Drug Discovery Using Stabilised GPCR Technology

Dr Eugenia Sergeev

11th November 2019 | What A Chemist Needs To Know About Biology 1: In Vitro Biology
Disclaimer

The material that follows is a presentation of general background information about Sosei Group Corporation and its subsidiaries (collectively, the “Company”) as of the date of this presentation. This material has been prepared solely for informational purposes and is not to be construed as a solicitation or an offer to buy or sell any securities and should not be treated as giving investment advice to recipients. It is not targeted to the specific investment objectives, financial situation or particular needs of any recipient. It is not intended to provide the basis for any third party evaluation of any securities or any offering of them and should not be considered as a recommendation that any recipient should subscribe for or purchase any securities.

The information contained herein is in summary form and does not purport to be complete. Certain information has been obtained from public sources. No representation or warranty, either express or implied, by the Company is made as to the accuracy, fairness, or completeness of the information presented herein and no reliance should be placed on the accuracy, fairness, or completeness of such information. The Company takes no responsibility or liability to update the contents of this presentation in the light of new information and/or future events. In addition, the Company may alter, modify or otherwise change in any manner the contents of this presentation, in its own discretion without the obligation to notify any person of such revision or changes.

This presentation contains “forward-looking statements,” as that term is defined in Section 27A of the U.S. Securities Act of 1933, as amended, and Section 21E of the U.S. Securities Exchange Act of 1934, as amended. The words “believe”, “expect”, “anticipate”, “intend”, “plan”, “seeks”, “estimates”, “will” and “may” and similar expressions identify forward looking statements. All statements other than statements of historical facts included in this presentation, including, without limitation, those regarding our financial position, business strategy, plans and objectives of management for future operations (including development plans and objectives relating to our products), are forward looking statements. Such forward looking statements involve known and unknown risks, uncertainties and other factors which may cause our actual results, performance or achievements to be materially different from any future results, performance or achievements expressed or implied by such forward looking statements. Such forward looking statements are based on numerous assumptions regarding our present and future business strategies and the environment in which we will operate in the future. The important factors that could cause our actual results, performance or achievements to differ materially from those in the forward looking statements include, among others, risks associated with product discovery and development, uncertainties related to the outcome of clinical trials, slower than expected rates of patient recruitment, unforeseen safety issues resulting from the administration of our products in patients, uncertainties related to product manufacturing, the lack of market acceptance of our products, our inability to manage growth, the competitive environment in relation to our business area and markets, our inability to attract and retain suitably qualified personnel, the unenforceability or lack of protection of our patents and proprietary rights, our relationships with affiliated entities, changes and developments in technology which may render our products obsolete, and other factors. These factors include, without limitation, those discussed in our public reports filed with the Tokyo Stock Exchange and the Financial Services Agency of Japan. Although the Company believes that the expectations and assumptions reflected in the forward-looking statements are reasonably based on information currently available to the Company’s management, certain forward looking statements are based upon assumptions of future events which may not prove to be accurate. The forward looking statements in this document speak only as at the date of this presentation and the company does not assume any obligations to update or revise any of these forward statements, even if new information becomes available in the future.

This presentation does not constitute an offer, or invitation, or solicitation of an offer, to subscribe for or purchase any securities. Neither this presentation nor anything contained herein shall form the basis of any contract or commitment whatsoever. Recipients of this presentation are not to construe the contents of this summary as legal, tax or investment advice and recipients should consult their own advisors in this regard.

This presentation and its contents are proprietary confidential information and may not be reproduced, published or otherwise disseminated in whole or in part without the Company’s prior written consent. These materials are not intended for distribution to, or use by, any person or entity in any jurisdiction or country where such distribution or use would be contrary to local law or regulation.

This presentation contains non-GAAP financial measures. The non-GAAP financial measures contained in this presentation are not measures of financial performance calculated in accordance with IFRS and should not be considered as replacements or alternatives profit, or operating profit, as an indicator of operating performance or as replacements or alternatives to cash flow provided by operating activities or as a measure of liquidity (in each case, as determined in accordance with IFRS). Non-GAAP financial measures should be viewed in addition to, and not as a substitute for, analysis of the Company’s results reported in accordance with IFRS.

