Development of AB-201, a novel allogeneic anti-HER2-specific CAR-NK cell therapy for the treatment of HER2+ tumors

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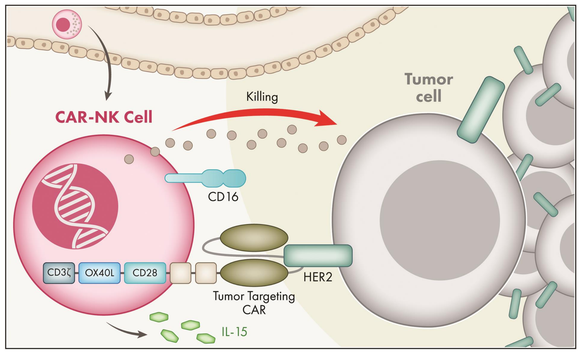
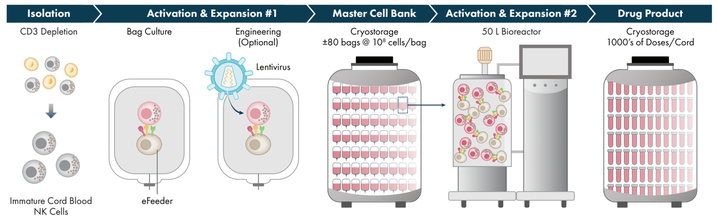
Introduction

Human Epidermal Growth Factor Receptor 2 (HER2) is a receptor tyrosine kinase that is highly expressed on the surface of many solid tumors. While many patients with advanced HER2+ cancers derive meaningful benefit from HER2 targeted therapies, they typically progress beyond approved therapies, and treatment of these patients remains a great unmet medical need. Currently, while there are eight approved HER2 directed therapies, there are no approved cellular therapies targeting HER2[1]. Over the past decade, cellular therapy has been shown to be a viable treatment option in different cancer types. Here we present AB-201, an off-the-shelf, cryopreserved cord blood (CB) derived HER2-CAR NK cell therapy with the potential to be an active and readily available option for patients with HER2+ solid tumors.



AB-201 (CAR-NK) has a unique HER2-specific targeting domain combined with our proprietary NK-specific co-stimulatory domains. Wild type IL-15 is co-expressed from the CAR lentiviral construct for enhanced NK cell activity and persistence.

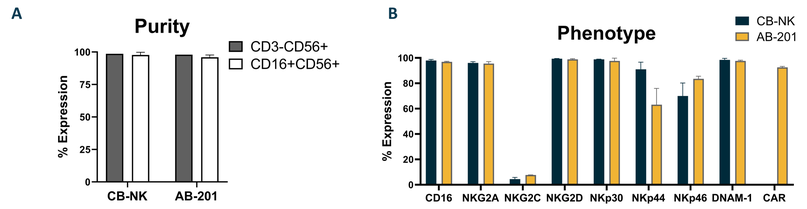
Methods



- AB-201 is comprised of *ex vivo* expanded allogeneic CB-derived NK cells that have been genetically modified to express a HER2-directed CAR and is a cryopreserved, infusion-ready product
- Manufacturing utilizes a feeder-cell line engineered to express factors specifically identified as supportive to NK cell expansion and a lentiviral transduction step to introduce the HER2 CAR construct
- Manufacturing has the potential to yield 1000s of clinical doses of the CAR-NK product from each CB unit

