CD19 And CD22 Dual-Target CAR-T Therapy Via CRISPR/Cas9 Engineering for Relapsed B-Cell Acute Lymphoblastic Leukemia

Aiqi Chen *

College of Life and Environmental Sciences, Minzu University of China, Beijing, China

* Corresponding Author Email: 23011928@muc.edu.cn

Abstract. Relapsed/refractory(B-cell)acute lymphoblastic leukemia (R/R (B-ALL) remains the most recalcitrant hematologic malignancy owing to clonal divergence and therapy resistance, and also due to antigen escape, which is a very intransigent challenge. Though it was successful for many in achieving remission, CD19-directed CAR-T cell therapy can relapse due to loss or modulation of the antigen. Dual-targeted CAR-T targeting CD19 and CD22 is now an option to avoid escape and expand the tumor area that is covered. At the same time, CRISPR / Cas9 gene modification can give us another way to make CAR-T safer and get better results, as well as to raise its duration and scale. This paper includes up-to-date information about childhood B-ALL epidemiology and treatment, information about recent progress of CAR-T, and problems associated with the single antigen approach. It investigated the design principles for CD19/CD22 dual-target CARs, contrasted conventional gene transfer methods against the CRISPR variety, then provided a rundown of current-day-to-day research. Lastly, there are the strengths, limitations, and future directions of the combination of the double-target strategy and CRISPR in R-ALL. And these approaches take us on a map forward to a long-term, right, and achievable immunotherapeutic approach that a lot more people can have a whole bunch of things that they don't have a lot of cures for.

Keywords: CAR-T, CD19, CD22, CRISPR/Cas9, B-cell Acute Lymphoblastic Leukemia.

1. Introduction

B-cell ALL, also referred to as a malignant tumor formed by mature B-lymphocytes when it is first born. It occurs more commonly in children, but also in adolescents and adults, and it is the most common childhood cancer. While there are great advances in therapy in the last couple of decades intensive combination chemotherapy protocols and hematopoietic stem cell transplantation, not everything is completely resolved because some will either relapse or be predominantly treatment resistant. And in those cases, it looks bleak for us humans to survive. Current data show that even in pediatric patients, cure rates might still be as high as 80-90%, but relapse/refractory disease appears to account for nearly every failure at less than 30% survival.

CAR-T has changed the way we handle B – cell – related cancers. To change the genetic material passed down from the parent cells on the inside of the patient, the new patient-derived T-lymphocytes will have an entirely new synthetic receptor that will bind only to proteins found on tumor cells, allowing the power of CAR-T therapy to happen without requiring MHC. The most commonly used target for B-ALL is CD19, which is an all B-Cell marker expressed by all developmental stages of B-cells. Regarding Anti-CD19 CAR-T, its efficacy is good for relapsed/refractory B-ALL. Over 70 - 80% at the beginning, as early as trials [1], but it has limited long-term remission due to antigen escape. Relapsing from leukemia clones that can no longer express or downregulate CD19 is another mechanism. Thus, it inactivates the CD19-targeted CAR T cells. This is likewise one of the more thoroughly recorded escape techniques, having been seen in many relapses.

For such of problem, scientists will develop CAR–T product which can recognize different tumors at the same time, among which, there is the most clinical attention on dual targeting of CD19 and CD22. CD22 is another B-cell surface protein that is often retained on CD19-negative relapse disease as well [2]. Dual-target CD19 and CD22 CAR-T cells will cover more with lower chances of

escape. And maybe longer-lasting remissions [3]. Some of these trials have shown some promise with bispecific, or tandem, targeting 2 molecules [4].

While innovations in CAR arise, at the same time, the technologies of gene editing are changing the engineering ways of cellular therapies. It's traditionally retrovirally or lentivirally; it sort of just integrates in there. When it works, it's kind of dangerous for introducing a mutation; it also expresses differently, and we do not know where it attaches to the DNA. CRISPR/Cas9 has a solution. Scientists can tell the Cas9 nuclease where to go in the genome, precisely put the CAR transgenes in certain spots, so they would all turn on the same way and have a low risk [5]. We can also use CRISPR for some multiplex editing by knocking out endogenous TCRs or immune checkpoint receptors to have universal "off the shelf" CAR-T products.

