Potassium channel modulators for the treatment of autoimmune disorders
Autoimmune disorders

- During normal immune responses white blood cells protect the body from antigens such as bacteria, viruses, toxins, cancer cells
  - The cellular immune system attacks infected cells with CD4 (helper) and CD8 (cytotoxic) T cells
  - The humoral system responds to bacteria and viruses by instigating attack by immunoglobulins produced by B cells
- In patients with an autoimmune disorder the immune system cannot distinguish between foreign antigens and healthy tissue, resulting in destruction of tissue or abnormal growth patterns
- Many different organ or tissue types may be affected
  - Blood vessels, connective tissue, nerves, joints, muscles, skin
- More than 80 discrete autoimmune disorders have been identified
- The aggregate prevalence of AI disorders is ~5000 per 100,000
  - Incidence is higher in women than men
- Different AI disorders have different molecular phenotypes
### Autoimmune phenotypes

*Effector memory T cells and class switched B cells predominate*

<table>
<thead>
<tr>
<th>Disease</th>
<th>Target organ</th>
<th>Autoreactive lymphocyte</th>
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<tbody>
<tr>
<td>Psoriasis</td>
<td>Skin</td>
<td>CD45RO+CD45RA- CCR7- TEM cells</td>
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<tr>
<td>Grave disease</td>
<td>Thyroid</td>
<td>IgD-IgG+ memory B cells</td>
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<tr>
<td>Rheumatoid arthritis</td>
<td>Joints</td>
<td>CD28nullCD45RA-CCR7- TEM cells</td>
</tr>
<tr>
<td>Hashimoto disease</td>
<td>Thyroid</td>
<td>CD45RA- memory T cells</td>
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<tr>
<td></td>
<td></td>
<td>IgD-IgG+ memory B cells</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>Skin, mucous membranes</td>
<td>CD45RO+ memory T cells</td>
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<tr>
<td>Crohns disease</td>
<td>Digestive tract</td>
<td>CD45RO+CD28null memory T cells</td>
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<td>Type I diabetes mellitus</td>
<td>Pancreas</td>
<td>CD28 costimulation-independent memory T-cells</td>
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<tr>
<td>Multiple sclerosis</td>
<td>CNS</td>
<td>CD28 costimulation-independent CD45RO+CD45RA-CCR7- TEM cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgD-CD27+ class-switched memory B cells</td>
</tr>
</tbody>
</table>
Prevalence of AI disorders

- Third most common category of disease, after cancer and heart disease, affecting 4% of the population
- Global market reached $34Bn in 2010 and is expected to reach $55-77Bn by 2016 (CAGR ~10%)
- 14 drugs at or near blockbuster status (7 >$3Bn)
Leading product classes

- Large molecule therapies predominate
- *Intra venous* dosing is required for the majority
- Significant opportunity for a novel, orally bioavailable, small molecule approach
The autoimmune pipeline

Biologics still predominate
Opportunity for new targets providing small molecule therapeutics
T cell machinery overview

- Antigen presenting cell activates the T cell receptor
- PLCγ releases IP₃ which activates the IP₃ receptor to release Ca²⁺ from the ER
- T cell and B cell activation and proliferation are dependent on Ca²⁺
- Maintenance of this process depends on ion channel activity
Ion channels in T cells

- Ca\textsuperscript{2+} depletion in the ER leads to association of Orai1 and STIM1 to form the CRAC channel
- Ca\textsuperscript{2+} influx through CRAC depolarises the T cell
- K\textsuperscript{+} channels are recruited to the immune synapse and activated to induce repolarisation, maintaining the driving force for Ca\textsuperscript{2+} influx
- Elevated intracellular Ca\textsuperscript{2+} drives transcription factors such as nuclear factor of activated T cells

Selective block of K\textsuperscript{+} channels leads to membrane depolarisation, inhibits Ca\textsuperscript{2+} influx and shuts down cytokine production and immune cell proliferation
Role of ion channels in T cells

- At the level of the immune synapse, ion channels regulate local ionic concentrations, assembly of molecular aggregates that form signalling complexes and trans-synaptic signalling.

- At the level of the whole T cell, ion channels regulate membrane potential, Ca^{2+} influx, K^+ efflux and Cl^- efflux, leading to changes in gene expression, motility and cell volume.

- Expression of Ca^{2+} and K^+ channels can vary greatly following activation and differentiation, and may form a positive feedback loop sensitising T cells to produce a larger Ca^{2+} signal following repeat challenge with the same antigen.

