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ATL1102 treatment in non-ambulant boys with DMD modulates Latent TGF-beta-binding protein 4, and thrombospondin-1, two disease genetic modifiers of ambulant DMD, and CXCL16

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

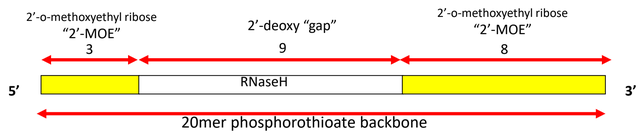
[1]Antisense Therapeutics Limited, Melbourne, Australia. www.antisense.com.au [2]Boulder Bioconsulting, Inc., Boulder, Colorado, 80303, USA . https://boulderbioconsulting.com [3] Murdoch Children's Research Institute, Melbourne, 3052, Australia, https://www.mcri.edu.au/ 4. SomaLogic, Inc., 2945 Wilderness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

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

Introduction

ATL1102

- ATL1102 is a 2'MOE gapmer antisense oligonucleotide drug to human integrin a_4 RNA (CD49d alpha 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) [1,2]
- ATL1102 administered at 25mg once weekly s.c for 24 weeks was safe and reduced CD3-CD49d+ NK lymphocytes and CD3+CD49d+ T lymphocytes, the latter rebounding at 28 weeks, 4 weeks post dosing [1,2]

6 Month Mean and Median lymphocyte and T-cell CD49d+ modulation

White blood cell type (X10^9 cells per litre)	Mean # and Change from baseline			Median % change from baseline	
	Baseline	24 weeks (end of dosing)	28 weeks	24 weeks (end of dosing)	28 weeks
Lymphocytes (most are CD3+ T cells)	3.68	-0.28	+0.19	-4.22%	+11.81%
CD3+ CD49d+ T cells (mostly CD3+CD4+CD49d+ and CD3+CD8+CD49d+)	2.44	-0.28	+0.11*	-9.78%	+9.93%
CD3+CD4+ CD49d+ T cells	1.20	-0.19	+0.01	-16.7%	+1.73
CD3+CD8+ CD49d+T cells	1.17	-0.05	+0.11	-5.79%	+13.37

*The mean # of CD3+CD49d+T cells at week 24 is statistically significantly lower vs week 28 (p= 0.030 paired T test).

Table 1. Shows the Lymphocyte and T cell CD49d+ cell modulation at week 24 versus baseline and week 28

- ATL1102 stabilized multiple parameters of disease progression, including performance of upper limb function (PUL2.0), muscle strength (MyoGrip, MyoPinch), versus losses reported in the literature,[2,3,4] and stabilized the % fat in muscle compared to increases using corticosteroids.[2,3]

6 Month Mean PUL2.0, MyoGrip and MyoPinch stabilization

Efficacy Parameter	Description	Result (VS Baseline at Wk 24) Mean Change (95% CI)
PUL2.0	PUL2.0- Performance of Upper Limb Measure assesses the performance of a large group of muscles of the upper body of patients in 3 dimensions; the shoulder, elbow, and wrist-finger	+0.9 (-1.33, 3.11)
MyoGrip (dom) (Kg)	MyoGrip-evaluates the clamping force of the fingers	+0.2 (-0.25, 0.67)
MyoPinch (dom) (Kg)	MyoPinch- evaluates the thumb-index fingers	0.0 (-0.18, 0.19)

Table 2. Results of the ATL1102 Phase 2 24 week mean PUL2.0 and Grip & Pinch changes compared to baseline

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 3) and
- Provide insights on the mode of action and broader biological activity of ATL1102 in DMD (See Conclusions)

Methods

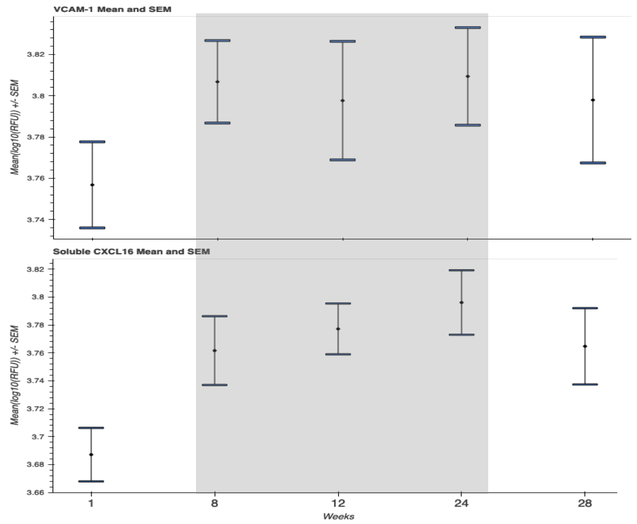
- Steroids (prednisolone, deflazacort) were not permitted within 24 hours of the plasma collections.
- Using the Somascan assay, a large scale, aptamer-based assay, patient plasma samples were tested and the normalized relative fluorescence units (nRFU) of over 7000 proteins determined including:
  - 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 Benjaminin-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 VCAM-1, CXCL16 and 2 DMD genetic modifier proteins

