CAR T-cell therapy for a relapsed/refractory acute B-cell lymphoblastic lymphoma patient in the context of Li-Fraumeni syndrome

Liting Chen [ID], Bin Xu, Xiaolu Long, Jia Gu, Yaoyao Lou, Di Wang, Yang Cao, Na Wang, Chunrui Li, Gaoxiang Wang, Ying Wang, Li Zhu, Jin Wang, Haiyun An, Min Xiao, Yi Xiao, Jianfeng Zhou

Department of Hematology, Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology, Wuhan, China

Correspondence to
Jianfeng Zhou;
jfzhou@tjh.tjmu.edu.cn

Yi Xiao; yixiao@tjh.tjmu.edu.cn

ABSTRACT

Background Li-Fraumeni syndrome (LFS) is characterized as an autosomal dominant cancer predisposition disorder caused by germline *TP53* gene mutations. Both primary and therapy-related hematopoietic malignancies with LFS are associated with dismal outcomes with standard therapies and even allogenic stem cell transplantation (SCT).

Case presentation We reported a relapsed/refractory acute B-cell lymphoblastic lymphoma (B-LBL) patient in the context of LFS. He was identified to harbor a *TP53* c.818G>A (p.R273H) germline mutation, and his family history was significant for rectal carcinoma in his father, an unknown cancer in his sister and acute lymphoblastic leukemia in his brother and one of his sons. The patient received murine monoclonal anti-CD19 and anti-CD22 chimeric antigen receptor (CAR) T-cell "cocktail" therapy and achieved complete remission with negative minimal residual disease (MRD), as assessed by morphology and multiparameter flow cytometry. Fifteen months after murine monoclonal CAR T-cell "cocktail" therapy, the patient's B-LBL recurred. Fortunately, a round of fully human monoclonal anti-CD22 CAR T-cell therapy was still effective in this patient, and he achieved CR again and continued to be followed. Each time after infusion, the CAR T-cells underwent extremely rapid exponential expansion, which may be due to the disruption of *TP53*, a gene that can functionally control cell cycle arrest. Grade 4 and grade 1 cytokine release syndrome occurred after the first and second rounds of CAR T-cell therapy, respectively.

Conclusions This case provides the first report of the use of CAR T-cell therapy in a hematologic malignancy patient with LFS. As traditional chemotherapy and allogenic SCT are not effective therapy strategies for patients with hematologic malignancies and LFS, CAR T-cell therapy may be an alternate choice.

TRIAL REGISTRATION NUMBERS

ChiCTR-OPN-16008526 and ChiCTR1900023922.

INTRODUCTION

Li-Fraumeni syndrome (LFS) is a prototypical cancer predisposition syndrome caused by germline *TP53* gene mutations and was first described in 1969 by Drs Frederick Li and Joseph Fraumeni.[1–4] Patients with LFS have a predisposition to multiorgan tumorigenesis and are at increased risk of cancer-related morbidity. The LFS primary tumor spectrum includes soft tissue sarcomas, breast cancers, brain tumors, osteosarcomas and adrenocortical carcinomas.[5] Other cancers associated with LFS include ovarian, gastrointestinal, pancreatic, genitourinary, skin, renal, thyroid, prostate and lung cancers, as well as hematopoietic malignancies including leukemia, lymphoma and MDS. The incidence of hematopoietic malignancies in LFS is ~4%.[6] In addition, therapy-related hematopoietic malignancies are common in patients with LFS.[7]

