The Libyan International Medical University

Faculty of Basic Medical Science

How to eliminate solid tumors by CAR T-cell therapy

Student name : Asma Abdul Salam Ahmed Ibrahim

Student number : 2094

Supervised by: Dr.Huda Gargoum

Assisted by: Dr. Nawar

Report Submitted to fulfill the requirements for Scientific Research Activity

Date of Submission: …/……/ 2020

Abstract

T cells expressing chimeric antigen receptors (CART) have shown significant promise in clinical trials to treat hematologic malignancies, but their efficacy in solid tumors has been limited ,It's because solid tumors don't seem to be the identical . Oncolytic viruses have the potential to act in synergy with immunotherapies because of their immunogenic oncolytic properties and also the opportunity of incorporating therapeutic transgenes in their genomes. hypothesized that an oncolytic adenovirus armed with an EGFR-targeting, bispecific T-cell engager (OAd-BiTE) would improve the end result of CART-cell therapy in solid tumors. demonstrated that OAd-BiTE–mediated oncolysis significantly improved CART-cell activation and proliferation, while increasing cytokine production and cytotoxicity, and showed an in vitro favorable safety profile. BiTEs secreted from infected cells redirected CART cells toward EGFR within the absence of FR-a, thereby addressing tumor heterogeneity. BiTE secretion also redirected CAR-negative, nonspecific T cells found in CART-cell preparations toward tumor cells. The combinatorial approach improved antitumor efficacy and prolonged survival in mouse models of cancer compared with the monotherapies, and this was the results of an increased BiTEmediated T-cell activation in tumors . these results demonstrated that the mixture of a BiTE-expressing oncolytic virus with adoptive CART-cell therapy overcomes key limitations of CART cells and BiTEs as monotherapies in solid tumors and encourage its further evaluation in human trials.

Introduction

TCRs (T-cell receptors) are expressed by T cells, and profoundly and actively influence lymphocyte activity in modulating the immune response. Manipulating T cell receptor properties and numbers to boost immune responses is a very active area of immunotherapy research. TILs (tumor-infiltrating lymphocytes) are being actively investigated for their anti-cancer properties, as tumor biopsies often contain immune cells conditioned to the tumor microenvironment. These cells can be harvested, studied, and subjected to chemokine fortification prior to reintroduction. So, CAR-T cells (chimeric antigen receptor-T cells) refers to genetically engineered T cells which express surface receptors specifically targeting cancer cell-expressed surface antigen. (1)

CARs are engineered fusion proteins that contain an extracellular antigen-binding domain composed of a single chain variable fragment derived from an antibody and intracellular signaling domains, which are involved in the initiation of T-cell signaling and downstream T-cell effector functions . First-generation CARs consisted of only the T-cell receptor complex CD3 chain domain and antigen recognition domains, showed minimal clinical success, and were characterized by very low levels of engraftment in patients Second-generation CARs containing costimulatory domains ,typically either CD28 , were hypothesized and shown to augment CAR T-cell survival and proliferation . The inclusion of a costimulatory domain dramatically increased the antitumor efficacy and persistence of CAR T cells . CAR modified T cells targeting CD19 have shown activity in case series of patients with acute and chronic lymphocytic leukaemia , B-precursor acute lymphoblastic leukaemia (B-ALL) is the most common malignancy in childhood and B-cell lymphomas . Although CAR T-cell therapies are on a fast track to approval by the FDA for B-cell malignancies there is active investigation into building better CAR T cells for treating hematologic malignancies and solid tumors.(2)

For now CD-19 is the most attractive target in this immunotherapy Encouragingly, T cells expressing the CD19-CARs have achieved unprecedented therapeutic efficacy in malignant hematological diseases with up to 90% complete remission rate in ALL and more than 60% in non-Hodgkin’s lymphoma (NHL) .(3)

While CD19-directed CAR T cells have been very effective for the treatment for B-cell lymphoid malignancies, the use of CAR T cells for solid tumors has not been as successful. The early CAR T cell studies in patients with solid tumors demonstrated feasibility with limited to minimal efficacy. For example, Kershaw et al., administered a first-generation Car T cells directed against the alpha-folate receptor (FR) followed by high dose IL-2 (HD IL-2) in patients with metastatic ovarian cancer. Patients tolerated the infusion well, however the trial was limited by poor persistence of the CAR T cell product, with the majority of patients having absent circulating CAR T cells by three weeks.(4)

Unfortunately, no objective responses were seen, and all patients had disease progression . While this first study likely did not demonstrate clinical responses secondary to the use of a first generation CAR T cell product, which were limited by a lack of co-stimulation, clinical outcomes for trials using CAR T cells in solid tumors even using high later generation products have also been largely disappointing , A key limitation of CART therapy in solid tumors is the immunosuppressive tumor microenvironment , which preferentially recruits regulatory T cells, tumor-associated macrophages (TAM), and myeloid-derived suppressor cells, which can mediate CART-cell inhibition. Another important hurdle encountered with CART cells is tumor immune evasion due to antigen loss. CD19-negative relapses have emerged as a major problem in patients with hematologic malignancies treated with CD19-directed immunotherapies.(4-5)

