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 expert target class knowledge and specialised screening technology such as automated patch clamp (APC) electrophysiology.

Here we outline our example where a Japanese pharma company interested in ion channels, but lacking expertise and screening platforms turned to Metrion Biosciences, a specialist ion channel focused CRO, to fill this knowledge gap.

In this example case study the Japanese pharma company had validated a plate-based screening assay, but 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 turnaround time

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

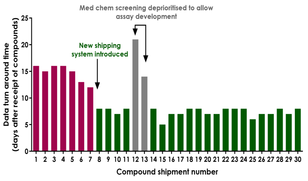


Figure 1: Automated patch clamp enables high quality, rapid turnaround compound screening
Example data turn around time for the first 30 weekly shipments received from Japan. Metrion adapted its compound handling process to ensure data was returned in a timely manner to keep pace with SAR in Japan. Data for tier one assays was returned to partner within five working days of compound receipt from Japan.

2. Consistent pharmacology

Positive control enables QC of assay performance and provides a benchmark to drive SAR

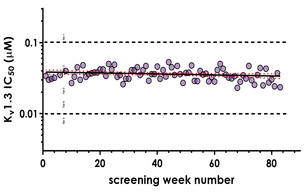


Figure 2: Consistent primary screening assay using QPatch 48
Consistent pharmacology achieved for positive control used in the QPatch $K_v1.3$ assay. Reproducibility well within industry standard (dashed lines show < three-fold variation) with low week to week variation (red dotted line shows 95% CI). Assay was stable so that potency achieved in week one for a specific compound would be repeated when tested eighty weeks later.

3. Using QPatch 48 to drive robust SAR

Potency targets met using QPatch 48 to drive SAR

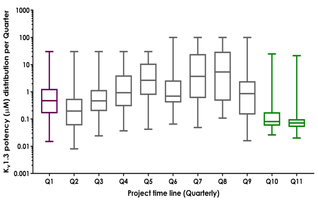


Figure 3: Using QPatch 48 to progress SAR development
Fast data turnaround times coupled with a robust assay enabled medicinal chemistry targets to be achieved. Shown is a box whisker distribution plot of potency values for compounds grouped per quarter. Initial SAR assessment (Q1) revealed an acceptable range of potencies, 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).

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

Establishing gene family counterscreens (Tier two)

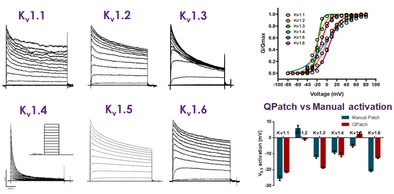


Figure 4: Biophysical characterisation of K_v1 family on QPatch 48
K_v1 family selectivity of compounds required assessment using the same platform to exclude platform bias. Therefore, full biophysical assessment was performed on QPatch 48 before compounds were progressed further through the screening cascade.

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

i) Rat $K_v1.3$ IV

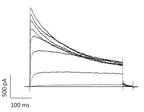
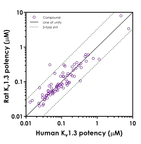


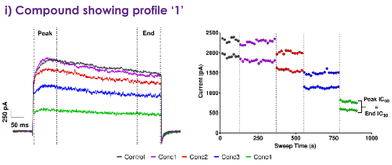
Figure 5: Determine species liability of lead compounds
A rat $K_v1.3$ cell line was generated, as rat models were principally used for *in vivo* testing (tier three and four of cascade) and due to the lack of commercial supplier.
(i) Example current voltage (IV) relationship for $rK_v1.3$ cell line.
(ii) Screening of compounds against rat $K_v1.3$ showed minimal difference due to species (< three fold).

ii) Species selectivity



Exploring mechanism of action using Qpatch 48 (Tier four)

i) Compound showing profile '1'



ii) Compound showing profile '2'

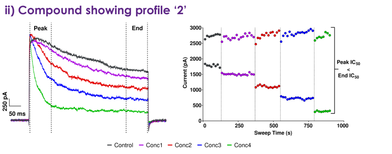
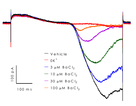


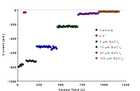
Figure 6: Potency and mechanism of action can be determined in the same experiment
Placing a variety of cursors on current trace provided important information in translation of data from models:
(i) a compound showing a state independent mechanism of block and **(ii)** an example compound showing state dependent block.

Extended cardiac panel testing (Tier five)

i) Current Trace



ii) Block by $BaCl_2$



iii) Pharmacology

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 7: Successful assay transfer of a cardiac ion channel from manual patch clamp platform to QPatch 48
(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 48 assays require full pharmacological 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})

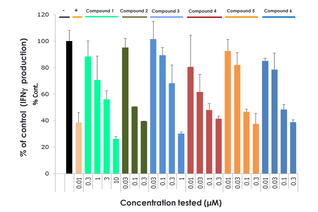


Figure 8: Example human T-cell *ex vivo* data generated by collaborator showing nM inhibition of stimulated IFNγ release

Collaboration structure and management

Screening cascade and partner contributions

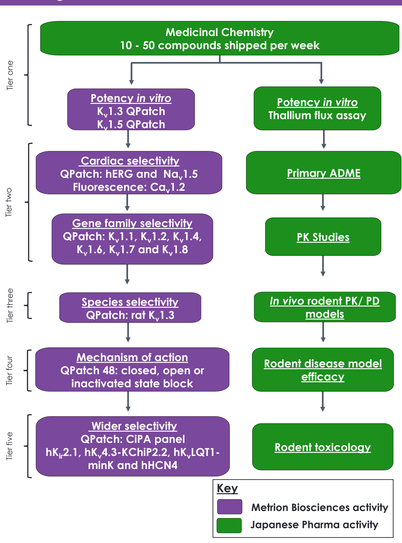


Figure 9: Screening cascade
The Japanese pharma partner provided the medicinal chemistry SAR which supplied compounds into a five tier screening cascade.

Metrion contributed:
APC assays using the QPatch 48 assay 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

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 and 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	**Three to six months** Two-day on site visit

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.**
- **Metrion Biosciences has acquired the $K_v1.3$ intellectual property rights from the Japanese partner and is currently further developing the lead compounds into preclinical assets using internal research resources and UK SME grant support.**

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Poster Presenter: Dr Andrew Southan (CEO)

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