References to “FY” in this presentation for periods prior to 1 January 2018 are to the 12-month periods commencing in each case on April 1 of the year indicated and ending on March 31 of the following year, and the 9 month period from April 1 2017 to December 31 2017. From January 1 2018 the Company changed its fiscal year to the 12-month period commencing in each case on January 1. References to “FY” in this presentation should be construed accordingly.

Sosei Heptares is the corporate brand of Sosei Group Corporation. Sosei, Heptares, the logo and StaR® are Trade Marks of Sosei Group Corporation.
Agenda

1. G Protein-Coupled Receptors
2. Sosei Heptares GPCR Drug Discovery Platform
3. Case Study: Negative Allosteric Modulators for mGlu₅
4. Case Study: Target Validation for GPR52 Inverse Agonism
5. Summary
G Protein-Coupled Receptors
G Protein-Coupled Receptor (GPCR) Family

- Largest super-family of transmembrane proteins in the human genome with ~800 members

- Characteristic structural features
  - 7 transmembrane domains (TM)
  - 3 intracellular loops (ICL)
  - 3 extracellular loops (ECL)

- Classification by phylogenetic relationship
  - Class A/Rhodopsin: Biggest family with 659 receptors (includes olfactory receptors)
  - Class B1/Secretin: Extracellular peptide hormone-binding domain
  - Class B2/Adhesion: Non-covalently associated N terminus
  - Class C/Glutamate: Dimeric quaternary structure
  - Class F/Frizzled: Wnt glycoproteins
  - Taste2: High sequence diversity
GPCR Signalling Cascade

1. Agonist-bound GPCR favours active conformation and facilitates association of heterotrimeric G protein

2. Depending on Gα subtype, different signalling cascades initiated

3. Phosphorylation of GPCR C terminus by GPCR kinases (GRKs) as part of downregulation cascade

4. Arrestin associates with phosphorylated GPCR C terminus and induces internalisation and/or signalling

➢ GPCR signalling highly complex with many different nuances
Modalities of GPCR Ligands

• Pharmacological profiles far beyond traditional orthosteric agonism and antagonism
  • Orthosteric binding site corresponds to endogenous ligand binding site
    – Agonist ligand binding induces receptor signalling
    – Antagonist ligand binding inhibits receptor signalling induced by constitutive activity or endogenous agonist binding
  • Allosteric ligands are classified by binding to a different site than orthosteric ligands and can have intrinsic activity and/or modulate the signalling of ligands binding to other sites (or have no effect on the receptor at all)
    – Negative allosteric modulator (NAM)
    – Positive allosteric modulator (PAM)
    – Silent allosteric modulator (SAM)

• Large portfolio of different ligand types allows variety of GPCR signalling modulation to suit respective needs in context of drug development
GPCRs as Drug Targets

- 475 approved drugs mediate effects via 108 GPCR targets
  - 27% of human non-olfactory GPCRs

- Aminergic receptors targeted by 67% of approved drugs

- Currently established GPCR drug targets used by ~10 distinct approved agents
  - Saturation of current target space

- Need to identify new druggable receptors to develop novel medications
  - Especially for diseases with large unmet medical needs and few current viable targets, e.g. Alzheimer disease

GPCRs as Drug Targets

  - Including 43 different GPCR targets
  - 25% of new GPCR targeting approvals were for first-in-class therapies
  - Majority of new GPCR approvals demonstrate improvements over existing agents in pharmacokinetics, selectivity & safety
- However, many notable GPCR drug failures (due to efficacy & safety)
  - CB1 (obesity), CGRP (migraine), mGlu5 (depression), GPR40 (diabetes)

![Graph showing FDA Drug Approvals](image)
Sosei Heptares GPCR Drug Discovery Platform
Sosei Heptares – Japan-listed biotech with R&D centre in the UK

**R&D CENTER**
CAMBRIDGE, UK

~120 EMPLOYEES

- Proprietary StaR® GPCR technology underpin
- Research, Drug Discovery and SBDD² Platform
- Translational and Early-Stage Clinical Development Expertise
- Business Development

