CLINICAL ONCOLOGY JOURNAL

Clinical Oncology Journal

ISSN: 2766-9882

Refining Immunotherapy of Human B-cell Non-Hodgkin's Lymphoma by CD19 CAR T Cell: Current and Future Perspectives

Mckenna Raeann Gleason and Ali R Jazirehi*

Department of Biological Sciences, College of Natural and Social Sciences, California State University, Los Angeles (CSULA), Los Angeles, CA 90032, USA

*Corresponding author: Ali R Jazirehi, CLS, Ph.D., Department of Biological Sciences, California State University, Los Angeles, BS 134, 5151 State University Drive, Los Angeles, CA 90032, USA. Tel: +1-323-343-2050; E-mail: ajazire@calstatela.edu

Article Type: Research Article

Compiled date: May 08, 2020

Volume: 1

Issue: 1

Journal Name: Clinical Oncology Journal

Journal Short Name: Clin Oncol J

Publisher: Infact Publications LLC

Article ID: INF1000032

Keywords: Immunotherapy; Resistance; Non-Hodgkin's Lymphoma; Chimeric antigen receptor; Suicide gene; HDCAi; Celebrex; Apoptosis

Cite this article: Mckenna RG, Ali RJ. Refining immunotherapy of human B-cell non-hodgkin's lymphoma by CD19 CAR T Cell: current and future perspectives. Clin Oncol J. 2020;1(1);1-6.

Abstract

Non-Hodgkin's Lymphoma (NHL) is responsible for 4% of all cancer cases in the United States, but its conventional treatment options have experienced limited success. Patients develop resistances to the standard chemotherapy, CHOP, as well as Rituximab, a chemosensitizing agent used in concert with CHOP. Chimeric antigen receptor T cell (CAR-T cell) therapy may be a viable option for patients with refractory/relapsed NHL. In this new immunotherapy, a CAR is genetically added to the surface of T cells, allowing the patient's own T cells to selectively recognize, bind, and eliminate malignant B cells. CARs are typically engineered to be CD19 specific as that is a common antigen on B cells, allowing the recognition of malignant B cells such as those present in NHL. However, there have been cases that show the development of resistances to CD19 CAR-T cells, as well as fatal cases of cytokine storm that result from cytokine release by active CAR-T cells after completion of the treatment. To address these problems and improve the efficacy of CAR-T cell immunotherapy, CAR therapy could be used in combination with FDA-approved drugs such as histone deacetylase inhibitors (HDACis) or celecoxib (Celebrex), or it could incorporate a suicide gene. The suicide gene may be implemented with gene-directed enzyme prodrug therapy (GDEP), CRISPR/iCasp9, or therapeutic mAb-mediated mechanisms.

Introduction

Non-Hodgkin's Lymphomas (NHL) are a heterogeneous group of malignancies that originate from lymphoid precursor cells [1]. The most common type of NHL is diffuse large B cell lymphoma (DLBCL) accounting for 30% of new cases, and the second most common type of NHL is follicular lymphoma (FL), accounting for 22% of new cases [2]. NHL can occur in patients of any age; however, over half of the newly diagnosed patients are 65 or older at their time of diagnosis. NHL accounts for 4% of all cancer cases in the United States [3].

NHL is categorized into two groups: high grade or low grade. Low-grade cases are slow growing and may be referred to as indolent lymphomas, the most common of which is FL. Other types of low-grade NHL include mantle cell lymphoma, marginal zone lymphoma, small lymphocytic lymphoma, lymphoplasmacytic lymphoma, and skin lymphoma [4]. Alternatively, high grade NHL is fast growing and may be referred to as aggressive lymphomas. The most common high-grade NHL types are DBCL, Burkitt, lymphoma, and peripheral T cell lymphoma [5].

Treatment options for NHL

Treatment plans for patients with NHL can vary greatly depending on the specific type, grade, and extent of NHL; however, the most common treatment plans may include radiotherapy, surgery, chemotherapy (CHOP), or chemotherapy with a sensitizing agent, such as rituximab.

