AdAlta

next generation protein therapeutics

i-bodies targeting complex membrane proteins identified by phage display screening

Discovery on Target, Boston 2016: *Antibodies Against Membrane Protein Targets*

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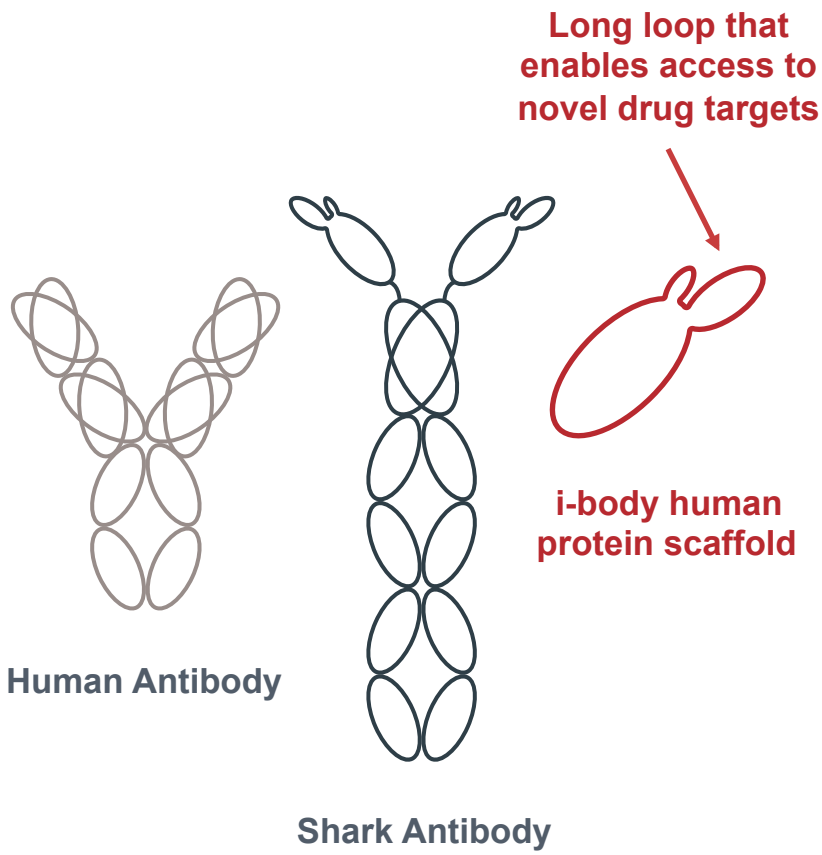
i-body technology

AdAlta is developing a new technology platform that produces unique proteins known as i-bodies, that mimic the shape of shark antibody binding domain and engineers their key stability features into a human protein, for therapeutic intervention in disease.

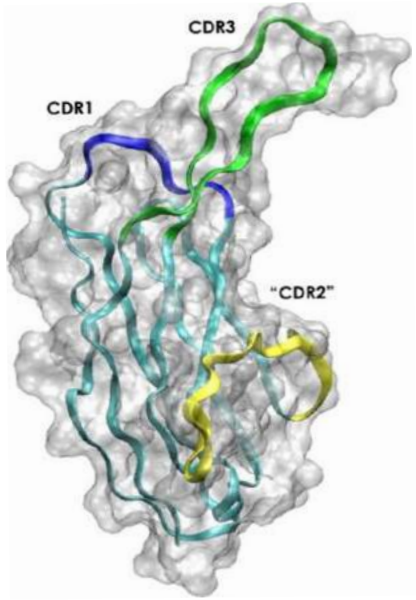
The single domain antigen binding region of shark antibodies is extremely stable and has a long binding loop not present in either human or next generation antibodies.

Advantages of i-bodies

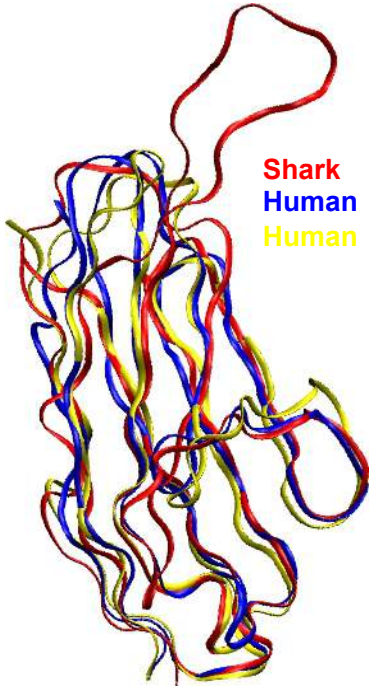
- High target specificity and high affinity for their target
- Small proteins; 10% the size of a typical human antibody
- Highly stable to proteases, high temperatures and low pH
- Long loop that can bind to a diverse range of therapeutically relevant targets including those that are difficult for current antibody therapies
- **Human protein** – reduced risk of immune response



i-bodies: human single domains

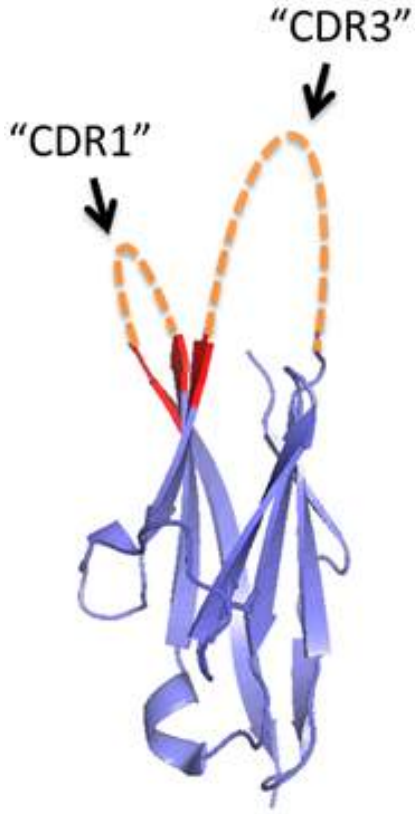


VNAR



Ribbon Overlay

NCAM Domain 1



i-body library

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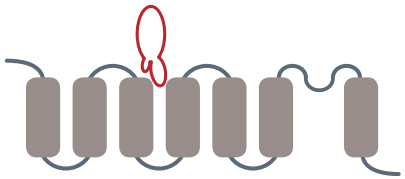
i-body technology advantages

Challenging targets



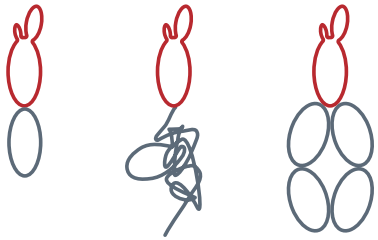
Because of the long binding loop of the i-body, that is lacking in traditional antibodies, i-bodies recognise and bind to a diverse range of different therapeutically-relevant targets including those that are difficult/intractable to access by current antibody therapies such as G-protein coupled receptors (GPCRs) and ion channels.

Multiple delivery routes

Inhalation Ocular Oral-to-topical

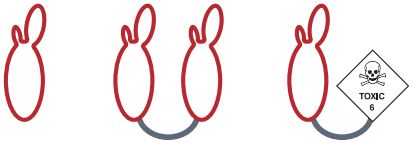
The small physical size and stable properties of i-bodies provides advantages for tissue and organ penetration as well as multiple delivery routes.

Customised half-life



As a result of their small size and exceptional stability i-bodies can serve as building blocks to engineer therapeutics with tailored pharmacokinetic properties.

Multi formatting



Can easily engineer unique differentiated i-body products in a variety of formats including monospecific and bispecifics as well as i-body drug conjugates (IDCs), thus tailoring them for different therapeutic purposes.

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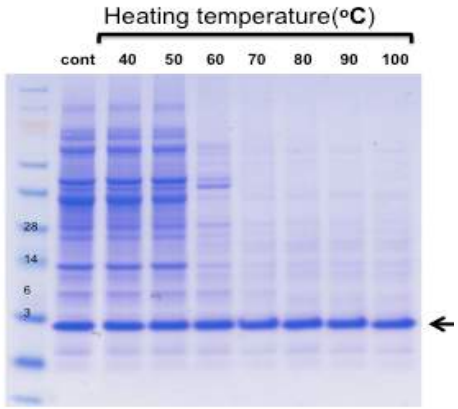
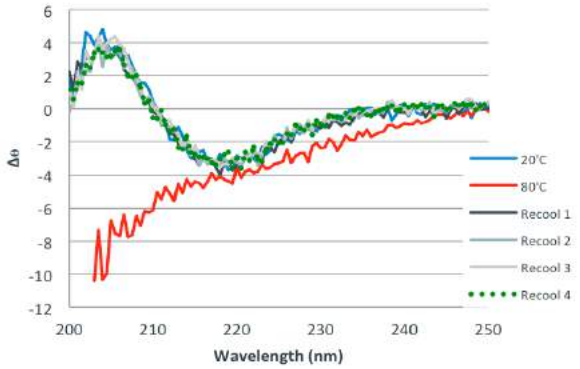
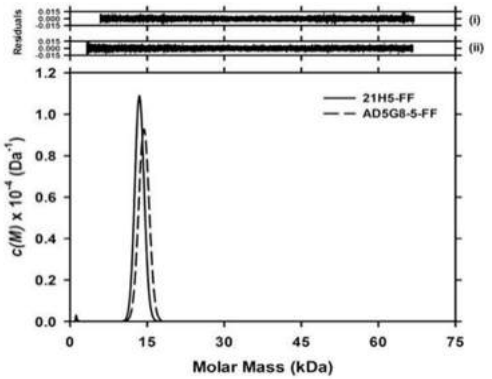
i-body monomeric and extremely stable to proteases, high temperatures and low pH



i-bodies are extremely stable at high temperatures, including boiling, while still retaining their activity. Here we show we can reduce a number of *E.Coli* proteins heating above 60°c.



