IFITM3, SAMHD1, and the IFN Response to Viruses

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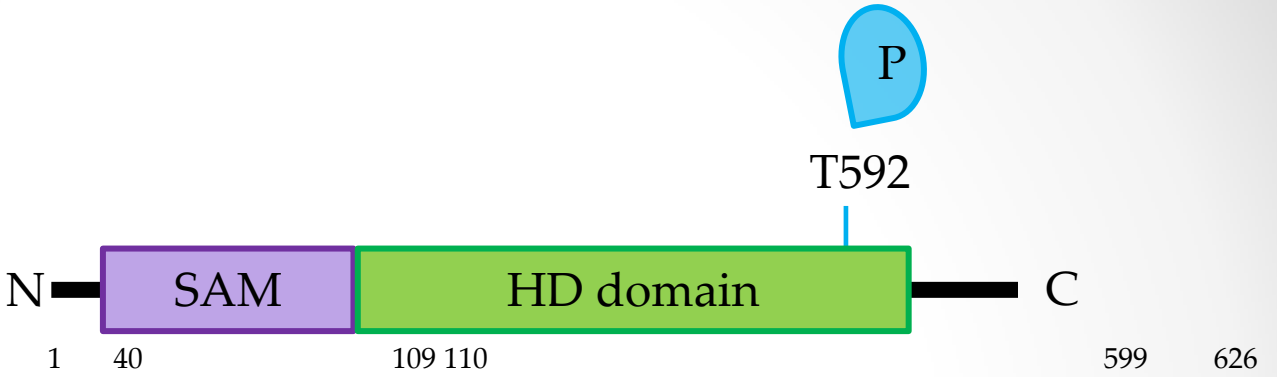
Research on HIV-1

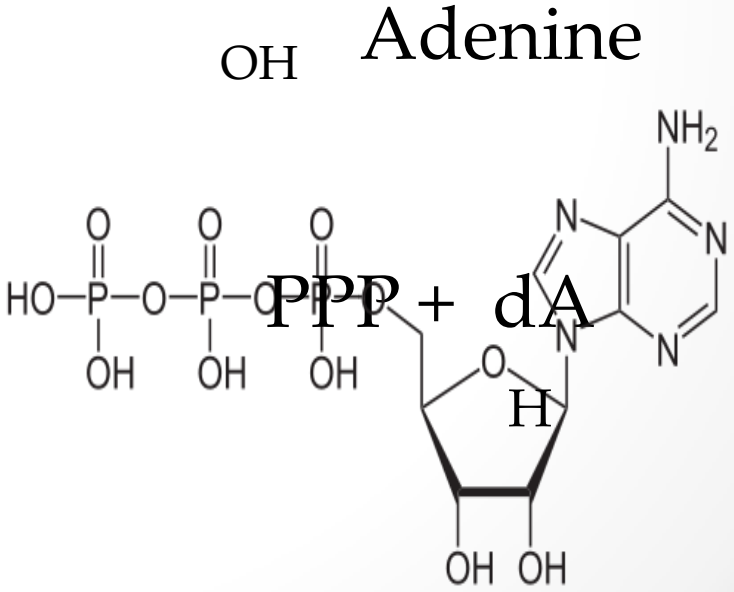
- No preventative vaccine
- 37.9 million infected, 1.7 million new cases/year*
- Gene therapy through vector technology
- IFITM3 and SAMHD1
- Planelles Lab

*Mahy, Mary; Marsh, Kimberly; Sabin, Keith; Wanyeki, Ian; Daher, Juliana; Ghys, Peter D. HIV estimates through 2018, AIDS: December 15, 2019 - Volume 33 - Issue - p S203-S211 doi: 10.1097/QAD.0000000000002321

Viral Restriction Factor: SAMHD1



- T592-phosphoacceptor, deactivation site
- Only when active, in its dephosphorylated state



Does Infection with HIV-1 Result in a Protective "Bystander Effect"?

