Pre-Clinical Efficacy of AB-101, An Allogeneic Cord-Blood Derived Natural Killer (NK) Cell Therapeutic Candidate, in Combination with Anti-CD38 Antibodies in Models of Multiple Myeloma

Gina Chu*, Kun-Yu Teng*, Lisa Guerrettaz, Rodrigo Silva, Salvatore Rotondo, Claudia Palomino La Torre, Amanda Conerty, Peter Flynn‡, Heather Raymon, Anita Seto, Srinivas Somanchi

Artiva Biotherapeutics, San Diego, CA

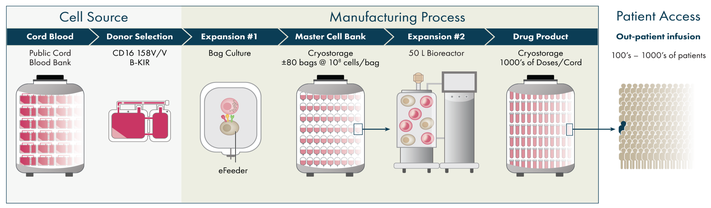
* Co-first authors and ‡ corresponding author (pflynn@artivabio.com)

Introduction

AB-101 is a non-engineered, allogeneic, off-the-shelf, cryopreserved cord blood-derived natural killer (NK)-cell therapy in development as a cancer therapeutic. A highly scaled manufacturing process enables the production of thousands of doses of AB-101 from a single donor cord blood unit (CBU). AB-101 is derived from CBUs selected for KIR-B haplotype and natural high-affinity variant of CD16 (158V/V), which are associated with improved anti-tumor activity and ADCC enhancement, resulting in a highly active NK cell product without the requirement for additional engineering. We are developing AB-101 for use in combination with monoclonal antibodies (mAbs) to enhance antibody-dependent cellular cytotoxicity (ADCC) in patients, initially in an ongoing Phase I clinical trial for NHL.

To further develop AB-101 as an ADCC enhancer, we assessed anti-tumor activity in combination with anti-CD38 mAbs against multiple myeloma (MM) cell lines. CD38 is a transmembrane glycoprotein that is uniformly and highly upregulated on MM cells. Anti-CD38 mAbs exert anti-tumor activity against MM through ADCC and other mechanisms. NK cells play a key role in ADCC responses through CD16 receptor signaling. However, the majority of NK cells express CD38 and are depleted by therapeutic CD38 mAbs, which could limit their therapeutic efficacy[1]. Combining NK cell therapies with anti-CD38 mAbs thus necessitates CD38 knockout to prevent fratricide. AB-101 has a naturally low percent and intensity of CD38 expression. Here we present data demonstrating that AB-101 is resistant to anti-CD38 mAb-induced fratricide due to their low CD38 expression (<23% of the drug product) and can elicit significant ADCC against MM cell lines both *in vitro* and *in vivo*. Since anti-CD38 mAbs are combined with glucocorticoids in the clinic, we assessed the impact of dexamethasone and methylprednisolone on ADCC by AB-101. Here we report robust ADCC by AB-101 following treatment with glucocorticoids.

Methods



AB-101 Manufacturing: Uses U.S.-licensed umbilical cord blood (CB) units with selected characteristics. Following isolation, NK cells are expanded utilizing a proprietary engineered feeder cell (eFeeder) to derive a master cell bank, followed by a bioreactor-based large-scale NK cell expansion and activation process to produce pure NK cells. High and consistent expression of the 158V/V CD16 is achieved in the AB-101 drug product without the requirement for engineering of the cells. The scale of production potentially enables hundreds to thousands of patients to be treated from a single donor CBU. CB-NK is derived from CBU without clinical attributes as a comparator.

CD38 Phenotype, Fratricide, and ADCC Assays: AB-101 was screened for CD38 expression and incubated with anti-CD38 mAb – daratumumab (Dara), isatuximab (Isa), or hIgG1 control (100 – 0.14 µg/ml) for 24 hours to assess fratricide. In vitro ADCC assays were performed at various effector to target (E:T) ratios against controls (Daudi and/or K562) and MM cell lines (NCI-H929, RPMI-8226, and MM.1S) using 2 µg/ml of Dara, Isa, or hIgG1. To study the effect of glucocorticoids, AB-101 was cultured in 10 µM of dexamethasone and 2 µg/ml of methylprednisolone for 48 hours in the presence of IL-2 prior to the ADCC assay.

In Vivo Fratricide and Efficacy study: In vivo fratricide of AB-101, CB-NK, and PB-NK was evaluated in naïve NSG mice with or without intravenous administration of 5mg/kg Dara. In vivo efficacy of AB-101 in combination with Dara was assessed in the intraperitoneal (IP) MM.1S-Luc xenograft tumor model in NSG mice. Tumor burden was assessed by bioluminescent imaging (BLI).