- At the level of the whole animal, manipulating ion channel currents in T cells could provide relief from inappropriate acute T cell activation (KCa3.1, CRAC) or chronic inflammatory and autoimmune disorders (Kv1.3) – why this differentiation?
T cell maturation changes K⁺ channel levels

- During the first stage of an immune response naïve T cells develop into naïve effector T cells in the lymph nodes
- These produce cytokines, proliferate, then most die
T cell maturation changes K+ channel levels

- Some naïve effector T cells differentiate into long-lived central memory T cells
- When activated by an antigen these produce cytokines and proliferate, then most die
T cell maturation changes K⁺ channel levels

- Repeated antigen stimulation, as in autoimmune disorders and chronic infections, causes $T_{CM}$ cells to differentiate into $T_{EM}$ cells.
- These do not need to home to the lymph nodes for activation.

**Diagram:***

- Naïve T cell: $CD4^+ CCR7^+ CD45^+$
- Naïve Effector: $CD4^+ CCR7^+ CD45^-$
- Effector memory: $CD4^+ CCR7^- CD45^-$
- Central memory: $CD4^+ CCR7^- CD45^-$

**Quiescent**

**Activated**
T cell maturation changes K\(^+\) channel levels

- These different types of T cells have different patterns of potassium channel expression
- Membrane potential in activated T\(_{EM}\) cells is dominated by Kv1.3

\[\begin{align*}
\text{Naïve T cell} & \quad \rightarrow \\
\text{Naïve Effector} & \quad \rightarrow \\
\text{Effector memory} & \quad \rightarrow \\
\text{Central memory} & \quad \rightarrow \\
\text{Quiescent} & \quad \rightarrow \\
\text{Activated} & \quad \rightarrow
\end{align*}\]
B cell maturation changes K⁺ channel levels

- A similar pattern of development and changes in potassium channel expression occurs for B cells

Kv1.3 > KCa3.1; KCa3.1 dominates
Kv1.3 < KCa3.1; KCa3.1 dominates
Kv1.3 >>> KCa3.1; Kv1.3 dominates

Quiescent        Activated
Targeting ion channels for AI disorders

- Ion channel expression patterns in different T cell subsets change with activation and differentiation
- Targeting KCa3.1 or CRAC channels will suppress differentiation of naïve T cells and B cells and suppress all immune responses
  - Currently in early lead optimisation with CRAC inhibitors
- Targeting Kv1.3 channels will suppress only terminally differentiated T cells and B cells, suppressing chronic and autoimmune responses
  - Advanced lead Kv1.3 inhibitors
  - Indicated for MS, rheumatoid arthritis, psoriasis, other AI disorders
Kv1.3 as a drug target

- The functional channel is composed of 4 $\alpha$ subunits encoded by *KCNA3*
- Kv1.3 expression is almost entirely confined to immune cells
- Inhibited by a number of potent and selective toxins - e.g. ShK (and derivatives) & ADWX-1
- Toxin and small molecule Kv1.3 blockers have been shown to reduce T cell and B cell proliferation and cytokine production
- Substantial validation in human disease
The role of Kv1.3 in Multiple Sclerosis

*CNS infiltrating T cells express high levels of Kv1.3*

- MS is characterized by CNS cell infiltrates of activated T cells and macrophages
- These display an effector memory phenotype in post-mortem examination
- Kv1.3 expression is elevated in these cells
The role of Kv1.3 in Multiple Sclerosis
inhibition of Kv1.3 reduces clinical score in an EAE model

- Proliferation of rat myelin basic protein-specific T cells is inhibited by Kv1.3 blocking toxins\(^1\)
- ShK-L5 reduces the clinical score in an EAE model of MS\(^2\)
- Kv1.3 KO mice have a lower incidence & severity of EAE\(^3\)

The role of Kv1.3 in Rheumatoid Arthritis

$T_{EM}$ cells from RA synovial fluid express high levels of Kv1.3

- Staining of synovial tissues from RA patients revealed infiltrating T cells express Kv1.3 but not CCR7, indicating a $T_{EM}$ cell phenotype

- T cells from synovial fluid of patients with RA expressed higher levels of Kv1.3 than those from patients with osteoarthritis (OA)

1) Beeton. PNAS (2006) PMID: 17088564
The role of Kv1.3 in Rheumatoid Arthritis

inhibition of Kv1.3 reduces disease severity in a RA model

- ShK-186 (SL5) reduced the number of joints affected in a pristane-induced model of RA\(^{(1)}\)

1) Beeton. PNAS (2006) PMID: 17088564
Kv1.3 Inhibitor pharmacology

- **Toxins**
  - Charybdotoxin, margatoxin
  - Sea anemone family of toxins: ShK, ShK-L5,

- **Antibodies**
  - E314 has high affinity, is selective, and produces functional inhibition

- **Natural product small molecules and analogues**
  - Khellinones, correolides, Psora family of compounds

- **Drug like small molecules**
  - Scaffolds identified by several groups; few combine potency, selectivity and sustained oral exposure
Discovery of small molecule Kv1.3 inhibitors

