25 September 2023
ASX Announcement

AD-214 CLINICAL DOSE SIMULATIONS SUPPORT TARGET IV DOSE REGIMEN, SUGGEST PATH TO ENHANCED SC REGIMEN FOR PARTNERS

Key points

- **A model of AD-214 bioavailability and receptor occupancy has been developed to estimate likely therapeutic effective doses in humans**
- **Supports potential efficacy of target intravenous doses of AD-214**
- **Suggests that subcutaneous administration could also be effective**
- **Combined results reduce Phase II risk and significantly enhance partnering potential**
- **Results presentation at international Discovery on Target conference in Boston, USA**

MELBOURNE Australia, 25 September 2023: AdAlta Limited (ASX:1AD), the clinical stage drug discovery company developing novel protein and cell therapeutic products from its i-body platform, is pleased to report results of dose simulation studies which support the potential efficacy of AD-214 in fibrotic disease at planned Phase II doses. The simulations also highlight the possibility that AD-214 could be delivered subcutaneously at lower doses, further enhancing the potential of the product.

Key findings

Intravenous (IV) delivery (direct infusion via canula into a vein during a hospital or clinic visit): the model suggests that a clinically feasible dosing regimen of 10 mg/kg of AD-214 administered IV every two weeks bind its target receptor, CXCR4, and maintains levels of receptor occupancy necessary to materially inhibit a model fibrosis process. This dosing regimen is being used in the current Phase I extension clinical trial and would likely be progressed in a Phase II clinical trial.

Subcutaneous (SC) delivery (administration under the skin via syringe, can be self-administered by patients at home): the model was used to investigate the feasibility of administering AD-214 subcutaneously, suggesting that target levels of receptor occupancy could be achieved at doses of 1-3 mg/kg weekly, and less than 0.1 mg/kg daily. Not only is SC administration more convenient for the patient, it also requires much less AD-214 (based on the simulations), thus substantially reducing cost of goods. More work is required to verify these results and develop a suitable formulation for subcutaneous use.

Dr Tim Oldham, CEO and Managing Director, commented:
"These simulations have resulted in two very important conclusions for AdAlta. Firstly, based on our target product characteristics for commercial success, the target dosing regimen for AD-214 has been 10 mg/kg IV every two weeks. The simulations further strengthen and support the potential efficacy of this dosing regimen.

"Secondly, we have, for the first time, been able to explore the potential efficacy of subcutaneously administered AD-214 under clinically convenient dosing regimens. While it still makes sense to move forward to Phase II studies using intravenous AD-214, these subcutaneous results add significant value to our partnering program by pointing the way to a lower cost product in a more convenient format that patients could self-administer at home. Our potential partners are genuinely excited by this method of delivery."

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The results of these dose simulation studies will be presented at the 20th Discovery on Target conference in Boston, 25-28 September 2023 in a poster titled "*Clinical dose estimation for anti CXCR4 i-body-Fc fusion AD-214 for the treatment of fibrotic diseases*". Organised by the Cambridge Healthtech Institute, Discovery on Target is the industry's pre-eminent event on novel drug targets and technologies. A copy of the poster is attached.

About the PK/PD model

Developing models of drug bioavailability (pharmacokinetics or PK) and target receptor engagement or occupancy (pharmacodynamics or PD) is a valuable tool to assist in selecting doses and dose regimens for clinical trials.

In collaboration with PK/PD modelling experts, Lyo-X, AdAlta has been able to develop a model of AD-214 bioavailability that can accurately reproduce the PK and receptor occupancy profiles observed in Phase I clinical studies.

Drug dosing regimens are selected to maintain a drug concentration and receptor occupancy level, necessary to materially inhibit the progression of disease. Recent AdAlta studies[1] showed that AD-214 receptor occupancy as low as 30% could materially inhibit a key fibrotic process (immune and inflammatory cell migration). AD-214 maximally inhibited immune and inflammatory cell migration at receptor occupancy of 60-85%.

Using these findings, the PK/PD model can be used to assess the likely efficacy of different dosing regimens for AD-214.

Next steps

The results reported here reinforce the target IV product profile that AdAlta, in consultation with its advisers, plans to continue through the current Phase I extension study and then into Phase II (with partners).

