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Active Specific Immunotherapy (ASI) in Cancer Treatment: Five Case Reports

Roni Moya[1,3], Mike KS Chan[1-3], Michelle BF Wong[1,3], Dmitry Klokol[1,3], Yvonne Cashinn Chia[2,3] and Shing Yi Pan[2,3]*

[1]FCTI Biotech R & D GmbH
[2]Baden Research and Testing Lab, Asia-Pacific
[3]European Wellness Academy, Germany

Abstract

Immunotherapy, including the complementary immunotherapy for cancer, can be categorized as either specific or nonspecific, both with the aim to enhance the immunity against tumors. A proper immunotherapy approach must address a patient's individual features of complexity such as tumor's immune suppressive and pro-inflammatory aspects, antigen heterogeneity and immunogenicity. The Active Specific Immunotherapy (ASI) is capable of rearming and boosting the immune system against cancer by exclusively regulating patient's own immune-modulatory molecules. This article presents five distinct clinical reports of cancer patients that have undergone the ASI treatment, combined with ozone therapy, vitamins infusion and several programs of chemotherapies. As a result, the ASI improved the immune status of all patients and their overall life quality by reducing pain, fatigue and infections recurrences. Furthermore, the ASI treatment did not cause any negative side effects nor interfere whatsoever with the chemotherapy protocol. In conclusion, although further studies are urgently required in the field, ASI offers an interesting and safe strategy as integrative personalized immunotherapy.

Keywords: Active specific immunotherapy; Cancer; Chemotherapy; Immunotherapy; Ozone therapy; Vitamins infusion; Biological regenerative medicine

Abbreviations: ASI: Active Specific Immunotherapy; APC: Antigens Presenting Cells; TCs: Autologous Tumor Cells; CTLA-4: Cytotoxic T Lymphocyte Antigen 4; DC: Dendritic Cell; GGT: Gamma-Glutamyl Transferase; LAK: Lymphokine-Activated Killer; MHC: Major Histocompatibility Antigens; mAbs: Monoclonal Antibodies; PD-L1: Programmed Death-Ligand 1; TME: Tumor Microenvironment Elements; TAA: Tumors Associated Antigens

Introduction

Immunotherapy has recently been described as the fourth pillar of cancer treatment besides surgical therapy, chemotherapy and radiation therapy [1]. Immunotherapy is based on the modulation of the immune system to attack malignant or hyper-reactive inflammatory cells [2]. The development of immune checkpoint inhibitors, such as anti-CTLA-4 monoclonal antibodies (mAbs) (ipilimumab, tremelimumab), anti-PD-1 mAbs (nivolumab, pembrolizumab) and anti-PD-L1 mAbs (atezolizumab, durvalumab, avelumab), has introduced a promising therapeutic approach for several types of cancer, including melanoma, lung, head and neck, kidney, bladder, etc. [3]. Cancer immunotherapy started in 1891, when William Coley discovered that patients could recover from osteosarcoma after developing a form of cellulitis known as erysipelas [4]. Nearly a century later the mechanism of dendritic cell's immune activation and pathogen recognition was better elucidated [1]. The immunotherapy can be divided into specific, targeting direct response against accurate antigens (ex. peptide therapy and dendritic cell therapy), or nonspecific, boosting the general immunity without aiming any particular biomarker (cytokine therapy and lymphokine-activated killer (LAK) cell therapy) [5]. The cancer-immunity cycle is a complex cascade of signaling interactions initiated by the releasing of tumor antigens, recognition and presentation by antigens presenting cells (APC) (such as the dendritic cells (DC)) and the major histocompatibility antigens (MHC-1 or MHC-2), activation of immune stimulatory cytokines and tumor microenvironment elements (TME) and the final induction of cancer cell killing and apoptosis [6]. The immune system's renowned feature to impair tumor growth is further supported by assays showing a direct association between intra-tumoral infiltration of T cells and patient survival across different cancer types [7]. In order to be successful, the optimal protective immune system must balance between elimination of foreign pathogens and tolerance of self-antigens. In the recent years, different molecules were pointed to be able either to stimulate (e.g. CD28 and ICOS) or inhibit the cellular immunity mediated by T-cell; including cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed death-ligand 1 (PD-L1). Co-inhibitory molecules such as CTLA-4 and PD-1 act as "immune checkpoints", suppressing the excessive inflammatory reactions and playing a fundamental role in protecting the body from tissue damaging [8]. The PD-L1 expression level on tumor cells have been linked to a favorable outcome of patients treated with checkpoint inhibitors [9]. But as a setback, due to cancer unlimited DNA replicative and auto-repairing potential, molecular diversity, stress adaptation, aggressive immune and apoptosis evasion and pernicious immunosuppressive or inflammatory effects, the classical immunotherapy alone may not be so effective in all patients and a wider individualized specific biomarkers selection is needed for the optimization of treatments. The idea of an advanced immunotherapeutic approach must also include and combine personalized agents that might induce a more specific immune response against malignant cells. Taking this argument, to increase the effectiveness response against cancer, the immunotherapy shall consider three main steps: (1st) Tumor exclusivity (natural and restricted presentation of self/individual tumor's antigen); (2nd) immunogenicity (natural presentation of the epitopes that can be rightly recognized by the individual's T cells) and (3rd) broad expression (the patient should highly express his tumor's own molecules) [2]. Subsequently of this

