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Creating CAR T therapies that don't cause cancer

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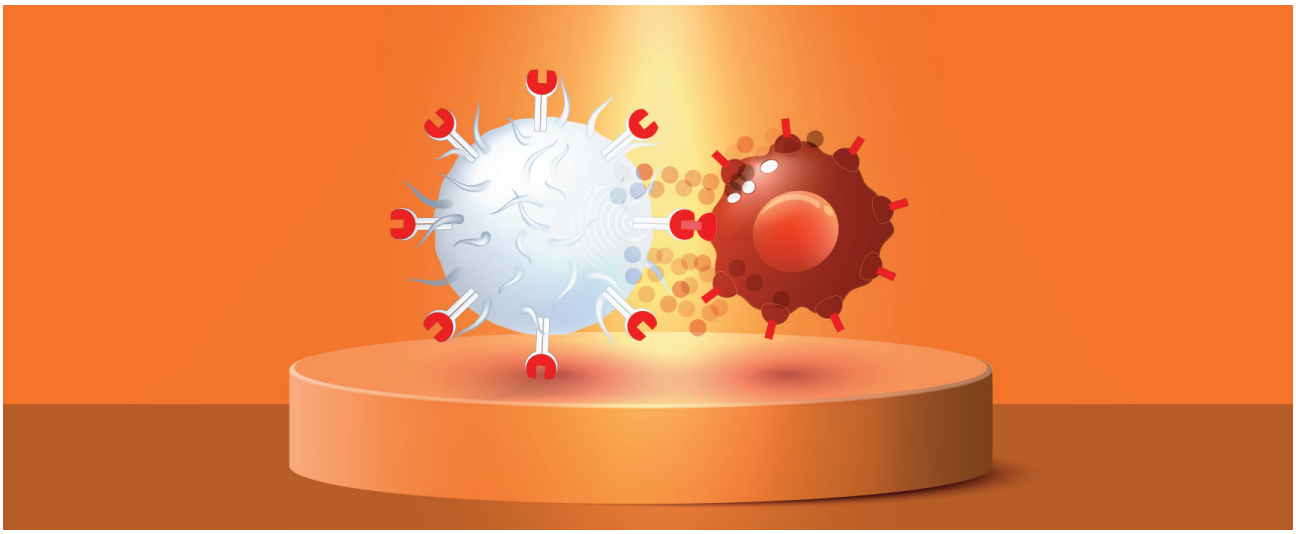

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The revelation that CAR T cell therapies designed to cure cancers can give rise to new malignancies jolted the industry and raised concerns about the breakthrough modality's ability to expand into new indications, but alternative CAR T technologies that avoid or reduce the risk are already in development.

With the understanding that the risk of secondary malignancy is real and not just theoretical, safety switches are back. Non-viral gene insertion technologies may also have a moment to shine.

In November, FDA's Center for Biologics Evaluation and Research (CBER) issued a statement on the risk of T cell malignancies following BCMA- or CD19-directed CAR T cell therapy. As of Dec. 31, the agency had received reports of 22 secondary malignancies, some of which were CAR-positive cancers, indicating that they arose from the CAR T cells.

The adverse events earned all eight CAR T cells with U.S. approval a boxed warning, including one — Tecartus brexucabtagene autoleucel from the Kite Pharma Inc. unit of Gilead Sciences Inc. (NASDAQ:GILD) — that hasn't directly been linked to cases of T cell cancers. Last month, FDA issued safety labeling change notifications to each manufacturer.

The risk is very serious — leading in some cases to fatal secondary cancers — but it is also very rare. As of the end of last year, about 27,000 patients had been treated with the modality in the U.S. It's likely that the number of cases of secondary malignancy will rise as FDA investigates, but CBER Director Peter Marks has reassured patients and developers that the benefits still far outweigh the risks in the advanced cancer settings in which the therapies are approved.

The fate of the modality is less certain in indications where tolerance for risk is lower, such as earlier cancer settings and autoimmune diseases.

That's where new technologies can step in. Because the secondary malignancy risk is related to the lentiviral delivery vehicle used to express the CAR in patients' T cells, non-viral technologies for gene expression may be able to sidestep the problem.

There's also an opportunity for safety switches to add a layer of protection to CAR T therapies. Kill switches, or suicide switches, are designed to eliminate the CAR T cells upon

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contact with an antibody or small molecule trigger, meaning that in those rare cases where the CAR T cells become cancerous, activating the kill switch should eliminate the secondary cancer.

