Gene therapy to control virus infection: RNA-directed silencing of HIV-1, SIV, HTLV-1 & SARS-CoV-2

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Background

- The first two gene therapies using RNA therapeutics was approved by the US Food and Drug Administration (FDA) in 2018/19 to treat liver diseases.
- Virus infections can also be treated using RNA therapeutics and gene therapy.
- We have developed a gene therapy using RNA therapeutics to target HIV-1 (human immunodeficiency virus type 1) and are using the same development platform to engineer novel RNA therapeutics targeting SIV (simian immunodeficiency virus), HTLV-1 (Human T-cell leukemia virus type 1) and SARS-CoV-2 (severe acute respiratory syndrome-coronavirus 2, which causes COVID-19 disease).
- Delivery of RNA therapeutics to the cells that need treatment is a major challenge for successful gene therapy.
- We are exploring two different RNA delivery platforms:
  i) lentiviral vectors and ii) nanoparticles

Projects on offer:

- Honours project developing RNA-directed silencing of HTLV-1
- Honours project developing RNA-directed silencing of SIV
- HDR project examining RNA-directed silencing of SARS-CoV-2 using nanoparticle delivery
- HDR project examining RNA-directed silencing of HIV-1 using nanoparticle delivery
- HDR project examining RNA-directed silencing of HIV-1 using lentiviral vector delivery

Methods & Techniques to learn

Honours/Masters/HDR projects:

- Computational sequence conservation analysis to determine the hot spots suitable to design RNAs to target the virus sequence.

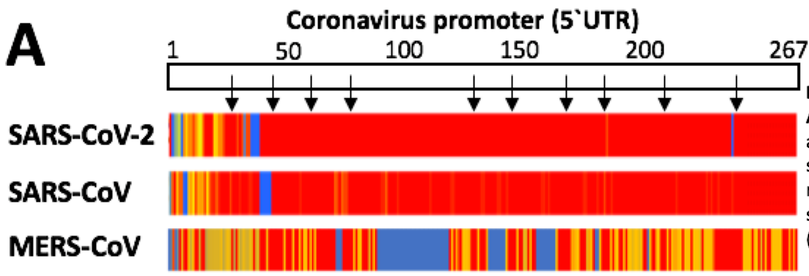

Figure 1. Sequence conservation analysis & siRNA screen. A) Sequence conservation analysis of SARS-CoV-2, SARS-CoV and MERS-CoV in the promoter (5`UTR) region. Heat maps show **red 'hot'** regions (high conservation), **blue 'cold'** regions (low conservation & high diversity) and **yellow** shows medium conservation. Designer antiviral **siRNAs** (**black arrows**) target **red 'hot'** highly conserved sequences.

- Molecular cloning methods to generate a reporter cell line to facilitate rapid siRNA screening.
- Cell culture and flow cytometry in order to screen designer short interfering (si)RNAs.

Masters/HDR projects:

- Molecular assays (RT-qPCR) in order to screen designer siRNAs.
- Protein assays (western blot analysis) to confirm siRNA antiviral activity.
- Multiplexing assays to asses and determine siRNA combinations for optimal antiviral effect.
- Microscopy to visualise siRNA and/or nanoparticle cell entry.

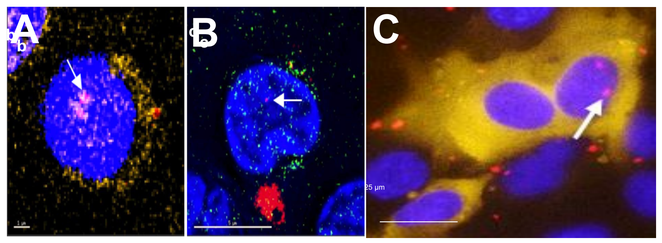

Figure 2. Nanoparticle delivery of siPromA into the nucleus of HIV-infected. A) activated, **B**) resting primary CD4+ T cells and **C**) SVG astrocytes. Primary human CD4+ T cells were infected with HIV-1 virus expressing VSVg pseudotyped HIV-1 mOrange or live HIV-1 GFP. Imaging 24 h post siPromA nanodelivery indicates successful nuclear entry of AlexaFluor647 labelled siRNA. Left bar 1 μm, middle bar 5 μm, right bar 25 μm. Magnification 60x.

- *In vivo* transgenic or humanised mouse studies (with collaborators at WEHI, Melbourne) to generate pre-clinical experimental data required for progressing into clinical trials.

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