Radiation is the most utilized treatment option for most lymphoma types, especially when diagnosed in its early stages. Low grade NHL may use radiation alone with typical dosages of 24 Gy in 12 fractions. High grade NHL uses combined modality treatments (CMT) of radiation with a typical dosage of 30 Gy in 15 fractions paired with chemotherapy [6]. In patients who present bulky masses, radiation can also be used after the completion of chemotherapy to consolidate the mass [6].

The standard chemotherapy used to treat NHL is the first-generation treatment, CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone). Cyclophosphamide, vincristine, and doxorubicin are administered intravenously, and prednisone is taken orally in the form of a tablet. As this treatment lacks the specificity to target cancer cells exclusively, CHOP treatment entails many adverse side effects including: increased risk of infection, bruising, bleeding gums, fatigue, indigestion, hair loss, swelling in the face, hands, and feet, diarrhea, loss of appetite, sore mouth, bladder inflammation and nail changes among others [7]. CHOP is often prescribed in 3–4 cycles; however, there is significant relapse when CHOP is administered alone because the body becomes refractory to CHOP alone over time. This refraction can be resolved with the addition of a sensitizing agent in concert with CHOP, such as rituximab.

Combination of immunotherapy and chemotherapy for the treatment of non-Hodgkin's Lymphoma

Rituximab is an anti-CD20 monoclonal antibody that chemosenitizes drug-resistant NHL cells through the downregulation of anti-apoptotic factors (such as Bcl-2, Bcl-xL) and inhibition of STAT-3 activity, resulting in better patient outcomes when paired with CHOP [8]. Rituximab is typically administered intravenously to patients with relapsed or refractory, CD20-positive B cell NHL at a dosage of 375 mg/m2 once weekly for four weeks [9]. In a recent study, the effects of CHOP and R-CHOP in patients with DLBCL were compared. Patients treated with a CHOP-like treatment exhibited 64% overall response (OR) and 39% complete remission (CR) while patients treated with R-CHOP exhibited 100% OR and 89% CR [10]. However, in patients with DLBCL, 30%–40% of patients treated with R-CHOP relapse and 10% are primary refractory. The NHL cells will eventually become resistant to R-CHOP as well, highlighting the need for a new treatment modality. After treatment with Rituximab or autologous stem cell transplantation, long term survival for these patients is 48% [11].

About 50% of relapsed DLBCL patients may continue the typical treatment plan which includes allogeneic hematopoietic stem cell transplantation (HCT). HCT is not always a viable treatment option due to the lack of compatible allogeneic donor, refractory disease, or cumulative toxicity from previous chemotherapy treatments [11].

Surgery can confirm or refute the diagnosis of NHL, but it is not often used to treat NHL because of the previous success of chemotherapy, radiation and HCT. Surgery can provide palliation and diagnosis for certain cases of NHL through the removal of an affected organ, but as NHL is a blood disease, surgery is not often used as a treatment option for NHL [12].

New Immunotherapy for NHL

A solution to refractory/relapsed NHL may be observed in the emerging immunotherapy, chimeric antigen receptor T cell (CAR-T) therapy. In this therapy, a patient's own T cells (or T cells from an allogeneic donor) are isolated, activated, and genetically modified to express a CAR on the surface of the T cell. This allows T cells to recognize and bind a specific antigen. CAR binding signals T cell proliferation, cytolysis, and cytokine secretion to kill the target cell expressing the complementary antigen [13]. CAR-T cell therapy addresses the problem of selectively eliminating cancer cells by circumventing the MHC-restriction requirement. Most tumors downregulate surface MHC to avoid their destruction, but the genetic addition of a CAR eliminates the need for MHC-mediated peptide presentation altogether [14].

Structure of Chimeric Antigen Receptor (CAR)

There are four generations of CARs. First-generation CARs have an extracellular binding domain, a hinge region, a transmembrane domain and an intracellular signaling domain with the CD3V chain domain as the intracellular signaling domain. The extracellular domain typically consists of single-chain variable fragments (scFvs) that are derived from tumor antigen-reactive antibodies. These first-generation cells exhibited anti-tumoral activity but lacked long term persistence [15]. The second-generation CARs exhibit improved proliferation, cytokine secretion, resistance to apoptosis and *in vivo* persistence due to an addition of co-stimulatory domains such as CD28 or 4-1BB. The third generation further improved *in vivo* persistence as well as effector functions. The fourth generation CARs (also referred to as TRUCKs or armored CARs) combine second-generation CAR expression with other anti-tumoral factors such as cytokines, additional co-stimulatory ligands or enzymes that assist in the degradation of solid tumors, enhancing its efficacy [15].

