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Tucaresol-Cyclophosphamide Combination Therapy: Further Support for A Safer, Affordable Alternative to Solid Tumor 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 immune T-cells thereby permitting the modified T-cells to recognize and eradicate tumors. Multiple hematological tumors have been treated with a significant increase in survival in comparison with prior, traditional cancer treatments. However, CAR T-cell therapy does not always provide a lasting anticancer response. Additionally, two negative issues accompany CAR T-cell therapy. First is the possibility of encountering any one of multiple side effects and the second is the high cost of the treatment. The issue of health care cost is increasingly problematic due to aging and increasing populations. Presented herein is the second of two publications regarding oral therapy with Tucaresol, up-regulates CD4+ and CD8+ T-cells, and low dose cyclophosphamide, down-regulates regulatory T (Treg) cells that protect the tumor. While the first publication focuses on our initial research that supports our proposal for Tucaresol - low dose cyclophosphamide combination therapy, this publication provides additional evidence from other sources that support combination of Tucaresol - low dose cyclophosphamide therapy for treatment of solid tumors. Of note is that Tucaresol facilitates a human immune T helper cell response in immune suppressed patients. Additionally, the convenience and affordability of our combination therapy merits exploration of approved CAR T-cell treatments for hematological cancers and in combination as an adjuvant for therapeutic cancer vaccines.

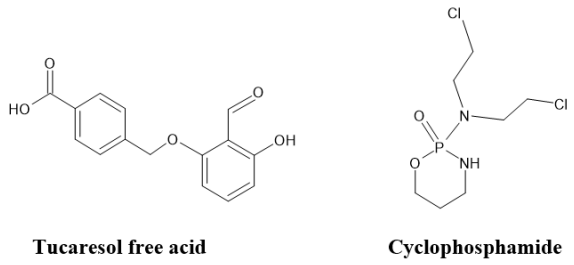
Keywords

Tucaresol; Cyclophosphamide; Cancer; CAR T-cell; Treg cell.

Introduction

Background

Chimeric Antigen Receptor (CAR) T-cell therapy, a method of immune T-cell stimulation by which to elicit a significant anticancer response, has achieved significant success regarding the treatment of hematological tumors. However, CAR T-cell therapy has obtained only limited success regarding the treatment of solid tumors. Regardless of the tumor type, CAR T-cell therapy has two potentially significant limitations. First is the possibility of a significant toxicity issue while second is the high cost of CAR T-cell therapy. To reduce risk of infection to the cancer patient while CAR T-cells are in preparation, work is underway to develop allogeneic or "off the shelf" CAR T-cell therapy. Off the shelf CAR T-cells avoid the need to send the patient's T-cells away for genetic modification and subsequent return to treat the cancer. However, use of allogeneic CAR T-cells (not from the patient) requires immunosuppression to reduce risk of rejection by the cancer patient of the allogeneic CAR T-cells. The usual source of allogeneic T-cells for modification to CAR T-cells is from donated blood. Subsequently, we proposed combination therapy with an orally active, small molecule T-cell stimulant candidate drug, Tucaresol (**Figure 1**), that was studied in an HIV clinical trial and a second orally active small molecule approved anticancer drug, low dose cyclophosphamide (**Figure 1**), as a potential alternative to CAR T-cell therapy especially in those cases where CAR T-cell therapy is partially successful (e.g. cancer remission) or side effects are significant [1].



Tucaresol free acid **Cyclophosphamide**

Figure 1: Structure of the two components that comprise the combination immune modulator drug therapy proposed herein; the candidate drug Tucaresol and approved cancer drug cyclophosphamide. Note the simple (low molecular weight) structure of both drugs which is reflected by their oral activity**.**

The Proposal

In view of the challenges regarding development of CAR T-cell therapy efficacious against solid tumors, likely the best path forward is one that targets a biochemical target. This could be achieved, for example, by selection of a well-studied small molecule that selectively binds and stimulates a receptor or co-receptor on a patient's T immune cell. This avoids removal of the patient's immune cells for gene modification, as occurs in the autologous CAR T-cell process, and patient vulnerability to infection during the period of T-cell depletion. Furthermore, synthetic routes that avoid lengthy (multi-step) pathways yield reliable, affordable quantities of the small molecule medicine.