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Matt Szaniawski
Elizabeth Williams

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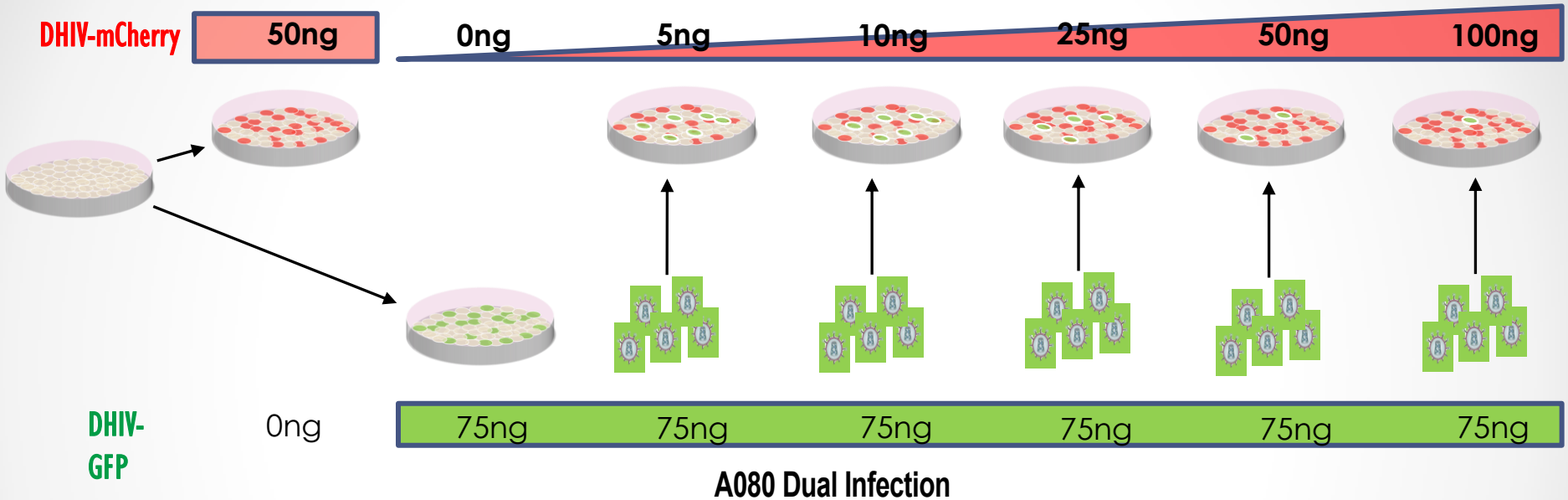

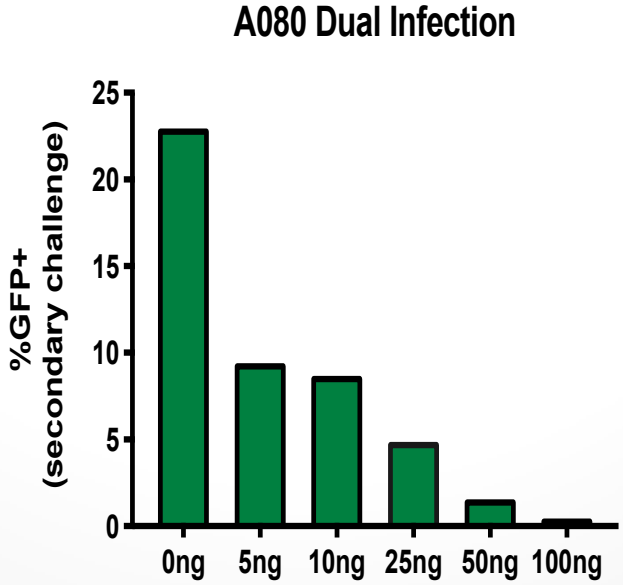

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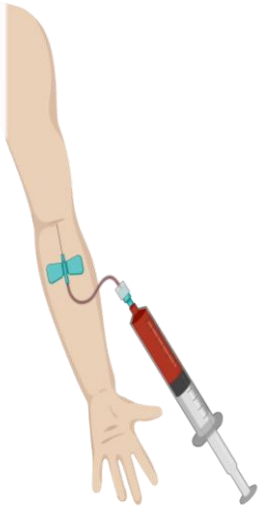
What Exactly is Causing the "Bystander Effect"?

