Corning® KBM581: Nutritious, Serum-free Media for CAR-T Cell Expansion

Application Note

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Introduction

T cells that express a tumor-associated chimeric antigen receptor (CAR) show potential as an alternative adoptive cell therapy treatment for tumor patients and patients with blood cancers[1]. When considering an adoptive cell therapy approach, the transfusion of a sufficient number of CAR-T cells with an appropriate phenotype is critical[2]. CAR-T cell expansion requires anti-CD3/CD28 microbeads combined with IL-2, as well as the use of a nutritious, serum-free medium to ensure the safety and efficacy of CAR-T cell therapy.

Corning KBM581 has been shown to be a nutritious, safe, and serum-free medium for other immune cell therapies, such as CIK and NK cells. With this track record, KBM581 has the potential to support lymphocytes expand to a high fold rate with an optimized phenotype.

To discover which medium among several commercialized media (including KBM581) is most appropriate for supporting CAR-T cell growth, the research group from Bioraid Bio generated CAR-T cells and analyzed the expansion folds and phenotype between different media. The study showed that KBM581 could efficiently expand CAR-T cells to a fold rate that was sufficiently high to transfuse B-ALL (acute lymphoid leukemia of B cells) patients for clinical trial. It also showed a high proportion of CD3+CD8+ T cell subsets (>80%), which indicated the cytotoxicity of the cultured cells.

Methods

CD3+ T Cell Isolation Day 1

Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation with a Corning lymphocyte separation medium (Corning Cat. No. 25-072-CI). CD3+ T cells were separated according to the datasheet supplied with the T cell isolation kit (Miltenyi Biotec).

Activation

CD3+ T cells were activated with anti-CD3/CD28 microbeads (Life Tech). IL-2 was added at a concentration of 1,000 IU/mL.

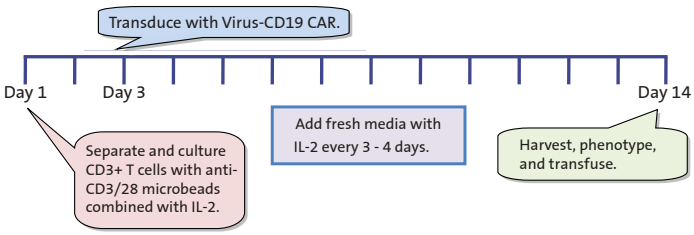
Virus Transduction

Lentivirus-CD19 CAR was transducted into CD3+ T cells at Day 3.

Cell Expansion

Cells were expanded for 11 to 14 days, fresh media with IL-2 were added every 3 or 4 days, and cells were diluted at 10^6/mL. Cells were then harvested between Day 13 and Day 14.

Timeline



Results

Researchers first compared the expansion folds of CAR-T cells in several commercialized media that had been activated with CD3/CD8 microbeads. PBMCs were isolated from the peripheral blood of patients who had previously received regular chemotherapy treatment. In a complete, serum-free culture system, KBM581 was determined to be the best medium for supporting cell expansion during the first two weeks of cell cultivation (Figure 1A). Cell viability was also determined to be highest in KBM581 (data not shown).

Cell subsets were then detected and measured at Day 14 (Figure 1B). The purity of T cells was high across all groups (>95%). The proportion of CD8+ T cells was over 80% in KBM581, Competitor G, and Competitor L media, while the proportion of CD4+ T helper cells was low.

Cell growth in KBM581 and Competitor G media was then compared against the starting material of normal B-ALL patients. After 11 days in culture, cells grown in KBM581 expanded to 42.8 fold, while cells grown in Competitor G expanded to 40.5 fold (Figure 2). The phenotype of the cell subsets were equivalent between the two media (data not shown). Researchers also tested and compared results in 10 other patients and healthy donors. No statistical difference was found between the two media in terms of cell expansion folds and phenotype.

