Empowering T cell therapies: the strategic use of LentiBOOST in CAR T and TCR modalities

The field of immunotherapy is rich with strategies aimed at leveraging the natural capacity of the immune system to fight disease. From cytokine-based interventions to antibody treatments and adoptive cell therapy (ACT), each offers considerable promise for future clinical applications. Among these, ACT modalities, such as chimeric antigen receptor (CAR) T, tumor-infiltrating lymphocytes, and engineered T cell receptor (TCR)-based therapies, stand out for their ability to exploit T cells and other immune elements to target disease.

A crucial step in the development of ACTs is the genetic modification of immune cells. One widely adopted method involves the use of lentiviral vectors, which are known for their efficiency in integrating therapeutic genes into target cell genomes. These vectors have several advantageous traits, including a relatively large cargo capacity and the ability to transduce both dividing and non-dividing cells. The FDA-approved therapy Kymriah® (tisagenlecleucel) is an example of this technique's success, relying on a lentiviral vector to transduce the therapeutic CAR into T lymphocytes.[1]

Boosting lentiviral transduction efficiency

While lentivirus-mediated gene transfer has shown great promise for the development of ACTs, researchers are seeking innovative ways to optimize the efficiency of lentiviral transduction. High transduction efficiency is crucial for the success of ACTs as it directly impacts the effectiveness, scalability, cost, and safety of these therapies. One strategy to improve transduction efficiency involves the use of lentiviral transduction enhancers, which facilitate viral entry into target cells, thereby increasing the likelihood of successful gene delivery and expression.

Revvity's LentiBOOST™ technology is a lentiviral transduction enhancer designed to improve lentiviral transduction, particularly in difficult-to-transduce human and other mammalian cells. LentiBOOST acts as a universal adjuvant, facilitating fusion of lentivirus with the cell membrane, increasing vector copy number, and improving transduction efficiency without the need for specific receptors (Figure 1). This attribute makes it compatible with a wide range of cell types, including CD34+ hematopoietic stem cells (HSCs),[2] primary T cells,[3] and NK cells.

The adoption of LentiBOOST has been growing in recent years among both academic and industrial organizations. Initially, its primary applications were with CD34+ HSCs, where it has been successfully integrated into clinical trials to help improve transduction protocols. More recently, there has been growing interest and clinical application of LentiBOOST in T cell research to overcome inherent transduction challenges. As the field continues to evolve, next-generation CAR T approaches are being developed, including those with more advanced receptor constructs, e.g., additional costimulatory domains, safety switches, and cytokine expression, or methods focusing

