8th December 2015

ATL1102 Cancer Data Presentation

Antisense Therapeutics Limited ("ANP") wishes to advise that data from the testing of ATL1102 in an animal cancer research study conducted at the Children's Hospital Los Angeles (CHLA) is to be presented today at The American Society of Hematology (ASH) 57th Annual Meeting in Orlando Florida. The data from this pilot animal study shows that ATL1102, an antisense drug targeting CD49d (VLA-4), led to the rapid mobilization of acute myeloid leukemia (AML) cells to the peripheral blood in mice that had been engrafted with human AML cells. Details of the study and results are outlined in the attached poster presentation. A new provisional patent application incorporating this data and covering ATL1102's potential application in AML and other leukemias has been filed by ANP.

The rationale for this study of ATL1102 is based on the bone marrow microenvironment having been shown to promote cell adhesion-mediated drug resistance in leukemia cells. Breaking the adhesive bonds of AML cells with their protective niche to mobilize them from the bone marrow to the peripheral blood may make drug treatment more effective. Studies have suggested the adhesion molecule CD49d is an anchor molecule for AML and certain other leukemia cells in the bone marrow and that drugs like ATL1102 which reduce CD49d expression may cause the release of these cancer cells from their protective environment to make the cancer cells more accessible to chemotherapy. No drug targeting CD49d is currently approved for use in leukemia.

AML is the most common acute leukemia in adults and the seventh most common pediatric malignancy comprising approximately one-fifth of pediatric leukemias. Treatment is dominated by generic chemotherapeutic drugs. In children, relapse following primary chemotherapy approaches 40%, and the 5-year event-free survival rate is only approximately 50%. Novel therapeutic strategies are highly warranted to eradicate residual disease.

Further animal studies are ongoing at the CHLA at their cost to more fully assess ATL1102's therapeutic potential in this disease setting.

Contact Information:
Website: www.antisense.com.au
Managing Director: Mark Diamond +61 (0)3 9827 8999

About Antisense Therapeutics Limited

Antisense Therapeutics Limited is an Australian publicly listed biopharmaceutical drug discovery and development company. Its mission is to create, develop and commercialise second generation antisense pharmaceuticals for large unmet markets. Antisense Therapeutics has 4 products in its development pipeline that it has in-licensed from Isis Pharmaceuticals Inc. (ISIS), a world leader in antisense drug development and commercialisation - ATL1102 (injection) which has successfully completed a Phase IIa trial in patients with relapsing-remitting multiple sclerosis (RRMS), ATL1103 drug designed to block GHr production which in a Phase II clinical trial reduced blood IGF-1 levels in patients with the growth disorder acromegaly, ATL1102 (inhaled) which is at the pre-clinical research stage as a potential treatment for asthma and ATL1101 a second-generation antisense drug at the pre-clinical stage being investigated as a potential treatment for cancer.

ATL1102 background Information

ATL1102 is a second generation antisense inhibitor of CD49d, a subunit of VLA-4 (Very Late Antigen-4). In inflammation, white blood cells (leukocytes) move out of the bloodstream into the inflamed tissue, for example, the Central Nervous System (CNS) in MS, and the lung airways in asthma. The inhibition of VLA-4 may prevent white blood cells from entering sites of inflammation, thereby slowing progression of the disease. Antisense inhibition of VLA-4 expression has demonstrated activity in a number of animal models of inflammatory disease including asthma and MS with the MS animal data having been published in a peer reviewed scientific journal. ATL1102 was shown by the Company to reduce MS lesions in a Phase IIa clinical trial in RRMS patients and the data have been published (Limmroth, V. et al Neurology, 2014; 83(20): 1780-1788).

Mobilizing acute myeloid leukemia cells using a novel integrin a4 targeting antisense, ATL1102.

Duchartre Y[1], Gang EJ[1], Kim HN[1], Shishido SN[1], Grishin A[3], Fabbri M[1,2], Parekh C[1,2], Abdel-Azim H[1,2], Bhojwani D[1,2], Wayne AS[1,2], Tachas G[4] and Kim YM[1,2]

[1] Division of Hematology, Oncology and Blood & Bone Marrow Transplantation, Children's Hospital of Los Angeles, University of Southern California, Los Angeles, CA, USA;
[2] Children's Center for Cancer and Blood Diseases, Children's Hospital Los Angeles, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA;
[3] Division of Pediatric Surgery, Children's Hospital Los Angeles, Los Angeles, CA 90027; Department of Surgery, Keck School of Medicine of the University of Southern California, Los Angeles, CA, USA;
[4] Antisense Therapeutics Ltd., Toorak, Victoria, Australia.

