Inflammation 2010

The discovery of CDP-323, a novel and potent inhibitor of the integrins α4β1 (VLA-4) and α4β7

Julien Brown

First report

Structure of Integrin, a Glycoprotein Involved in the Transmembrane Linkage between Fibronectin and Actin

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“We propose the name integrin for this protein complex to denote its role as an integral membrane complex involved in the transmembrane association between the extracellular matrix and the cytoskeleton.”

The integrins were first named by Tamkun, ~ 25 years ago.
Members of the gene family

• Followed by the identification of a whole family of glycoproteins exhibiting similar function.
• Each of these glycoproteins is a heterodimer comprising an alpha and beta subunit

More than 24 heterodimers identified – bind to a diverse range of ligands

Why $\alpha_4$ integrins?

Alpha 4 integrins are expressed on most leukocytes

Vascular cell adhesion molecule (VCAM-1 – Up-regulated by inflammatory cytokines)
Mucosal-addressin cell adhesion molecule (MAdCAM-1 – Expressed in Peyer’s patches)

Pivotal role in directing lymphocyte migration to inflamed tissue and mucosal lymphoid organs
A variety of diseases have some dependency on $\alpha_4$ integrins

Potentially useful in:

$\alpha_4\beta_1$ : Multiple Sclerosis, Rheumatoid Arthritis, Asthma

$\alpha_4\beta_7$ : Crohn’s Disease, Ulcerative Colitis, IBD

Transmigration - A Multistep Process

<table>
<thead>
<tr>
<th>STEP</th>
<th>Ligands on endothelium</th>
<th>Ligands on leukocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firm adhesion</td>
<td>VCAM-1, ICAM-1</td>
<td>$\alpha_1$, $\alpha_2$ and $\alpha_7$ integrins</td>
</tr>
<tr>
<td>Transmigration</td>
<td>VCAM-1, ICAM-1, PECAM-1</td>
<td>$\alpha_1$, $\alpha_2$ and $\alpha_7$ integrins, PECAM-1</td>
</tr>
</tbody>
</table>

Process of leukocyte rolling, adhesion and transmigration was described in the early 19th century.

Mechanism now unravelled and the concept of a multi-component adhesion cascade developed.
Integrin conformation

Activation is mediated by inside-out and outside-in signalling events
Binding to activated or inactivated form can have different outcomes

Integrin structure - binding domain

VLA-4 binds to VCAM-1 through the sequence Gln-Ile-Asp-Ser (QIDS) and to CS-1 segment of Fn through Leu-Asp-Val (LDV).

Wang PNAS (1995) 92 5714

Springer PNAS (1997) 94 65

Central aspartic acid crucial for binding, postulated to bind a divalent cation in the ligand binding region.
Validation of integrin therapy in inflammation

Natalizumab (Tysabri®; Biogen / Elan) humanised monoclonal antibody (mAb) to the \( \alpha_4 \) subunit, MS and Crohn’s disease.

Efalizumab (Raptiva®; Genentech), mAb to \( \alpha_L\beta_2 \) integrin, moderate to severe psoriasis

Both have been associated with cases of PML.

i) Tysabri was temporarily withdrawn  
   High efficacy and level of medical need led to reintroduction

ii) Efalizumab withdrawn 2009

Vedolizumab (MLN0002; Takeda), humanised mAb against the \( \alpha_4\beta_7 \) integrin receptor. PIII trials for ulcerative colitis and Crohn’s disease.

No orally active small molecule integrin inhibitors on the market

Progress to tyrosine analogues

Cyclic peptide lead structure (Tanabe Seiyaku Co. Ltd.)

\[ \text{\( \alpha_4\beta_1 \) cell IC}_{50} \text{ 3600nM} \]

- Arginine replaced by adamantyl
- Cysteine carboxylic acid essential
- Aspartic acid not required

Decyclisation – removal of alanine acid was not detrimental to potency

Removal of disulphide
- L to D thioproline switch
- Amide NH required

100 fold increase in potency on a simplified molecule

Efficacy in allergic sheep model of asthma

However, rapid biliary clearance in a number of species

Modification of amide

Find a replacement that retains the amide like character of the NH.

Squarate series retained cellular potency and more favourable clearance characteristics, however:
- AHP patent claimed these compounds
- Compounds racemised
- Enantiospecific clearance

N-Aryl series could not be adequately advanced - potency/clearance

Deconstructing the squareamide

This process led us to investigate aminocyclobutenones scaffold

Features
- Novel structure
- Three points of diversity around the ring allows derivatisation
Aminocyclobutenone – A tractable series?

