Inflammation 2010

The discovery of CDP-323, a novel and potent inhibitor of the integrins $\alpha4\beta1$ (VLA-4) and $\alpha4\beta7$

Julien Brown

Alison,
living with rheumatoid arthritis

First report

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

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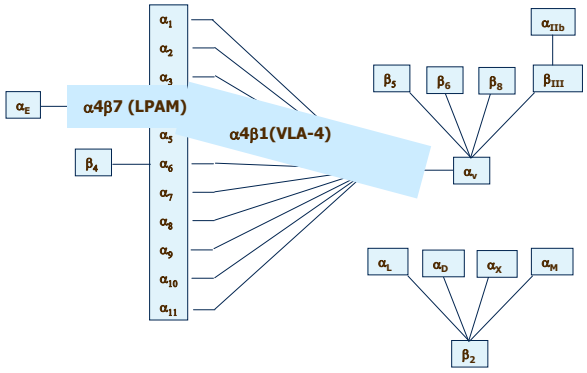
Cell, Vol. 46, 271–282, July 18, 1986,

"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

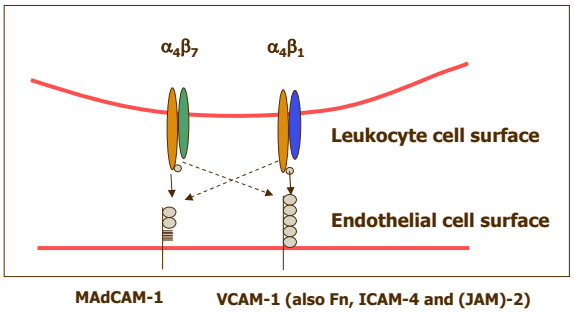
- Followed by the identification of a whole family of glycoproteins exhibiting similar function.
- Each of these glycoproteins is a heterodimer comprising an alpha and beta subunit



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

Why α4 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

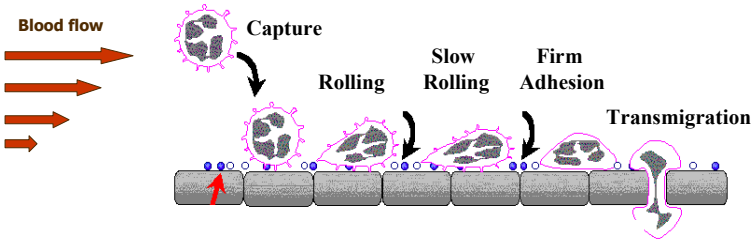
A variety of diseases have some dependency on α4 integrins

Potentially useful in:

α4β1 : Multiple Sclerosis, Rheumatoid Arthritis, Asthma

α4β7 : Crohn's Disease, Ulcerative Colitis, IBD

Transmigration - A Multistep Process

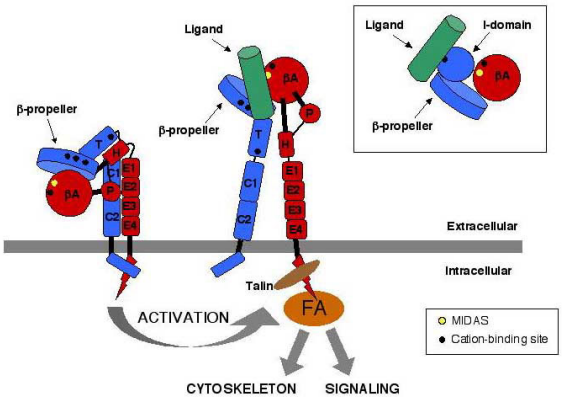


STEP	Ligands on endothelium	Ligands on leukocyte
Firm adhesion	VCAM-1, ICAM-1	β1, β2 and β7 integrins
Transmigration	VCAM-1, ICAM-1, PECAM-1	β1, β2 and β7 integrins, PECAM-1

