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Unanswered questions following reports of secondary malignancies after CAR-T cell therapy

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Reports of T cell malignancies after CAR-T cell therapy should be investigated, but existing data from follow-up studies suggest a low risk compared with other cancer treatments.

On 28 November 2023, the US Food and Drug Administration (FDA) announced T cell malignancies, including chimeric antigen receptor (CAR)-positive lymphoma, in patients who received treatment with BCMA- or CD19-directed autologous CAR-T cell immunotherapies. The FDA has determined that the risk of T cell malignancies is applicable to all approved BCMA-directed and CD19-directed genetically modified autologous CAR-T cell immunotherapies. These include tisagenlecleucel (Kymriah), lisocabtagene maraleucel (Breyanzi), axicabtagene ciloleucel (Yescarta), brexucabtagene autoleucel (Tecartus), idecabtagene vicleucel (Abecma) and ciltacabtagene autoleucel (Carvykti).

The FDA stated that the overall benefits of these products continue to outweigh their potential risks for their approved uses. However, it is investigating the identified risk of T cell malignancy with serious outcomes, including hospitalization and death, and is evaluating the need for regulatory action. The CAR-T products were approved with the requirement to conduct 15-year long-term follow-up observational safety studies to assess the long-term safety and the risk of secondary or subsequent malignant neoplasms occurring after treatment. The FDA now states that patients and participants of clinical trials receiving treatment with these products should be monitored life-long for new malignancies. The FDA hopes to provide the community with further information during early 2024.

Reported malignancies

The limited additional available information has only been disclosed through news reports, which quote the FDA as saying it is investigating 20 reports of T cell malignancy, including T cell lymphomas and leukemias. Manufacturers of CAR-T cells have treated more than 34,400 patients with these therapies. Information on the patients in which T cell malignancies have been observed and the products that they have been treated with has been scarce. An abstract at the 2023 American Society for Hematology (ASH) meeting reported on a CAR^+ T cell lymphoma after treatment with ciltacabtagene autoleucel[1]. The authors' conclusions from the analysis of this rare malignancy are that it was potentially driven by genetic mutations (such as TET2, NFKB2, PTPRB and/or JAK3), some of which may have existed in the form of a clone with malignant potential before cilta-cel manufacturing (for example, TET2 p.H1416R and JAK3 p.722I variants), meaning that it was present in the collected cells used to generate the CAR-T product. A potential contributory role of the CAR insertion in the 3′ untranslated region of *PBX2* to the development of T cell lymphoma remains unclear and cannot be excluded at this time.

Another 2023 ASH abstract on the clinical trial of a bi-specific CD19/CD20 CAR reported secondary or subsequent malignant neoplasms after CAR-T cell infusions observed in three patients[2]. Two of these were acute myeloid leukemia (AML) that occurred at 2 and 10 months after treatment, and the other was an Epstein–Barr virus (EBV)-positive cytotoxic T cell lymphoma that occurred after 8 months; the tumor biopsy was tested by quantitative PCR and the CAR transgene was negative. The authors state that none of these cases were related to the CAR product.

T cell homeostasis in vivo is controlled at the level of the T cell receptor by clonal competition and T cells are relatively resistant to genotoxicity. Under unusual settings, such as retroviral activation of JAK kinase[3], and at very high insertion copy numbers using a transposon system for CAR gene delivery[4], T cell lymphomas may be induced. The Center for International Blood and Marrow Transplant Research (CIBMTR) has captured 11,345 recipients of commercial CAR-T cells (at the time of writing), of whom 8,060 are enrolled in post-authorization safety studies that aim to detect secondary or subsequent malignant neoplasms. The median follow-up time of patients is 13 months, with a range from 0 to 69 months; 565 secondary or subsequent malignant neoplasms in 485 patients were reported in patients who had received commercial CAR-T cells, and 420 secondary or subsequent malignant neoplasms were identified in 357 patients from the post-authorization studies. As of 14 December 2023, there have been three reported T cell malignancies among these cases reported by the CIBMTR — one T cell large granular cell leukemia, one anaplastic T cell lymphoma, and one peripheral T cell lymphoma; none demonstrated aberrant expression of CD19 in the tumor cells based on routine clinical immunophenotyping.

The median time from CAR-T cell infusion to first subsequent neoplasm is 9 months, and the most common cancer types reported are non-melanomatous skin cancers (basal cell and squamous cell carcinoma) and therapy-related myelodysplasia or AML; these are expected based on the age and the prior treatment exposure of patients who receive CAR-T cells. Similarly, in a retrospective cohort of 420 children and young adults with B-cell acute lymphoblastic leukemia (B-ALL)

who received CD19 CAR-T cells between 2012 and 2019, the authors identified a secondary malignancy in 7 out of 420 (1.7%) patients. Diagnoses included cholangiocarcinoma, synovial sarcoma, malignant melanoma, papillary thyroid carcinoma and three myeloid neoplasms. The median time to diagnosis of secondary malignancies after the initial B-ALL diagnosis was 8.2 years, and after CD19 CAR-T cell infusion was 3.2 years[5].