This process led us to investigate aminocyclobutenones scaffold

• Can we make them?
• Limited synthetic precedent

Aminocyclobutenone – A tractable series?

Synthesis of diones and coupling to amino ester derivatives straightforward

Chemically stable (6M HCl, 3M NaOH; 24h, 38°C)

Stereochemistry when R1 ≠ R2 could not be controlled
Geminal substituents

Accessing lipophilic pocket enhances potency
Cyclohexyl and THP chosen for further study

Alkene substituents

Polar functionality not tolerated
Bromo cyclohexyl carried forward
Phenyl to pyridyl

R1
R2
N
H
OH
O
Cl
Cl
N
R3
O
SMe
Cl
Br
R3

Pyridyl analogues

Solubility is slightly improved
Potency tends to be less than the phenyl equivalent
Clearance is higher than phenyl analogues

Summary of bromocyclohexyl compound

IC_{50} (nM) Protein Assay

<table>
<thead>
<tr>
<th></th>
<th>α4β1</th>
<th>α4β7</th>
<th>α5β1</th>
<th>α5β2</th>
<th>LFA-1</th>
<th>avgIII</th>
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</thead>
<tbody>
<tr>
<td>α4β1</td>
<td>0.3</td>
<td>0.7</td>
<td>133</td>
<td>2150</td>
<td>207</td>
<td>&gt;50000</td>
</tr>
</tbody>
</table>

IC_{50} (nM) Cellular Assay

<table>
<thead>
<tr>
<th></th>
<th>α4β1</th>
<th>α4β7</th>
<th>α5β1</th>
<th>α5β2</th>
<th>LFA-1</th>
<th>avgIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>α4β1</td>
<td>1</td>
<td>34</td>
<td>45210</td>
<td>19750</td>
<td>7970</td>
<td>&gt;50000</td>
</tr>
</tbody>
</table>

pKa 2.85
PSA 118
LogD 0.09
PPB > 95%
Sol 5.4 mg/mL (pH 6.8)

CYP Inhibition (μM)

<table>
<thead>
<tr>
<th></th>
<th>1A2</th>
<th>2C9</th>
<th>2C19</th>
<th>2D6</th>
<th>3A4</th>
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</thead>
<tbody>
<tr>
<td>α4β1</td>
<td>&gt;100</td>
<td>19</td>
<td>24</td>
<td>&gt;100</td>
<td>20</td>
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</table>

IC_{50} (nM) WITH SERUM ALBUMINS

<table>
<thead>
<tr>
<th></th>
<th>α4β1/VCAM</th>
<th>α4β7/VCAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCS</td>
<td>6</td>
<td>34</td>
</tr>
<tr>
<td>HSA</td>
<td>13</td>
<td>63</td>
</tr>
<tr>
<td>MSA</td>
<td>65</td>
<td>208</td>
</tr>
<tr>
<td>RSA</td>
<td>62</td>
<td>25</td>
</tr>
<tr>
<td>HWB</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>MBW</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>BSA</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>HSA</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>RSA</td>
<td>208</td>
<td></td>
</tr>
<tr>
<td>HAS</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>
Top group modifications

GSK compound demonstrated that altering top group of amino acid derivatives could give potent and bioavailable compounds

<table>
<thead>
<tr>
<th></th>
<th>α4p/VCAM 1IC50 (nM)</th>
<th>α4p7/VCAM 1IC50 (nM)</th>
<th>F%</th>
<th>Clearance (mL/min/kg)</th>
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<tbody>
<tr>
<td>SB683698</td>
<td>92</td>
<td>620</td>
<td>60</td>
<td>&gt;100 (R)</td>
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<table>
<thead>
<tr>
<th></th>
<th>H</th>
<th>228</th>
<th>1369</th>
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<tbody>
<tr>
<td>Br</td>
<td>30</td>
<td>780</td>
<td>38</td>
</tr>
</tbody>
</table>