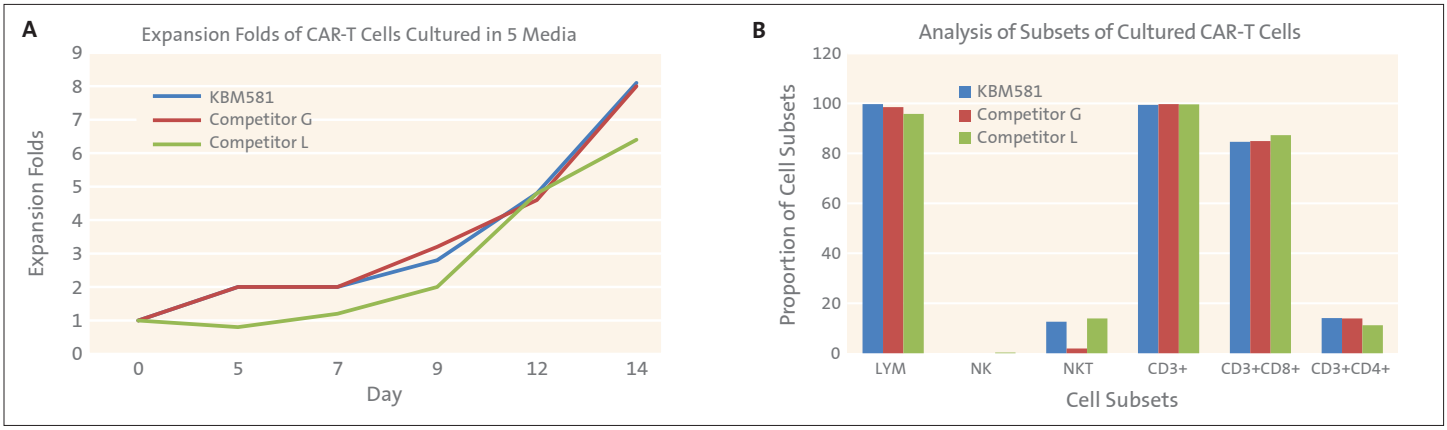


Figure 1. KBM581 supported the highest expansion fold rate, and over 80% CTL proportion, of CAR-T cells. PBMCs were isolated from B-ALL patients who had received chemotherapy and were then stimulated with CD3/CD28 microbeads. Cells expanded 8-fold in KBM581 and Competitor G media (Figure 1A). The proportion of CD45+ WBC, CD3+ T lymphocytes, CD3-CD56+ NK cells, CD3+CD56+ NKT cells, CD3+CD8+ CTL, and CD3+CD4+ T helpers was detected with a flow cytometer (Figure 1B). The end products of all groups were shown to have a very high purity of T cells, and the proportion of CTL (over 80%) was similar in KBM581, Competitor G, and Competitor L.

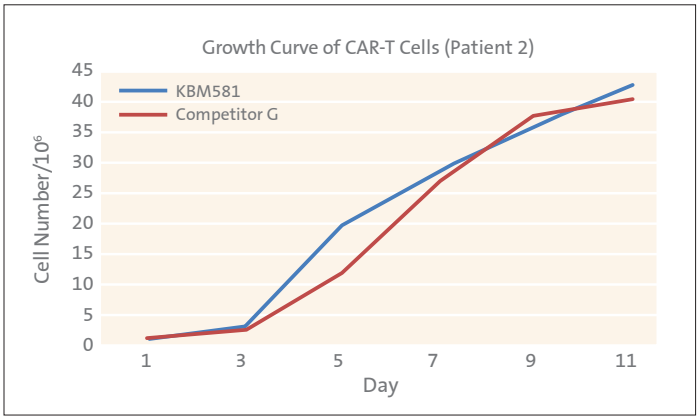


Figure 2. KBM581 achieved a higher cell growth number and higher expansion fold, than Competitor G after 11 days of cell culturing. PBMCs were isolated from a B-ALL patient who had not received chemotherapy before. The growth curve indicates a prolonged exponential growth phase in KBM581.

Conclusions

A sufficient number of CAR-T cells can be generated in a KBM581 serum-free culture system in combination with CD3/CD28 microbeads. Furthermore, the performance of this medium in a CAR-T cell culture medium is shown to be greater than in other media, indicating that KBM581 may be a better alternative tool for researchers conducting T cell therapy.

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References

1. Michael Kalos, et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci. Transl. Med. 3, 95ra73 (2011).
2. Yixin Li and Roger J Kurlander. Comparison of anti-CD3 and anti-CD28-coated beads with soluble anti-CD3 for expanding human T cells: Differing impact on CD8 T cell phenotype and responsiveness to restimulation. J. Transl. Med. 8:104 (2010).

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