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

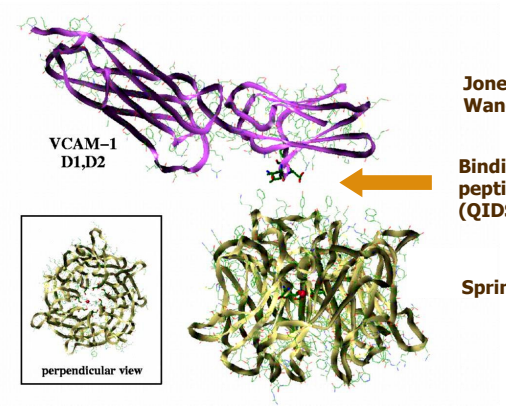


Activation is mediated by inside-out and outside-in signalling events

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).



Jones et al Nature (1995) 373 539
Wang PNAS (1995) 92 5714

Binding region, peptide sequence (QIDS)

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 α4 subunit, MS and Crohn's disease.

Efalizumab (Raptiva®; Genentech), mAb to αLβ2 integrin, moderate to severe psoriasis

Both have been associated with cases of PML.

i) Tysabri was temporarily withdrawn

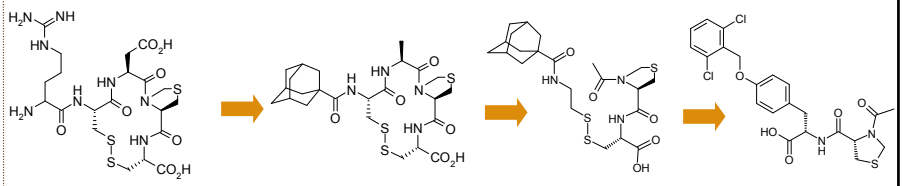
High efficacy and level of medical need led to reintroduction

ii) Efalizumab withdrawn 2009

Vedolizumab (MLN0002; Takeda), humanised mAb against the α4β7 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



α4β1 cell IC_{50} 3600nM

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

α4β1 cell IC_{50} 330nM

Arginine replaced by adamantyl

Cysteine carboxylic acid essential

Aspartic acid not required

α4β1 cell IC_{50} 280nM

Decyclisation – removal of alanine acid was not detrimental to potency

α4β1 cell IC_{50} 35nM

Removal of disulphide

L to D thioproline switch

Amide NH required

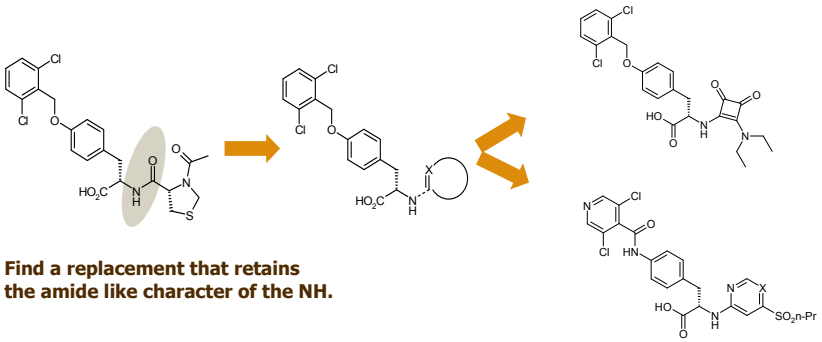
100 fold increase in potency on a simplified molecule

Efficacy in allergic sheep model of asthma

However, rapid biliary clearance in a number of species

Lead reference: Porter et al *Bioorg Med Chem Lett* **2003, 13, 805**

Modification of amide



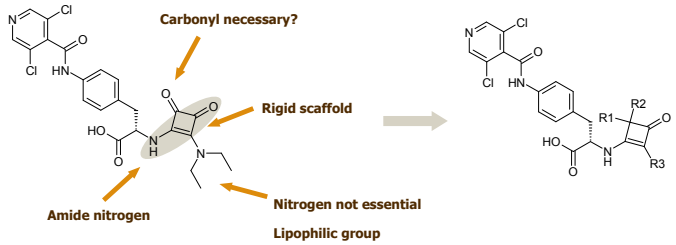
Find a replacement that retains
the amide like character of the NH.

