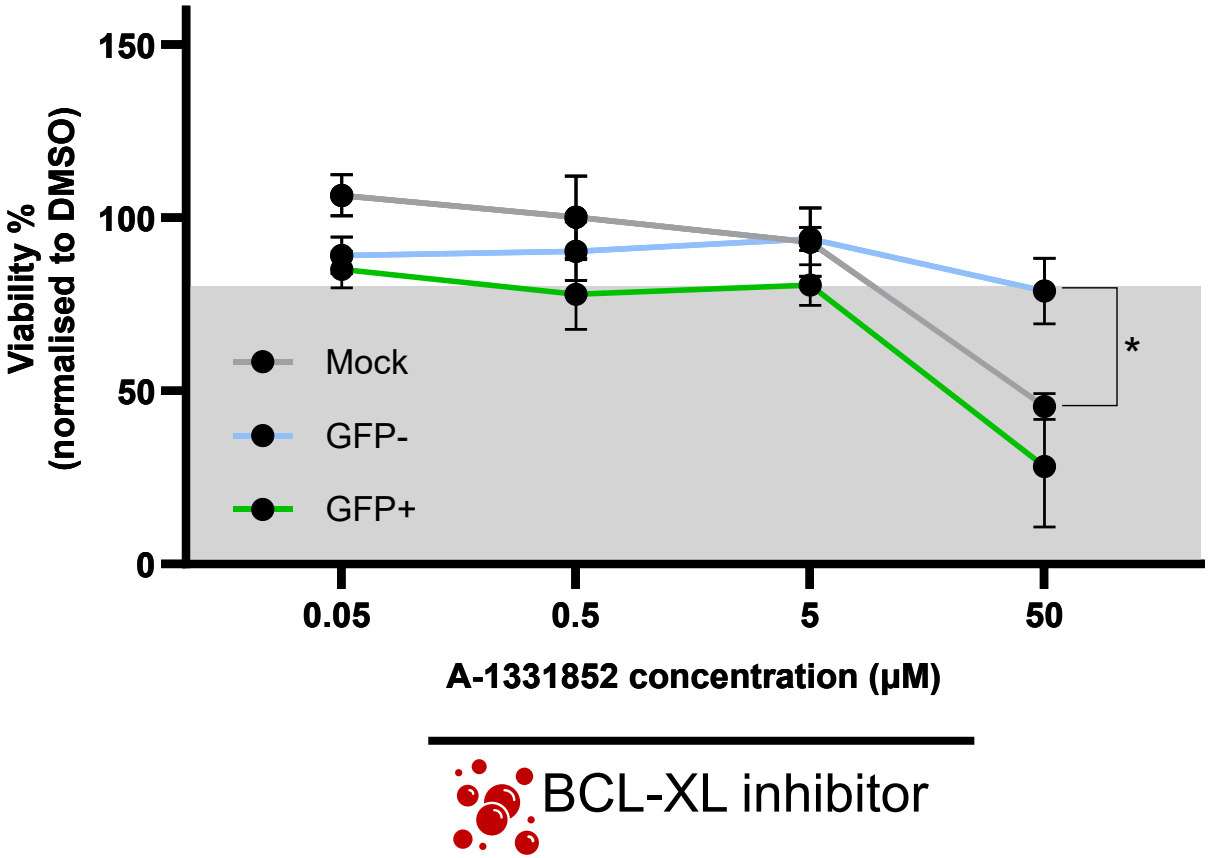



BCL-2 inhibitor

AlvMDM n=6, MDM n=5; RM two-way ANOVA with Geisser-Greenhouse correction, *p<0.05, **p<0.01