Tucaresol, 4-[(2-formyl-3-hydroxyphenoxy) methyl] benzoic acid, Molecular Weight = 272, is an orally active small molecule candidate immune restorative drug with good oral bioavailability (70%) and ambient temperature stability. Tucaresol displays immunomodulatory activity against HIV in humans as was demonstrated in a phase 1b/2a clinical trial [2]. Of note was the observation that among the different treated groups (HAART therapy and/or treatment with Tucaresol) significance ($P<0.05$) was achieved in

immune memory CD4+ T lymphocytes, naive CD4+ T lymphocytes and interferon-gamma secreting cytotoxic CD8+ T lymphocytes during the treatment period. It later followed that Tucaresol was evaluated for safety, tolerability and immunologic activity as an oral candidate drug in patients with advanced malignant melanoma [3]. Tucaresol induces a T-cell costimulatory signal that preferentially augments a Th1 cellular immune response. However, detailed clinical data did not appear in the public domain. As regards our proposal for combination therapy with Tucaresol, it is encouraging that this earlier work demonstrates Tucaresol interacts with and stimulates human T-cells since our proposal includes administration of low dose cyclophosphamide with Tucaresol to specifically down regulate Treg cells within the tumor microenvironment. Treg cells function to protect the tumor from destruction by cytotoxic CD8+ T-cells. Initially, Tucaresol was under development by Glaxo Wellcome for palliation of sickle cell anemia and preclinical data including PK/PD is available [4]. For example, acute toxicity data in healthy volunteers indicated Tucaresol is routinely tolerated up to an oral dose of 3.6 grams while the plasma half-life at this dose is approximately 12 days. Lower doses of Tucaresol yield shorter half-lives. Tucaresol was also 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%. However, data is not available for treatment of tumors with Tucaresol - cyclophosphamide combination therapy. Specifically, no reports have appeared regarding the anticancer activity of Tucaresol combined with low dose cyclophosphamide in mice.

In-vivo demonstration of significant oral antitumor activity with combination Tucaresol - cyclophosphamide versus Tucaresol alone would demonstrate an important role of cyclophosphamide in this treatment protocol, However, combination therapy mouse studies versus Tucaresol alone are usually long due to the need for separate animal facilities, veterinarian and animal committee participation and lengthy data acquisition (usually months). Additionally, caution has been expressed that positive murine anticancer data is not always replicated in human trials. Reasons for discrepancy between murine and human in-vivo studies include differences in the genetics of mouse and human cancer disease and cancer mismatch such as a localized tumor in mice versus a metastatic tumor in humans. Therefore, in view of the time and cost for evaluation of Tucaresol in an animal combination versus single agent study it may be more appropriate to proceed with a small (e.g. phase 1b/2a) cancer clinical trial. This latter decision is supported by the safety data acquired over the years with Tucaresol which, unlike many immunostimulants, does not indiscriminately stimulate normal immune status individuals thereby preventing a potentially harmful immune response. As noted above, human T-cells are stimulated by Tucaresol only in immunosuppressed individuals such as occurs in cancer patients. This fact avoids unnecessary in-vivo work to determine that Tucaresol does not significantly stimulate human T-cells in healthy patients. However, a more recent approach apparently exists for determination of the probability that Tucaresol - cyclophosphamide therapy would induce synergistic anticancer activity without need for prior animal combination therapy evaluation.

Artificial Intelligence (AI) progresses with increased acceptance regarding computational techniques for the determination of potential drug synergy pertaining to treatment of cancer. One goal of AI research is identification of potential synergistic drug combinations for treatment of tumors that do not completely respond to approved drugs in a timely manner to avoid drug resistance and/or eventual drug toxicity.