CAR-T cells are commonly engineered to be CD19 specific to treat B-cell malignancies such as NHL, meaning that the scFv is specifically the CD19 receptor [16]. CD19 is typically expressed in pre-B cells and is maintained through maturity but is absent on plasma cells. This allows CAR-T cells to recognize all B cell malignancies with specificity, thus, preventing peripheral cytotoxicity [17]. Patients can be preconditioned with chemotherapy before CAR-T cell infusion. The preconditioning

regimen commonly consists of radiation with high-dosage (2.5 mg/m^2 x 2 d) cyclophosphamide or cyclophosphamide combined with fludarabine (25 mg/m^2 x 5 d) [18]. The chemotherapy is used to reduce cancer burden as much as possible before the infusion and to deplete endogenous leukocytes that could inhibit the antitumor ability of the transferred CAR-T cells [17]. CD19 CAR-T cells have produced impressive results in clinical trials with NHL patients.

Two CAR-T cell products have been tested and approved by the FDA: Axicabtagene ciloleucel (Yescarta) for DLBCL and tisagenlecleucel (Kymrial) for adult DLBCL subtypes. Clinical trials of these products have exhibited high efficacy, increased homogeneity of the treatment, more predictable toxicities and more consistent proliferation following administration. A recent clinical trial treated 14 patients with DLBCL and 14 patients with FL using tisagenlecleucel (Kymrial), a complete response (CR) of 43% of the DLBCL patients and 71% of the FL patients was achieved. Moreover, at a median follow up of 28.6 months, 86% of patients with DLBCL who had a response and 89% of patients with FL who had a response had maintained the response, suggesting that Kymrial could be a good long-term solution for NHL patients [19]. However, only modest to low response rates have been observed overall in clinical trials of CD19 CAR-T cell therapy for patients with NHL [20].

Mechanisms of Resistance to CD19 CAR-T cells

The decreased efficacy of CAR-T cell therapy in NHL patients is proposed to be the result of cell-induced apoptosis-resistance in patients. CD19 expression may be downregulated following CD19 CAR-T cell infusion. One trial reported that patients with low to undetectable levels of CD19 had experienced a frameshift mutation that caused the deletion ofa C-terminal tyrosine critical for signal transduction through CD19 [21]. Patients who had a defect in the CD81 gene were also unresponsive to CD19-redirected CAR-T cell therapy as CD81 works in conjunction with CD19/CD21 complex in B cell receptor (BCR) signaling and could affect CD19 expression [22]. NHLs may also utilize alternative differentiation and signaling pathways to evade recognition by CD19 CAR T cells [20]. The apoptotic resistance may also be caused by abnormal levels of pro- and anti-apoptotic proteins expressed by tumor cells, physical barriers or an immunosuppressive tumor microenvironment [20]. Many cancers follow a trend of decreased proapoptotic factors (Bax and Bak) and increase anti-apoptotic factors (Bcl-2, Mcl-1, Bcl-xL, and Bfl-1) [23,24]. Physical barriers may include a lack of receptors necessary for the adhesion of T cells to tumor cells [20]. And finally, tumor cells can release immunosuppressive cytokines such as transforming growth factor (TGF)-β and interleukin (IL)-10 to inhibit T-cell proliferation [25].

One of the leading issues with CAR-T cell therapy is long-term toxicities due to the persistence of activated CAR-T cells that remain in circulation after treatment ends. These activated CAR-T cells can release large quantities of cytokines. Increased cytokine levels may cause cytokine release syndrome or cytokine storm if they become increasingly high. A cytokine storm develops a positive feedback loop that promotes additional cytokine production though proinflammatory signals and causes the entire body to develop severe inflammation [20]. Cytokine storm is normally successfully treated with neutralizing antibodies or steroids, but it can be fatal. Patients treated with CAR-T cell therapy have also been reported to experience neurological toxic effects such as delirium, aphasia, hallucinations and seizure-like activity [25].