The results also provide encouragement that an enhanced SC product profile is achievable. Additional product development would be required to confirm these findings and develop a subcutaneous form of AD-214 suitable for clinical use. Based on the positive response from pharmaceutical companies with whom AdAlta has been able to share these findings, the Company believes that a partner could potentially prepare a SC formulation of AD-214 ready for introduction into Phase III clinical trials.

Authorised for lodgement by:
Tim Oldham
CEO and Managing Director
September 2023

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[1] ASX release 7 July 2023

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Notes to Editors

PK/PD model results in more detail

The model was first asked to identify IV doses that could maintain greater than 30% receptor occupancy for two weeks, the target interval between IV infusions required for reasonable clinical adoption.

As described earlier, the results show that a dose of 10 mg/kg maintains greater than 30% receptor occupancy (potential to materially inhibit fibrosis) throughout the two-week interval between doses and greater than 60% receptor occupancy (potential to maximally inhibit fibrosis) for at least 75% of that time (Figure 1A). This result supports the potential therapeutic efficacy of this dosing regimen.

SC administration brings the possibility of patient self-administration at home which is generally more convenient than IV infusions, which usually take place in clinics or hospitals. The PK/PD model was therefore adapted to simulate SC administration and asked to identify SC AD-214 dose regimens that could match or exceed the receptor occupancy profile achieved with 10 mg/kg IV every two weeks. The simulations focused on weekly and daily intervals between doses, the interval between SC injections required for reasonable clinical adoption and high compliance.

The simulation results showed that 1-3 mg/kg weekly (Figure 1B) and 0.1 mg/kg or lower daily (results not shown) could achieve or exceed the target receptor occupancy believed necessary to inhibit fibrosis. These dosing regimens offer the added benefit of requiring less total AD-214 over a year of therapy, substantially reducing the cost of goods. This is particularly valuable in markets other than the USA where market prices for antifibrotic drugs are lower.

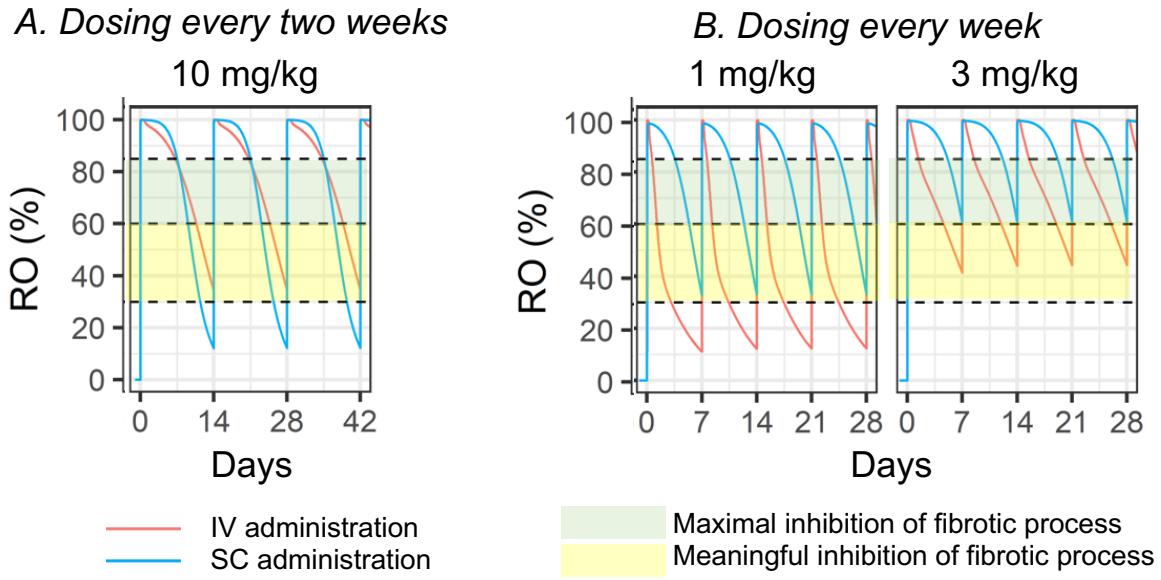

Figure 1: Simulated CXCR4 receptor occupancy following IV (red) and SC (blue) administration of AD-214 doses. Panel A simulates 10 mg/kg AD-214 administered every two weeks. Panel B simulates 1 mg/kg (left) and 3 mg/kg (right) AD-214 administered every week. Shading represents receptor occupancy (RO) required for maximal (green) and meaningful (more than 50%, yellow) inhibition of a model fibrotic process in ex vivo *experiments.*

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About AdAlta

AdAlta Limited is a clinical stage drug development company headquartered in Melbourne, Australia. The Company is using its proprietary i-body technology platform to solve challenging drug targeting problems and generate a promising new class of single domain antibody enabled protein and cell therapeutics with the potential to treat some of today's most challenging medical conditions.