Oncolytic adenoviruses (OAd) may mitigate these challenges to T-cell therapy within the tumor microenvironment. By selectively infecting and replicating in malignant cells, OAds may provide the twin good thing about debulking the tumor through selective lysis and providing a viral danger signal that would create a more appropriate environment for T-cell expansion and functionality . OAds may be genetically modified to selectively express a therapeutic transgene within the tumor microenvironment . Bispecific T-cell engagers (BiTE) are immunotherapeutic molecules consisting of an anti-CD3 single-chain variable fragment (scFv) fused to an antitumor-associated antigen scFv via a versatile linker. Blinatumomab, a BiTE targeting CD19, was approved by the FDA for treatment of acute lymphoblastic leukemia, and a number of other other BiTEs targeting various antigens . has reported the generation of ICO15K-cBiTE, an OAd that secretes an EGFR-targeting BiTE (OAd-BiTE) upon infection of malignant cells and show that the OAd-BiTE induces robust and specific T-cell activation and proliferation upon infection of cancer cells enhancing the antitumor efficacy of the virus in mouse xenograft models of cancer. but exogenous administration of BiTEs to treat solid tumors has several drawbacks, including limited capacity to penetrate into the tumor and a short serum half-life, thus requiring continuous systemic infusions that can lead to increased toxicities .(5)

Aim of the study test the hypothesis that combining CART cells targeting folate receptor alpha (FR-a) with OAd-BiTE could improve CART-cell therapy .

Materials and methods

search in the google scholar using a combination of the following keywords: “CAR-T therapy,” “chimeric antigen receptor T cell,” “solid tumor,” and “Oncolytic Virus".

Used Cells

SKOV3, HCT116, Panc-1, and NCI-H226 ..

Generation of CART cells

Primary lymphocytes from normal donors .Briefly, CD4þ and CD8þ T cells were cultured separatelywith CD3/CD28-activating Dynabeads (Invitrogen) at a bead-t cell.

Oncolytic adenoviruses

The OAds ICOVIR15K and ICOVIR15K-cBiTE (OAd-BiTE) .

Cytotoxicity assays

Cytotoxic killing of target cells was assessed using the xCELLigence Real-Time Cell Analyzer System (ACEA Biosciences).

Xenograft models

mice were purchased from The Jackson Laboratory and bred and housed in the vivarium at the University of Pennsylvania in pathogen-free conditions.Xenograft tumors were established by subcutaneous injection of indicated tumor cells into the flanks of NSG mice.After mean tumor volume reached 100 mm3, mice were treated with one intratumoral injection. After 3 or 5 days, mice were treated with one intravenous injection CAR T cells (50%CARþ, 1:1CD4:CD8),a secondCART-cell injection was performed after one week. Tumors were measured once or twice a week.

Bioluminescent imaging

T cells expressing CBR luciferase were used to detect trafficking of the T cells to the tumor. Anesthetized mice were imaged using a Xenogen Spectrum system and Living software. Mice were given an intraperitoneal injection and imaged at the peak of photon emission . Tumor radiance was measured around the tumor contour.(5)

Results

CART-cell therapy of solid tumors induces tumor escape To assess the killing capacities of CART cells in vitro. CART cells exhibited dose-dependent and antigen-specific cytotoxicity.However, total tumor cell clearance was observed only in the cell line . animals bearing SKOV3 tumors were treated with two doses of CART cells or left untreated. CART-cell treatment significantly delayed tumor growth, but eventually, tumors grew exponentially (Fig. 1F). Several areas of the tumor expressed FR-a and contained large numbers of CD3þ T cells, suggesting that, despite successfully infiltrating the tumors, CART cells had become hypofunctional.

also found areas of the tumors with no FR-a expression in both treated and untreated tumors, suggesting that heterogeneity of antigen expression could also be a mechanism for tumor escape.

A BiTE-armed oncolytic adenovirus improves tumor killing of CART cells

hypothesized that combining CART cells with a BiTE-armed oncolytic virus could enhance tumor killing by using a multimodal killing mechanism. To test this, FR-CART cells were used in combination with OAd-BiTE . so the One of the main challenges of CART cells for the treatment of solid tumors is the selection of cancer-specific targets to avoid the on-target . Overall, the combination of OAd- BiTE and FR-CART cells resulted in improved killing of cancer cells and showed a favorable safety profile.(5)

Discussion

CART cells face several hurdles in solid tumors, including tumor-antigen heterogeneity and an immunosuppressive tumor microenvironment . this studies demonstrate that the combined action of virus, BiTE, and CART cells leads to a synergistic control of the tumor growth and increased overall survival without compromising safety. The combination of CART cells with OAd-BiTE enhanced the activation, proliferation, and killing activity of CART-cell preparations in vitro. These enhanced effector functions are due, in part, to the BiTE-mediated engagement of the untransduced (CAR–) T cells present in the CART-cell preparations. this results demonstrated that redirection of the UTD cells within CART-cell preparations toward tumor antigens can maximize the antitumor effects of the CART therapy. this study reports a strategy that exploits the effector potential of CAR-negative cells found in CART-cell preparations. also show that BiTE expression by an OAd can redirect CARþ T cells toward a secondary tumor antigen in the event of loss or lack of expression of the CAR-targeted antigen.