***Corresponding author:** Shing Yi Pan, Researcher, European Wellness Academy, Klosterstraße 205, 67480 Edenkoben, Germany, Tel: +60143708083; E-mail: dr.pan@european-wellness.com

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hypothesis, an integrative immunotherapeutic tool that could support a more personalized strategy is the Active Specific immunotherapy (ASI). The Active Specific Immunotherapy potentially reach the genomic heterogeneity of the tumor cells through the use of live, metabolically active, autologous tumor cells or patient's own blood that contains the entire antigenic diversity of the primary tumor and its signaling immunosuppressive or inflammatory molecules [10].

As reviewed in our previous publication, the basic concept of ASI is to identify the tumors associated antigens (TAA) in order to develop an individualized vaccine, targeting the exact anti-idiotypic antibodies formed against the tumor's variable regions, in which is expected to form the ligand complex of tumor-antigen-binding-antibodies and therefore, release a systematic innate and adaptive immune chain reaction against the cancer cells [11,12]. ASI involves generating a massive, cell-mediated, cytotoxic immune reaction against tumor cells capable of rearming the immune system against cancer [10]. In summary, on one hand the ASI job is to identify and boost the patient's immune cells and cytokines to enhance recognition, signaling and attack of the malignant cells; on the other hand and by another side inhibit the tumor immunosuppressive potential, pro-inflammatory adaptation and apoptosis evasion. In contrast with some other immunotherapies available and because of its individualized focus, the ASI does not promote liver overcharge, drugs interaction or negative side effects to the patient apart from rare cases of typical symptoms of immune modulation such as slight fever or light provisory tiredness. Due to the ASI good tolerance and safety, it can be combined with other immunotherapies as well as with conventional anti-cancer therapies with immunomodulatory properties, such as chemotherapy and radiotherapy and oncogene-targeted therapies [13]. These techniques can directly deplete certain pro-tumor immune populations or indirectly activate anti-tumor immune response through increased antigen availability and induction of immunogenic cell death [14,15]. In order to achieve the benefits and limits of this specific immunotherapy, this article presents and discusses short five cases reports of different cancer patients that have undergone the ASI combined treatment.

ASI Preparation and Protocol

After three days clotting, 30 ml of the buffy-coat of the patient's peripheral blood was isolated under the GMP laminar-flow-technique and the immune molecular elements were separated by different patented biochemical and physical steps, followed by proprietary oxygenation biotherapy. Afterwards a culture of immune activating additives substances was then added to the buffy-coat to prepare 30 vials of vaccine (1.1 ml each for subcutaneous injections). ASI vaccines were administered three times per week for a total of 10 weeks [11].

