A drug discovery collaboration between Japanese pharma and a UK SME CRO successfully developed novel small molecule inhibitors of the $K_v1.3$ channel to treat autoimmune disease

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Introduction

Ion channels represent 15 – 20% of historic drug approvals and recent drug discovery projects. Many ion channel families (Na_v, Ca_v, TRPx and GABA) are validated as therapeutic targets based on human genetics, animal models and selective pharmacology. However, ion channels are challenging targets requiring specialist target class knowledge and screening technology such as automated patch clamp (APC) electrophysiology.

Here we outline our example where a pharma company interested in ion channels, but lacking expertise and screening platforms turned to a specialist CRO to fill this knowledge gap. In our case a Japanese pharma company with plate-based assay data wanted to expand medicinal chemistry SAR by accessing high quality APC and ion channel expertise.

During the collaboration selective $K_v1.3$ modulators with nM potency and efficacy against human T-cells were identified.

1. Fast data turn around time

Efficient shipping system and integration into compound management at Metrion ensured rapid data turn around

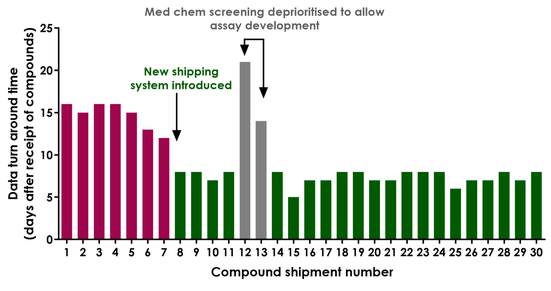


Figure 2: Using automated patch clamp allows rapid screening
Example data turn around time for the first 30 weekly shipments received from Japan. Metrion adapted its compound handling process to ensure data was returned in a timely manner to keep pace with SAR in Japan. Data for tier 1 assays was returned to partner within 5 working days of compound receipt from Japan.

2. Consistent pharmacology

Use of positive control allows QC of assay performance and a benchmark to drive SAR

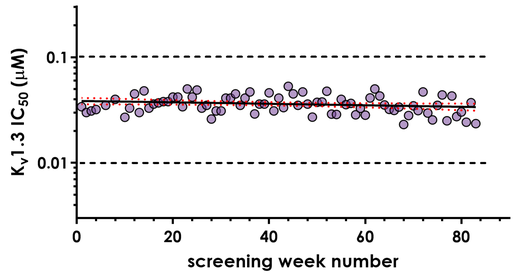


Figure 3: Consistent primary screening assay using the QPatch
Consistent pharmacology achieved for positive control used in QPatch $K_v1.3$ assay. Reproducibility well within industry standard (dashed lines show <3-fold variation) with low week to week variation (red dotted line shows 95% CI). Assay was stable so that potency achieved in week 1 for a specific compound would be repeated when tested 80 weeks later.

3. Using QPatch to drive robust SAR

Using QPatch to drive SAR meeting potency targets

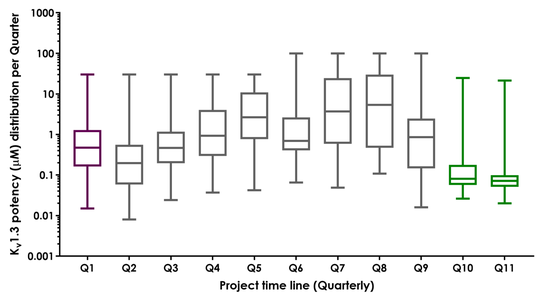


Figure 4: Using QPatch to progress SAR development
Fast data turn around times coupled with a robust assay assisted meeting medicinal chemistry targets. Shown is a box whisker distribution plot of potency values for compounds grouped per quarter. Initial SAR assessment (Q1) showed good range in potency, however, optimisation of other properties was required (grey Q2 – Q9) before the target potency (IC_{50} < 0.1 µM) could be achieved (green - Q10 and Q11).

Collaboration structure and management

Screening cascade and partner contributions

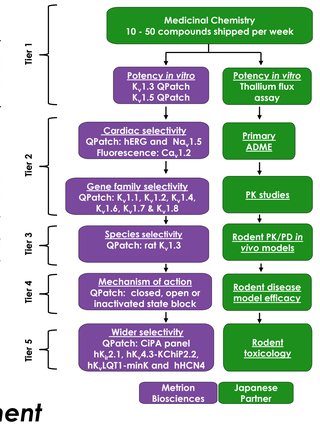


Figure 1: Screening cascade
The Japanese pharma partner provided the medicinal chemistry SAR which supplied compounds into a 5 tier screening cascade.

Metrion contributed: APC assays using the QPatch system for all tiers. The first tier the primary assessment of potency against $hK_v1.3$ and a gene family member, $hK_v1.5$.