- 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%), soluble CXCL16 (29.9%), and VCAM-1 (18.0%) 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 had CXCL16 increases at week 24 and 8 of 9 participants had similar positive or negative changes referred to below for the 3 other proteins

6 Month Mean changes in the CD49d ligand VCAM-1 and CXCL16



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

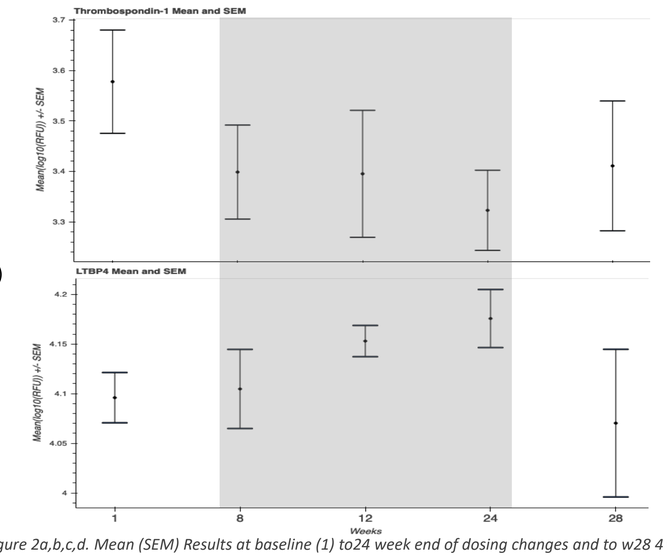


Figure 2a,b,c,d. Mean (SEM) Results at baseline (1) to24 week end of dosing changes and to w28 4 weeks past dosing

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

Control	Median (95% CI)	ATL1102	BL Median	Week 24 Median (95% CI)
• VCAM-1	6513 (4019,10496)	• VCAM-1	5000	6041 (5147, 6867)
• CXCL16	5417 (3882, 7635)	• CXCL16	4921	6385 (5713,6927)
• THBS1	2859 (427,10079)	• THBS1	2983	2017 (1470,3375)
• LTBP4	16248 (9553, 25477)	• LTBP4	11397	14067(13172,17364)

Table 3. 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 further insight into the mode of action and biological activity of ATL1102 in DMD
- DMD patients with more progressed disease and severe disease have a greater number of circulating T cells expressing high levels of CD49d[5]
- VLA-4 antibody blocks DMD patient T cell endothelial (VCAM-1) and fibronectin driven migration ex vivo.[5]
- ATL1102 induced increase in plasma VCAM-1 over 24 weeks of treatment is supportive of the ATL1102 antisense mechanism of action of reducing CD49d on the surface of cells to which soluble VCAM is bound
  - Soluble VCAM-1 is bound to various cells including NK cells and certain T cells, and can be released upon T cell apoptosis[6]
- ATL1102 induced increase in CXCL16, a chemokine with a role in muscle regeneration via satellite cells in mice, is potentially related to the observation of reduced % fat fraction and increase lean muscle mass[2]
- ATL1102 also induced positive LTBP4 increases and positive THBS1 decreases in plasma, 2 known DMD disease genetic modifier proteins with opposite effects on TGF-beta involved in modifying the rate of loss of ambulation (LoA);[7,8]
  - LTBP4 sequesters TGF-beta and THBS1 activates Latent TGF-beta and both are involved in fibrosis
  - A minor THBS1 allele with reduced expression appears protective against DMD progression.
  - A rare recessive LTBP4 allele in 12% of patients with greater levels is associated with mild DMD providing 1-2 years delayed LoA[8]
  - Over-expression of murine LTBP4 leads to amelioration of the dystrophic process in the mdx mouse.
- Analysis of the proteomics data continues which may further elucidate ATL1102's biological effects and thereby direct the drug's development into other disease settings

Conclusions

- ATL1102 modulation of (i) VCAM-1, a CD49d ligand, is supportive of ATL1102's antisense mechanism of action in reducing CD49d and in reducing inflammation, (ii) CXCL16, a chemokine with a role in muscle regeneration, appears to align with the positive effects on muscle structure observed under MRI in DMD patients treated with ATL1102, and (iii) the two DMD disease genetic modifiers known to impact TGF beta and the rate of LoA in DMD patients, support ATL1102's potential to reduce fibrosis in human disease.
- These together reflect the drugs reported positive effects in stabilizing muscle function and strength seen in non-ambulant patients treated with ATL1102
- Modulation of these plasma proteins has, to our knowledge not, previously been reported with the use of corticosteroid drugs in non-ambulant DMD patients
- ATL1102 stabilization of multiple muscle disease progression parameters together with these positive effects on the above proteins, positions 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**