**HEADQUARTERS**
TOKYO, JAPAN

~30 EMPLOYEES

- Late-Stage Japanese Development Expertise
- Access to Capital and also Royalty Income from Novartis

Japan-anchored, with a fully integrated global discovery and development business in Cambridge, UK, driving enhanced science, productivity, and collaboration and partnership opportunities

---

¹ Stabilized receptor technology
² Structure-based drug design
Generation of GPCR Stabilised Receptors (StaR®)

1. Unstable Native GPCR
2. Select Conformation
3. Mutagenesis
   - AATCAGC
   - GTGC
4. Recombination
5. Thermostability
6. Pharmacology
7. Screening
   - Biacore kinetics
   - Crystallisation
   - Antibody Generation
8. StaR®
Application of StaR® GPCRs in Drug Discovery

Stabilised Receptor (StaR®)

- Increased stability
- Validated pharmacology

GPCR Structure Determination

- Landmark structure reveals new drug binding site in Family B GPCRs

GPCR Fragment-Based Design

- FBDD of mGlu5 modulator with <nM affinity

Controlling Receptor Kinetics

- Drug kinetics by StaR® using SPR related to X-ray crystal structures

Antigens for mAb Discovery

- Functional or blocking mAbs generated using StaR® antigens
Developing of Therapies for Areas of High Unmet Medical Need

Sources: World Health Organization, EvaluatePharma, Management Estimates. Notes: Market sizes represent global sales of products targeted at any aspect of the market. Sosei Heptares may choose to only target a segment of the specific market. 1 Represents market size for Cushing’s syndrome, rather than Cushing’s disease.
# Sosei Heptares Partnered Pipeline

<table>
<thead>
<tr>
<th>Product/Program</th>
<th>Modality</th>
<th>Indication</th>
<th>Partner</th>
<th>Discovery</th>
<th>Preclinical</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Marketed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Japan Marketed Products (Out-licensed to Marketing / Distribution / Commercialization Partners)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NorLevo®</td>
<td>SME</td>
<td>Emergency contraception</td>
<td>ASKA Pharmaceutical Co. Ltd.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORAVI®</td>
<td>SME</td>
<td>Oropharyngeal candidiasis</td>
<td>FUJIFILM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Partnered Pipeline - Respiratory Products (Traditional out-licensing)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seebri*/Ultibro*</td>
<td>SME</td>
<td>COPD</td>
<td>NOVARTIS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QVM149</td>
<td>SME</td>
<td>Asthma</td>
<td>NOVARTIS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Partnered GPCR Pipeline (Traditional out-licensing/collaboration projects)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| A2a antagonist  | SME      | Multiple solid tumors           | AstraZeneca        | | | | | | ADV
| A2a antagonist  | SME      | EGFRm NSCLC                      | AstraZeneca        | | | | | | ADV
| M₄ agonist      | SME      | Alzheimer’s disease              | Allergan           | | | | | | ADV
| M₄ agonist      | SME      | Alzheimer’s disease              | Allergan           | | | | | | ADV
| M₄/M₁ dual agonist | SME | Alzheimer’s disease              | Daiichi-Sankyo     | | | | | | ADV
| Single target   | SME      | Pain                             |                    | | | | | | ADV
| Multiple targets| SME      | Multiple indications             |                    | | | | | | ADV
| Multiple targets| mAb      | Inflammation                     |                    | | | | | | ADV
| Multiple targets| SME/LME  | Multiple indications             |                    | | | | | | ADV
| **Partnered GPCR Pipeline (Co-development/profit share)** | | | | | | | | | |
| CXCR4 mAb       | mAb      | Immuno-oncology                  | kymab              | | | | | | ADV
| Single target   | mAb      | Immuno-oncology                  | kymab              | | | | | | ADV
| Single target   | Peptide  | Inflammation                     |                    | | | | | | ADV
| **Asset-centric Companies** | | | | | | | | | |
| Orexin agonists | SME      | Narcolepsy                       | Orexia             | | | | | | NEW
| Orexin agonists | SME      | Narcolepsy                       | INESSA             | | | | | | NEW

*Note: SME = small molecule; LME = large molecule; mAb = monoclonal antibody*
# Sosei Heptares In-House (Pre-Partnered) Pipeline

