# ATL1102 treatment in non-ambulant boys with DMD modulates plasma proteins with roles in TGF-β mediated fibrosis, and cartilage and bone physiology

George Tachas[1], Christopher Mueller [2], Robert Kirk DeLisle[4], Ian R. Woodcock [3], Monique. M. Ryan [3], Advait Padhye[1] and Nuket Desem[1]

[1]Antisense Therapeutics Limited, Melbourne, Australia. www.antisense.com.au [2]Boulder Bioconsulting, Inc., Boulder, Colorado, 80303, USA . https://boulderbioconsulting.com [3]Murdoch Children's Research Institute, Melbourne, 3052, Australia, https://www.mcri.edu.au/ [4]SomaLogic Operating Company, Inc., 2945 Wilderness PI. Boulder, Colorado 80301, USA. www.somalogic.com

**With a potential role in the positive stabilization of upper limb muscle function, strength and MRI observed with ATL1102 in the Phase 2 trial in non-ambulant DMD**

## Introduction

### ATL1102 and DMD

- Children with DMD have dystrophin deficient muscles susceptible to injury which triggers the immune system, exacerbating muscle damage, and are currently treated with corticosteroids[1]
- DMD patients with more progressed disease or severe disease have a greater number of circulating T cells expressing high levels of integrin CD49d, despite corticosteroid (CS) treatment[2]
- ATL1102 is a 2'MOE gapmer antisense oligonucleotide drug to human integrin CD49d RNA, alpha 4 subunit of VLA-4, an adhesion molecule expressed widely in human leukocytes, except neutrophils
- ATL1102 is an immunomodulatory drug, and has been studied in a completed, successful Phase 2 trial in 9 adolescent non-ambulant patients with Duchenne Muscular Dystrophy (DMD) [1,3,4]
- ATL1102 administered at 25mg once weekly s.c for 24 weeks was safe and reduced CD3-CD49d+ NK cells (p=0.018 mixed model of repeat week 8,12 & 24 measurements) and CD3+CD49d+ T cells at week 24 vs week 28 (p=0.010 paired T test*), 4 weeks post the end of dosing (EoD) [1,3]

### 6 Month Mean and Median, Lymphocyte, T-cell CD49d and NK CD49d reductions

| White blood cell type (X10⁹ cells per litre) | Mean # and Change from baseline | | | Median % change from baseline | |
|---|---|---|---|---|---|
| | Baseline | 24 weeks (EoD) | 28 weeks | 24 weeks (EoD) | 28 weeks |
| Lymphocytes | 3.68 | -0.28 | +0.19 | -4.22% | +11.81% |
| CD3+ (CD4+ or CD8+) CD49d+ T cells | 2.44 | -0.28 | +0.11* | -9.78% | +9.93% |
| CD3- (CD56+CD16+) CD49d+NK cells | 0.45 | -0.10 | -0.10 | -25. 9% | -7.28 |

*Table 1. Shows the lymphocyte, T-cell CD49d+ and NK cell CD49d+ cell modulation at week 24 vs baseline and week 28*

- ATL1102 stabilized multiple parameters of disease progression, including performance of upper limb function (PUL2.0), muscle strength (Myogrip, Myopinch) [1,3,4], versus losses reported in the literature, [3-6] and stabilized the % fat in muscle compared to increases using corticosteroids. [3,5].

### 6 Month Mean PUL2.0, MyoGrip and MyoPinch stabilization using ATL1102

**PUL 2.0** a key endpoint: measures 3 dimensions of upper limb function – the shoulder, elbow and wrist/hand

ATL1102 treated patients showed an overall improvement from baseline to week 24 in total PUL2.0 score of **0.89* (-1.33, 3.11)**

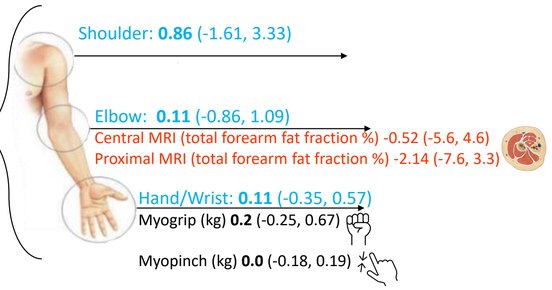



*Figure 1. Results of the ATL1102 Phase 2 24 week mean PUL2.0*, MRI, and Grip & Pinch changes from baseline * 0.89 (SD 2.89) vs a loss in PUL2.0 of -2.00 (SD 3.018 ) in the matched external control group (n=20, 39) p=0.01.[4]*

## Objectives

- To conduct a proteomics analysis of over 7000 plasma proteins from samples in the Phase 2 Study
- To compare changes to an external healthy subject control (See Table 2) and
- Provide insights on the mode of action and broader biological activity of ATL1102 in DMD (See Conclusions)