Although patients with *TP53* germline mutations are being identified in a rapidly increasing number because of improved genetic screening techniques and the increased acceptability of clinical testing for germline mutations, there are more patients with hereditary cancer predisposition syndromes than we thought, especially children.[6 8] In a large genomic profiling study of 124 patients with childhood hypodiploid ALL, *TP53* alterations were observed in 91.2% of low-hypodiploid patients. Moreover, in 43.3% of the *TP53* mutation-carrying patients, the same mutations were also present in their non-tumor cells, suggesting that these were germline alterations and that this portion of patients had LFS.[9] In adult low-hypodiploid acute lymphoblastic leukemia (ALL) patients, the incidence of germline *TP53* mutations is not as high.[10] The obvious disparity in *TP53* germline mutation incidence between children and adult ALL patients suggests an early onset of disease, reflecting a high risk of cancer development.
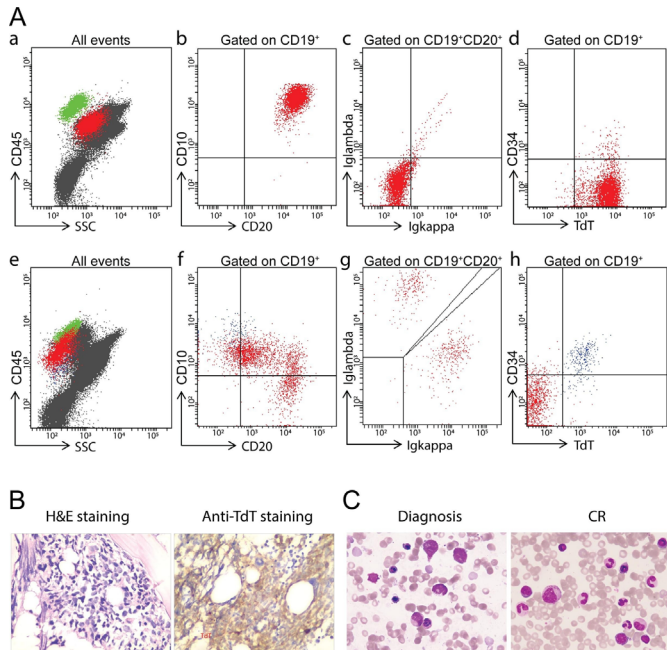
Figure 1 Immune and cytological analysis. (A) Phenotypic analysis of the bone marrow aspirate at diagnosis (a–d) and after fully human CAR T-cell infusion (e–h). Red dots represent CD19+ cells; green dots represent mature lymphocytes; blue dots represent progenitor B-cells; gray dots represent all the other cells. (B) Histological analysis of bone marrow sections by hematoxylin and eosin (H&E) and anti-TdT staining at diagnosis. (C) H&E staining of bone marrow aspirate slides at diagnosis (left) and at CR (right). CAR, chimeric antigen receptor; TdT, terminal deoxynucleotidyl transferase.
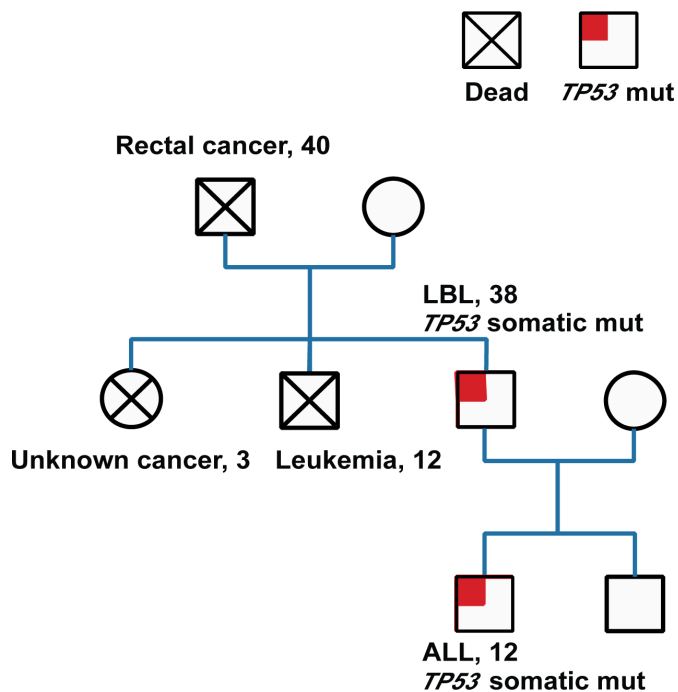

Figure 2 Family history studies. Pedigree charts. The number indicates the age at which the symptomatic tumor was detected. ALL, acute lymphoblastic leukemia; LBL, lymphoblastic lymphoma.