<table>
<thead>
<tr>
<th>Product/Program</th>
<th>Modality</th>
<th>Indication</th>
<th>Originator</th>
<th>Discovery</th>
<th>Preclinical</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Marketed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In-house (pre-partnered) GPCR pipeline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M₁ agonist¹</td>
<td>SME</td>
<td>DLB (Japan)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mGlùs NAM</td>
<td>SME</td>
<td>Neurology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSTR agonist</td>
<td>Peptide</td>
<td>Endocrine disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGRP antagonist</td>
<td>SME</td>
<td>Migraine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLP-1 antagonist</td>
<td>Peptide</td>
<td>Metabolic diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLP-2 agonist</td>
<td>Peptide</td>
<td>Intestinal failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orexin-1 antagonist</td>
<td>SME</td>
<td>Cocaine-use disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apelin agonist</td>
<td>Peptide</td>
<td>PAH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPR35 agonist</td>
<td>SME</td>
<td>Inflammatory bowel disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H4 antagonist</td>
<td>SME</td>
<td>Atopic dermatitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAR2 mAb</td>
<td>mAb</td>
<td>Atopic dermatitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Multiple programs targeting a broad range of GPCRs</strong></td>
<td>SME/ LME/ mAb/ Peptide</td>
<td>Multiple indications across Neurology, GI / Inflammation, Immuno-oncology and rare / specialty diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Note: SME = small molecule; LME = large molecule; mAb = monoclonal antibody

² Phase 2 trial of HTL0018318 for DLB in Japan remains under voluntary suspension. The Group plans to resubmit a new clinical trial notification for HTL0018318 (or another novel M1 agonist) to the Japanese Pharmaceuticals and Medical Devices Agency (PMDA) in the future.

Multiple candidates entering clinical development and next wave of targets in advanced discussions

16
Case Study: Negative Allosteric Modulators for mGlu$_5$
StaR®-Enabled Target-Based Drug Discovery Process

- Target Selection and Validation
- StaR® Generation and Structure Determination
- StaR®-Enabled Hit Identification
- Lead Optimisation
- Preclinical Studies
- Clinical Studies
StaR®-Enabled Target-Based Drug Discovery Process

- Target Selection and Validation
- StaR® Generation and Structure Determination
- StaR®- Enabled Hit Identification
- Lead Optimisation
- Preclinical Studies
- Clinical Studies
Metabotropic Glutamate Receptor 5 (mGlu$_5$)

- Class C GPCR
- Family contains eight mGlu receptors that are subdivided based on sequence similarity, pharmacology and signalling
  - Group I: mGlu$_1$ and mGlu$_5$ (G$\alpha_q$)
  - Group II: mGlu$_2$-$3$ (G$\alpha_{i/o}$)
  - Group III: mGlu$_4$ and mGlu$_6$-$8$ (G$\alpha_{i/o}$)

- Conserved orthosteric glutamate binding site across mGlu family

Modulators of mGlu$_5$ as Medicines

- Increasing evidence for mGlu$_5$ negative allosteric modulators (NAMs) in a range of diseases
  - Anxiety/depression (e.g. basimglurant; Roche/Chugai)
  - Fragile X syndrome (e.g. mavoglurant; Novartis)
  - Dystonia and dyskinesia (e.g. dipraglurant; Addex)

- Glutamate (orthosteric) binding site is difficult to drug
  - Orthosteric ligands are typically glutamate mimics
  - Poor PK properties, often incompatible with CNS exposure
  - Difficult to achieve selectivity (e.g. quisqualate, mGlu$_{1/5}$, also AMPA)

- Allosteric site in the TM bundle with problematic history
  - Limited range of chemotypes many with toxic liabilities
  - Poor PK properties including active metabolites
  - Mode switching between PAMs and NAMs
  - Lack of X-ray structures for Class C GPCRs
StaR®-Enabled Target-Based Drug Discovery Process

- Target Selection and Validation
- StaR® Generation and Structure Determination
- StaR®-Enabled Hit Identification
- Lead Optimisation
- Preclinical Studies
- Clinical Studies
Superposition and Comparison of mGlu$_5$ with Rhodopsin and CRF$_1$R

