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Advances in Engineering CAR-T cell therapies

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Abstract. The remarkable success of Chimeric Antigen Receptor (CAR-T) cells in B-cell malignancies has not yet translated to solid tumors. Several factors explain this gap. Solid tumors actively exclude and suppress infiltrating lymphocytes through a complex microenvironment. Their surface antigens are often heterogeneous, making it hard to find a target expressed uniformly on all malignant cells. And the current manufacturing paradigm, autologous and ex vivo, remains logistically challenging and expensive. Recent engineering efforts have taken different tacks. One approach aims at off-the-shelf CAR-T cells, using gene editing to eliminate alloreactivity while preserving function. Another seeks to redesign the CAR itself, for instance with logic-gated circuits that sharpen tumor recognition and limit off-tumor toxicity. A third strategy targets the Tumor Microenvironment (TME) directly, either by remodeling it or by using delivery systems that improve T cell infiltration. Meanwhile, in vivo CAR-T generation, which reprograms T cells inside the patient with targeted nanoparticles or viral vectors, could eventually bypass ex vivo manufacturing altogether. These lines of work are gradually converging, and together they point toward a future in which CAR-T therapy might become effective, safe, and accessible for a wider range of cancers.

Keywords: CAR-T, in vivo CAR-T cell engineering, off-the-shelf CAR-T cells, on-target/off-tumor toxicity

1. Introduction

The 2017 approval of Kymriah marked a turning point in cancer therapy. For the first time, a living cell was approved as a drug, engineered from a patient's own T cells to recognize and eliminate cancer. The results in certain blood cancers were striking. Patients with relapsed or refractory B cell malignancies, who had few options left, went into remission. But extending this success to solid tumors has proven more difficult than many anticipated. Solid tumors account for about 90 percent of cancers, and to date no CAR-T product has been approved for them. The reasons are not mysterious. Solid tumors erect a physical and biochemical barrier that excludes T cells. They create an environment that exhausts those cells that do manage to infiltrate. Their antigens are often expressed heterogeneously, so a target present on one part of the tumor may be absent on another. And even when a suitable target exists, it may also turn up on normal tissues, leading to off-tumor toxicity. On top of all this, the manufacturing process itself presents challenges. It takes weeks, it costs hundreds of thousands of dollars, and it sometimes fails because the patient's own T cells are compromised by prior treatments or the disease itself. The field has responded by rethinking the approach. Attention is shifting from hematologic to solid tumors, from personalized products to off-the-shelf alternatives, from ex vivo manufacturing to in vivo generation. Gene editing is being used to create universal CAR-T cells that could be

manufactured in advance and stored. Novel delivery systems are being explored to generate CAR-T cells directly inside the patient, bypassing the need for cell collection and manufacturing altogether. And various strategies are being tested to overcome the barriers posed by the TME, some targeting the microenvironment itself, others redesigning the CAR to better navigate it. This review will walk through these developments, focusing on the engineering strategies that are beginning to make headway against obstacles that have long seemed intractable.

2. In vivo CAR-T cell engineering

The idea behind in vivo CAR-T generation is straightforward. Instead of collecting a patient's cells, engineering them in a clean room, and infusing them back weeks later, researchers have been trying to deliver the genetic cargo directly to T cells inside the body. Several groups have been working on this. The approaches fall into two broad categories. One uses viral vectors, lentivirus or adeno-associated virus, to deliver the CAR transgene. The other uses non-viral platforms, mostly lipid or polymer nanoparticles. Both have their trade-offs [1]. Viral vectors are efficient and can integrate into the genome for long-term expression, but they raise concerns about insertional mutagenesis and immunogenicity. Nanoparticles are safer in some respects, but getting them to target T cells specifically without also hitting liver hepatocytes has been a challenge.

A recent clinical trial from China showed what might be possible. Investigators used a lentiviral vector to generate anti-BCMA CAR-T cells directly in patients with relapsed or refractory multiple myeloma. The response rate was 100 percent, which is remarkable for a heavily pretreated population [2]. But the field is not there yet for solid tumors. mRNA-based approaches, while safer, typically express CAR protein for only days to weeks. This duration is probably not long enough for sustained antitumor activity [3]. Integrating vectors solve the durability problem but bring their own risks [4]. And then there is the question of delivery. Most nanoparticles, including the LNPs that made mRNA vaccines possible, have a natural tropism for the liver. That works well for targeting hepatocytes but not for reaching T cells circulating in blood or sitting in lymph nodes.

Some groups are trying to get around this with clever design. Inspired by cardiolipin, a team at Peking University developed a new class of lipids called CAMPs and used them to make PL40 LNPs [5]. These particles self-assemble into structures with rigid surfaces and internal phase separation. These features let them interact with lipid rafts on T cell membranes. Through membrane perturbation and macropinocytosis, they deliver mRNA to T cells without needing antibodies for targeting. It is still early, but the approach suggests that engineering the particle itself might be enough to overcome the liver problem.

