Review

Immunotherapeutic Intervention against Sarcomas

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Received: 2011.05.02; Accepted: 2011.06.03; Published: 2011.06.13

Abstract

Advances in systemic therapy for sarcoma have produced, over the last two decades, relatively short-term benefits for the majority of patient. Among the novel biologic therapeutics that will likely increase our ability to cure human cancer in the years to come, immunotherapy is one of the most promising approaches. While past attempts to use immunotherapy have failed to dramatically shift the paradigm of care for the treatment of patients with sarcoma, major advances in basic and translational research have resulted, in more recent years, in clinical trial activity that is now beginning to generate promising results. However, to move from “proof of principle” to large scale clinical applicability, we need well-designed, multi-institutional clinical trials, along with continuous laboratory research to explore further the immunological characteristics of individual sarcoma subtypes and the consequent tailoring of therapy.

Key words: Immunotherapy, sarcoma

Introduction

Sarcomas constitute an extremely heterogeneous group of diseases, both in terms of histology and of biological and clinical behaviour [1]. Progress in the systemic treatment of sarcoma has been frustratingly slow. Prognosis of patients with metastatic or recurrent disease is poor and most of them will die from tumor progression. In such patients, with significant differences depending on histology subtype and age at disease onset, the overall median survival is around one year and about 10% of cases are alive at 5 years. Treatment of patients failing conventional treatments is mainly palliative as so far novel therapeutic approaches have not had a significant impact, with the exception of GIST, on the prognosis of these patients. This is in contrast with major advances in the understanding of the biology of this group of diseases.

Immunotherapy has long been discussed as a promising method for the treatment of patients with solid tumors but thus far its exact role in sarcoma remains to be defined. Previous reports have suggested that immune-based treatments may be effective in sarcoma, but such approaches have not yet become part of standard clinical practice. We now know that some promising targets for immunotherapy including cancer testis antigens are frequently expressed in certain sarcoma subtypes. [2,3]. Here we review prior trials of immunotherapy including nonspecific immunomodulators, vaccines, and adoptive T-cell therapy.

Nonspecific Immunomodulation

Nonspecific immunomodulation refers to ap-
proaches of therapy aimed to induce antitumor immunity without exposing the patient to a target molecule.

Six sarcoma patients were included in one early high-dose interleukin 2 (IL-2) trial used in combination with limphokine-activated killer (LAK) cells [4]. None of the patients responded. More recently, high-dose IL-2 was given in a pediatric population including 4 patients with osteosarcoma and 2 patients with Ewing’s sarcoma [5]. Two osteosarcoma patients had complete responses (CR) that were durable, which represents an encouraging finding that warrants more investigation focused on this sarcoma subtype.

Muramyl tripeptide phosphatidylethanolamine (MTP) is a synthetic analogue of a bacterial cell wall that has been studied clinically as a nonspecific immune modulator (Bacillus Calmette–Guerin). Based on early studies of a potential benefit of liposomal MTP in sarcoma [6,7], the Children’s Oncology Group's Intergroup-0133 conducted a randomized trial in patients with newly diagnosed osteosarcoma. The study showed that ifosfamide added to the benefit seen with cisplatin and doxorubicin in the adjuvant setting, but only when the ifosfamide was given with liposomal MTP [8]. A subsequent report suggested that improvements in outcomes may also be seen in patients with metastatic disease although this analysis was not powered to demonstrate a statistically significant benefit in either event-free or overall survival [9]. To date, liposomal MTP has not secured FDA approval but is available at a number of centers for compassionate use.

Since the seventies interferon (IFN) has been employed in several sarcoma subtypes, particularly osteosarcoma, with contrasting results. Published studies (10-15, summarized in table 1) do not allow to draw conclusion on the potential benefit of IFN in patients with sarcoma. In patients with localized osteosarcoma, who have had a good histological response to neoadjuvant chemotherapy, the European and American Osteosarcoma Study Group trial is conducting a randomized trial (EURAMOS 1) of postoperative systemic therapy consisting of methotrexate, doxorubicin, and cisplatin with or without pegylated IFN α-2b. The pegylated preparation of IFN α has an extended half-life and consequently can be administered less frequently with higher dose delivery. The results of this study will help to define the role of IFN in the adjuvant treatment of osteosarcoma.