On heating and re-cooling the i-body we show by Circular dichroism spectroscopy that the b-sheet of the i-body unfolds and re-folds. This process was repeated eight times.



The i-body is a monomeric protein.

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i-bodies combine benefits of small molecules and conventional antibodies

	Small Molecule	Conventional Antibody	AdAlta i-body
High selectivity-specificity		●	●
Low toxicity: no off target effects		●	●
Cavity binding and new epitopes	●		●
Stability	●		●
Alternative routes of administration	●		●
Easy to manufacture	●		●
Speed & risk of development		●	●

Long loop that enables access to novel drug targets

i-body human protein scaffold

i-bodies offer a new and potentially more effective approach to the treatment of a wide range of human diseases.

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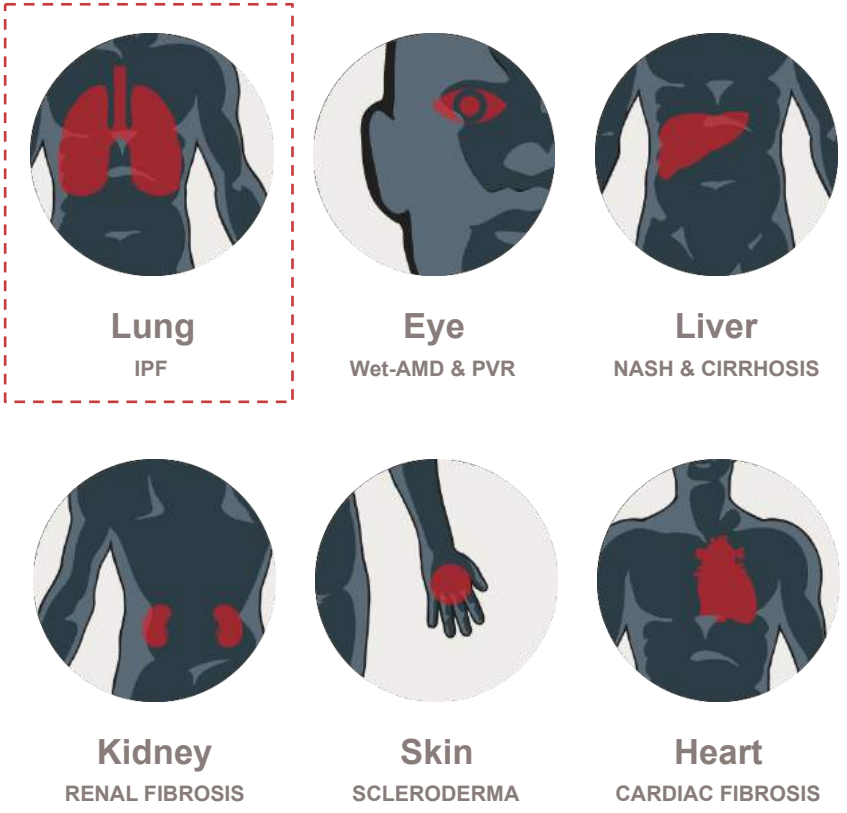
Fibrosis: unmet medical need with multiple indications

- Developing i-bodies as improved therapies for the treatment of fibrosis
  - a condition that is prevalent in 45-50% of all diseases
- Fibrosis can occur in many tissues of the body as a result of inflammation or damage
  - it can result in scarring of vital organs causing irreparable damage and eventual organ failure
- AdAlta's initial focus is on lung fibrosis

Collectively fibrosis represents a large unmet clinical need



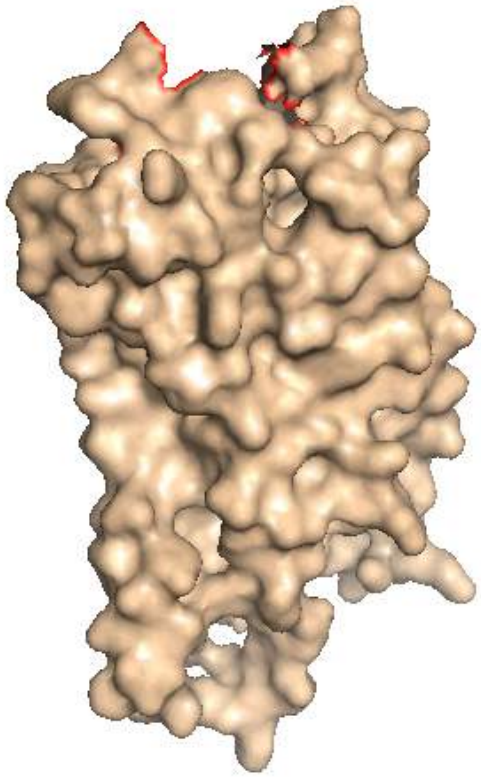
CXCR4 is involved in fibrosis and other disease states

CXCR4 is important in maintaining stem cells in bone marrow with Mozobil (AMD3100) approved for single use.

HIV-1 uses CXCR4 as a co-receptor for viral entry into host cells and CXCR4 has been associated with more than 23 types of cancers

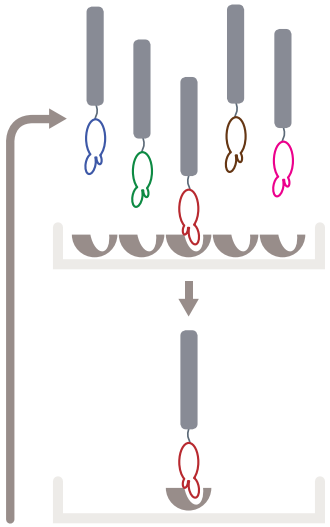
CXCR4 has more recently been recognised as a critical player in development of a number of areas of fibrosis including:

- Lung
- Kidney
- Heart
- Eye
- Skin



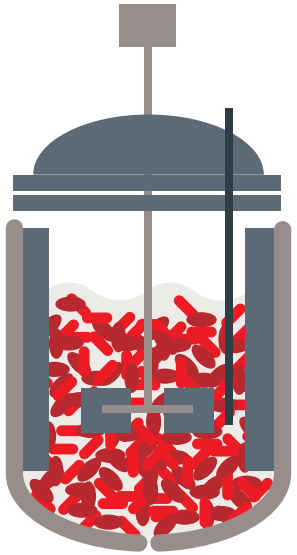
Selecting i-bodies against CXCR4

Large diverse synthetic library of 2 billion i-body protein compounds that can bind to a broad range of therapeutically relevant targets



i-body identified by rapid screening on CXCR4 lipoparticles

i-body affinity matured to enhance target binding and generate lead i-body candidate



Manufactured in microbial systems; more cost-effective and easier than conventional monoclonal antibodies. Potential for direct peptide synthesis.

Round 1 affinity maturation

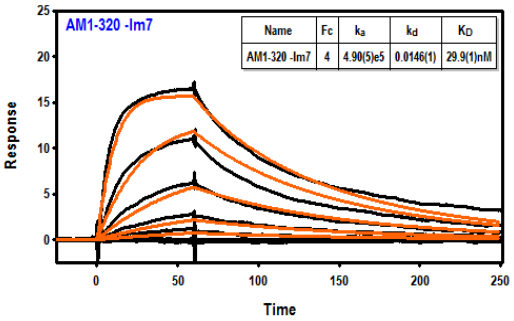
Single point mutations were made in the CDR1 and CDR3 binding regions only of the lead i-body identified

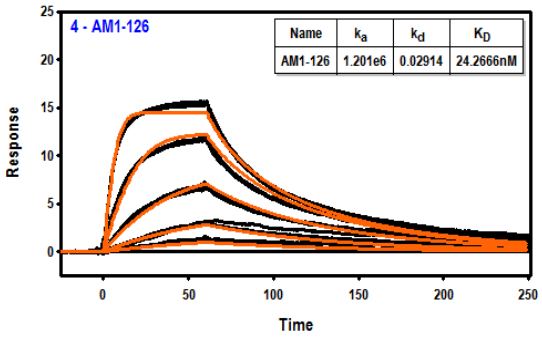
```
                    10        20        30        40        50        60        70        80        90        100
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AD99AM WT           LQVDIVPSQGEISVGESKFFLCQVAGSGSDIRISWFSPNGEKLTPNQQRISVVWNDDSSSTLTIYNANIDDAGIYKCVVYRTGGYRHRALVLGEATVNVK
324                 ..........................................................................................Y..........
320                 ...........................K..............................................................Y..........
113                 ...........................Y..............................................................Y..........
518                 ............................G.............................................................................
545                 ............................G.....................................................V.....W..................
557                 ............................G.....................................................W..................
126                 ...........................H.....................................................W..................
Cont36              ..............................Y...................................................W..................
```

The sequences of i-bodies identified above demonstrated binding to CXCR4 lipoparticles.

Affinities of the two highlighted sequences were 30nM and 25nM.

The first round of affinity maturation improved affinities from 700nM to 25nM with single point mutations.





