DEVELOPMENT OF A ROBUST MANUFACTURING PROCESS FOR AB-1015, AN INTEGRATED CIRCUIT T CELL (ICT) PRODUCT, USING TARGETED, CRISPR INTEGRATION OF TRANSGENES BY ELECTROPORATION (CITE) EDITING

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Background AB-1015 is an autologous, integrated circuit T cell (ICT) product for the treatment of platinum-resistant ovarian cancer. AB-1015 includes a transgene cassette with two functional modules: an "AND" logic gate designed to limit off-tumor toxicity through dual tumor antigen recognition, and a dual shRNA-miR designed to resist TME suppression and to improve ICT cell function. ICT cells are generated via CRISPR integration of transgenes by electroporation (CITE), a non-viral and site-specific integration approach that provides enhanced safety, increased cargo capacity, and reduced cost. A scalable, semi-closed, and semi-automated manufacturing process was developed to support GMP manufacture of AB-1015 for Phase 1 clinical evaluation.

Methods Clinical-scale, end-to-end runs were performed in healthy donors using Arsenabio’s manufacturing process for AB-1015. On Day 0, CD4 and CD8 positive cells were isolated from fresh apheresis from healthy donors. Cells were activated using CD3/CD28 stimulation, electroporated with Cas9 protein, sgRNA targeting a safe harbor site (GS94), and plasmid DNA encoding the transgene. Cells were expanded until harvest and formulated into drug product. Frozen cell drug product was thawed and characterized using flow cytometry, in vitro functional assays, and in vivo tumor efficacy models.

Results Processing of healthy donor apheresis from more than 20 donors using the AB-1015 manufacturing process resulted in average knock-in efficiencies of approximately 25% and total therapeutic yields exceeding 2.5e9 transgene positive cells. In addition to robust IFN-γ production and tumor cell killing in dual antigen-specific co-culture, AB-1015 ICT cells retained favorable memory phenotype (CCR7+) at harvest. Furthermore, AB-1015 ICT cells demonstrated potent anti-tumor responses in a xenograft in vivo model.

Conclusions A robust, 10-day manufacturing process was successfully developed for AB-1015 that enables high knock-in efficiencies of a large (> 6 kb) transgene using a site-specific, non-viral integration approach. Billions of ICT cells can be generated from a single healthy donor in a semi-closed, semi-automated fashion and these ICT cells display potent anti-tumor activity in vitro and in vivo with high specificity. AB-1015 will be evaluated in clinical trials for treatment of platinum-resistant ovarian cancer.