Approaches to Increase CD19 CAR T-cell Efficacy

The use of FDA-approved drugs, such as histone deacetylase inhibitors (HDACi) and celecoxib, combined with CAR T-cell therapy or the incorporation of a suicide gene system within the CAR T-cell could potentially help to address the current resistance mechanisms and adverse side effects of CAR T-cell therapy, thereby improving its efficacy.

Histone deacetylase inhibitors (HDACis) can specifically target histone deacetylases and alter gene transcription selectively [26]. There are four classes of HDACs: class III are NAD-dependent and Classes I, II, and IV are zinc-dependent [27]. Suberoylanilide hydroxamic acid (SAHA) orZolinza™ can inhibit classes I, II, and IV deacetylases [28]. HDCAis are strong anti-tumor agents because they help regulate the expression of anti-apoptotic genes. HDCAis upregulate tumor necrosis factor-related apoptosis-induced ligand (TRAIL) death receptor-5 (DR5), which induces apoptosis in tumor cells, and HDCAisplus TRAIL induce Bid cleavage and activate a caspase cascade that promotes extrinsic apoptotic pathway [29]. HDCAis also promote intrinsic apoptosis by reducing the expression of anti-apoptotic proteins and increasing expression of proapoptotic proteins (Bim, Bax, Bak) [30]. The HDACipanobinostat, also referred to as LBH589, can inhibit classes I, II, and IV HDACs and depresses tumor angiogenesis [31].

Celecoxib, also referred to as Celebrex, is a nonsteroidal anti-inflammatory drug (NSAID) that inhibits cyclooxygenase-2 (Cox-2) [32]. Cox-2 promotes tumor growth through its enzymatic product, prostaglandin E2 (PGE2) [33]. PGE2 promotes the expression of IL-6 and upregulates Mcl-1, providing optimal conditions for tumor growth [34]. Therefore, Celebrex can indirectly inhibit PGE2 to depress tumor growth. Celebrex can also initiate intrinsic apoptosis as it downregulates Mcl-1, downregulates survivin (a member of the IAP family that inhibits caspase activation) and promote caspase-dependent apoptosis in Cox-2 deficient gastric cancer cells [35–37].

The incorporation of a suicide gene within the CAR-T cell could prevent cytokine release syndrome. In this method, a genetically encoded molecule capable of inducing apoptosis is added to selectively destroy the infused CAR-T cells [38]. This reaction would ensure that all CAR-T cells would be eliminated after the cessation of the treatment before unwanted toxicities could

manifest.

The suicide system may be implemented using gene-directed enzyme prodrug therapy (GDEP), which causes nontoxic compounds in CAR-T cells to turn into toxic compounds using metabolic machinery [39]. This method was utilized in the activation of the Herpes simplex thymidine kinase (HSV-TK)/ ganciclovir (GCV) suicide system. HSV-TK phosphorylates acyclovir and GCV [40]. The phosphorylation results in its incorporation into a growing DNA chain via DNA polymerase and ultimately causes chain termination and apoptosis [41]. This approach allows alloreactive T cells to be eliminated in allogeneic environments; however, as HSV-TK has a viral origin, it could trigger an immune system and signal the rejection of CAR-T cells, limiting the success of this approach [20].

Suicide genes may also be induced using inducible caspase 9 (iCasp9). The genome-editing technology, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 are able to produce CD19-redirected CAR T-cells directed to T-cell receptor (TCR) α constant (TRAC) locus in a mouse model of ALL. This resulted in uniform CAR T-cell expression in human peripheral blood T-cells as well as an increase in T-cell proliferation and cytotoxic potential [42]. iCasp9 can be used in combination with AP 1903 (a chemical inducer of dimerization) to induce the cleavage of caspase 9, which will activate caspase 3, resulting in apoptosis [43].