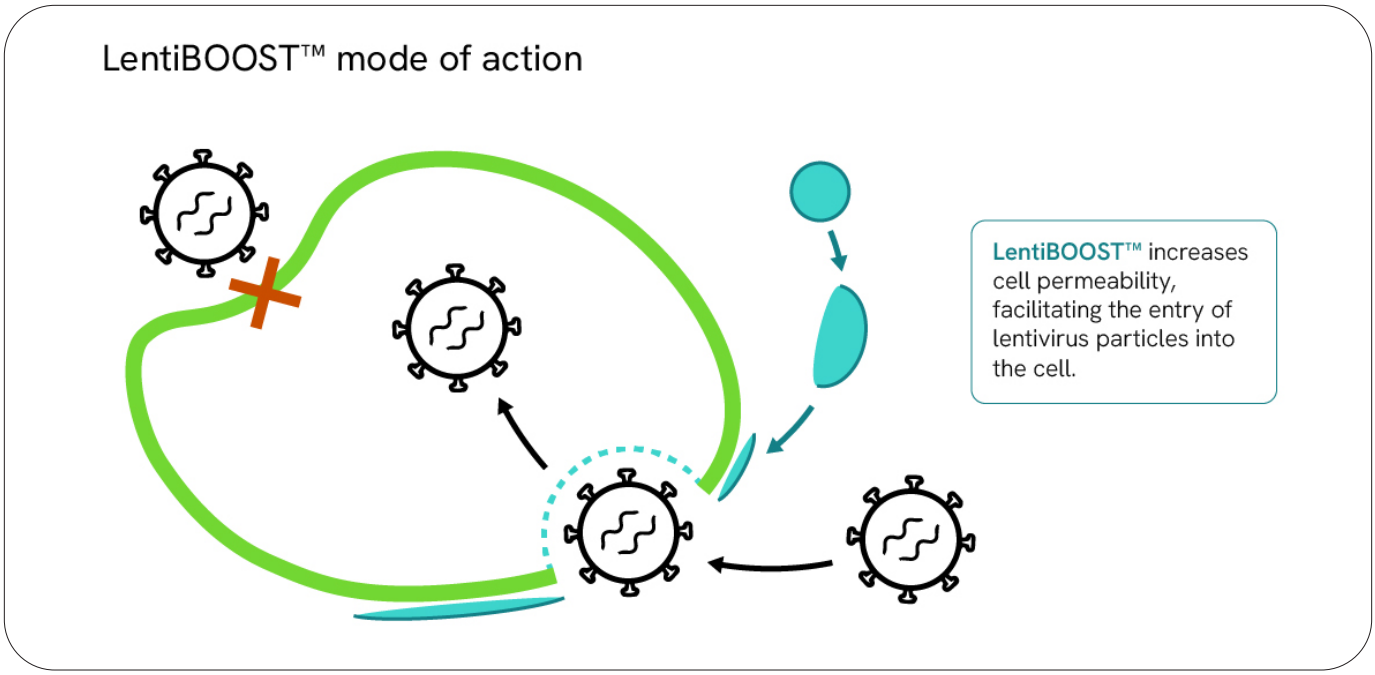

Figure 1: LentiBOOST mode of action: LentiBOOST acts as a universal receptor-independent adjuvant which facilitates fusion of lentivirus with the cell membrane, increases vector copy number, and improves lentivirus transduction efficiency.

on different cell types such as regulatory T cells and NK cells. These approaches are being honed for a broad range of indications, spanning oncology to autoimmune diseases.[4] This article aims to explore several applications of LentiBOOST, highlighting the success of the technology in advancing T cell research.

Applications of LentiBOOST transduction enhancer

Optimizing allogeneic CAR T-cell production

Autologous CAR T-cells targeting specific antigens expressed on tumor cells have shown efficacy against several types of blood cancers. However, factors such as prior chemotherapy treatments, the cancer environment within the host, and the burden of the cancer itself can compromise the quality of autologous T cells harvested from patients. These issues can significantly affect the final CAR T-cell product. To overcome these limitations, the focus is shifting to alternative approaches that leverage allogeneic T cells derived from healthy donors.

Led by Young-In Kim-Hoehamer, a research team based at St. Jude Children's Research Hospital in Tennessee has developed a cGMP-compliant method for manufacturing allogeneic CD19-CAR T-cells from memory T cells.[3]

Central to this study was the optimization of lentiviral transduction efficiency using LentiBOOST. Memory T cells were activated and then transduced with clinical-grade lentiviral vectors encoding a CD19-CAR. The team tested various LentiBOOST concentrations (0.5, 1, and 2 mg/mL) to determine the optimal concentration in combination with several multiplicities of infection (MOI) levels of the vector. The research revealed that LentiBOOST significantly improved CD19-CAR expression and vector copy number (VCN) at an MOI of 5 or 10 (Figure 2) without affecting T cell phenotype, expansion, or functionality. Furthermore, higher LentiBOOST concentrations led to a marked increase in VCN and transduction efficiency, with no impact on viability (Figure 3).

