ASX Release

17 April 2023

ALA-101 CONFERS SIGNIFICANT ANTI-TUMOUR EFFECT AND SURVIVAL BENEFIT IN AGGRESSIVE LEUKEMIA MODEL

- **Arovella has presented new data at the American Association for Cancer Research (AACR) Annual Meeting for CAR19-iNKT cells produced using a new 3rd-generation lentiviral vector as planned for clinical trials (ALA-101).**
- **The data indicate that ALA-101 has the potential to be a novel 'off-the-shelf' cell therapy to treat CD19-expressing leukemias and lymphomas. Key highlights include:**
  - **iNKT cells could be well expanded (~5000-fold) during manufacture, and cryopreserved (frozen) for future use, without compromising the potency of the cells.**
  - **ALA-101 effectively killed tumour cells that express CD19, including primary patient-derived tumour cells.**
  - **ALA-101 significantly extended the lifespan of mice transplanted with aggressive human B-Cell Acute Lymphoblastic Leukemia (B-ALL) that does not produce CD1d, indicating the activity of ALA-101 through the engineered CD19 CAR.**
  - **Following ~5,000-fold increase in cell numbers during manufacture, CAR19-iNKT cells retained the ability to multiply further when exposed to tumour cells that express CD19.**
  - **The data confirmed that the proposed manufacturing process maintained the effectiveness of cryopreserved ALA-101 when used 'off-the-shelf' and after thawing.**
- **Arovella will present the data to investors during a webinar to be held at 11 AM (AEST) on Wednesday 19th of April 2023.**

MELBOURNE, AUSTRALIA 17 April 2023: Arovella Therapeutics Ltd (ASX: ALA), a biotechnology company focused on developing its invariant Natural Killer T (iNKT) cell platform to treat cancer, today presented new data at the American Association of Cancer Research (AACR) Annual Meeting in Orlando, Florida. The poster was the Company's first presentation at a major international conference and described Arovella's lead product, ALA-101, as a novel treatment for CD19-expressing leukemias and lymphomas. A copy of the poster is attached to this announcement and is also available on the Company's website.

Arovella's CEO and MD, Dr Michael Baker, commented, "We are delighted with the data we are generating for ALA-101 and pleased to have progressed to the point of having a vector suitable for clinical development. The fact that we see a statistically significant lifespan extension in an aggressive leukemia animal model provides excellent data to indicate our product's potency and support the ongoing development of ALA-101 and progression to human Phase 1 clinical trials in 2024."

Arovella's newly appointed Chairman, Dr Tom Duthy, commented, "This is an exciting time for Arovella. The AACR Annual Meeting is one of the preeminent cancer meetings held each year, providing Arovella with significant exposure to clinicians and researchers alike. The data we have presented at AACR this year confirms my belief in the potential of our proprietary and highly differentiated iNKT cell platform with applicability to several haematological cancers utilising our off-the-shelf production methodology, which is

expected to provide higher commercial returns with fewer logistical challenges than current autologous cell therapies."

The data presented used iNKT cells expressing a Chimeric Antigen Receptor (CAR) that targets CD19 (ALA-101). These cells were manufactured using Arovella's new lentiviral vector, as planned for use in clinical trials. The vector is a 3rd-generation lentiviral vector manufactured by Lentigen Technology, Inc., a world-leading manufacturer of lentiviral vectors for cell and gene therapies. This new lentiviral vector is expected to pass regulatory requirements more easily as Arovella seeks to initiate first-in-human clinic trials.

The 3rd-generation vector is considered safer than previous lentiviral systems. It is more accepted by global regulatory agencies, including the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). The new vector was also optimised to contain a well-known promoter with a proven safety profile.

During manufacture, ALA-101 cells could be expanded ~5,000-fold and retain functionality. A high level of expansion is critical for producing multiple clinical doses of iNKT cells from a single batch, thereby reducing costs and enabling use as an efficient, off-the-shelf product for cancer treatment.