The lentiviral issue

The source of the new CAR T safety concerns is almost certainly the lentiviral vectors used to express the CAR. Lentiviruses are the delivery vehicles of choice for CAR Ts due to their efficiency and ability to transduce the sensitive T cell population without causing excessive damage. Unfortunately, the way lentiviruses integrate into the genome can also cause cancer.

Lentiviruses work by inserting their transgene cargo into a random site in the host genome. Integration at the wrong site can lead to cancer by, for example, disrupting a critical tumor suppressor.

In recent years, the cancer risk has gone from theoretical to material in other therapeutic contexts as well. In 2022, Skysona elivaldogene autotemcel from bluebird bio Inc. (NASDAQ:BLUE) was approved in the U.S. despite multiple cases of treatment-related cancer.

Viral vectors that don't integrate into the genome such as AAVs do exist, but integration is important for CAR T cell efficacy.

To fight cancers, CAR T cell therapies need to replicate when they encounter cancer cells. If the CAR gene hasn't integrated into the genome, it won't be replicated and passed on during cell division, and the number of CAR-expressing T cells capable of attacking the tumor won't rise.

According to FDA leaders, it may be time to advance technologies that can integrate CAR sequences into the DNA at specific rather than random sites.

In a New England Journal of Medicine Perspective piece on the secondary cancer risk published last month, Marks and Nicole Verdun, director of CBER's Office of Therapeutic Products, wrote that "moving forward, particularly as the use of CAR T cells for indications outside hematology and oncology is considered, new strategies involving targeted insertion of the CAR construct to specific loci might help reduce the risk of cancers due to integration of the CAR construct at an oncogenic loci within the genome."

A wide array of non-viral technologies may be able to help with that.

> **"LENTIVIRUSES INTEGRATE INTO HOT SPOTS OF THE GENOME, INCREASING THE POTENTIAL FOR TRANSFORMATION OF THE CELLS."**
>
> **HELEN SABZEVARI, PRECIGEN**

Non-viral next steps

Among the next-generation CAR T cell manufacturing technologies in development, gene editing may be the only one that can deliver truly site-specific CAR expression.

The challenge with gene editing in this application is that expressing a CAR requires insertion of its genetic sequence, but the current versions of the technology are better suited for gene disruption than gene insertion. That's because when CRISPR-cas9 or related tools cut the DNA, the gene repair pathway that's most likely to fix the cut does so in a way that causes gene knockout. By contrast, homology-directed repair, the pathway needed to insert a gene, is far less likely to be activated.

What drug developers have discovered, however, is that the efficiency of CAR insertion achieved by current gene editing protocols may be sufficient, given the ability to identify and select the CAR-expressing cells.

Several companies are charging ahead with CRISPR-based CAR insertion, including two of the pioneering CRISPR gene editing companies, CRISPR Therapeutics AG (NASDAQ:CRSP) and Caribou Therapeutics Inc. (NASDAQ:CRBU). At least six other companies have also disclosed programs.

In most cases, these companies are inserting the CAR into a location in the genome that not only avoids the cancer risk but can contribute a secondary benefit.

The most common locus for insertion is the TRAC gene, which must be disrupted in the process of making the cell allogeneic, thereby saving an engineering step. Another example is insertion of the CAR in the gene encoding PD-1, which disrupts the checkpoint's expression and promotes CAR T cell persistence.

Other companies are sticking with lentiviruses to express the CAR but using gene editing to knock out TRAC, PD-1 or other genes. Among these are Cellectis S.A. (Euronext:ACLCS; NASDAQ:CLLS) and Allogene Therapeutics Inc.

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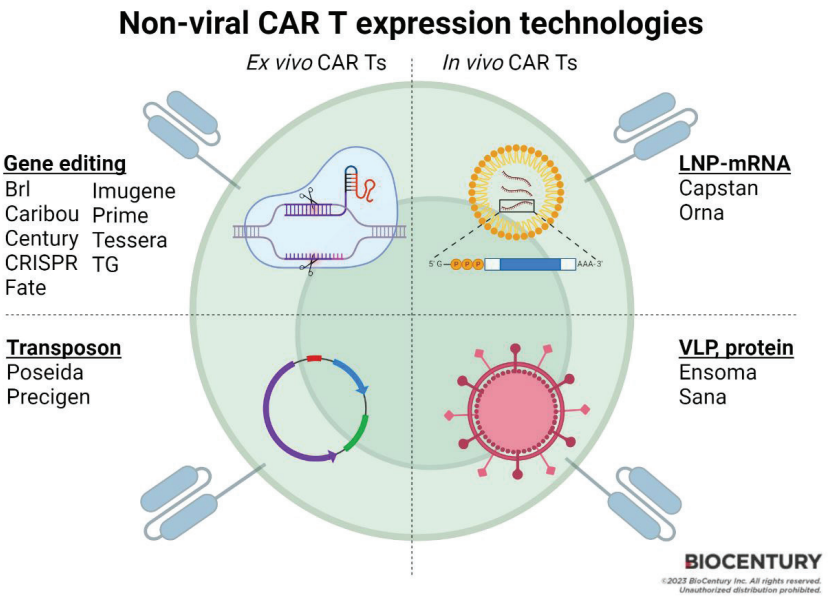
(NASDAQ:ALLO), which are using TALEN-based gene editing rather than CRISPR.

