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Short Communication

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Multimodal Approach with CAR-T and CAR-NK Cells for Synergistic Action to Treat Cancer

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Recent years have seen a craze about the T-cell immunotherapy for B-cell malignancies, and there is growing interest in NK-CAR therapy. In this paper, I discuss the similarities and differences between the CARs in the NK and T-Cells. Further, explore the possible theoretical concept of a multimodal approach towards treating cancer. Whether the two genetically modified cells could provide an added advantage acting synergistically to complement each other's therapeutic qualities or counteract each other's downfall. This short communication is an attempt to pique interest to understand the mechanisms of NK cell and T cell biological interaction in the presence of a therapeutic weapon "CAR" to target tumor.

T cells are the adaptor immune cells that recognize foreign antigens generating a specific immune response to attack the foreign molecule. T cells have the ability to differentiate into memory T cells for long term protection [1]. For generating a CAR-T product, autologous T cells are separated out from the patient blood and genetically modified using Lentiviral systems under closed GMP conditions and multiplied in numbers. These genetically engineered CAR-T cells are reinfused into the patient after preconditioning with lympho-depleting regimen [2].

The initial development of CAR T-cell therapies were focused largely on Acute Lymphoblastic Leukemia (ALL), the most common cancer in children [45]. ALL arises in B cells that express CD 19 receptor [3]. CD19 is primarily expressed on both benign and most malignant B cells with extremely limited non-B cell expression [4]. The T-cells derived from patient are genetically wired to target the CD19 receptors to kill. The T-cell CAR came into limelight when the CD 19 T-CARs were tested in clinical trial with adult B-cell lymphoma patients [5, 6, 7]. 80% of the 63 patients tested achieved complete remission after median follow-up of 2 years [8].

The most famous case of a patient who benefitted from CAR T cell therapy is probably that of Emily Whitehead, a child suffering from recurrent acute lymphoblastic leukemia (ALL) [9]. CAR-T therapy provides alternative for patients whose cancer returns after chemotherapy or is non-responsive to stem cell transplantation.

Since the success seen in B-ALL patients [10, 11], the CD 19 CAR is registered in several clinical trials to treat several other cancers such as, AML, acute myeloid leukemia; DLBCL, diffuse large B cell lymphoma [12]; HL, Hodgkin lymphoma; Non-Hodgkin lymphoma [13], MM, multiple myeloma [14]. In 2016 alone, 62 new CAR-T cell clinical trials have been entered into ClinicalTrials.gov. While the CAR-T has seen a lot of success with B-cell ALL, the clinical effectiveness of the CAR-T is not pronounced in solid tumors yet [15]. Although the CD 19 CAR is labeled as a living/miracle drug, for patients whose cancer refracted after bone marrow transplantation or after two or three lymphodepleted chemotherapeutic treatments, it is not without its fair share of adverse reactions such as on/off target effects, neurotoxicity and excessive cytokine release from dying tumor cells. 4 out of 5 patients survive these adverse effects through control regimes for these side effects. In some cases, the CD 19 is downregulated or mutated in tumor cells rendering resistance to CD19 expressing cells from the CAR- T cells [16, 17]. Scientists are trying to relieve these unwanted side effects by synthesizing SMART CARs that harbor equipment/modules to deliver the functionalities that decrease the side effects. Of those strategies include incorporating elements in the chimeric antigen receptor that facilitate the binding of exogenous small molecule when excess cytokines such as IL-6, TNF-α are released from the tumor cells to induce suicide signaling [20]. Another approach might be to engineer another targetable cell-surface antigen, such as CD20 or epidermal growth factor receptor, to the CAR itself. This would make the CAR-T cell susceptible to rituximab and cetuximab, respectively, similarly making possible a selective apoptosis. Such control over the CAR-T cells may allow modulation of toxicity and make cellular immunotherapy more broadly applicable to patients who are not likely to tolerate severe CRS (cytokine release syndrome) [18, 21].

In the same category are fourth-generation CARs, so-called TRUCKs or armored CARs, combine the expression of a second-generation CAR with factors that enhance anti-tumoral activity, such as cytokines, co- stimulatory ligands, or enzymes that degrade the extracellular matrix of solid tumors [19]. To enhance the safety of CAR-T cell therapy, so-called smart T cells which are either equipped with a suicide gene or include synthetic control devices

are under non-clinical and clinical investigation [20].

NK cells are natural effector cytotoxic cells of innate immune system that do not require a specific antigenic signal to kill [22]. This makes them unique in their capability to be used as biological weapons. Several studies have shown pre-clinically that the NK cells harbor a greater capacity to kill the tumor than the T-cells [23, 24]. However, in vivo, due to their short life span in peripheral blood, the inability to localize in the tumor, the short coming in the inhibitory tumor microenvironment makes them ineffective in treating cancer. The process of isolating NK cells and growing them in culture, genetically modifying the NK cells to express the CAR is as time bearing and tedious process as the culture and manipulation of T cells [25, 26].