• StaR® generated in presence of non-selective mGlu$_5$ NAM MPEP
• Modifications to facilitate crystallisation
  • Flexible domains removed from N and C terminus
  • T4-lysozyme inserted into intracellular loop 2

• Superimposition across TM positions best across the intracellular halves
  • Structural constraints of G protein coupling
• Highest levels of structural diversity across the extracellular portions
• Position of TM5 most prominent difference between receptors – entire helix positioned further inwards by approximately 6Å
  • Contributes to the narrow entrance to the allosteric cavity

Structural Insight into mGlu Subtype Selectivity

Wu et al. 2014. Science. 344:58 (mGlu1 Structure); Bennett et al. 2015. Curr Opin Pharmacol. 20:1-7. (Structure Comparison)
The mGlu₅ Allosteric Modulator Binding Site – Role of Water

- Ligand binding traditionally defined by interaction with amino acid side chains, but may not be the ‘whole story’
- Water molecules play an essential role in the structure and function of biological systems
- Displacement of waters from a binding site is a key component of ligand binding
  - Binding energy, and thus potency, often gained from the entropic gain of the displacement
  - Stabilisation or destabilisation of waters or water networks within a binding site can also enable control of activity and even function of a receptor

- Repercussions for ligand modelling & design
  - Pharmacophore models may be wrong
  - Docking to protein structure may be wrong
  - Opportunities to make ligands smaller with different properties by utilising water

Role of Water in mGlu$_5$ Ligand Function

- Hydrogen bonds between water molecule and Y659, T781 and S809

- Network of interactions which contribute to the activation state of the receptor

- Can change molecule from negative allosteric modulator to silent allosteric binder to positive allosteric modulator by simple methoxy to chloro to fluoro change

- Interactions only with water network

Vary 3-Methyl: Methoxy > Chloro > Fluoro
Ligand Modality: NAM > Neutral > PAM
StaR®-Enabled Target-Based Drug Discovery Process

- Target Selection and Validation
- StaR® Generation and Structure Determination
- StaR®-Enabled Hit Identification
- Lead Optimisation
- Preclinical Studies
- Clinical Studies
Fragment Screening for Drug Discovery

• Fragment characteristics
  • Low molecular weight compounds (MW<300)
  • High chemical diversity
  • Bind very weakly but can ‘grow’ to increase affinity at target
    - Fragments have extremely high affinity considering their size (high ligand efficiency)

• With structural information it is possible to incorporate large element of design in fragment optimisation into high-affinity lead
  • Growing of additional binding groups
  • Linking two fragments together

High Concentration Fragment Screening for mGlu$_5$

- mGlu5 StaR® generation carried out with a NAM – receptor is stabilised in conformation favouring NAM binding
  - Dramatic increase in expression with the StaR®
- Due to low affinity of fragments requirement to screen at high concentrations - protein needs to withstand high concentrations of solvent (dimethyl sulfoxide)
  - StaR® has significantly higher DMSO tolerance
- Heptares fragment library and bespoke Class C GPCR fragment set yielded tractable hits for mGlu$_5$ and mGlu$_2$ (6-8% hit rate, >30% cut off)
StaR®-Enabled Target-Based Drug Discovery Process

Target Selection and Validation
StaR® Generation and Structure Determination
StaR®-Enabled Hit Identification
Lead Optimisation
Preclinical Studies
Clinical Studies
Screening for Improved mGlu$_5$ NAM Ligands