Here, I'm looking at whether dual goal CAR-T therapy mixed in with CRISPR/Cas9 is shaping up when it comes to B-ALL relapse. First, talk about CAR-Ttech biology, compared to traditional/CRISPR tech, then the reason for CD19/CD22 and some recent progresses, also talk about possible improvements, some current obstacles, and future directions for combined strategies to help with one of the hardest problems with both child and adult blood cell cancers.

2. CAR-T Technology Basics and CAR-T Cell Structure and Signaling Mechanisms

CAR-T consists of extracellular parts, which are usually single-chain fragment variable(scFv), associated with the antigen and transmembrane part, and intracellular signaling parts. First gen had CD3z, and it wasn't that persistent. Second and third gen CARs added costimulatory domains such as CD28 or 4 - 1BB for better proliferation, survival, and cytokine production. Generatio CAR known as Trucks are in the 4th generation of CARs, which contain cytokines or inducible elements, so that the form is more potent. When the cells bind to an antigen, it will activate a signal pathway, causing NFAT activation, NF-kB, MAPK pathway that leads to toxicity. Make the dual-target CAR better.

In relapsed B-ALL, antigen density/distribution affects CAR-T activity. Too much is full activation, and too little means no action. Thus, we need to design CARs with the right binding affinity, not too high and not too low; otherwise, it may cause off-target toxicity and lack of efficiency.[6] Dual-target also needs to do this for CD19 and also have CD22 [7].

Traditional viral methods are random genomic insertions, so I can't know if they will change how something functions or damage your body. On the other hand, CRISPR/Cas 9 would knock in at a nice, safe harbor but still have CAR on. CRISPR might allow for more than one knock-in. And knockout PD-1 or CTLA-4 to counteract exhausted T cells; knockout HLA to make allogeneic CAR-T cells. Targeting just CD19 works but is prone to antigen escape, with CD22 still often expressed on many CD19 relapses. Dual targeting cuts the chance of it coming back, it can make it more spread out, and it might help it last longer.

CRISPR enables several innovations: (1) insertion of CARs into the TRAC locus to replace endogenous TCR signaling, yielding uniform CAR expression and reduced GvHD in allogeneic use; (2) multiplex knockouts of inhibitory receptors like PD-1, TIM-3, or Fas, which prolong T cell persistence; (3) deletion of HLA class I/II molecules to reduce host rejection. Preclinical studies and early-phase trials demonstrate feasibility, with low rates of chromosomal translocations. One trial reported universal CD19/CD22 CAR-T cells generated with CRISPR achieved >80% remission in r/r B-ALL.

For building dual-targeted CAR-T, these would be: (a) co-transduction with two vectors expressing 2 different CARs, which will result in heterogeneous CAR expression, (b) Bicistronic vectors where both CARs are expressed from the same promoter, ensuring uniform co-expression, (c) Tandem CARs that have two scFVs linked as a single molecule to improve synapse formation, may face steric hindrance, (d) Logic-gated CARs that require both CD19 AND CD22 to induce activation (AND-gates). A clinical trial with a bispecific construct showed a good response rate, but it also points to more antigen density recognition and less fratricide needed.