Results

Figure 1. Purity, Phenotype, and CD38 Expression of AB-101

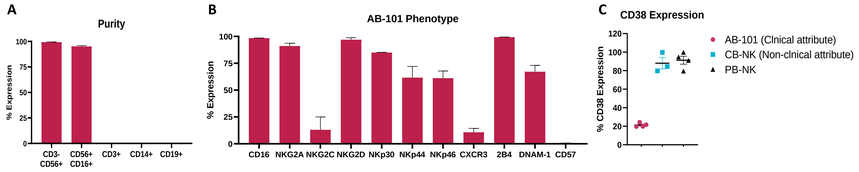


Figure 1. A. Purity of AB-101 characterized as $CD3^-CD56^+$ and $CD56^+CD16^+$ NK cells **B.** Phenotypic characterization of NK cell receptors on AB-101 ($CD3^-CD56^+$ cells). **C.** Low CD38 expression level (~20%) was found in AB-101 with clinical attributes compared to high CD38 expression in CB-NK without clinical attributes and PB-NK. Data is shown as Mean ± SEM.

Figure 2. AB-101 In Vitro and In Vivo Fratricide

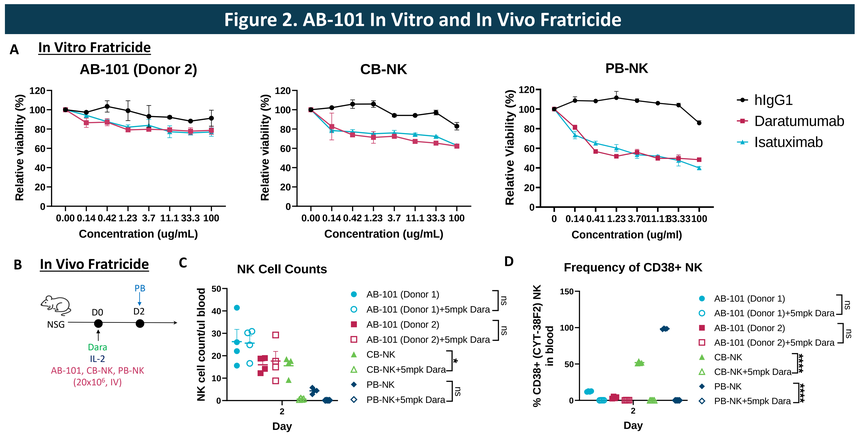
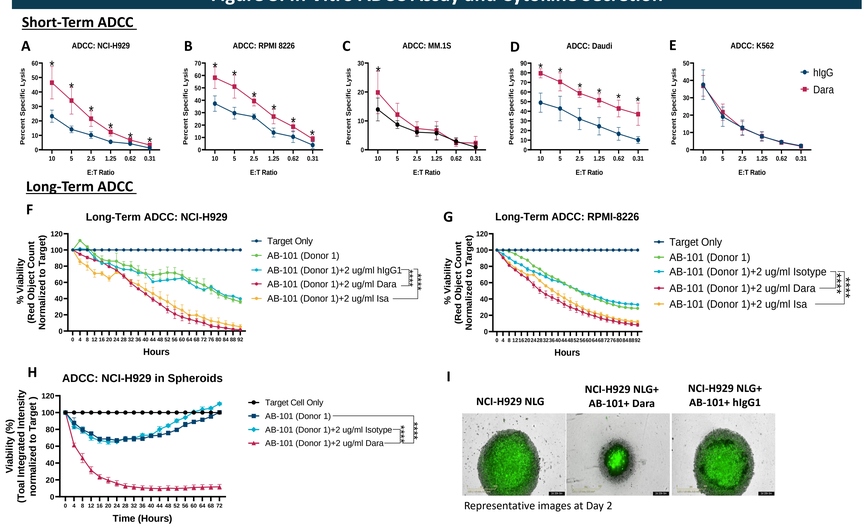


Figure 2. A. In vitro fratricide of AB-101, CB-NK, and PB-NK induced by Dara and Isa overnight was measured by CellTiter-Glo assay, and data is presented as relative viability (%) by normalizing to hIgG1 control. **B.** Schema of in vivo fratricide study design. NK cells were administered intravenously on Day 0 in naïve NSG mice with or without intravenous injection of 5 mg/kg (mpk) Dara. Peripheral blood (PB) was collected on Day 2 for fratricide analysis by flow cytometry. The impact of Dara on the levels of AB-101, CB-NK, and PB-NK present in the peripheral blood on Day 2 is shown by **(C)** NK cell count per µl of blood and **(D)** frequency of CD38+ NK cells. In vitro and in vivo fratricide correlated with CD38 expression levels on the NK cells. Data is shown as Mean ± SEM.