Abstract

BACKGROUND: Acute myeloid leukemia (AML) patients have a poor prognosis with a low (30-50%) disease-free survival rate. Novel therapeutic strategies are highly warranted. The bone marrow (BM) microenvironment has been shown to promote cell adhesion-mediated drug resistance (CAM-DR) in leukemia cells. However, current treatment regimens give little attention to the role of the BM in chemoprotective interactions with AML cells. Breaking adhesive bonds of AML cells with their protective niche may make drug treatment more efficient. Studies including our own have identified the adhesion molecule integrin a4 as an anchor molecule for ALL and AML cells in the bone marrow. However, as of today, no FDA-approved drug is available to target integrin alpha4 in the clinic for mobilization purposes. Here, we evaluate a novel integrin a4 targeting antisense, ATL1102, which has shown efficacy in Phase 1 and 2 studies of Multiple Sclerosis patients, in human AML cells.

METHODS: We determined integrin a4 expression in human AML HL-60 treated with an integrin a4 targeting antisense ATL1102 and antisense control by qPCR and flow cytometry. Annexin V/DAPI and BrdU stainings were used for viability determination and cell cycle assay respectively by flow cytometry. A NOD/SCID IL2Rγ-/- xenograft model of human HL60 was used for an *in vivo* mobilization assay.

RESULTS: To assess the on-target effect of ATL1102 on CD49d, HL60 cells were nucleoporated with either ATL1102 or control antisense. CD49d expression on mRNA level was significantly decreased by integrin a4 antisense ATL1102 treatment in HL-60 human AML cell line (85.2%±15.4 expression inhibition using ATL1102 1μM after 24h compared to control, p<0.001) as assessed by RT-PCR. The FACS analysis reveals a significant decrease of surface expression of CD49d in a dose-dependent manner (57.8%± 7.2 ATL1102 (10μM) vs 99.7%±0.1 for control antisense (30 μM), P<0.001, n=3, 72h after ATL1102 treatment). No significant effect on apoptosis or cell cycle of HL60 cells was observed after ATL1102 treatment. We also evaluated the in vivo effect of ATL-1102 on mobilization of leukemia cells in a pilot experiment. For this purpose, HL-60 cells (AML (5x106/per mouse) were injected via the tail vein in sublethally irradiated NSG mice. Presence of human cells (hCD45) was determined weekly by flow cytometry of white blood cells isolated from peripheral blood (PB). 23 Days post-leukemia injection, mice were treated with either antisense control (CTRL) (150mg/kg, n=3), ATL1102 (50mg/kg, n=4) or ATL1102 (150mg/kg, n=3). PB was drawn before and 24 ours after ATL1102-treatment. ATL1102 induced a strong mobilization of AML cells to the peripheral blood of leukemia-recipient mice compared to control antisense treated-mice (52.8% ±45.4% vs 9.8% ±15.9% at 24h after treatment using 50mg/kg ATL1102, n=3). The mobilized cells show a marked decrease of surface expression of CD49d (16.8%±9.2% vs 32.8%±16.7%, n=3) and CD29 (integrin β1, 11.9%±5.8% vs 25.9%±15.7%). Experiments to determine where the AML cells are mobilized from and whether there is synergism with chemotherapeutic treatment are in progress.

CONCLUSION: We demonstrate that ATL1102 can efficiently decrease CD49d expression in AML cell line in vitro and in vivo, and that ATL1102 leads to mobilization of AML cells to the peripheral blood.

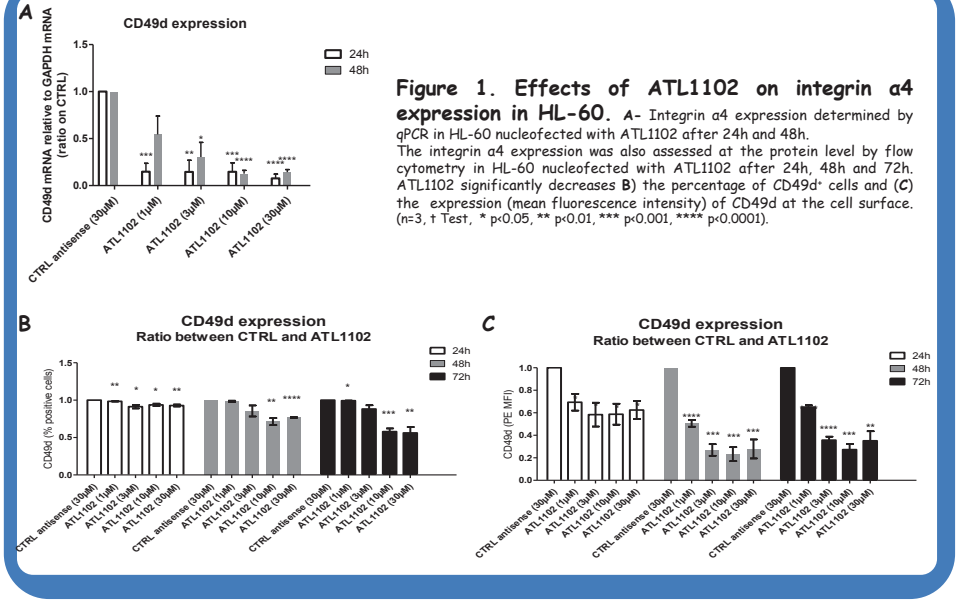

Figure 1. Effects of ATL1102 on integrin α4 expression in HL-60. **A-** Integrin α4 expression determined by qPCR in HL-60 nucleofected with ATL1102 after 24h and 48h.
The integrin α4 expression was also assessed at the protein level by flow cytometry in HL-60 nucleofected with ATL1102 after 24h, 48h and 72h. ATL1102 significantly decreases **B)** the percentage of CD49d$^+$ cells and (**C**) the expression (mean fluorescence intensity) of CD49d at the cell surface. (n=3, t Test, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

