

## Fact Sheet – Autoimmune Disease

Over 50 different autoimmune diseases have been identified, affecting 3-5% of the population of Europe and North America. Autoimmune disease treatment has traditionally represented a field characterised by the use of dated therapeutics that sub-optimally treat their target diseases, often providing symptomatic relief rather than disease modification. An aberrant T-cell response is now known to underlie a number of major autoimmune diseases, including rheumatoid arthritis, psoriasis, multiple sclerosis, and inflammatory bowel disease, and the preferred approach for new treatments for these disorders is modulation of T-cell function. The advent of biologics has initiated a transformation in the therapeutic approach to immunology. However, this new wave of medicines has benefited some conditions more than others, and, given that many biologics suffer from significant shortcomings such as very high cost, inconvenient administration, hepatotoxicity, and increased risk of infection and malignancy, there is a widely recognised need for alternative, orally bioavailable, small-molecule therapies. Xention has a particular interest in developing new therapies for rheumatoid arthritis, multiple sclerosis and psoriasis. Rheumatoid arthritis (RA) is a progressive chronic inflammatory disease of the peripheral joints affecting ~6 million people worldwide, of which fewer than 50% are ultimately unable to continue to work or function normally. Multiple sclerosis (MS) is a chronic immune-mediated disease characterized by focal T-cell and mononuclear cell infiltrates, demyelination, and the development of sclerotic plaques within the CNS. There are around 2.5 million sufferers worldwide and the current market value for MS is estimated to be >\$7 billion per year. Psoriasis is a skin disease that affects approximately 2.5% of the world's population. The disease is characterised by epidermal hyperproliferation, vascular dilatation, and inflammation, leading to the clinical features of red, elevated, scaly plaques.

These diseases represent a very substantial unmet medical need. To address this need Xention is developing well-tolerated, orally bioavailable, inhibitors of two of the ion channels known to play a key role in the proliferation of T-cells. These are the Kv1.3 potassium channel and the CRAC channel. By developing drugs that inhibit these channels it will be possible to target specific autoimmune responses without promoting generalised immunosuppression.

### **Kv1.3 – A Target for Autoimmune Disease**

The role of autoreactive late-stage effector-memory T-cells ( $T_{EM}$ ) in the pathogenesis of a variety of autoimmune diseases including rheumatoid arthritis, psoriasis, multiple sclerosis, IBD and others is well established. Activation of these cells is followed by substantial up-regulation of Kv1.3 channel expression and, as a result, Kv1.3 becomes the predominant route of potassium efflux from the cell. Thus, selective blockade of Kv1.3 causes membrane depolarisation and inhibition of  $Ca^{2+}$  influx, leading to inhibition of cytokine production and cell proliferation. Experimental evidence has shown that inhibition of Kv1.3 selectively suppresses  $T_{EM}$  cell function while leaving other T-cell populations intact. This promotes suppression of disease relevant T-cells without a global generalised immunosuppression. This is expected to result in new safe, effective and well-tolerated treatments for a number of autoimmune disorders.

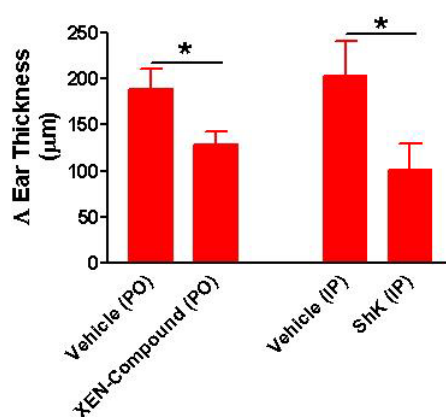
### ***Xention's Kv1.3 Programme***

We have applied our ion channel approach to discover and optimise modulators of Kv1.3 that have high potency and excellent selectivity against non-target channels. Using our approach