Additionally, the use of a therapeutic mAb-mediated mechanism can be used to help CAR-T cells evade immunogenic responses. This therapy most commonly uses the chimeric mouse anti-human CD20 mAb (Rituximab) [20]. Its methodology consists of the genetic manipulation of CAR-T cells to express CD20 receptors, followed by the transfer of the adopted T-cells, followed by anti-CD20 mAb administration, and finally the elimination of unwanted/excess CAR-T cells [44-46]. However, as CD20 is also expressed on healthy T-cells, this treatment can weaken humoral immunity and cause off-target toxicity [47].

CAR-T cells can also be engineered to express or not express other receptors that improve their efficacy. For example, adenosine is produced in immunosuppressive concentrations in the tumor microenvironment [48]. In clinical trials, the efficacy of CAR-T cell therapy was greatly improved when the adenosine receptor A2AR was targeted in a murine model of HER2+ self-antigen tumors. The efficacy was only further improved when used in combination with other checkpoint inhibitorslikemAbs directed against program death (PD)-1 [49].

Conclusion

CAR-T cell therapy has experienced significant success in treating NHL; however, its efficacy can be further improved. A general improvement could come from optimizing the number of infused transgenic CAR-T cells, but there are also particular subsets of NHL that require additional advancements for CAR-T cell therapy to remain a viable option for them. The subset of NHL cases that either inherently possess or develop CD19 CAR-T cell resistances require a new means to circumvent the resistance, the most promising of which include the addition of celecoxib or HDACisused in combination with CAR-T cell therapy.

Furthermore, in CAR-T cell therapy, T cells persist after treatment cessation resulting in delirium, aphasia, hallucinations, seizure-like activities, fever, hypotension, and hypoxia. To eliminate unwanted CD19 CAR-T cells after treatment completion, a suicide gene should be incorporated into the genetically modified T-cells. This suicide gene could be implemented using GDEP, CRISPR/iCasp9 or mAb-mediated mechanisms.

Competing Interests

The authors claim no conflicts of interest.

References

1. Blosser N, Jupp J, Yau P, Stewart D. Clinical Pharmacokinetic and Pharmodynamic Considerations in Treating Non- Hodgkin Lymphoma. Clin Pharmacokinet. 2016. Available from: https://link.springer.com/article/10.1007%2Fs40262-019-00807-8
2. Marcus R, Hagenbeek A. The therapeutic use of rituximab in non-Hodgkin's lymphoma. Eur J Haematol Suppl. 2007;(67):5–14.
3. Key Statistics for Non-Hodgkin Lymphoma. American Cancer Society. 2019. Available from: https://www.cancer.org/cancer/non-hodgkin-lymphoma/about/key-statistics.html.
4. Low grade NHL. 2019. Available from: https://www.cancerresearchuk.org/about-cancer/non-hodgkin-lymphoma/types/low-grade.
5. High grade NHL. 2019. Available from: https://www.cancerresearchuk.org/about-cancer/non-hodgkin-lymphoma/types/high-grade.
6. DM Jayalathike, A Stevens, S Paneesha, S Chaganti, Y Hassan, AM Zarkar. Treatment outcomes using involved field and involved site radiotherapy for NHL and HL: Retrospective analysis from a large UK Radiotherapy center. Hematol Oncol. 2017;35(2):333.
7. Treatment for high grade non-Hodgkin lymphoma. 2019. Avaliable from: https://about-cancer.cancerresearchuk.org/about-cancer/non-hodgkin-lymphoma/treatment/high-grade?_ga=2.25058170.1572411530.1578872137-1431857555.1577786953
8. Ali R Jazirehi, Benjamin Bonavida. Cellular and molecular signal transduction pathways modulated by rituximab (rituxan, anti-CD20 mAb) in non-Hodgkin's lymphoma: implications in chemosensitization and therapeutic intervention. Oncogene. 2005;(13):2121–2143.
9. Plosker G, Figgitt, D. Rituximab: A Review of its Use in Non-Hodgkin's Lymphoma and Chronic Lymphocytic Leukemia. Drugs. 2003;(8):803–843.
10. Oliver C, Guillermo C, Martínez P, Díaz L. Comparison between