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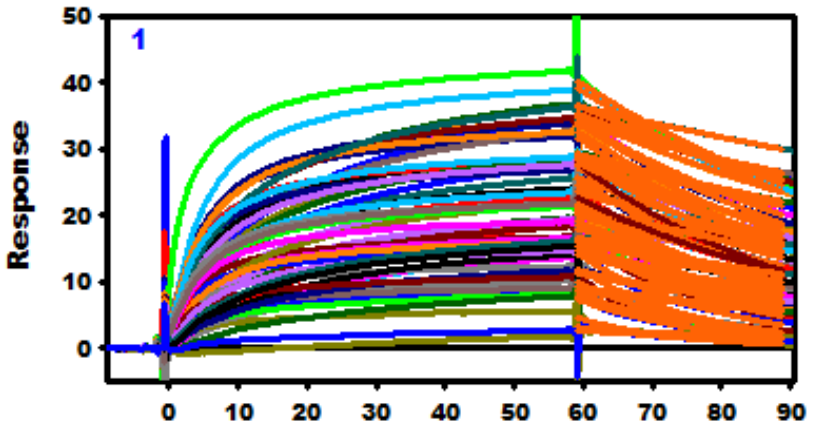
Round 2 affinity maturation

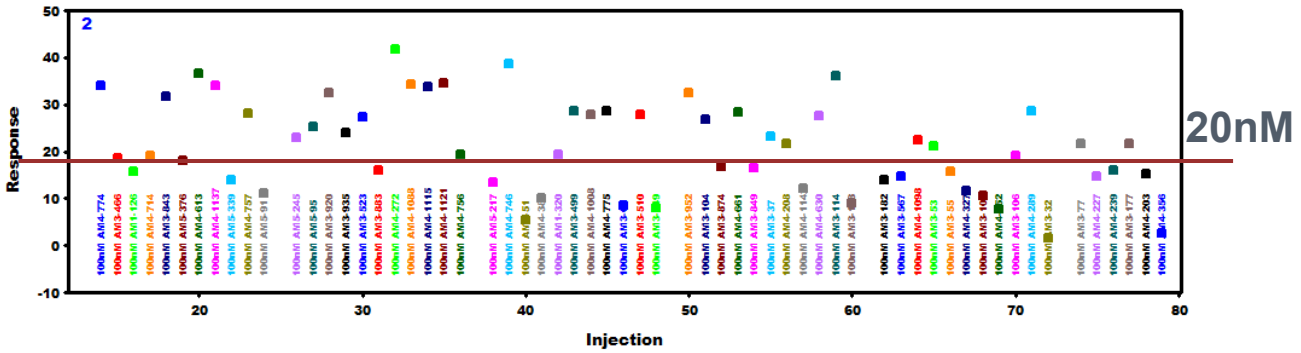
A mutant library was made changes from the two lead candidates from affinity maturation round 1.

Mutant binders in round 2 affinity maturation were ranked with regards to their off rate.

I-bodies were identified with improvements in the second round of affinity maturation from 20nM to 1nM



Rank	Clone	kd	Rank	Clone	kd	Rank	Clone	kd
1	**AM3-114**	**0.0066**	22	AM4-756	0.01667	43	AM3-53	0.0273
2	**AM3-466**	**0.00784**	23	AM4-746	0.01686	44	AM4-1008	0.02771
3	**AM3-920**	**0.00817**	24	AM5-339	0.01697	45	AM3-177	0.02862
4	**AM4-661**	**0.00955**	25	AM4-630	0.01718	46	AM3-104	0.02865
5	**AM3-523**	**0.00998**	26	AM4-1137	0.018	47	AM3-510	0.02913
6	**AM4-774**	**0.0105**	27	AM4-327	0.01897	48	AM3-77	0.02963
7	**AM4-1121**	**0.01153**	28	AM4-775	0.01899	49	AM3-55	0.03097
8	**AM4-613**	**0.01236**	29	AM5-217	0.01948	50	AM3-106	0.03213
9	AM4-208	0.01283	30	AM4-1115	0.01973	51	AM3-849	0.03716
10	**AM4-1088**	**0.01349**	31	AM5-376	0.02008	52	AM3-549	0.0395
11	AM4-239	0.01356	32	AM3-499	0.02012	53	AM3-874	0.04054
12	AM3-32	0.0136	33	AM3-935	0.02026	54	AM3-883	0.0421
13	**AM5-245**	**0.01445**	34	AM5-91	0.02042	55	AM4-356	0.0456
14	AM4-757	0.01488	35	AM3-952	0.02114	56	AM3-567	0.0466
15	AM4-386	0.015	36	AM1-126	0.02232	57	AM3-10	0.0481
16	AM4-352	0.0152	37	AM4-714	0.02236	58	AM3-62	0.0519
17	AM3-182	0.01562	38	AM4-289	0.02264	59	AM4-1142	0.0546
18	AM1-320	0.01589	39	AM3-843	0.02283	60	AM3-183	0.0626
19	AM4-203	0.01621	40	AM4-1098	0.02306	61	AM3-51	0.0678
20	AM4-272	0.01631	41	AM3-37	0.02339			
21	AM5-95	0.01663	42	AM4-227	0.02576			



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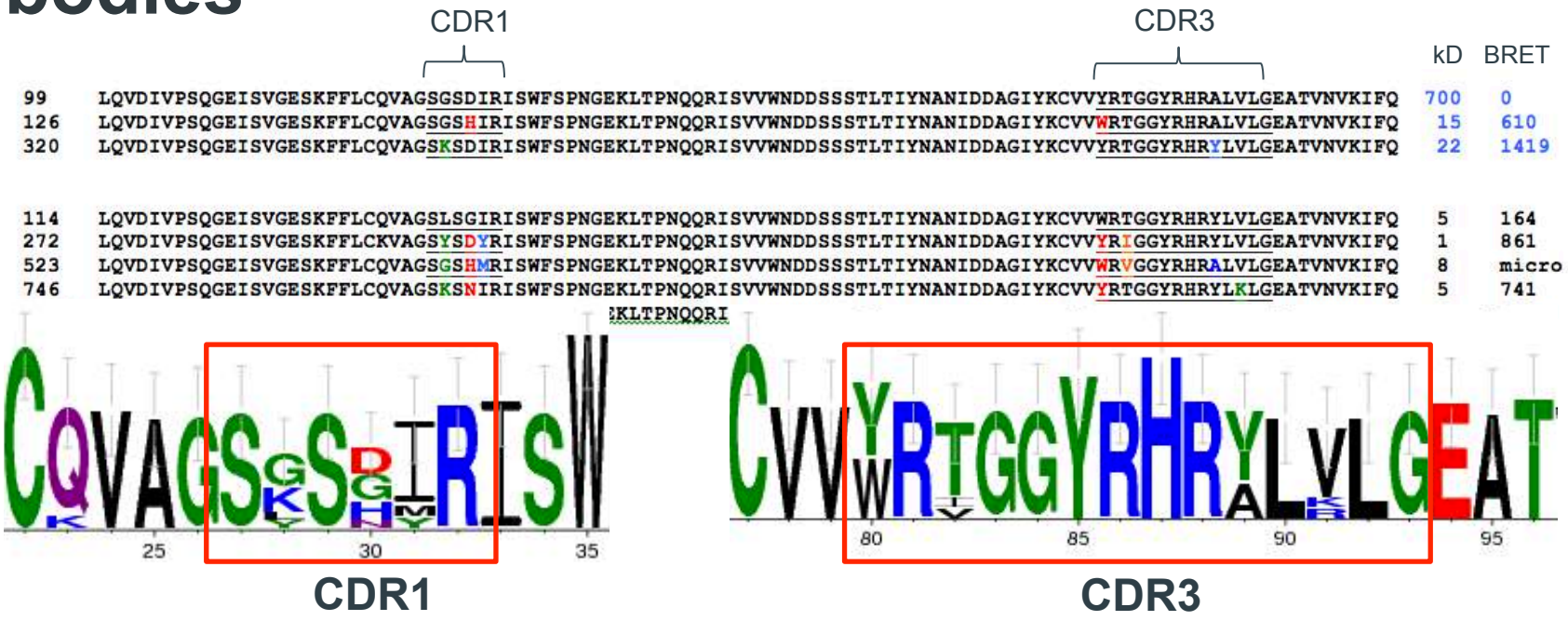
Sequences of affinity matured CXCR4 binding i-bodies



The affinity maturation process allowed identification of residues in the CDR1 and CDR3 that were important for binding to CXCR4.

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Family of CXCR4 binding i-bodies

i-bodies from the affinity maturation process were evaluated in several *in vitro* assays including

- β-arrestin BRET assay
- Calcium flux assay
- cAMP assay

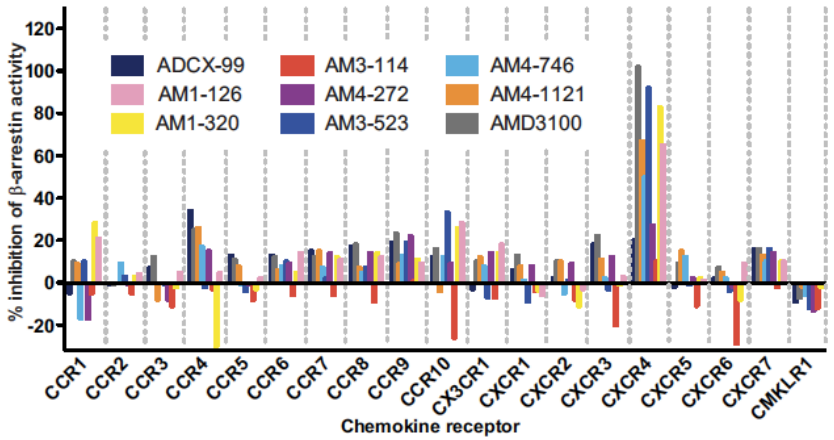
1-3 single point mutations could dramatically effect the β-arrestin activity, while still having the same affinity for the receptor CXCR4

None of the i-bodies identified had any effect on calcium flux

Protein	Affinity to CXCR4 (nM)	IC50 (nM) in b-Arrestin BRET assay
ADCX-99	700	No activity
AD-320	21.8	1419
AD-245	14.88	~uM
AD-126	14.75	610
AD-466	13.83	6322
AD-661	9.53	1544
AD-523	8.55	12713
AD-613	7.83	1826
AD-1121	7.21	796
AD-920	7.18	~uM
AD-746	5	741
AD-114	4.85	164
AD-272	1.6	861

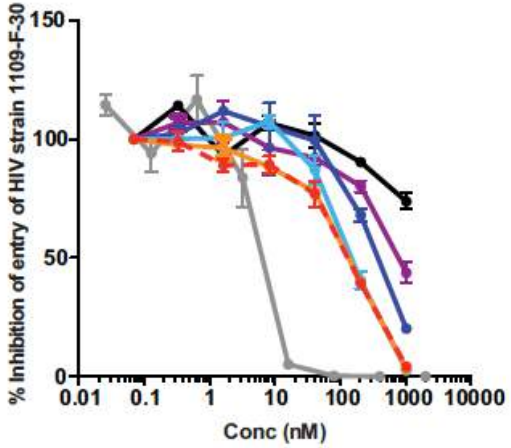
Panel of i-bodies against CXCR4 specific and have *in vitro* activity

I-bodies are specific to CXCR4



When tested against 167 GPCRs, the 10 affinity matured i-bodies specifically antagonized CXCR4. There was no agonist activity on any of the GPCRs.

