## 274 DEVELOPMENT OF CTX112 A NEXT GENERATION ALLOGENEIC MULTIPLEXED CRISPR-EDITED CART CELL THERAPY WITH DISRUPTIONS OF THE TGFBR2 AND REGNASE-1 GENES FOR IMPROVED MANUFACTURING AND POTENCY

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**Background** CTX110, a CD19-directed first-generation CRISPR-edited CAR T cell therapeutic candidate for B-cell lymphoma, has induced clinical responses and durable remissions (2+ years) in some patients (CARBON trial, NCT04035434). CTX112 contains the same edits as CTX110 to disrupt the B2M locus for immune evasion and the TRAC locus to prevent GvHD and allow for CAR transgene integration. Additionally, CTX112 contains disruptions in the TGFBR2 gene to avoid immune suppression of CAR T activity by cells of the tumor environment and the Regnase-1 gene to increase functional persistence. This increase in functional persistence was also assessed for increased manufacturing yields.

**Methods** CTX112 was produced utilizing CRISPR/Cas9 gene editing to disrupt genomic loci, and AAV6 vector to introduce donor template for CAR integration. Manufacturing was performed similarly to CTX110 but CTX112 was also used as a seed bank to generate upwards of 20X more product. Functional assessment of standard and extended cultures of CTX112 was performed in vitro as well as in vivo for anti-B-cell lymphoma/leukemia activity against several cell lines, including Nalm6, Raji and Jeko-1. Safety and immunogenicity of the engineered cells were assessed in vitro by cytokine independent growth and in vivo in toxicology studies in NSG mice.

**Results** CTX112 is at least 10x more potent than CTX110 in xenograft models. CTX112 cells secrete high levels of a broad array of cytokines, resist TGFβ inhibition, have a higher proliferative potential and are more sensitive to cytokines. CTX112 does not display cytokine independent growth but displays greater expansion during manufacturing.

**Conclusions** CTX112 is a more potent next generation anti CD19 CAR T cell that also shows improved manufacturability. The lack of class I MHC on CTX112 could enable rejection by patient NK cells but such cells are rare and show little activity at tumor sites. Thus, we hypothesize that CTX112 will show increased functional persistence at the site of tumor and will continue to kill cancer cells for longer as compared to other allogeneic CAR T cell products through its persistent effector function and TME suppression resistance. CTX112 is being evaluated in a clinical trial for B cell malignancies (NCT05643742).

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0274