Also, as noted above, animal combination drug studies are time demanding and expensive. Therefore, successful application of AI for prediction of potential drug synergy between two lower molecular weight (well characterized smaller structure) drugs such as Tucaresol and cyclophosphamide would permit the exploration of a broader range of conditions such as drug concentration(s) and solvent composition (salt and buffer component(s), concentration and pH) to predict optimal conditions that yield maximum therapeutic efficacy than obtained with in-vivo studies. The importance regarding use of AI to predict drug synergy is significantly increasing as illustrated by a review article focused on AI for prediction of drug combination synergy during treatment of malignant disease [5]. However, more impact may result from the ability of AI to predict with good accuracy the success of clinical trials, including combination studies. A prelude to this opportunity occurred this year, 2026, with the announcement that the AI company BioinvestGPT successfully predicted the outcome of 5 of 6 clinical trials.

In 2000, we published significant in-vivo anticancer results regarding combination therapy with our small molecule CD8+ T-cell immunostimulant BCH-1393, a purine analogue, in combination with low dose cyclophosphamide [6]. Low dose or metronomic cyclophosphamide refers to use of smaller amounts of cyclophosphamide administered as a single dose or on a continuous or frequent schedule to deplete regulatory T-cells and stimulate an immune response rather than directly kill tumor cells. Low dose cyclophosphamide combination therapies generally cause less cases of severe bone marrow suppression or hair loss than associated with full dose cyclophosphamide. BCH-1393 alone weakly stimulates CD8+ T-cells but in combination with low dose cyclophosphamide yields significant anticancer activity. Thus, for example, with combination BCH-1393, 50 mg/kg, and sub therapeutic single iv bolus injection of cyclophosphamide, tumor outgrowth was prevented in 70% - 80% of treated mice. In the remaining mice there was a nearly complete inhibition (90%) of tumor growth. However, since BCH-1393 is an inhibitor of the adenosine receptor, the potential exists for development of drug toxicity due to the widespread occurrence of the adenosine receptor that includes neurological pathways.

In the case of the interleukin 12 - low dose cyclophosphamide combination anticancer studies, interleukin 12 was introduced by gene therapy. Based on work where it was demonstrated that a potent antitumor effect was obtained against gastrointestinal carcinoma in mice with a single low dose of cyclophosphamide and interleukin 12 gene therapy, a follow-up study was undertaken to treat a murine model of colorectal cancer with interleukin 12 gene therapy and metronomic cyclophosphamide [7]. This combination therapy reversed induced immunosuppression by decreasing the number of Treg cells. However, cyclophosphamide delivered as a single low dose displayed a superior antitumor profile than cyclophosphamide administered by a metronomic schedule. Another study was undertaken that included a triple combination consisting of interleukin 12 gene therapy, low dose (metronomic) cyclophosphamide and a DNA cancer vaccine [8]. This study consisted of five groups which were the control group (no treatment), the control gene vector group, intratumoral interleukin 12 gene therapy, intratumoral gene therapy and metronomic cyclophosphamide and intratumoral interleukin 12 gene therapy and metronomic cyclophosphamide and human tyrosinase DNA (cancer) vaccine. All treatment groups showed increased survival and higher cure rates relative to the control groups. Survival of non-cured mice was increased when the metronomic cyclophosphamide was combined with interleukin 12 gene therapy. Furthermore, mice that received metronomic cyclophosphamide had a lower percentage of regulatory T