Another group took a completely different tack. Instead of delivering genetic material, they designed magnetic bispecific nanobodies that bind circulating T cells and redirect them to tumors [6]. The system works like this. Anti-CD3 and anti-PD-L1 antibodies are assembled on magnetic nanoparticles. Injected into the bloodstream, they latch onto T cells via CD3. An external magnet then guides the whole complex to the tumor, where PD-L1 recognition provides additional targeting. The approach does not involve genetic modification at all, just physical redirection. In mice, it worked. Whether it scales to humans remains to be seen, but it is a reminder that there are many ways to engineer T cells, not all of them genetic.

3. Off-the-shelf CAR-T cells

The idea of off-the-shelf CAR-T cells is appealing from a manufacturing standpoint. Make the cells in advance, characterize them, freeze them in batches, and ship them to hospitals when needed. No waiting, no apheresis, no concern about whether the patient's own T cells are any good. But getting there means solving a

problem that autologous CAR-T cells avoid entirely immune rejection. Two directions of rejection have to be dealt with. One is graft-versus-host disease, where the donor T cells recognize the recipient's tissues as foreign and attack. This is mediated by the T cell receptor, and the standard fix is to knock out TRAC, the gene encoding the TCR alpha chain [7]. The other direction is host-versus-graft, where the recipient's immune system recognizes the donor cells as foreign and clears them. This is mainly about HLA mismatch, and the usual approach is to knock out B2M or CIITA to eliminate HLA class I and II expression [8].

But here is the catch. Knock out HLA completely, and the donor cells become targets for Natural Killer (NK) cells. NK cells are programmed to kill anything that fails to present self HLA, a mechanism known as missing-self recognition. So the elimination strategy has built-in limitations. The cells are stripped of the very molecules that provoke rejection, but they are also stripped of the molecules that protect them from another arm of the immune system.

Some groups are trying more refined approaches. Rather than eliminating TCR and HLA entirely, they are looking for ways to make them less visible. One recent study took this route by targeting SPPL3, a protease in the Golgi apparatus [9]. Knock out SPPL3, and multiple glycosyltransferases become aberrantly active. The result is a dense layer of glycans on the cell surface, a sort of sugar coating that physically shields the TCR complex and HLA molecules without removing them. T cells modified this way are less likely to trigger allorecognition, and they also seem less susceptible to NK killing. The work is early, and it is not clear whether glycan shielding will prove durable or complete enough in humans. But it points to a different philosophy. Instead of deleting targets, hide them.

Another line of work has moved beyond simple knockout altogether. Traditional CRISPR-Cas9 editing cuts both strands of DNA, and while that works, it can also cause translocations or larger deletions. Base editing and prime editing, sometimes called CRISPR 2.0, avoid double-strand breaks altogether. They chemically convert one base to another or write small sequences into the genome. One group used adenine base editors to introduce six edits simultaneously into CAR-T cells, targeting TRAC, B2M, CIITA, PDCD1, TGFBR2, and ADORA2A [10]. The editing efficiencies were high, above 90 percent for each locus. The resulting cells resisted multiple suppressive signals in vitro and killed better than single-edit controls. Whether such heavily modified cells will persist and function in patients remains to be seen, but the technical feat is notable.

The field is moving away from brute-force knockout toward more nuanced engineering. Epigenetic regulation, post-translational modifications, multiplex base editing. These are the tools now being brought to bear. The goal is no longer simply to eliminate what causes rejection, but to tune the cells so they strike some balance between invisibility to the host and functionality in the tumor. Whether that balance exists, and where it lies, is what the next few years of research will have to figure out.

4. Novel strategies to overcome solid tumor barriers

Solid tumors present obstacles that go beyond what CAR-T cells were originally designed to handle. The microenvironment is not just physically dense. It is packed with immunosuppressive cells, myeloid-derived suppressor cells, regulatory T cells, tumor-associated macrophages. These cells secrete cytokines and metabolites that blunt T cell function. Even when CAR-T cells make it into the tumor, they often find themselves in an environment that pushes them toward exhaustion.

One approach has been to target those suppressive elements directly. A recent study focused on TREM2, a marker enriched on immunosuppressive macrophages in tumors. The group engineered CAR-T cells to recognize TREM2, but they added another layer of control. The CAR construct included a synthetic promoter responsive to T cell activation, so that when the CAR-T cell engaged its target, it also turned on expression of

IL-12. This localized cytokine production remodeled the TME, recruited endogenous immune cells, and led to tumor regression in mouse models without the systemic toxicity usually associated with IL-12 [11]. The strategy is clever. Instead of trying to make CAR-T cells resist suppression, it sends them in to clear out the suppressors first.

