Poster 2848

Boris Engels, PhD
boris.engels@novartis.com

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

Boris Engels,[1] Xu Zhu,[1] Jennifer Yang,[1] Andrew Price,[1] Akash Sohoni,[1] Andrew M. Stein,[1] Lana Parent,[1,*] Michael Greene,[1,*] Matthew Niederst,[1] Jeanne Whalen,[1] Elena J. Orlando,[1] Louise M. Treanor,[1] Jennifer L. Brogdon[1]

[1]Novartis Institutes for BioMedical Research, Cambridge, MA, USA
*Current affiliation iVexSol, Inc., Worcester, MA, USA

INTRODUCTION

- Extended T-cell culture periods *in vitro* deplete the chimeric antigen receptor (CAR)-T cell final product of naive and stem cell memory T-cell (T_{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®), an approved CAR-T cell therapy for pediatric/young adult B-cell acute lymphoblastic leukemia (B-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™, which reduces the manufacturing process time to <2 days
- The new T-Charge™ 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)

METHODS

Study Design

YTB323 Manufacturing (T-Charge™) vs 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)

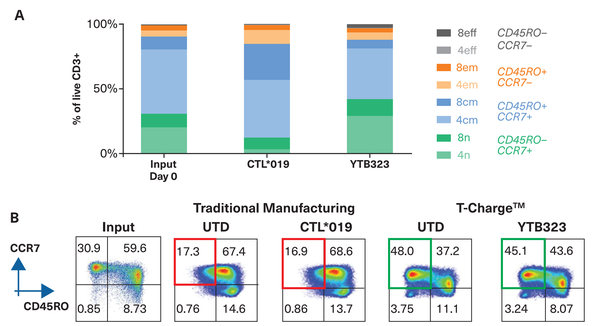
Post-Manufacturing YTB323 CAR-T *In Vitro* and *In Vivo* Assessments

- 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^{null}) 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

RESULTS

- YTB323 CAR-T cell products generated via this novel expansionless manufacturing process retained the immunophenotype of the input leukapheresis material
  - Naive/T_{scm} cells (CD45RO–/CCR7+) were retained as shown by flow cytometry (**Figure 1**)
- In contrast, the TM process with *ex vivo* expansion generated a final product consisting mainly of central memory T cells (T_{cm}) (CD45RO+/CCR7+)

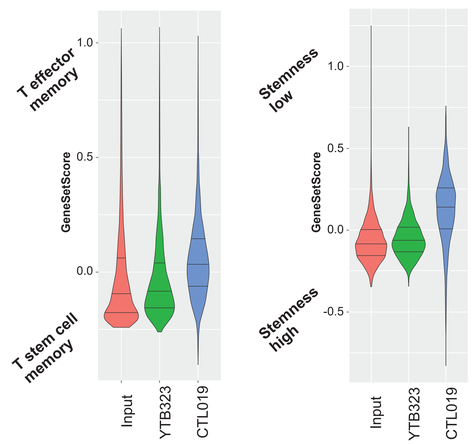
Figure 1. Immunophenotype of CAR-T Cells Generated by Traditional Manufacturing (CTL*019) and T-Charge™ (YTB323)



(**A**) T cell phenotype of leukapheresis (Input) and CTL*019 and YTB323 CAR-T products. The percentage of the respective populations (colors) refer to % of CD3+ cells. 4 and 8, CD4+ and CD8+, respectively; eff, effector; em, effector memory; cm, central memory; n, naive. Data are representative of 3 full-scale runs with 3 different healthy donors (n= 3) and several small-scale runs used to optimize the process. (**B**) Gating strategy on live CD3+ events used to determine T cell subsets (CCR7 vs. CD45RO). For YTB323 and CTL*019 bulk cultures are shown (CAR positive and negative). "UTD" refers to untransduced T cells manufactured according to the T-Charge™ and the TM processes. Numbers in quadrants indicate % of CD3+ population. Red boxes indicate loss of naive and T_{scm} phenotypes by TM process. Green boxes indicate the maintenance of naive and T_{scm} cells by the T-Charge™ process.

- Single-cell RNA sequencing of YTB323 and CTL*019 CAR-T cell products and starting material (input) was performed (**Figure 2**)
- Results further demonstrated the preservation of the T-cell phenotype of the starting material
- YTB323 CAR-T cells show similar T_{scm} and stemness gene signatures as the input material

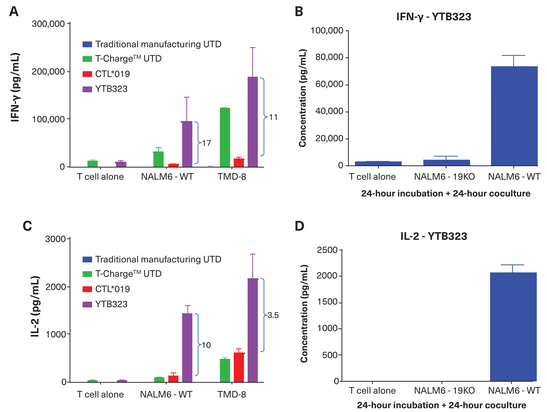
Figure 2. Single-Cell RNA Sequencing of YTB323 and CTL*019 Products



Single cell gene set enrichment for input (leukapheresis), YTB323, and CTL*019 cells. Gene signature sets for **Left:** T effector memory compared to T stem cell memory and **Right:** stemness were assessed for gene set enrichment in each cell in the three samples. Violin plots compare the gene set enrichment scores between the 3 samples with lines indicating the 25%, 50%, and 75% quantiles. RNA, ribonucleic acid.