### Vaccine Trials

Vaccines expose patients to tumor antigens in order to evoke an antitumor immune response usually in the presence of adjuvant and occasionally in combination with immunomodulation [3,16]. In sarcoma patients, a number of small trials have been conducted using a variety of different vaccines, some with targeted well-defined antigens, others have targeted tumor lysate (summarized in table 2). In one trial [17], patients received an intradermal injection of irradiated autologous tumor cells along with either IFN gamma or GM-CSF as an adjuvant. Median survival was doubled among patients who were delayed-type hypersensitivity (DTH) skin test responders compared to those who were DTH nonresponders, but no measurable responses were reported. Among 10 pediatric patients treated with the same vaccination approach, one patient with fibrosarcoma had a partial response to the treatment which included the CR of several sizable pulmonary metastases [18].

### Table 1: Reports of nonspecific immunomodulation with interferon (IFN) in sarcoma

| Treatment          | # pts/Histology | Clinical setting     | Outcome                          | Reference                  |
|--------------------|-----------------|----------------------|----------------------------------|---------------------------|
| Leukocyte IFN      | 3 / osteosarcoma| Metastatic disease   | 2/3 partial responses            | Ito, 1980 [10]            |
| r-IFN alfa-2a      | 20 / bone sarcomas| Advanced disease    | 3/20 short-lasting partial responses | Edmonson,1987 [11]       |
| IFN beta           | 158 / osteogenic sarcoma | Adjuvant  | 74% 2.5 year disease free      | Winkler, 1984 [12]       |
| r-IFN alfa-2b      | 1 / clear cell sarcoma | Metastatic disease | CR lasting 17 mo               | Steger, 1991 [13]        |
| Leukocyte IFN alfa | 19 /osteosarcoma | Adjuvant            | 12/19 5-year disease free       | Strander, 1995 [14]      |
| r-IFN alfa         | 178 /osteosarcoma | Adjuvant            | 39% 10 year recurrence-free survival | Muller, 2005 [15]       |
Table 2: Reports of vaccine-based studies in sarcoma

| Vaccine                        | # pts/Histology/clinical setting                                      | Outcome                                           | Reference                        |
|-------------------------------|---------------------------------------------------------------------|---------------------------------------------------|----------------------------------|
| Irradiated autologous tumor cells | 16/various pediatric / advanced disease                         | Improved survival in skin test responders (16.6 vs 8.2 mo). No tumor response | Dillmann, 2004 [17] |
| Dendritic cells pulsed with Tumor lysate | 10/various pediatric/ advanced disease                         | One measurable response                           | Geiger, 2001 [18] |
| DC pulsed with tumor-specific peptides | 16/Ewing-rhabdo/ Advanced, bulky                                  | One mixed response                                | Dagher, 2002 [20] |
| DC pulsed with tumor lysate (#=3) SYT-SSX2 or EWS-FLI-1 peptides (#=2) | 5/ various / (residual tumor post auto TX)                       | One complete response 77mo+ (Ewing)              | Suminoe, 2009 [21] |
| 105AD7 (against CD55)         | 28/osteosarcoma / advanced, post conventional chemotherapy         | T cell response in vivo (13/28); 1 long-lasting response | Pritchard–Jones, 2005 [23] |

The largest dendritic cell vaccine trial to date enrolled 52 patients with t(2;13) or t(11;22) translocation positive, recurrent, or metastatic Ewing’s sarcoma family of tumors or alveolar rhabdomyosarcoma [19]. All patients underwent prechemotherapy cell harvest via apheresis for potential receipt of immunotherapy. Following completion of standard multimodal therapy, 30 patients ultimately underwent immunotherapy with dendritic cells pulsed with peptides derived from tumor-specific translocation breakpoints and E7, a peptide known to bind HLA-A2. Toxicity was minimal. Intention-to-treat analysis suggested a longer overall survival for patients who received immunotherapy compared to all patients apheresed. While the results provided by this study are intriguing, a firm conclusion of the efficacy of this approach can only be drawn from a prospective randomized trial.

Other series of vaccine-based treatment including a limited number of patients have been reported [20-25], none providing clear evidence of a potential benefit of this approach in sarcoma patients.

There is an on-going randomized placebo controlled multicenter Phase II trial of a trivalent peptide vaccine to the gangliosides GD2, GD3, and GM2 in patients with advanced stage sarcoma rendered disease free by surgical resection. These gangliosides, thought to play a role in cell adhesion and cell-cell interactions, may be expressed in some sarcomas [26-28] and one report suggests that soft tissue sarcoma patients develop an antibody response to GD2 more frequently than healthy subjects [29]. On the other hand, it is worth noting that a randomized trial of gangliosidein in melanoma failed to demonstrate improvement in survival [30].

