Targeting MLKL for the development of novel anti-inflammatory therapeutics

- Novel pathway to treat inflammatory diseases
- Small molecule inhibitors of MLKL exhibit nM activity in vitro

Team

Catalyst Therapeutics
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The opportunity

The necroptosis (programmed necrosis) pathway has emerged as an essential mediator of inflammatory diseases and innate immunity. This pathway operates as a failsafe mechanism in conditions where the apoptosis machinery is incapable of signalling for cell death. Several studies have demonstrated the importance of necroptosis in chronic inflammation and pathology of infection, thereby implicating this pathway as an unexplored therapeutic opportunity for these conditions.

Walter and Eliza Hall Institute researchers have made breakthrough discoveries to recognise crucial components of the necroptosis pathway, which have advanced the understanding of two of the key effectors: Receptor Interacting Protein Kinase 3 (RIPK3), and the pseudokinase domain-containing protein, Mixed Lineage Kinase Domain-Like (MLKL). MLKL contains a kinase-like domain lacking enzymatic activity, however, this pseudokinase enzyme is an essential “molecular switch” component of the pathway. Phosphorylation by RIPK3 induces a conformational change in MLKL, leading to necroptotic cell death. Despite lacking kinase activity, MLKL contains a distinct nucleotide-binding pocket that can bind ATP in vitro and, as such, is a novel target for the development of ATP-competitive small molecules that modulate MLKL signaling and flux via the necroptosis pathway.

Existing anti-inflammatory drugs offer only short-term solutions and are often associated with a range of unfavorable side effects. Specifically targeting necroptotic signalling via inhibition of the pseudokinase MLKL may prevent side effects, owing to MLKL's unique active site properties.

The technology

Catalyst Therapeutics is currently developing small molecule inhibitors of MLKL.

In genetic studies, MLKL-deficient mice compellingly demonstrate that targeting this pathway offers an exciting and previously unexplored avenue for therapeutic intervention in inflammatory diseases, such as psoriasis (Figure 1).

Figure 1. Deletion of MLKL blocks development of a psoriasis-like disease in Sharpin<sup>cpdm</sup>/Mlkl<sup>-/-</sup> mice.
Proof-of-concept data demonstrates that small molecules targeting MLKL’s pseudo-active site effectively blocks necrototic cell death in tissue culture, providing a sound basis for the development of anti-MLKL compounds.

To this end, Catalyst Therapeutics, in collaboration with the Walter and Eliza Hall Institute and SYNthesis Med Chem, has commenced two programs to identify novel and potent small molecule inhibitors of MLKL: (i) biomolecular screen; and (ii) fragment-based screen. Lead optimisation studies are underway, in which the team: (i) has demonstrated cellular activity (anti-necroptotic, low nM); and (ii) is improving potency, selectivity and pharmacokinetics.

Applications
MLKL has been shown to modulate necroptotic signalling as a means of tackling a range of inflammatory diseases, including psoriasis, Crohn’s disease, inflammatory bowel disease and rheumatoid arthritis.

Furthermore, these small molecules will serve as invaluable tools in advancing the understanding of necroptosis.

Intellectual property
Catalyst Therapeutics has filed PCT and provisional applications that claim composition of matter and methods of use.

Opportunity for partnership
Catalyst Therapeutics is seeking a partner to co-invest in the development of compounds exhibiting inhibition of the necroptosis pathway. The ultimate goal is to develop clinical candidates that possess appropriate potency, safety and pharmacokinetic profiles.

The Walter and Eliza Hall Institute and SYNthesis Med Chem are extensively experienced and have successful track records in high-throughput screening and medicinal chemistry campaigns focused on hit-to-lead and lead optimisation.

Research within the Walter and Eliza Hall Institute has led to the development of distinctive protein structure data, cell lines, expression constructs, recombinant proteins and mouse models that have positioned the institute at the forefront of necroptosis research.

Key publications

Figure legend
Figure 1: Deletion of MLKL blocks development of a psoriasis-like disease in Sharpin<sup>cpdm/cpdm</sup> mice. (A) Sharpin<sup>cpdm/cpdm</sup> mice develop a severe skin inflammation at 12 weeks that is rescued by concomitant loss of Mlkl (B). (C) Like loss of MLKL, deletion of its immediate upstream regulator, RIPK3, leads to reduced disease severity at 12 weeks of age.