## Methods

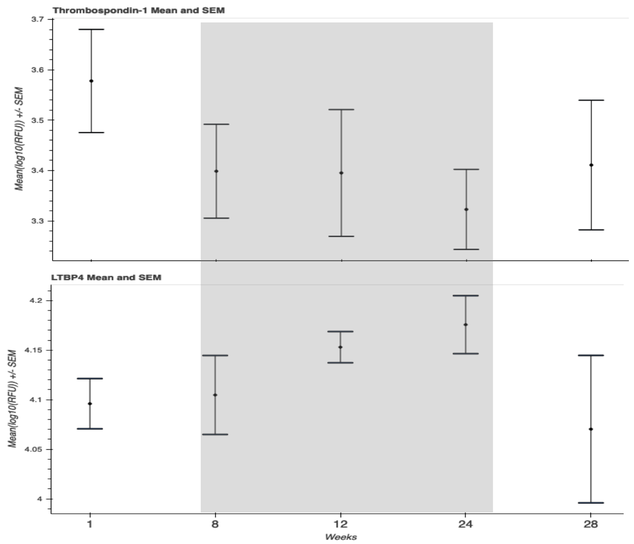
- Steroids (prednisolone, deflazacort) were not permitted within 24 hours of the plasma collections.
- Using the SomaScan® assay, a large scale, aptamer-based assay, patient plasma samples were tested and the normalized relative fluorescence units (nRFU) of over 7000 proteins determined including:
  - baseline, weeks 8, 12, and 24 (each 3 days post the previous ATL1102 dose) and at week 28, 4 weeks past the last ATL1102 dose; the times the CD49d T cell and NK cell changes were assessed
- Linear mixed effects models relating time post-dose to SomaScan-detected protein levels, with p-values adjusted using the Benjamini-Hochberg false discovery rate (FDR), was used to identify proteins of interest, for which we computed the mean % change at weeks 8, 12, and 24 vs baseline
- Plasma proteins with a significant 6 month change and FDR of zero (<0.0005) were identified.

## Results

### ATL1102 modulates LTBP4, Thrombospondin-1, BMP-5 and BMP-6

- In the mixed effect dataset at 24 weeks, ATL1102 treated patients demonstrated a statistically significant mean reduction of Thrombospondin-1 (-49%), and increases of LTBP4 (+20.7%), BMP5 (+46.3%), and BMP6 (+34.4%) compared to baseline levels (FDR p-value <0.0005).
- Consistent mean changes during treatment were seen from week 8 to week 24 (shaded below) for 3 proteins, with LTBP4 increasing from week 12 to 24, all trending back towards baseline levels at 4 weeks post dosing (Figure 2 below)
- All 9 treated participants showed an increase for both BMP-5 and BMP-6 at week 24, and 8 out of 9 participants showed a similar positive change for LTBP4 and a negative change for THBS-1

### 6 Month mean changes: of genetic modifiers LTBP4 and Thrombospondin-1




### 6 Month mean changes: of BMP5 and BMP6

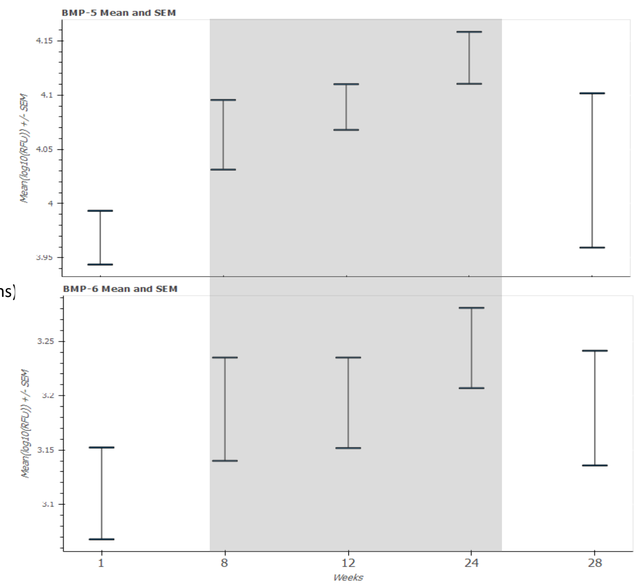



*Figure 2a,b,c,d. Mean (SEM) Results at baseline (1) to24 week end of dosing changes and to w28 4 weeks past dosing*

### Healthy Control nRFU compared to ATL1102 Phase 2 Participant Baseline and w24

| Control | Median (95% CI) | ATL1102 | BL Median | Week 24 Median (95% CI) |
|---|---|---|---|---|
| • BMP-5 | 14160 (7775,23580) | • BMP-5 | 8919 | 13866 (12371,15202) |
| • BMP-6 | 1748 (1185,2805) | • BMP-6 | 1160 | 1720 (1446, 2178)) |
| • THBS1 | 2859 (427,10079) | • THBS1 | 2983 | 2017 (1470,3375) |
| • LTBP4 | 16248 (9553, 25477) | • LTBP4 | 11397 | 14067(13172,17364) |