The combination of CD19/CD22 dual-target CAR-T and CRISPR / Cas9 gene editing has distinct superiority in terms of efficacy, safety, and manufacturing. (a) It can reduce the recurrence caused by escaping. CD19 and CD22, CAR-T cells will remain active even if they lose one of the antigens or downregulate one antigen. So, it covers a wide range of tumors in heterogeneous B-ALL. b) It also extends its functional life. Dual engagement brings on-going excitement without getting tired quickly. Combined with CRISPR knockout of inhibitory receptors such as PD-1 or LAG-3, T cell survival and activity would be retained. (C) CRISPR, the exact, safe genetic editing. Different from viruses that randomly integrate, CRISPR enables better targeted knock-ins, e.g., to the TRAC locus, and stable expression, thus mitigating the risk of insertional mutagenesis. (d) It can better create "off-the-shelf" CAR-T cells. And after removing the TCR and HL gene from our body by CRISPR, we can now produce the allogeneic CAR-T product now and also reduce cost and production time(e). It's both modular and safe. CRISPR could be used as an incorporation of a safety switch as well as logic-gated control circuitry to help with clinical management of cytokine-related toxicity, as well as future design choices. However, several obstacles remain. When designing for dual targets, perhaps this causes interference of signaling/signaling receptor balance, CD22 expression is variable, with low expression associated with relapse. In CRISPR editing, there is a problem of off-target effects and genome stability; the safety must be validated strictly. And make. The manufacturing process is also complex and expensive. The size and follow-up time of the data are limited [8].

Existing outcomes bring about thoughts on therapy, as well as it having been said that there is an obstacle due to antigen modifications. Based on the data from our clinic, we have found that the bispecific CD19 / CD22 CAR is giving very high rates of remission with safety. Tandem CARs did have some remission, but it is hard to hold onto those remissions when the disease is not CD22 positive, so they had a phase 1 trial, which showed [9]: In preclinical studies, it shows that CRISPR universal CARs are potent and scalable. Then there are novel constructs, such as CAR–T cells that express Bispecific Engagers.

r/r B - ALL - dual - target CAR-T is a big help, especially with CRISPR. It might work for people who had a relapse after CD19 - CAR.T - treatment. And universal products could become more accessible than an autologous production, cutting down on cost as well as treatment time. As for the future direction of work, we can try multiple combinations of antigens, add on-off switches, and also combine with checkpoint blockades.

3. Discussion

The combined approach of dual-target CAR-T and CRISPR/CAS9 is one of the most promising front-line cell-based immunotherapies. Dual-targeting is for antigen escape, the #1 reason why CD19-directed CAR-T has relapse. Adding it in CD22 makes it less probable that a leukemic clone would escape from us, and increases the odds of treating everything. There is some clinical evidence that this is safe and possible: There were a few early trials when the full remission rates would get super high.

CRISPR/Cas9 has added this with an exact editing toolbox. Against viral transduction, we could do CRISPR for insertions, multiple knockouts, and universal products. It is both more effective and more logistically convenient as a form of Allogeneic CAR-T. Universal CRISPR-CARs are also bankable, distributable, low-cost cost and accessible.

Nonetheless, important bottlenecks remain. 1st no long-term follow-ups: We don't know whether targeting 2 things blocks all the way so that the antigen can avoid the immune attack, given that changing CD22 when the gene "came back" was observed, and second, that CRISPR off-targets are still just theoretical. Although there isn't much data at this point, we should do a more thorough safety examination in big scale. The third point is that making more dual CAR-Ts with CRISPR will be hard and pricey in the beginning. Last, and most important, we still need to consider the immunodepleted toxicities, e.g., [CRS[ICANs] [10].

As for prospects, it maybe will contain the improvement of antigen combination, multiple logic CAR, suicide switch, and combine CAR-T with checkpoint inhibitors [11], microenvironment modulation, nonviral delivery, and development of base editing. Tech. And then after that, to combine the bioinformatic and single-cell sequencing so that we can further fine-tune our antigens, and we can also predict and see which one is the resistant pathway.

In conclusion, although dual target CRISPR – CAR – T is just at the beginning, it is the next wave of highly personalized precision oncology. More and more continuous innovations and clinical evaluations can uncover where it will fit in the armory against relapsed B-ALL.

4. Conclusion

Dual-targeting CD-19 / CD-22 CAR-T is a crucial advance in fighting relapsed/refractory B-ALL. Single-antigen escape can be overcome with dual-targeting, which gives broad, long-lasting remission. CRISPR/Cas9 tech is an improvement on all these, using engineering well, inserting safely, and the prospect of universal CAR-T stuff. They all lead to more dependable, scalable, and patient-friendly Immonutheries.

