Published in final edited form as:

Immunotherapy. 2012 March ; 4(3): 283–290. doi:10.2217/imt.12.3.

Cellular immunotherapy for soft tissue sarcomas

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SUMMARY

Soft tissue sarcomas are rare neoplasms, with approximately 9,000 new cases in the United States every year. Unfortunately, there is little progress in the treatment of metastatic soft tissue sarcomas in the past two decades beyond the standard approaches of surgery, chemotherapy, and radiation. Immunotherapy is a modality complementary to conventional therapy. It is appealing because functional anti-tumor activity could affect both local-regional and systemic disease and act over a prolonged period of time. In this report, we review immunotherapeutic investigative strategies being developed, including several tumor vaccine, antigen vaccine, and dendritic cell vaccine strategies.

Keywords

immunotherapy; dendritic cell; intratumoral; radiation therapy; neoadjuvant; soft tissue sarcoma; cytotoxic T lymphocytes; immune-specific response

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Conflict of Interest Statement: none of the authors have a conflict of interest.

FINANCIAL DISCLOSURE

No financial relationships to disclose

Clinical challenges in the treatment of soft-tissue sarcomas

Sarcomas are relatively rare neoplasms, with approximately 9,000 new cases of soft tissue sarcomas in the United States every year. Sarcomas affect all age groups: 15% are found in children younger than 15, and 40% occur after age 55. These tumors arise from smooth and skeletal muscles, tendons, fat, fibrous tissue, synovial tissue, vessels, and nerves. Approximately 60% of soft tissue sarcomas arise in the extremities.

There has been little progress in the treatment of metastatic soft tissue sarcomas over the past two decades. In general, soft tissue sarcomas arising in the extremities or trunk metastasize to the lungs. The standard treatment for these patients is systemic chemotherapy. There is no evidence that combination chemotherapy is more effective than single agents in producing improved overall survival, however, tumor response rates are higher with the combination of doxorubicin and ifosfamide. This drug combination often requires hospitalization for infusion and may produce significant toxicity [1]. In a subset of the patients, metastasectomy or careful observation of asymptomatic lesions can be considered. Resection of lung metastases may be performed if the patient can tolerate the surgery and if the number of metastatic lesions is low. Often, this surgery is performed after chemotherapy, so that responsiveness to chemotherapy can be determined with measurable disease in place. The response rate to combination chemotherapy is 30–60% depending on the agents and doses used, as well as the histology, resulting in a median overall survival of 1 to 2 years. Salvage chemotherapy with gemcitabine and docetaxel at the time of progression can be offered. However, many of the patients on second line therapy eventually succumb to the disease.

Biology and challenges of cancer immunotherapy

During the past decade, intensive studies resulted in the identification of many tumor-associated antigens (TAA) expressed by different neoplasms, including melanoma, cervical, colon, lung, and breast cancers [2]. Several groups, including ours, have demonstrated clearly the induction of an antitumor immune response by immunization of animals with appropriate antigens [3]. The immune cells responsible for tumor cell killing appear to be primarily major histocompatibility complex (MHC)-restricted cytotoxic T lymphocytes (CTL) or CD8⁺ cells. Such CTLs, via their T cell receptor (TCR) detect target cells by recognizing short peptide fragments of endogenous proteins, which are presented to them in complex with MHC class I molecules on the target cell's surface. Induction of CTL responses requires effective cooperation between antigen-presenting cells (APCs), T-helper (CD4⁺) cells, and CTLs. The APCs are responsible for effective presentation of antigen and delivery of co-stimulatory signals to CD4⁺ and CD8⁺ cells. The most potent APCs known are dendritic cells (DCs).

Dendritic cells arise from the bone marrow and can be isolated from peripheral blood, lymph nodes, spleen, and skin or can be generated in vitro from progenitors from these sources. Relatively immature DCs have a high potential to acquire, process, and present soluble antigens. They are also capable of taking up apoptotic cells and apoptotic bodies and to then process and effectively present antigens from these apoptotic sources [4,5]. Dendritic cells

are the only cells capable of stimulating primary immune responses, including cytotoxic T cell responses [6,7], and so play a crucial role in antitumor immune response. Moreover, they are extremely effective in the stimulation of secondary immune responses. After contact with antigens and activation, DCs migrate to draining lymph nodes where they encounter and activate naïve T cells. Efficient presentation of antigens requires upregulation of MHC class II and co-stimulatory molecules. In vivo, this activation of the APC takes place in tissues after contact with cytokines produced by activated macrophages and T cells, as well as during the direct contact with T cells. It is the DCs, and not tumor cells themselves, now thought to be responsible for the induction of antitumor immunity in a tumor-bearing host [8,9].

