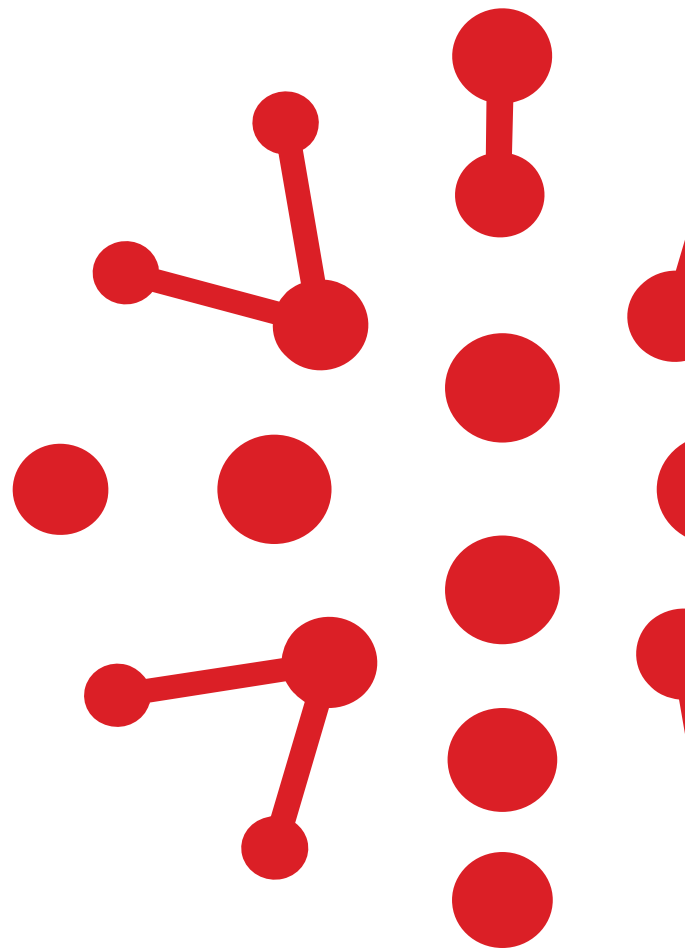



Secondary Malignancies Following CAR-T Cell Therapy

What Could We Learn from
the FDA Investigation?





On November 28, 2023, the U.S. Food and Drug Administration (FDA) announced that it had launched an investigation into the risk of T-cell malignancies following autologous Chimeric Antigen Receptor (CAR-)T cell immunotherapies^[1]. While the Agency stated that T cell malignancies observed in clinical trials as well as post-marketing adverse event monitoring programs were the trigger for this investigation, their announcement did not provide further information. This has led to a wave of confusion among the Cell and Gene Therapy Community. We address key areas and questions concerning the FDA's recent announcement in the interest of our scientific and research community.

CAR-T Cell Therapy and Potential Risks + Benefits

Chimeric Antigen Receptors (CARs) are synthetic receptors that enable T cells to recognize antigens on malignant cells and thus direct CAR-T cells to kill these target cells. To date, the FDA has approved six CAR-T cell products targeting B cell cancers and multiple myeloma refractory to previous therapies. In all six products, patient T cells are genetically engineered with integrating lentiviral or retroviral vectors to express CARs targeting common B cell tumor markers CD19 and B Cell Maturation Antigen (BCMA). After activation and expansion *ex vivo*, the CAR-T cells are reinfused back into the same patient to attack malignant B cells. After clearance of the tumor cells, CAR-T cells can remain in the body for years and suppress potential re-emergence of malignant cells. Multiple clinical trials showed impressive complete response (CR) rates several years after CAR-T cell infusion in patients with various B cell malignancies^[2]. In some patients, CAR-T cells were still detectable even after a decade^[3]. Because of this property, they are hailed as living drugs, changing the perspective on how we view therapeutics.

Despite unprecedented benefits to patients, CAR-T cell therapies also pose considerable risks. Notable adverse events described in FDA approval summaries are cytokine release syndrome (CRS) and neurological toxicities^{[4]-[7]}, which typically occur within the first month of CAR-T cell treatment. Due to the frequency and severity of these adverse events, black

box warnings in the labeling of these cell therapy products have been issued, and management of these syndromes by healthcare professionals is a well-established priority. However, the long-term risks of secondary malignancies from CAR-T cells were undefined at the time of FDA approval.

How do T Cell Malignancies Occur After Administration of CAR-T Immunotherapy?