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

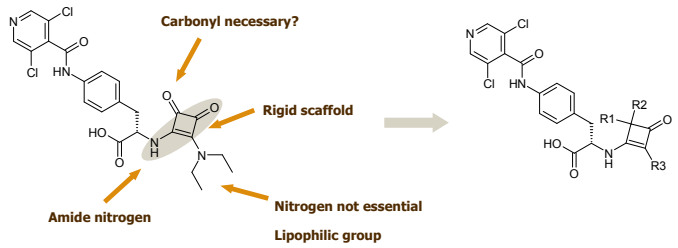


<u>This process led us to investigate aminocyclobutenones scaffold</u>

Features

- **Novel structure**
- **Three points of diversity around the ring allows derivatisation**

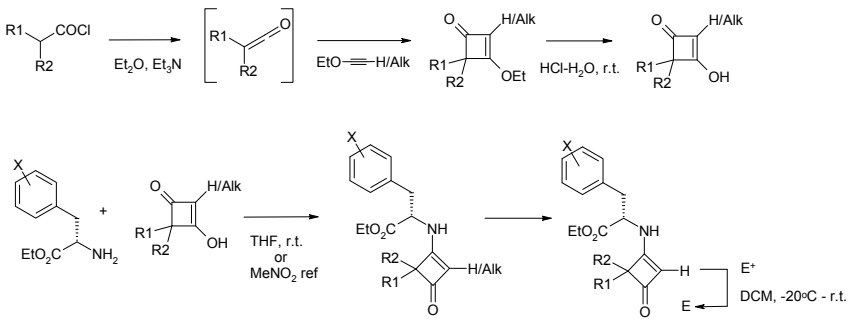
Aminocyclobutenone – A tractable series?



This process led us to investigate aminocyclobutenones scaffold

- **Can we make them?**
- **Limited synthetic precedent**

Aminocyclobutenone – A tractable series?

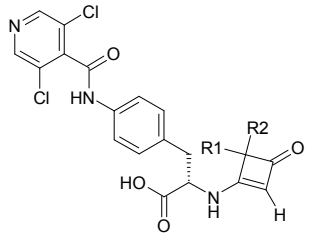


Synthesis of diones and coupling to amino ester derivatives straightforward

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

Stereochemistry when R1≠ R2 could not be controlled

Geminal substituents

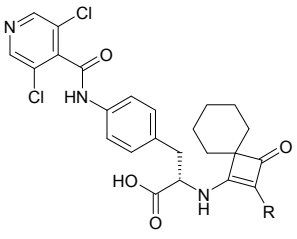


	R1R2	α4β1/VCAM IC_{50} (nM)	α4β7/VCAM IC_{50} (nM)	Clearance (mL/min/kg)
ACYCLIC	Di-methyl	175	921	
	Di-propyl	44	313	
	Di-Bn	3320	> 10,000	
	Me, Bn	45	3540	
	Me, Pr	20	277	
CYCLIC	cyclopentyl	30	401	23
	cyclohexyl	9	142	36
	cycloheptyl	10	120	54
	THP	4	241	
	NAc piperazine	112	417	

Accessing lipophilic pocket enhances potency

Cyclohexyl and THP chosen for further study

Alkene substituents

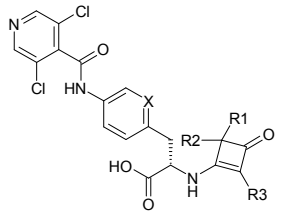


R	α4β1/VCAM IC50 (nM)	α4β7/VCAM IC_{50} (nM)	Clearance (mL/min/kg)
H	9	142	36 (R)
Br	1	34	12 (R) 18 (M)
Cl	3	27	21 (R) 17 (M)
Me	4	80	25 (R) 21 (M)
S^iPr	0.35	2	48 (R) 72 (M)
CH_2NMe_2	>5000	> 5000	
3-pyridyl	4	56	
D-Phenylalanine configuration			
Br	120	1026	

