Small Molecule TLR7 Agonists for the Treatment of HCV Infection

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Outline

- **HCV**
  - Current standard of care
  - Where might a TLR7 agonist fit in and why?

- **TLR7 agonists**
  - Mechanistic details of immune stimulation
  - Chemistry starting points
  - Challenges

- **Identification of a development candidate**
  - Potency
  - Solubility
  - Solving an aldehyde oxidase liability

- **Clinical data with PF-4878691**
  - A question of therapeutic index
Hepatitis C virus

- HCV is a *flaviviridae* +ve stranded RNA virus
  - >190 million individuals infected worldwide
  - Virus replicates primarily in the liver
  - 85% of patients develop a chronic infection
  - 20% of these go on to develop cirrhosis in 10-30 years

- Current standard of care is PEG-IFNα2a + ribavirin
  - 48% SVR in genotype 1 patients with 12 month therapy
  - Flu-like symptoms, depression, anemia, high discontinuation rate

Ribavirin

Developing new treatments for HCV

- Direct acting antivirals
  - HCV Protease (NS3), HCV RNA polymerase (NS5B)
  - Other non-structural proteins (NS5A, NS4B, NS4A)
  - Structural proteins (Core, p7 channel)
  - Combination of IFNα and selective inhibitor limited by IFNα side-effects

- Indirect antivirals
  - Using the innate immune response to boost production of endogenous IFN’s, mediated by Toll-like receptors (TLR’s)
  - Induction of the whole suite of IFN’s, not just one

Seeking an oral TLR7 agonist to be used in IFN-sparing regimens with improved efficacy and reduced side effects relative to IFNα2a
Toll-like receptors and TLR7

- Large family of type 1 trans-membrane proteins
  - Expressed on immune cells, especially dendritic cells
  - Recognise pathogenic components and via signalling cascade, induce inflammatory response
  - Induces cytokines, including interferons

Science, 2004, 303, 1481
Small molecule TLR7 agonists

- Precedented agents

Imiquimod (3M)
Topically administered as a 5% cream, Aldara
Launched for HPV 1997
MOA found in 2002!
EC₅₀ 1µM
Low solubility

Isatoribine (Anadys)
Anti-HCV activity with IV formulation
Oral prodrug pursued
EC₅₀ 50µM
Low solubility
Poor absorption

Sumitomo (AZ)
Pre-clinical IFN response data
Highly potent IFN stimulator
Inhaled indications
EC₅₀ 20nM
Reasonable solubility + absorption

J Med Chem. 2002, 45(25), 5419
Purine analogues

- Retain small alkyl substituent
- Retain equivalent of the 8-oxo group
- Investigate core changes around a bicyclic heterocycle
- Retain the pendant aryl group

MedChemComm, 2011, 2(3), 185-189
3-Deazapurines

EC$_{50}$ 829nM
MWt. 255
LogD 2.3
HLM <7μL/min/mg

EC$_{50}$ 1540nM
MWt. 254
LogD 2.3
HLM <7μL/min/mg

EC$_{50}$ >4000nM
MWt. 240
LogD 1.9
HLM <7μL/min/mg

EC$_{50}$ 304nM
EC$_{50}$ 480nM
EC$_{50}$ >4000nM
EC$_{50}$ 870nM

EC$_{50}$ 102nM
MWt. 308
LogD 3.3
HLM <7μL/min/mg
A solubility issue

- The first set of compact deazapurines were very low solubility
  - Initial potency was good
  - Pharmacokinetics were encouraging

Rat PK
Cl 33mL/min/kg
V<sub>dss</sub> 2.7L/kg
T<sub>1/2</sub> 1h

Dog PK
Cl 6mL/min/kg
V<sub>dss</sub> 2.7L/kg
T<sub>1/2</sub> 5.5h

Predicted human PK
Cl 8mL/min/kg
V<sub>dss</sub> 2.4L/kg
T<sub>1/2</sub> 3h

EC<sub>50</sub> 102nM
MWt. 308
LogD 3.3
HLM <7μL/min/mg
Aq. sol. ~1μg/mL
T<sub>m</sub> ~365°C

OPRD, 2011, 15(4), 788-796
Solubilizing SAR

- Increasing solubility investigated on three fronts
  - Basic groups *(potent but permeability + clearance issues)*
  - Introduction of heteroatoms to disrupt hydrocarbon packing
  - Introducing 3D shape to disrupt packing and facilitate solvation

Retain the core deazapurinone structure

Investigate pendant groups on the core that disrupt packing

Introduce heteroatoms into the benzyl group + explore other heterocycles here

Methyl pyridyl substituent was one of the more potent, with reasonable solubility.

EC<sub>50</sub> 100nM
Aq. sol. 1μg/mL

EC<sub>50</sub> 833nM
Sol. 23μg/mL

EC<sub>50</sub> 1440nM
Sol. 37μg/mL

EC<sub>50</sub> 50nM
Sol. 22μg/mL
Sol. 152μg/mL

EC<sub>50</sub> 103nM
Sol. 4μg/mL

EC<sub>50</sub> 193nM
Sol. 35μg/mL

EC<sub>50</sub> 79nM
Sol. 8μg/mL

C2 SAR

- C2 heterocycles looked the most promising for both potency and solubility
  - Subtle ether SAR provided some back-up options

\[ \begin{align*}
\text{EC}_{50} & = 102 \text{nM} \\
\text{Sol.} & = 66 \mu\text{g/mL}
\end{align*} \]