Japanese partner contributed: ADME, PK, ex- and *in vivo* efficacy and toxicology studies

Collaboration management

Reporting level	Responsibilities	Personnel	Frequency
Project management team	• Review screening data generated before shared back with Chemists in Japan • Discuss assay development progress • Communicate updates from studies in Japan • Ad-hoc modifications to screening priorities	• Metrion project manager • On site Japanese pharma representative	**Weekly** In person at Metrion
Science meeting	• Science exchange from both partners • Ensure targets set at JRC level are on schedule	• Metrion and Japan lab scientists & project management teams	**Quarterly** By telecom
Joint Research Committee (JRC)	• Ratify decisions made at science meeting • Nominate compounds for progression to different tiers of the screening cascade • Assess resourcing needs • Agree screening cascade and priorities for next quarter • Decide whether project milestones had been achieved	• **Three nominated from each partner** **Metrion:** • Project manager, Chief Scientific Officer and Chief Operating Officer **Japan:** • Research co-ordinator and senior directors of biology and chemistry	**3 – 6 months** 2-day on site visit

4. Metrion's APC expertise used successfully to support the collaboration at each tier

Establishing gene family counterscreens (Tier 2)

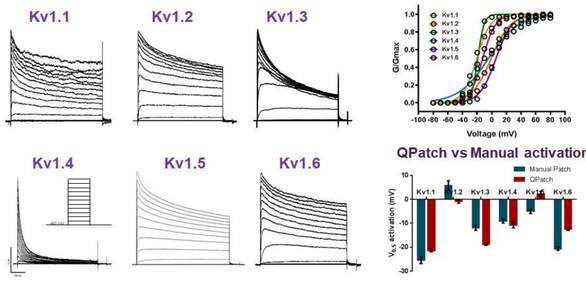


Figure 5: Biophysical characterisation of K_v1 family on QPatch
Important that selectivity of compounds was assessed using the same platform to exclude platform bias. Therefore, full biophysical assessment was performed on QPatch before testing compounds.

Rat $K_v1.3$ cell line required for cascade (Tier 3)

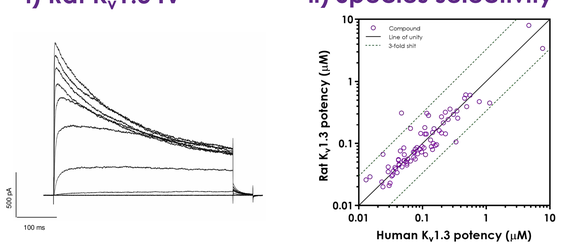
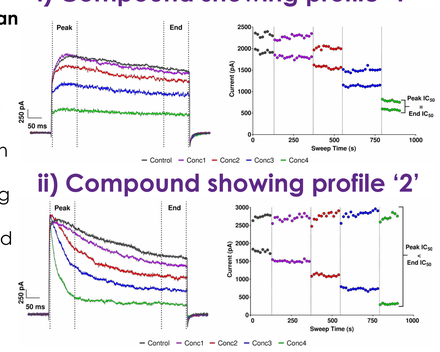


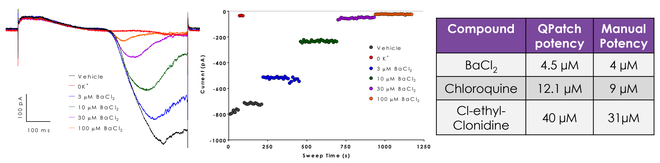
Figure 6: Determine species liability of lead compounds
A rat $K_v1.3$ cell line was generated as rat models were principally used for *in vivo* testing (tier 3 and 4 of cascade) and due to the lack of commercial supplier. **(i)** Example current voltage (IV) relationship for $rK_v1.3$ cell line. **(ii)** screening of compounds against rat $K_v1.3$ showed minimal difference due to species (< 3 fold).

Exploring mechanism of action using QPatch (Tier 4)

Figure 7: Potency and mechanism of action can be delineated in the same experiment
Placing a variety of cursors on current trace provided important information in translation of data from models:
(i) a compound showing a state independent mechanism of block and
(ii) an example compound showing state dependent block.



Extended cardiac panel testing (Tier 5)



Compound	QPatch potency	Manual Potency
$BaCl_2$	4.5 µM	4 µM
Chloroquine	12.1 µM	9 µM
Cl-ethyl-Clonidine	40 µM	31µM

Figure 8: Successful assay transfer of a cardiac ion channel from manual patch clamp platform to QPatch
(i) Example of assay transfer from manual to APC for $hK_v2.1$ **(ii)** shows an example concentration and time dependent block by $BaCl_2$ **(iii)** QPatch assays require full pharmacology validation.

5. Optimised $K_v1.3$ molecules show nM potency in human T-cells

Potent inhibition of IFNγ production from human CD4 effector memory T-cells (T_{EM})

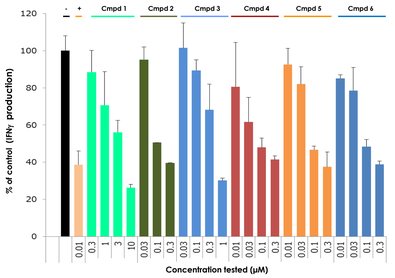


Figure 9: Example human T-cell *ex vivo* data generated by collaborator showing nM inhibition of stimulated IFNγ release

Conclusions

- **Metrion's ion channel expertise combined with the use of automated patch clamp successfully supported a screening cascade over three years that led to identification of potent and selective compounds that demonstrated *ex vivo* human T-cell and *in vivo* animal model efficacy.**
- **A novel outcome of this collaboration was the licencing by Metrion of the $K_v1.3$ IP from the Japanese partner to further develop into preclinical assets using internal R&D resources and UK SME grant support.**

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