In regard to treatment, Pepper *et al* found that *TP53*-mutated lymphocytes in LFS patients had an intrinsic resistance to conventional chemotherapeutic drugs.[11] Moreover, a recent case series study showed that both primary and therapy-related hematopoietic malignancies with LFS were associated with dismal outcomes with standard therapies and even allogenic stem cell transplantation (SCT).[5] The cohort of patients in that study included five patients with a history of solid tumors and a known LFS diagnosis who developed therapy-related acute myeloid leukemia (AML) or MDS during follow-up surveillance, as well as two patients who were diagnosed with de novo acute leukemia with an LFS diagnosis established during leukemia therapy. They had no responses or very short responses to induction therapy. Five patients received allogenic SCT after chemotherapy but did not achieve long-term remission. All of them had extremely poor outcomes. In summary, standard therapy and allogenic SCT do not provide long-term control of primary or therapy-related hematopoietic malignancies that are associated with LFS, and more efficient treatment methods are needed for these situations. Here, we report a relapsed/refractory acute B-cell lymphoblastic lymphoma patient in the context of LFS who was identified to harbor a *TP53* c.818G>A (p.R273H) germline mutation. After failure of several lines of chemotherapy, this patient received murine monoclonal anti-CD19 and anti-CD22 chimeric antigen receptor (CAR) T-cell "cocktail" therapy and achieved complete remission (CR). Fifteen months after murine monoclonal CAR T-cell "cocktail" therapy, the patient's B-cell lymphoblastic lymphoma (B-LBL) recurred. Fortunately, a round of fully human monoclonal anti-CD22 CAR T-cell therapy was still effective in this patient, and he achieved CR again. Before this study, immunotherapeutic strategies had not been studied in LFS. The present study showed a potential therapeutic strategy for LFS with hematologic malignancies.

CASE REPORT

A 38-year-old man presented to a local hospital in April 2017 with pain throughout his body that had been occurring for a month. He was diagnosed with diffuse large B-cell lymphoma (DLBCL) at stage IV and had an international prognostic index (IPI) score of 3. The patient was started on induction chemotherapy with four courses of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and carboplatin) and then attained partial remission by positron emission tomography-CT. He then underwent two courses of R-ICE (rituximab, ifosfamide, carboplatin, and etoposide), two courses of DHAP (dexamethasone, high-dose-Ara-C, and platinol) and one course of R-MA (rituximab, methotrexate, and Ara-C). He did not achieve CR.

To pursue CAR T-cell therapy, the patient was referred to our hospital in January 2018. The multiparameter flow cytometry results showed that 2.9% of nucleated cells expressed surface CD20, CD19, CD10, CD38, and CD22,
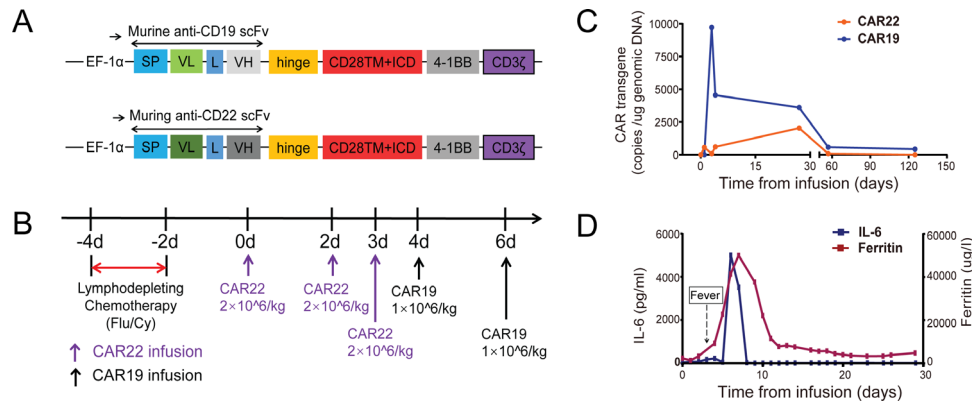
Figure 3 The protocol and response for murine monoclonal anti-CD19 and anti-CD22 CAR T-cell "cocktail" therapy. (A) Schematic diagram of murine anti-CD19 and anti-CD22 CAR vectors. SP, signal peptide; VH, variable H chain; L, linker; VL, variable L chain. (B) The protocol of murine CAR22 and CAR19 "cocktail" infusion in combination with chemotherapy. Chemotherapy included fludarabine and cyclophosphamide. (C) Murine CAR22 and CAR19 transgene copy numbers detected by ddPCR. (D) Levels of IL-6 and ferritin after murine CAR22 and CAR19 infusion. CAR, chimeric antigen receptor; ddPCR, droplet digital PCR.

as well as intracellular CD79a and TdT (terminal deoxynucleotidyl transferase), while Igκ and Igλ were negative. The immunophenotypic analysis is partially illustrated in figure 1A a–d. In addition, immunohistochemistry staining also showed that the bone marrow sections were TdT positive (figure 1B). According to these phenotypes, the patient was newly diagnosed with relapsed/refractory acute B-LBL.[12 13]