\[ \begin{align*}
\text{EC}_{50} & = 633 \text{nM} \\
\text{Sol.} & = 45 \mu\text{g/mL}
\end{align*} \]

\[ \begin{align*}
\text{EC}_{50} & > 4000 \text{nM} \\
\text{Sol.} & \text{ND}
\end{align*} \]

\[ \begin{align*}
\text{EC}_{50} & = 474 \text{nM} \\
\text{Sol.} & = 1 \mu\text{g/mL}
\end{align*} \]

\[ \begin{align*}
\text{EC}_{50} & = 99 \text{nM} \\
\text{Sol.} & = 17 \mu\text{g/mL}
\end{align*} \]

\[ \begin{align*}
\text{EC}_{50} & > 4000 \text{nM} \\
\text{Sol.} & \text{ND}
\end{align*} \]

\[ \text{EC}_{50} = 50 \text{nM} \]

\[ \text{Aq. sol.} = 22 \mu\text{g/mL} \]
An unexpected in vivo profile

The 2nd generation agents had better solubility, physchem properties, *in vitro* ADME and retained potency.

So what's gone wrong?
Aldehyde oxidase activity

- Heterocyclic analogues from within this series were substrates for AO, especially rat AO

Rat hepatocytes

Major metabolite

Oxidised metabolite not picked up in microsomes or in human hepatocyte systems

Generation of metabolite by AO confirmed using a specific AO inhibitor, Raloxifene
Aldehyde oxidase

- AO is a Mo-cofactor containing enzyme found mainly in cytosol; known to oxidise heterocycles

Merck GABA_\text{A} inverse agonist
No AO turnover in dog + rat cytosol
Major route in rhesus and human

Carbazeran
No AO turnover in dog cytosol
Major route in human

Gsk D3 antagonist
No AO turnover in dog + rat cytosol
Major route in rhesus and human

\textbf{J. Med.Chem., 2010, 53(24), 8441-8460}
Direct AO-susceptibility is predictable

- AO liability is very substitution + electronics dependent
- Beware species differences with AO
  - Usually more prevalent in human than preclinical species but not with our TLR7 systems!

Rapid turnover in rat cytosol

Rapid to moderate turnover in rat cytosol

Stable in rat cytosol

DARU, 2003, 13(3)
But AO liability can be tuned remotely

- Recognition by AO can be tuned by making remote alterations away from the susceptible group
- Can be rationalised through modelling

Rapid to moderate turnover in rat cytosol

Stable in rat cytosol
- Docked structure rationalises turnover of CF$_3$ agent
- THP analogue clashes with Ser1020
A development candidate with fit for purpose potency, pharmacokinetic and physchem credentials

Rat PK
- Cl 36mL/min/kg
- $V_{dss}$ 1.3L/kg
- $T_{1/2}$ 0.4h

Predicted human PK
- Cl 16mL/min/kg
- $V_{dss}$ 2.1L/kg
- $T_{1/2}$ 1.5h

Measure IFN levels in mouse

Modelled PK-PD in human

EC$_{50}$ 99nM
MWt. 369
LogD 2.1
HLM <7μL/min/mg
Aq. sol. ~20μg/mL

>100 x selective in a broad panel of kinases, receptors, ion channels and enzymes

30mg human dose predicted to match exogenous IFN

Antimicrob Agents Chemother., 2010, 54(3), 1179
A new player enters

- **Pfizer acquires Coley Pharmaceuticals and with it PF-4878691**
  - Already investigated at single doses from 2-20mg in healthy volunteers with transient, dose-dependent increases in white blood cells
  - Most expeditious route to POM with this compound

- **Healthy volunteer study**
  - Twice-weekly oral dosing
  - 2 weeks
    - days 1, 4, 8 and 11
  - 3, 6 and 9mg doses
  - Biomarkers monitored
    - 2,5-oligo adenylate synthetase
    - direct response to IFN’s

EC$_{50}$ ~100nM
MWt. 361
LogD 1.2
HLM <7µL/min/mg
Adverse events observed

- Biomarker induction in a dose-dependent and dose-frequency related manner
  - Consistent with anticipated TLR7 agonism expected to provide an antiviral response
- Two subjects in the 9mg dose group had serious adverse events
  - Flu like symptoms, hypotension, lymphopenia
  - Study was discontinued
- TLR7 stimulation at doses predicted to be efficacious were associated with adverse events with this compound
- A single non-responder was identified in the study
  - Single polymorphism in the IFNα receptor 1 subunit; V168L
Effects of repeat TLR7 agonist dosing

- TLR7 expression increases in response to TLR7 agonism in a dose-dependent manner
- Consequently, IFN-stimulated biomarkers + cytokine levels also increase in a dose-dependent manner
  - Positive feedback loop, very low TI for systemic applications

![TLR7 RNA expression graph]

* Clin. Pharmacol. Ther., 2011, 89(6), 821-829
Summary

- Seeking a novel, oral, potent TLR7 agonist for HCV
- Initial investigations into novel core structures identified the 3-deazapurinone template
  - Encouraging potency and pharmacokinetics
  - Solubility issues
- Attempts to improve solubility uncovered aldehyde oxidase liabilities
  - Recognition by rat AO in particular, however a simple preclinical prediction path was preferred
  - SAR identified direct and remote strategies to avoid AO activity
- Quality CAN identified
- Clinical data obtained with an acquired compound
  - Very narrow TI over pro-inflammatory cytokines
  - Dose-dependent auto-induction of the TLR7 receptor in vivo
  - Programme halted
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