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Tucaresol-Cyclophosphamide Combination Therapy: Proposal for a Safe, Affordable Alternative to CAR T-Cell Therapy

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Abstract

Chimeric Antigen Receptor (CAR) T-cell therapy is a newer immunotherapeutic process in which genetic engineering is used to incorporate a receptor protein into a patient's T-cells thereby permitting the modified T-cells to recognize and eradicate tumors. Initially, CAR T-cell therapy was reserved as a last resort when standard cancer treatments failed to provide significant efficacy but subsequently, CAR T-cell therapy is finding use against earlier stage cancers. Since 2017, seven CAR T-cell therapies have attained FDA approval for treatment of hematological cancers. The latest approval, November 8, 2024, is for treatment of B-cell acute lymphoblastic leukemia. However, CAR T-cell therapy does not always provide a lasting anticancer response which leads to loss of tumor remission. The percent loss of tumor remission depends upon the type of hematological cancer being treated. Although currently limited to hematological cancers, CAR T-cell therapy provides cancer patients a significant increase in survival unattainable with traditional cancer treatment regimens. However, two significant issues accompany CAR T-cell therapy. The first is multiple toxicity issues which although occurring individually in a low percentage of patients, taken together constitute a significant probability of encountering a potentially fatal side effect. The second problem is the high cost of CAR T-cell therapy starting at $450,000 US per treatment. Contributing to both of these problems is the fact that CAR T-cell therapy is labor intensive which will exacerbate existing clinical facilities already challenged by a myriad of mutating pathogens and an aging population. Against this setting is proposed therapy consisting of clinical stage and FDA approved anticancer drugs with excellent safety records. Proposed herein is combination therapy with Tucaresol, up-regulates CD4+ and CD8+ T-cells, and cyclophosphamide, down-regulates Treg cells, as a convenient, cost effective cancer treatment.

Keywords: Tucaresol, Cyclophosphamide, Cancer, CAR T-cell, Treg cell

1. Background

Decades ago, the search began for small molecules that safely stimulate the immune system sufficiently to function as anticancer drugs. A few low molecular weight drugs such as Levamisole, Tilorone and Imiquimod found limited use as interferon inducers but their market is stagnant or disappeared due to toxicity issues that became apparent with increased use. There is a fine line between small molecule cancer drug immunostimulant activity and drug toxicity which has been difficult to separate over the years. Therefore, efforts were expanded to develop large protein molecules including anticancer antibodies (monoclonal, bispecific, single chain and drug conjugate antibodies) with the expectation that these proteins should be less toxic since they bind with high specificity to their biochemical target and, as endogenous proteins, are generally well tolerated. Additionally, antibodies can adapt to capture malignant cells that evade drug treatment by mutating such that anticancer drugs lose their activity with time.

Incorporating the concept of highly specific and therefore anticipated safer protein binding to target the cancer cell, CAR T-cell therapy uses genetic engineering to introduce a highly specific protein sequence that binds to either the CD19 (Cluster of Differentiation cell surface receptor) or BCMA (B-Cell Maturation Antigen). CD19 and BCMA are specific B-cell markers that serve to attract the altered T-cell to the B-cell blood cancer. However, other specific B-cell markers are under study either in combination with CD19 or BCMA CAR T-cells or as alternative markers to attract T-cells to cancerous B-cells. For example, CD22 is expressed by the majority of blast cells present in B-cell acute lymphoblastic leukemia (B-ALL) and so CD22 CAR T-cell therapy is being evaluated for treatment of B-ALL. Early work indicates that CD22 CAR T-cell therapy is effective and well tolerated but most patients experience relapse. Also, a Tandem CAR T-cell therapy that targets both CD19 and CD22 may be more effective than CD19 CAR T-cell therapy.