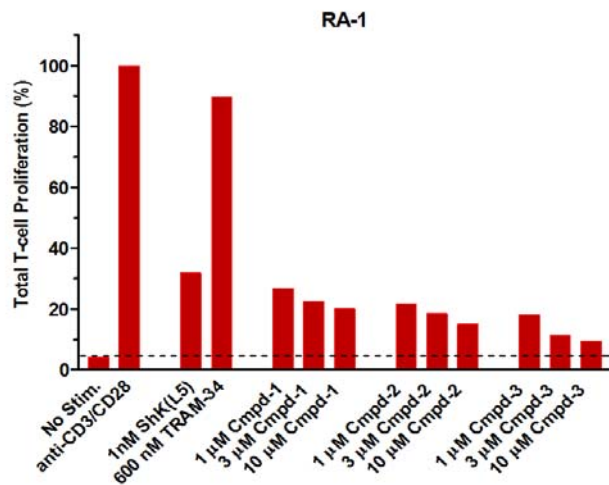
of incorporating electrophysiology into the lead optimisation process we have optimised three lead series and identified compounds not only with excellent potency and selectivity characteristics, but also compounds with good ADME and bioavailability properties that are suitable for further development.

### ***In Vitro and in vivo Proof of Concept Studies***

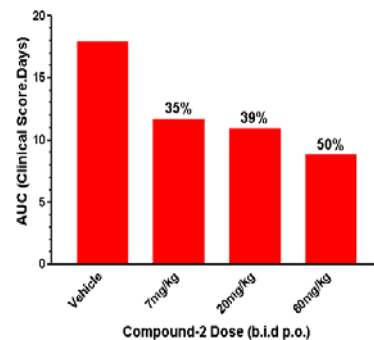
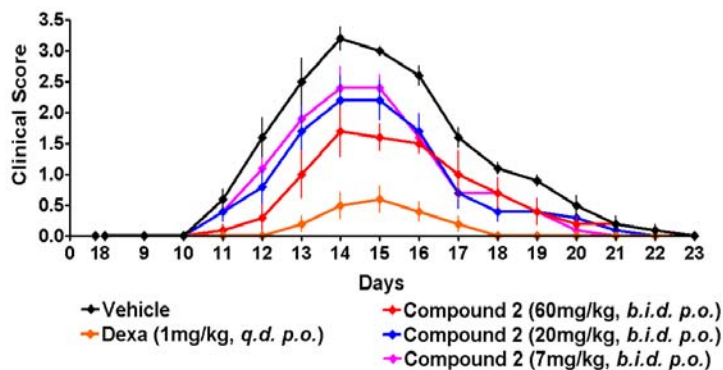
We have used the *in vivo* acute-contact dermatitis (ACD) model to examine the efficacy of our small molecules in the delayed-type hypersensitivity skin reaction mediated by T<sub>EM</sub>-cells. In the rat ACD model, following initial sensitization, rechallenge with oxazolone results in Kv1.3<sup>HIGH</sup> CD8+CD45RC- T<sub>EM</sub>-cells infiltrating the ear causing it to swell. Similar to psoriasis, the model is characterised by IFN $\gamma$  production and epidermal hyperplasia. Compound 1, when dosed orally, was effective in significantly reducing ear-swell in this model by a similar magnitude as the Kv1.3-selective peptide-toxin ShK (see below). The ACD assay is not only a model of psoriasis but also other type 1-cytokine pattern autoimmune diseases such as rheumatoid arthritis and MS. These data provide proof-of-concept data for an orally active Xention compound that is potentially effective in the treatment of psoriasis and other autoimmune diseases.