Hematological malignancy-targeted deep sequencing was performed on his plasma cell-free DNA and revealed a *TP53* c.818G>A (p.R273H) mutation with a mutation allele fraction (MAF) of 88.8% by droplet digital PCR. online supplementary material To test whether the patient harbored a germline *TP53* mutation, we analyzed his saliva samples and cells obtained by buccal swabbing. The *TP53* germline analysis revealed the same mutation. Family studies were initiated, and the patient's father, sister and brother had died from rectal carcinoma, an unknown tumor and ALL, respectively. Additionally, the same *TP53* variant was also detected in one of the patient's

sons, who suffered from ALL 18 months later. Given these results, the patient was considered to have LFS (figure 2).

To stop tumor progression, the patient was given decitabine ($25\,mg/m^2$) and liposomal doxorubicin before CAR T-cell therapy. Then, he received lymphodepleting chemotherapy with fludarabine ($25\,mg/m^2$) and cyclophosphamide ($300\,mg/m^2$) for 3 days (days −4 to −2). Subsequently, he received murine monoclonal anti-CD19 and anti-CD22 CAR T-cell "cocktail" therapy (figure 3).[14] Autologous CD22-targeted CAR T-cells (CAR22) ($2*10^6$ cells/kg) were infused on day 0, day +1, and day +2, followed by CD19-targeted CAR T-cells (CAR19) ($1*10^6$ cells/kg) on day +3 and day +5 (figure 3B). After day +5, he suffered a serious case of cytokine release syndrome (CRS) of grade 4 according to the grading system published by Lee *et al*, which lasted for 4 days and was characterized by a sustained high fever at 39°C to 40°C and hypoxia.[15] At the same time, he also developed headache, dizziness, aphasia, dyscalculia and delirium lasting for a week, which fit the criteria for immune effector
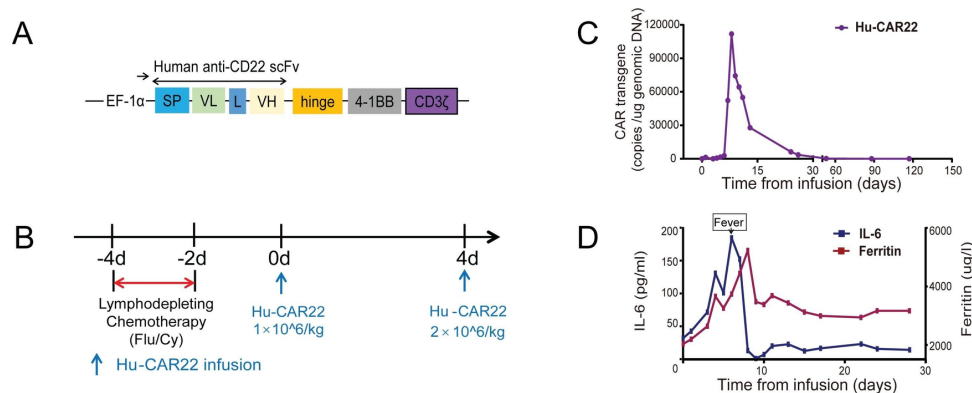

Figure 4 The protocol and response for the fully human monoclonal anti-CD22 CAR T-cell therapy. (A) Schematic diagram of fully human anti-CD22 CAR vectors. SP, signal peptide; VH, variable H chain; L, linker; VL, variable L chain. (B) The protocol of Hu-CAR22 infusion in combination with chemotherapy. Chemotherapy included fludarabine and cyclophosphamide. (C) Hu-CAR22 transgene copy numbers detected by ddPCR. (D) Levels of IL-6 and ferritin after Hu-CAR22 infusion. CAR, chimeric antigen receptor; ddPCR, droplet digital PCR.

cell-associated neurotoxicity, grade 3.[16 17] These symptoms were attenuated after plasmapheresis and steroid treatment. Both the CAR19 and CAR22 transgene copy numbers were tracked (figure 3C). At day +27, the patient achieved CR with negative MRD, as assessed by morphology and multiparameter flow cytometry of bone marrow aspirate samples.