ALA-101 produced using this method displayed robust killing of multiple CD19-expressing tumour cell lines, including cell lines that do not express CD1d. ALA-101 also eliminated more than 90% of viable cells that express CD19 in a lymphoma patient sample. The dual targeting potential of ALA-101 via the CAR and the invariant T Cell Receptor was demonstrated by the killing of unpulsed and α-GalCer-pulsed C1R-CD1d cells, respectively. ALA-101 cells also dramatically upregulated cytokines that promote tumour killing (granzyme B, TNF-α and perforin) when exposed to CD19-expressing leukemia cells.

Significantly, a relatively low dose of ALA-101 was effective in an aggressive NSG mouse model of human B-Cell Acute Lymphoblastic Leukemia (B-ALL). Tumour burden after 26 days in ALA-101-treated animals was ~90% lower than in the untreated control animals. ALA-101 also significantly enhanced the survival of the mice over untreated controls ($p<0.005$). The model used tumour cells originating from a patient with an aggressive form of B-ALL.

Data presented also demonstrates that ALA-101 cells retain their ability to multiply in response to CD19, as binding of the CD19 target on tumour cells promoted further expansion of the ALA-101 cells. This antigen-induced proliferation is expected to occur when ALA-101 cells bind to tumour cells in a patient's bloodstream, thereby enhancing the persistence of the cells and potentially improving efficacy.

Together, this data indicates ALA-101 has the potential to be a novel, 'off-the-shelf' cell therapy to treat CD19-expressing leukemias and lymphomas.

Arovella will hold a webinar to present the data at 11 AM (AEST) on Wednesday, the 19th of April. Shareholders, investors, and other interested parties can register and attend via the following link. Further details on how to attend will be provided by email following registration.

https://us02web.zoom.us/webinar/register/WN_uF1SlhqXSvO0ra9_C4qr-g

A recording of the webinar will be made available via the Company's website and social media channels following the event.

Questions can be submitted on the day or sent in advance to investor@arovella.com.

Release authorised by the Managing Director and Chief Executive Officer of Arovella Therapeutics Limited, Dr Michael Baker.

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NOTES TO EDITORS:

About Arovella Therapeutics Ltd

Arovella Therapeutics Ltd (ASX: ALA) is a biotechnology company focused on developing its invariant natural killer T (iNKT) cell therapy platform from Imperial College London to treat blood cancers and solid tumours. Arovella is also expanding its DKK1-peptide targeting technology licenced from MD Anderson and used in conjunction with its iNKT cell therapy platform. Arovella's lead product is ALA-101. ALA-101 consists of CAR19-iNKT cells that have been modified to produce a Chimeric Antigen Receptor (CAR) that targets CD19. CD19 is an antigen found on the surface of numerous cancer types. iNKT cells also contain an invariant T cell receptor (iTCR) that targets α-GalCer bound CD1d, another antigen found on the surface of several cancer types. ALA-101 is being developed as an allogeneic cell therapy, which means it can be given from a healthy donor to a patient. For more information, visit www.arovella.com.

Glossary: iNKT cell – invariant Natural Killer T cells; **CAR** – Chimeric Antigen Receptor that can be introduced into immune cells to target cancer cells; **TCR** – T cell receptors are a group of proteins found on immune cells that recognise fragments of antigens as peptides bound to MHC complexes; **B-cell lymphoma** – A type of cancer that forms in B cells (a type of immune system cell); **CD1d** – Cluster of differentiation 1, which is expressed on some immune cells and cancer cells; **aGalCer** – alpha-galactosylceramide is a specific ligand for human and mouse natural killer T cells. It is a synthetic glycolipid.

The Company is also commercialising ZolpiMist™ to treat short-term insomnia.