The optimal LentiBOOST concentration in their setting was determined to be 1 mg/mL, with the resulting CD19-CAR(Mem) T cells demonstrating a potent ability to recognize and kill CD19+ target cells *in vitro*. They also showed significant antitumor activity in an acute lymphoblastic leukemia (ALL) xenograft model. These promising findings and optimized manufacturing processes laid the groundwork for an ongoing clinical trial (MEMCAR19, NCT04881240) evaluating the safety and efficacy of these engineered T cells in targeting relapsed or refractory leukemia.[5] They also underscore

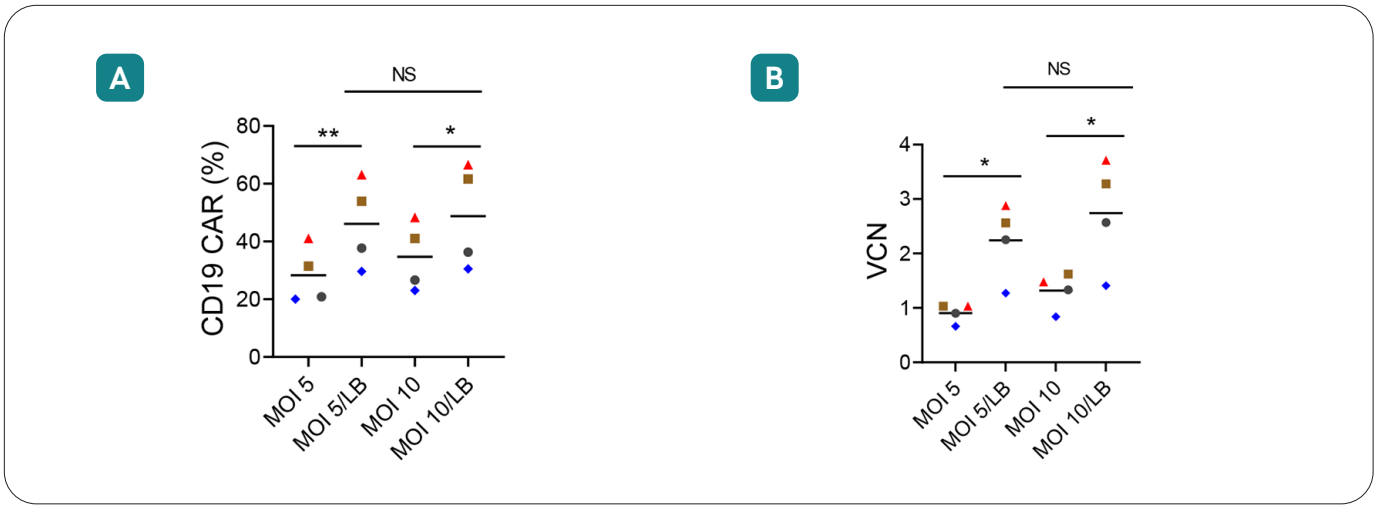

Figure 2: Optimization of transduction of memory T cells with a clinical-grade lentiviral CD19-CAR vector. (A) CAR expression based on flow cytometry (n=4 donors; mean values depicted as bars). (B) Vector copy number (VCN; n=4 donors; mean values depicted as bars). Image credit: Kim-Hoehamer Y-I, Riberdy JM, Zheng F, Park JJ, Shang N, Métais J-Y, et al. Development of a cGMP-compliant process to manufacture donor-derived, CD45RA-depleted memory CD19-CAR T-cells. Gene Therapy. 2022 Jan 8;30(3-4):222-31. Used under a Creative Commons Attribution 4.0 International License.