Transposons come of age

Transposon-based gene insertion offers an alternative way to express a CAR, but the approach may not entirely eliminate the risk of secondary malignancies.

Transposons are genetic elements that naturally move to new sites within the genome, with the help of enzymes called transposases that cut and paste the genetic material. Companies including Precigen Inc. (NASDAQ:PGEN) and Poseida Therapeutics Inc. (NASDAQ:PSTX) have been working for years on using DNA transposon systems to drive CAR expression in T cells.



The process involves electroporation rather than viral transduction to deliver a DNA plasmid encoding the transposon and the CAR sequence to T cells.

Once inside the cell, insertion isn't completely random, but it's also not selectively targeted. Transposons move to new DNA sites with certain characteristics, which are often found in non-coding regions. In theory, it shouldn't matter if a DNA integration event disrupts a sequence that won't be transcribed.

By contrast, lentiviruses integrate into actively transcribed genes to ensure that their viral DNA is expressed.

"Lentiviruses integrate into hot spots of the genome, increasing the potential for transformation of the cells. In contrast, the chance of non-viral transposons being inserted into a hot spot is significantly lower," Helen Sabzevari, president and CEO of Precigen, told BioCentury.

Kristin Yarema, president and CEO of Poseida, said that the preference of transposons for non-transcribed, open chromatin also means "you will get a low copy number per cell." A lower copy number means a lower risk for oncogenesis.

Sabzevari told BioCentury that when Precigen launched in 1998, non-viral platforms weren't in favor because the technology had deficiencies that needed to be overcome. She noted that Precigen's work to increase viability and transfection efficiency solved some of those issues.

More than 25 years later, Precigen has established an overnight manufacturing process based on the technology and has advanced three CAR T programs into the clinic.

At last year's American Society of Hematology (ASH) meeting, Poseida reported that P-BCMA-ALLO1 led to an overall objective response (ORR) of 82% with nine of 11 patients achieving a response. The program, which is partnered with Roche (SIX:ROG; OTCQX:RHHBY), is Poseida's lead candidate in hematological malignancies. It remains to be seen whether the design will solve the durability challenge other allogeneic CAR T cell therapies have faced.

The next-generation of transposon-based medicines marries the technology to CRISPR gene editing. Using the CRISPR guide machinery to direct the DNA transposition machinery, companies including Prime Medicine Inc. (NASDAQ:PRME) and Tessera Therapeutics Inc. aim to write the CAR sequence into a specific locus.

In vivo solutions

While the first generation of CAR T cell therapies are all made outside the body using lentiviral vectors, there appears to be more flexibility in delivery methods that can be used to create CAR T cells inside patients — a version of the modality called in vivo CAR T.

Instead of removing T cells from the patient to engineer them in the lab, in vivo CAR T production involves administering the CAR transgene directly to the patient using a vector that can seek out and selectively express the CAR in the patient's T cells.

Some companies, including Kelonia Therapeutics Inc. and Interius BioTherapeutics Inc., are sticking with lentiviruses for in vivo CAR T production, systemically delivering

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versions of the viral therapies designed to restrict CAR expression to T cells.

Others are looking to mRNA to drive transient expression of the CAR. Capstan Therapeutics Inc. and Orna Therapeutics Inc. are each using nanoparticles to deliver CAR-encoding RNA to T cells in vivo. The RNA won't integrate into the genome and be propagated to daughter T cells, but it can be delivered as a simple injection that's repeated as necessary to create more CAR Ts or a more durable response.

Ex vivo CAR T strategies involve a long and logistically complicated manufacturing and delivery process and therefore can't be readily re-dosed. Viral vectors delivered systemically also lack re-dosing potential because the initial dose generates neutralizing antibodies that will quickly eliminate subsequent doses.

Orna and Capstan are preclinical companies developing in vivo CAR T therapies for cancer, with the latter company also exploring the therapies for autoimmunity, fibrosis and other indications.