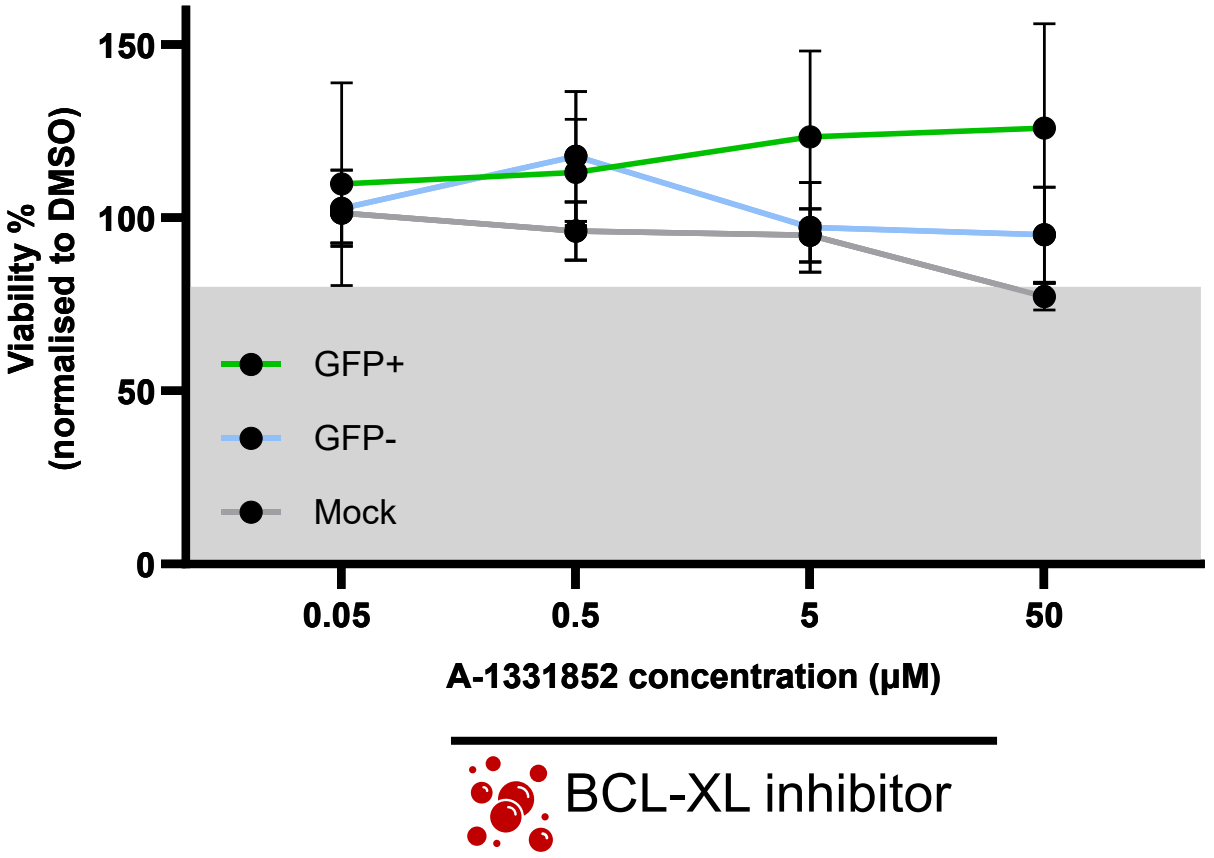
# HIV-infected MDM show **resistance** to BCL-XL inhibition