The i-body technology mimics the shape and stability of a unique and versatile antigen binding domain that was discovered initially in sharks and then developed as a human protein. The result is a range of unique proteins capable of interacting with high selectivity, specificity and affinity with previously difficult to access targets such as G-protein coupled receptors (GPCRs) that are implicated in many serious diseases. i-bodies are the first fully human single domain antibody scaffold and the first based on the shark motif to reach clinical trials.

AdAlta is extending Phase I clinical studies for its lead i-body candidate, AD-214, that is being developed for the treatment of Idiopathic Pulmonary Fibrosis (IPF) and other human fibrotic diseases for which current therapies are sub-optimal and there is a high unmet medical need. Preparation for Phase II clinical studies is also underway. AdAlta has a second target in discovery research, also in the field of fibrosis and inflammation.

The Company is also entering collaborative partnerships to advance the development of its ibody platform. It has a collaboration with Carina Biotech to co-develop precision engineered, i-body enabled CAR-T cell therapies (i-CAR-T) to bring new hope to patients with cancer. It has an agreement with GE Healthcare to co-develop i-bodies as diagnostic imaging agents (iPET imaging) against Granzyme B, a biomarker of response to immuno-oncology drugs, a program now in preclinical development.

AdAlta's strategy is to maximise the products developed using its next generation i-body platform by internally discovering and developing selected i-body enabled product candidates against GPCRs implicated in fibrosis, inflammation and cancer and partnering with other biopharmaceutical companies to develop product candidates against other classes of receptor, in other indications, and in other product formats.

Further information can be found at: https://adalta.com.au

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Clinical dose estimation for anti CXCR4 i-body-Fc fusion AD-214 for the treatment of fibrotic diseases

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Background

- i-bodies are small, stable, human scaffolds inspired by shark single domain antibodies. i-body-Fc-fusion AD-214 has specificity and picomolar affinity for CXCR4, a GPCR upregulated in fibrosis.
- AD-214 has been specifically designed for fibrotic disease, has demonstrated anti-fibrotic activity in several preclinical models and was found to be well tolerated in a Phase I clinical trial.
- AD-214 maintained >60% receptor occupancy (RO, blocking) for up to three weeks after IV infusion however this had not been correlated with efficacy, making dose estimation challenging

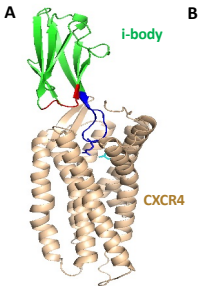


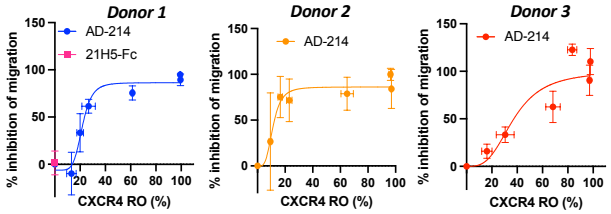
***Fig. 1 (A)** Ribbon diagram showing i-body bound to CXCR4. **(B)** Graphical Abstract.*

Aims

- To determine levels of RO required to achieve efficacy
- To determine clinically convenient IV and SC dosing regimens (two weeks between IV doses, daily or weekly SC doses) that maintain the RO levels required to achieve efficacy

Maximal inhibition of T cell migration can be achieved with just 60-85% CXCR4 receptor occupancy and low serum concentrations of AD-214

- *Ex vivo*, SDF-1α induced migration of primary human T cells models migration of inflammatory cells, a key fibrotic process on which AD-214 acts.
- The correlation of inhibition of migration with CXCR4 receptor occupancy (RO) enables a target RO for therapeutic efficacy to be estimated
- 1 nM of AD-214 (serum concentration detectable in clinical trial participants 72h after IV, 10 mg/kg AD-214) achieves high (57-85%) CXCR4 occupancy and maximally inhibits SDF-1α induced T cell migration.
- Meaningful (>50%) inhibition can be achieved at 0.1-0.2 nM AD-214