(Treg) cells. Addition of the human DNA vaccine significantly increased the cure rate and resulted in increased survival compared to other treatment groups. A humoral memory response against the tumor cells was observed in all the groups that received interleukin 12 gene therapy. Similar cancer studies followed that pursued the treatment regimen consisting of a CD8+ T cell stimulant to attack tumor cells combined with low dose cyclophosphamide to reduce the number of Treg cells that protect the tumor. In fact, the safety of this two component drug therapy protocol often permits addition of a third component to yield a more potent three drug cocktail, this third component being an anticancer chemotherapeutic. One of the older three component drug treatments for human breast cancer is metronomic cyclophosphamide with 5-Fluorouracil (stimulates CD8+ cytotoxic T cells) and methotrexate [9]. However, the majority of two drug anticancer combinations serve to stimulate the activity of the third component; an anticancer therapeutic vaccine. The two drug components function as a vaccine immunoadjuvant. Examples of earlier murine anticancer vaccine immunoadjuvant combinations are referenced above [8, 9]. The most recent example of an anticancer vaccine, immunoadjuvant combination was reported in 2026 [10]. In this study, the anticancer vaccine consisted of two components which were chimpanzee adenovirus and modified vaccinia Ankara, two viral vectors with established clinical safety profiles, in combination with CarboTaxol (combination Carboplatin and Taxol). It was demonstrated that CarboTaxol expands TGF1+ CD8+ T-cells. However, low dose cyclophosphamide was not included in this study to deplete Treg cells in the tumor microenvironment.

Tucaresol and cyclophosphamide are similar size, well characterized molecules with respective Molecular Weights of 272 and 261. They are not large complex structures that require multi-step synthetic routes. Tucaresol may be synthesized by two practical, improved synthesis developed by us, starting with 2,6-dimethoxybenzaldehyde or resorcinol [11]. Cyclophosphamide is a well-established, available anticancer drug approved by the US FDA in 1959. Similar to Tucaresol, cyclophosphamide may be synthesized from simple materials; phosphorus oxychloride, bis (2-chloroethyl) amine and 3-aminopropanol; US patent, 3,018,302 (1962).

Oral bioavailability of low dose cyclophosphamide is high but a portion of the drug must undergo first pass metabolism (oxidation) in the liver to form the active drug: 4-hydroxycyclophosphamide. This cyclic drug, after cancer cell penetration, degrades to acrolein and phosphoramide mustard alkylator which targets and halts cancer DNA replication. Taken together, additional safety of Tucaresol includes the available clinical data and low dose cyclophosphamide combination therapy in which patients exhibit less bone marrow suppression or hair loss than patients administered regular dose cyclophosphamide. Additionally, since the product of first pass hepatic metabolism and not the orally administered cyclophosphamide degrades to the in situ active alkylator, it is expected that our Tucaresol - cyclophosphamide combination cancer therapy, either alone or as an immunoadjuvant combined with a therapeutic cancer vaccine, will exhibit superior safety in comparison with prior drug - therapeutic cancer vaccines.

Discussion

Two years ago we proposed a novel, safer and affordable oral immunotherapy cancer treatment with a clinical candidate drug, Tucaresol, and low dose FDA approved cyclophosphamide [1]. At that time, a few

labor intensive, expensive autologous CAR T-cell therapies for treatment of hematological tumors were approved but not for treatment of solid tumors. As a partial solution to the issue of patient safety and treatment affordability, work was undertaken regarding the development of "off the shelf" or allogeneic CAR T-cell therapy thereby eliminating the need for genetic engineering of the patient's T-cells to express a CAR receptor. However, this effort has not led to success regarding the development of allogeneic CAR T-cell therapy for treatment of hematological and solid tumors or autologous CAR T-cell therapy for treatment of solid tumors. As of two years ago, millions of dollars continue to be spent regarding research and development of CAR T-cell therapy against solid tumors. Efforts to date to develop an allogeneic CAR T-cell clinical therapy with significant anticancer activity remain elusive. Recently, for example, was the discontinuation by Caribou Biosciences of its clinical-stage pipeline amid a financial freeze in its CAR T-cell program. Galapagos NV reduced its CAR T-cell technology and cell therapy programs to rebrand as Lakefront Biotherapeutics NV in 2026. Nonetheless, with an increasing and aging population along with occurrence of cancer at an earlier age, there is an increased need for additional safe cancer therapies. In order to offer a broad range of different therapeutic options, it is proposed that our combination Tucaresol - low dose cyclophosphamide cancer treatment be evaluated for use in pharmacoeconomic cancer immunotherapies and therapeutic cancer vaccines.

Declarations

No animal studies or human trials were undertaken by the author for this project.

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Conflicting interest

The author declares that there is no conflict of interest.

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