Preservation of T-Cell Stemness with a Novel Expansionless CAR-T Manufacturing Process, Which Reduces Manufacturing Time to Less Than Two Days, Drives Enhanced CAR-T Cell Efficacy

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Introduction

• Extended T-cell culture periods in vitro deplete the chimeric antigen receptor (CAR)-T final product of naive and stem cell memory T-cell (T\text{scm}) subpopulations that are associated with improved antitumor efficacy\(^1\)

• YTB323 is an investigational, autologous CD19-directed CAR-T cell therapy
  – YTB323 expresses the same validated CAR as tisagenlecleucel (Kymriah\textsuperscript{®}), an approved CAR-T cell therapy for pediatric/young adult acute lymphoblastic leukemia (ALL) and relapsed or refractory (r/r) diffuse large B-cell lymphoma (DLBCL)\(^2,3\)
  – YTB323 is produced using a simplified and innovative platform called T-Charge\textsuperscript{™}, which reduces the manufacturing process time to <2 days

• The new T-Charge\textsuperscript{™} manufacturing platform was evaluated in a preclinical setting compared to traditional manufacturing (TM) of a CAR-T product using the same lentiviral vector (CTL*019)

CAR, chimeric antigen receptor; CD, cluster of differentiation; T\text{scm}, stem cell memory T cell.
Methods

YTB323 Manufacturing (T-Charge™) Versus CTL*019 Manufacturing (TM Process)

- Using the T-Charge™ platform, T cells were enriched from healthy donor leukapheresis, followed by activation and transduction with a lentiviral vector encoding for the same CD19-targeting CAR used for tisagenlecleucel before final harvest, wash and formulation (YTB323).

- Using TM, T cells were enriched from the same healthy donor leukapheresis, activated and transduced with an identical lentiviral vector. CAR-T cells were then expanded for 9 to 11 days before being washed and formulated (CTL*019).

Post-Manufacturing YTB323 CAR-T In Vitro and In Vivo Assessments

- YTB323 and CTL*019 CAR-T cell products were analyzed by flow cytometry and single-cell RNAseq.

- CAR-T cell products were assessed in T-cell functional assays in vitro:
  - Co-culture experiments with a pre-B-ALL cell line (NALM6), a CD19-knockout variant of NALM6 (NALM6-19KO), and a DLBCL line (TMD-8).
  - Repetitive stimulation assays with NALM6-RFP using IncuCyte fluorescence readout.

- CAR-T cell products were assessed for antitumor activity and expansion in vivo:
  - Immunodeficient NOD scid gamma (NSG) mice (NOD- scid IL2Rgamma<sup>null</sup>) inoculated with a pre-B-ALL cell line (NALM6) were utilized to evaluate antitumor activity and CAR-T cell expansion, measured by flow cytometry of mouse blood samples.

ALL, acute lymphoblastic leukemia; CAR, chimeric antigen receptor; CD, cluster of differentiation; KO, knockout; NSG, NOD scid gamma; RFP, red fluorescent protein; RNA, ribonucleic acid; seq, sequencing; TM, traditional manufacturing.
YTB323 CAR-T Cell Products, Generated Via T-Charge™, Retained the Immunophenotype of the Input Leukapheresis Material

• Naive/T_{scm} cells (CD45RO−/CCR7+) were retained as shown by flow cytometry.

• In contrast, the TM process with ex vivo expansion generated a final product consisting mainly of central memory T cells (T_{cm}) (CD45RO+/CCR7+)..

• Single-cell RNA sequencing of YTB323 CAR-T cell products further demonstrated the preservation of the T-cell phenotype of the starting material compared with the gene signature of CTL*019 CAR-T cell products.

CAR, chimeric antigen receptor; T_{cm}, central memory T cells; TM, traditional manufacturing; T_{scm}, stem cell memory T cell; UTD, untransduced T cells.
YTB323 Cells Showed Superior Anti-Tumor Activity in Vitro

- Persistence and exhaustion of the YTB323 CAR-T cell products was tested using a tumor repeat stimulation assay.
- YTB323 cells were able to control the tumor at a 30-fold lower effector:tumor cell ratio and for a minimum of 5 or more stimulations in the repeat stimulation assay compared to CTL*019 cells.

CAR, chimeric antigen receptor; RFP, red fluorescent protein.

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YTB323 Controlled NALM6 B-ALL Tumor Growth at a Lower Dose Compared to CTL*019

• YTB323 CAR-T cells were able to fully reject NALM6 cancers at a dose of $0.1 \times 10^6$ CAR+ cells
• CTL*019 required $0.5 \times 10^6$ CAR+ cells for full cancer control
• This ability of YTB323 to control the tumor at lower doses confirms its enhanced potency also in vivo

B-ALL, B-cell acute lymphoblastic leukemia; CAR, chimeric antigen receptor; DLBCL, diffuse large B-cell lymphoma; NSG, NOD scid gamma; UTD, untransduced T cells.
YTB323 Showed Higher Expansion ($T_{\text{max}}$ and $AUC_{0-21d}$) Compared With CTL*019

- Expansion of CD3+/CAR+ T cells in blood was analyzed weekly by flow cytometry for up to 4 weeks postinfusion.
- Dose-dependent expansion ($C_{\text{max}}$ and $AUC_{0-21d}$) was observed for both YTB323 and CTL*019. $C_{\text{max}}$ was ≈40-times higher and $AUC_{0-21d}$ was ≈33-times higher for YTB323 compared with CTL*019 across multiple doses.
- YTB323 peak expansion ($T_{\text{max}}$) was delayed by at least 1 week compared with CTL*019.

AUC, area under the curve; B-ALL, B-cell acute lymphoblastic leukemia; CAR, chimeric antigen receptor; $C_{\text{max}}$, maximum concentration; $T_{\text{max}}$, time to peak expansion.
Conclusions

• YTB323 CAR-T cells generated via T-Charge™ retained the naive/T_{scm} immunophenotype of the input leukapheresis

• The ability of YTB323 to control tumor growth in vivo and at lower doses compared to CTL*019 confirms its proliferative capacity and potency

• Compared to approved CAR-T cell therapies, YTB323 has the potential to achieve improved clinical efficacy at its respective lower doses

• YTB323 is currently being investigated in a first-in-human trial for the treatment of patients with relapsed/refractory diffuse large B-cell lymphoma (Flinn I, et al. ASH 2021. Oral 740)

• The T-Charge™ platform is also being evaluated in the context of a BCMA-targeting CAR-T (Dexiu B, et al. ASH 2021. Poster 2770; Sperling A, et al. ASH 2021. Poster 3864)
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