*Table 2. Shows median nRFU of external control (95%CI), baseline median and week 24 nRFU (95% CI)*

- Compared to healthy adult control, nRFU, baseline median levels of the proteins in the Phase 2 DMD study were near the median for Thrombospondin-1 (THBS1) and below median for the other 3 proteins, with ATL1102 treatment modulating these 3 other proteins towards the external control median.
- The healthy control values are a robust point estimate generated during assay validation of the aptamers, and values are the median of 1000 individuals from an adult US population, both males and females, ages varying between 18-80; there is no healthy adolescent dataset matching the DMD patients

## Discussion

- Proteomics analysis of blood samples from the non ambulant DMD patients treated with ATL1102 was undertaken, to provide insight into the mode of action and biological activity of ATL1102 in DMD
- ATL1102, as previously reported, induced modulation of plasma sVCAM-1, a CD49d ligand, which is supportive of ATL1102's antisense mechanism of action in reducing CD49d and in reducing inflammation[7] .
- ATL1102 also induced positive LTBP4 increases and positive THBS1 decreases in plasma, 2 known DMD disease genetic modifier proteins with opposite effects on TGF- β1 involved in modifying the rate of loss of ambulation (LoA);[7,8,9] DMD (dystrophin deficiency) progresses in a variable way with a few genetic factors
  - A recessive LTBP4 allele in only 12% of patients with DMD, with greater levels of LTBP4, is associated with mild DMD providing 1-2 years delayed LoA[9].
  - A minor THBS1 allele with reduced expression appears protective against DMD progression.
  - LTBP4 sequesters TGF- β1 and THBS1 activates Latent TGF- β1 and both are involved in fibrosis
- ATL1102 induced increases in BMP-5 and BMP-6, which are members of the TGF-β1 superfamily[10,,11]
  - BMP-5 and 6 have been shown to play an important role in cartilage and bone formation[12,13]
  - Serum BMP6 levels are reportedly associated with improved elbow flexion in DMD patients.[14]

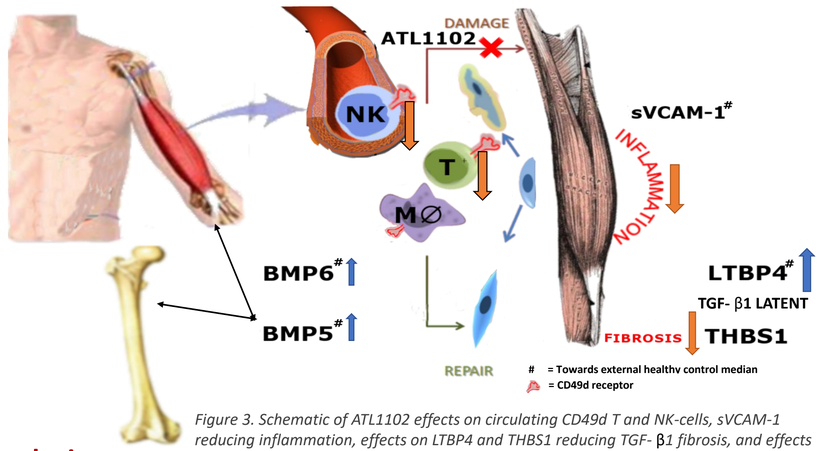



*Figure 3. Schematic of ATL1102 effects on circulating CD49d T and NK-cells, sVCAM-1 reducing inflammation, effects on LTBP4 and THBS1 reducing TGF- β1 fibrosis, and effects on BMP's which may improve bone density. BMP & TGF-β cross talk effects[10] are not shown*

## Conclusions

- ATL1102 modulates two DMD disease genetic modifiers, LTBP4 and THBS1, known to impact TGF-β1 and the rate of LoA in DMD patients, supporting ATL1102's potential to reduce fibrosis in DMD.
- ATL1102 modulates BMP-5 and BMP-6, two members of TGF- β family, returning both levels towards the healthy controls, and suggests a potential for improved bone density in non-ambulant patients
- ATL1102 effects on lymphocytes and these proteins may have a role in the observed ATL1102 benefits of stabilizing muscle function, strength and MRI seen in non-ambulant patients treated with ATL1102
- ATL1102 stabilization of multiple disease progression parameters together with these effects position ATL1102 as an exciting prospect for the treatment of both non ambulant and ambulant DMD patients

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**Corresponding Author**

George Tachas, Director Drug Discovery & Patents
Email: **George.Tachas@antisense.com.au**