I-bodies have *in vitro* activity



The i-bodies were evaluated in a number of *in vitro* assays. In this assay we demonstrated that a panel of i-bodies with affinity to CXCR4 <20nM could inhibit inhibition of entry of the HIV virus.

REF: Griffiths et al JBC June 2016

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Epitope mapping: core residues on CXCR4

CDR1 CDR3

```
114   LQVDIVPSQGEISVGESKFFLCQVAG SLSGIR ISWFSPNGEKLTPNQQRISVVWNDDSSSTLTIYNANIDDAGIYKCVV WRTGGYRHRYLVLG EATVNVKIFQ
272   LQVDIVPSQGEISVGESKFFLCQVAG SYSDYR ISWFSPNGEKLTPNQQRISVVWNDDSSSTLTIYNANIDDAGIYKCVV YRIGGYRHRYLVLG EATVNVKIFQ
523   LQVDIVPSQGEISVGESKFFLCQVAG SGSHMR ISWFSPNGEKLTPNQQRISVVWNDDSSSTLTIYNANIDDAGIYKCVV WRVGGYRHRALVLG EATVNVKIFQ
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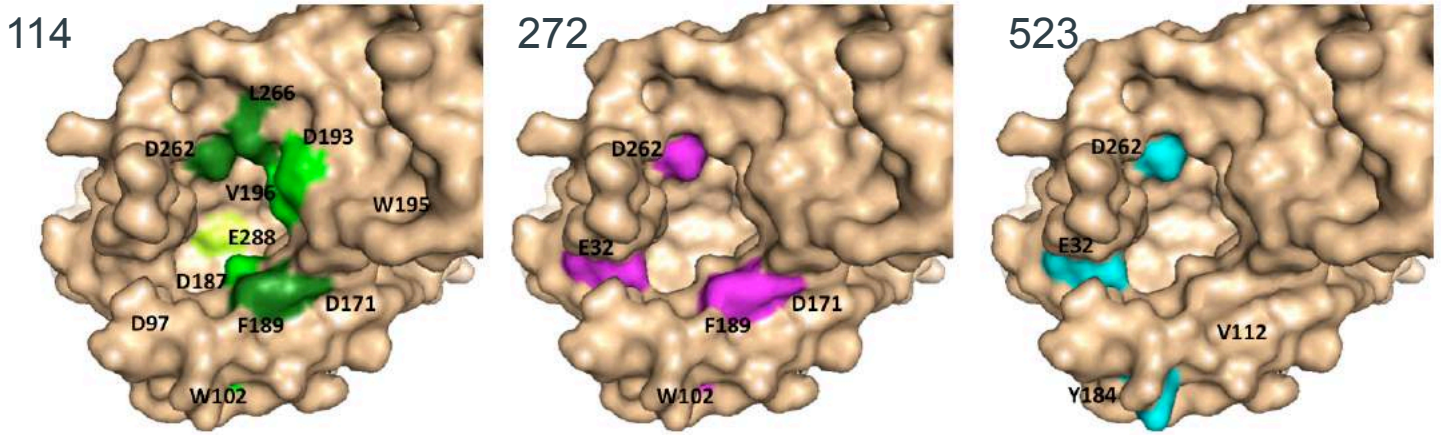

High β-arrestin
High cAMP

Low β-arrestin
High cAMP

Medium β-arrestin
Medium cAMP

Epitope mapping of three i-bodies to CXCR4, demonstrated that residue E262 was common to all i-bodies. E262 which is deep in groove of CXCR4, also binds AMD3100. All three i-bodies had unique binding sites reflecting their different functionalities.

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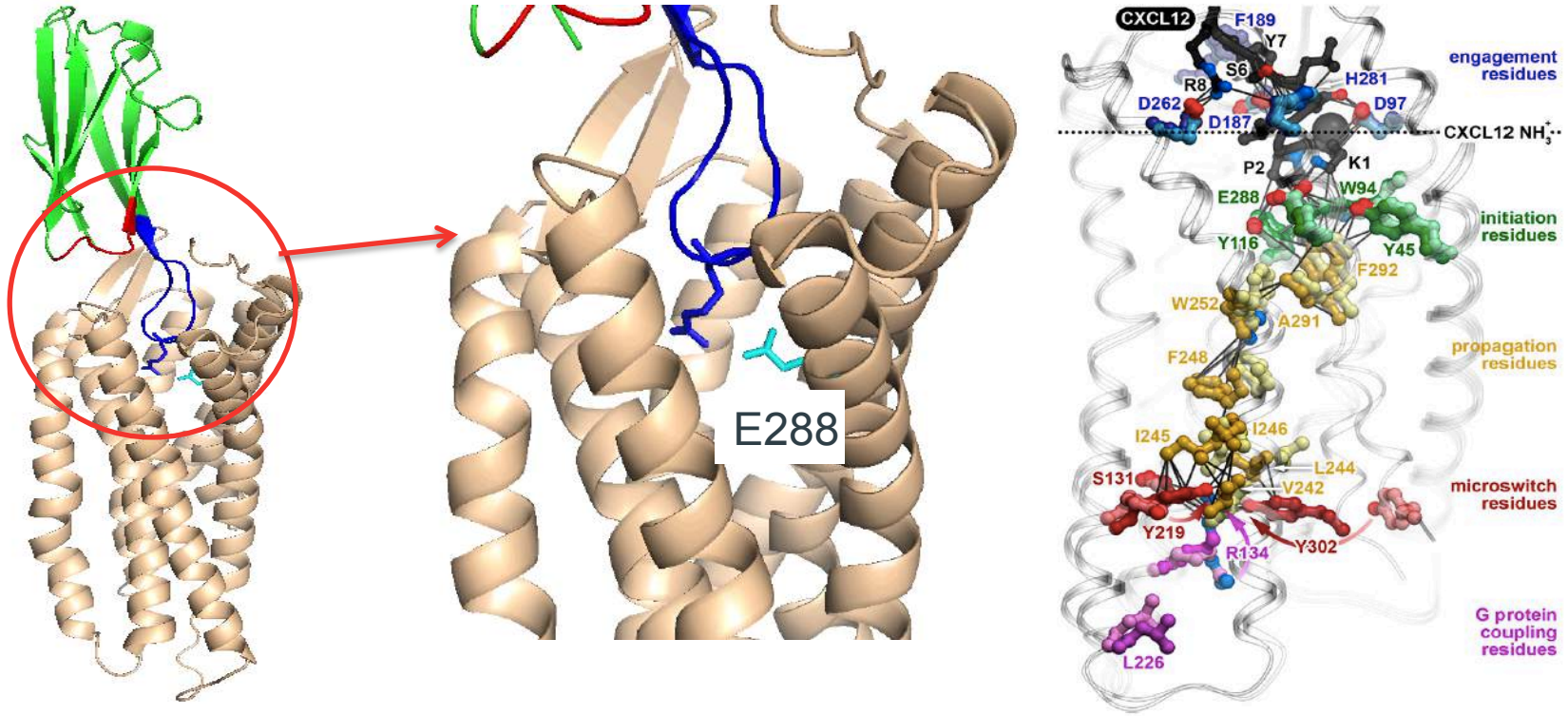
Homology model of i-body and CXCR4



Side chain CDR3 contact residues also demonstrate that the long loop of the i-body, binds deep in the ligand binding pocket of CXCR4

REF: Wescott et al PNAS 2016

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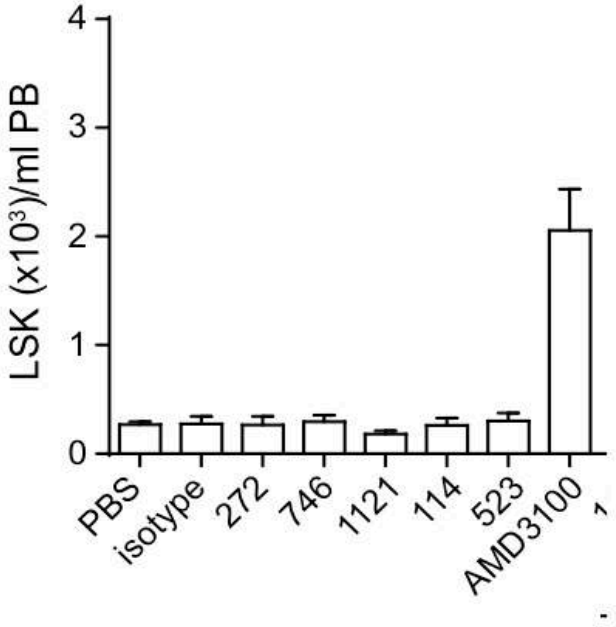
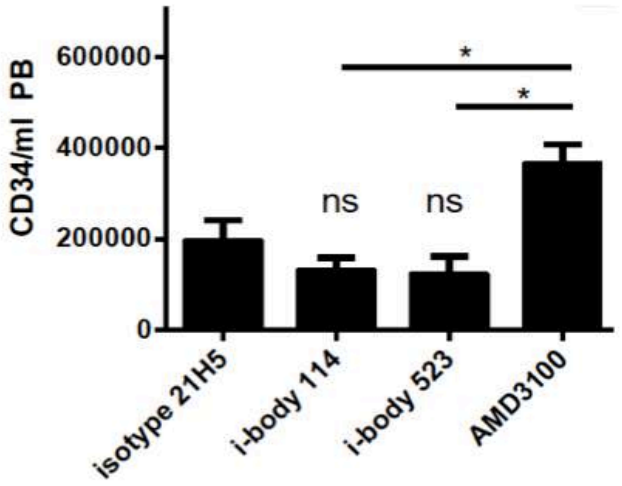
i-bodies do not mobilize stem cells *in vivo*

i-bodies do not mobilize stem cells in mouse model (left) or humanised mouse model (right) of stem cell mobilization, unlike the small molecule AMD3100





REF: Griffiths et al JBC June 2016

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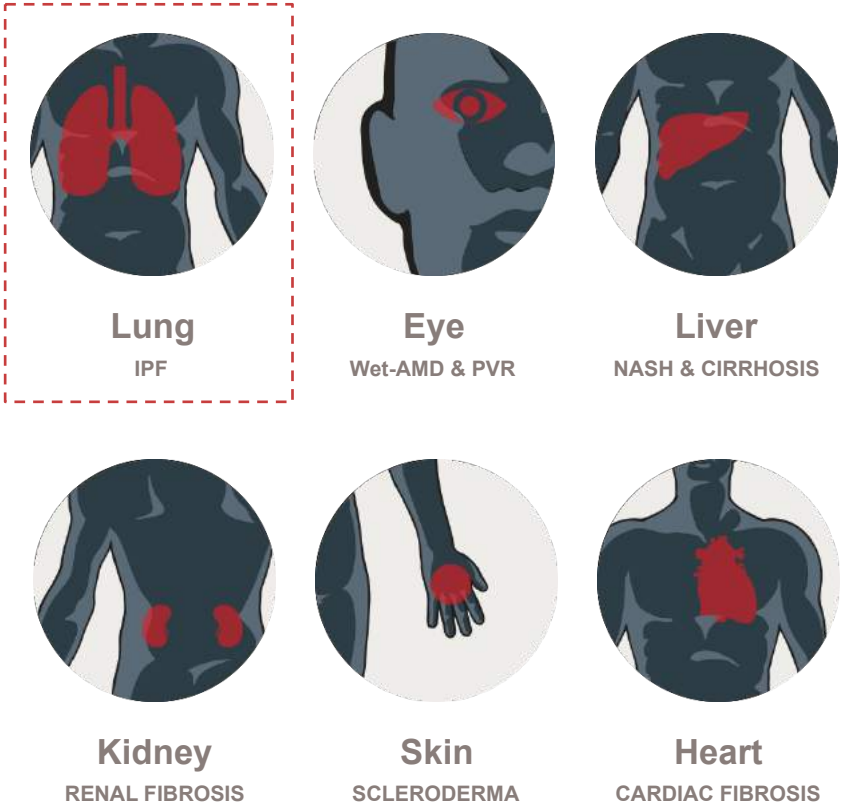
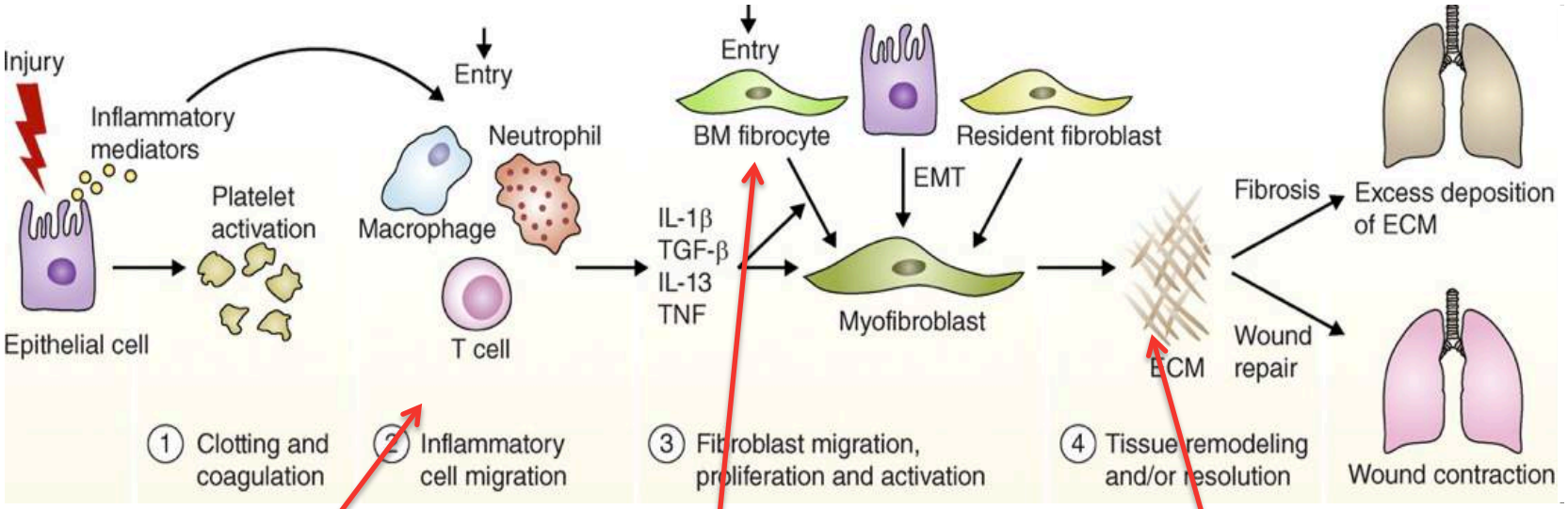
Fibrosis: unmet medical need with multiple indications

- Developing i-bodies as improved therapies for the treatment of fibrosis
  - a condition that is prevalent in 45-50% of all diseases
- Fibrosis can occur in many tissues of the body as a result of inflammation or damage