Adoptive T-Cell Therapy (ATCT)

ATCT involves the expansion either ex vivo (for later reinfusion) or in vivo, of immune effector cells capable of tumor killing. This may be nonspecific, as in the case of allogeneic hematopoietic stem cell transplantation (HSCT), or cytokine-induced killer (CIK), or may use tumor/antigen-specific ex vivo cultures or genetically engineered cells to have tumor-directed specificity.

Non-specific ATCT

Allogeneic Stem Cell Transplantation

Evidence of an immune-mediated effect against sarcoma in experimental animal models of allogeneic HSCT has been reported since the 80’ [31,32]. Based on these preclinical results single case reports and small series of patients with sarcoma treated with allogeneic HSCT from HLA-matched sibling donors have been reported with contrasting results [33,34]. A retrospective analysis of adult patients with soft tissue sarcoma registered at the EBMT database [35] was not able to draw firm conclusions about a possible role of allogeneic transplantation in advanced STS, mainly because of the heterogeneity of the patient population.

Recently, Thiel et al [36] retrospectively analyzed data of 87 Ewing sarcoma patients from various registries treated with allogeneic HSCT and evaluated the outcome regarding the use of reduced-intensity conditioning (RIC) and high-intensity conditioning (HIC) regimens as well as human leukocyte antigen (HLA)-matched and HLA-mismatched grafts. There was no improvement of survival with RIC compared with HIC due to increased relapse incidence after RIC despite less transplant-related mortality (TRM) incidence. HLA mismatch was not generally associated with a greater antitumor effect. These results suggest general absence of a clinically relevant Graft-versus-Sarcoma effect.

Allogeneic HSCT can be viewed, in perspective, as a platform for additional approaches of adoptive...
immunotherapy [37]. The donor immune system can in fact permit the repeated infusion of alloimmune lymphocytes, tumor-specific T cells or NK/CIK cells from the donor without risking their rejection.

**Cytokine-Induced Immune Effector Cells**

LAK cells are cytotoxic effector lymphocytes whose cytolytic activities are not restricted by major histocompatibility complex (MHC) and have the ability to kill fresh tumor cells and NK-resistant tumor cell lines [38]. LAK cells are generated from blood lymphocytes following expansion in the presence of IL-2 for a 5-day culture period. LAK cells demonstrated potent in vitro cytotoxicity against susceptible tumor cells and led to the regression of established tumors in animal models [39-41]. In clinical studies, LAK cells had shown modest efficacy in solid tumors such as renal cell carcinoma, melanoma and hepatocellular carcinoma [42,43] and no data are available in the setting of sarcoma.

Closely related to LAK cells, CIK cells are polyclonal T effector cells generated in vitro by incubation of peripheral blood lymphocytes with anti-CD3 monoclonal antibody, IL-2, IL-1 alpha, and interferon-gamma [44]. This unique subset of non-MHC-restricted CD3+CD56+ T cells was referred to as NK-like T cells since, similar to the NK cells, they do not require prior specific sensitization to induce the recognition of target cells. CIK cells have a high rate of proliferation and demonstrate a potent cytolytic activity in vitro against a variety of tumor targets, including sarcomas [45,46]. However, data on the efficacy of CIK cells in vivo are limited [47].

CIK cells show only limited graft-versus-host effects in various mouse models [48] which suggest their potential use as adoptive immunotherapy following allogeneic transplantation [49,50] i.e as an effective alternative to classic donor lymphocyte infusion [51].

**Targeted ATCT**

A strategy that has proven effective in increasing the efficacy of antitumor cell therapy protocols is the *ex vivo* identification of autologous or allogeneic lymphocytes with antitumour activity, which are then administered to cancer patients. A number of different approaches have been so far employed to obtain tumor-specific T cells, such as: *ex vivo* selection TIL based on their capacity to recognize autologous tumor cells, repeated *in vitro* stimulation with tumor-associated antigens (TAA)/whole tumor cells, or, more recently, genetic modification of T-cells using T-cell receptors encoding retroviruses, that can convert normal lymphocytes into cells with specific anti-cancer activity.