Fifteen months after CAR T-cell "cocktail" therapy, the patient's B-LBL unfortunately recurred. In June 2019, he received fully human anti-CD22 CAR T-cell therapy after lymphodepleting chemotherapy with fludarabine and cyclophosphamide (figure 4). The cells carrying the fully human CAR transgene exhibited efficient expansion. The Cmax (maximum expansion) value reached $1.1*10^5$ copies/μg genomic DNA at day +8 (figure 4C). At day +30, the patient achieved CR again (figure 1A e–h, C). He is currently in remission 4 months after fully human CAR T-cell therapy and continues to be followed.

DISCUSSION

An international consensus on the components of a cancer screening program for *TP53* mutation carriers is lacking, and therefore, the personalized guidance for *TP53* mutation carriers varies across institutions, regions, and countries. However, this situation has improved in recent years. In 2015, the MD Anderson Cancer Research Center announced the beginning of a program named LEAD (Li-Fraumeni Syndrome Education and Early Detection) for patients with LFS, which offers a comprehensive screening plan for LFS patients to detect cancers as early as possible. Furthermore, several surveillance protocols for *TP53* mutation carriers have been proposed in Australia, the USA, and Canada.[4 18–20] Nevertheless, in most countries, effective risk management approaches in carriers of *TP53* germline mutations remain to be established, particularly in children.

In the present patient, screening for *TP53* germline mutations was not performed, and LFS was not established during diagnosis and the beginning of treatment. The presence of his *TP53* germline mutation was considered when the MAF of the *TP53* c.818G>A (p.R273H) mutation detected in his plasma circulating tumor DNA was consistently above 50%, even when he achieved complete remission by multicolor flow cytometry. Thus, lipid biopsy may be applied as a method to discover germline mutations.

Patients with DLBCL that is refractory to chemotherapies or that has relapsed after SCT have a poor prognosis. Two CAR T-cell products were approved for use in DLBCL. Axi-cel is the first CAR T-cell product to be FDA approved for DLBCL. The overall response rate was 83%, with 58% of patients having complete responses.[21] The second anti-CD19 CAR T-cell product to receive FDA approval for DLBCL is tisa-cel. The overall response rate to tisa-cel in adult relapsed or refractory DLBCL was 52%; 40% of subjects had complete responses.[22] CAR T-cell therapy also showed promise in children and adults with refractory and relapsed B-cell ALL. In a reported trial, after receiving tisa-cel, 90% of patients achieved CR with 6-month event-free survival and OS values of 67% and 78%, respectively.[23]

Antigen escape-related relapse is a major challenge for long-term disease control in CAR T-cell therapy. Our group previously reported that treatment of relapsed/refractory (r/r) ALL patients with a cocktail of both CAR19 and CAR22 prevented antigen escape of CD19-CD22+blasts0.[24] In this case, we first administered sequential CAR T-cell infusions targeting CD19 and CD22 to prevent antigen escape. This CAR T-cell "cocktail" was efficient, and the patient achieved CR with negative MRD, which lasted for 15 months. Furthermore, this study also indicated that a fully human CAR may still work even if the patient relapses after infusion of T-cells carrying a murine monoclonal antibody-based CAR.

This case provides evidence for the use of CAR T-cell therapy in hematologic malignancy patients with germline *TP53* mutations. Each time after infusion, the CAR T-cells underwent extremely rapid exponential expansion. A recent study reported that *TET2*-disrupted anti-CD19 CAR T-cells exhibited growth advantages and displayed a central memory phenotype, which suggested that some key gene mutations may alter the function of CAR T-cells.[25] In this case, we supposed that the extremely rapid exponential expansion was due to the disruption of *TP53*, a gene that can functionally control cell cycle arrest.

In conclusion, this is the first time that immunotherapeutic strategies have been used in LFS. Since LFS is associated with dismal outcomes with standard therapies and even allogenic SCT, CAR T-cell immunotherapy may be a promising treatment method. We suggest that CAR T-cell therapy be considered in LFS patients with r/r B-cell lymphoma or leukemia. As *TP53* mutations will be present in CAR T-cells derived from LFS patients, attention should be paid to potential extremely rapid exponential expansion and CRS.

ORCID iD
Liting Chen http://orcid.org/0000-0002-7786-1226

REFERENCES

1 Li FP, Fraumeni JF. Soft-Tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? *Ann Intern Med* 1969;71:747–52.

2 Chompret A, Abel A, Stoppa-Lyonnet D, *et al*. Sensitivity and predictive value of criteria for p53 germline mutation screening. *J Med Genet* 2001;38:43–7.