  - it can result in scarring of vital organs causing irreparable damage and eventual organ failure
- AdAlta's initial focus is on lung fibrosis

Collectively fibrosis represents a large unmet clinical need



Fibrosis is a complex disease



Inflammation

Fibrocyte migration

Deposition of collagen/ECM

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CXCR4 and idiopathic pulmonary fibrosis (IPF)

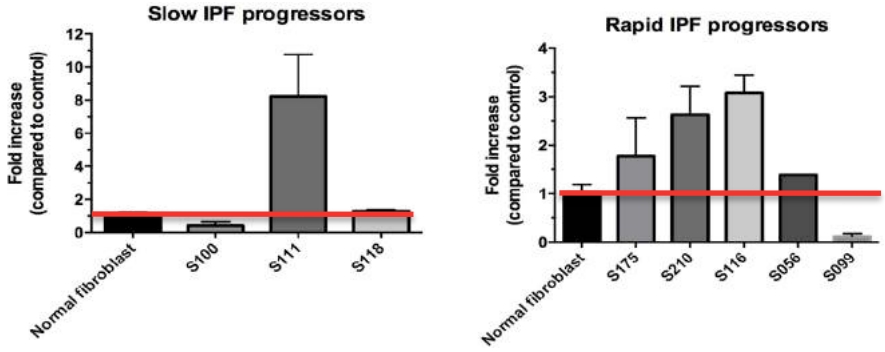
Patients that rapidly progress express more CXCR4 compared to slow IPF progressors

CXCR4 +ve cells (fibrocytes) significantly elevated in stable IPF patients, and further increased during acute exacerbations

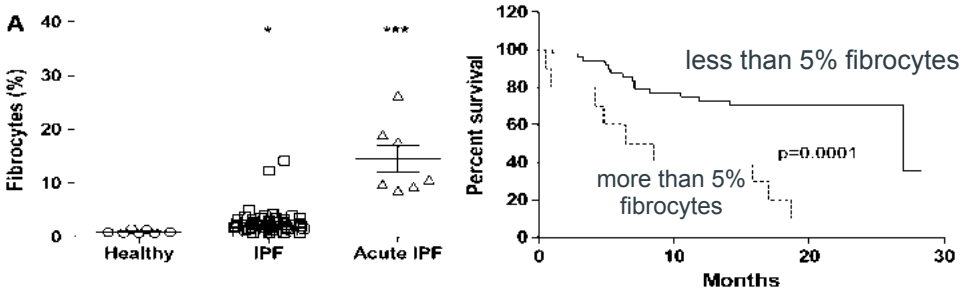
Fibrocytes not correlated with lung function but an independent predictor of early mortality

- 7.5 months with more than 5% fibrocytes
- 27 months with less than 5% fibrocytes

CXCR4 expression increased in fast progressing IPF patient tissue



Fibrocyte numbers predict mortality



REF: Moeller, et al. Am J Respir Crit Care Med Vol 179. pp 588–594, 2009

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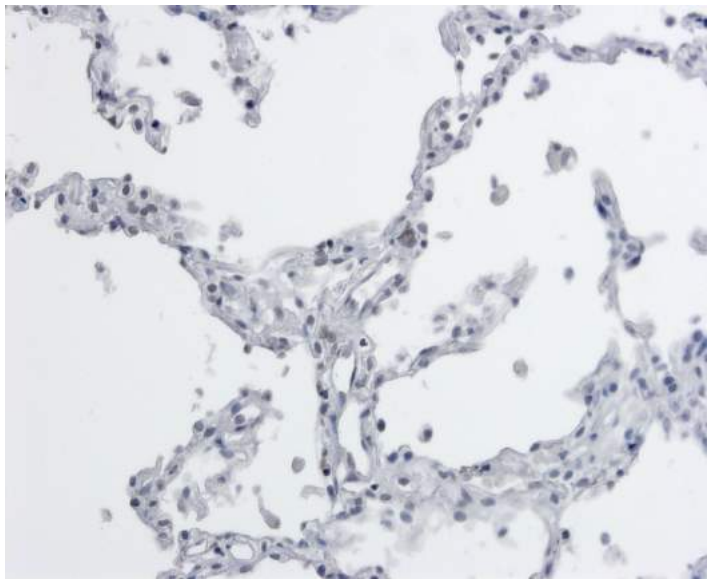
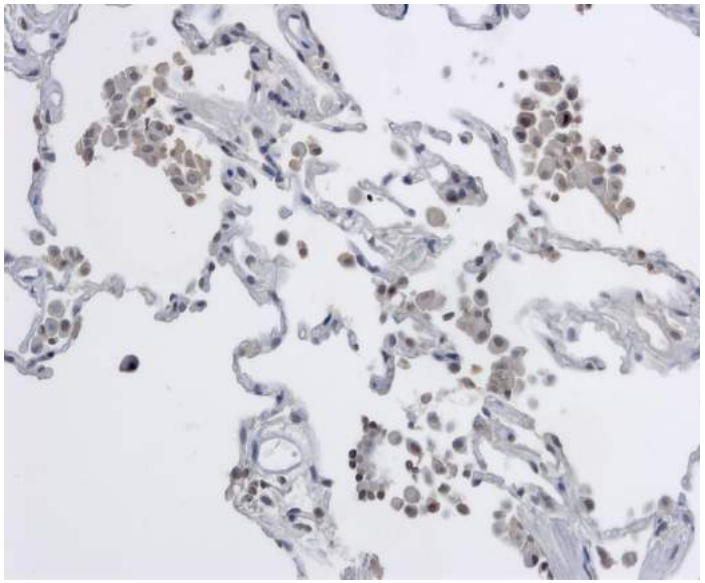
AD-114 binds to lung tissue from patients with fibrosis

The i-body AD-114 was used for Immunohistochemical (IHC) staining of normal and diseased lung tissues to verify expression of CXCR4 *in situ*



AD-114 does not bind lung tissue from normal lungs



AD-114 binds to lung tissue from fibrosis

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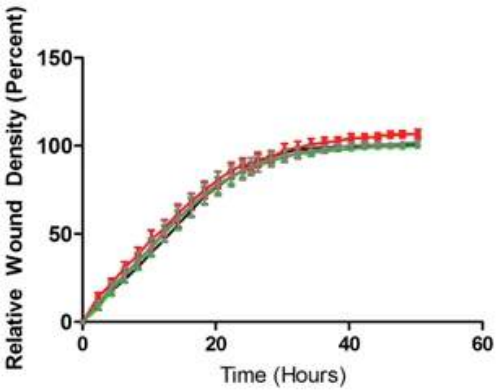
Migration/invasion specifically reduced with IPF lung fibroblasts

i-bodies specifically inhibited migration of slow and rapid IPF fibroblast migration but did not have any effect on normal fibroblasts.

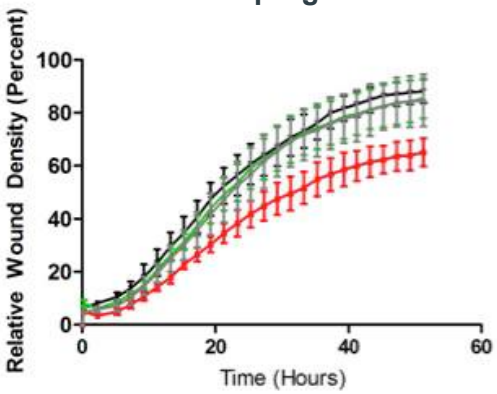
AD-114 has greater *in vitro* efficacy compared to the only approved therapies Nintedanib and Pirfenidone for IPF treatment.

MIGRATION	No effect on normal fibroblasts	Inhibits slow IPF progressors	Inhibits fast IPF progressors
i-body AD-114	✔	✔	✔
Nintedanib (Boehringer)	✗	✔	✔
Pirfenidone (Roche)	✔	✗	✗