This announcement contains certain statements which may constitute forward-looking statements or information ("forward-looking statements"), including statements regarding negotiations with third parties

and regulatory approvals. These forward-looking statements are based on certain key expectations and assumptions, including assumptions regarding actions of third parties and financial terms. These factors and assumptions are based upon currently available information and the forward-looking statements contained herein speak only as of the date hereof. Although the expectations and assumptions reflected in the forward-looking statements are reasonable in the view of the Company's directors and management, reliance should not be placed on such statements as there is no assurance that they will prove correct. This is because forward-looking statements are subject to known and unknown risks, uncertainties and other factors that could influence actual results or events and cause actual results or events to differ materially from those stated, anticipated or implied in the forward-looking statements. These risks include, but are not limited to: uncertainties and other factors that are beyond the control of the Company; global economic conditions; risk associated with foreign currencies; and risk associated with securities market volatility. The Company assumes no obligation to update any forward-looking statements or to update the reasons why actual results could differ from those reflected in the forward-looking statements, except as required by Australian securities laws and ASX Listing Rules.

Engineering allogeneic 'off-the-shelf' CD19-directed CAR-iNKT cells without additional genetic manipulations for the treatment of hematological malignancies

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Background

- invariant Natural Killer T (iNKT) cells are a unique subset of T cells that naturally target and kill cancer cells[1]
- iNKT cells express an invariant T cell receptor (iTCR) that recognizes glycolipids presented in the context of the monomorphic, MHC-class I related molecule, CD1d
- Engineering a Chimeric Antigen Receptor (CAR) makes iNKT cells dual targeting, thereby enhancing cytotoxicity[2]
- iNKT cells promote anti-tumor activity by reprogramming the immunosuppressive tumor microenvironment to be immunostimulatory[3]
- iNKT cells can target cancers without the risk of graft-versus-host disease (GvHD), circumventing the need to delete or knock out the endogenous TCR for an allogeneic product[4,5]

Allogeneic Off-the shelf CAR-iNKT cells

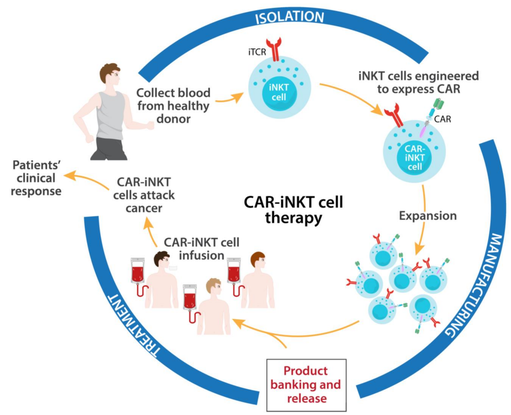
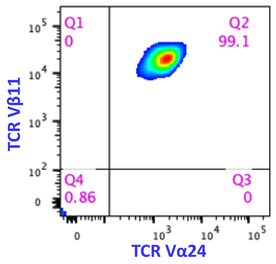


Fig 1. Schematic representation of CAR-iNKT cell manufacturing using lentiviral vector

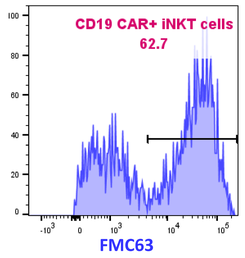
Methods

Briefly, peripheral blood-derived iNKT cells were isolated from healthy donors and engineered to express a CD19 CAR using a 3rd generation lentiviral vector. Cells were then expanded for 21 days. To demonstrate CAR19-dependent and independent anti-tumor activity, CAR19-iNKT cells **(ALA-101)** were compared *in vitro* against non-transduced (NT) iNKT cells in cytotoxicity assays and for cytokine secretion. Finally, the anti-tumor activity of cryopreserved CAR19-iNKT cells (ALA-101) were evaluated in an established aggressive NSG mice model of SEM-luc, a B cell lymphoblastic leukemia cell line expressing luciferase.