Figure 3. In Vitro ADCC Assay and Cytokine Secretion



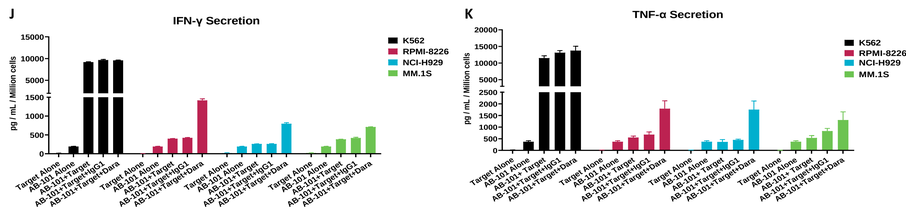


Figure 3. Dara induced significant short-term (4 hours) ADCC by AB-101 cells against **(A)** NCI-H929, **(B)** RPMI 8226, **(D)** Daudi at various E:T ratios. ADCC efficacy against **(C)** MM.1S and **(E)** control K562 was not significant. AB-101 in combination with Dara or Isa induced significant long-term ADCC against **(F)** NCI-H929 and **(G)** RPMI-8226 compared to isotype control (hIgG1) at a 1:1 E:T ratio over 92 hours measured by Incucyte. **H.** AB-101 + Dara demonstrated significant ADCC against NCI-H929 in a tumor spheroid model. **I.** Representative images of nuclear green-labelled NCI-H929 (NCI-H929 NLG) spheroids mixed with AB-101 and AB-101 + Dara or hIgG1. Dara induced AB-101 secretion of **(J)** IFN-γ and **(K)** TNF-α. Data was normalized to secretion/million cells. Statistical Analysis for short-term ADCC was performed using Multiple Wilcoxon matched-pairs signed rank test (* p<0.05). Long-term ADCC was analyzed by two-way ANOVA using Tukey's multiple comparisons test (**** p<0.0001).

Figure 4. Impact of Steroids on ADCC

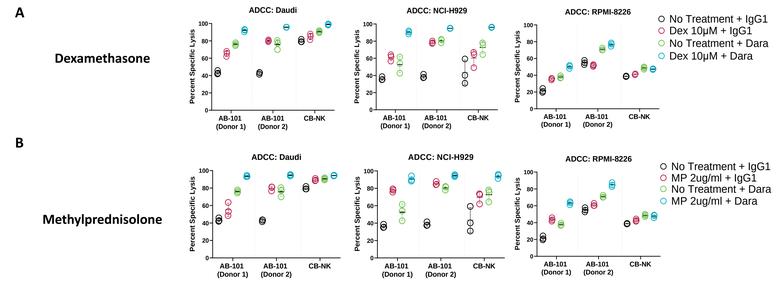


Figure 4. Effect of glucocorticoids on AB-101 mediated ADCC. AB-101 was cultured for 48 hours in 10 µM of **(A)** Dexamethasone (Dex) and 2 ug/ml of **(B)** Methylprednisolone (MP) in the presence of IL-2, followed by ADCC assessment against control (Daudi) and NCI-H929 and RPMI-8226 MM lines. AB-101 or CB-NK was seeded with tumor cells at E:T ratio of 5:1. ADCC against Daudi and MM lines was not negatively impacted by Dex or MP. Data is shown as Mean ± SD.

Figure 5. AB-101 + Dara Enhanced Anti-Tumor Activity in MM.1S Xenograft Tumor Model

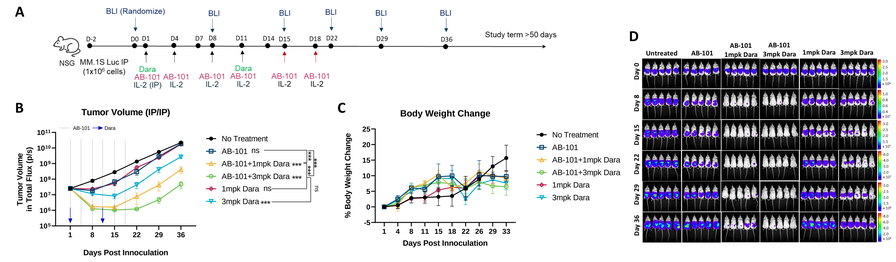


Figure 5. A. Study design of AB-101 efficacy in MM.1S tumor model. **B.** AB-101 + Dara significantly delayed MM.1S tumor progression compared to no treatment, AB-101 ($10x10^6$) and Dara (1mpk) monotherapies. **C.** Body weight change was calculated on the day NK cells were injected. **D.** Representative BLI images of mice bearing MM.1S-Luc tumors at multiple timepoints following administration of tumor cells, mean total [flux photons/second] ± SEM for each group of mice is shown. ***p<0.0001, two-way ANOVA with Tukey's multiple comparison test.

Conclusions

AB-101 has high and consistent expression of CD16, low CD38 expression, and is resistant to anti-CD38 mAb-induced fratricide and hence can be combined with daratumumab (Darzalex) or isatuximab (Sarclisa) without additional engineering (such as CD38 knockout). Anti-CD38 mAbs are combined in the clinic with glucocorticoids, immunomodulatory drugs (IMiDs), and proteosome inhibitors (PI). While IMiDs and PIs are reported to enhance NK cell function, glucocorticoids have been shown to diminish NK cell cytotoxicity. Data presented here suggests that the ADCC potential of AB-101 is not negatively impacted by glucocorticoids. Taken together, the *in vitro* and *in vivo* data support further clinical development of AB-101 in combination with anti-CD38 mAbs to treat MM patients.

Reference

1. Casneuf, T., et al., Effects of daratumumab on natural killer cells and impact on clinical outcomes in relapsed or refractory multiple myeloma. Blood Adv, 2017. 1(23): p. 2105-2114.