CCR2 antagonists for the treatment of neuropathic pain

The discovery and development of AZD2423

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Outline

- Rationale: CCR2 and neuropathic pain
- Project objectives
- Medicinal chemistry
- Profile of AZD2423
- Pre-clinical efficacy
- Clinical development
- Conclusions
CCR2 and its ligand CCL-2 (MCP-1)

- CCR2 is the receptor for CCL-2, CCL-7, CCL-8 and CCL-13
  - CCR2 is only known receptor for CCL-2 (MCP-1) and CCL-13 (MCP-4)
  - CCL-2 binds preferentially to CCR2 with high affinity ($K_i$ 2 nM)
- CCL-2 is chemotactic for monocytes, dendritic cells, memory T cells and basophils
- CCL-2 knock-out studies indicate that it has a non-redundant role for monocyte recruitment
Neuropathic pain

- Heterogeneous group of disorders having in common nerve injury
  - spinal nerve root pain (radicular pain)
  - pain after nerve trauma (post traumatic neuralgia, PTN)
  - painful diabetic neuropathy (PDN)
  - pain after shingles (post herpetic neuralgia, PHN)
- Total prevalence at least 1%
- Approved treatments: gabapentin, pregabalin, and duloxetine
- Limited efficacy (1/3 of subjects) and CNS side effects
Peripheral Nerve Injury

DRG Primary Afferent Neurons

Spinal or Peripheral Tissue

Site of CCL2 release

CCL2 release from peripheral injury site or central PAF terminals

Recruited monocyte

Spinal Site of Action

Peripheral Site of Action

Peripheral Nerve Injury

Vasculature

CIRCULATING MONOCYTE

CCR2 antagonists inhibit chemotaxis and infiltration
Peripheral Nerve Injury

Infiltrating Monocytes

Vasculature

CCL2 release

Recruited monocyte

Site of CCL2 release

Spinal or Peripheral Tissue

CCR2 antioxidant

Attenuation of Immune Cell Infiltration and Release of Sensitising Factors

Normalisation of Peripheral and Spinal Sensitisation

Attenuation of Immune Cell Infiltration and Release of Sensitising Factors

Spinal Site of Action

Peripheral Site of Action

Peripheral Nerve Injury

DRG

Primary Afferent Neurons

Infiltrating Monocytes

Spinal Cord

CCR2 antagonists inhibit chemotaxis and infiltration
Project goals

• Potent CCR2 antagonist
• Selective vs other chemokine receptors and GPCRs (>100 x)
• Predicted human PK consistent with once daily oral dosing (< 1 mg/kg)
• hERG IC\textsubscript{50} > 20 µM and hERG margin (vs free C\textsubscript{max}) >300
  - Assuming human C\textsubscript{max}/C\textsubscript{min} = 3, requires \textit{in vitro} margin CCR2b/hERG >3000x [>3.5 log units]
• Determination of CNS exposure
• Rodent or other non-human species potency to demonstrate activity in disease models and set target C\textsubscript{ss}
Lead generation

Potency and hERG correlate with lipophilicity

Improving hERG margin by modulating LLE

Δ CCR2 = +0.4
Δ hERG = −1.1
Δ logP = −1.5
Δ logD₇.₄ = +0.6

CCR2 binding IC₅₀ = 3.5 nM
CCR2 Ca²⁺ flux IC₅₀ = 5.8 nM
hERG binding IC₅₀ = 17 µM
Good solubility and rat PK
CYP 3A4/2D6 IC₅₀ > 10 µM
Rat CCR2 Ca²⁺ flux IC₅₀ = 200 nM

• Elevation of CYP1A1 after 7 day rat oral tolerability study
• Flag for arylhydrocarbon receptor (AhR) agonism

pKa = 10.0

pKa = 7.8
Compound 2
Toxicities associated with AhR agonism

CYP1A1 mediated
• Drug-Drug Interactions
• Activation of carcinogens (PAHs)
• Increased metabolism of endogenous ligands?

