Combination therapy

Capturing the promise of p38 MAPK inhibition

Often in drug discovery, as our biological understanding increases, cellular pathways emerge that offer great potential for discovery of new drugs that can intervene with therapeutic effect. The challenge then is to develop those drugs and prove their safety and durable efficacy in clinical trials.

This article describes how the biotech company Kinarus Therapeutics revisited a family of cell signalling kinases that have long been of interest in the pharmaceutical industry, and discovered that a combination drug approach may be able to overcome previous obstacles to targeting this important pathway.

The family of kinases is the p38 Mitogen-Activated Protein Kinases (p38 MAPKs) which have been the subject of intense pharmaceutical research from 2005 to 2010. The p38 MAPKs consist of four related kinases transmitting environmental cues from receptors at the cellular membrane. Once activated, p38 MAPK phosphorylates downstream protein substrates leading to increased expression of genes that may lead to chronic pro-inflammatory and pro-fibrotic responses underlying autoimmune disorders such as rheumatoid arthritis (RA), psoriasis, and lupus – all conditions where patients are in urgent need of improved treatment options.

In RA, for example, macrophage-like and fibroblast-like synoviocytes are present in the joints of patients. The activation of these cells via p38 and other kinase signalling pathways leads to synovial hyperplasia and progressive joint destruction. The existing treatments are biological therapies that block tumour necrosis factor alpha (TNF-alpha), a cytokine which has a key role in driving the pathogenesis and persistence of RA. For decades, blocking TNF-alpha has been the mainstay of treatment with injectable drugs Remicade (infliximab - Johnson & Johnson) and Humira (adalimumab - AbbVie). The blockade of TNF has substantial therapeutic efficacy but about 40% of RA patients do not respond to treatment.

So, while TNF-alpha therapeutics are successful, there is clearly an important persisting unmet need. Indeed, considerable effort has gone into developing p38 MAPK inhibitor drugs over the last 20 years. Pharma companies optimized many p38 MAPK inhibitors to directly target the kinase catalytic pocket. Among these molecules are Novartis' acumapimod, GSK's losmapimod, Roche's pamapimod, and Vertex's neflamapimod. Having shown impressive acute efficacy in early trials these promising candidates, however, did not demonstrate sustained and significant efficacy in longer duration mid-stage clinical testing in their initial indications. Two of these drugs are being repositioned in other indications and a third, pamapimod, was in-licensed by Kinarus from Roche.

A study published in 2013, on Pfizer's p38 MAPK inhibitor PH-797804 in chronic obstructive pulmonary disease, singled out tachyphylaxis as the explanation for highly variable clinical data. Tachyphylaxis is either the short-term response to a drug, or the onset of drug tolerance that leads to a declining response over time.[1]

The short-term efficacy of the p38 MAPK inhibitors appears to be mechanistic in nature, which is to say, the feedback upregulation of the p38 signalling pathway itself. In a way, the cell tries to respond by stepping on the gas pedal while the brake is still engaged. This is illustrated by data for Bristol Myers Squibb's BMS-582949, a unique p38 MAPK inhibitor, also previously in development for the treatment of RA.

In contrast to inhibitors that bind only within the kinase pocket, the BMS drug interacted with the p38 protein differently. It blocked not only p38 kinase activity, but also its own phosphorylation by upstream kinases (MKK3/6) that tend to be overexpressed in response to the presence of an inhibitor. BMS-582949 demonstrated prolonged efficacy in RA patients who achieved the highest exposure to the drug. Although development of BMS-582949 was halted due to highly variable drug exposure, its example serves to demonstrate that alternative ways to target p38 may ultimately overcome the Achilles heel of earlier generation inhibitors.

At Kinarus, we set out to find a way to improve upon the efficacy and durability of p38 inhibition. In 2015, Kinarus obtained a licence to pamapimod, a clinically advanced p38-alpha inhibitor originally developed by Roche. Our research led us to the concept of combining pamapimod with pioglitazone, an approved insulin-sensitising drug used for the treatment of type 2 diabetes. The new entity is known as KIN001.

Why should a diabetes drug be able to overcome the limits of p38 inhibitors? It has long been recognised that individuals with insulin resistance or type 2 diabetes exhibit chronic systemic inflammation. These individuals often have increased levels of glucose, free fatty acids, and inflammatory cytokines, to which cells respond by activation of p38 signalling. Pioglitazone is an agonist of the peroxisome proliferator-activated receptor gamma (PPAR-gamma), a type II nuclear receptor that directly binds to the promoters of target genes to increase expression. PPAR-gamma was first recognised as a ligand-activated regulator of gene transcription in adipose tissue. PPAR-gamma promotes uptake of free fatty acids increasing adipocyte insulin sensitivity. It also regulates adiponectin, a signalling protein that acts as a homeostatic factor for regulating glucose level, lipid metabolism, and inflammation. Simply put, by improving metabolic control through PPAR-gamma activation, drugs such as pioglitazone can damp down the p38 pathway.