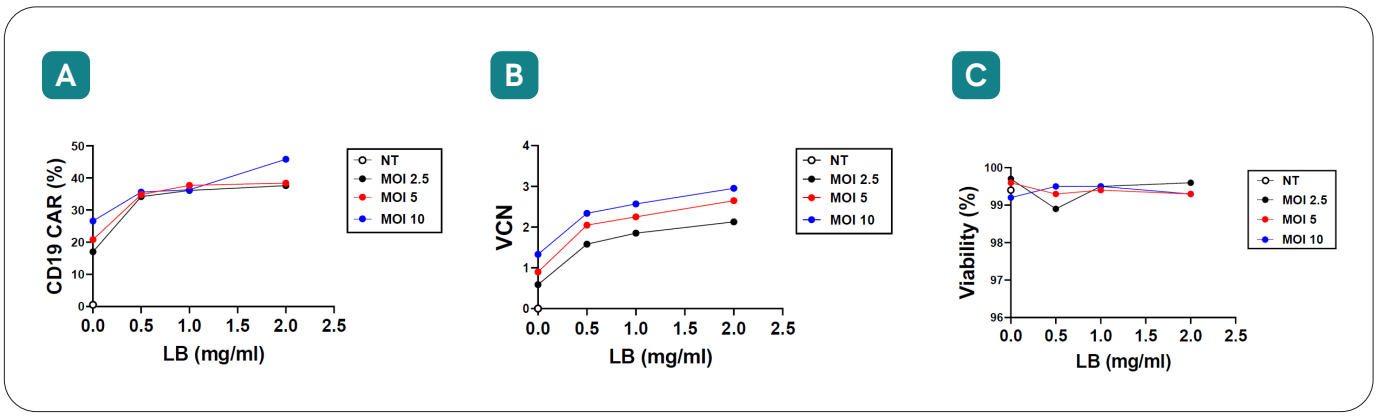

Figure 3: LentiBOOST enhances lentiviral transduction of memory T cells. Apheresis mononuclear cells are transduced with CD19-CAR lentiviral vector at different MOI in the presence of various LentiBOOST concentrations (0, 0.5, 1, and 2 mg/mL) for 18-24 hr. After transduction, the cells were transferred into G-Rex for expansion. The cells were harvested on day 7. (A) CD19-CAR expression by flow cytometry, (B) VCN, (C) Viability. Image credit: Kim-Hoehamer Y-I, Riberdy JM, Zheng F, Park JJ, Shang N, Métais J-Y, et al. Development of a cGMP-compliant process to manufacture donor-derived, CD45RA-depleted memory CD19-CAR T-cells. Gene Therapy. 2022 Jan 8;30(3-4):222-31. Used under a Creative Commons Attribution 4.0 International License.

the value of LentiBOOST in optimizing the production of allogeneic CAR T-cells.

Optimizing CAR T-cells targeting ovarian cancer

The use of LentiBOOST has also shown promising results in the manufacturing of CAR T-cells targeting ovarian cancer, particularly against the MUC16 antigen. In a study led by Jing Guo, MSLN-CAR T-cells produced with the aid of LentiBOOST demonstrated potent cytotoxicity against MUC16-positive ovarian cancer cells (OVCAR3) and their stem-like cells *in vitro*.[6] Moreover, *in vivo* studies using mouse models showed that both systemic and local

administration of MSLN-CAR T-cells led to a significant reduction in OVCAR3 xenograft tumors.

Overall, the study highlights the potential of LentiBOOST-enhanced CAR T-cell therapies in targeting and eliminating MUC16-positive ovarian cancer cells. The ability of the CAR T-cells to shrink tumors in mice also illustrates the potential clinical relevance of these findings for future investigation.

Improving CAR-Treg transduction

The effectiveness of LentiBOOST extends beyond specific types of T cells or genetic modifications, proving valuable across a variety of T cell subsets. This includes regulatory T cells (Tregs), which can be less amenable to transduction than other T cell types. A study conducted by Baptiste Lamarthée and colleagues evaluated the effects of CAR tonic signaling on CAR-Treg phenotype and function.[7] The team utilized LentiBOOST to enhance lentiviral transduction and the expression of CAR constructs on Treg surfaces. Tonic signaling refers to the activation of a CAR signaling pathway even in the absence of target antigen engagement. This persistent activation can lead to CAR T-cell dysfunction.

The researchers compared the impact of different CAR designs on Tregs, with a particular focus on the effects of 4-1BB costimulatory domain (CSD)-associated tonic signaling compared to CD28 CSD. Adding 0.25 mg/mL of LentiBOOST during transduction resulted in robust expression of the CAR constructs on Treg surfaces, facilitating further investigation of tonic signaling in CAR-Tregs. Their findings revealed that 4-1BB tonic signaling adversely affects CAR-Treg biology, compromising their suppressive function. However, this adverse effect was counteracted by transient mTOR inhibition, which rescued the Tregs' suppressive functions. By enabling efficient transduction and expression of advanced CAR constructs, this study demonstrates the critical role of LentiBOOST, particularly in challenging T cell subsets like Tregs.

