# Sex differences in replication kinetics of primary HIV-1 isolates in monocyte-derived macrophages.

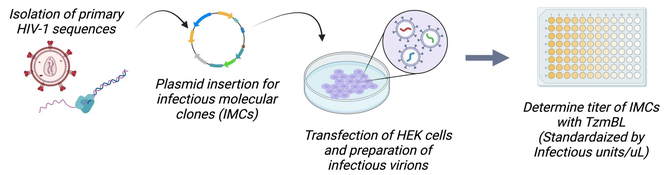
Jake A. Robinson[1], Gregory Whitehill[1], Hannah Schrader[1], Ryan Krause[1], Frederic Bibollet-Ruche[1,2], and Katharine J Bar[1]

[1] *Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA*

[2]*Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA.*

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## Infectious molecular clones of HIV-1 primary isolates



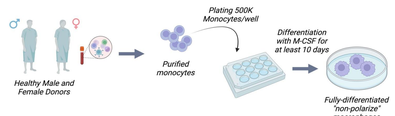

Primary isolates of HIV-1 from people with HIV were isolated, reverse-transcribed, mutagenized to original isolate sequence, and packaged into plasmids for generation of infectious molecular clones (IMCs). IMC plasmids were transfected into HEK-293T cells and supernatant was collected, which contains infectious virions. Infectivity (infectious units/uL) was determined by Tzm-BL reporter cells.

| Viral Grouping | Virus | Tropism | IFN Resistance in CD4 T cells |
|---|---|---|---|
| Rebound Virus | 9201r1 | R5 | ++++ |
| | A08 1A5 | X4 | ++++ |
| | 601r1 | R5 | ++++ |
| | A09 1A2 | R5 | ++++ |
| Transmitted-Founder Virus | CH58 | R5 | +++ |
| | CH77 | R5/X4 | +++ |
| | THRO | R5 | +++ |
| | WITO | R5 | +++ |
| Chronic Virus | MM33-13 | R5 | + |
| | MM33-17 | R5 | + |
| | YU2c | R5 | + |
| | CH167 | R5 | - |
| | MCST | R5 | - |
| Clade D Virus | D-190882 | | |
| | D-191647 | | |
| | D-191859 | | |
| | D-191727 | | |

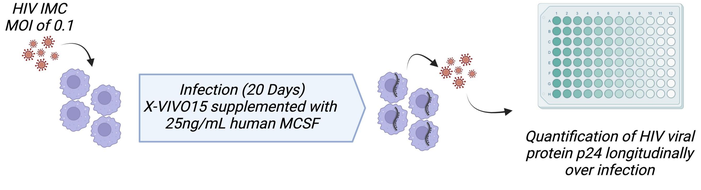
Primary isolates were grouped by isolation origin and clade of virus. Rebound, transmitted-founder and chronic viruses were all clade B or C viruses. Rebound viruses were isolated after treatment interruption from people with HIV on ART. Transmitted-founder viruses were isolated from pre-ART viremic people with HIV. Chronic viruses were isolated from people with HIV with chronic viremia. As Clade D viruses are considered myeloid-tropic, Clade D viruses were included in experimental outputs. Viruses were previously characterized for interferon-resistance in CD4 T cells, showing rebound and TF viruses to be resistant to IFN-based antiviral responses in CD4 T cells. Infectivity was reported for each virus and used for standardizing infections in MDM experiments.

## Monocyte differentiation from healthy donors

| | Donor ID | HIC ID | Age | Sex |
|---|---|---|---|---|
| 1 | 37574 | ND307 | 51 | Male |
| 2 | 37575 | ND362 | 27 | Male |
| 3 | 37576 | ND647 | 30 | Male |
| 4 | 37577 | ND616 | 61 | Male |
| 5 | 37763 | ND648 | 35 | Female |
| 6 | 37765 | ND410 | 60 | Female |
| 7 | 37767 | ND500 | 30 | Female |
| 8 | 38107 | ND624 | 24 | Female |
| 9 | 38108 | ND608 | 27 | Female |