Dimethoxy aryl compound has high bioavailability

Incorporation of this head group did not offer us necessary potency

• gave impetus to try alternative top groups

Amide to naphthyridine

Constrain within aromatic ring

Naphthyridines synthetically accessible
## Naphthyridine SAR

<table>
<thead>
<tr>
<th></th>
<th>R1</th>
<th>R2</th>
<th>α4/β7/VLA4 cell IC50 nM</th>
<th>α4/β7/VLA4 cell IC50 nM</th>
<th>Clearance ml/min/kg</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Br</td>
<td>5</td>
<td>194</td>
<td>41 (M)</td>
<td>21 (R)</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>20</td>
<td>678</td>
<td>23 (M)</td>
<td>26 (R)</td>
</tr>
<tr>
<td></td>
<td>Br</td>
<td>2</td>
<td>61</td>
<td>31 (M)</td>
<td>32 (R)</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>20</td>
<td>194</td>
<td>12 (R)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Br</td>
<td>11</td>
<td>220</td>
<td>39 (M)</td>
<td>15 (R)</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>25</td>
<td>509</td>
<td>24 (M)</td>
<td>9 (R)</td>
</tr>
<tr>
<td></td>
<td>Br</td>
<td>7</td>
<td>76</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>40</td>
<td>236</td>
<td>34 (M)</td>
<td>46 (R)</td>
</tr>
</tbody>
</table>

Enone SAR of naphthyridine tracked that of amides

2,6 Naphthyridines shown to be strong substrate for S9 metabolism – abandon

O- linked compounds proved prone to racemisation - abandon

3-Methyl did not offer advantage over unsubstituted

## Carboxylic acids characterised by:

- Poor absorption in all species – benzamides and naphthyridines
- Low permeability in *in vitro* cell systems
- Elimination by biliary uptake and hepatic clearance
- Low bioavailability

Develop pro-drugs to address these issues

Approach followed by other groups
**Prodrug summary**

<table>
<thead>
<tr>
<th>X</th>
<th>R</th>
<th>IC_{50, \alpha4\beta1} (nM)</th>
<th>IC_{50, \alpha4\beta7} (nM)</th>
<th>AUC h·ng/mL</th>
<th>mu-F% 50mg/kg</th>
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</thead>
<tbody>
<tr>
<td>H</td>
<td></td>
<td>13</td>
<td>220</td>
<td>370</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Et</td>
<td></td>
<td>-</td>
<td>-</td>
<td>6890</td>
<td>45</td>
</tr>
<tr>
<td>HOEt</td>
<td></td>
<td>-</td>
<td>-</td>
<td>2473</td>
<td>17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>X</th>
<th>R</th>
<th>IC_{50, \alpha4\beta1} (nM)</th>
<th>IC_{50, \alpha4\beta7} (nM)</th>
<th>AUC h·ng/mL</th>
<th>mu-F% 50mg/kg</th>
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</thead>
<tbody>
<tr>
<td>H</td>
<td></td>
<td>1</td>
<td>34</td>
<td>420</td>
<td>2</td>
</tr>
<tr>
<td>Et</td>
<td></td>
<td>-</td>
<td>-</td>
<td>4821</td>
<td>25</td>
</tr>
<tr>
<td>HOEt</td>
<td></td>
<td>-</td>
<td>-</td>
<td>7041</td>
<td>43</td>
</tr>
</tbody>
</table>

Approx 25 prodrugs made, ethyl and hydroxyethyl showed most benefit

Decision taken to evaluate two compounds
- Naphthyridine ethyl ester
- Dichloropyridyl amide hydroxyethyl ester

Both active in mouse CIA
Naphthyridine preferred as better exposure across range of species
10x better exposure in rat

Progress naphthyridine – CDP-323 (Free acid UCB1212874) and hold amide
Summary of CDP-323 (Acid and Ester)

**IC₅₀ (nM) Protein / Protein Assay**

<table>
<thead>
<tr>
<th>α-M</th>
<th>α-β</th>
<th>α-β</th>
<th>LFA-1</th>
<th>α/βⅢ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>0.6</td>
<td>126</td>
<td>6792</td>
<td>1502</td>
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</table>

**IC₅₀ (nM) Cellular Assay**

<table>
<thead>
<tr>
<th>α-M</th>
<th>α-β</th>
<th>α-β</th>
<th>LFA-1</th>
<th>α/βⅢ</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>220</td>
<td>3260</td>
<td>&gt;10000</td>
<td>11506</td>
</tr>
</tbody>
</table>