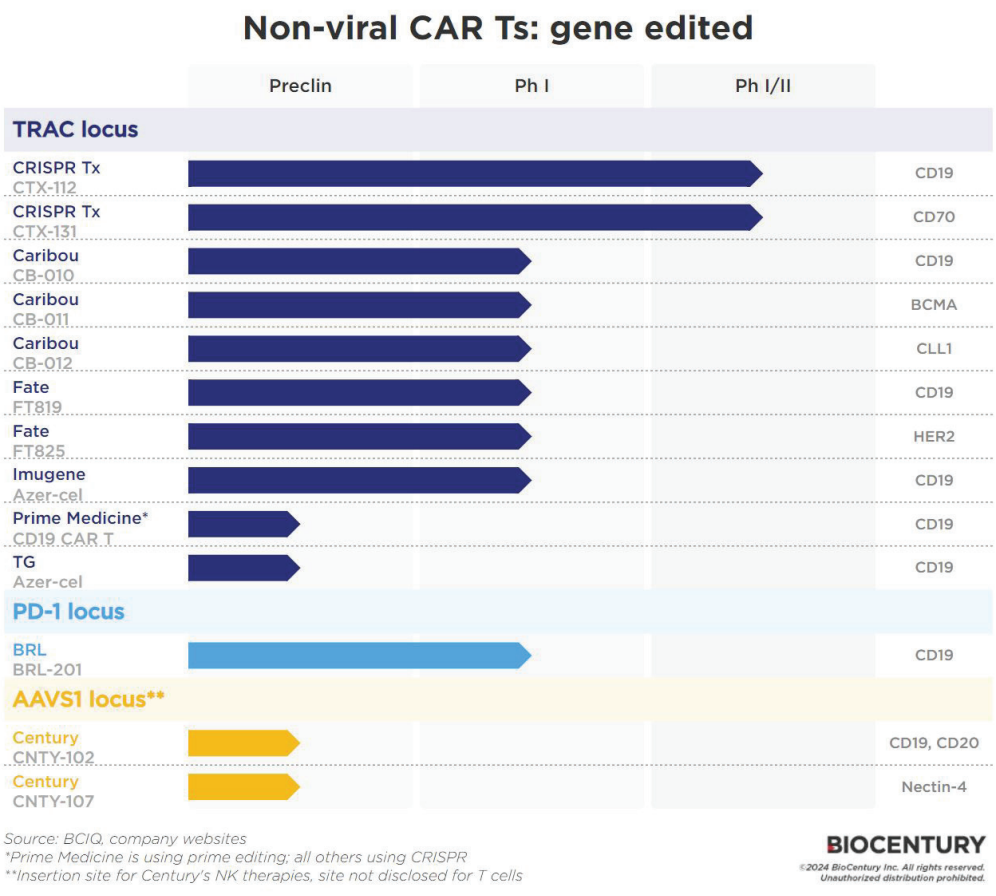
Haig Aghajanian, co-founder and VP of research at Capstan, thinks the transient CAR expression achieved by the company's antibody-targeted lipid nanoparticles will be an advantage in autoimmune indications. "In the case of autoimmune diseases, you don't want the durability seen with autologous CAR Ts." The hypothesis is that rapidly eliminating existing B cells, and then letting the cells repopulate, will help reset the immune system to treat autoimmune disease.

Other in vivo techniques include the fusosome cell-targeting proteins used by Sana Biotechnology Inc. (NASDAQ:SANA), and the adenovirus vector-based virus-like particles used by Ensoma Inc. Both strategies evade immune detection, but like mRNA, they don't integrate into the genome.

Backup plan

Regardless of the vehicle selected for CAR expression, companies can also consider incorporating a safety switch into their products.

Safety switches had a moment where they became a popular next-generation design feature of CAR Ts, but were then deprioritized by some companies. No CAR T cells approved in the U.S. are equipped with safety switches.



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One reason may be that the technology was designed to address safety concerns that were largely solved by other methods. CAR T cells carry risks of serious cytokine release syndrome and neurotoxicty, and it was once considered a priority to be able to shut off the cells if the serious adverse reactions occurred. However, hospitals have since established treatment protocols for reducing and managing the risks.

Eliminating a secondary cancer that arises from a CAR T therapy is a new application for switch technology.

Precigen is among the companies using the approach, and has a "safety switch which allows for elimination of these CAR T cells if something goes wrong," said Sabzevari.

Some of the safety switches in development work by turning CAR expression on or off, but eliminating secondary cancers requires kill switches that destroy the CAR Ts.