Polar functionality not tolerated

Bromo cyclohexyl carried forward

Phenyl to pyridyl



X = N or C

R1R2	R3	α4β1/VCAM IC_{50} (nM)	
		Phenyl	Pyridyl
Dimethyl	Br	24	155
Cyclohexyl	Br	1	8
Cyclohexyl	Cl	3	16
Dimethyl	SMe	2	20

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

α4β1	α4β7	α5β1	αMβ2	LFA-1	αvβIII
0.3	0.7	133	2150	207	> 50000

IC_{50} (nM) Cellular Assay

α4β1	α4β7	α5β1	αMβ2	LFA-1	αvβIII
1	34	45210	19750	7970	> 50000

CYP Inhibition (μM)

1A2	2C9	2C19	2D6	3A4
>100	19	24	>100	20

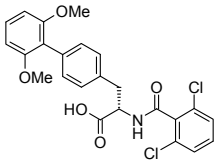
pKa	2.85
PSA	118
LogD 7.4	0.09
PPB	> 95%
Sol	5.4 mg/mL (pH 6.8)

IC_{50} (nM) WITH SERUM ALBUMINS

α4β1/VCAM						α4β7/VCAM			
FCS	HSA	MSA	RSA	HWB	MWB	BSA	HSA	RSA	HAS
6	13	65	62	4	20	34	63	208	25

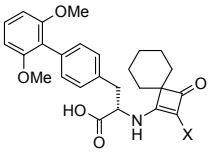
Top group modifications

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



SB683698 (TR14035)

	α4β1/VCAM IC_{50} (nM)	α4β7/VCAM IC_{50} (nM)	F% Rat	Clearance (mL/min/kg)
SB683698	92	620	60	>100 (R)



X				
H	228	1369		
Br	30	780		38 (M)

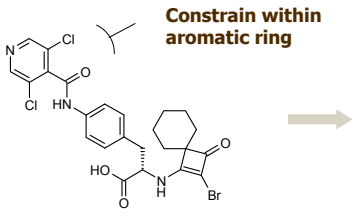
Dimethoxy aryl compound has high bioavailability

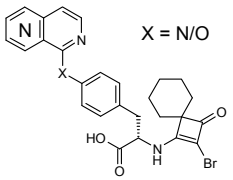
Incorporation of this head group did not offer us necessary potency

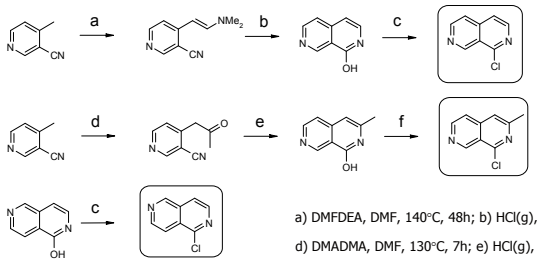
- **gave impetus to try alternative top groups**

Amide to naphthyridine

Constrain within aromatic ring





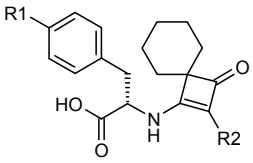


a) DMFDEA, DMF, 140°C, 48h; b) HCl(g), AcOH, H_2O, 40°C, 18h; c) $POCl_3$, 110°C, 24h.

d) DMADMA, DMF, 130°C, 7h; e) HCl(g), AcOH, rt, 18h; f) $POCl_3$, 130°C, 3h

Naphthyridines synthetically accessible

Naphthyridine SAR



R1	R2	α4β1/VLA4 cell IC_{50} nM	α4β7/VLA4 cell IC_{50} nM	Clearance ml/min/kg	
	Br	5	194	41 (M)	21 (R)
	H	20	678	23 (M)	26 (R)
	Br	2	61	31 (M)	32 (R)
	H	20	194		12 (R)
	Br	11	220	39 (M)	15 (R)
	H	25	509	24(M)	9 (R)
	Br	7	76		
	H	40	236	34 (M)	46 (R)

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

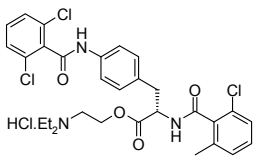
3-Methyl did not offer advantage over unsubstituted