In total, 20 cases of T cell malignancy have been reported out of 8,000 cases in the FDA Adverse Events Reporting System (FAERS) database. Given that an estimated 34,400 patients have received commercially available CAR-T cells so far, the rate of T cell malignancies observed is far lower than that seen with some other treatments. T cell malignancy has been observed, if rarely, after immune checkpoint blockade. In a case report of T cell lymphoma secondary to therapy with the pembrolizumab checkpoint inhibitor, the authors also reviewed the FAERS database. The incidence of T cell lymphoma secondary to pembrolizumab, nivolumab or ipilimumab was found to be 0.02%[6].

Before the approvals of CAR-T cell therapies, investigators interrogated the National Cancer Institute (NCI) Surveillance, Epidemiology, and End Results Program (SEERS) database and determined that patients with a first B cell lymphoma, an indication diagnosis for four of the six approved CAR-T cell therapies, had a 4.7-fold higher standardized incidence ratio of developing a second primary T cell lymphoma, with notable increased bi-directional risks for certain subtypes such as diffuse large B-cell lymphoma and angioimmunoblastic T cell lymphoma[7].

Safety monitoring

The fundamental question is whether there is a causal link between CAR-T therapy and these rare cases of T cell malignancy. Much is unknown about the T cell lymphomas in the cases reported to the FDA, including important patient characteristics such as age, prior therapies (including prior autologous or allogeneic stem cell transplantation), immune status, and other clinical features as well as the time from CAR-T infusion to the development of T cell lymphoma.

The clinical status of the patients in terms of immunosuppression, previous therapy, conditioning chemotherapy, and evidence of prior clonal hematopoiesis is also unknown. Related to the product, the vector copy number, integration site analysis and detection of the CAR transgene in the T cell lymphomas are all crucial information for analysis. These data are critical to determine any possible biological or causal relationship with CAR-T cells but can be difficult to obtain as measurements of CAR expression or integration are not commercially available as clinical diagnostic assays.

Although the CAR-T products were approved with the requirement for 15 years of monitoring, an unknown number of patients may not be in follow-up as recommended, and there may therefore be undetected or unknown early warning signs. The framework of the post-authorization safety studies requires patients to sign an informed consent to participate in follow-up studies. Reporting responsibilities reside with the CAR-T treatment center; however, both FAERS and CIBMTR reporting is voluntary, meaning that there is neither a reliable numerator nor a denominator to assess. It is estimated that over 34,400 patients have received commercial FDA-approved CAR-T cells worldwide, yet the FDA FAERS database included outcomes on just under 8,000 patients, despite FDA requirements for mandatory 15-year follow-ups.

How many cases have been reported to neither the FDA nor the CIBMTR, to one or the other, or to both? The CIBMTR estimates that it captures 65% of all commercial CAR-T cell treatments in the USA. Thus, the number of cases of T cell lymphoma might be underreported. Alternatively, the number of cases may be overestimated due to reporting bias; a case resulting in T cell malignancy is much more likely to be reported to the FDA than a case of another type of cancer.

Relative risk

Risks from CAR-T cell therapy can be put into context by comparison with other therapeutic modalities. Secondary or subsequent malignant neoplasms are well characterized after standard chemotherapy and radiotherapy[8]. These widely used therapies have substantial long-term side effects, including genotoxicity and predisposition to secondary cancers at a much higher rate than the rate suggested by the reported T cell malignancy cases following CAR-T cell therapy.

Hematopoietic stem cell transplant increases the risks of secondary cancers, although such risks are variable and impacted by patient-specific factors (such as age), underlying disease, prior therapies, type of transplant (autologous versus allogeneic), and post-transplant therapies. For patients with Hodgkin lymphoma and non-Hodgkin lymphoma (NHL) who undergo autologous stem cell transplantation, the risk of secondary hematological malignancies has been estimated to be as high as 8–29%[9], and serves as an example of how therapy before transplant may influence the risk of secondary or subsequent malignant neoplasms after transplant. However, most of these secondary cancers are myeloid-derived (AML and myelodysplastic syndromes). In patients with Hodgkin lymphoma, the relative risk of developing secondary NHL varies by inclusion of radiation in primary therapy, but is estimated to be 11- to 17-fold higher than an untreated population, and the increased risk has not plateaued after 40 years of observation[10].

Clonal selection of hematopoietic stem cells after gene therapy for sickle cell disease has recently been reported, in which an increased frequency of potential driver mutations associated with myeloid neoplasms or clonal hematopoiesis was observed in both genetically modified and unmodified cells[11]. An increased mutation rate in some patients with sickle cell disease and positive selective pressure on hematopoietic stem cells that contain pre-existing driver mutations could be mechanisms that increase leukemia risk in gene therapy trials for this disease.