Dual-targeting CAR T-cell manufacturing

LentiBOOST has also been used in the production of dual-targeting CAR T-cells, as demonstrated in a study aimed at enhancing the persistence and efficacy of BCMA CAR T-cells in multiple myeloma.[8] By successfully co-expressing a BCMA CAR with a CD19 CAR, UCL researchers, led by Lydia Sarah Hui Lee, are now evaluating the therapeutic outcomes of the resulting CAR T-cells in the MCARTY trial, a Phase 1 dose-escalation study evaluating their safety and efficacy in patients with relapsed/refractory multiple myeloma.[9]

The initial manufacturing process involved the double transduction of autologous CD4/CD8-selected T cells with lentiviral vectors, utilizing LentiBOOST to enhance the transduction process. This method successfully produced three distinct populations of CAR T-cells for further investigation. Early results from the study are promising, indicating well-tolerated doses, robust expansion, and positive clinical responses.[8]

Additionally, scientists from Cellectis recently published preclinical data on the use of LentiBOOST with an allogeneic dual CD20/CD22 CAR.[10] The results demonstrated that these dual-targeting CAR T-cells exhibited robust, sustained, and dose-dependent activity both *in vitro* and *in vivo*, effectively targeting primary B-cell non-Hodgkin lymphoma samples with heterogeneous levels of CD20 and CD22. This research supports the development of dual-targeting CAR T-cell therapies to overcome current resistance challenges in the treatment of relapsed/refractory B-cell lymphoma.

Together, these findings highlight LentiBOOST's potential in developing CAR T-cells engineered to express multiple CAR constructs. The use of LentiBOOST in both autologous and allogeneic settings also showcases its versatility and value in advancing CAR T-cell therapies across various applications.

Improving TCR gene delivery and function in cord blood-derived T cells

An alternative gene-modification strategy for ACT is to generate TCR-engineered T cells. Compared to CAR T-cells, which target surface antigens through an engineered synthetic receptor, TCR-engineered T cells are generated by introducing a transgenic tumor-associated antigen (TAA)-specific TCR. This TCR recognizes intracellular antigens presented as peptides on the cell surface by major histocompatibility complex (MHC) molecules. In a study led by Vania Lo Presti, UMC Utrecht, researchers investigated whether LentiBOOST could improve gene delivery into umbilical cord blood (CB) CD8+ T cells.[11] These cells are of particular interest for allogeneic T cell research due to their favorable characteristics compared to peripheral blood T cells.

As shown in Figures 4A and 4B, the addition of LentiBOOST significantly increased the proportion of successfully transduced T cells. This was even evident at a reduced MOI, where a significant increase in green fluorescent protein (GFP)-positive T cells was observed. Lentiviral VCN analysis confirmed these findings, demonstrating an increase in VCN at both MOI of 10 and 1 with the application of LentiBOOST (Figure 4C).

Further experiments involved transducing CB CD8+ T cells with a TAA-specific TCR in the presence of LentiBOOST. The results, as illustrated in Figures 5A and 5B, showed a significant increase in the efficiency of TCR transduction without compromising the functionality of the engineered T cells. Notably, the LentiBOOST-treated cells exhibited increased cytotoxicity, as shown in Figure 5C, and secreted higher levels of cytotoxic cytokines compared to their untreated counterparts. These findings support the use of LentiBOOST for improving gene delivery, while also maintaining the cellular functions of the T cells and boosting their ability to target and destroy cancer cells.

Conclusion

LentiBOOST has emerged as a valuable tool for enhancing lentiviral transduction efficiency, making it a useful asset in the development of advanced T cell therapies across various applications. Multiple studies have demonstrated its ability to enhance lentiviral transduction, including optimizing both allogeneic and autologous CAR T-cell production and improving gene delivery in cord blood-derived T cells. The research presented here demonstrates LentiBOOST's versatility and compatibility with various cell types, offering a means to overcome transduction challenges. The high transduction efficiency facilitated by LentiBOOST is crucial for the success of ACTs as it directly impacts their effectiveness, scalability, cost, and safety. Furthermore, its successful integration into multiple clinical trials underscores its value in the manufacturing of potential T cell-based therapies for a wide range of cell types and diseases.