AlvMDM




BCL-XL inhibitor

MDM

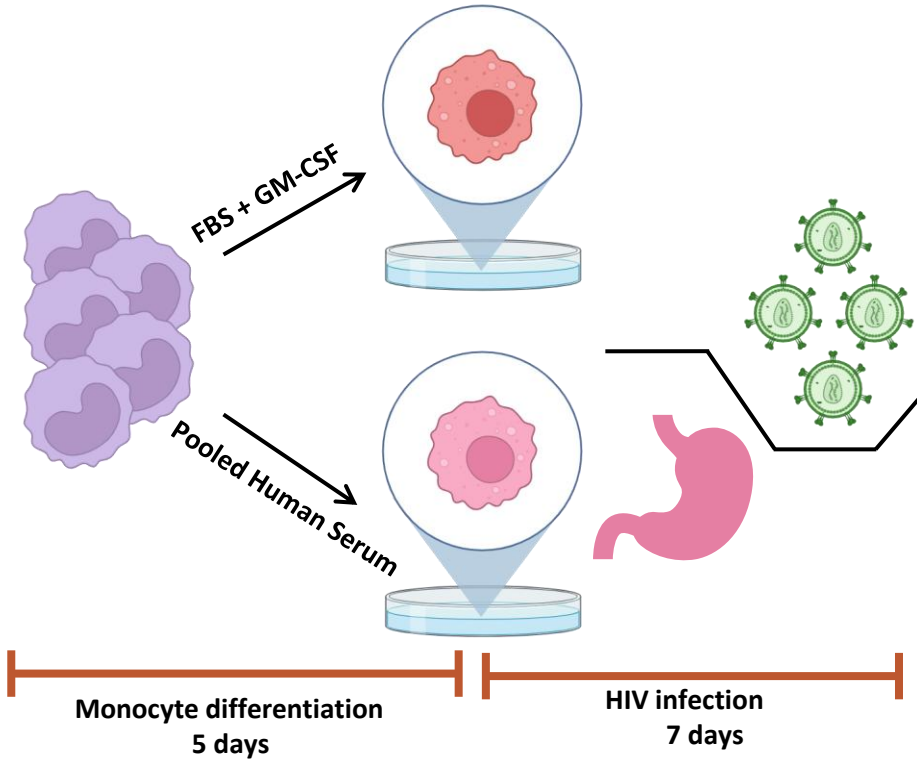


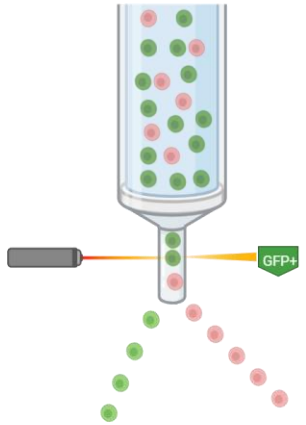

BCL-XL inhibitor

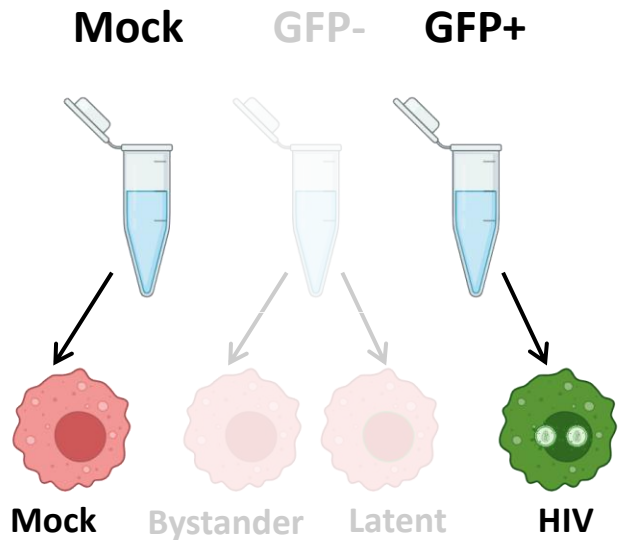
AlvMDM n=6, MDM n=5; RM two-way ANOVA with Geisser-Greenhouse correction, *p<0.05