Indeed, with multiple demonstrations of promising activity against hematological cancers, work has subsequently been undertaken to develop allogeneic or "off the shelf" CAR T-cell

therapy. Use of "off the shelf" CAR T-cells avoids the need to send out the cancer patient's T-cells for genetic modification to incorporate the B-cell antigen (CD19 or BCMA) into their T-cells. During the period of T-cell depletion, the patient is susceptible to infection and potentially lethal septicemia. It follows that if harvesting the patient's T-cells for genetic modification and subsequent T-cell modification is replaced by transfusion with recently, genetically modified allogeneic CAR T-cells (not from the patient) then risk of infection or septicemia is significantly reduced. The usual source of allogeneic T-cells for prior modification to CAR T-cells is from donated blood. Use of allogeneic T-cells may offer more availability of CAR T-cells but requires immunosuppression to reduce the risk of rejection by the cancer patient. Problematic is the relative lack of progress regarding the development of an effective CAR T-cell technology for treatment of solid tumors. Lack of progress against solid tumors confines CAR T-cell therapy to treatment of hematological cancers. While the cancer hematology market is estimated by Global Data to be lucrative at $37 billion in 2028, this is only a portion of the total market for cancer cell therapy. Multiple issues need be addressed regarding treatment of solid tumors. These include immune escape due to low density and heterogeneity of tumor antigens, restricted entry and/or limited persistence of CAR T-cells in the tumor microenvironment (TME) and the presence of immunosuppressive molecules or cells in the TME. These factors are discussed in detail; reference [1]. Nonetheless, 2024 has seen the first FDA approval treatment for solid tumors. On February 16, 2024 the FDA approved Amtagvi as the first T-cell therapy for treatment of a solid tumor; accelerated approval for advanced melanoma. Subsequently, on August 2, 2024, the FDA approved Tecelra as the first engineered treatment of a solid tumor; approval for synovial sarcoma. Both solid tumor treatments replace a synthetic CAR unit of a T-cell with either a naturally occurring T-cell receptor (TCR), Amtagvi which uses a Tumor Infiltrating Lymphocyte (TIL) T-cell receptor from TIL cells, or a modified (genetically engineered) T-cell receptor, Tecelra. However, both variations, like CAR T-cell technology, are expensive with a list price of $515,000 US per treatment for Amtagvi and $727,000 US per one time treatment for Tecelra. Additionally, both variations like CAR T-cell technology are accompanied by significant, potentially fatal, toxicity issues as discussed below.

2. The Challenge

CAR T-cell therapy and the newer T-cell receptor therapy are accompanied by significant, potentially fatal, side effects that require attention. Toxicity issues may be divided into two categories; those associated with the cancer including relapsed disease and those not associated with the cancer. As regards deaths not due to relapsed disease, non-relapsed mortality (NRM), a collection of data from approximately 7,600 patients reveals that NRM accounts for about 11% of deaths. Approximately half of these deaths were due to infections. Multiple factors account for these infections which include the immunosuppressive effects of the cancer, molecules and cells present in the TME, the CAR T-cell drug attack on B cells and in the case of autologous CAR T-cell therapy the prolonged T-cell depletion during the genetic engineering of the patient's CAR T-cells. The conclusion was that the risk of infection is the single biggest problem followed by CAR T-cell specific complications such as neurotoxicity, immune-effector cell associated neurotoxicity syndrome (ICAMS), and cytokine release syndrome [2]. Rare is the occurrence of T-cell malignancies after completion of the CAR T-cell protocol.

Currently, CAR T-cell therapy is primarily used as a last resort cancer treatment which, in view of the labor intensive protocol and need for aseptic conditions, may be difficult to provide if this technology becomes mainstream. Most hospitals are filled to capacity and if CAR T-cell therapy becomes a preferred treatment then expansion of this technology will require significant capital investment to accommodate the increased demand on medical facilities. This view regarding the need to face an infrastructure reality check should CAR T-cell technology significantly expand was published earlier this year [3]. Additionally, cost of the treatment will likely not significantly decrease in the near future. For example, it is noted above (see Abstract) that the price of CAR T-cell therapy starts at $450,000 US. The cost increases to $515,000 for treatment of an advanced melanoma solid tumor with the newer TIL T-cell receptor therapy, Amtagvi, and $727,000 for treatment of synovial sarcoma, a solid tumor treated with the newest T-cell receptor therapy, Tecelra.

