Oncolytic Viruses as a Potential Approach to Eliminate the HIV Reservoir

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HIV Reservoirs

- Viral Reservoirs
  - Cell type or anatomical site
  - Replication-competent form of virus accumulates and persists

- Implications
  - A major factor contributing to our inability to cure HIV
Recombinant Maraba virus (MG1)*

- Oncolytic virus (OV) which selectively targets cancer cells
- Double mutant
  - G protein (Q242R) and M protein (L123W)
- Undergoes complete lifecycle in cytoplasm → No genotoxicity

*Kindly provided by Dr D. Stojdl and Dr J Bell
## IFN-related Abnormalities Shared by Cancer Cells and HIV-infected Cells

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Cancer Cells</th>
<th>HIV-infected Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>• IFN-α/β receptor (IFNAR) expression</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>• IFN-mediated signaling (STAT 1 &amp; STAT2)</td>
<td>✔️</td>
<td>✔️</td>
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<tr>
<td>• IFN-inducible genes (protein kinase RNA (PKR))</td>
<td>✔️</td>
<td>✔️</td>
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<tr>
<td>• IFN regulatory factor (IRF3)</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>• and others...</td>
<td></td>
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Rationale: HIV & Oncolytic Virotherapy

• Both cancer cells and HIV-infected cells have abnormal interferon signaling pathways, and thus differ from normal, healthy cells.

• Similar to OVs as a cancer therapeutic, OVs can be designed to selectively target HIV-infected cells with these abnormalities, while sparing healthy cells.
Hypothesis

Oncolytic viruses will have a greater propensity to target and kill HIV-infected cells compared to HIV-uninfected cells.
Objectives

1) To determine whether MG1 exerts greater killing in HIV-infected than non-HIV infected cells

2) To determine whether there is selective replication of MG1 in primary cells infected with HIV, with resultant cell death and decrease of HIV production
<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>Lineage</th>
<th>Copies of HIV Proviral DNA</th>
<th>Mechanism of HIV Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>U1 (U937)</td>
<td>Monocytic</td>
<td>2</td>
<td>Mutations in transcription activator Tat</td>
</tr>
<tr>
<td>ACH-2 (A3.01)</td>
<td>Lymphocytic</td>
<td>1</td>
<td>Mutations in Tat-associated gene region (TAR)</td>
</tr>
<tr>
<td>J1.1 (Jurkat)</td>
<td>Lymphocytic</td>
<td>1</td>
<td>Defective Ca2+ mobilization and IL-2 secretion</td>
</tr>
<tr>
<td>OM-10 (HL-60)</td>
<td>Monocytic</td>
<td>1</td>
<td>Disruption in protein kinase 2nd messenger pathway</td>
</tr>
</tbody>
</table>
Cell Lines: Experimental Outline

- **Cell Lines**
  - U1.1/U937
  - ACH-2/A3.01
  - OM-10/HL-60
  - J1.1/Jurkats

- **P24 Antigen Production**
  - ELISA

- **Infectivity**
  - Flow Cytometry for GFP Expression

- **Viability**
  - MTT Assay

- **P24 Antigen Production**
  - ELISA (cells and supernatants)

- MG1 at various MOIs ± TNF-α

- TNF-α

Diagrams represent experimental protocols involving cell lines and various assays related to HIV-1 infection and characterization.
GFP Expression as Gated on Live Cells
Percentage of GFP-Positive U937 and U1 Cells 24 hours Post-MG1 Infection (Gated on Live Cells)

U937

- 24 hours-no virus: <0.5%
- MG1 0.001: 34%
- MG1 0.01: 59%

U1

- 24 hours-no virus: <0.5%
- MG1 0.001: 97%
- MG1 0.01: 99%
Effect of HIV on MG1 Infectivity in U1 vs U937 Cells

18 hours post MG1 Infection

24 hours post MG1 Infection

MOI of MG1

% GFP+ cells

Unstimulated U937 cells
Unstimulated U1 Cells

MOI of MG1

% GFP+ cells

Unstimulated U937 cells
Unstimulated U1 Cells
Effect of HIV on MG1-induced Cell Death in U1 vs U937 Cells

18 hours post MG1 Infection

24 hours post MG1 Infection
Other Cells Lines

• ACH-2 and A301 cells:
  • Infectivity and cell death profiles similar

• OM-10 and HL-60 cells:
  • Infectivity and cell death profiles similar

• J1.1 and Jurkat cells:
  • Infectivity profiles similar
  • J1.1 more resistant to MG1-mediated cell death
Objective 2

• To determine whether there is selective replication of MG1 in primary cells infected with HIV, with resultant cell death and decrease of HIV production
Primary Cell Experiments

PBMCs from HIV patients on HAART with VL ≤40 copies/mL

CD4+HLADR-CD25- cells by negative selection

MG1 Infection

(Uninfected control cells, MOI 0.01 ± MOI 0.001)

Cells

(24 hours post MG1 infection)

- Infectivity (GFP by Flow)
- Viability (MTT Assay)
- anti-CD3 + IL-2 Co-culture
  - p24 antigen (ELISA)
  - HIV RNA (RT-PCR)
- Total HIV DNA (PCR)
Infectivity and Viability of CD4+CD25-HLADR-Cells from HIV+ Patients (N=20)

- Cells not infected by MG1
- Viability not reduced
p24 antigen concentrations in patient supernatants (N=20)

• below level of assay detection
PCR for Total HIV DNA on Patient Cells and Total HIV RNA on Supernatants (N=9)
Caution regarding PCR data

• Unclear whether our assay is sensitive enough to detect such low levels of events

• PCR-based assays do not differentiate between HIV that is replication-competent or not

• Unclear whether PCR is an effective method to monitor eradication strategies

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Concluding Thoughts....*The Road Ahead*

- Results to date very preliminary
- Combination therapy will likely required
Acknowledgements

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- Module G and Clinical Investigation Unit Nurses
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- Canadian HIV Trials Network (CTN)
- University of Ottawa Department of Medicine
- Ontario HIV Treatment Network (OHTN)
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