CHOP-like and R-CHOP in diffuse large B cell and follicular lymphoma. Rev Med Chile. 2013;(7):844–852.
11. Hopfinger G, Jäger U, Worel N. CAR-T Cell Therapy in Diffuse Large B Cell Lymphoma" Hype and Hope. Hemasphere. 2019;3(2):e185.
12. OrockE. Surgery for non-Hodgkin's lymphoma. Oncol Rev. 2015;(9):1.
13. Jackson HJ, Rafiq S, Brentjens RJ. Driving CAR-T cells forward. Nat Rev Clin Oncol. 2016;13(6):370–383.
14. Cheadle EJ, Gilham DE, Thistlethwaite FC, Radford JA, Hawkins RE. Killing of non-Hodgkin lymphoma cells by autologous CD19 engineered T cells. Br J Haematol. 2005;129:322–332.
15. Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR-T cells-challenges and opportunities in translating innovative treatment concepts. EMBO Mol Med. 2017;9(9):1183–1197.
16. Sommermeyer D, Hill T, Shamah S. Fully human CD19-specific chimeric antigen receptors for T-cell therapy. Leukemia. 2017;31(10):2191–2199.
17. Kochenderfer JN, Dudley ME, Kassim SH. Chemotherapy refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol. 2015;33(6):540–549.
18. Davila ML, Brentjens R, Wang X, Rivière I, Sadelain M. How do CARs work? Early insights from recent clinical studies targeting CD19. Oncoimmunology. 2012;1(9):1577–1583.
19. Schuster SJ, Svodoba J, Chong EA, et al. Chimeric antigen receptor T cells in refractory B-cell lymphomas. N Engl J Med. 2017;377(26):2545–2554.
20. Ali R Jazirehi and Tam NM Dinh. Approaches to Improve Clinical Efficacy of CD19-redirected Chimeric Antigen Receptor (CD19 CAR) T Cell Immunotherapy of Non-Hodgkin's Lymphoma.Canc Therapy & Oncol Int J. 1017;6(2):555682.
21. Van Zelm MC, Reisli I, van der Burg M, Castaño D, van Noesel CJ, van Tol MJ, Woellner C, Grimbacher B, Patiño PJ and van Dongen JJ. An antibody-deficiency syndrome due to mutations in the CD19 gene. N Engl J Med. 2006;354:1901–1912.
22. Cherukuri A, Shoham T, Sohn HW, Levy S, Brooks S, Carter R, et al. The tetraspanin CD81 is necessary for partitioning of coligated CD19/CD21-B cell antigen receptor complexes into signaling-active lipid rafts. J Immunol. 2004;172:370–380.
23. Bentires-Alj M, Dejardin E, Viatour P, Van Lint C, Froesch B, Reed JC, et al. Inhibition of the NF- κB transcription factor increases Bax expression in cancer cell lines. Oncogene. 2001;20:2805–2813.
24. Placzek WJ, Wei J, Kitada S, Zhai D, Reed JC, Pellecchia M. A survey of the anti-apoptotic Bcl-2 subfamily expression in cancer types provides a platform to predict the efficacy of Bcl-2 antagonists in cancer therapy. Cell Death Dis. 2010;1(5):e40.
25. Onea AS, Jazirehi AR. CD 19 chimeric antigen receptor (CD 19 CAR)-redirected adoptive T-cell immunotherapy for the treatment of relapsed or refractory B-cell Non-Hodgkin's Lymphomas. Am J Cancer Res. 2016;6(2):403–424.
26. Gui CY, Ngo L, Xu WS, Richon VM, Marks PA. Histone deacetylase (HDAC) inhibitor activation of p21WAF1 involves changes in promoter-associated proteins, including HDAC1. Proc Natl Acad Sci, USA. 2004;101:1241–1246.
27. Smith KT, Workman JL. Histone deacetylase inhibitors: anticancer compounds. Int J Biochem Cell Biol. 2009;41:21–25.
28. Xu WS, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of action. Oncogene. 2007;26:5541–5552.
29. Nakata S, Yoshida T, Horinaka M, Shiraishi T, Wakada M, Sakai T. Histone deacetylase inhibitors upregulate death receptor 5/TRAIL-R2 and sensitize apoptosis induced by TRAIL/APO2-L in human malignant tumor cells. Oncogene. 2004;23:6261–6271.
30. Zhang XD, Gillespie SK, Borrow JM, Hersey P. The histone deacetylase inhibitor subericbishydroxamate regulates the expression of multiple apoptotic mediators and induces mitochondria-dependent apoptosis of melanoma cells. Mol Cancer Ther. 2004;3:425–235.
31. Atadja P. Development of the pan-DAC inhibitor panobinostat (LBH589): successes and challenges. Cancer Lett. 2009;280:233–241.
32. Gupta RA, DuBois RN. Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. Nat Rev Cancer. 2001;1:11–21.
33. Greenhough A, Smartt HJ, Moore AE, Roberts HR, Williams AC, Paraskeva C, et al. The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. Carcinogenesis. 2009;30:377–386.
34. Gallouet AS, Travert M, Bresson-Bepoldin L, Guilloton F, PangaultC,Caulet-Maugendre S, et al. COX-2–Independent Effects of Celecoxib Sensitize Lymphoma B Cells to TRAIL-Mediated Apoptosis. Clin Cancer Res. 2014;20: 2663–2673.
35. Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, et al. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science. 2001;292:727–730.
36. Jendrossek V. Targeting apoptosis pathways by Celecoxib in cancer. Cancer Lett. 2013;332:313–324.
37. Pang RP, Zhou JG, Zeng ZR, Li XY, Chen W, Chen MH, et al. Celecoxib induces apoptosis in COX-2 deficient human gastric cancer cells through Akt/GSK3β/NAG-1 pathway. Cancer Lett. 2007;251:268–277.
38. Jones BS, Lamb LS, Goldman F, Di Stasi A. Improving the safety of cell therapy products by suicide gene transfer. Frontiers in Pharmacology. 2014;5:254.
39. Springer CJ, Niculescu-Duvaz I. Prodrug-activating systems in suicide gene therapy. The Journal of clinical investigation. 2000;105(9):1161–1167.