3. The Proposal

In view of the challenge regarding development of CAR T-cell drugs efficacious against solid tumors, it is not difficult to envisage that the best way forward is development of low molecular weight, small molecule antagonists of biochemical processes mandatory for cancer cell growth. The advantage of development of small molecule drugs versus large molecule (protein) drugs is reasonably obvious. The issue is that in a comparison of the in-vivo activity of large molecule T-cell anticancer drugs versus small molecule T-cell anticancer drugs, the small molecule drug can be effective although this most often is not the case. Advantages of small molecule drugs include greater penetration of the tumor environment, the TME, and they are generally easier and less expensive to manufacture. Therefore, it is little surprise that considerable effort has been made regarding development of small molecule T-cell anticancer drugs. However, not many significant leads have been forthcoming from these efforts. One initially promising anticancer drug class is derived from the immunosuppressive small molecule, adenosine. Present in high concentrations in the TME, adenosine binds to adenosine receptors present on immune cells where it functions as an immunosuppressant thereby providing protection to the tumor. Supporting the high concentration of adenosine at the TME is de novo synthesis of adenosine from ATP. A few adenosine analogs with nM in-vitro activity as adenosine receptor antagonists have been studied in early clinical trials but demonstrated moderate clinical activity, as summarized in the recent review article, reference [4]. It was noted that there are many cell types that express adenosine receptors thereby increasing the potential for "off-target" binding of the adenosine analog to receptors located on non-cancerous tissue.

Years ago, we published significant in-vivo anticancer results

regarding combination therapy with a small molecule CD8+ T-cell stimulant, BCH-1393, in combination therapy with low dose cyclophosphamide [5]. Low dose cyclophosphamide selectively decreases regulatory T cells (Tregs) when administered iv or orally, including in drinking water, to mice or humans [6, 7]. In our study, BALB/c mice were injected subcutaneously with mouse breast carcinoma (DA-3) on Day 0. Treatment with BCH-1393, 25 mg/kg and 50 mg/kg ip, was undertaken alone or following administration with cyclophosphamide on Day 0 as a single 100 mg/kg iv bolus injection. BCH-1393 was administered daily from Day -2 to Day 1 and then every day or every other day to Day 21. Cyclophosphamide was administered alone by the same method and dose as in combination with BCH-1393. Mice were monitored for tumor incidence, tumor size and body weight. A weak antitumor effect was observed with either dose of BCH-1393 or with single dose cyclophosphamide alone. However, a significant inhibition of tumor outgrowth and suppression of established tumor growth was observed when BCH-1393 was administered in combination with sub-therapeutic cyclophosphamide. Thus, for example, with combination therapy at a dose of 50 mg/kg BCH-1393 tumor outgrowth was prevented in 70% - 80% of treated mice. In the remaining 20%-30% of remaining mice that developed tumors, a nearly complete inhibition of tumor growth (90%) was observed at days 22-24 post tumor implant. We published another paper that demonstrated PBI-1393 significantly increases interleukin-2 and interferon-γ production in human activated T-cells and the conclusion from this study was that PBI-1393 modulated (inhibits) adenosine receptor activity [8]. The BCH-1393 in-vivo results are impressive for two reasons. First, as noted above, there is ubiquitous expression of adenosine receptors by many cell types thereby increasing the probability of off-target binding and subsequent low drug potency accompanied by side effects. As such, subsequent in-vivo studies with adenosine receptor inhibitors have demonstrated moderate antitumor activity [4]. However, in this study, a major antitumor activity, prevention of tumors in the majority of mice, was observed with combination BCH-1393 and low dose cyclophosphamide. Second, while millions of dollars have been spent on targeting CAR T-cell therapies towards treatment of solid tumors with little positive data forthcoming from this effort, combination of an apparently safe and affordable lead compound and low dose of an FDA approved drug prevented solid tumor growth in 70%-80% of the treated mice and significant retardation of tumor growth in the remainder of the mice .This combination of cyclophosphamide to down regulate (neutralize) Tregs within the cancer TME and the ability of BCH-1393 to inhibit activation of the adenosine receptor within the TME provides the basis for a promising, safe, affordable small molecule approach for treatment of solid tumors. However, the widespread occurrence of the adenosine receptor common to various functional pathways provides a warning regarding potential toxicity issues that may not be uncovered until appropriate but expensive, large (phase 3) clinical trials take place.