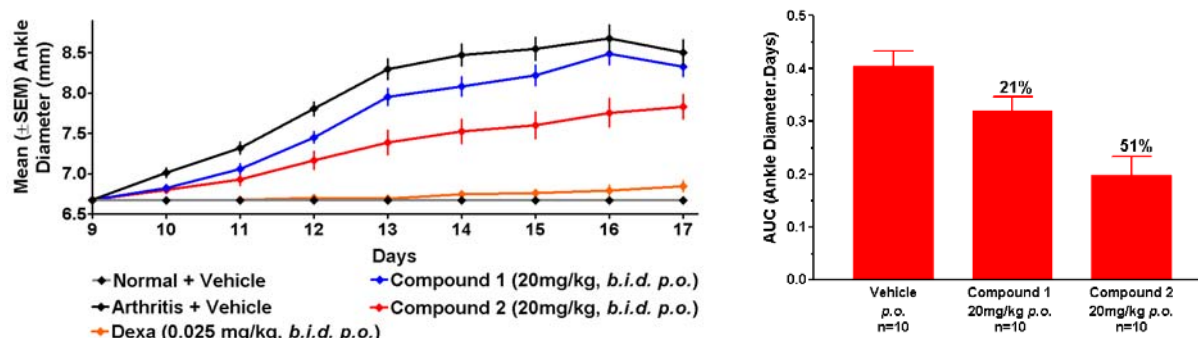
We have built on the above data set by looking closely at the usefulness of our Kv1.3 blockers for the treatment of multiple sclerosis and rheumatoid arthritis. In an initial set of experiments we studied the impact of our molecules on the anti-CD3/CD28 stimulated proliferation of T-cells purified from the synovial fluid of RA patients. Stimulation of RA T-cells with anti CD3/CD28 results in activation and proliferation. Incubation with a range of concentrations of Xention Kv1.3 inhibitors profoundly inhibited proliferation. This effect was not reproduced by the IKCa1 inhibitor TRAM-34, and exceeded the effects of the prototypical Kv1.3 channel inhibitor toxin ShK(L5).



To further demonstrate the efficacy of these compounds we have examined their effects using *in vivo* rat models of multiple sclerosis and rheumatoid arthritis. The rat Experimental Autoimmune Encephalomyelitis (EAE) model is a well established predictor of human efficacy in this disease area. Xention has carried out a detailed evaluation of a proprietary Kv1.3 inhibitor in this model, examining multiple dose levels using the oral route of delivery. The rats are evaluated for clinical score during the progression of the disease over a 3-week period. Our data showed a dose-dependent improvement in clinical score, reaching a 50% reduction at the top dose tested.



In a second *in vivo* efficacy model, for rheumatoid arthritis using the collagen-induced arthritis model in the rat, the two Xention Kv1.3 inhibitors tested showed a significant reduction in the clinical end point for this assay (mean ankle diameter).



## I<sub>CRAC</sub> as a target for autoimmune disorders

The I<sub>CRAC</sub> calcium current is the predominant route of calcium flux into non-excitable cells, including immune cells such as lymphocytes and mast cells, as well as blood platelets. Although the presence of store-operated signalling in immune cells has been known for many years, key proteins in the pathway have only recently been identified. The regulatory protein, Stim1 (stromal interaction molecule), which senses depletion of calcium from the endoplasmic reticulum and Orai1, which forms the pore of the plasma membrane calcium channel, are now known together to form the CRAC channel. In lymphocytes and mast cells, antigen or Fc receptor activation causes release of calcium ions from intracellular stores leading to influx of calcium ions through CRAC channels in the plasma membrane. The resulting increase in intracellular calcium levels activates calcineurin, a phosphatase that regulates the transcription factor, NFAT (nuclear factor of activated T-cells). In response to infection, and during transplant rejection, NFAT translocates to the nucleus and initiates a cascade of events leading to T-cell proliferation and an active immune response, including release of pro-inflammatory cytokines such as interleukin-2, interleukin-4 and interferon-gamma.

Xention, together with its research partner (Axxam SpA, Milan) has begun a screening and lead optimisation programme to identify novel, orally bioavailable, small molecule inhibitors of the I<sub>CRAC</sub> current as new autoimmune therapies for the treatment of diseases such as rheumatoid arthritis and multiple sclerosis and also to prevent transplant rejection.