Other CXCR4 drug (Sanofi)	✔	✗	✗

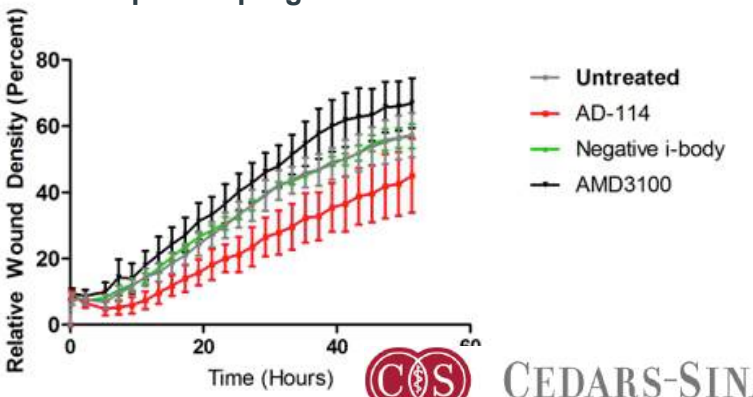
Normal fibroblasts



Slow/stable IPF progessor fibroblasts



Rapid IPF progressor fibroblasts



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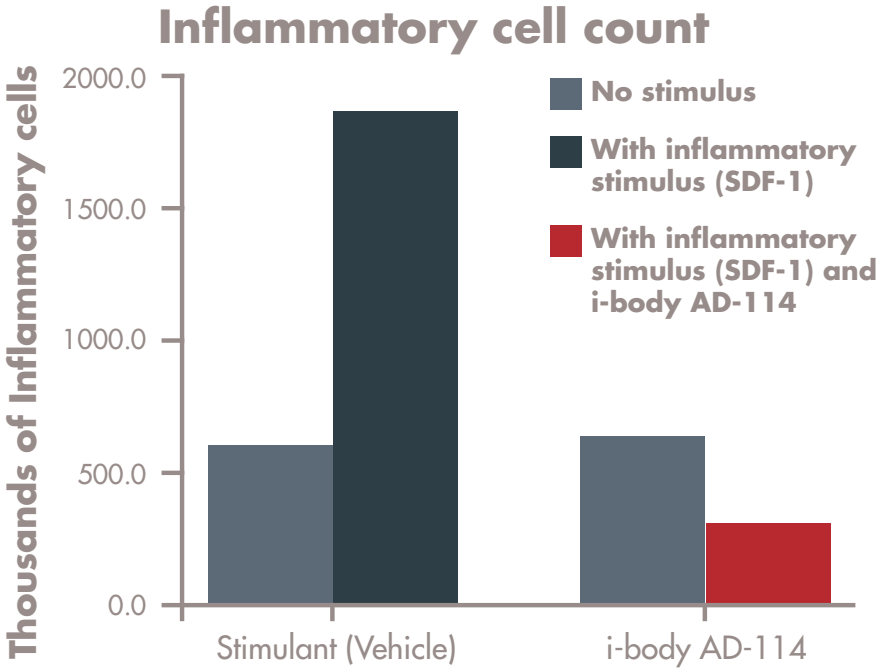
i-bodies block infiltration of leukocytes

AD-114 has also been evaluated for its anti-inflammatory activity. Anti-inflammatory activity is important for preventing fibrosis.

In this model, an inflammatory stimulant known as SDF-1 is added to an air-pouch created on the back of a mouse, which results in a dramatic increase in the amount of inflammatory cells counted in the air-pouch (black column). When a single dose of AD-114 is injected into the mouse, the migration of the inflammatory cells to the air-pouch is blocked (red column).



REF: Griffiths et al JBC June 2016

AdAlta

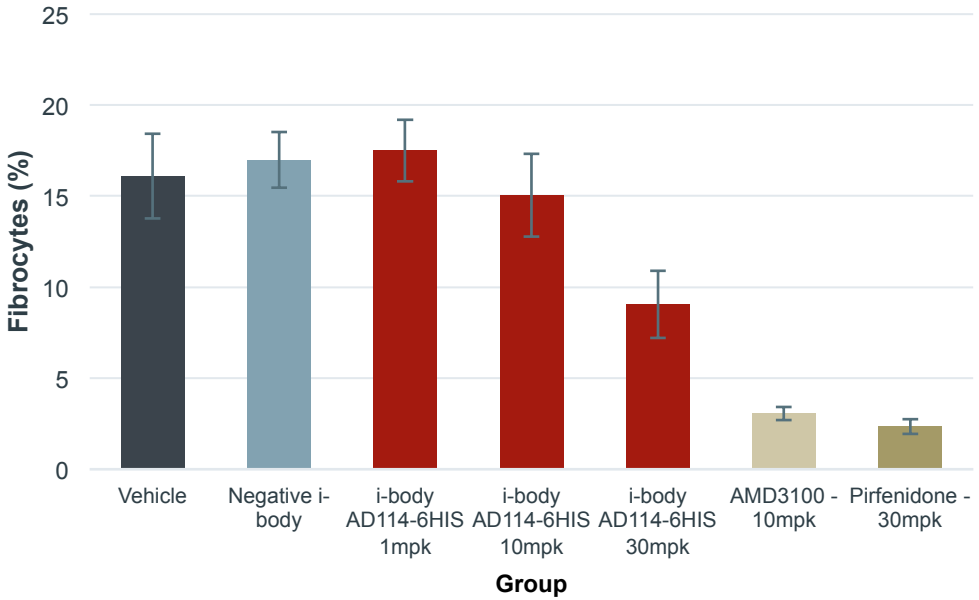
Bleomycin mouse model

Administration of Bleomycin is the most common animal model for the assessment of candidate drugs for the treatment of IPF. The Bleomycin treated mouse lung shows extensive collagen deposition and inflammatory cell infiltration.

- Mice received intratracheal instillation of Bleomycin at 2U/kg/mouse
- Groups treated starting on Day 0 of the study 1 hr prior to Bleomycin installation, with selected test compounds
- i-body was dosed at three levels,1mg/kg, 10mg/kg and 30mg/kg daily
- At Day 4 the number of fibrocytes (CXCR4+, Col1+ and CD45+ cells) in the lungs were measured by flow cytometry, RNA levels of collagen and collagen content were measured
- At Day 19 whole lungs were assayed for Hydroxyproline and histology (Masson's Trichrome) and Ashcroft score completed to analysis collagen content (Ashcroft, T., J.M. Simpson, and V. Timbrell. 1988. J. Clin. Pathol. 41:467-470). Body weights were also evaluated

AD-114 reduces fibrocytes in the Bleomycin mouse model

Mice challenged with Bleomycin and treated with AD-114 had reduced levels of fibrocytes in their lungs when compared to the mice treated with the negative control i-body



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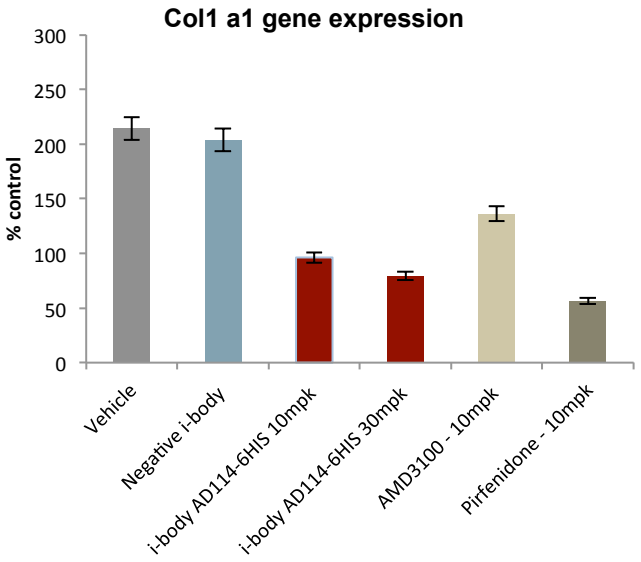
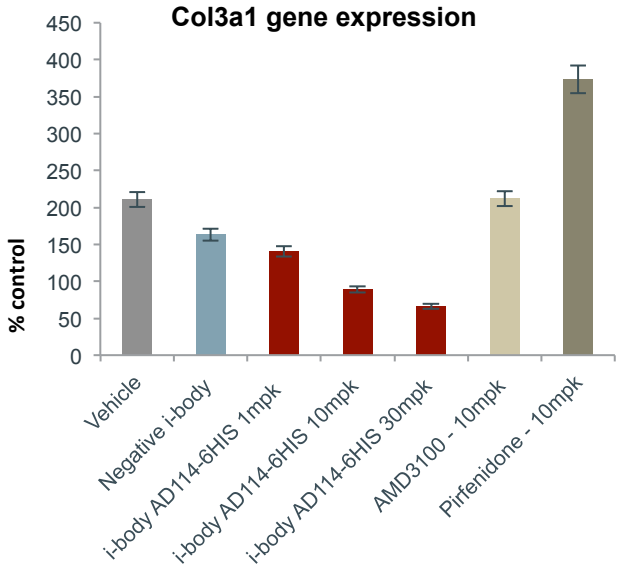
