ASX Announcement

26 July 2023

Positive new DMD Combination Therapy Data in mdx mice

Key highlights:

- Study results suggest the potential for ATL1102 in combination with dystrophin restoration drugs to improve therapeutic outcomes in patients with Duchenne muscular dystrophy;
- Improved muscle strength was detected following combination of a mouse CD49d antisense oligonucleotide with a dystrophin exon skipping restoration agent; and
- RNA-seq transcriptomics results support the role of ATL1102, as monotherapy and in combination with a dystrophin exon skipping restoration agent, in reducing inflammation and augmenting muscle recovery.

Antisense Therapeutics Limited [ASX:ANP | US OTC:ATHJY | FSE:AWY] (ANP or Company) is pleased to announce the new results from the Duchenne muscular dystrophy (DMD) mdx animal study, following previously released positive muscle function data, supporting the potential for ATL1102 (to human CD49d) in combination with a dystrophin exon skipping restoration (DSER) agent[1]. The study compared:

i. antisense oligonucleotide to mouse CD49d (ASO);
ii. a control mismatch oligonucleotide incapable of reducing CD49d;
iii. the DSER targeting exon 23;
iv. a combination of the control oligonucleotide and the DSER; and
v. a combination of the mouse CD49d ASO with the DSER (ASO+DSER).

Agents (i) to (v) were compared with one another and with a saline control.

The extensor digitorum longus (EDL) muscle, which is the limb muscle responsible for stretching toes and bending paws, was used to test muscle function responses. The study demonstrated positive muscle function in response to the ASO+DSER combination, compared with saline control, following application of a specific maximum force to the EDL muscle. A further test, measuring eccentric muscle force remaining in the EDL muscle following induced damage, was conducted after 1 and 10 lengthening muscle contractions. After 10 lengthening contractions significantly improved effects were noted in the ASO+DSER combination group compared with the DSER agent used alone, the DSER versus the saline control as well as with the mouse CD49d ASO compared with the saline and the control oligonucleotide. These results suggest there may be a clinical benefit of using ATL1102 together with a dystrophin exon skipping restoration agent in patients with DMD.

To understand the mechanisms behind the improved muscle strength, tests were performed to assess (i) the muscle dystrophin protein levels, (ii) the dystrophin fluorescence intensity in the fibres and (iii) changes in cellular markers. For these additional evaluations the quadricep muscle was used, which is the main bulk of the thigh and one of the most powerful muscles in the body, involved in hip flexor and knee extensor function.

Dystrophin protein and fibre data

The results of the dystrophin protein levels, following re-analyses using a more sensitive assay method, demonstrated similar low levels in the ASO+DSER combination and the DSER agent groups (both less than 1% of healthy mice levels) with virtually no dystrophin protein in the control group as expected in mdx mice. In ambulant DMD boys dosed with the dystrophin restoration exon skipping drugs, Eteplirsen and Golodirsen resulted in 0.44%[2] and 1%[3] of healthy dystrophin protein levels respectively at 48 weeks.

A study to assess the dystrophin fluorescence intensity in the fibres in the mdx study identified similar low percent dystrophin fluorescence in both the ASO+DSER combination and the DSER agent treated mice (both less than 2.5% of healthy mice levels) with virtually no dystrophin fluorescence in the saline control which is not unexpected in mdx mice.

Muscle RNA-seq transcriptomics data

It is interesting to note that even with low levels of dystrophin protein, the mdx mice still had improved muscle function after receiving ASO+DSER combination compared to the DSER agent.

To identify possible mechanisms behind the muscle functional benefits in the mouse CD49d ASO and ASO+DSER combination groups, the mdx quadricep muscle tissue was processed for RNA collection. Genes found in the cell nucleus and made up of DNA, are copied to messenger RNA (carrying instructions from the DNA), and the mRNA sequence is used for protein synthesis. The RNA was assessed with RNA-seq transcriptomic studies, a technique to evaluate both every RNA sequence present and quantity of RNA. RNA-seq only detects RNA associated with mutated dystrophin protein.

The transcriptomic results identified several proteins in the mouse CD49d ASO and additional proteins in the ASO+DSER combination groups which support the observed improved muscle function in mdx mice. The ASO monotherapy modulated specific RNA transcripts. One of the specific proteins, GM2a, is associated with the retention of anti-inflammatory cells in muscle, namely M2 macrophages. The M2 macrophages plays an important role in tissue repair and are involved in activating quiescent muscle stem cells, also important for muscle repair and regeneration. Another identified protein, Triadin, is thought to facilitate calcium release in the muscle which is useful for contraction.

The ASO+DSER combination group was associated with further transcriptomic effects on proteins involved in (i) immune response (BTLA*, PPML1, TNFSM13), (ii) fat and lipid breakdown (FAPBP4*, G0S2), (iii) IGF-1 pathway (IGFBP-7, Calu, ASB15), and (iv) muscle cell & muscle stem cell function (ADAM10, Mt-Tp, MyoM1). MyoM1 is a key skeletal muscle structural protein (up 33%) suggesting the presence of more muscle. BTLA* and FABP4* were modulated in both the monotherapy and in the combination group.

These mdx muscle RNA-seq transcriptomics results support the role of ATL1102, as monotherapy or in combination with a DSER, in reducing inflammation and augmenting muscle recovery both critical aspects for slowing the progressive decline in patients with DMD[4].

These changes and promising transcriptomic effects will be useful for the patent application titled "Combination Compositions and Methods for Treatment of Muscular Dystrophy" previously filed to seek protection of the combination of ATL1102 with the dystrophin restoration/exon skipping drugs to 2044. This is beyond the patent life of the dystrophin restoration drugs like Eteplirsen (Exondys-51) and Golodirsen (Vyondy-53) conditionally approved for use in exon 51 and exon 53 affected ambulant DMD subjects respectively.

Dr George Tachas, the Director of Drug Discovery and Patents said "The Company continues to be encouraged by the positive EDL functional benefits observed with the ASO monotherapy and ASO dystrophin restoration combination therapy approach and by the new RNA observations in the quadricep indicating the underlining biological mechanisms with ASO monotherapy and the apparent synergistic

mechanisms observed in the combination. The quadricep muscle is known to become rapidly weaker as children with DMD age, causing loss of ambulation, and whilst corticosteroid treatment prolongs ambulation for 2 to 3 years, better treatments are needed. These study results are exciting and suggest the potential for ATL1102 in combination with dystrophin restoration drugs to improve therapeutic outcomes in DMD patients".

This announcement has been authorised for release by the Board.

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About Antisense Therapeutics Limited [ASX: ANP | US OTC: ATHJY | FSE: AWY] is a publicly listed biotechnology company developing and commercializing antisense pharmaceuticals for rare diseases with significant unmet medical need. The company's lead program is ATL1102, an antisense inhibitor of the CD49d receptor, which is currently the subject of an ongoing international Phase IIb trial for non-ambulant subjects with Duchenne Muscular Dystrophy. The drug previously reported highly promising results from an exploratory Phase II trial in non-ambulant subjects DMD.

1. https://announcements.asx.com.au/asxpdf/20230201/pdf/45l635k5wctnzd.pdf
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3. https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/211970s002lbl.pdf
4. Rosenberg A, Woodcock J et al. Sci Transl Med. 2015 August 05; 7(299)