Case Series

Case report 1

The patient is a male, 58 years old, 1.85 m and 65 kg. In March 2017 he was diagnosed with stomach cancer. The tumor was extracted on May 30th and a first sequence of chemotherapy with docetaxel + cisplatin + 5-fluorouracil was administered every three weeks until July 2018. CT scan showed aggressive liver metastasis progression with peripheral neuropathy. He started a continuous cycle of palliative chemotherapy care with 5-fluorouracil every three weeks. In July 16th 2018, the integrative protocol was then initiated with ozone therapy 60 mcg/ml/200 ml and intravenous vitamin C 50g every week. The ASI treatment, 3 times a week during 10 weeks was started and integrated with the previous protocol on February 2019. In January 4th 2019, he had already undergone 20 sessions of the palliative chemotherapy with just a slight anemia (Hb 11.6 g/dl) and almost normal immune population profile with just a lower CD3 688.38 (normal range 1000 – 2780). He referred no pain or neurophatic symptoms and no pain killer drugs was necessary. The liver enzyme gamma-glutamyl transferase (GGT) was 72 U/l, slightly over the limit (normal range 12-64 U/l). Patient's overall well-being was good, with energy, healthy nutritional habits and even physical exercise practice. CT scan from February 22nd 2019 showed normal liver metastasis (6.8 cm and 5 cm). CT scan from April 16th 2019 showed presence of tumor and metastasis stabilization with no further growth. He received the combined treatment with a relative good state of health, immune profile with no further liver toxicity, digestive problems, anemia or pain.

Case report 2

The patient is a female, 31 years old, 1.65 m and 50 kg. Diagnosed in 2015 with an osteosarcoma. After several different chemotherapy protocols, dendritic cells and vitamins infusions as integrative immunotherapy, she entered in remission. In November 2018 she suffered a relapse of the same osteosarcoma with lung metastasis and immediately started a conventional protocol of gemcitabine + irinotecan I.V. every 15 days, with cyclophosphamide as oral immune suppressor daily. From April 2019 onwards, she received a combined conventional chemotherapy in conjunction with the ASI protocol with 60 mcg/ml/200 ml of ozone therapy as well as general hyperthermia once a month. Since then, CT scan shows no worsening in general, with normal-immune hemogram and phenotyping profile, as well as absence of anemia or liver toxicity. Although minor pain were managed with drugs, patient remains stable with good prognosis.

Case report 3

The patient is a male, 58 years old, 1.70 m and 60 kg. In May 2018, he was diagnosed with pancreatic adenocarcinoma stage IV with liver metastasis. The tumor genotype shows resistance to classical immunotherapy (PDL-1 10-15%). After several no positive results with chemotherapeutic protocols with paclitaxel + gemcitabine, he entered in the palliative care with folfirinox in combination with liposomal irinotecan (nal-IRI) every 15 days. On June 19th 2019 he was administered with ASI therapy with multivitamins infusions. He suffered from *B*-thalassemia (erythrocytes 3.9 and Hb 8.4 g/dl). The general state was weakness, fatigue and edema. As the ASI integrative treatment is still ongoing, it is too soon to get more results, The patient is stable, with little weakness and fatigue, no pain, less edema and no further side effects.

Case report 4

The patient is a female, 63 years old, 1.55 m and 63 kg. Multiple myeloma diagnosed on May 2019 with multiple inflammatory bone focuses. Patient was presented with overall joint pain, obstipation and fatigue, and decided to immediately start the ASI protocol with Vitamin C 50g infusions, even before the conventional hospital protocol established. On June 24th 2019, three weeks after the ASI initiation, she was already full of energy, sleeping well and active. The obstipation was better and pain was reduced to only in the hips and right arm. No painkillers were needed. The *kappa/lambda* ratio was slightly elevated (2.45) (normal range 0.310 – 1.560). Impressively, the CT scan from June 2019 couldn't identify any compatible multiple myeloma lesions and she was placed under surveillance with no further treatment.

Case report 5

The patient is a female, 75 years old, 1.55 m and 60 kg. Multiple myeloma Stage III with bone metastasis; diagnosed in January 2019.

Several pain focuses, including in the lower back and legs, treated with morphine patches and orally, with almost no positive effects on the pain. Patient with metabolic syndrome associated with several drugs protocols. Due to her age, no stem cell transplantation can be programmed and the prognosis is very poor. The only multiple myeloma treatment is with erythropoietin. On July 1st she started the ASI protocol with ozone therapy 60 mcg/ml/200 ml every week. Although is too soon to get any results, in few weeks she has been presented with better mobility, energy and relative good immunity state checked at the immune phenotyping profile. We are still waiting for a future *k chain* profile and a PET scan.