**IC₅₀ (nM) WITH SERUM ALBUMINS**

<table>
<thead>
<tr>
<th>α-M</th>
<th>α-β</th>
<th>α-β</th>
<th>LFA-1</th>
<th>α/βⅢ</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCS</td>
<td>HSA</td>
<td>HSA</td>
<td>RSA</td>
<td>HWB</td>
</tr>
<tr>
<td>13</td>
<td>378</td>
<td>466</td>
<td>270</td>
<td>83</td>
</tr>
</tbody>
</table>

**Aqueous solubility µg/mL**

<table>
<thead>
<tr>
<th>pH</th>
<th>2</th>
<th>4.5</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>754</td>
<td>5</td>
<td>n.d</td>
<td>n.d</td>
</tr>
</tbody>
</table>

**CDP-323: DMPK**

**Oral Bioavailability**
- Rat F ~ 20%
- Mouse F ~ 45%
- Dog F ~ 30%

Excreted as free acid via hepatic uptake & biliary excretion

No significant CYP450 isoform inhibition. Low potential for drug:drug interactions

**CYP Inhibition (µM)**

<table>
<thead>
<tr>
<th>CYP</th>
<th>1A2</th>
<th>2C9</th>
<th>2C19</th>
<th>2D6</th>
<th>3A4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td>&gt;100</td>
<td>20</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

No Induction of CYP3A4, low induction of 1A1/2 @ 100µM

Good exposure achievable for safety/toxicology studies in mouse and rat. Approximately dose proportional in these species

Data consistent with 1 or 2 times daily dosing in man.
CDP-323 - Primary pharmacology and secondary in vivo efficacy models

Primary models

Anti arthritic effect in mouse CIA
Multiple sclerosis model using EAE in rodents

Secondary in vivo/in vitro biological effect models

$\alpha_4\beta_1$ and $\alpha_4\beta_7$ dependent trafficking
  - Murine thioglycollate mononuclear cell recruitment.
  - Rat T-cell trafficking to Payer’s Patches

Murine Intravital Microscopy (IVM)

Therapeutic CDP-323 in murine CIA

CDP-323 reduced clinical score in murine CIA

Error bars removed for clarity
Prophylactic study showed that CDP-323 (100mg/kg b.i.d.) reduced disease incidence, delayed disease onset and reduced disease severity.

Prophylactic CDP-323 in murine CIA

Therapeutic CDP-323 in EAE model of MS

Lymphocyte migration to inflamed regions of the CNS is strongly correlated with their cell surface expression of α4β1.

Therapeutic dosing of CDP-323 produced a significant reduction in clinical score and delay of onset.

Prophylactic dosing significantly reduced disease severity and disease incidence.
**CDP-323 in murine Thioglycollate Model**

In vivo trafficking assay dependant on α4β1.

![Graph showing peritoneal lavage monocytes](image)

Compound dosed orally at -20min and at +2h, thioglycollate given at t = 0 (animals pretreated with anti-LFA-1 mAb)

ED$_{50}$ ~ 10mg/kg

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**CDP-323 inhibited recruitment to Peyer’s Patches**

Organised mucosal secondary lymphatic tissue located in small intestine

Multiple B-lymphocyte follicles separated by interfollicular regions containing T-lymphocytes

Immune surveillance

Oral tolerance
CPD-323 α4β7 Rat T-cell trafficking model

Acid delivered by mini pump to achieve steady state plasma levels.
Indium labelled cells given iv to allow quantification of trafficking
UCB1212874 caused concentration dependant inhibition to Peyer’s patches.
Trafficking to peripheral lymph nodes is unaffected

—

CPD-323 inhibits leukocyte adhesion in-vivo

Epifluorescence intravital microscopy to study effects of UCB1212874 (10mg/kg iv) on lymphocyte behaviour in Peyer’s patch HEV

No effect on percentage of lymphocytes rolling – but did significantly increase rolling velocity
Large suppression of % of lymphocytes adhering to the high endothelial venules
Pharmacology: Summary

Inhibition of murine collagen-induced arthritis. ED$_{50}$ ~50mg/kg
Significant reduction of clinical score in EAE model of MS
Inhibition of thioglycollate-induced monocyte recruitment  ED$_{50}$ 10mg/kg p.o.
Inhibition of T–cell trafficking to Payers patches in rat (Steady-state 300ng/ml)
Visualised inhibition of $\alpha 4$ mediated adhesion in mouse IVM studies