The induction of an antitumor immune response that is clinically useful, unfortunately, is seriously limited by the defects in the host immune system, attributable to the cancer cells acting directly or indirectly on the immune cells. Defects in both T cells and DCs have been described in animal tumor models and in cancer patients [10]. We and others recently demonstrated that presentation of antigens by functionally competent DCs may overcome some aspects of this immune deficiency in murine model systems. Frequent (every 4–5 days) administration of DCs that were pulsed with tumor-specific antigens resulted in a substantial antitumor effect [10]. This, and the lack of toxicity observed in DC-based immunotherapy trials suggests that DCs might be useful, even for treatment of patients with advanced cancer. This is very attractive for clinical trial development. For therapeutic cellular immunization, DCs have some ideal features.

However, the mere presence of DCs in the vicinity of tumors is insufficient to induce an antitumor response; even though tumor cells themselves are the best TAA source, and apoptosis can occur during normal development, it is an immunologically weak or even tolerizing environment [14]. The optimal presentation of antigens from tumor cells appears to require two conditions: 1) phagocytosis of apoptotic cells by immature DCs -- to provide peptides for MHC class I and II molecule-related presentation -- and 2) a maturation-inducing signal – this may be delivered by exposure to necrotic tumor cells or their products [15].

Apoptosis, or programmed cell death (distinguished from necrosis), is a complex enzymatic pathway culminating in condensation of chromatin, DNA fragmentation, and death. The apoptotic pathway is an active process that depends on RNA and protein synthesis by the dying cell and can be triggered by a variety of stimuli. Gamma-irradiation induces DNA damage and was one of the first methods found to induce apoptosis in vitro [16]. However, most tumors contain hypoxic cells, and they hypoxic environment can protect against ionizing radiation and subsequently the protected cells can repopulate the tumor after radiation [17]. Thus, γ -radiation may provide a good method of producing apoptotic bodies bearing tumor-derived, for induction of antitumor immune responses using DCs.

Novel immunotherapy combined with standard therapy

During the past couple of years, several groups reported successful results in animal tumor models by combining apoptosis-inducing treatment with intratumoral administration of DCs.

Candido et al. demonstrated that injection of DCs into tumors with high rate of spontaneous apoptosis resulted in an antitumor effect [18]. Using a mouse sarcoma tumor model, in our group it was demonstrated that combining γ -radiation with local DC administration into the tumor site resulted in the induction of tumor-specific immune responses, which had a significant antitumor effect [19]. Teitz-Tennenbaum and colleagues reported confirmation of these observations and showed that radiotherapy potentiates the efficacy of intratumoral DC administration [20]. Similar effects were observed when chemotherapy was used as the apoptosis-inducing agent. Tong et al. showed that combining chemotherapy and intratumoral DC administration leads to a potent antitumor effect [21]. In other work in our group, it was demonstrated that although repeated cycles of chemotherapy have negative cumulative effects on immune system function, chemotherapy does not preclude the generation of potent antitumor immune responses in tumor-bearing mice [22]. Recently, Song and Levy reported that intratumoral injection of DCs after chemotherapy led to complete, long-term regression of mouse lymphoma [23]. These studies have established proof-of-concept and demonstrated the therapeutic potential of this approach of placing dendritic cells and in situ apoptotic tumor to induce significant anticancer response for the host. To test clinically the safety and immunological efficacy of this approach, we initiated pilot clinical trials in sarcoma cancer patients with high risk of recurrence and in prostate cancer patients receiving primary definitive-intent radiotherapy.

Survivin as a surrogate marker of antitumor immune responses

Adequate evaluation of antitumor immune responses is a critical problem in development and assessment of cancer immunotherapeutic strategies [24]. It is clear that adequate evaluation of immune responses to cancer vaccines is impossible without measuring immune response to tumor-specific antigens. Unfortunately, in many tumors, such antigens have not been identified. This is especially true for soft tissue sarcoma. One of the most common approaches to solving this problem is to use whole tumor cell lysates as a source. This approach is feasible in patients with large soft tissue sarcomas, and we have employed it in our clinical trials in sarcoma. However, it would be highly beneficial to complement this analysis using defined antigens specific for the tumor cells. Survivin is a protein that can be very effective in this role.

Survivin is a recently described antiapoptotic protein that belongs to the inhibitor of apoptosis protein (IAP) family. It is a short-lived protein with a half-life of about 30 minutes [25]. Its intracytoplasmic degradation is regulated by a ubiquitin-proteasome pathway, which links peptide products of protein degradation and class I MHC peptides in cell surface MHC-epitope complexes, in different cells. Overexpression of survivin in the cytoplasm of tumor cells and its rapid degradation by proteasome-related mechanism may result in increased expression of survivin epitopes surface in association with MHC class I molecules on the tumor cell surface, thus representing tumor-specific targets for CTL lysis. Immune responses to survivin in cancer patients include the generation of antibodies [26] and the activation of type 1 T helper cells as well as CTL [27,28]. Human HLA-A2-positive DCs pulsed with survivin-derived HLA-A2 matching peptides were shown to induce CD8⁺ effector T cells against target cells that were transfected with survivin DNA, or against non-tumor targets pulsed with survivin-derived peptide [28]. Furthermore, utilizing a

functionally dominant-negative, altered surviving protein as the stimulating protein for immunotherapy, our group showed it is possible to generate a survivin-specific immune response in cells of healthy volunteers and of cancer patients [29].