Revvity's LentiBOOST technology is well-established in the market and readily available as a research and GMP-grade product, supporting users from development up to commercial stages of cell therapy programs. This positions LentiBOOST as an accessible solution for researchers that can be utilized throughout cell therapy pipelines. Additionally, Revvity provides support for academic developers of cell therapies through a special royalty-free license, encouraging innovation and collaboration in this field.

References

1. FDA approval brings first gene therapy to the United States [Internet]. FDA; [cited 2024 May 22]. Available from: https://www.fda.gov/news-events/press-announcements/fda-approval-brings-first-gene-therapy-united-states

2. Hauber I, Beschorner N, Schrödel S, Chemnitz J, Kröger N, Hauber J, et al. Improving lentiviral transduction of CD34+ hematopoietic stem and progenitor cells. Human Gene Therapy Methods. 2018 Apr;29(2):104–13. doi:10.1089/hgtb.2017.085

3. Kim-Hoehamer Y-I, Riberdy JM, Zheng F, Park JJ, Shang N, Métais J-Y, et al. Development of a cGMP-compliant process to manufacture donor-derived, CD45RA-depleted memory CD19-CAR T-cells. Gene Therapy. 2022 Jan 8;30(3–4):222–31. doi:10.1038/s41434-021-00307-0

4. Tomasik J, Jasiński M, Basak GW. Next generations of CAR-T cells - new therapeutic opportunities in hematology? Front Immunol. 2022 Oct 28;13:1034707. doi: 10.3389/fimmu.2022.1034707

5. Study of CD19-directed allogeneic memory T cell therapy for relapsed/refractory CD19+ leukemia [Internet]. [cited 2024 May 22]. Available from: https://classic.clinicaltrials.gov/ct2/show/NCT04881240

6. Guo J, Zeng X, Zhu Y, Yang D, Zhao X. Mesothelin-based CAR-T cells exhibit potent antitumor activity against ovarian cancer. Journal of Translational Medicine. 2024 Apr 18;22(1). doi:10.1186/s12967-024-05174-y

7. Lamarthée B, Marchal A, Charbonnier S, Blein T, Leon J, Martin E, et al. Transient mTOR inhibition rescues 4-1BB CAR-Tregs from tonic signal-induced dysfunction. Nature Communications. 2021 Nov 8;12(1). doi:10.1038/s41467-021-26844-1

8. Lee LS, Lim WC, Mactier C, Dias Alves Pinto J, Pereira V, Vaughan M, et al. Development of a phase 1 study evaluating the activity of modular CAR T for multiple myeloma (MCARTY) targeting BCMA and CD19 for improved persistence. Blood. 2023 Nov 2;142(Supplement 1):350–350. doi:10.1182/blood-2023-185085

9. A new study evaluating the activity of Modular CAR T for Myeloma [Internet]. [cited 2024 May 22]. Available from: https://classic.clinicaltrials.gov/ct2/show/NCT04795882

10. Aranda-Orgilles B, Chion-Sotinel I, Skinner J, Grudman S, Mumford B, Dixon C, et al. Preclinical evidence of an allogeneic dual CD20XCD22 CAR to target a broad spectrum of patients with B-cell malignancies. Cancer Immunology Research. 2023 May 31;11(7):946–61. doi:10.1158/2326-6066.cir-22-0910

11. Lo Presti V, Cornel AM, Plantinga M, Dünnebach E, Kuball J, Boelens JJ, et al. Efficient lentiviral transduction method to gene modify cord blood CD8+ T cells for cancer therapy applications. Molecular Therapy - Methods & Clinical Development. 2021 Jun;21:357–68. doi:10.1016/j.omtm.2021.03.015