Via other mechanisms
• Characteristic wasting syndrome
• Chloracne
• Immunosuppression
• Teratogenicity
• Endocrine disturbances
• Neuro-behavioural effects

Dioxin (TCDD)

Yushchenko endured dioxin poisoning, likely by political foes, which, along with nearly killing him, left his skin severely disfigured. WILLisms.com
Mitigation of AhR agonism liability

- No adverse effects observed in-life or at pathology
- Piperazine fragments detected in vivo (~2% relative to parent)
  - induce CYP1A1 in rat but not human hepatocytes
  - weak activity in rat AhR agonism reporter assay
  - inactive vs human AhR
- Parent compounds showed no activity in any of the in vitro assays

• Effect appears to be rat-specific and low risk for development
• Straightforward to monitor in pre-clinical tox studies
From isopropyl to tert-butyl: synthesis

(i) ArNCO or ArNHCO₂Ph
(ii) TFA

DMTMM, NMM piperazine

RI, Na₂CO₃

R = tBu

Acetone, H₂O
KCN, TsOH, 25°C

MeMgBr
THF, 0°C

s-BuLi, (−)-sparteine
CO₂, Et₂O, −78°C

HATU, DIPEA,
Benzyl piperazine
DMF, 25°C

H₂, Pd/C, EtOH

McDermott, B. P., Campbell, A. D., Ertan, A. Syn. Lett. 2008, 6, 875-879
From isopropyl to tert-butyl: SAR

\[ \Delta \log D_{7.4} = +0.13 \]

Matched pair analyses:

**potency**

**hERG**
Thiazole series SAR

<table>
<thead>
<tr>
<th>R</th>
<th>X</th>
<th>CCR2 Ca(^{2+}) flux (nM)</th>
<th>hERG (µM)</th>
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<tbody>
<tr>
<td>(^i)Bu</td>
<td>H</td>
<td>330</td>
<td>74</td>
</tr>
<tr>
<td>H</td>
<td>Cl</td>
<td>160</td>
<td>&gt;100</td>
</tr>
<tr>
<td>CF(_3)</td>
<td>H</td>
<td>150</td>
<td>&gt;100</td>
</tr>
<tr>
<td>H</td>
<td>Br</td>
<td>68</td>
<td>&gt;100</td>
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<tr>
<td>H</td>
<td>Ph</td>
<td>68</td>
<td>30</td>
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<tr>
<td>CF(_3)</td>
<td>Me</td>
<td>20</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Me</td>
<td>Cl</td>
<td>7.8</td>
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<tr>
<td>Ph</td>
<td>H</td>
<td>2.7</td>
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<tr>
<td>CF(_3)</td>
<td>Cl</td>
<td>1.3</td>
<td>26</td>
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<table>
<thead>
<tr>
<th>Compound 3</th>
<th>CCR2</th>
<th>0.65 nM</th>
<th>hERG</th>
<th>&gt;100 µM</th>
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</thead>
<tbody>
<tr>
<td>AZ889</td>
<td>CCR2</td>
<td>0.46 nM</td>
<td>hERG</td>
<td>16 µM</td>
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<tr>
<td></td>
<td>Rat CCR2</td>
<td>1.3 ±0.2 nM</td>
<td>Rat tool compound</td>
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</table>
Unexpected chemical reactivity (1)

**In vitro glutathione reactivity study**

<table>
<thead>
<tr>
<th>R</th>
<th>$t^{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me</td>
<td>17</td>
</tr>
<tr>
<td>Et</td>
<td>23.5</td>
</tr>
<tr>
<td>iPr</td>
<td>30</td>
</tr>
<tr>
<td>tBu</td>
<td>33</td>
</tr>
<tr>
<td>cPr</td>
<td>2</td>
</tr>
<tr>
<td>H</td>
<td>no reaction</td>
</tr>
<tr>
<td>CF$_3$</td>
<td>no reaction</td>
</tr>
</tbody>
</table>

reactivity: $X = Br > Cl > F$

$S_N$Ar mechanism?
Metabolism study identified reactive metabolites

- Parent −4H + cysteine observed in rat urine
- \(N\text{-tBu} > N\text{-iPr} > N\text{-Et/N-Me}\) (in vitro microsomal GSH trapping)
- Consistent with Merck MC4R agonist (MB243) metabolism

\[\text{GSH} \rightarrow \text{SG} \rightarrow \text{Glu}\]

Can we ‘design out’ reactive metabolites?