A plausible alternative was identified by Klingemann H.G. group to use a lymphoma patient derived cell line NK-92 [27]. These are less expensive alternative than the patient derived NK cells and can be grown and manipulated at a minimal cost. In vitro studies and mouse models using NK-92 CARs have shown significant killing of tumors by these genetically engineered NK-92 cells [28, 29, 30].

The presence of CAR overcomes the inhibitory signals at the tumor microenvironment largely because of the independent activation of the CAR through binding the tumor antigen. The presence of the inhibitory signals that downplay the action of NK cells do not alter this activation at the CAR receptor. Many mouse models have shown enhanced killing by the presence of CAR on the NK cells [31, 32, 33, 34].

The clinical significance of the same effect is yet to be seen in clinical trials. In humans, increasing doses of up to 5 billion cells of anti-CD33 CAR NK-92 CAR did not elicit any adverse reactions in the patients with relapsed acute myeloid leukemia. In contrast, although it was shown that the biological markers of tumor proliferation decreased transiently, overtime the patients relapsed and no significant improvement was observed with the administration of CAR NK-92 cells [36].

While T cell pose the risk of inducing the Graft vs host disease [51], and NK Cells could possibly augment the T-cell alloreactivity with donor lymphocyte infusion [52]. NK-92 cell line on the other hand, offers a bright future in that they are cultured cell lines that can be grown in numbers, avoiding the complex process of isolating and expansion from the patient blood, as well as preventing the GVHD risk with autologous NK cells [53, 54].

Thus, NK-92 cells offer opportunities to produce an off-the-shelf allogenic product that could be readily available for immediate clinical use. The most significant aspect of NK cells is their cytotoxic effect with no specific activation signals required [36]. The question then becomes could these NK-92 cells be modified to increase their lifespan in the peripheral blood so the cytotoxic effects could be prominent?

Confounding factors to achieve efficient cytotoxicity against tumor such as immune suppression by the tumor microenvironment, short peripheral life span, and tissue penetration although glaring is being worked on by several groups [37]. For example, therapeutic approaches to enhance the cytotoxic effect using interferon IL-15 is under investigation in clinical trials. This is achieved by engineering NK cells to express cytokines for boosting NK cell toxicity [35, 38, 39]. Many other strategies to improve NK cells use in cancer immunotherapy are very well discussed by the Katayoun Rezvani group [40].

Liu E et.al., discuss the generation of CAR-CD19+ NK cells that are believed to address all the limitations described above. CB-derived NK cells are genetically engineered with a retroviral vector (iC9/CAR.19/IL15) [41]. that (1) incorporates the gene for CAR CD19 to redirect specificity to CD19; (2) ectopically produces IL-15, a cytokine crucial for NK cell survival and proliferation [42]. and (3) expresses a suicide gene, inducible caspase-9 mnhb (iC9), that can be pharmacologically activated to eliminate transduced cells as needed [43]. These genetic modifications enabled the engineered NK cells to persist in sufficient numbers with impressive functional competence to effectively kill B cell leukemia or lymphoma cells in xenograft mouse models [41, 48, 49].

Now that we have seen and understood in action the CAR-T immunotherapy and the budding CAR- NK promise to the cure, I am intrigued to see could these two therapies work together to create a synergistic environment cancelling out the negativities of each other and bringing a greater effect than each by itself?

First challenge with CAR-T cells is the downregulation or mutation of the target receptor on the tumor cells. NK cells do not operate that way. Upon recognition of stressed or tumor cells, the expression of activating receptors such as NKp30, NKp46, and NKG2D increases on the NK cells resulting in enhanced antitumoral activity of NK cells [44, 46]. Studies indicate that the life span of NK cells is dependent on the maintenance of steady levels of IL-15 cytokine. Further, the IL-15 stimulation is essential for expression of MCL-1 (Myeloid cell leukemia -1) to prolong the life span of NK cells [47].

Another major challenge with CAR-T cells is the CRS which can be severe and may cause fatalities. Unlike IL-6 produced with CAR-T, the NK cells produce IFN-γ, macrophage inflammatory protein (MIP)-1α (CCL3), GM-CSF, and moderate levels of TNF-α. This cytokine/chemokine profile induces immune cell infiltration and promote endogenous antitumor immunity. This release of cytokines could serve as trigger for the infused CART-T cells to infiltrate into the tumor site.

As it is widely accepted that the NK cells have higher cytotoxic killing potential than the T-cells, this potential advantage of genetically modified NK cells could be explored further in solid tumors. Studies have shown that selective expression of peptide harmon relaxin (Rlx) inhibits the collagen formation associated with tumor development. Incorporating expression of Rlx into CAR-T cells could offer dual powers to enhance the efficacy of T- cells in solid tumors [50]. Novel concept could use genetically modified T-cells to invade the tumor microenvironment and start degrading the extracellular matrix. Following then, with the targeted NK cells could show a greater destruction of the tumor tissue by infiltration and homing into the environment.

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