<u>Carboxylic acids characterised by:</u>

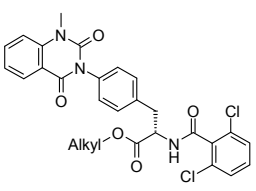
- **Poor absorption in all species – benzamides and naphthyridines**
- **Low permeability in *in vitro* cell systems**
- **Elimination by biliary uptake and hepatic clearance**
- **Low bioavailbility**

Develop pro-drugs to address these issues

Approach followed by other groups

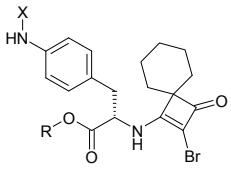


R411



AJM300

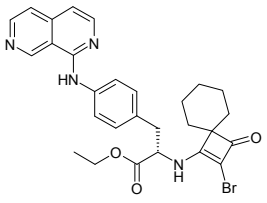
Prodrug summary

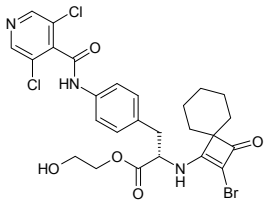


X	R	α4β1/VCAM IC_{50} (nM)	α4β7/VCAM IC_{50} (nM)	AUC h.ng/mL	mu-F% 50mg/kg
	H	13	220	370	<1
	Et	-	-	6890	45
	HOEt	-	-	2473	17

X	R	α4β1/VCAM IC_{50} (nM)	α4β7/VCAM IC_{50} (nM)	AUC h.ng/mL	mu-F% 50mg/kg
	H	1	34	420	2
	Et	-	-	4821	25
	HOEt	-	-	7041	43

Approx 25 prodrugs made, ethyl and hydroxyethyl showed most benefit





<u>Decision taken to evaluate two compounds</u>

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)

IC_{50} (nM) Protein / Protein Assay

α4β1	α4β7	α5β1	αMβ2	LFA-1	αvβIII
0.4	0.6	126	6792	1502	>10000

IC_{50} (nM) Cellular Assay

α4β1	α4β7	α5β1	αMβ2	LFA-1	αvβIII
13	220	32620	> 10000	11506	> 50000

IC_{50} (nM) WITH SERUM ALBUMINS

α4β1/VCAM						α4β7/VCAM	
FCS	HSA	MSA	RSA	HWB	MWB	BSA	RSA
13	378	466	270	83	113	220	144

Aqueous solubility µg/mL

pH	2	4.5	6	8
	734	5	n.d	n.d

pKa	5.5, 1.6
m.p.	194°C
PSA	108 (acid) 91 (ester)
LogD 7.4	0.74 (acid) 6.8 (ester)
PPB	> 98%

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)

	1A2	2C9	2C19	2D6	3A4
Acid	>100	20	>100	>100	>10

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

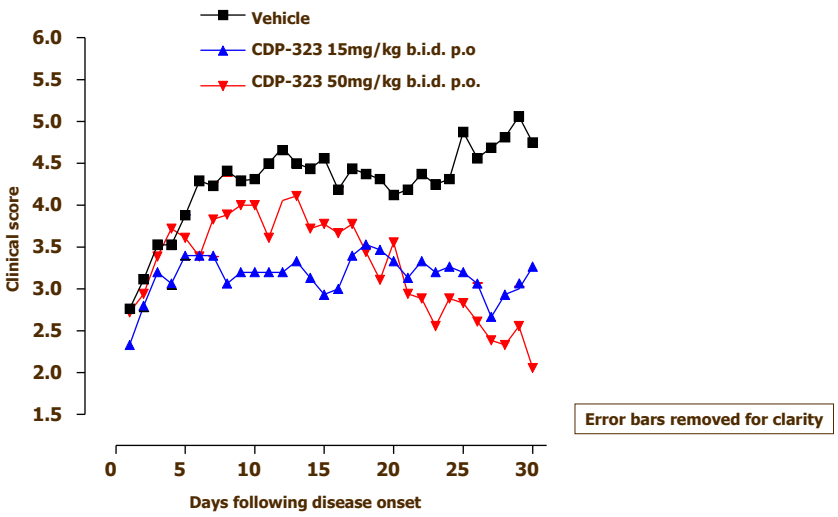
Secondary *in vivo/ in vitro* biological effect models