This reciprocal relationship between insulin resistance and inflammation suggests that p38 MAPK may be a key link between the two. The cellular crosstalk between p38 and PPAR-gamma is significant. Stimulation of p38 induced inflammation has been shown to promote cytoplasmic

retention of the PPAR-gamma protein, limiting its ability to induce its own transcriptional programme. Pioglitazone has been shown to oppose this effect, promoting PPAR-gamma shuttling into the nucleus. Similarly, pioglitazone promotes retention of phosphorylated p38 in the nucleus that occurs as a consequence of activation of the pathway.[2] This is an important aspect of crosstalk between p38 and PPAR-gamma. The retention of p38 in the nucleus allows sustained inhibition by a small molecule inhibitor since it sequesters p38 from cytoplasmic upstream kinases that would otherwise override the blocking activity of an inhibitor.

Repositioning in new indications

To capitalise on this approach, we undertook a systematic effort to explore the potential of the pamapimod/pioglitazone combination in diseases beyond RA. We identified several indications, supported by a strong scientific rationale, and then tested the combination against the single drugs, benchmarking against the current standard-of-care, in relevant preclinical disease models. Our data led to our current clinical pipeline for KIN001 in idiopathic pulmonary fibrosis (IPF), wet age-related macular degeneration (wet AMD), and Covid-19.

These efforts represent a traditional drug repurposing approach with a slight but important twist – the combination sidesteps tachyphylaxis, showing prolonged robust efficacy in preclinical testing.

The advanced clinical stage of pamapimod and the extensive experience with pioglitazone allows the direct launch of meaningful, fully powered, long duration clinical trials in patients. Pamapimod itself (not combined with pioglitazone) has been evaluated in 10 clinical studies to date, including two large Phase 2 trials in RA. In addition, pamapimod has completed all safety assessments comprising a complete long-term toxicology package that is sufficient to support a New Drug Application (NDA). Pioglitazone has been used in the medical management of type 2 diabetes for over 20 years with more than 30 million estimated patient years of use. These factors substantially reduce safety and other technical risks, leading to increased probability for success in Phase 3 and shorter time to regulatory approval.

And indeed, a compulsory three-month GLP toxicology study in rats showed that the combination of pamapimod and pioglitazone revealed no findings beyond those expected for pamapimod and pioglitazone alone. With a full package in-hand, regulators have authorised Phase 2 clinical testing in three indications.

Strong IP protection remains important in drug repurposing in order to create the incentive to invest in development, regulatory approvals and marketing, all costly requirements for gaining reimbursement and broad access for patients.

The composition of matter patent on pamapimod expired in 2022. However, because of the inventiveness of the new combination, Kinarus obtained new composition of matter protection for the KIN001 combination in the form of issued patents in the US, EU, China, and other countries. Additional use patents in various indications are also being pursued.

Idiopathic pulmonary fibrosis (IPF)

Idiopathic pulmonary fibrosis (IPF) is a relentlessly progressive disease with a median survival of two to four years from diagnosis. The current standard-of-care slows functional decline and disease progression but is also associated with significant safety and tolerability issues. Therefore, the unmet need in IPF is high. Drugs with anti-inflammatory and broad - rather than targeted - antifibrotic properties are more likely to be efficacious, as in IPF, a plethora of redundant and overlapping fibrogenic pathways are believed to contribute to disease pathogenesis.

We compared the effects of KIN001 vs. pioglitazone, pamapimod and pirfenidone, one of the two existing anti-fibrotic drugs on lung fibrosis in the bleomycin-induced pulmonary injury model in mice. Both once daily pioglitazone and once daily pamapimod, dosed individually, and in combination, significantly reduced normalised lung weights and fibrosis scores compared to vehicle-treated controls. The KIN001 combination of pamapimod/pioglitazone showed greater improvement in the extent of lung fibrosis compared with the groups receiving the single agents.

To explore the possible additive or synergistic mechanisms of the drug combination, we evaluated mRNA expression changes in lung tissue from similar experiments in mice. The results revealed that the KIN001 combination has synergistic activity to significantly downregulate multiple inflammatory cytokines and chemokines in comparison to the single drugs.