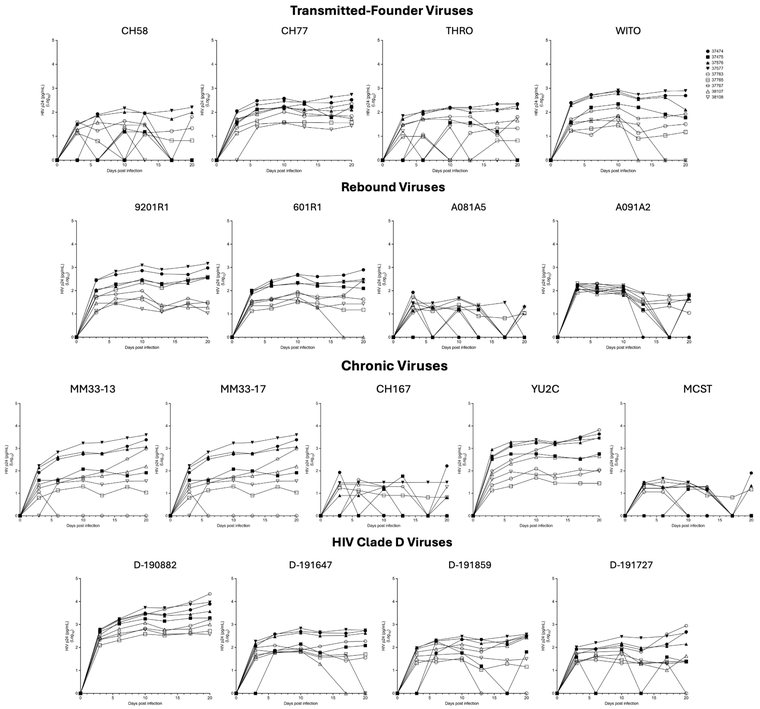



Male and female healthy donors were recruited by the Human Immunology Core at Penn. All experiments were performed in serum-free conditions. Monocytes were plated at 500,000 cell/well and differentiated to macrophages (MDMs) by stimulation with recombinant monocyte colony stimulating factor (M-CSF) for at least 10 days.



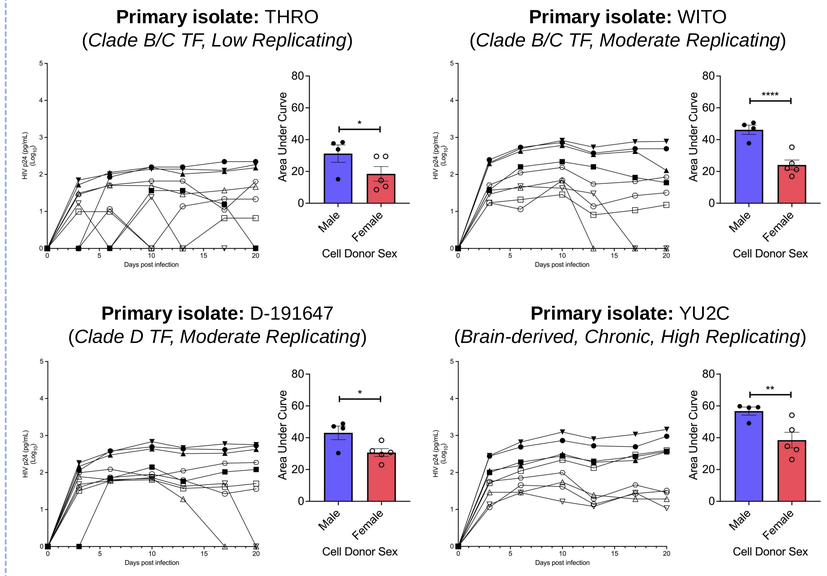

MDMs were washed to remove non-adherent cells and exposed to primary HIV-1 isolates at multiplicity of infection (MOI) of 0.1 in half-volume media for 24 hours. Viral media was replaced with fresh media. Samples of media were collected every 3-4 days (50uL) and replaced with same amounts of media with fresh M-CSF. Infection experiments were completed after 20 days of infection.

## Replication kinetics of primary HIV-1 isolates in MDMs



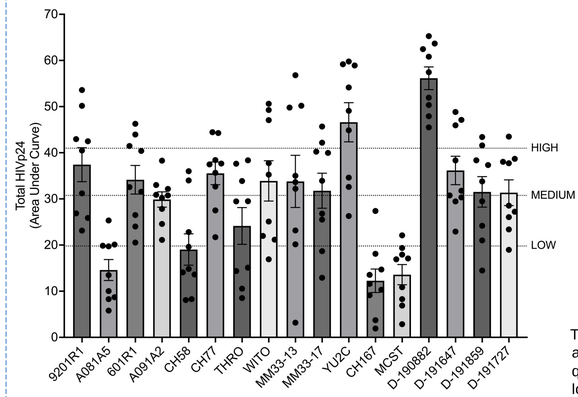

MDMs were infected with MOI 0.1 of HIV IMCs. HIVp24 was longitudinally quantified by AlphaLISA for all primary isolates over the course of 20 days of infections ($log_{10}$ transformed). Closed circles are male donor MDMs, and open circles are female donor MDMs.