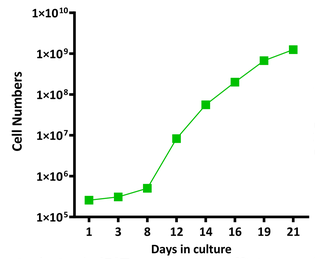
A. iNKT Cell Purity



B. Transduction Efficiency



C. Growth Curve



D. Fold Expansion

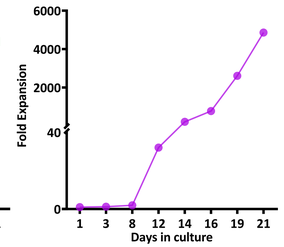
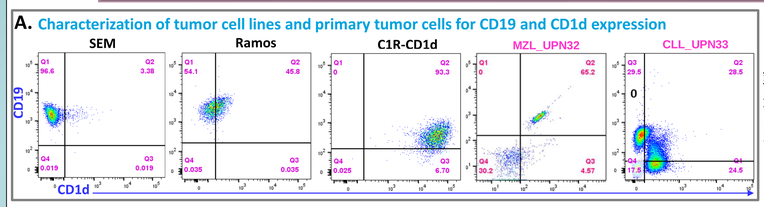


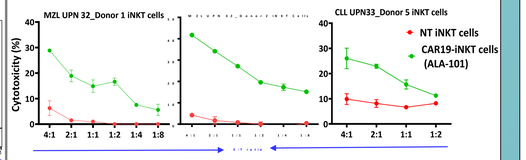
Fig 2. (A) iNKT purity after isolation (0.17% of CD3+ cells in PBMC to 99.1% after isolation), **(B)**Transduction efficiency of purified iNKT cells, CAR19-iNKT cell (ALA-101) research scale **(C)** Growth Curve & **(D)** Fold Expansion calculated as change from Day 1.

CAR19-iNKT cells (ALA-101) are cytotoxic to CD19+/CD1d+/- tumor cell lines and primary tumor cells

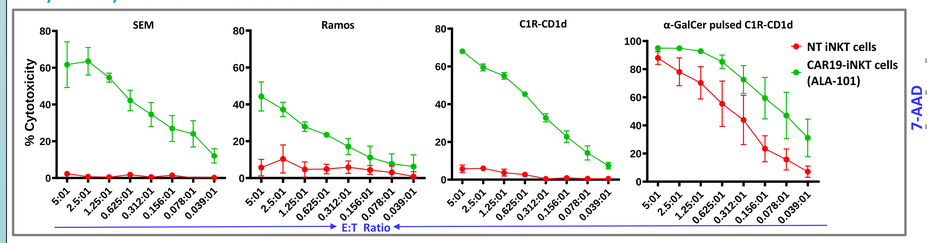
A. Characterization of tumor cell lines and primary tumor cells for CD19 and CD1d expression



B. I. Cytotoxicity to primary MZL and CLL cells



C. Cytotoxicity of CAR19-iNKT cells to tumor cell lines



II. Elimination of CD19+ cells in MZL sample by Donor 2 derived NT iNKT cells and ALA-101

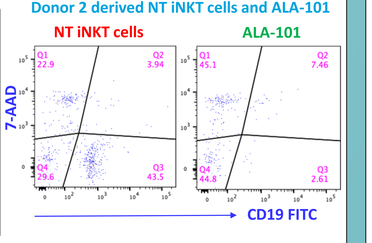
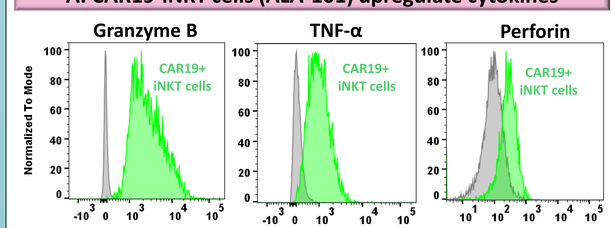


Fig 3. (A) Characterization of tumor cell lines & primary marginal zone B cell lymphoma (MZL) and chronic lymphocytic leukemia (CLL) cells. **(B)** Cytotoxicity of ALA-101 to **(I)** primary tumor cells, **(II)** FACS plots showing elimination of CD19+ cells **(C)** Cytotoxicity of ALA-101 (n=2) to tumor cell lines in 20 h co-culture.