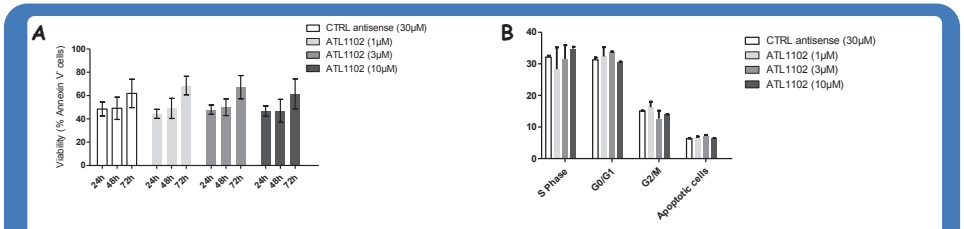

Figure 2. Effects of ATL1102 on viability and cell cycle in HL-60. **A-** Viability of control antisense or ATL1102-treated HL-60 24h, 48h and 72h after treatment (determine by AnnexinV/DAPI staining assay). **B- C**ell cycle assay on control antisense or ATL1102-treated HL-60 at 48h after treatment (determined by BrdU staining). ATL1102 treatment does not affect viability or cell cycle of HL-60 (n=3, t-Test).

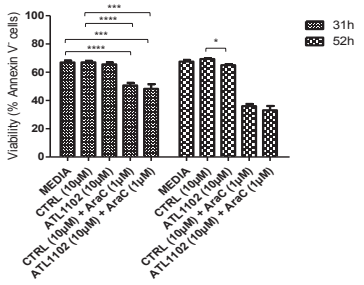

Figure 3. Conventional chemotherapy combined with ATL1102 treatment effects on HL-60 in vitro. Viability of HL-60 singled treated with AraC or ATL1102 and combination was assessed by the apoptosis assay. No additive or synergistic effect was observed with the combination treatment (n=3, t Test, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

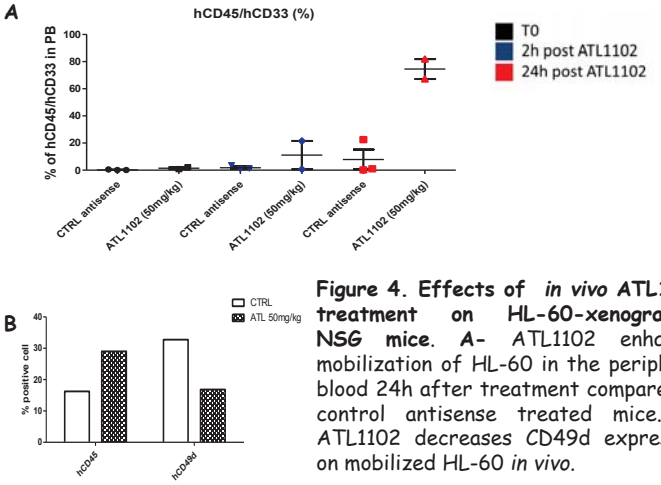

Figure 4. Effects of *in vivo* ATL1102 treatment on HL-60-xenografted NSG mice. **A-** ATL1102 enhances mobilization of HL-60 in the peripheral blood 24h after treatment compared to control antisense treated mice. **B-** ATL1102 decreases CD49d expression on mobilized HL-60 *in vivo*.

Conclusion / Prospects

We demonstrated here that ATL1102, a novel CD49d antisense, can efficiently decrease CD49d expression *in vitro* and *in vivo* in an AML cell line model. ATL1102 treatment in vitro induces a rapid and significant decrease of CD49d at the RNA and cell surface protein levels. The loss of integrin alpha4 does not affect the viability or the cell cycle of HL-60 cells. The combinatorial treatment with conventional chemotherapy AraC does not show any synergestic effect on cell viability *in vitro*. Interestingly, *in vivo* ATL1102 leads to a fast and substantial mobilization of bone marrow HL-60 to the peripheral blood in treated mice compared to control antisense-treated mice. These results could be of great relevance in the treatment of AML and other leukemias where relapse after treatment can occur potentially because of the chemoprotective effect of the bone marrow stroma. Further animal experiments are required to more fully assess and characterize the ATL1102 mobilization of AML and other leukemic cells to the peripheral blood.

Disclosures: ***Tachas:*** *Antisense Therapeutics Ltd: Employment, Equity Ownership, Patents & Royalties.* ***Kim:*** *Patents & Royalties.*