Table 1. In Vitro Profile of Compounds 6–17

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>R$^1$</th>
<th>R$^2$</th>
<th>R$^3$</th>
<th>mGlu$_5$ pK$_i$</th>
<th>mGlu$<em>5$ pIC$</em>{50}$</th>
<th>RLM $t_{1/2}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>N</td>
<td>N</td>
<td>CN</td>
<td>H</td>
<td>F</td>
<td>H</td>
<td>7.2</td>
<td>6.4</td>
</tr>
<tr>
<td>7</td>
<td>N</td>
<td>N</td>
<td>CN</td>
<td>F</td>
<td>H</td>
<td>H</td>
<td>6.6</td>
<td>nd$^*$</td>
</tr>
<tr>
<td>8</td>
<td>N</td>
<td>N</td>
<td>CN</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>6.1</td>
<td>nd</td>
</tr>
<tr>
<td>9</td>
<td>N</td>
<td>N</td>
<td>H</td>
<td>H</td>
<td>F</td>
<td>H</td>
<td>5.1</td>
<td>nd</td>
</tr>
<tr>
<td>10</td>
<td>N</td>
<td>N</td>
<td>OMe</td>
<td>H</td>
<td>F</td>
<td>H</td>
<td>&lt;4.2</td>
<td>nd</td>
</tr>
<tr>
<td>11</td>
<td>N</td>
<td>N</td>
<td>CONH$_2$</td>
<td>H</td>
<td>F</td>
<td>H</td>
<td>&lt;4.2</td>
<td>nd</td>
</tr>
<tr>
<td>12</td>
<td>N</td>
<td>N</td>
<td>CN</td>
<td>H</td>
<td>Me</td>
<td>H</td>
<td>8.4</td>
<td>7.9</td>
</tr>
<tr>
<td>13</td>
<td>N</td>
<td>N</td>
<td>CN</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>8.4</td>
<td>8.3</td>
</tr>
<tr>
<td>14</td>
<td>N</td>
<td>N</td>
<td>CN</td>
<td>F</td>
<td>Cl</td>
<td>H</td>
<td>9.3</td>
<td>8.6</td>
</tr>
<tr>
<td>15</td>
<td>N</td>
<td>CH</td>
<td>CN</td>
<td>H</td>
<td>Me</td>
<td>H</td>
<td>7.6</td>
<td>7.4</td>
</tr>
<tr>
<td>16</td>
<td>CH</td>
<td>N</td>
<td>CN</td>
<td>H</td>
<td>Me</td>
<td>H</td>
<td>7.7</td>
<td>7.7</td>
</tr>
<tr>
<td>17</td>
<td>N</td>
<td>N</td>
<td>CN</td>
<td>H</td>
<td>Cl</td>
<td>F</td>
<td>7.7</td>
<td>7.1</td>
</tr>
</tbody>
</table>

$^*$nd = not determined.

Christopher et al. 2015. J Med Chem. 58:6653-64.
Lead Optimisation

**mavoglurant**
- mGlu₅ pKᵢ 8.0
- clogP 3.1
- LE 0.47, LLE 4.9
- CNS MPO 5.2
- Acetylene containing
- Poor PK (rat F 22%)

**HTL14242**
- mGlu₅ pKᵢ 9.3
- clogP 3.0
- LE 0.57, LLE 6.3
- CNS MPO 5.5
- Good PK
  (F%>80% - 2 species)
- High RO
  (ED₅₀ 0.3 mg/Kg)
- Clean off-target profile

**Advanced homology modelling**
- Significant LLE & LE enhancements
  Sub optimal metabolic stability

**X-ray driven SBDD**
- Novel non-acetylene containing chemotype
  Sub optimal potency & LLE

**Fragment Screen**

Christopher et al. 2015. J Med Chem. 58:6653-64.
StaR®-Enabled Target-Based Drug Discovery Process

- Target Selection and Validation
- StaR® Generation and Structure Determination
- StaR®-Enabled Hit Identification
- Lead Optimisation
- Preclinical Studies
- Clinical Studies
Glutamate/mGlu$_5$ in Amyotrophic Lateral Sclerosis (ALS)

- ALS prevalence rate 3.9 per 100,000 persons; ages 40-70; av. survival from onset to death is 2-4 years (10% 10-year survival)
- Pathology involves death of motor neurons which control voluntary muscles resulting in gradually worsening weakness due to muscles decreasing in size and difficulty in speaking, swallowing, and eventually breathing
- Two different types of ALS
  - Sporadic: 90-95% of cases in U.S., may affect anyone, anywhere – undefined cause
  - Familial: Inherited, accounts for 5-10% of all cases in the U.S. (eg. SOD1$^{G93A}$ mutation)
- Significant unmet need, no cure – Riluzole may extend life 2-3 months
- Glutamate-mediated toxicity is recognised as a mechanism of neuronal injury
  - Glial cells reduced capacity to uptake glutamate
  - Increased glutamate receptor expression post-synaptically
- Evidence of neuroinflammation – activation of glial cells (astrocytes and microglia)
  - mGlu$_5$ expression in ALS spinal cord glia correlated with markers of glial activation (GFAP)
  - Partial knockdown of mGlu$_5$ receptor increases motor performance and survival in mouse models (Bonficino et al. 2017)
HTL0014242 Efficacy Study in SOD1\textsuperscript{G93A} Mouse Model of ALS