For all cell and gene therapies using integrating viral vectors, the potential risk of secondary cancers is well-recognized^[8] and included as a class warning in the U.S. Prescribing Information (USPI). Therefore, one requirement for the FDA approval of CAR-T therapies is 15-year post-marketing follow-up to observe the potential development of secondary malignancies. In the approval summary reports, the FDA was especially wary of two possibilities:

- 1) integrating viral vectors may change the expression of oncogenes and tumor suppressor genes by insertional mutagenesis, resulting in CAR-positive malignant T cells.
- 2) CAR-T cell-associated production of replication-competent lenti- or retrovirus in the body, which can cause secondary malignancies in other tissues.

Cases of insertional mutagenesis and detectable clonal outgrowth of CAR-T cells have been described in two patients, albeit without development into cancers^{[9],[10]}. In long-term observational studies, secondary cancers were observed, but CAR-positivity in those cancers was not detected, and T cell malignancies derived from CAR-T cells were not found^[2]. No significant correlation was seen between the incidence of secondary malignancies and retroviral genetic modification of immune cells^[11], and there have been no cases of CAR-T cell-related replication-competent lentivirus in the clinic^[12].

It is worth noting that cancer patients can have genetic predispositions which may raise the risk of developing secondary malignancies^[11]. First-line treatment of patients with chemo- and radiotherapy is also widely understood to induce mutations^[13] as well as immunosuppression, which can increase the likelihood of developing subsequent cancers. Furthermore, the suppression of the bone marrow by these treatments can lead to so-called clonal hematopoiesis, a process in which stem cells harboring mutations that

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provide a growth advantage preferentially expand. This can increase the patient's risk of developing blood cancers^[14]. Emerging evidence suggests that myelosuppression and clonal hematopoiesis can also be caused by CAR-T cell immunotherapies^[15], possibly due to the use of chemotherapy agents for lymphocyte depletion, which is a necessary step for CAR-T cell engraftment.

In their initial announcement, the FDA mentioned the testing of samples from secondary T cell malignancies for CAR transgene, which may provide hints as to what extent each of these factors could play a role. Further details of the investigation have not been disclosed^[1].

How Might This FDA Investigation Affect Current and Future CAR-T Cell Products?

If the risk of CAR-T cell-related malignancies is found to be serious, the existing class warning may be elevated to a black box warning. This would likely have little impact on current CAR-T cell products, which are already labeled with black box warnings for more frequent severe adverse events, such

as CRS and neurological toxicity. New CAR-T cell products currently in development for cancer treatment are also unlikely to face unexpected obstacles from this investigation.

Only in the worst-case scenario, CAR-T therapies may become more restricted to certain groups of patients with worse prognosis, more limited treatment options, or those who have already undergone multiple failed treatments, which would impede the potential of this technology. In addition, more stringent scrutiny may be placed on future CAR-T products for non-life-threatening conditions, such as autoimmune diseases.

How Can We Mitigate the Risk of Secondary T cell Malignancies in CAR-T Cell Immunotherapy?

If insertional mutagenesis is identified as a serious risk factor, more emphasis may be placed on controlling the conditions for viral transduction to minimize vector copy integrations. Transposon systems have been developed as alternatives to viral vectors, which do not bear the risk of becoming

replication-competent.

Nevertheless, they have the same drawback of random integration, and two cases of lymphomas derived from transposon-engineered CAR-T cell have been described^[16]. Adverse effects of random integration may be mitigated

with genome editing using TALEN or CRISPR/Cas^[9] technology, for instance. However, risks of off-target or repeated cutting, DNA double-strand breaks, and chromosomal rearrangements remain^[17].

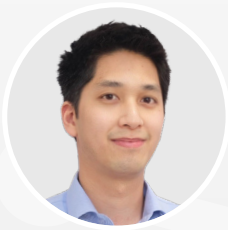
Allogeneic CAR-T therapies may see increasing support if genetic predisposition and pretreatment of patients with chemo- and radiotherapy are deemed more concerning. The use of healthy donor cells to produce CAR-T cells could circumvent these problems. The pre-infusion conditioning of patients may also see potential improvements. Even though aggressive lymphocyte depletion using cyclophosphamide and fludarabine is essential for the success of CAR-T therapy^[18], reducing myelotoxicity and mutagenicity may become a consideration for future changes to this treatment.

Closing Statement

The FDA's decision to investigate now, after 30,000 patients have received CAR-T cell therapy, left many people including top researchers in the cell and gene therapy field perplexed^[19]. In our opinion, this investigation represents the coming of age of CAR-T cell therapy, rather than an unnecessary attempt to hold back potential therapies. Since CAR-T cells are becoming more widely available to cancer patients and beyond, the FDA is merely playing its role as the protector and educator

of patients and healthcare workers by identifying potentially serious risks of CAR-T cell products. As they stated, "the overall benefits of these products continue to outweigh their potential risks"^[1]. We hope that the FDA will continue to work closely with healthcare professionals, but also with drug makers and the research community, to ensure safety and efficacy of CAR-T cell therapy.

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About Theragent

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