Inhibition of CD3+ T cell migration		Donor 1	Donor 2	Donor 3
Maximal inhibition	%RO	57%	57%	85%
	[AD-214]	0.7 nM	0.8 nM	1 nM
IC_{50}	%RO	22%	11%	37%
	[AD-214]	0.15 nM	0.07 nM	0.1 nM

***Table 1** Concentrations of AD-214 and CXCR4 RO required to achieve the indicated levels of migration inhibition.*

***Fig. 2 (A)** Primary human CD3+ T cells treated with a concentration gradient of AD-214 for 15 mins at 37 °C were loaded onto a transwell plate with AD-214 present or washed and analysed for CXCR4 occupancy and migration. AD-214 was detected using AF647-conjugated anti-H+L secondary antibody and free CXCR4 using a competing anti-CXCR4 (clone 12G5)-BrilliantViolet421 antibody. %RO = 100 x (normalizedOCCUPIED / normalizedOCCUPIED + normalizedFREE). For migration assays, SDF-1α (10 nM) induced migration across the transwell was quantified 2.5 h later and results were expressed as % inhibition by AD-214 relative to minimum (zero SDF-1) and maximum (+SDF-1) in each donor. Error bars denote SEM; technical replicates: n = 3 for donors 1 and 3, n = 2 for donor 2.*

Clinically feasible, two weekly or longer IV dosing regimens can support sufficient receptor occupancy (RO) to inhibit fibrotic processes

- *Ex vivo* RO and T cell migration results suggest target RO levels for anti-fibrotic efficacy of 30% (minimum 50% inhibition of fibrosis at trough) to 60%-85% (range of RO required to maximally inhibit fibrosis in all donors at trough)
- Maintaining efficacious receptor occupancy levels is the objective of dose selection
- Phase I clinical trials show that these efficacious receptor occupancy levels can be maintained for at least two weeks after an IV infusion in humans, a clinically viable dosing regimen

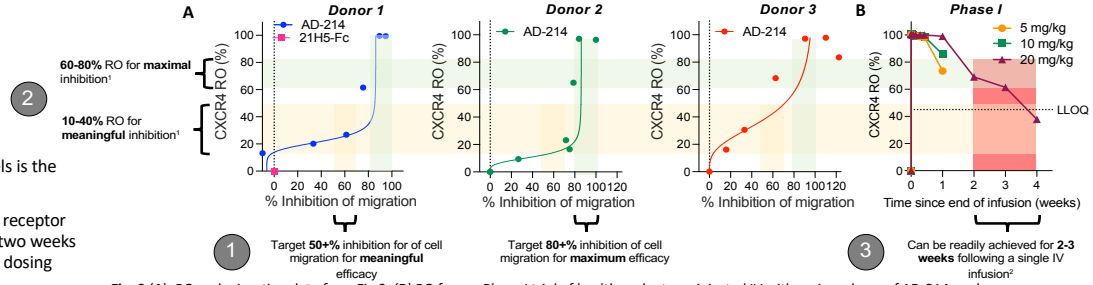


***Fig. 3 (A)**. RO and migration data from Fig 2. **(B)** RO from a Phase I trial of healthy volunteers injected IV with various doses of AD-214 as shown.*

PK/PD model for AD-214 dose estimation

- A model of IV AD-214 administration was developed and validated using SPR derived binding data and Phase I human PK, RO data (Model A) and additionally CXCR4 expression data (Model B).
- Preclinical PK results and reasonable assumptions allowed this to be extrapolated to SC AD-214 administration.

