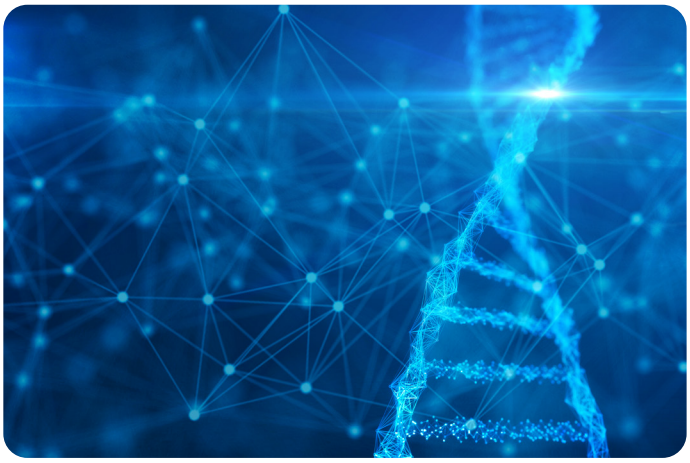
The future of immunotherapy gets brighter with base editing

Researchers and clinicians have long sought to leverage the human body's immune system against diseases such as cancer. With recent advances in genomic engineering, this goal is now being realized. The 2017 US Food and Drug Administration (FDA) approval of Axicabtagene ciloleucel (Yescarta®) and tisagenlecleucel (Kymriah®), two genetically engineered T cell therapeutics targeting B cell malignancies, marked the arrival of a new era of anticancer immunotherapy. Yescarta and Kymriah are unique in that they cannot be manufactured without a patient suffering from the very malignancy which they treat. To produce these therapeutics, T cells are harvested from a patient and subsequently genetically engineered to express a chimeric antigen receptor (CAR) targeting CD19, a protein present on the cell surface of B cell malignancies. These CAR-T cells are then re-infused into the patient from whom they originated, thereby introducing immune cells which target B cell malignancies in a CD19-directed fashion.



Autologous CAR-T therapy

CAR-T cell therapy is a form of adoptive cell therapy, wherein the immune system is harnessed to target malignant cells. In autologous CAR-T cell therapy, T cells are extracted from a patient, transported to a laboratory where they are genetically modified to express a protein-targeted CAR, and then re-infused into the same patient. Yescarta, Kymriah, and other FDA-approved CD19-directed CAR-T therapies fall into this category. Because the T cells are harvested from and re-infused into the same patient, there is no risk of rejection. However, the process of harvesting, genetically engineering, and re-introducing these autologous CAR-T cells is complex and costs hundreds of thousands of dollars. Efficient and highly coordinated logistical and experimental protocols must be in place, and the patient must have enough healthy T cells and be able to withstand an interim of at least three weeks while the transportation and engineering procedures are completed. The difficulties of this process have been borne out in clinical trials, as some studies have reported that nearly 30% of patients were unable to receive their engineered cells. When these processes are carried out successfully, initial patient responses are high. Unfortunately, durable remission is not observed for many; half of those who initially responded to Yescarta or Kymriah saw their cancers return within one year of treatment.

Allogeneic CAR-T therapy

While the promise of CAR-T therapy is evident, the logistical complexities of producing autologous cells and the undesirable rates of relapse following treatment represent significant barriers to widespread use. To overcome these concerns, researchers have pursued allogeneic cell therapies where T cells are harvested from healthy donors rather than from the patients themselves. While this approach requires genetic modification of the cells to prevent host-mediated rejection events, it also removes the time constraints and T cell quality concerns associated with autologous CAR-T therapies. CRISPR/Cas9 has become the preferred method for genetic editing of allogeneic CAR-T cells, as it is more efficient, scalable, and procedurally simple compared with early gene editing tools such as zinc finger nucleases (ZFNs) and transcription-activator-like effector nucleases (TALENs).

Allogeneic CAR-T cells have shown promise in clinical trials thus far, and the prospect of developing "off the shelf" CAR-T therapies has spurred significant research efforts. As these cells lack the supply and time constraints associated with their autologous counterparts, increasingly complex genetic modifications have been explored with goals of improving effector cell persistence and efficacy, as well as limiting adverse effects such as cytokine release syndrome. While studies have demonstrated progress in each of these areas, they have also revealed a separate concern; as more genes are manipulated, the risk of off-target edits increases while the efficiency of on-target edits decreases.

Safety concerns related to gene editing

For CAR and similar immunotherapies to succeed in the clinic, any genetic manipulations performed must be highly specific. Lack of specificity can lead to unintended off-target effects, mutagenesis in the genome, and safety concerns. By improving the molecular components and delivery of CRISPR/Cas9, researchers have been able to reduce, though not eliminate, the occurrence of off-target edits. The DNA double-strand breaks introduced by CRISPR/Cas9 and similar nuclease-based gene editing tools is inherently problematic, as they can activate p53-dependent DNA damage response pathways. This can lead to cell cycle arrest, apoptosis, and mutagenesis events such as large insertions or deletions, duplications, inversions, translocations, or chromosomal rearrangements. Furthermore, as multiple genetic modifications are made simultaneously, the resulting free DNA ends can repair and anneal in undesired and unpredictable configurations.

Base editing addresses the shortcomings of other gene editing technologies

While the DSB-based mechanism of action of CRISPR/Cas9 may preclude its use in wide-scale production of CAR-T immunotherapies, an alternative strategy has been developed by the research groups of David Liu (Harvard University), Akihiko Kondo (Kobe University), and Shengkan Jin (Rutgers University). Their approach, termed base editing, alters single nucleotides without introducing DSBs by exploiting DNA mismatch and base excision repair pathways. Base editors (BEs), primarily composed of a catalytically impaired Cas nuclease for targeted DNA binding and a single-stranded effector protein for nucleotide deamination, chemically alter single nucleotides and thus transform them into different bases. Cytidine BEs (CBEs) catalyze the conversion of C-G base pairs to T-A base pairs through deamination of C to T via a uridine intermediate, while adenine BEs (ABEs) deaminate A to G via an inosine intermediate.

By targeting a single nucleotide, base editing can be used to introduce premature stop codons in the coding regions of targeted genes, effectively inactivating them. Without the risks of DNA DSB-based rearrangements associated with CRISPR/Cas9, base editing can be used to knock out multiple target genes simultaneously with high efficiency and without detectable translocation events. Modifications of the single-stranded deamination effector proteins have helped prevent unwanted DNA and RNA base edits, while transient delivery of base editing components through mRNA or ribonucleotide protein complexes has largely ameliorated off-target effects. The ability to precisely and simultaneously inactivate multiple genes in allogeneic T cells allows the engineering of CAR

therapies which have longer persistence and greater efficacy, while the lack of off-target edits improves safety.

While unbiased genome- and transcriptome-wide safety profiling of BEs is currently underway, it is clear that base editing will have a significant impact upon the field of immunotherapy. Leukemia-targeting CAR-T cells generated with BEs are already advancing towards clinical trials, with preliminary reports finding no evidence of genomic rearrangement or p53 activation. Solid tumors, which have proven to be recalcitrant to immunotherapies thus far, may soon be viable targets of highly-specific CAR-T cells produced through base editing. Despite still being in its infancy, the field of base editing has rapidly expanded and progressed; the future is bright for this technology as well as for BE-based immunotherapy.

Reference

1. Harbottle, J. A. (2021). Immunotherapy to get on point with base editing. Drug Discovery Today, 26(10), 2350-2357. https://doi.org/10.1016/j.drudis.2021.04.003

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