

ANTI-VIRAL DRUG DISCOVERY PLATFORM

A novel, high-throughput assay focused on specifically inhibiting nuclear import of viral proteins. The assay has identified a number of anti-viral drug candidates targeting an evolutionarily conserved (but market differentiated) mechanism of action across numerous viral species.

- **Proprietary assay to identify potential 'First in class' or 'Best in Disease' antivirals with a novel and differentiated Mechanism of Action**
- **Specifically targets viral/host protein interface**
- **Applicable to various viral pathogens**
- **Potent exemplar molecules identified**

Using a selective counter screen to eliminate compounds that target host factors, the assay has identified a small number of potent anti-viral 'hits' from a library of just 1300 known compounds.

Exemplar molecules have been found to inhibit HCV*, HIV, dengue virus, West Nile Virus*, chikungunya virus* and Venezuelan Equine Encephalitis virus.^{2,3,4}

By way of example, the team (led by Dr Johanna Fraser) derived from the assay that the clinically tested synthetic retinoid derivative N-(4-hydroxyphenyl) retinamide (4-HPR; also known as Fenretinide), is efficacious against all 4 serotypes of dengue virus in cell culture infections with a similar EC₅₀ to the benchmark clinical compound balpiravir⁵ (Hoffman-La Roche). 4-HPR has potent efficacy in a dengue viremia mouse model, turning 100% lethality into a 70% survival rate at sub-optimal doses (Fig.1).

The screen has applicability to other indications such as cancer, where the nuclear localisation of specific factors is essential to disease progression.

THE OPPORTUNITY

Monash seek partners that wish to develop novel and differentiated anti-viral medicaments.

THE CHALLENGE

Nuclear entry of key viral components is essential for the efficient replication of many viruses. In the case of HIV, nuclear trafficking is required for genomic integration, while other viruses, such as dengue and RSV, require nuclear trafficking of viral proteins to restrict the host cell immune response. These events are critical to virus replication and survival. Molecules greater than 45 K_D require a nuclear localisation signal (NLS) to gain nuclear entry. Classically, this requires the recognition of a NLS in the cargo protein by a host importin receptor, which subsequently facilitates entry into the nucleus.

Transport of viral proteins from the cytoplasm through the nuclear envelope-embedded nuclear pore complex and into the nucleus remains a highly attractive although largely overlooked target for the development of differentiated anti-viral therapeutics.

THE TECHNOLOGY

Monash researchers, Professor David Jans and Dr Kylie Wagstaff have developed a novel high throughput screening assay for identifying inhibitors of nuclear import based on a roboticised amplified luminescent proximity homogeneous assay technology (AlphascreenT).¹ The assay screens for compounds that block association of a specific viral protein with a host importin receptor (e.g. NS5 protein from dengue virus with importin, or Integrase from HIV with importin).

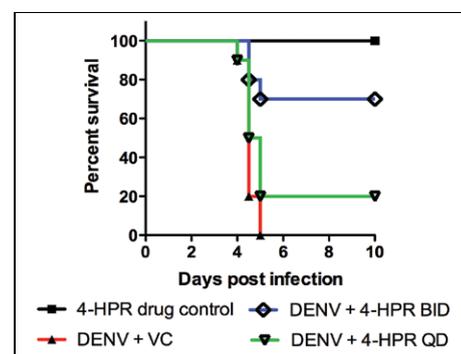


Figure 1: 4-HPR protects mice against a lethal challenge with dengue virus. AG129 mice (Schul et al., JID, 2007) were dosed once (QD) or twice (BID) daily with 20 mg/kg of 4-HPR for 5 days, starting at day 0. Mice were scored for viability until day 10 (10 mice per VC, BID and QD groups; VC – vehicle control).

References

1. An AlphaScreen®-based assay for high-throughput screening for specific inhibitors of nuclear import. Wagstaff *et al.* (2011) *J Biomol Screen*. Feb; 16 (2): 192-200.
2. Nuclear import and export inhibitors alter capsid protein distribution in mammalian cells and reduce Venezuelan Equine Encephalitis Virus replication. Lundberg *et al.* (2013) *Antiviral Res*. Dec; 100 (3):662-72.
3. Nuclear localization of dengue virus (DENV) 1-4 nonstructural protein 5; protection against all 4 DENV serotypes by the inhibitor Ivermectin. Tay *et al.* (2013) *Antiviral Res*. Sep; 99 (3):301-6.
4. Ivermectin is a specific inhibitor of importin α/β -mediated nuclear import able to inhibit replication of HIV-1 and dengue virus. Wagstaff *et al.* (2012) *Biochem J*. May 1; 443 (3):851-6.
5. Ten years of dengue drug discovery: progress and prospects. Lim *et al.* (2013) *Antiviral Res*. Nov; 100 (2):500-19

* Unpublished data

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