- YTB323 cell potency was assessed *in vitro* through co-culture with cancer cells and measurement of cytokine secretion
  - Co-cultures were set up immediately after thawing CAR-T final products and cytokines were measured after 24h or 48h of co-culture at an effector to target (E:T) ratio of 1:1
- YTB323 T cells exhibited 11- to 17-fold higher levels of IFN-γ (**Figure 3A**) and 3.5- to 10-fold higher levels of IL-2 (**Figure 3C**) secretion upon CD19-specific activation compared with CTL*019
- YTB323 T cells show background levels of cytokine secretion after a 24-hour rest period while retaining CD19-specific activation and cytokine secretion (**Figures 3B and 3D**)

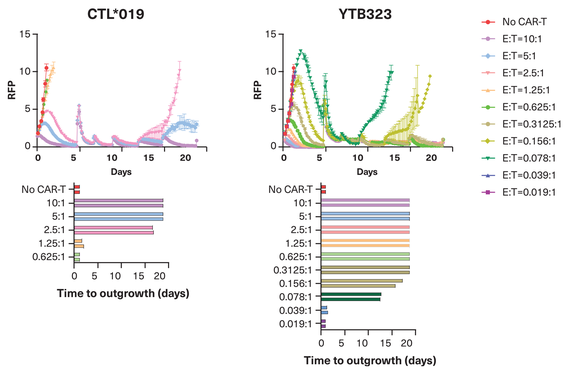
Figure 3. In Vitro Secretion of IFN-γ and IL-2 by YTB323



Cytokine concentration in cell culture supernatants. IFN-γ (**A and B**) and IL-2 (**C and D**). Data shown are derived from 2 healthy donor T cells and is representative of 2 experiments with 3 donors total. (**A and C**) CTL*019, YTB323, and respective UTD were co-cultured with NALM6-WT (ALL), TMD-8 (DLBCL), or without cancer cells (T cells alone). Supernatant was collected 48h later. (**B and D**) YTB323 was co-cultured with NALM6-WT, NALM6-19KO (CD19-negative) or alone. To assess antigen-specific cytokine secretion, YTB323 were cultured alone for 24h, washed and then co-cultured with target cells for 24h. IFN, interferon; IL, interleukin; KO, knockout; TM, traditional manufacturing; UTD, untransduced T cells; WT, wild-type.

- Anti-tumor activity of YTB323 and CTL*019 was tested *in vitro* in a stress test, using a cancer repeat stimulation assay (**Figure 4**)
  - 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

Figure 4. Anti-tumor activity of YTB323 and CTL*019 CAR-T Cell Products

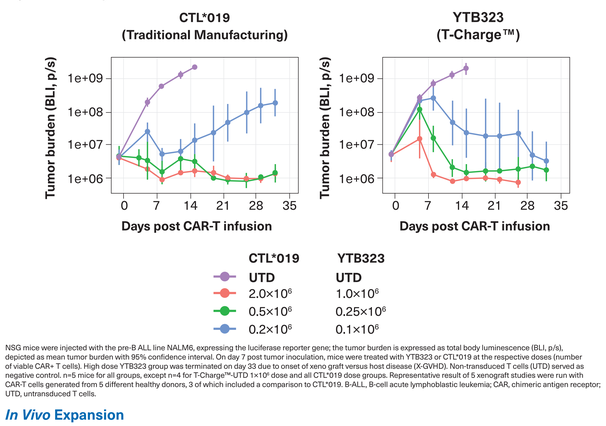


Red fluorescent protein (RFP) labeled NALM6 ALL cells were co-cultured with the CTL*019 or YTB323 CAR-T cells at the indicated effector to target (E:T) ratios in a repeat-stimulation assay. Media with fresh cancer cells was replaced (CAR-T cells were left behind) shortly after clearance of previous stimulation. **Top:** Total RFP signal was measured every 4 hours via the IncuCyte S3. **Bottom:** Time to outgrowth of cancer cells is plotted for each condition. Individual bars represent duplicate wells. CAR, chimeric antigen receptor; RFP, red fluorescent protein.