- Interchanging viral envelopes
  - Creating pseudotyped virus
    - HIV (JRFL), VSVg, AMLV, GMTR, Lassa virus, RD114, LCMV
    - Same core

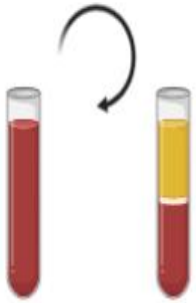
Bacterial Transformation, Cell Splitting and Viral Transfection

- Transformation
  - Plasmid with antibiotic resistance gene
  - Culture with E. coli
  - Maxi prep to select for viral plasmid
- Splitting
  - Thawing HEK293FT cells
  - Around 3-5 days later, split cells for more viruses
  - Continue splitting every 2-3 days
- Transfection
  - Using PEI and Lipofectamine 3000
    - Allows plasmids to enter cells, replication to occur, and virus to enter media
  - Collect media, freeze for storage, and thaw for experiments

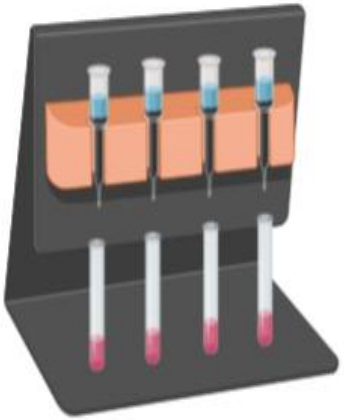
Monocyte Derived Macrophage model of HIV-1 infection



Draw 120-180 mL blood from healthy donors

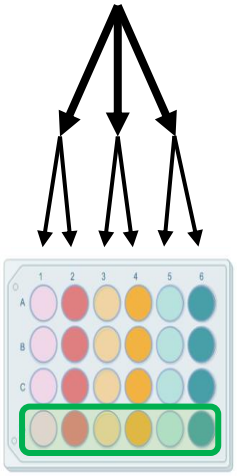


Centrifuge and collect PBMCs



Isolate CD14+ monocytes

Differentiate into MDMs over 5 days in pooled human serum



Day 7: First infection

Day 8: Second infection

Day 10: Stain cells for flow cytometry

Elizabeth Williams

Results

	1st (GFP) ↓ / 2nd (mCherry) →	JRFL	VSVg	AMLV	GMTR	Lassa	RD114	LCMV
		0.21	33.4	5.89	0.49	0.11	0.15	2.56
JRFL	0.44	0.17	17.6	2.73	0.21	0.082	0.14	1.86
VSVg	41.7	0.1	6.67	0.12	0.098	0.067	0.13	0.14
AMLV	4.88	0.21	20.5	1.94	0.16	0.099	0.11	0.98
GMTR	0.14	0.12	18.4	2.61	0.2	0.052	0.11	2.18
Lassa	0.22	0.19	24.9	5.23	0.35	0.1	0.12	1.52
RD114	0.32	0.19	33.4	5.88	0.29	0.045	0.14	1.61
LCMV	5.2	0.17	30.8	4.58	0.49	0.072	0.15	1.64

Future Plans

- Significance of IFITM3 role and protecting cells from infection
  - After isolating CD14+ monocytes, could use neon electroporation to CRISPR out IFITM3
- Changing cores
  - Expect to see no difference in bystander effect
    - dHIV vs MLV
- Initial infection had a range of around 41% with VSVg, 5% with AMLV, and 5% with LCMV
  - Control for the amount of initial infection

Acknowledgements

- Department of Pathology
- Vicente Planelles
- Elizabeth Williams