3 Zerdoumi Y, Aury-Landas J, Bonaïti-Pellié C, *et al*. Drastic effect of germline TP53 missense mutations in Li-Fraumeni patients. *Hum Mutat* 2013;34:453–61.

4 McBride KA, Ballinger ML, Killick E, *et al*. Li-Fraumeni syndrome: cancer risk assessment and clinical management. *Nat Rev Clin Oncol* 2014;11:260–71.

5 Swaminathan M, Bannon SA, Routbort M, *et al*. Hematologic malignancies and Li-Fraumeni syndrome. *Cold Spring Harb Mol Case Stud* 2019;5. doi:10.1101/mcs.a003210

6 Bougeard G, Renaux-Petel M, Flaman J-M, *et al*. Revisiting Li-Fraumeni syndrome from TP53 mutation carriers. *J Clin Oncol* 2015;33:2345–52.

7 Valdez JM, Nichols KE, Kesserwan C. Li-Fraumeni syndrome: a paradigm for the understanding of hereditary cancer predisposition. *Br J Haematol* 2017;176:539–52.

8 Wasserman JD, Novokmet A, Eichler-Jonsson C, *et al*. Prevalence and functional consequence of TP53 mutations in pediatric adrenocortical carcinoma: a children's Oncology Group study. *J Clin Oncol* 2015;33:602–9.

9 Zhang J, Walsh MF, Wu G, *et al*. Germline mutations in predisposition genes in pediatric cancer. *N Engl J Med* 2015;373:2336–46.

10 Holmfeldt L, Wei L, Diaz-Flores E, *et al*. The genomic landscape of hypodiploid acute lymphoblastic leukemia. *Nat Genet* 2013;45:242–52.

11 Pepper C, Thomas A, Hoy T, *et al*. Leukemic and non-leukemic lymphocytes from patients with Li Fraumeni syndrome demonstrate loss of p53 function, Bcl-2 family dysregulation and intrinsic resistance to conventional chemotherapeutic drugs but not flavopiridol. *Cell Cycle* 2003;2:52–7.

12 Loghavi S, Kutok JL, Jorgensen JL. B-Acute lymphoblastic leukemia/lymphoblastic lymphoma. *Am J Clin Pathol* 2015;144:393–410.

13 Lassoued K, Nuñez CA, Billips L, *et al*. Expression of surrogate light chain receptors is restricted to a late stage in pre-B cell differentiation. *Cell* 1993;73:73–86.

14 Wang N. Anti-CD19 and anti-CD22 CAR T-cell "cocktail" therapy for Patients with Refractory/Relapsed B-Cell MalignanciesBlood. *Blood*.

15 Lee DW, Gardner R, Porter DL, *et al*. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood* 2014;124:188–95.

16 Neelapu SS, Tummala S, Kebriaei P, *et al*. Chimeric antigen receptor T-cell therapy — assessment and management of toxicities. *Nat Rev Clin Oncol*.

17 Rice J, Nagel S, Randall J, *et al*. Chimeric antigen receptor T cell-related neurotoxicity: mechanisms, clinical presentation, and approach to treatment. *Curr Treat Option Ne* 2019;21.

18 Tempero MA, Malafa MP, Al-Hawary M, *et al*. Pancreatic adenocarcinoma, version 2.2017, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw* 2017;15:1028–61.

19 Villani A, Tabori U, Schiffman J, *et al*. Biochemical and imaging surveillance in germline TP53 mutation carriers with Li-Fraumeni syndrome: a prospective observational study. *Lancet Oncol* 2011;12:559–67.

20 Villani A, Shore A, Wasserman JD, *et al*. Biochemical and imaging surveillance in germline TP53 mutation carriers with Li-Fraumeni syndrome: 11 year follow-up of a prospective observational study. *Lancet Oncol* 2016;17:1295–305.

21 Locke FL, Ghobadi A, Jacobson CA, *et al*. Long-Term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1-2 trial. *Lancet Oncol* 2019;20:31–42.

22 Schuster SJ, Bishop MR, Tam CS, *et al*. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med* 2019;380:45–56.

23 Maude SL, Frey N, Shaw PA, *et al*. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med* 2014;371:1507–17.

24 Wang N, Hu X, Cao W, *et al*. Efficacy and safety of CAR19/22 T-cell cocktail therapy in patients with refractory/relapsed B-cell malignancies. *Blood* 2020;135:17–27.

25 Fraietta JA, Nobles CL, Sammons MA, *et al*. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. *Nature* 2018;558:307–12.