RIPK2 inhibitor for the treatment of multiple sclerosis and other inflammatory diseases

- Validated target in inflammation with an important role in multiple sclerosis
- Potent compound with a selective kinase profile
- In vivo single agent efficacy in a multiple sclerosis model

Scientific team
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Background
Diseases associated with aberrant activation of the innate immune system are an increasing burden on healthcare systems worldwide. Underlying many conditions is the dysregulation of inflammatory signalling pathways. Receptor-interacting serine/threonine-protein kinase 2 (RIPK2) plays a central role in regulating the innate immune system by acting downstream of the NOD bacterial peptidoglycan receptors. The RIPK2 signalling pathway has been causally linked with a number of inflammatory diseases including multiple sclerosis, inflammatory bowel diseases and rheumatoid arthritis. To date, no RIPK2-specific inhibitors have advanced to clinical trials, and, as such, there is a first-in-class opportunity to address these significant unmet needs.

With a focus on inflammatory conditions, researchers at the Walter and Eliza Hall Institute have developed a small molecule inhibitor of RIPK2, WEHI-345, that demonstrates:

- In vitro efficacy
- In vivo efficacy
- Favorable toxicity profile and oral bioavailability

The technology
In vitro
WEHI-345 exhibits potent and specific inhibitory activity against RIPK2 when tested in in vitro kinase assays. Purification and identification of WEHI-345 targets from cell lysates using quantitative mass spectrometry demonstrated that WEHI-345 is highly specific for RIPK2 (Figure 1). Furthermore, in RIPK2-dependent inflammation models, WEHI-345:
(i) suppresses in vitro production of inflammatory cytokines; and (ii) does not demonstrate cytotoxicity.

In vivo
WEHI-345 inhibits RIPK2 signalling in vivo, reducing levels of inflammatory cytokines in the serum of mice following stimulation of NODs (the principal upstream activator of RIPK2). Administration of WEHI-345 also resulted in a reduction in clinical score in experimental autoimmune encephalomyelitis (EAE), the gold standard model of multiple sclerosis (Figure 2).

Pharmacokinetics
Pharmacokinetic analysis of WEHI-345 demonstrated bioavailability in mice following administration by intraperitoneal injection or oral gavage with no adverse effects detected. There was no pathology or changes to white blood cell counts observed at the maximum tolerated dose.

Applications
WEHI-345 has the potential to treat a number of inflammatory diseases including multiple sclerosis, Crohn’s disease and rheumatoid arthritis.

In addition, WEHI-345 will serve as an invaluable tool to advance the understanding of RIPK2 and associated signalling proteins in the control of inflammation.
The institute has a successful track record in medicinal chemistry programs focused on hit-to-lead and lead optimisation. Moreover, the development team includes world experts in RIPK2 biology and inflammatory diseases.

### Key publications


### Figure Legend

**Figure 1.** WEHI-345 binds specifically to RIPK2. Quantitative SILAC proteomics was used to determine proteins bound to Sepharose-WEHI-345 from cell lysates. The six strongest binding kinases are shown.

**Figure 2.** WEHI-345 ameliorates symptoms in the gold standard *in vivo* model of multiple sclerosis. The compound was tested in the gold standard model: autoimmune encephalomyelitis (EAE) model. Following induction of EAE and treatment with WEHI-345 twice daily (20 mg kg⁻¹) for six consecutive days starting on day 9 after disease induction, clinical score was assessed on day 23.

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**Walter and Eliza Hall Institute of Medical Research**

The Walter and Eliza Hall Institute is Australia’s longest-serving medical research institute, celebrating its centenary in 2015. Our multidisciplinary research teams are focused on solving complex biological questions, and effectively linking laboratory research to the clinic. With a strong collaborative focus, the institute is a founding member of Biomedical Research Victoria, Cancer Trials Australia, BioGrid Australia and the Victorian Comprehensive Cancer Centre.

#### Research

- 750 research staff trained nationally and internationally
- 81 research laboratories

The institute’s scientists are driving innovative programs aimed at understanding, preventing and treating:

- Cancer: especially leukaemia, lymphoma, breast cancer, bowel cancer, ovarian cancer, lung cancer, pancreatic cancer and stomach cancer
- Immune disorders: including diabetes, rheumatoid arthritis, coeliac disease, rheumatic fever and other inflammatory conditions
- Infectious diseases: focusing on malaria, HIV, tuberculosis and hepatitis viruses

#### Research outcomes

- More than 300 publications annually
- 74% of publications in biomedical journals with an impact factor >15
- 34% of publications in the top 10% of their field

#### Strong translational focus

- Discovery pipeline fed by more than 300 projects
- Institute research is the basis of more than 100 clinical trials, and has resulted in 4 start-up companies and 5 spin-out companies
- Institute research has benefited more than 20 million people worldwide
- More than 470 collaborative partners across 120 cities in 43 countries

#### Opportunities for partnership

- Therapeutics
- Biomarkers and diagnostics
- Unique pre-clinical models
- Access to platform technologies including
  - bioinformatics
  - clinical translation centre
  - drug discovery including high-throughput screening and medicinal chemistry
  - systems biology including proteomics

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