In Vivo Antitumor Efficacy

- Antitumor efficacy of YTB323 against B-cell tumors was assessed in immunodeficient NSG mouse models at multiple doses
- YTB323 controlled NALM6 B-ALL tumor growth at a lower dose of 0.1×10^6 CAR+ cells compared to 0.5×10^6 CAR+ cells required for CTL*019 (**Figure 5**)
- In the DLBCL model TMD-8, only YTB323 was able to control the tumors, while tumor progression was observed with CTL*019 at the respective dose group (data not shown)

Figure 5. Efficacy of YTB323 in a B-ALL Tumor Model

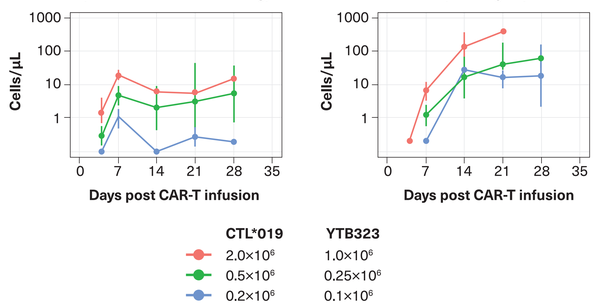


NSG mice were injected with the pre-B ALL line NALM6, expressing the luciferase reporter gene; the tumor burden is expressed as total body luminescence (BLI, p/s), depicted as mean tumor burden with 95% confidence interval. On day 7 post tumor inoculation, mice were treated with YTB323 or CTL*019 at the respective doses (number of viable CAR+ T cells). High dose YTB323 group was terminated on day 33 due to onset of xeno graft versus host disease (X-GVHD). Non-transduced T cells (UTD) served as negative control. n=5 mice for all groups, except n=4 for T-Charge™ UTD 1×10^6 dose and all CTL*019 dose groups. Representative result of 5 xenograft studies were run with CAR-T cells generated from 5 different healthy donors, 3 of which included a comparison to CTL*019. B-ALL, B-cell acute lymphoblastic leukemia; CAR, chimeric antigen receptor; UTD, untransduced T cells.

In Vivo Expansion

- YTB323 cell potency was also examined through comparison with CTL*019 regarding *in vivo* expansion at multiple doses
- Expansion of CD3+/CAR+ T cells in blood was analyzed weekly by flow cytometry for up to 4 weeks postinfusion (**Figure 6**)
- Dose-dependent expansion (maximum concentration [C_{max}] and area under the curve from Day 0 to Day 21 [$AUC_{0\text{-}21d}$]) was observed for both YTB323 and CTL*019. C_{max} was ≈40 times higher and $AUC_{0\text{-}21d}$ was ≈33 times higher for YTB323 compared with CTL*019 across multiple doses
- YTB323 time to peak expansion (T_{max}) was delayed by at least 1 week compared with CTL*019, supporting that increased expansion was driven by the less differentiated T-cell phenotype of YTB323

Figure 6. CAR-T Expansion of YTB323 and CTL*019 in a B-ALL Tumor Model



Time course of CAR+ T cell concentrations in NALM6 tumor-bearing mice treated with CTL*019 or YTB323. Blood samples were taken at 4, 7, 14, 21 and 28 days post CAR-T cell injection; CAR+ T cell concentrations were analyzed by flow cytometry, depicted as mean cells with 95% standard deviation. CAR, chimeric antigen receptor.

CONCLUSIONS

- The novel, expansionless T-Charge™ platform utilized to manufacture YTB323 is simplified and shortened
- YTB323 CAR-T cells generated via T-Charge™ retained the naive/T_{scm} immunophenotype of the input leukapheresis
  - YTB323 CAR-T cells showed similar T_{scm} and stemness gene signatures as the input material
- The ability of YTB323 to control tumor growth *in vivo* and at lower doses compared to traditionally manufactured 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 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 (Bu D, et al. ASH 2021. Poster 2770; Sperling A, et al. ASH 2021. Poster 3864)

Presented at the 2021 ASH Annual Meeting and Exposition; December 11-14, 2021; Atlanta, GA, and Virtual.

Acknowledgments

Medical writing support was provided by Jacqueline R. Ward, PhD, of Healthcare Consultancy Group, LLC, and funded by Novartis Pharmaceuticals Corporation. This study was sponsored by Novartis Pharmaceuticals Corporation.

Declaration of Interests

BE reports employment, holds stocks, and holds patents with Novartis. **XZ** and **MN** report employment and hold stocks in Novartis. **JY, AP, AS, AMS, JW,** and **EJO** report employment with Novartis. **LP** reports employment with iVexSol and former employment in the last 24 months with Novartis. **MG** reports employment with and is a current equity holder, holder of individual stocks, and holder of stock options in iVexSol. **LMT** reports employment, is a current holder of individual stocks, divested equity in the past 24 months, and holds patents with Novartis. **JLB** reports employment with, is a current holder of stock options, and holds patents with Novartis.

References

1. Fraietta JA, et al. *Nat Med.* 2018;24(5):563-571.
2. Maude SL, et al. *N Engl J Med.* 2018;378(5):439-448
3. Schuster SJ, et al. *N Engl J Med.* 2019;380(1):45-56.