# Methodology for single-cell RNA-Seq

*Dr. Michelle Wong*



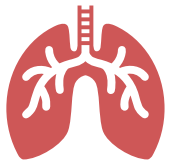




*Dr. Mark Zeimann*

*Laura Rikard-Bell*

**Bioinformatics**

# HIV infection **induces transcriptomic** changes to macrophages



## AlvMDM

Downregulated in HIV ← 1016

Upregulated in HIV → 1301

**Total: 2317**

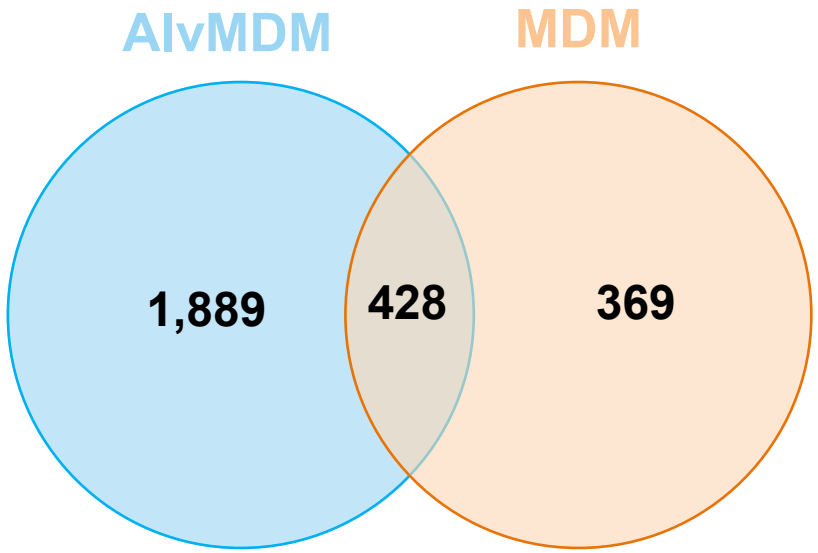
## MDM

Downregulated in HIV ← 482

Upregulated in HIV → 315

**Total: 797**

Overlap between DEGs between macrophage types



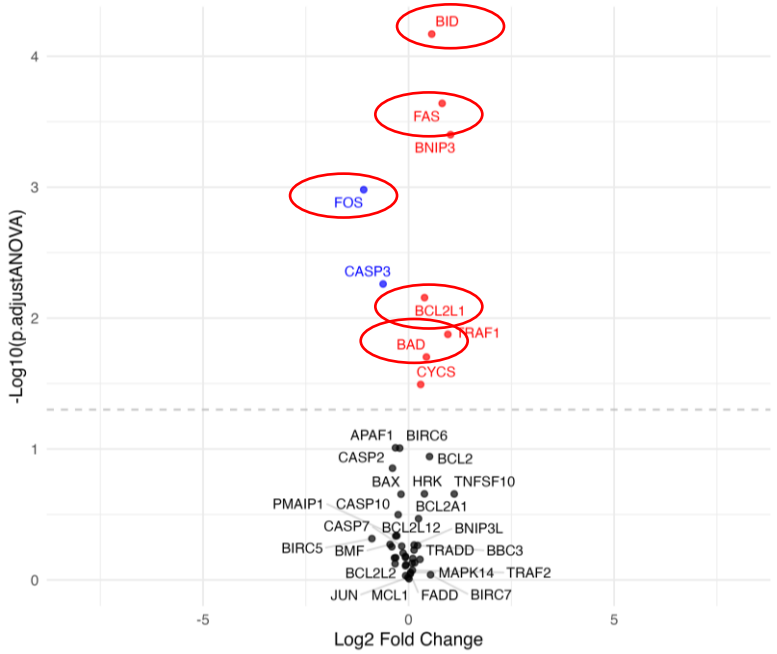

**↑ proteasomal pathways in MDM**

**↑ immune-response pathways in AlvMDM.**

FDR<0.05, log2 fold-change >0.5

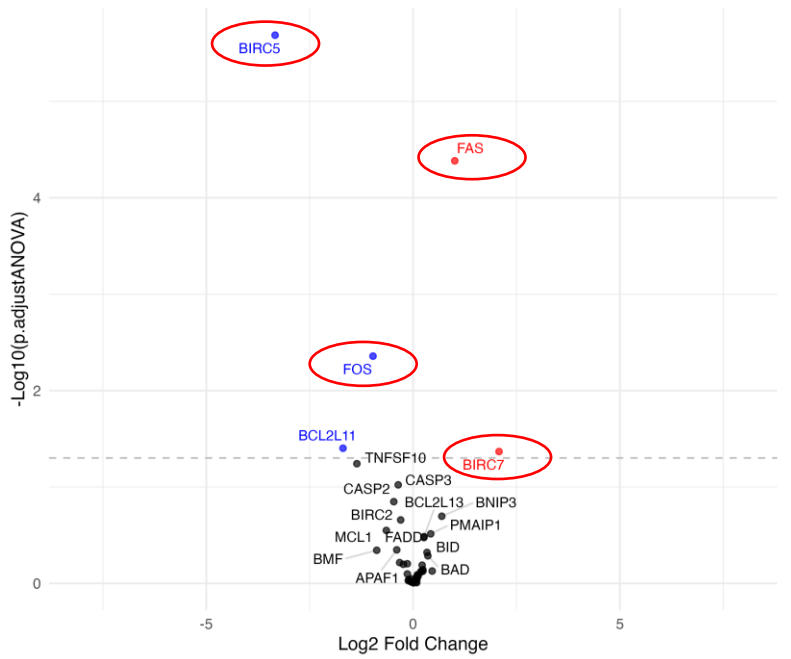
# HIV infection **modulates apoptosis-associated** transcriptome

## AlvMDM




Downregulated in HIV ← → Upregulated in HIV

## MDM




Downregulated in HIV ← → Upregulated in HIV

- ↑ in FAS receptor gene for both macrophage types.
- ↓ in FOS gene for both macrophage types
- Pro-apoptotic proteins BID and BAD upregulated in AlvMDM.
- BIRC genes (Inhibition of Apoptosis pathway) modulated in MDM
- No significant difference in BCL-2 gene. ↑ in BCL2L1 in AlvMDM.

**HIV modulates apoptosis-associated gene and induces heterogenous transcriptomic changes within macrophages types.**

FDR<0.05, log2 fold-change >0.5

FDR<0.05, log2 fold-change >0.5

# Summary

- HIV-infected MDM, but not AlvMDM, demonstrated **enhanced cell survival** *in vitro.*
- HIV-infected AlvMDM were **sensitive to BH3 mimetics targeting BCL-2 proteins**, while MDM were resistant to BH3 mimetics at non-toxic doses.
- scRNA-seq demonstrated transcriptional differences between HIV infected macrophage types, and highlighted some novel apoptosis targets for further investigation.

Combined, these findings highlight differences between HIV-infection in macrophage populations and provide important insights for developing strategies to eliminate HIV reservoirs.

# Thank you


Hearps lab

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Hannah Russel
Lauren Cooney

Dr. Michelle Wong
Dr. Mark Zeimenn


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