But it may have some temperament of excitement. ClinData is scanty for now, but attention should be paid to the long-term safety of edited CRISPR CAR T-cells. Potential Risks: off-target antigens beyond CD19/CD22； off-targets of genome editing; ongoing immune toxicity. And the process is complex and expensive as well.

The fate of CAR–T for B-ALL will be the combination of dual and new gene editing, and also off-the-shelf, all combined with anything else you can do to boost the immune system. Keep learning and working with translational science, clinical studies on how rules work, two types of CRISPR CAR-T might help more people who got B-ALL that didn’t get well when other medicines were used for the second time or first get long-lasting fixes when there aren’t many good things to take care of them.

References

[1] Maude S L, Frey N, Shaw P A, et al. Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia[J]. The New England Journal of Medicine, 2014, 371(16): 1507–1517. DOI:10.1056/NEJMoa1407222.

[2] Fry T J, Shah N N, Orentas R J, et al. CD22-Targeted CAR T Cells Induce Remission in B-ALL That Is Naive or Resistant to CD19-Targeted CAR Immunotherapy[J]. Nature Medicine, 2018, 24(1): 20–28. DOI:10.1038/nm 4441.

[3] Maude S L, Laetsch T W, Buechner J, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Acute Lymphoblastic Leukemia[J]. The New England Journal of Medicine, 2018, 378(5): 439–448. DOI:10.1056/NEJMoa1709866.

[4] Yin Y, Boesteanu A C, Binder Z A, et al. A Bispecific Anti-CD19/CD22 CAR T Cell Therapy for Relapsed or Refractory B Cell Malignancies[J]. Frontiers in Immunology, 2023, 14: 1165502. DOI:10.3389/fimmu.2023.1165502.

[5] Eyquem J, Mansilla-Soto J, Giavridis T, et al. Targeting a CAR to the TRAC Locus with CRISPR/Cas9 Enhances Tumor Rejection[J]. Nature, 2017, 543(7643): 113–117. DOI:10.1038/nature21405.

[6] Sterner R C, Sterner R M. CAR-T Cell Therapy: Current Limitations and Potential Strategies[J]. Blood Cancer Journal, 2021, 11(4): 69. DOI:10.1038/s41408-021-00459-7.

[7] Wang N, Hu X, Cao W, et al. Efficacy and Safety of CD19/CD22 Chimeric Antigen Receptor T Cell Cocktail Therapy in Patients with Relapsed or Refractory B-Cell Malignancies[J]. Blood, 2020, 135(1): 17–27. DOI:10.1182/blood.2019001642.

[8] Wang X, Riviere I. Clinical Manufacturing of CAR-T Cells: Foundation of a Promising Therapy[J]. Molecular Therapy - Oncolytics, 2016, 3: 16015. DOI:10.1038/mto.2016.15.

[9] Zhou X, Einsele H, Danhof S. CD19/CD22 Dual-Targeted CAR-T Cells for the Treatment of Relapsed/Refractory B Cell Acute Lymphoblastic Leukemia: A Phase I Trial[J]. Nature Medicine, 2023, 29(1): 50–59. DOI:10.1038/s41591-022-02029-4.

[10] Lee D W, Kochenderfer J N, Stetler-Stevenson M, et al. T Cells Expressing CD19 Chimeric Antigen Receptors for Acute Lymphoblastic Leukaemia in Children and Young Adults: A Phase 1 Dose-Escalation Trial[J]. The Lancet, 2015, 385(9967): 517–528. DOI:10.1016/S0140-6736(14)61403-3.

[11] June C H, O'Connor R S, Kawalekar O U, et al. CAR T Cell Immunotherapy for Human Cancer[J]. Science, 2018, 359(6382): 1361–1365. DOI:10.1126/science. aar6711.