Collagen gene expression reduced with i-body AD-114

- RNA extracted and analyzed for collagen gene expression
- Both *COL1A1* and *COL3A1* reduced in mice treated with i-body AD-114 in the Bloemycin mouse model
- The negative control i-body had no effect on either *COL1A1* or *COL3A1*





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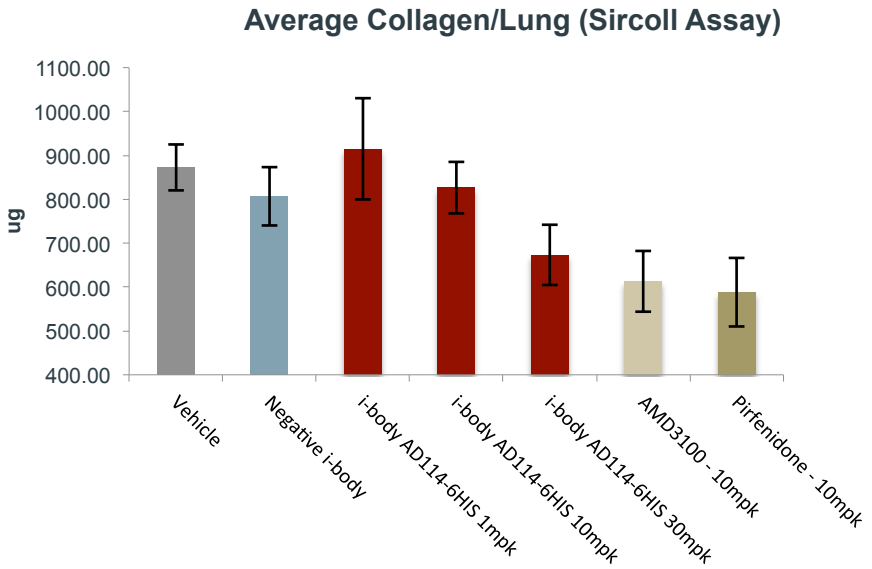
Collagen content reduced with i-body AD-114

- Collagen-1 content was measured by Sircol assay
- AD-114 reduced the level of collagen protein deposited in the lung of Bleomycin-treated mice
- Negative control i-body had no effect on collagen deposition



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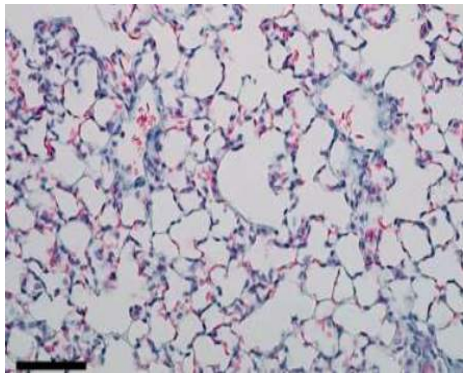
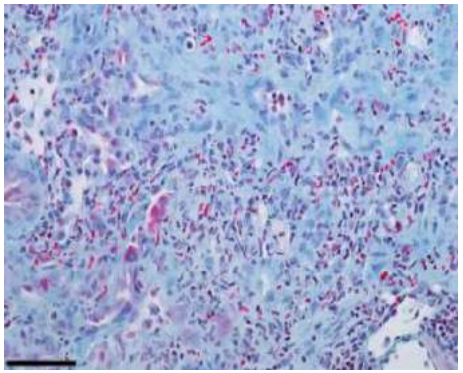
AD-114 prevents lung fibrosis in disease models

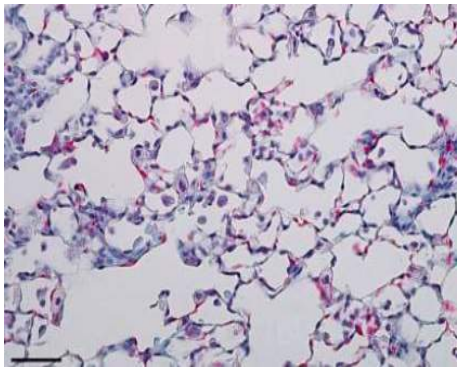
Extensive pre-clinical AD-114 studies have demonstrated positive *in vitro* (in the lab) and *in vivo* (in animals) data



Normal lung tissue



IPF lung tissue
(lung disease mouse model)

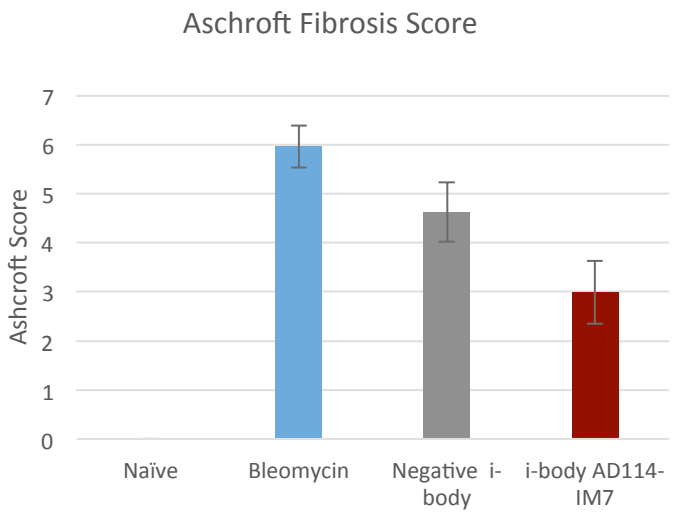


IPF lung tissue + AD-114 dosed for 21 days
(lung disease mouse model)

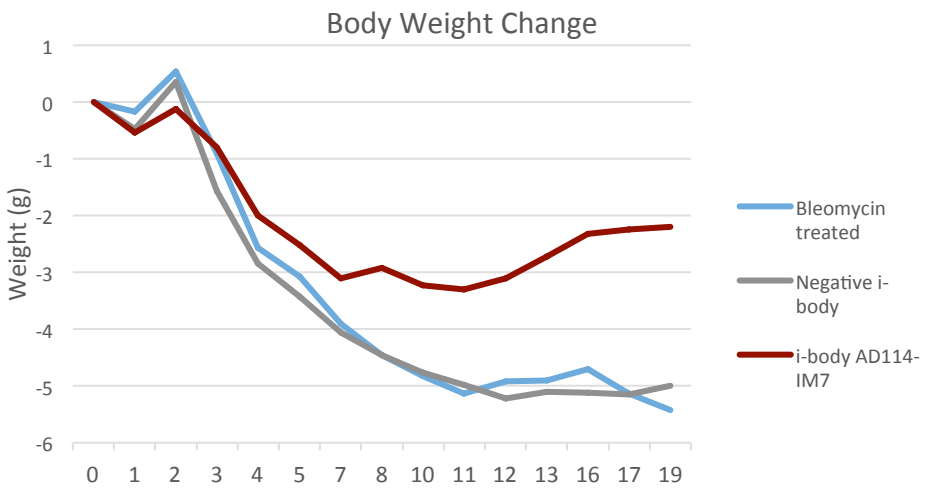
AD-114 reduces collagen content and inflammatory cell infiltration and demonstrates a similar architecture to that of the normal lung in the Bleomycin mouse model

AD-114 prevents lung fibrosis in disease models

AD-114 reduction in collagen deposition confirmed by quantitation using Ashcroft scores



Loss of body weight due to Bleomycin is prevented with daily dosing of i-body AD-114 10mg/kg



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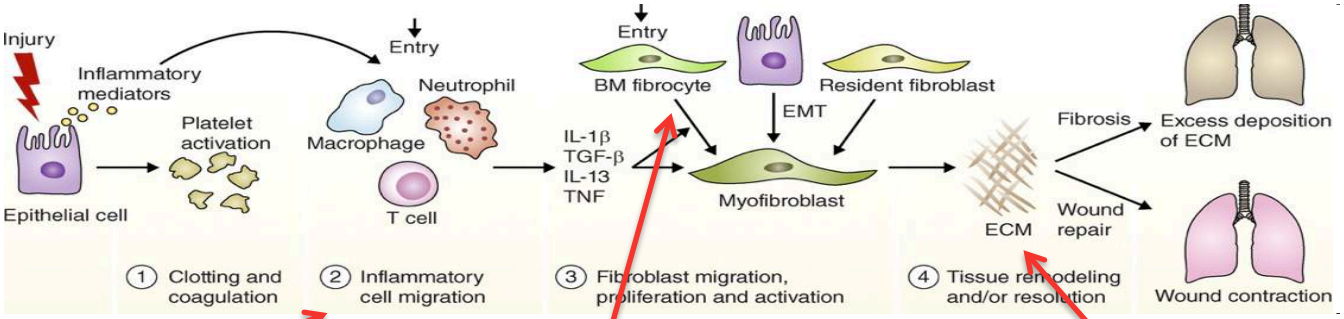
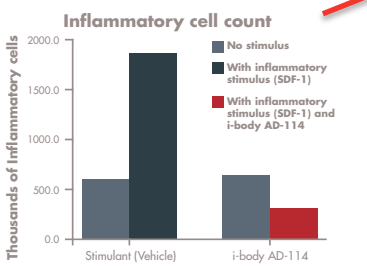
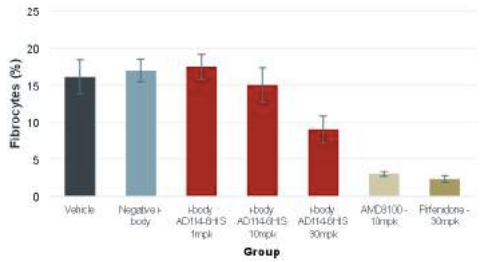
AD-114 inhibits key features of the fibrogenic pathway with novel MOA

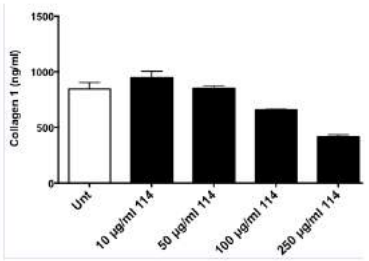




Modulate aspects of inflammation



Block fibrocyte recruitment into the damaged lung



Reduce ECM deposition during tissue remodeling

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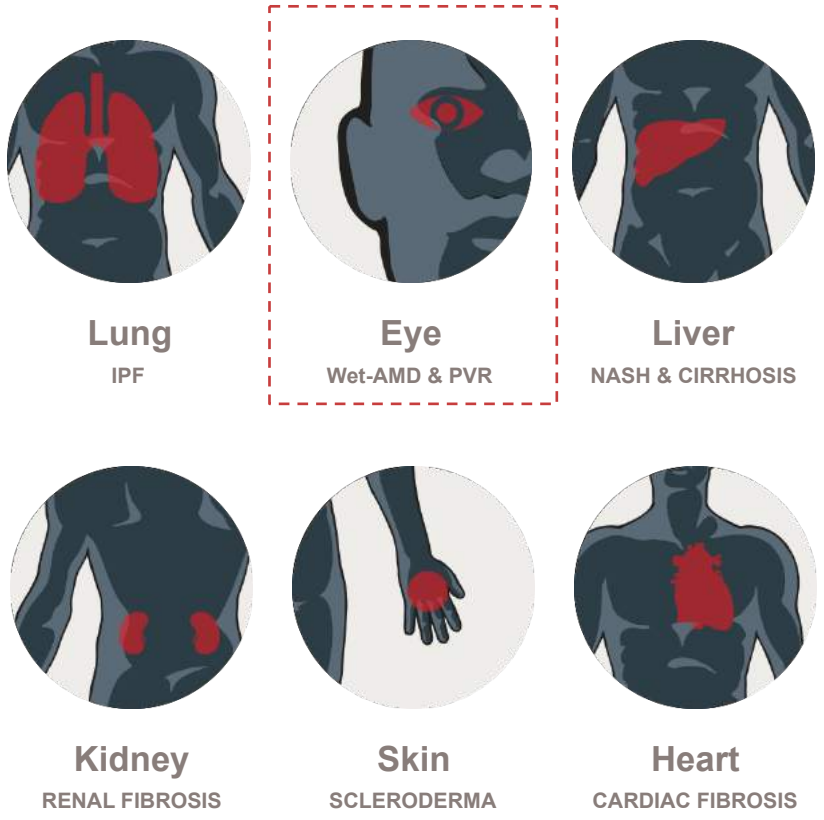
Fibrosis: unmet medical need with multiple indications