40. Bonini C, Ferrari G, Verzeletti S, Servida P, Zappone E, Ruggieri L, et al. HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. Science. 1997;276:1719–1724.
41. Moolten FL. Tumor chemosensitivity conferred by inserted herpes thymidine kinase genes: paradigm for a prospective cancer control strategy. Cancer Res. 1986;46(10):5276–5281.
42. Eyquem J, Mansilla-Soto J, Giavridis T, van der Stegen SJ, Hamieh M, Cunanan KM, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature. 2017;543(7643):113–117.
43. Gargett T, Brown MP. The inducible caspase-9 suicide gene system as a "safety switch" to limit on-target, off-tumor toxicities of chimeric antigen receptor T cells. Front Pharmacol. 2014;5:235.
44. Introna M, Barbui AM, Bambacioni F, Casati C, Gaipa G, Borleri G, et al. Genetic modification of human T cells with CD20: a strategy to purify and lyse transduced cells with anti-CD20 antibodies. Hum Gene Ther. 2000;11(4):611–620.
45. Serafini M, Manganini M, Borleri G, Bonamino M, Imberti L, Biondi A, et al. Characterization of CD20-transduced T lymphocytes as an alternative suicide gene therapy approach for the treatment of graft-versus-host disease. Human Gene Therapy. 2004;15(1):63–76.
46. Griffioen M, van Egmond EH, Kester MG, Willemze R, Falkenburg JF, Heemskerk MH. Retroviral transfer of human CD20 as a suicide gene for adoptive T-cell therapy. Haematologica. 2009;94(9):1316–1320.
47. Minagawa K, Zhou X, Mineishi S, Di Stasi A. Seatbelts in CAR therapy: How safe are cars? Pharmaceuticals. 2015;8(2):230–249.
48. Ohta A, Gorelik E, Prasad SJ, Ronchese F, Lukashev D, Wong MK, et al. A2A adenosine receptor protects tumors from antitumor T cells. Proc Natl Acad Sci, USA. 2006;103(35):13132–13137.
49. Beavis PA, Henderson MA, Giuffrida L, Mills JK, Sek K, Cross RS, et al. Targeting the adenosine 2A receptor enhances chimeric antigen receptor T cell efficacy. J Clin Invest. 2017;127(3):929–941.