Discussion

The immune system has the potential to cure cancers, as evident by the occurrence of spontaneous regression of tumors following allogeneic stem cell transplantation in hematological malignancies and other immunotherapies approaches, either specific or non-specific [16]. Antigen-specific immunotherapy is a rapidly growing field providing promising new interesting options in cancer therapy and as the research publications multiplies, the idea that individualized treatment is possible becomes more distinct aiming the instability of tumors as well as the enhancement of the host immune system, without the common known conventional treatment's negative side-effects. Of course there are several problems to fulfill in cancer therapy, such as the limitless tumor's DNA repairing system; oxidative stress, chemical and radiation adaptation and resistance; metastatic potential; chronic inflammatory with severe immunosuppressive features, angiogenesis, genetic heterogeneity, etc. It has been acknowledged that autologous tumor cells (TCs) may be a good source of tumor-associated antigens (TAA) for active specific immunotherapy (ASI) [17]. This proper patient's source of TAA addresses the commented checkpoints of tumor heterogeneity, quantity and exclusivity of antigens and therefore, immunogenicity [18,19]. The aim of this article was to present five short cases of cancer patients who have undergone our ASI protocol. The common similarity of all five cases is that each patient's own blood with all its particularity, antigens and immune signaling molecules could be isolated, boosted and cultured to become a personalized integrative vaccine. In cases 1, 2, 3 and 5; we have different degrees of metastasis malignancy, but most of all, they are advanced and aggressive with a rather poor prognosis. In cases 1, 2 and 3 the patients went through and still are receiving several chemotherapeutic protocols. In case 2, the primary osteosarcoma had been already treated with dendritic cells infusions and even so, relapsed with lung metastasis. In case 5, a 75 years old woman with an advanced multiple myeloma and poly-medicated for metabolic syndrome received no conventional treatment other than erythropoietin. In summary and every aspect, the patients have their own peculiarity such as age, weight, clinical history, medical protocols, etc. But when the ASI is given, the first positive point is that no one felt any negative side effects. Secondly, immunophenotype analysis profile showed improvement although patients were under immunosuppressive medication. No collateral infections were noted. Reduction in pain experienced by patients resulted in better life quality and wellbeing. Another interesting point is that the patients did not need to stop their conventional chemotherapeutics or other kind of therapies when ASI was applied. No worsening of symptoms, imaging or lab tests results were further noticed. On the contrary, as previously described, most of them responded positively to ASI therapy. In case 1, following long term chemotherapy with no positive outcomes, CT scan showed stabilization of metastasis progression and tumors size after the initiation of ASI therapy. In case 4, no related active multiple myeloma was confirmed by the last CT scan and the patient stayed only under hospital surveillance control. In all cases, the ASI was always combined with multivitamins and ozone infusions in order to improve the health quality of patients, as intravenous vitamin C and ozone therapy have an immunomodulatory effect on cancer [20-22]. It can be argued that those treatments should have their positive effects reported even without the ASI therapy. This statement is valid, but in the cases 1, 2 and 3 the patients had been previously treated with a similar protocol without the ASI and a different not so positive outcome was presented, mainly in the immune profile and imaging aspects [23-27]. Although this current report focuses on a small group of patients with severe malignancies receiving short-term ASI therapy, the aim of this study is to highlight some ideas about the relevancy of integrative specific immunotherapy as a considerable approach in treating cancer patients. We propose that ASI therapy, an individualized autologous immunomodulating therapy, in conjunction with other anti-cancer tools can provide a smarter way to address oncological cases [28-31].

Conclusion

The Active Specific Immunotherapy, in conjunction with other anti-tumor treatments may support patients to acquire a better life quality and immune profile. It is imperative to publish further studies and explore even wider immunological anti-tumors biomarkers to highlight the cancer diagnostic and treatment with a more personalized and effective medicine applied.

Conflict of Interest

Authors declare no potential conflict of interests.

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