**Tumor-Infiltrating Lymphocytes**

TIL therapy can be considered a targeted T cell therapy as they are ex vivo selected for their capacity to recognize autologous tumor cells. Transfusion of TIL has emerged as the most effective treatment for patients with metastatic melanoma, a decisive improvement in their efficacy coming with the introduction of an immunodepleting preparative regimen given before the adoptive transfer, which resulted in the clonal repopulation of patients with anti-tumour T cells [52]. Though some early work did seem to demonstrate that TIL can be grown in culture from patients with sarcoma [53,54], with variable yield, no clinical data are available. We believe that this may represent an area of future development.

**T-Cell Lines Specific for TAA**

Over the last decade, progress in the field of biotechnology has allowed for the characterization of tumor cells, with identification of tumor-specific or tumor associated antigens. However, the number of TAA identified so far is relatively limited if compared to the plethora of molecules present on tumor cells that may contribute to stimulate a protective immune response. To overcome this problem, during the past few years, the use of dendritic cells pulsed with whole tumor cell preparations, to cross-prime cytotoxic T-lymphocytes (CTLs) has been investigated [55-57]. Montagna et al. demonstrated the feasibility of obtaining large quantities of autologous anti-tumor specific CTLs generated by stimulation of patients’ peripheral blood mononuclear cells with dendritic cells pulsed with apoptotic tumor cells [58]. In a pilot study [59], the same authors have shown that anti-tumor CTLs can be administered safely in patients with advanced solid malignancies, including sarcoma, and can improve the immunological status of recipients against tumor. The clinical efficacy of such immunotherapeutic approach will be investigated further in a phase II study.

Very recently, It has been shown that cancer stem-like cells/cancer-initiating cells of bone malignant fibrous histiocytoma are recognized by autologous CTLs in the tumor microenvironment and peripheral circulating lymphocytes [60] which support the hypothesis that CTL-based immunotherapy could target cancer stem cells of bone sarcoma.

**ATCT with T-Cells Specific for Viral Antigens**

A rare example of solid cancer setting in which tumor-specific T cells have been employed with suc-
cess is virus-related tumors. In particular, independent phase I-II studies demonstrated that clinical and immunological responses can be obtained in patients with radiotherapy- and chemotherapy-resistant, EBV-related nasopharyngeal carcinoma by administration of EBV-specific autologous polyclonal CTL therapy [61-64]. No clinical data are yet available in the setting of virus-related sarcomas and this may well be an area of future development in selected patients [65-66].

**T-Cells Modified to Express Chimeric Receptors**

A strategy to broaden the reactivity against shared cancer-associated antigens present on multiple tumour types consists in grafting specificities for antigens expressed on tumour cells through genetic manipulation [67]. Investigators have developed artificial T-cell receptors, also referred to as chimeric antigen receptors, isolated from high avidity T cells that recognize cancer antigens, and retroviral or lentiviral vectors have been used to redirect lymphocyte specificity to these cancer antigens. Clinical studies in B-cell haematological malignancies [68] and subsequently in solid tumors [69-72] demonstrated that normal human lymphocytes genetically engineered to express a TAA, can mediate cancer regression in vivo. Very recently, Robbins et al [73] reported on the ability of adoptively transferred autologous T cells transduced with a T-cell receptor (TCR) directed against the cancer testis antigen NY-ESO-1 to mediate tumor response in metastatic synovial cell sarcoma. Objective clinical responses were observed in four of six patients with synovial cell sarcoma including a near CR lasting 18 months. This represents the first demonstration of the successful treatment of a non-melanoma tumor using TCR-transduced T cells. The NY-ESO-1 antigen is expressed in 80% of synovial sarcoma but also in 15% to 50% of highly prevalent tumors that include breast, lung, prostate, and ovarian cancer [74,75]. Therefore, effective therapies that target NY-ESO-1 could potentially be applied to the large population of cancer patients.

**Conclusions**

Over the years immunotherapeutic approaches have shown signals of great potential in selected patients with sarcoma. As an example, the dramatic responses to T-cell therapy recently demonstrated in synovial cell sarcoma. Studies like this just scratch the surface of what might be feasible for patients with sarcomas in the future, since as many as 25% of sarcomas have reproducible genetic changes.

To move from “proof of principle” to large scale clinical applicability we need well-designed, multi-institutional clinical trials, along with continuous laboratory research to explore further the immunological characteristics of individual sarcoma subtypes and the consequent tailoring of therapy.

While past attempts to use immunotherapy have failed to dramatically shift the paradigm of care for the treatment of patients with sarcoma, a great opportunity now exists to increase the therapeutic options available in this challenging group of diseases.

**Conflict of Interest**

The authors have declared that no conflict of interest exists.

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