Another group took a different angle. Rather than targeting the microenvironment, they targeted the tumor cells themselves and tried to change how the microenvironment sees them. Using a bioinformatics platform called CancerPAM, they screened patient tumor sequencing data to find safe sites for gene insertion. Then they used CRISPR to knock genes for inflammatory chemokines, CXCL10, CXCL11, and IFNG, directly into the tumor cell genome [12]. In mouse models of neuroblastoma, tumors engineered this way started secreting chemokines that attracted T cells. When CAR-T cells were given later, they infiltrated better and controlled tumors more effectively. The tumor cells themselves became signaling beacons.

These two strategies are conceptually different but potentially complementary. One clears out suppressive elements. The other sends out recruitment signals. Together, they could create a loop where more T cells get in, clear more suppressors, and those activated T cells then recruit more. In theory, that could drive deeper and more durable responses than either approach alone. But combining them is not straightforward. Both strategies involve adding powerful positive feedback loops to an already complex biological system. Too much inflammation could trigger toxicity. Too much recruitment could exhaust the available T cell pool. The signaling pathways might interfere with each other in ways that are hard to predict. And engineering cells to do multiple things at once raises questions about metabolic burden and manufacturing feasibility. The logic is appealing, but the execution will require careful titration and probably some trial and error.

5. On-target/off-tumor toxicity

One of the hardest problems in CAR-T therapy, particularly for solid tumors, is that the same antigens found on cancer cells sometimes show up on normal tissues. Targeting the antigen may kill the tumor, but it may also kill healthy cells. The consequences depend on where that normal expression occurs. Hit CD19 on B cells and the result is B cell aplasia, which is manageable with immunoglobulin replacement [13]. Hit an antigen in the gut or the lung or the heart, and things can go badly wrong. Claudin18.2 is a case in point. It is expressed on gastric cancer cells, which makes it an attractive target. But it is also expressed at low levels on normal gastric mucosa. Several groups have seen gastric toxicity in preclinical models, and the problem appears to be independent of exactly how the CAR is designed. Birocchi and colleagues recently set up a mouse model that recapitulates the human expression pattern of CLDN18.2. They showed that CAR-T cells expand in the gastric tissue, driven by antigen, and that this expansion causes damage. They then tested an AND-gate strategy, requiring both CLDN18.2 and mesothelin for full activation. The logic worked. Antitumor activity was preserved, but gastric toxicity was largely eliminated [14].

Around the same time, Carstens and colleagues were looking at the same problem from a different angle. They found that toxicity correlated with binding affinity. Higher affinity CARs killed better, but they also caused more damage to normal tissues. So they developed low-affinity, fully human VH-only CARs. One of these, called 5795-VH, maintained good antitumor activity in preclinical models but with much less gastric toxicity [15]. The trade-off between potency and safety could be managed by tuning the receptor itself.

These two approaches are conceptually distinct. One solves for specificity, making sure the T cell only activates when it sees the right combination of targets. The other solves for signal strength, turning down the volume so that transient or low-level encounters with normal tissue do not trigger full activation. In principle, they could be combined. A low-affinity AND-gate CAR might be safer than either modification alone. But

engineering that kind of system is not trivial. Each additional component adds complexity to the construct, and screening for the right balance of affinity and logic becomes a high-dimensional problem. The work so far shows that the toxicity can be managed. Whether it can be managed while keeping enough potency to matter in patients is the next question.

6. Conclusion

CAR-T therapy has clearly transformed the treatment of blood cancers, but solid tumors have proven more stubborn. The reasons are now well understood. The TME excludes and exhausts incoming T cells. Antigen expression is patchy and unpredictable. And the targets that do exist are often shared with normal tissues, so killing the tumor can mean damaging healthy organs. Manufacturing adds another layer of difficulty. The current process is slow, expensive, and not always feasible for patients with compromised immune systems. None of these problems have been fully solved, but the past few years have seen real progress on multiple fronts. Lentiviral vectors work but carry risks of insertional mutagenesis. Base editing avoids double-strand breaks but raises its own questions about off-target activity.

The microenvironment can be targeted directly, as with TREM2-directed CAR-T cells that clear suppressive macrophages, or indirectly, by engineering tumor cells to send out recruitment signals. Logic-gated circuits offer a way to sharpen specificity, and affinity tuning can dial down unwanted activation on normal tissues. Off-the-shelf and in vivo-generated CAR-T cells point toward a future where manufacturing is no longer a bottleneck. Trade-offs are everywhere. AND-gate CARs are more specific but harder to design. Low-affinity receptors are safer but may miss tumors with low antigen density. Heavily edited cells might resist rejection but could carry unforeseen risks from multiple genetic modifications. The field is moving from simple solutions to more nuanced ones, from single-target efficacy toward systems that integrate logic control, functional enhancement, and smarter manufacturing.

Whether these approaches will work in patients remains to be seen. Preclinical models are helpful but imperfect. The human TME is more complex than anything in a mouse, and the human immune system is harder to fool. The direction is clear. The next generation of CAR-T therapies will not just be more potent. They will be more precise, more controllable, and more accessible. Getting there will take more time and more trial and error, but the pieces are starting to come together.

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