## Donor cell sex is a determinant of viral kinetics in MDMs




HIVp24 was longitudinally quantified 20 days of infections ($log_{10}$ transformed) and total HIVp24 was determined by area under the curve analysis to compare sex differences of viral kinetics. Closed circles are male donor MDMs, and open circles are female donor MDMs.

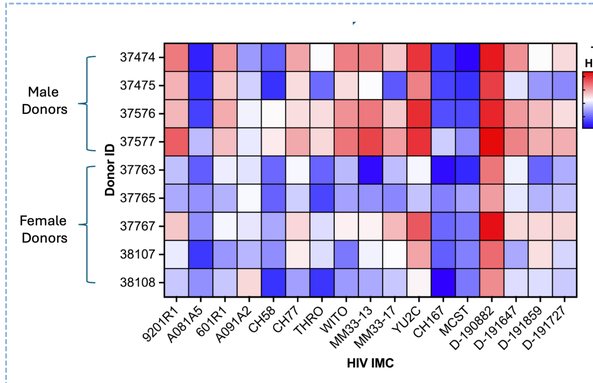
## Categorization of MDM replication of HIV-1 isolates




| MDM Replication | Viruses |
|---|---|
| *Non-productive* | A081A5, CH58, CH167, MCST |
| *Low* | A091A2, THRO |
| *Moderate* | 9201R1, 601R1, CH77, WITO, MM33-13, MM33-17, D-191647, D-191859, D-191727 |
| *High* | YU2C, D-190882 |

Total HIVp24 was determined by area under the curve analysis which allowed for grouping viruses by quartiles, establishing categories for non-productive, low, moderate, and high infection in MDMs.

## Donor cell sex comparison across all primary HIV-1 isolates




| Comparison | Source | Variation (%) | *P* |
|---|---|---|---|
| Two-way ANOVA | Interaction | 8.5 | 0.0013 |
| | Donor Sex | 10.0 | <0.0001 |
| | Virus | 59.8 | <0.0001 |

| Comparison | Virus | Significant | *Q* |
|---|---|---|---|
| Male v. Female Post-hoc (FDR) | 9201R1 | Yes | 0.0014 |
| | A081A5 | No | 0.46 |
| | 601R1 | Yes | 0.0040 |
| | A091A2 | No | 0.54 |
| | CH58 | No | 0.20 |
| | CH77 | No | 0.075 |
| | THRO | Yes | 0.021 |
| | WITO | Yes | <0.0001 |
| | MM33-13 | Yes | <0.0001 |
| | MM33-17 | No | 0.33 |
| | YU2C | Yes | 0.0011 |
| | CH167 | No | 0.44 |
| | MCST | No | 0.28 |
| | D-190882 | No | 0.078 |
| | D-191647 | Yes | 0.024 |
| | D-191859 | No | 0.26 |
| | D-191727 | No | 0.29 |

Total HIVp24 was determined by area under the curve analysis for the entirety of 20 days of infection. Comparison of male and female donor cell infections showed a general trend of higher HIV replication in male donor cells over female donor cells (Heat-map). Two-way ANOVA of all primary isolates showed a significant interaction between infection and donor cell sex (Interaction, P<0.0013), with donor cell sex accounting for 10% variation in all infections (Donor sex, P<0.0001). In post-hoc analysis, donor cell sex was a significant determinant of MDM replication in 7/17 primary HIV-1 isolates (FDR, Q<0.05), and among these viruses, the average percent difference between replication in male and female donors was 39.5% difference.

## Summary, conclusions, and future directions

*Summary:*

Minimal variation was observed among Clade B/C transmitted-founder viruses, rebound viruses, and chronic viruses in replication kinetics in MDMs from health donors. The well-characterized brain-derived YU2 virus and Clade D viruses were generally better at replicating in MDMs, which agrees with the observations that brain derived viruses are myeloid tropic.

*(1) Clade D and brain-derived viruses efficiently replicated in MDMs*

*(2) Donor sex was a significant determinant of viral replication in MDMs from health donors.*

*Future Directions:*

- Distinct myeloid host restriction factors may differentially regulate viral replication of primary isolates.
- Donor sex is an important and understudied variable in infection studies of HIV-1 isolates and should be heavily considered in future *in vitro* and clinical HIV cure and reservoir studies.
- While we observed interesting results in MDMs, future experiments should incorporate models of tissue macrophages and microglia to generate a broader understanding of HIV replication in myeloid cells.

## Acknowledgements

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