Poster 3481

KITE-753: An Autologous Rapid Manufactured Anti-CD19/CD20 CAR-T Product for the Treatment of B-cell Malignancies

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BACKGROUND

- Although anti-CD19 chimeric antigen receptor (CAR) T-cell therapies have made transformative impacts on the lives of patients with B cell malignancies, relapse due to antigen escape remains a key obstacle.
- Hence, a Phase 1, first-in-human, open-label, multicenter study is currently ongoing to evaluate the safety and efficacy of KITE-363, a dual-targeting anti-CD19/CD20 CAR T-cell therapy, for the treatment of patients with relapsed/refractory B-cell lymphomas (NCT04989803).
- In conjunction with multi-antigen targeting, optimizing the CAR T-cell product attributes through next-generation manufacturing platforms provides another opportunity of improving the CAR T-cell products to potentially enhance patient responses.
- The shortening of the ex vivo expansion period yields an increased proportion of juvenile (naïve and early memory) T cells in the final product, which has been linked to enhanced efficacy and improved toxicity profile[1, 2, 3].
- Accordingly, KITE-753 is an autologous rapid manufactured CAR-T cell product transduced with a bicistronic lentiviral vector with resultant expression of an anti-CD19 CAR and anti-CD20 CAR.
- Here we evaluate and compare the in vitro and in vivo functionality of KITE-753 (Rapid) and KITE-363 (Traditional) autologous CAR T-cell products from a common healthy human donor. Both CAR T-cell products express the same anti-CD19 and anti-CD20 CARs using the same bicistronic lentiviral vector (LVV) and differing only in the manufacturing process.

Dual-targeting structure and mode of action

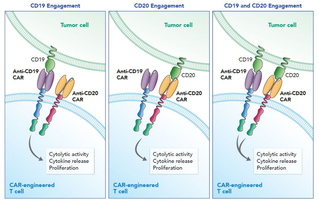

Figure 1. CD19/CD20 dual-targeting with 2 individual CARs. The bicistronic lentiviral vector encodes for an anti-CD19 CAR, containing the FMC63 binder and CD28 costimulatory domain, and anti-CD20 CAR, containing a novel CD20 binder and 41BB costimulatory domain. Transduction of T cells results in expression of both the anti-CD19 CAR and anti-CD20 CAR on the cell surface, allowing recognition and elimination of tumor cells expression CD19 and/or CD20.

KITE-363 demonstrates enhanced expansion without increase in pro-inflammatory cytokines

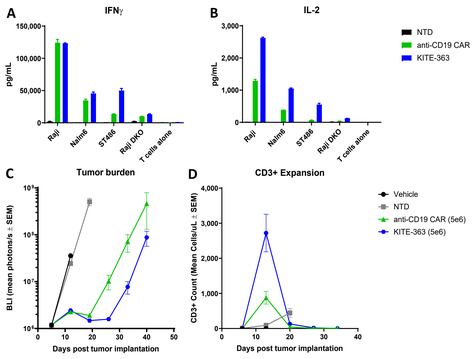

Figure 2. In vitro and in vivo function of KITE-363. (A-B) After 24 hours of co-culture with Raji ($CD19^{hi}CD20^{hi}$), Nalm6 ($CD19^{hi}CD20^{lo}$), ST486 ($CD19^{lo}CD20^{lo}$), and Raji DKO ($CD19^{KO}CD20^{KO}$) (1:1 E:T), **(A)** IFNγ and **(B)** IL-2 production was measured via MSD assay. Cytokine levels and trends between anti-CD19 CAR and KITE-363 varied across different donors (data not shown). **(C-D)** NSG mice received luciferase-expressing Raji tumor cells, intravenously, on day 0, and NTD or CAR T cells on day 5. On Days 5-8, mice were injected IP bid with 36μg of rhIL-2. Tumor burden was monitored with bioluminescence imaging and recorded as photons/second **(C)**. Blood was collected and analyzed for $CD3^+$ cells/μL blood by flow cytometry **(B)**. Abbreviations: NTD, nontransduced; DKO, double knockout, BLI, bioluminescence imaging.

RESULTS

Rapid manufacturing of KITE-753 product preserves juvenile T cells

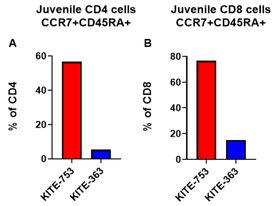

Figure 3. Assessment of phenotype at the end of manufacturing. Both CAR T-cell products were assessed for $CCR7^+/CD45RA^+$ juvenile populations in both **(A)** $CD4^+$ and **(B)** $CD8^+$ T cell compartments at the time of harvest.