An important issue with the use of T-cell stimulants, either small or large (protein) molecules, is that their use as therapeutics requires the correct balance between sufficient stimulation of T-cells to effectively destroy cancer cells but not be so active that they destroy healthy cells. Indeed, the hallmark of hyperstimulation of T-cells is cytokine release syndrome (CRS) arising from an excessive proinflammatory response from the rapid release of proinflammatory cytokines, notably interleukin-2 and interferon-γ. If not rapidly treated CRS can be fatal. Mild to fatal CRS is a common toxicity issue with CAR T-cell therapy. Ideally, combination anticancer therapy with low dose cyclophosphamide to down regulate (neutralize) Tregs would be best accomplished with a safe, conveniently administered, reasonably available (affordable) T-cell stimulant. A potential small molecule, clinical stage, orally active candidate drug is Tucaresol.

Tucaresol, 4-[(2-formyl-3-hydroxyphenoxy)methyl] benzoic acid Molecular Weight=272, is an orally active candidate immune restorative drug with good oral bioavailability (70%), ambient temperature stability and positive activity against HIV as shown in a phase 1b/2a clinical trial. Tucaresol was under development years ago by Glaxo Wellcome for palliation of sickle cell anemia. Preclinical data, including PK/PD, is available. Tucaresol functions as a host targeted antiviral or anticancer agent by the ability to provide a costimulatory signal to CD4+ T helper cells in immune suppressed individuals. Important to note is that Tucaresol does not function to indiscriminately stimulate normal immune status individuals thereby preventing a hyperactive and potentially fatal response such as cytokine storm (CRS). In fact, the ability to stimulate a controlled immune response in an immune deficient mammal such as may occur during a viral infection or cancer is a key tenet of the intellectual property of Tucaresol as defined in the patents issued to GSK Wellcome (patents expired). A more detailed overview regarding Tucaresol, including mechanistic information pertaining to antiviral activity, is available in our recent publications [9, 10].

In view of the above, ***it is proposed herein that the combination of Tucaresol with low dose cyclophosphamide be investigated for treatment of solid tumor or hematological cancers by stimulation of CD4+ T helper cells/CD8+ T cytotoxic cells and suppression of Tregs.***

Tucaresol has been evaluated in the mouse colon adenocarcinoma (solid tumor) model (MC38); US patent 5,958,980 (1989); Glaxo Wellcome. The percent reduction in tumor growth in C57BL/6 mice (mean tumor weight of excised tumors) was 46% without treatment with low dose cyclophosphamide (reduction of Tregs). Although not strictly comparable, a similar in-vivo assay was undertaken with BCH-1393 and the MC38 tumor model in C57BL/6 mice [5]. BCH-1393 administered alone was ineffective against early established tumors while mice treated with combination BCH-1393 low dose cyclophosphamide displayed a highly significant delay in tumor progression compared to control (saline treated) mice; p=0.0009, Treated/Control=13%.

Taken together, the above results suggest that the combination of Tucaresol and low dose cyclophosphamide should yield

significant antitumor activity devoid of serious toxicity.

4. Discussion

Millions of dollars continue to be spent on the research and development of expensive gene and cell therapies treatment of cancer but extending into other therapeutic areas as exemplified by CAR T-cell therapy for treatment of autoimmune diseases such as lupus [11]. Additionally, a variety of cancer antibodies exemplified by monoclonal antibodies, bispecific antibodies, single chain antibodies and antibody drug conjugates are commercially available with more in the pipeline. However, these antibody variants are priced at approximately $100,000 or more annually. In going forward with this broad array of biologics it is important that a pharmacoeconomic perspective is taken into consideration. We still need a blend of newer but accessible, efficacious, safe, affordable drugs along with more sophisticated and therefore expensive gene and cell therapies to expand our armament of anticancer drugs or face the prospect of an eventually unaffordable and ultimately unsustainable health care system.. With this objective in mind, we offer a proposal for a novel but safe, pharmacoeconomic T-cell anticancer drug [12].

Declarations

No animal studies or human trials were performed by the authors for this project.

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Author Contributions

Project conceptualization CP, BZ, Drafting of original manuscript CP, Review and editing CP, JSD, Literature collection and analysis CP, BZ, Project administration CP, JSD, BZ, Methodology JSD, BZ, Experimentation JSD

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Conflicting Interest

The authors declare that there is no conflict of interest.

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12. Presented herein is the case for a significant safe stimulation of one's own (autologous) T-cells by combination therapy as opposed to over stimulation of the patient's T-cells and accompanying toxic effects. As we do not have the resources for a proof of concept study, we welcome a collaboration and/or other inquiries of interest.