Similarly, pre-existing mutations in patients receiving CAR-T cell therapies may in rare cases lead to secondary malignancies, with 'lineage switch' from ALL to AML in patients with KMT2A rearrangements being a notable example[12]. A recent report[13] found clonal hematopoiesis in 86% of patients receiving CAR-T cells before treatment among those with prolonged cytopenias, and this may place them at increased risk of secondary or subsequent malignant neoplasms. However, as indicated above, those rare cases in patients receiving CAR-T cells at present seem to be occurring at a much lower rate than conventional and long-accepted regimens of chemotherapy.

A review of 340 patients with various primary diagnoses, ages and prior or follow-on therapies who received gene-modified T cells, found no increased risk of subsequent malignancy in patients treated with retroviral genetically modified T cells compared with a control group of patients. In patients receiving retrovirally modified cells who did develop secondary or subsequent malignant neoplasms, assessment of 11 out of 16 subsequent tumors (where biopsies were available) showed no sample that was transgene positive. Replication-competent retrovirus testing of peripheral blood mononuclear cells was negative[14]. Several clinical centers with extensive experience in gene-modified

T cell clinical trials reported on absence of replication-competent retrovirus or lentivirus in dozens of vector lots, in more than 1,000 patient products, and in patient follow-up samples[15].

Recommendations

Considering the advanced relapsed or refractory hematological malignancies in patients receiving CAR-T cell therapies, the true nature and frequency of the risk of genotoxicity due to retroviral or lentiviral insertion is unknown and deserves thorough investigation and transparency. As current and past presidents of the American Society for Transplantation and Cellular Therapy (ASTCT), the European Society for Blood and Marrow Transplantation (EBMT), and the International Society for Cell & Gene Therapy (ISCT), senior scientific director of the Center for International Blood and Marrow Transplant Research (CIBMTR), chief scientific officer of the Parker Institute for Cancer Immunotherapy (PICI), committee chairs of these organizations, and immunotherapy developers and clinicians, it is our view that the benefits of CAR-T therapies continue to outweigh the potential risks in the vast majority of cases.

While we wait for the results of the FDA investigation, centers with expertise in cell therapy should continue to make commercial CAR-T products available to patients when this appears to be the best option possible (based on the most up-to-date and confirmed safety information). All those who administer CAR-T cell therapy should support the FDA long-term follow-up recommendations by participating in post-authorization safety studies, and promptly report subsequent malignancies, recognizing the FDA announcement states that patients and participants of clinical trials receiving treatment with these products should now be monitored life-long for new malignancies as well as leading, developing and conducting research in this area based on the most up to date and confirmed safety information.

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Competing interests

B.L.L. is an inventor on patents and/or patent applications licensed to Novartis Institutes of Biomedical Research and receives license revenue from such licenses; is a scientific founder of Tmunity Therapeutics and Capstan Therapeutics; and a member of the scientific advisory boards of Akron Biotech, Avectas, Capstan Therapeutics, Cellula Therapeutics, Immuneel Therapeutics, Immusoft, In8bio, Kite Gilead, Ori Biotech, Oxford Biomedica, ThermoFisher Pharma Services, UTC Therapeutics and Vycellix. M.C.P. received research support from Novartis, Kite Pharma, Bristol Myers Squibb, Janssen Consulting: Novartis and Gilead. J.E.C. is a cofounder of Twain Therapeutics, director at Dispatch Therapeutics and 3T Biosciences, and receives research funding from Janssen. D.L.P. reports stock and other ownership interests from Genentech, Roche (spouse former employment); consulting or advisory roles from Novartis, Kite/Gilead, Gerson Lehrman Group and Janssen (Johnson & Johnson), Bristol-Myers Squibb, Bluebird Bio, Angiocrine, Mirror Biologics, Capstan Therapeutics, Instill Bio, Sana Biotechnology and Verismo Therapeutics; received research funding from Novartis and Bristol-Myers Squibb; and reports royalties and other intellectual property from Novartis and Tmunity. D.L.P. is a patent inventor for use of CAR-T cells in CD19$^+$ malignancies, and was chair of the board of directors of the National Marrow Donor Program from October 2018 to October 2020. J.J.B. reports consulting fees from Sanofi, Sobi, SmartImmune, Immusoft, BlueRock, Advanced Clinical, Merck and Bluebird Bio. S.A.G. declares research and/or clinical trial support from Novartis, Servier, Vertex, Cellectis and Kite; and was a member of steering committees, scientific advisory boards or held consulting positions at Novartis, Allogene, Adaptimmune, GlaxoSmithKline, Vertex, Jazz, Kyttaro and Cabaletta. M.V.M. is an inventor on patents related to adoptive cell therapies, held by Massachusetts General Hospital (some licensed to Promab) and the University of Pennsylvania (some licensed to Novartis);

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