Block oxidation on piperazine (c.f. Merck paper)

Potency OK

expand to homopiperazine

Lose potency
Unexpected chemical reactivity (2)

\[
[M+H]^+ = 476.2238
\]

Major species in blood and urine \( m/z = 488.2238 \) \([M+C+H]^+\)

Found in pre-dose urine spiked with compound

Formaldehyde (0.1 mM in rat blood)

\[ ^1H \text{ NMR (COSY & ROESY)} \]

Martin, S. et al. Drug Metabolism and Disposition 2012, 40, 1478-1486
Potent and selective inhibitor of CCR2 with properties suitable for development and testing of clinical hypothesis.
Pre-clinical rat neuropathic pain models
Chronic constriction injury (CCI)-induced mechanical hypersensitivity

AZ889

$E_{\text{max}} > 100\%$
$EC_{50} \text{ plasma} = 191 \text{ nM (total), 33 nM (free)}$
$EC_{50} \text{ brain} = 63 \text{ nM (total), 3.8 nM (free)}$

AZD2423 in Chung heat hyperalgesia model

AZD2423

Serrano, A. et al. Molecular Pain 2010, 6, 90
AZD2423 Ph2a trials in neuropathic pain patients

- Randomized, double-blind, placebo-controlled multi-centre trials
- 20 mg or 150 mg AZD2423 or placebo, once daily for 28 days

PTN 133 patients

PDN 134 patients

Kalliomäki, J. et al. *Pain* 2013, 154, 761-767
**Summary of AZD2423 receptor occupancy**

AZD2423 is a non-competitive negative allosteric modulator

<table>
<thead>
<tr>
<th>Rat pain model (80% reversal of hyperalgesia in CCI)</th>
<th>Estimated based on IC50 and exposure</th>
<th>Peripheral (80%)</th>
<th>Central (17%)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>PDN and PTN Phase 2a studies (C_{ss,avg} 150 mg)</th>
<th>Inhibition of CCL2 clearance <em>in vivo</em></th>
<th>96%</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Binding <em>ex vivo</em> in peripheral blood monocytes</td>
<td>93%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Calculated based on K_B and exposure</td>
<td>97%</td>
<td>90%</td>
</tr>
</tbody>
</table>

- Both exposure and receptor occupancy exceed efficacious levels in rat pain models
- Therefore conclude that the hypothesis that AZD2423 is analgesic in PDN and/or PTN patients has been tested
Conclusions

- Novel, potent and selective series of CCR2 antagonists identified from screening of ‘GPCR ligand motif’ library
- Separation of CCR2 and hERG activity achieved through structural modifications without increasing molecular weight
- CYP1A1 induction and reactive metabolite liabilities investigated
- Unexpected chemical reactivities observed within series
- AZD2423 selected as candidate for clinical development
- Good efficacy in pre-clinical models of neuropathic pain
- Two Phase 2a trials in neuropathic pain patients failed to show efficacy against primary endpoints despite good evidence of target engagement
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CCR2 literature validation for neuropathic pain

- CCL-2 induces pain when injected peripherally or into spinal cord
- Over-expression of CCL-2 in the spinal cord induces pain
- CCR2/CCL-2 elevated in the spinal cord after nerve injury
- CCL-2 can directly activate DRG neurons
- CCL-2/CCR2 key regulators of microglia recruitment/biology
- CCR2 KO mice show absence of hyperalgesia after nerve injury (Merck)
- Ablation of CCR2 centrally & peripherally is required for analgesia (Ji Zhang)
- A CCR2 antagonist is active in mouse neuropathic pain model (Merck)
### CCR2 antagonists in clinical development

**Highest phase: Ph2**

<table>
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<th>Compound</th>
<th>Structure</th>
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<tr>
<td>BMS-741672</td>
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<tr>
<td>Cenicriviroc (CCR2+CCR5)</td>
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