Kinarus is planning a one-year Phase 2 trial in recently diagnosed IPF patients. The primary endpoint is reduction in decline of lung function as measured by forced vital capacity (FVC) at one year. The unmet need in IPF remains high. Many drugs in development are tested in small Phase 2 trials of short duration. The ability to perform a longer-term study with KIN001 is an advantage of repurposing of late-stage candidates.

Wet age-related macular degeneration (AMD)

Wet age-related macular degeneration (wet AMD) is a common and progressive eye disorder that affects central vision and is caused by the abnormal growth of blood vessels that leak fluid into the macula of the retina. The causes of AMD are multifactorial. Several inflammatory pathways and mechanisms related to oxidative stress have been associated with AMD. Oxidative stress and reactive oxygen species (ROS), leading to apoptosis of retinal epithelial cells, have been shown to activate signalling pathways including the MAPKs. These data suggest that MAPK inhibitors may be useful for treatment of AMD and other retinal diseases.

KIN001 and each of its individual components were tested in two animal models of AMD. In a mouse model of AMD, KIN001 reduced pathological choroidal neovascularisation (CNV) of the eye after laser damage. KIN001 reduced CNV lesion leakiness as determined by fluorescein angiography (FA), and reduced retinal neovascularisation and fibrosis, as determined by histology for markers of endothelial cells and fibrotic tissue. In a second study in a non-human primate CNV model, KIN001 treatment reduced the development of clinically relevant advanced retinal lesions. These data supported our plan to conduct a Phase 2 clinical trial in wet AMD patients.

The current mainstay of treatment for wet AMD are Eylea and Lucentis, biologics that block the action of VEGF, a key factor that drives the pathological neovascularisation of the retina. These drugs are directly injected into the eye as often as monthly. In addition to the unpleasantness of intraocular injections, the burden on patients and caregivers is high, often leading to poor long-term compliance and continued vision loss. The real-world effectiveness of these therapies is insufficient, mainly due to sub-optimal durability as well as issues with compliance, cost, and access limitations. There is a clear unmet medical need for new or improved therapies.[3]

Our clinical strategy is to develop KIN001 as an oral treatment that complements anti-VEGF agents. Our KIN001-201 Phase 2 trial will assess the potential of KIN001 to decrease the number of anti-VEGF injections by providing an effective oral background therapy. Pharmacoeconomically, a reduction in the number of injections by 30% would offer a profound improvement for patients and payers. KIN001, as a more affordable and convenient treatment option, may also increase compliance and broaden patient access to an effective therapy.

Covid-19

SARS-CoV-2 infection upregulates components of the p38 MAPK pathway through the increased phosphorylation of several p38 substrates. A prototypical p38 MAPK inhibitor often employed in research studies, SB203580, demonstrated significant antiviral activity against SARS-CoV-2 *in vitro*, coincident with decreased expression of inflammatory cytokines such as interleukin-6 (IL-6) and TNF-alpha. A small *in vivo* study in SARS-CoV-2 infected mice showed that SB283580 also increased survival.

We recently published results of *in vitro* studies which showed that the KIN001 combination has potent and synergistic antiviral activity against SARS-CoV-2 replication.[4] Antiviral potency was similar across several cultured cell types and for important variants including SARS-CoV-2 Wuhan strain and the variants of concern (VoCs) Alpha, Beta, Gamma, Delta, and Omicron. These data support the combination of pamapimod and pioglitazone as a potential therapy to reduce duration and severity of disease in Covid-19 patients.

We launched two clinical studies in Covid-19: KINETIC in hospitalised patients to reduce the need for respiratory support, and KINFAST in ambulatory patients with mild to moderate disease to reduce severity and duration of symptoms. KINETIC was suspended after an interim analysis showed it was underpowered to demonstrate efficacy, potentially because of the trend towards less severe disease with newer variants and improved hospital treatment regimens. Notably, the KIN001 combination was safe and well-tolerated in KINETIC. KINFAST is currently ongoing and recruiting patients.

Outlook

The p38 MAPKs remain of high interest in many diseases beyond those mentioned in this article, but have continued to frustrate efforts toward drug development. New efforts, such as the approach at Kinarus, offer the potential to recapture the promise of p38 inhibition.

Other companies are also exploring new ways to tackle p38: several molecules that target the interaction between p38 and MK2, a downstream effector, have shown promise in preclinical and early clinical testing. The potential rewards are significant in terms of addressing high unmet medical needs. The next few years promise, once again, to be an exciting time in p38 MAPK drug development.

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