- SOD1\textsuperscript{G93A} is one of the mutations linked to the development of familial ALS
- Familial ALS only accounts for 5-10\% of ALS cases but SOD1\textsuperscript{G93A} mouse model is a useful preclinical model
  - Replicates genetics of the human disease
  - Shows similar phenotypes to human disease (e.g. selective vulnerability of motor neurones)
  - Robust and reproducible

### Summary of mGlu5 Effects in SOD1\textsuperscript{G93A} Mouse Model

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Vehicle</th>
<th>HTL0014242 25D Cohort</th>
<th>HTL0014242 75D Cohort</th>
<th>Riluzole</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effect on onset of clinical signs of disease</strong></td>
<td>✗</td>
<td>✗</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Increased number of motor neurons at 90D</strong></td>
<td>✗</td>
<td>✗</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td><strong>Reduction in GFAP staining at 90 days in SC</strong></td>
<td>✗</td>
<td>✓</td>
<td>✓</td>
<td>✗</td>
</tr>
<tr>
<td><strong>Reduction in Iba1 staining at 90 days in SC</strong></td>
<td>✗</td>
<td>✓</td>
<td>✓</td>
<td>✗</td>
</tr>
<tr>
<td><strong>Improvement in motor function as seen on rotarod</strong></td>
<td>✗</td>
<td>✗</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td><strong>Effect on survival</strong></td>
<td>✗</td>
<td>✗</td>
<td>✗</td>
<td>✗</td>
</tr>
</tbody>
</table>
StaR®-Enabled Target-Based Drug Discovery Process

- Target Selection and Validation
- StaR® Generation and Structure Determination
- StaR®-Enabled Hit Identification
- Lead Optimisation
- Preclinical Studies

HTL0014242 Ph 1 Study 2019
Double blind placebo controlled single ascending dose in healthy volunteers
Case Study: Target Validation for GPR52 Inverse Agonism
StaR®-Enabled Target-Based Drug Discovery Process

- Target Selection and Validation
- StaR® Generation and Structure Determination
- StaR®-Enabled Hit Identification
- Lead Optimisation
- Preclinical Studies
- Clinical Studies
GPR52 and Huntingtin Protein Regulation Hypothesis

- GPR52 is a class A orphan with no known endogenous ligand but interesting biology
- Huntington's disease (HD) is caused by a mutated form of the huntingtin (Htt) gene
  - Excessive (>36) CAG repeats result in formation of huntingtin protein with abnormally long polyglutamine tract at N-terminus
- GPR52 activation may prevent Huntingtin protein degradation via Rabgap1
- GPR52 knock down and inverse agonism has reportedly lowered mutant huntingtin protein in multiple cell and animal models
  - Mouse striatal cell line (STH/dh Q7/Q111 cells)
  - Mouse primary striatal and cortical neurons (Hdh Q140/Q140 mice) and brain tissue from GPR52 KO animals
  - HD patient stem cell-derived striatal-like neurons
  - In vivo in GPR52 knockout HD model mice (Hdh Q140/Q140 GPR52KO mice)

Mouse STH/dh Cell Model and GPR52 Expression

- Immortalised cell line derived from mouse striatum engineered to contain human Huntingtin (HTT) or mutant Huntingtin (mHTT) protein
- GPR52 expression probed on messenger RNA (mRNA) level
  - Quantitative reverse transcription PCR (RT-qPCR)
  - Whole genome messenger RNA sequencing

\[ Q = \text{Number of Glutamines in Huntingtin Protein} \]
Literature Evidence for GPR52 Role in Regulating Htt Levels