- Objective is a potent, selective, orally bioavailable small molecule with a PK profile that provides sustained Kv1.3 inhibition
  - Essential to sustain T cell suppression for long periods in order to achieve a significant therapeutic effect
- Starting points identified by HTS, computational approaches, and re-profiling of a substantial internal Kv1.x dataset
- Screening using information-rich electrophysiology assays
  - Target, gene family and cardiac safety screening conducted on the same automated patch clamp platform
- Progression into T cell electrophysiology and proliferation assays, \textit{in vivo} testing in psoriasis, multiple sclerosis and rheumatoid arthritis assays
Automated patch clamp screening

- Kv1.3 QPatch assay is reliable & pharmacologically validated

- Seal and whole-cell parameters determine success rate & data quality
APC assays provide additional information

- Kv1.3 primary assay can identify different mechanisms-of-action
  - Multiple cursors to assess different types of block; enrich SAR
  - Charge transfer to measure inhibition independently of mechanism
Structural information is available

1J95 --> Kcsa Bacterial

2A79 --> Kv1.2 Rat, Open Channel, 2.90A (2005)

2R9R --> Rat, Chimeric, Kv2.1 Voltage Sensor, 2.40A (2007)
Computational models support SAR

HTVS

- Model is sufficiently defined to allow virtual screening of large real and virtual compound libraries
- Iterative *in silico* screening followed by real ‘wet’ electrophysiology rapidly refines the computational model
- Similar approach applied to Kv1.1/1.2 to identify novel hits with *in vivo* activity in a model of MS nerve damage
Current status

- Multiple series of potent small molecule Kv1.3 inhibitors
  - Potency in the low nM range
  - Pharmacophores developed and validated for Kv1.3 and other gene family members
  - Binding site hypothesis developed using homology models
- Selective molecules identified
  - Gene family selectivity
  - Cardiac selectivity (hERG, Nav1.5, Cav1.2)
- Good ADME properties
- Compounds inhibit human $T_{EM}$ cell Kv1.3 currents and proliferation
- Compounds are active in established *in vivo* models of autoimmune disease
Lead compound overview

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; Kv1.3 (nM)</th>
<th>hERG</th>
<th>Nav1.5</th>
<th>Kv1.5</th>
<th>Sol (µM)</th>
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<th>DLM (%)</th>
<th>HLM (%)</th>
<th>Cmax (ng/ml)</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt; (min)</th>
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- Potent small molecule Kv1.3 inhibitors identified
- Most examples have good physicochemical and ADME properties
- SAR for gene family selectivity developed using electrophysiology data

![xent.png](attachment://xent.png)
# Lead compound overview

**Gene family selectivity**

<table>
<thead>
<tr>
<th>Compd</th>
<th>IC$_{50}$ Kv1.3 (nM)</th>
<th>hERG x Fold</th>
<th>Nav1.5 x Fold</th>
<th>Kv1.5 x Fold</th>
<th>Kv1.1 x Fold</th>
<th>Kv1.2 x Fold</th>
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</table>

SAR for gene family selectivity developed
In vivo proof of concept
rat oxazolone-induced contact dermatitis (DTH) model

- Initial treatment with oxazolone triggers T cell activation and sensitisation
- Kv1.3 blocker (po) significantly attenuated ear swelling following second oxazolone challenge
- Similar magnitude of effect to that elicited by ShK (ip)
In vivo proof of concept
rat EAE model

- Kv1.3 blocker produced a significant, dose proportional, attenuation of clinical score in this multiple sclerosis model
In vivo proof of concept
rat collagen-induced arthritis model

- Kv1.3 blocker produced a significant attenuation of arthritic symptoms
**Xention Kv1.3 programme**

*Summary*

<table>
<thead>
<tr>
<th>PROPERTY</th>
<th>TARGET</th>
<th>STATUS</th>
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<tbody>
<tr>
<td>Potency</td>
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<tr>
<td>Kv1.x gene family selectivity</td>
<td>&gt;30-fold</td>
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<tr>
<td>Cardiac Selectivity</td>
<td>&gt;150-fold (hERG, Na\textsubscript{v}1.5, Ca\textsubscript{v}1.2)</td>
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<td>ADME</td>
<td>Drug-like</td>
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<td>Bioavailability</td>
<td>Orally bioavailable</td>
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<tr>
<td><em>Ex Vivo Efficacy</em></td>
<td>Human synovial T\textsubscript{EM}-cell proliferation assay</td>
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<tr>
<td><em>In Vivo Efficacy</em></td>
<td>Disease relevant animal models (DTH, EAE and CIA)</td>
<td>✓</td>
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</table>

Many thanks to the research team at Xention