A. CAR19-iNKT cells (ALA-101) upregulate cytokines



B. Antigen specific proliferation of CAR19-iNKT cells (ALA-101)

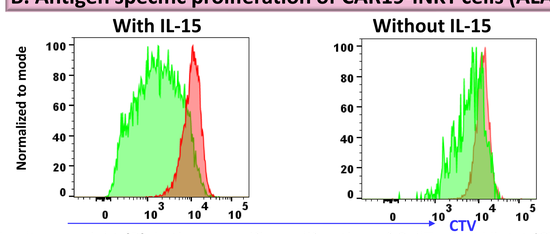
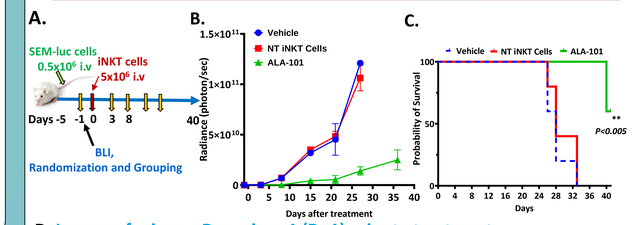
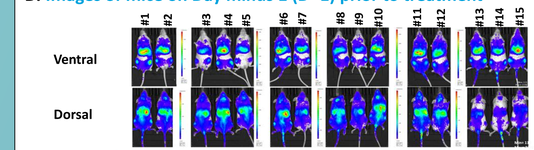


Fig 4. **(A)** Intracellular cytokine staining of CAR19-iNKT cells co-cultured with SEM cells overnight **(B)** Antigen specific proliferation of CellTrace™ Violet (CTV) labeled CAR19+ iNKT cells upon exposure to 3 rounds of irradiated tumor cell challenge with **SEM (CD19+)** or **K562 (CD19-)** cells every 24h for 3 days. Based on the dilution of CTV, proliferation of CAR19+ iNKT cells in the presence or absence of exogenous IL-15 was assessed on day 7 following the first stimulation.

ALA-101 Mediated Antitumor Activity & Survival Benefit in an Aggressive Disseminated NSG Mouse Model of Acute Lymphoblastic Leukemia (SEM-luc)



D. Images of mice on Day minus 1 (D -1) prior to treatment



E. Images of mice Day 8 (D8) post treatment

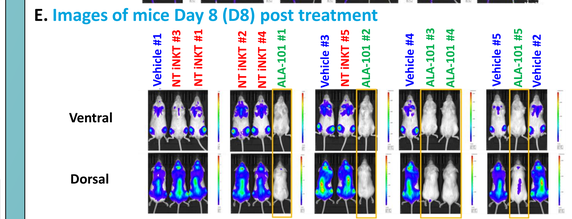


Fig 5. (A) NSG mice were injected i.v on D -5 with 0.5x10^6 SEM-luc tumor cells. On D -1 mice were imaged, & randomized. On D0, mice (n=5) were treated with 5x10^6 Non-transduced (NT) iNKT cells, or CAR19-iNKT cells (ALA-101), or vehicle. **(B)** Total flux of the tumor at different time points based on the quantification by bioluminescence imaging (BLI). **(C)** Survival curve. **(D)** Images of mice on D -1 and **(E)** D8 post treatment.

Summary & Conclusions

- iNKT cells can be isolated from PBMC with a high purity (≥ 99.1%) and can be efficiently transduced to express CD19 CAR
- CAR19-iNKT cells (ALA-101) can be expanded to ~ 5000- fold
- ALA-101 is cytotoxic to CD1d- (SEM), CD1d+ (Ramos) and CD1d+++ (C1R-CD1d) cell lines and primary MZL and CLL tumor cells
- ALA-101 displays excellent proliferative response after at least 3 rounds of serial killing of CD19+ tumor cells
- ALA-101 prolongs survival and mediates anti-tumor activity in an aggressive CD1d-negative ALL model of SEM-luc *in vivo*
- ALA-101 has the potential to treat CD19+ malignancies

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