KITE-753 exhibits high expression of both CARs

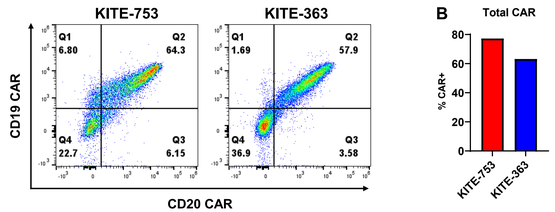

Figure 4. Evaluation of CAR expression at the end of manufacturing. Both CAR T-cell products were analyzed for anti-CD19 CAR and anti-CD20 CAR expression **(A)**. Total CAR expression was measured by the sum of total anti-CD19 CAR and/or anti-CD20 CAR expression **(B)**.

KITE-753 shows comparable cytotoxicity in vitro against various antigen-positive cell lines

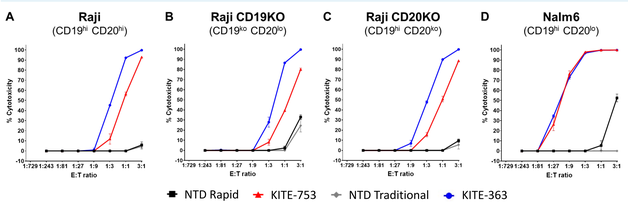

Figure 5. Analysis of antigen-specific cytotoxicity in vitro. CAR-T products and NTD controls were co-cultured with various CD19 and CD20 antigen-positive cell lines [**(A)** Raji ($CD19^{hi}CD20^{hi}$), **(B)** Raji CD19KO ($CD19^{KO}CD20^{hi}$), **(C)** Raji CD20KO ($CD19^{hi}CD20^{KO}$), and **(D)** Nalm6 ($CD19^{hi}CD20^{lo}$)] at various effector to target ratios (E:T). After 4 days, cytotoxicity was measured via luciferin viability read-out. No killing was observed in the K-562 antigen-negative cell line for both CAR-T products (data not shown). Abbreviations: NTD, nontransduced; KO, knockout.

KITE-753 exhibits enhanced proliferation in response to antigen

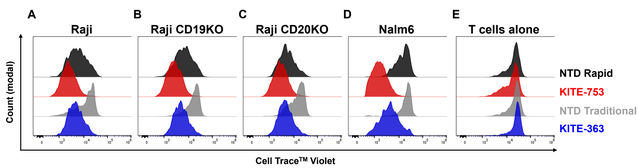

Figure 6. Evaluation of antigen-specific proliferation in vitro. After 4 days of co-culture with **(A)** Raji ($CD19^{hi}CD20^{hi}$), **(B)** Raji CD19KO ($CD19^{KO}CD20^{hi}$), **(C)** Raji CD20KO ($CD19^{hi}CD20^{KO}$), and **(D)** Nalm6 ($CD19^{hi}CD20^{lo}$) (1:1 E:T), proliferation was measured by CTV (Cell Trace™ Violet) dye dilution. T cells were cultured in the absence of target cells to assess background homeostatic proliferation **(E)**. Abbreviations: NTD, nontransduced; KO, knockout.

Enhanced IL-2 production by KITE-753 in response to antigen-positive cell lines

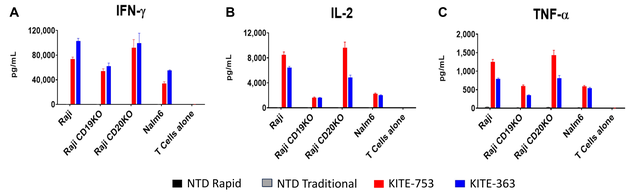

Figure 7. Measurement of antigen-specific cytokine production in vitro. After 24 hours of co-culture with Raji ($CD19^{hi}CD20^{hi}$), Raji CD19KO ($CD19^{KO}CD20^{hi}$), Raji CD20KO ($CD19^{hi}CD20^{KO}$) and Nalm6 ($CD19^{hi}CD20^{lo}$) (1:1 E:T), **(A)** IFN-γ, **(B)** IL-2, and **(C)** TNF-α production was measured via MSD assay. Cytokine levels and trends between KITE-753 and KITE-363 varied across different donors (data not shown). Abbreviations: NTD, nontransduced; KO, knockout.

KITE-753 demonstrates superior antitumor efficacy and increased peripheral CAR-T peak expansion

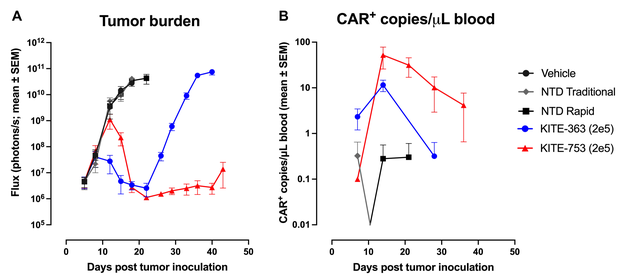

Figure 8. Antitumor efficacy and in vivo expansion in a B-ALL xenograft mouse model. NSG-MHC I/II DKO mice received luciferase-expressing Nalm6 tumor cells, intravenously, on day 0, and NTD or CAR T cells on day 6. Tumor burden was monitored with bioluminescence imaging and recorded as photons/second **(A)**. Blood was collected and analyzed for CAR^+ copies/μL blood by ddPCR **(B)**. An arbitrary value of 0.01 was assigned to allow plotting on logarithmic y-axis when the mean value was 0. Abbreviations: NTD, nontransduced; KO, knockout.