Expression of survivin at a high level is observed in many common human cancers but not in normal, terminally differentiated adult tissues [25,30–36]. Overexpression of survivin in tumors correlates with more aggressive disease and worse survival [37,38]. This expression of survivin was evaluated specifically in malignant tissue samples from 63 soft tissue sarcoma patients as well as from a panel of tumor cell lines [39,40]. The survivin protein levels were quantified by a novel ELISA and by Western blot analyses. High levels of survivin were detected in tumor samples from more than 75% of patients with stage II and from more than 90% of patients with stage III soft tissue sarcoma [39]. In contrast, none or only weak expression of survivin protein was found in the nonmalignant specimens. As a clinical predictor, elevated survivin content in tumor tissue extracts was a significant and independent negative factor for patients with soft tissue sarcomas [39]. The presence of increased survivin expression and its correlation to prognosis also were confirmed in a variety of soft tissue sarcoma subtypes, including leiomyosarcoma, synovial sarcoma, and pleomorphic liposarcoma [41]. Thus, it can be stated that survivin is overexpressed in a large proportion of cancers, and particularly of soft tissue sarcoma patients. Its defined HLA-A2-bound epitopes are recognized by CTL, and this makes surviving and acquisition of increased titer of anti-survivin CTL) an attractive surrogate marker for evaluation of immune responses in patients with soft tissue sarcoma.

Pre-clinical experiments using mouse tumor models

In preliminary studies using animal tumor models, our group addressed the hypothesis that combining apoptosis-inducing therapy with intratumor administration of DCs can result in a potent antitumor response [19,22]. We used mouse models to prove the concept that DCs injected after γ -irradiation of tumor 1) migrate to the tumor site, 2) engulf apoptotic tumor cells, and 3) induce an antitumor immune response. Two well-characterized mouse sarcoma models carrying defined MHC class I-restricted tumor-specific antigens were used: MethA sarcoma and C3 tumor. MethA is a sarcoma induced by methylcholanthrene, developed in BALB/c mice and passaged as an ascitic tumor. It is a relatively immunogenic tumor that carries a mutant endogenous p53 gene. The C3 sarcoma was obtained by transfecting MEC cells of C57BL/6 mice with EJ-ras and plasmid containing human papillomavirus type 16.

MHC class I restricted peptides (tumor-specific and control) were used to evaluate immune responses in both tumor models. DCs were generated from bone marrow of syngeneic mice using GM-CSF and interleukin-4 (MS-A). MethA or C3 cells ($4\text{--}5 \times 10^5$) were injected subcutaneously into the hind legs of BALB/c or C57BL/6 mice, respectively. Treatment was started on the 13–15th day, when tumors reached at least 5–7 mm in diameter. The γ -irradiation (30 or 50 Gy total) was delivered in 3–5 doses of 10 Gy each, over 2–3 weeks. Apoptosis of tumor cells was verified by TUNEL assay at 3, 18, and 48 hours after γ -irradiation [19]. The γ -irradiation was delivered only to the tumor site, and the rest of body was shielded. DCs were injected intravenously (two-thirds of mice) into the tail vein and subcutaneously (one-third of mice) in close proximity to the tumor 3–4 hours after each dose

of irradiation. Mice were sacrificed on the 14–16th day of treatment in order to evaluate immune responses or, when tumors became bulky, for evaluation of an antitumor response.

Using fluorescence labeling, we observed that DCs indeed accumulate at the tumor site after irradiation and they engulf apoptotic tumor cells in situ [19]. In the same study, the immune response to TAA in treated mice was evaluated. Tumors were established subcutaneously, and treatment was started when tumors reached 5–7 mm in diameter. Four independent experiments were performed, with four mouse groups for each: γ irradiation only, DC administration only, both, or neither. Mice were sacrificed 3–4 days after the third irradiation (3 weeks after the start of the treatment), splenocytes were isolated, and the presence of peptide-specific interferon- γ -producing CD8⁺ T cells was evaluated using ELISPOT assays. Only mice treated with combined therapy demonstrated a significant response to the specific peptide [19]. These results were confirmed in experiments with antigen-specific tetramer staining of CD8⁺ T cells [19]. Thus, γ -irradiation in combination with administration of DCs induces tumor-specific immune responses in tumor-bearing mice. Further, using ex vivo-generated, transgenic DCs with defective antigen presentation, we demonstrated that these observed effects required cross-presentation by donors' DCs [19].