α4β1 and α4β7 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



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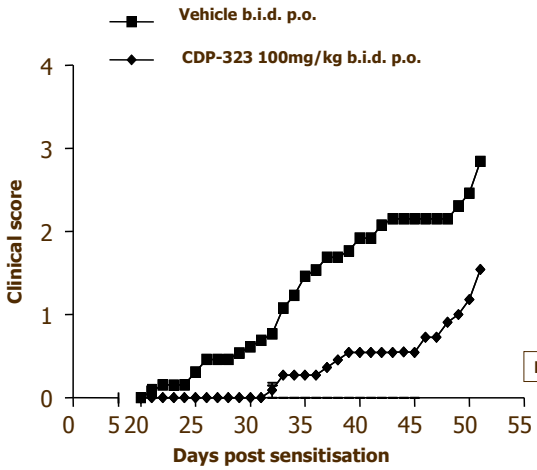
CDP-323 reduced clinical score in murine CIA

Prophylactic CDP-323 in murine CIA



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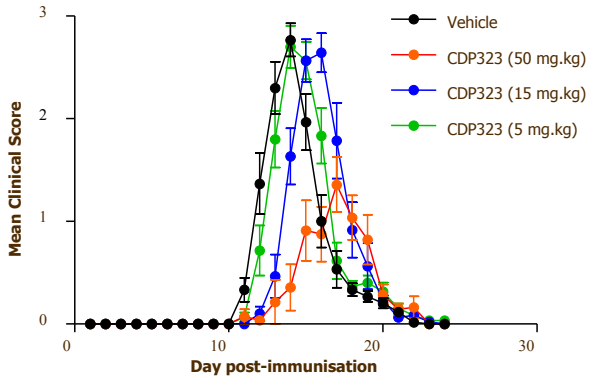
Prophylactic study showed that CDP-323 (100mg/kg b.i.d.) reduced disease incidence, delayed disease onset and reduced disease severity

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

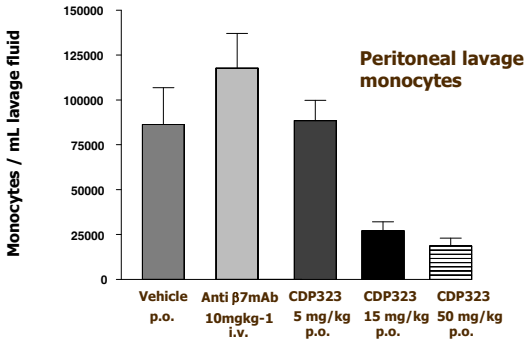


Therapeutic dosing of CDP-323 produced a significant reduction in clinical score and delay of onset

Prophylactic dosing significantly reduced disease severity and disease incidence

CDP-323 in murine Thioglycollate Model

In vivo **trafficking assay dependant on α4β1.**

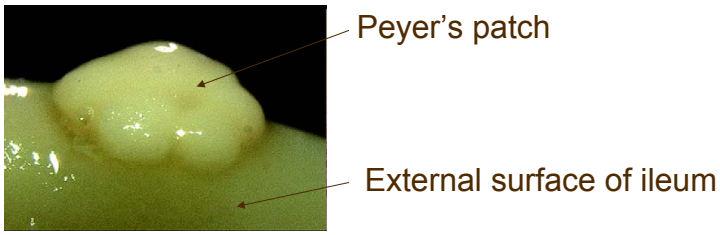


Compound dosed orally at -20min and at +2h, thioglycollate given at t = 0

(animals pretreated with anti-LFA-1 mAb)