- Employed methodology
  - siRNA knockdown: Use of small interfering RNA that targets the RNA-induced silencing complex to degrade GPR52 mRNA
  - Western blot: Specific antibody-based detection of proteins in cell lysates that have undergone polyacrylamide gel electrophoresis and were transferred onto a membrane
  - Htt detection by HTRF: Fluorescently-labelled antibody-based method that enables detection of binding using plate reader

GPR52 inverse agonist E7 reduced Htt protein levels (Song et al. 2018)

Increasing GPR52 levels increased Htt protein level (Yao et al. 2015)
In-House Data Does Not Replicate Literature Observations

- We have not been able to replicate work by Boxun Lu’s group at Fudan University
  - Successful GPR52 siRNA knockdown (54-88%) and inverse agonism did not reduce Htt levels in STH/dh Q7/Q111 mouse striatal cells
  - Also unable to demonstrate the proposed mechanism in the opposite direction - increasing GPR52 expression did not increase Htt levels
  - Two selective GPR52 inverse agonists with different chemotypes were identified and have greater potency and efficacy than the previously described inverse agonist E7

GPR52 knockdown and E7 treatment did not result in reduction of mHtt levels

Overexpression of GPR52 did not increase mHtt protein levels

GPR52 Inverse Agonism

hGPR52 BacMam %

Htt Western blot quantification

Band density relative to Gapdh

Htt

mHtt
(PolyQ)

Ctrl
siRNA
Gpr2
siRNA
DMSO
E7

siRNA knockdown (qPCR)

Gpr52 expression (vs GAPDH)

Band density relative to Gapdh

Htt

mHtt
(PolyQ)

0.00000
0.00001
0.00002
0.00003
0.00004

Ctrl
siRNA
Gpr52
siRNA
DMSO
E7

Htt Western blot quantification

GPR52-CHO cAMP signal

(GPRT2-CHO cAMP signal vs basal & 32nM HTL29632)

Compound (Log M)

E7

HTL-A

HTL-B

hGPR52 BacMam

hGPR52 BacMam

Normalised protein expression vs GAPDH

hGPR52 BacMam %

Htt

mHtt
(PolyQ)
StaR®-Enabled Target-Based Drug Discovery Process

- **Target Selection and Validation**
- **Requirement to Identify Alternative Rationale for Targeting GPR52 with Inverse Agonists Prior to Proceeding with Cascade (If Appropriate)**
- **Lead Optimisation**
- **Preclinical Studies**
- **Clinical Studies**

Requirement to Identify Alternative Rationale for Targeting GPR52 with Inverse Agonists Prior to Proceeding with Cascade (If Appropriate)
### Summary

1. GPCRs are a tractable family of drug targets

2. Sosei Heptares StaR® technology is utilized in drug discovery for GPCR targets and significantly enables hit identification and lead optimisation

3. mGlu$_5$ NAM HTL0014242 was developed at Sosei Heptares using a StaR®-based fragment-screening approach followed by structure-based drug design

4. HTL0014242 shows encouraging signs of efficacy in the SOD1$^{G93A}$ mouse model of ALS (neuroprotective effect & reduction in neuroinflammation) and has entered clinical trials

5. Role of GPR52 in modulating levels of Htt shown in literature is not reproducible – demonstrates importance of thorough target validation prior to advancing to hit identification stage

---

StaR® technology facilitates discovery of specific ligands for difficult GPCR targets and can aid the development of tool ligands for orphan GPCRs to enable target validation
Acknowledgements

- Sosei Heptares Departments
  - Pharmacology
  - Chemistry
  - Computational Chemistry
  - Biomolecular Structure
  - Protein Engineering
  - Translational Sciences
  - Development

- SITRaN Sheffield
  - Dr Richard Mead
  - Dr Heledd Brown-Wright
  - Dame (Prof) Pamela Shaw
Thank you for your attention!

SOSEI HEPTARES

PMO Hanzomon 11F
2-1 Kojimachi, Chiyoda-ku
Tokyo 102-0083
Japan

Steinmetz Building
Granta Park, Cambridge
CB21 6DG
United Kingdom

North West House
119 Marylebone Road
London NW1 5PU
United Kingdom