- Developing i-bodies as improved therapies for the treatment of fibrosis
  - a condition that is prevalent in 45-50% of all diseases
- Fibrosis can occur in many tissues of the body as a result of inflammation or damage
  - it can result in scarring of vital organs causing irreparable damage and eventual organ failure
- AdAlta's initial focus is on lung fibrosis

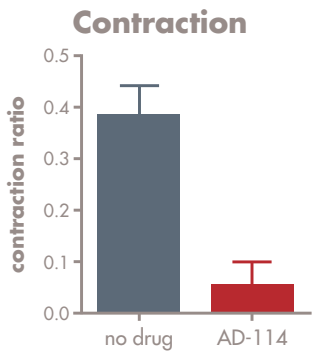
Collectively fibrosis represents a large unmet clinical need

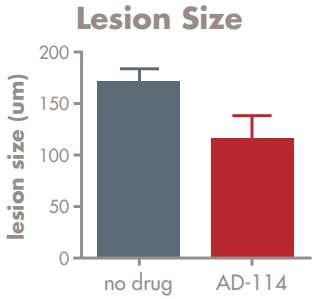


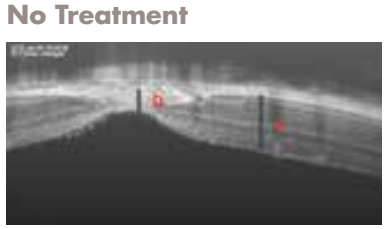
AD-114 prevents eye fibrosis

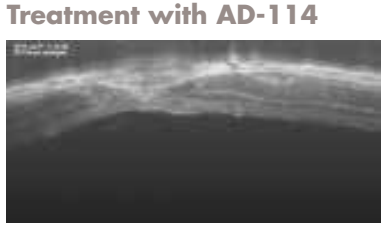
- Mouse chorodial neo-vascularization model: laser burn to the retina
  - Induces subretinal haemorrhage
  - Contraction of retinal tissue
  - Alteration in microglia and glial response
  - Alteration in gene expression
- IVT injection of single dose of i-body
  - Improves retinal retraction and reduces lesion size
  - Fibrosis gene expression reduced

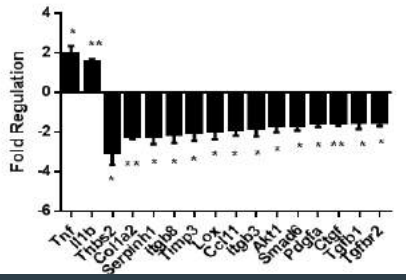
AD-114 reduces contraction and lesion size in eye fibrosis mouse model











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AdAlta

AdAlta summary

- **Powerful proprietary technology platform to develop a pipeline of i-bodies for the treatment of a wide range of human diseases**
  - Extreme stability of i-body similar to single domain shark antibody
  - Long loop of i-body binds deep in GPCR pocket and has functional activity
- **Advanced lead candidate AD-114 with significant pre-clinical validation**
  - has specificity for diseased human tissue with effects only shown on IPF tissue and no effects displayed on normal lung tissue nor any evidence of off target effects;
  - is more effective than existing IPF approved drugs showing greater *in vitro* efficacy compared to the only approved therapies Nintedanib and Pirfenidone;
  - demonstrates both anti-fibrotic and anti-inflammatory effects in multiple animal models in multiple areas of fibrosis; and
  - is a novel mechanism of action for fibrosis making AD-114 a potential “first in class” therapy.

AdAlta

next generation protein therapeutics

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