ED_{50} ~ 10mg/kg

CDP-323 inhibited recruitment to Peyer's Patches



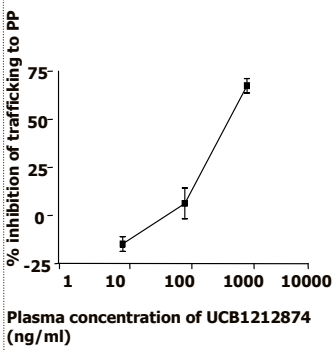
Organised mucosal secondary lymphatic tissue located in small intestine

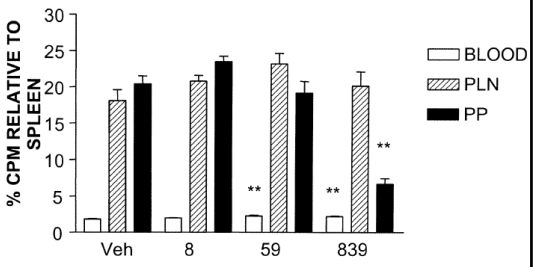
Multiple B-lymphocyte follicles separated by interfollicular regions containing T-lymphocytes

Immune surveillance

Oral tolerance

CDP-323 α4β7 Rat T-cell trafficking model





** P<0.01 by ANOVA with Dunnett's post-test

Acid delivered by mini pump to achieve steady state plasma levels.

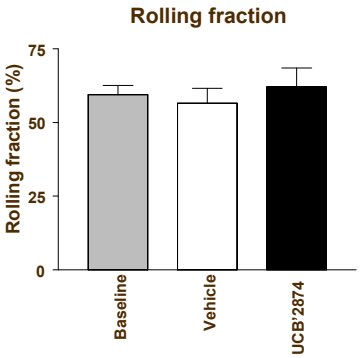
Indium labelled cells given iv to allow quantification of trafficking

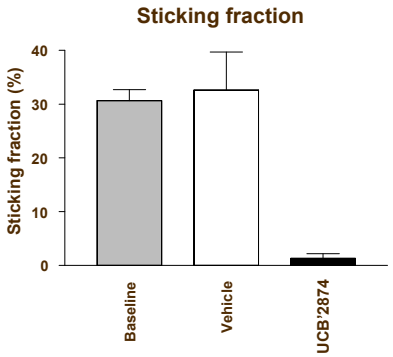
UCB1212874 caused concentration dependant inhibition to Peyer's patches.

Trafficking to peripheral lymph nodes is unaffected

CDP-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 α4 mediated adhesion in mouse IVM studies

Phase I data

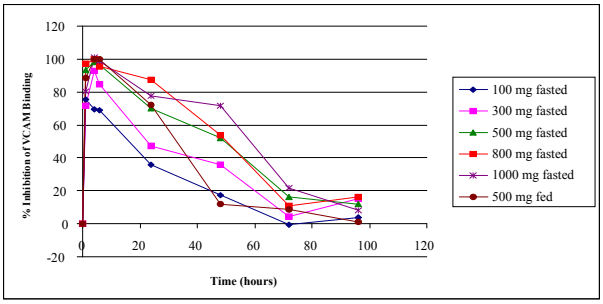
CDP323-001	Single ascending oral dose (24 male volunteers) Up to 1000mg		Well tolerated. Data suggests dose proportionality
CDP323-002	Multiple dose (27 male volunteers). 250/500/1000mg	6 day b.i.d., single dose day 7	Well tolerated, steady state reached day 2
CDP323-003	Single dose PK/ PD gender comparison	500mg	No statistical male / female difference

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

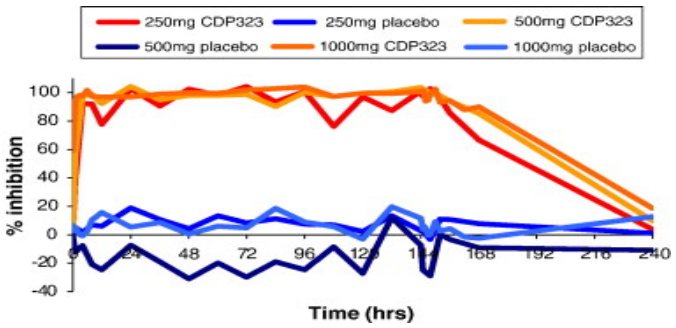
Degree and duration ~ dose dependant

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

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-Stuart Bailey

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Pharmacology

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-Neil Gozzard

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-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 Mn^{2+}
Cross screen for αvβ3, LFA-1, αvβ5, Mac-1, aIIbβ3, 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, aIIbβ3, α5β1