KITE-753 demonstrates enhanced potency when dosed as low as 4e4 CAR^+ cells

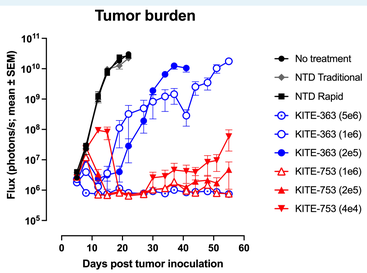

Figure 9. CAR-T potency in a xenograft B-ALL tumor model. NSG mice received luciferase-expressing Nalm6 tumor cells, intravenously, on day 0, and NTD or CAR T cells on day 6. Tumor burden was monitored with bioluminescence imaging and recorded as photons/second. Abbreviations: NTD, nontransduced; KO, knockout.

CONCLUSIONS

- **In comparison to the traditionally manufactured KITE-363, rapid manufacturing of the KITE-753 product enriches for a more juvenile, less differentiated T-cell population, resulting in superior antitumor efficacy and potency with enhanced CAR-T expansion in vivo.**
- **In addition to the potential to prevent and rescue CD19 negative relapses via CD19/CD20 dual-targeting, the improved product potency of the rapid manufactured KITE-753 product may enable the use of lower CAR-T doses in the clinic.**
- **A Phase 1, first-in-human, open-label, multicenter study is currently ongoing to evaluate the safety and efficacy of KITE-753 in patients with relapsed/refractory B-cell lymphomas (NCT04989803).**

METHODS

A total of 4-5 different healthy donors were utilized for all studies but representative data from 1 healthy donor is shown.

Pre-clinical manufacturing:

- Using a bicistronic lentiviral vector and healthy human donor T cells, rapid manufactured anti-CD19/CD20 CAR-T cells (KITE-753) was generated.
- In parallel, anti-CD19/CD20 CAR-T cells were manufactured with a traditional process (KITE-363) using the same bicistronic lentiviral vector and healthy donor T cells and served as the benchmark comparator.
- Rapid and traditional T cells that were not transduced and do not express a CAR (NTD Traditional and NTD Rapid) were also manufactured in parallel and served as controls.
- Phenotype and transduction efficiency of CAR T-cell products were analyzed by flow cytometry on the day of harvest.

In vitro functional characterization:

- T cell products were co-cultured with various cell lines, including Raji ($CD19^{hi}$ $CD20^{hi}$), Raji CD19KO ($CD19^{KO}$ $CD20^{hi}$), Raji CD20KO ($CD19^{hi}$ $CD20^{KO}$), and Nalm6 ($CD19^{hi}$ $CD20^{lo}$).
- Functional read-outs included cytotoxic activity (luciferin) on days 1 and 4, cytokine production (MSD assay) on day 1 and T cell proliferation (Cell Trace™ Violet dye dilution by flow cytometry) on days 4.

In vivo efficacy models:

- Nalm6 B-ALL model: NSG or NSG-MHC I/II DKO female mice (The Jackson Laboratory) received luciferase-expressing Nalm6 tumor cells, intravenously (IV), on Day 0. Tumor burden was monitored with bioluminescence imaging and recorded as photons/second. On Day 6, after randomization on tumor burden, mice were injected IV with NTD or CAR T cells. Blood was collected 24 hours post T-cell infusion and weekly thereafter to analyze CAR^+ copies/μL blood by ddPCR.
- Raji Burkitt's lymphoma model: NSG female mice (The Jackson Laboratory) received luciferase-expressing Raji tumor cells, intravenously (IV), on Day 0. Tumor burden was monitored with bioluminescence imaging and recorded as photons/second. On Day 5, after randomization on tumor burden, mice were injected IV with NTD or CAR T cells. On Days 5-8, mice were injected IP bid with 36μg of rhIL-2. Blood was collected 24 hours post T-cell infusion and weekly thereafter to analyze $CD3^+$ cells/μL blood by flow cytometry.

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ACKNOWLEDGEMENTS:

We would like to thank the following people for their guidance, support, and contributions:

- Stacey Valny and Alessandro Calo, members of the Kite Research Automation Core.

FUNDING:

Funded by Kite, a Gilead company

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