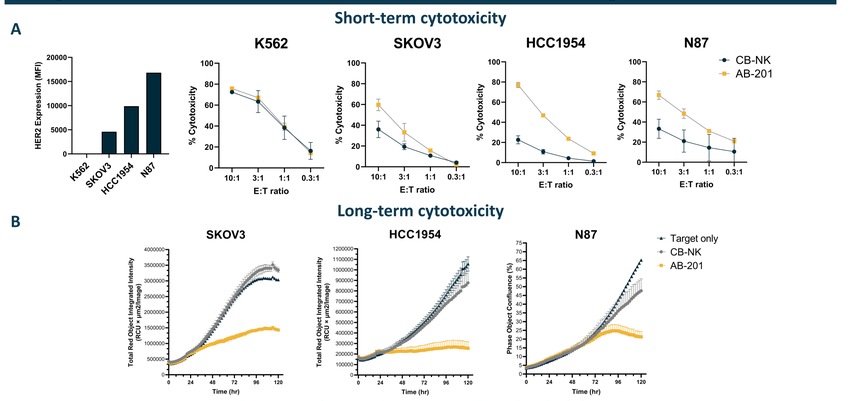
Results

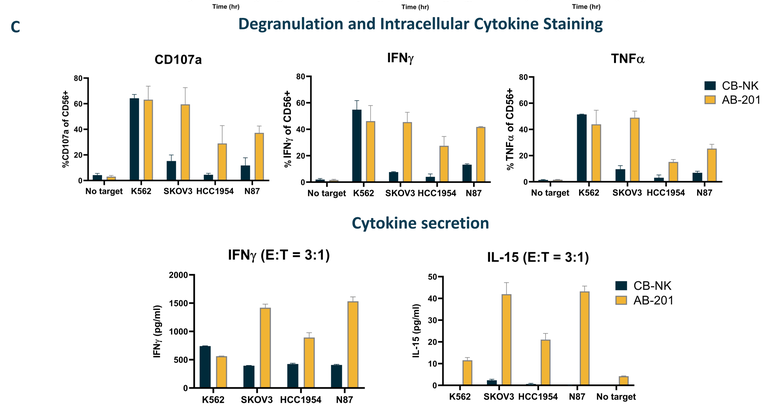
Figure 1. AB-201: HER2 CAR-NK Phenotypic Characterization



A. Characterization and frequency of NK cells (CD3-CD56+), (CD16+CD56+) in CB-NK and AB-201
B. Frequency of CD16+, NKG2A+, NKG2C+, NKG2D+, NKp30+, NKp44+, NKp46+, DNAM-1 was analyzed on control CB-NK cells and AB-201 gating on CD3-CD56+ cells. Data are plotted as the mean ± SD

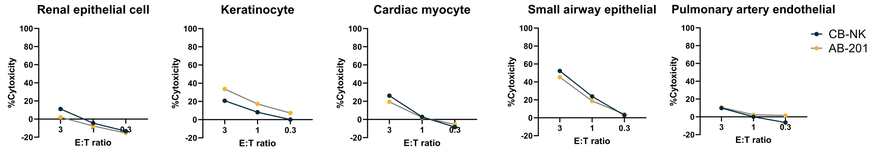
Figure 2. AB-201 Demonstrates Enhanced Effector Function Against HER2+ Tumors





A. Her2 expression (Mean fluorescence intensity=MFI) on tumor cell lines and short-term cytotoxicity of AB-201 and control CB-NK cells.
B. Long-term cellular cytotoxicity of AB-201 and CB-NK cells against HER2+ tumor cell lines over 120 hours, E:T ratios of 3:1, 1:1 or 0.3:1 are shown for SKOV3, HCC1954 or N87, respectively.
C. AB-201 or CB-NK were stimulated for 24 hours with tumor cell lines at a 1:1 E:T ratio. Following stimulation, degranulation (CD107a)/cytokine secretion and soluble cytokine levels (ELISA) were measured. Data are plotted as mean ± SEM.

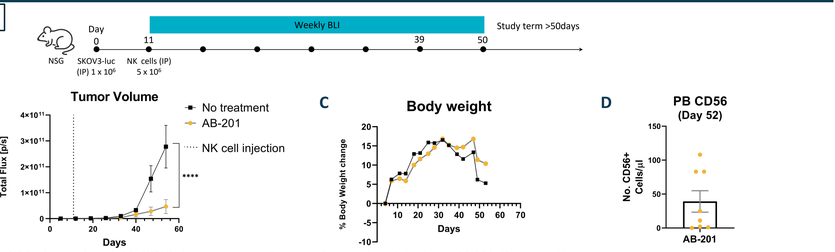
Figure 3. No HER2-dependent Cytotoxicity was Observed with AB-201 on Primary Cells



Cytotoxicity of primary cells (non-tumor) was measured following co-culture of AB-201 or control CB-NK cells with pulmonary artery endothelial cells, keratinocytes, renal epithelial cells, cardiac myocytes and small airway epithelial cells for 4 hours at Effector: Target (E:T) ratios of 3:1, 1:1, or 0.3:1.