Consistent with the enhanced tumor cell killing shown in vitro the combination of CART cells and OAd-BiTE also displayed an improved antitumor effect in vivo when compared with each monotherapy. found that human CART cells alone were able to accumulate to high numbers in the tumor tissue and initially induced tumor regression. However, despite persisting in the tumor microenvironment, CART cells lost their capacity to control tumor growth during the course of the treatment. These findings are consistent with previous reports indicating that CART cells undergo rapid loss of functional activity in solid tumors as a result of a multifactorial process, which includes the expression of T-cell–inhibitory enzymes and surface inhibitory receptors and loss of CAR expression by the T cells . in this report demonstrated that in the presence of OAd-BiTE, T cells showed enhanced in vivo activation 15 days after T cell administration. observed an increased percentage of T cells expressing the proliferation marker Ki67. Different factors can account for this increased T-cell activation and proliferation.

In the presence of the EGFR-targeting BiTE, CART cells can be activated throughCD3in the event of CAR expression loss by T cells or lack of FR-a expression by tumor cells. OAds can induce tumor debulking, which may create a more suitable environment for T-cell activation. In this regard,OAds can also activate the innate immune response through the release of tumor antigens, pattern recognition receptor ligands, and danger signals. One of the main limitations of this study is that the use of NSG mice precludes the study of the effects of the interaction between the OAd and the tumor immune cells, which could further enhance the antitumor effects of the combination therapy. Solid tumors exhibit antigen heterogeneity, making it challenging to identify a universal target expressed throughout the whole tumor. found that tumor xenografts contained areas with low , suggesting that tumor heterogeneity could have a key role in tumor escape.

Tumor cells expressing the CAR-targeted antigen can also escape T-cell therapy by losing the expression of the antigen especially when targeting nondriving or nononcogenic proteins Various mutations in the CD19 locus, in addition to alternative splice variants of CD19, have been associated with the development of CART19-resistant acute lymphocytic leukemia . Diminished CD22 surface expression on B-cell ALL cells has also been recently identified as a mechanism for relapse following CD22-CAR therapy. The simultaneous targeting of different tumor antigens has been reported as a promising solution for antigen loss in hematologic malignancies and solid tumors, with dual and bispecific CART cells providing superior potency than pooled combination of CART cells (34, 36, 41). A main drawback of these approaches and other targeted therapies is that selecting safe solid tumor targets can be challenging due to on-target, off-tumor side effects .One advantage of our therapy is the restricted BiTE expression from the oncolytic virus in cancer cells. The combination of OAd-BiTE with FR-CART showed a favorable in vitro safety profile in keratinocytes and fibroblasts compared with EGFR-targeting CART cells alone or in combination with FR-CARTs. Thus, the localized BiTE expression in the tumor microenvironment adds a level of selectivity which is lacking in CART cells or BiTEs as monotherapies.(5)

Conclusion

shown that anti–FR-a CART cells and a BiTE-expressing OAd displayed synergistic antitumor effects in vivo by enhancing T-cell activation, which resulted in prolonged survival of tumor-bearing mice. These data provide the rationale to test this combination therapy in human trials in patients with solid tumors.

Reference

1. Turtle, C. J., Hanafi, L. A., Berger, C., Gooley, T. A., Cherian, S., Hudecek, M., ... & Robinson, E. (2016). CD19 CAR–T cells of defined CD4+: CD8+ composition in adult B cell ALL patients. The Journal of clinical investigation, 126(6), 2123-2138.
2. Maus, M. V., & June, C. H. (2016). Making better chimeric antigen receptors for adoptive T-cell therapy.
3. Maude, S. L., Frey, N., Shaw, P. A., Aplenc, R., Barrett, D. M., Bunin, N. J., ... & Mahnke, Y. D. (2014). Chimeric antigen receptor T cells for sustained remissions in leukemia. New England Journal of Medicine, 371(16), 1507-1517.
4. Heyman, B., & Yang, Y. (2019). Chimeric antigen receptor T cell therapy for solid tumors: current status, obstacles and future strategies. Cancers, 11(2), 191.
5. Wing, A., Fajardo, C. A., Posey, A. D., Shaw, C., Da, T., Young, R. M., ... & Guedan, S. (2018). Improving CART-cell therapy of solid tumors with oncolytic virus–driven production of a bispecific T-cell engager. Cancer immunology research, 6(5), 605-616.