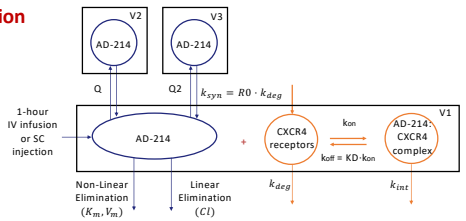


***Fig 4**. A 3-compartmental model with parallel linear and non-linear elimination (Michaelis-Menten approximation) as well as explicit receptor binding + complex internalization was able to fit the PK and CXCR4 RO observations more accurately than any simpler model*

Predicted CXCR4 RO at trough after q1d, q1w, q2w and q3w IV and SC doses

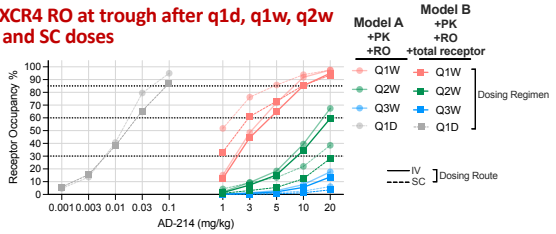


***Fig 5.** Predicted steady-state CXCR4 RO at trough in a typical 70-kg individual for different repeated SC and IV dose regimens. Horizontal dashed lines are the target RO of 30%, 60% and 85%. Model A, fitted to PK + RO data. Model B, fitted to PK + RO + total CXCR4 data.*

q2w ≥5 mg/kg IV or SC predicted to achieve target plasma [AD-214] and RO

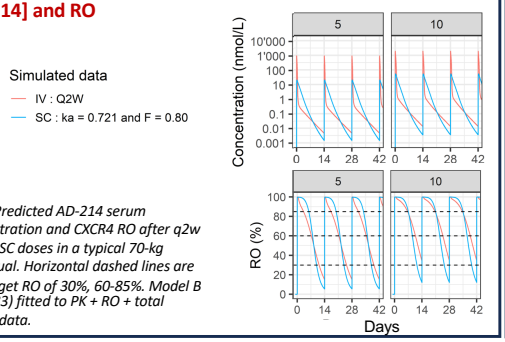


***Fig 6.** Predicted AD-214 serum concentration and CXCR4 RO after q2w IV and SC doses in a typical 70-kg individual. Horizontal dashed lines are the target RO of 30%, 60-85%. Model B (run 083) fitted to PK + RO + total CXCR4 data.*

q1w ≥1 mg/kg IV or SC predicted to achieve target plasma [AD-214] and RO

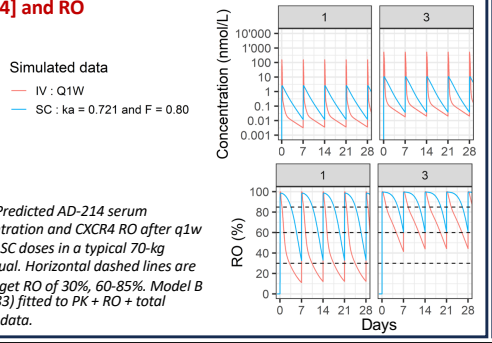


***Fig 7.** Predicted AD-214 serum concentration and CXCR4 RO after q1w IV and SC doses in a typical 70-kg individual. Horizontal dashed lines are the target RO of 30%, 60-85%. Model B (run 083) fitted to PK + RO + total CXCR4 data.*

q1d ≥0.01 mg/kg SC predicted to achieve target plasma [AD-214] and RO

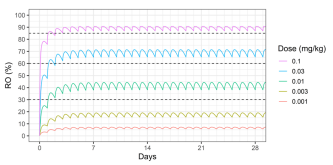


***Fig 8.** Predicted CXCR4 RO after q1day SC doses of 0.001 to 0.1 mg/kg in a typical 70-kg individual. Horizontal dashed lines are the target RO of 30%, 60-85%. Model B (run 083) fitted to PK + RO + total CXCR4 data.*

Conclusions and future directions

- *Ex vivo* studies correlating receptor occupancy and inhibition of a model fibrotic process (cell migration) at serum concentrations achieved during Phase I clinical studies are reported, alongside PK and receptor occupancy simulations.
- Phase I clinical results and simulation experiments support the hypothesis that clinically feasible q2w intravenous dosing regimen is likely to be efficacious in fibrotic disease.
- Further, an even more convenient q1d or q1w SC administration regimen may also be efficacious.
- AD-214 multidose Phase I extension clinical study underway evaluating safety PK and PD of multiple 10 mg/kg doses (final results Q1 2024):
  - ✓ Strengthens safety profile: establishes safety and tolerability at likely maximum dose to be used in Phase II studies
  - ✓ Further validates PK/PD model to better inform Phase II dose regimen selection: further explores PK, PD and safety trends observed in Phase I