Phase I data

<table>
<thead>
<tr>
<th>Study</th>
<th>Dose and Details</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDP323-001</td>
<td>Single ascending oral dose (24 male volunteers) Up to 1000mg</td>
<td>Well tolerated. Data suggests dose proportionality</td>
</tr>
<tr>
<td>CDP323-002</td>
<td>Multiple dose (27 male volunteers). 250/500/1000mg</td>
<td>Well tolerated, steady state reached day 2</td>
</tr>
<tr>
<td>CDP323-003</td>
<td>Single dose PK/ PD gender comparison</td>
<td>No statistical male / female difference</td>
</tr>
<tr>
<td></td>
<td>500mg</td>
<td></td>
</tr>
</tbody>
</table>

Package of non-clinical data adequately supported progression to the clinic

PK, safety and tolerability in 75 healthy volunteers in three Phase I studies
Inhibition of VCAM binding

CDP323-001 data

CDP-323 causes decreased ability of lymphocytes to bind VCAM-1

Degree and duration ~ dose dependant

800mg dose of CDP-323 did not change expression level of a4

Fasted and fed levels at 500mg similar

CDP-323 inhibition of VCAM binding, repeat dosing

CDP323-002 data

- Good plasma exposure
- Potent and prolonged inhibition of VCAM-1 binding in whole blood assays
- Within 24 h of dosing cessation, VCAM-1 inhibition has dropped to < 50%, consistent with a rapid wash out of the small molecule.
- Clear PD marker
Phase IIa data

**Sept 2006** - UCB and Biogen Idec announced that they would co-develop CDP-323 for the treatment of MS.

**May 2007** - Phase IIa clinical trial in MS was initiated
234 subjects at 70 centers in Europe, the US and Canada. (Subjects were required to have at least one documented clinical relapse during the 12 months preceding screening and to have failed prior treatment with either β-interferon or copaxone due to lack of efficacy or intolerability).

**June 2009** - Interim analysis showed that the primary endpoint of cumulative newly active lesions did not provide the level of efficacy expected for an α4 integrin inhibitor and the program was prematurely terminated.

Summary

Developed potent, mixed α4 antagonist, traced back to cyclic peptide

- Clearance profile problematic
- Ester pro-drug gave adequate bioavailability

Phase I data

- Good plasma exposure, potent and prolonged inhibition of VCAM binding

Phase II data

- Interim analysis, did not reach required level of efficacy – program terminated
Acknowledgements

Chemistry
- Stuart Bailey
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- Martyn Robinson
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- Alisa Webster

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- Roly Foulkes
- Neil Gozzard
- Adrian Moore
- Roger Palframan
- Kate Tomlinson
- Alex Vuglar

DMPK
- Mark Baker
- Kirstie Childs
- David Critchley
- Paul Green
- Hanna Hailu
- David Howat
- Lloyd King
- Ted Parton

Supplementary slides
**α4β1 and α4β7 protein-protein assay** –
High throughput fluorescence based assay which measures the interaction of purified integrin with VCAM-1 (or MadCAM-1). Performed under high affinity conditions using 2mM Mn²⁺
Cross screen for αvβ3, LFA-1, αvβ5, Mac-1, αIIβ3, αvβ1, αEβ7

**Cell-based adhesion assay** –
Measure adhesion of E6.1 Jurkats to VCAM-1. In presence of serum albumin (1% human, rat, mouse)
Cross screen for αvβ3, LFA-1, αvβ5, Mac-1, αIIβ3, α5β1

**Whole blood ligand-binding assay** –
FACS-based assay using modified VCAM-1 to whole blood in the presence of 1mM Mn²⁺.
A human whole blood assay was developed and also an assay to measure inhibition of VCAM-1 binding ex vivo following p.o. dosing (mouse and human) (BEVVI)

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**Safety Pharmacology**

**Cerep profiling** – free acid and ethyl ester profiled. At 10mM (acid 77% binding to K⁺ATP channel, ester 80% binding to PAF receptor)

**Cell proliferation** – no effect in JY proliferation up to 100mM.

**Genotoxicity** – Ames negative in the presence and absence of S9.

**hERG K channel** – no significant blockade at 5 or 50mM.

**Irwin screen** – No CNS related effects at 15 and 150mg/kg (ester).

**GI safety evaluation** – No GI effects at 15 and 150mg/kg (ester).

**Safety & toleration** – NOAEL > 2g/kg single dose, >500mg/kg 28 days

Safety package supported dosing at 500mg/kg