Whole blood ligand-binding assay –

FACS-based assay using modified VCAM-1 to whole blood in the presence of 1mM Mn^{2+}.

A human whole blood assay was developed and also an assay to measure inhibition of VCAM-1 binding *ex vivo* folowing p.o. dosing (mouse and human) (BEVVI)

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.

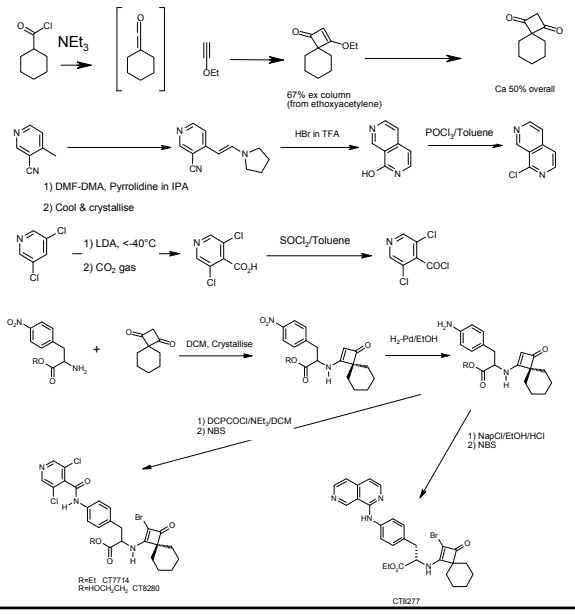
hERG K channel – no significant blockade at 5 or 50mM.

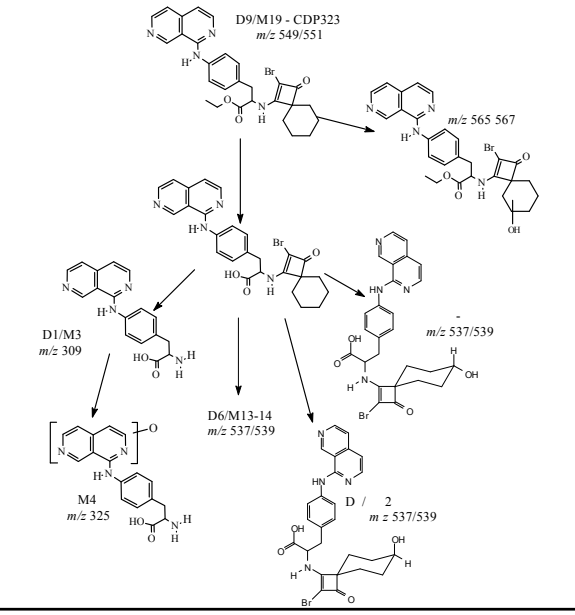
Irwin screen – No CNS related effects at 15 and 150mg/kg (ester).

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


NEt3
67% ex column
(from ethoxyacetylene)
Ca 50% overall
HBr in TFA
POCl3/Toluene
1) DMF-DMA, Pyrrolidine in IPA
2) Cool & crystallise
1) LDA, <-40°C
2) CO2 gas
SOCl2/Toluene
DCM, Crystallise
H2-Pd/EtOH
1) DCPCOCl/NEt3/DCM
2) NBS
1) NapCl/EtOH/HCl
2) NBS
R=Et CT7714
R=HOCH2CH2 CT8280
CT8277


D9/M19 - CDP323
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m/z 565 567
D1/M3
m/z 309
D6/M13-14
m/z 537/539
m/z 537/539
M4
m/z 325
D / 2
m z 537/539