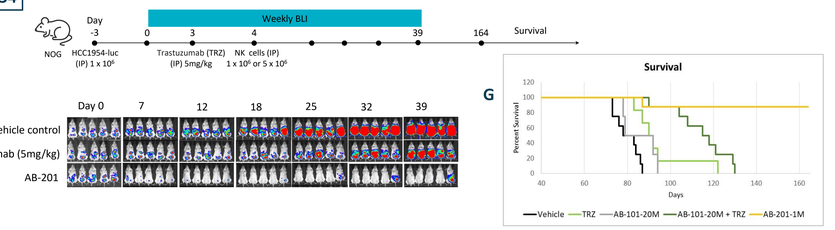
Figure 4. AB-201 Demonstrates Enhanced *in vivo* Antitumor Activity Against HER2+ Tumors

SKOV3



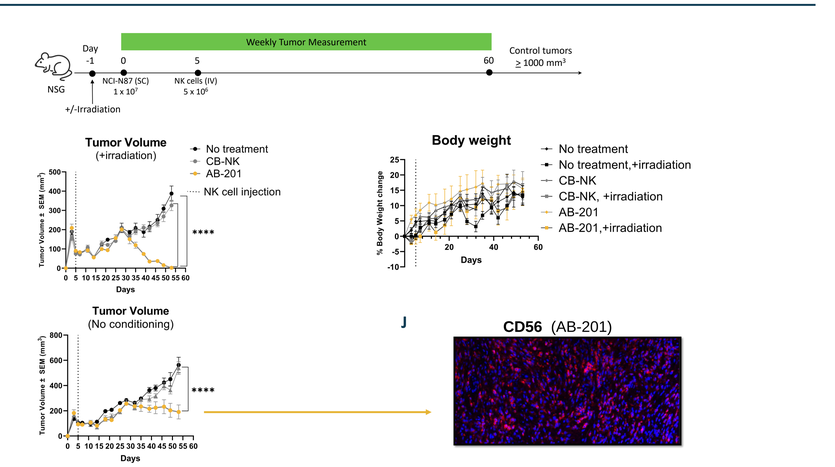
A. NSG mice received 1x10[6] SKOV3- Luc tumor cells (IP) on day 0 and a single injection of AB-201 (IP) on day 11.
B. Bioluminescence (BLI) measurements of SKOV3-Luc, mean total flux ±SEM for each group of mice are shown. ****p<0.0001, two-way ANOVA.
C. No observed difference in body weight between groups.
D. Persistence of AB-201 in peripheral blood (PB) by flow cytometry at day 52 (gated on human CD45+CD56+)

HCC1954



E. NOG mice received 1x10[6] HCC1954-Luc tumor cells (IP) on day -3 and a single injection of AB-201 (1,5x10[6]), AB-101, AB-101+ Trastuzumab (TRZ) (IP), TRZ alone (5mg/kg) (IP). AB-101 = Non-genetically modified CB-NK
F. BLI assessment demonstrates single administration of AB-201 resulted in tumor remission in 4 of 5 animals
G. In a separate survival study, single administration of AB-201 lead to 7/8 mouse survival up to 164 days

N87



H. NSG mice received +/-irradiation (1.5Gy) on day –1 and were inoculated with 1x10[7] N87 tumor cells (SC) on day 0. On day 5, mice received a single injection of CB-NK or AB-201 (IV).
I. AB-201 demonstrated significant efficacy over no treatment and CB-NK (P<0.0001, Two-way ANOVA) in irradiated mice (top) and unconditioned mice (bottom) was well tolerated based on no change in body weight across groups (right graph).
J. AB-201 infiltration in tumor. Representative image of CD56 immunofluorescence staining of tumor section (40x magnification) from AB-201 group indicated by arrow. CD56 is indicated in red (Alexafluor-647) and DAPI (nuclear counterstain) in blue.

Conclusions

These preclinical findings suggest that AB-201, a highly scaled, cryopreserved HER2-directed CAR NK cell product, has potential to be an effective therapy in the treatment of HER2+ tumors.

References

1. Oh, DY., Bang, YJ. HER2-targeted therapies — a role beyond breast cancer. *Nat Rev Clin Oncol* **17,** 33–48 (2020).