Kill switches can be triggered by antibodies or small molecules, and rituximab has emerged as a leading trigger. Some therapies, such as preclinical candidate ALLO-819 from Allogene Therapeutics Inc. (NASDAQ:ALLO), incorporate a rituximab-binding region into the CAR. When the antibody binds the CAR, it attracts immune cells that kill the CAR-carrying T cell.

Others approaches involve expressing a rituximab binding region on the T cell surface. For example, Autolus Therapeutics Inc. (NASDAQ:AUTL) uses a retrovirus to express a compact CD20 epitope on the cell surface.

One challenge of adding off-switches is that their sequences take up space in the vector. Lentiviral carrying capacity for genetic cargo is limited, and leaves little room for additional sequences beyond a CAR.

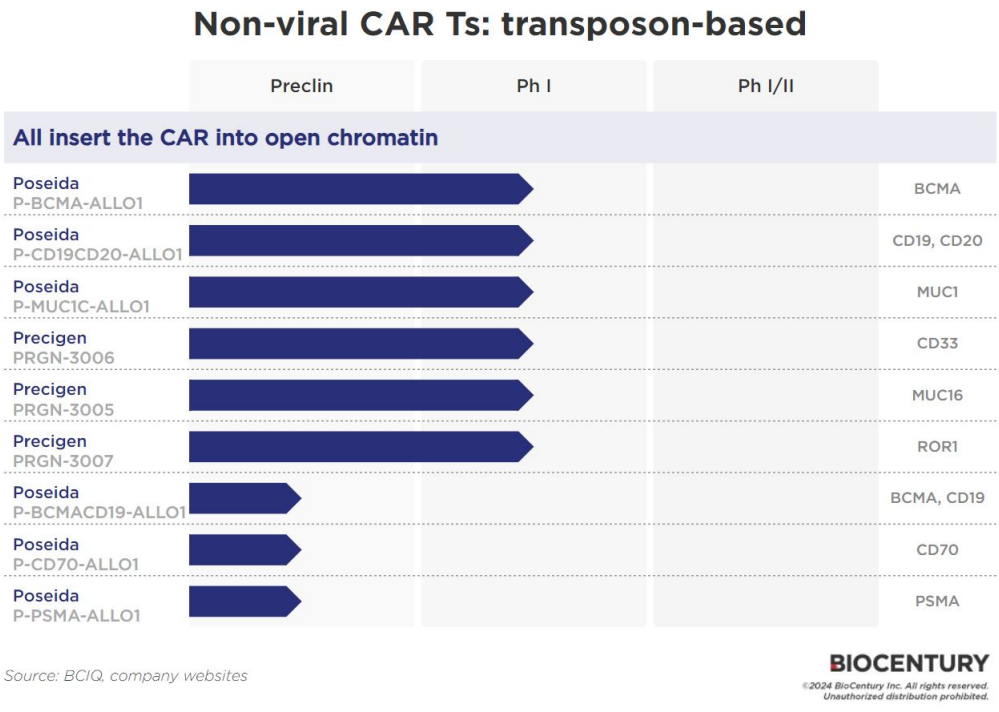
For that reason, companies have turned to alternative vectors to express the switch, with Precigen using its transposon and Autolus a retroviral vector.

Sana has a different kill switch design built into its hypoimmune T cell platform. The company relies on CD47 expression to help hide its allogeneic T cells from the innate immune system. Therefore, treatment with an anti-CD47 antibody exposes the cells and leads to their detection and elimination.

Small molecule triggers are also making their way into the pipeline, and because they aren't dependent on immune cells to eliminate the CAR Ts, they may act more quickly.

"With a single administration of an appropriate agent, you can completely eradicate the signal in those cells in a matter of minutes to hours," said Yarema. "For anti-CD20 as a triggering agent, it's more like a timescale of days. If you're looking at something like a secondary malignancy, you can debate whether that's meaningful, but there are other situations where speed of action is very important," such as severe cytokine release syndrome.

Autolus also has a rapamycin-based switch in preclinical development. When rapamycin binds the site, it



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heterodimerizes with caspase-9 to activate a cell death cascade within the T cell.

Poseida also uses a small molecule trigger for its switch, which can be readily expressed in CAR Ts due to the large carrying capacity of its transposon plasmids.

Yarema told BioCentury that kill switches may have an added benefit as CAR Ts move into autoimmune applications, where a short duration of activity is preferred. "We don't know the length of time we really want a CAR to stick around," she said, adding "the safety switch could play a role in the active management."

"I think it's a fantastic insurance policy," she said. "People ask us, 'have you used this?' No, but it's there. It's kind of a silent passenger if there ever is a reason to want to turn off the cells."

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