To evaluate the antitumor effects of the combined therapy, mice were observed up to 45 days from the start of the treatment and sacrificed when tumors became bulky. Again, in each experiment, the four groups were used: γ irradiation only, DC administration only, both, or neither. Each group included 3–6 mice, and experiments were repeated 3–4 times. MethA sarcoma quickly progressed in non-treated mice. Gamma-radiation and DCs delivered separately only slightly delayed the tumor progression. In contrast, by day 14, ten of twelve mice in the combination treatment rejected the tumors, and growth slowed in the other two. [19].

A more prolonged experiment was performed in the poorly immunogenic C3 tumor model. Mice were treated 5 times with γ -irradiation, or 6 times with DCs, or with both and then observed for 45 days from the start of the treatment. Again, tumor growth was significantly reduced in the combined treatment group; by day 20, 4 out of 10 mice in this group rejected tumors [19]. Thus, these data demonstrate that the combination of local γ -irradiation of tumor and DC administration resulted in the generation TAA-specific CTLs in tumor-bearing mice, and a significant immune response was following the combined treatment.

The effect of combined administration of apoptosis-inducing therapy and DCs was investigated in another experimental model, using chemotherapy as the trigger for immunotherapy [22]. Currently, chemotherapy is the most frequently used treatment for most advanced-stage cancers, with conventional chemotherapy given as several cycles over a relatively long period of time. While it is known that chemotherapy is associated with systemic immune suppression, the salient question is whether this context will still be permissive for induction of an effective anticancer immune response.

To address this question, work in our group was conducted with experiments utilizing mice bearing mammary adenocarcinoma expressing a model tumor antigen, influenza virus HA

(DA3-HA), and parental tumor (DA3), without that antigen. The experimental animals received different doses of paclitaxel, with or without additional administration of DCs. Paclitaxel was injected 3 times weekly, and DCs were injected either intravenously or into tumor site 36 hours after each injection of paclitaxel. Apoptosis was measured using Annexin V binding or TUNEL assays. The CD8-mediated response of T cells to HA-derived peptide epitope was measured with ELISPOT and the CD4-mediated response of T cells to HA-derived peptide was measured by a proliferation assay. The combined treatment resulted in induction of HA-specific CD8-mediated response in all 9 tested mice and CD4-mediated responses in 4 of 6 treated mice [22]. These effects were observed only if DCs were injected into tumor site, but not when injected intravenously. No specific responses were found in mice treated with either chemotherapy or DCs alone. Injection of dexamethasone together with paclitaxel (to mimic the frequent usual coadministration of corticosteroids with taxanes in clinical practice) did not affect the induction of immune responses. A significant antitumor effect with combined treatment was observed both for the DA3-HA or the DA3 tumor [22].

Initial results of pilot clinical trial in soft tissue sarcoma patients

The above murine data and results from other groups have provided the foundations for a clinical trial to combine radiation and intratumoral injection of DCs in cancer patients. At Moffitt Cancer Center, we conducted a phase I/II clinical trial in patients with high-grade, large (> 5 cm diameter) extremity soft tissue sarcoma (NCT00365872), a group for whom there about 50% risk of developing recurrent metastatic disease. In addition, these patients generally benefit (in terms of size reduction and potentially smaller tumor) from neoadjuvant external beam radiation therapy (EBRT) to the tumor prior to resection. Goals of the trial were to address the safety of combined administration of EBRT and intratumoral placement of DCs in the neoadjuvant setting, and additionally to evaluate sarcoma-specific immune responses (Figure 1) [42–45].

Seventeen eligible patients were treated in the neoadjuvant setting with EBRT, combined with four weekly intratumoral injections of 10^7 DCs followed by complete resection. Patients first underwent leukapheresis to collect peripheral blood mononuclear cells (PBMCs) for the generation of DCs. The PBMCs were stored frozen in aliquots and were thawed 1 week before each injection so that freshly generated DCs would be used. For the EBRT, megavoltage equipment with effective photon energies from 4 to 23 MV was utilized. Any field arrangement that covered the target volume was allowed. All fields were treated every day. The clinical target volume (CTV) was the gross tumor as per planning study(s) plus 5–10 cm longitudinal margin. The planning target volume (PTV) was the CTV plus 2.0 cm. Cerrobend blocks or multi-leaf collimators were used to limit the dose to nearby normal structures. The radial CTV could be limited in order to spare a required strip of skin (2–3 cm) but had to cover the tumor with a minimum of 2 cm. The PTV was treated to 50.4 Gy in 1.8-Gy fractions (total of 28 fractions), given five times per week. The prescribed dose was defined on or near the central axis. The PTV was covered by the 95% isodose line; a significant hot spot did not exceed 110%.

One week after the start of radiation therapy, the autologous PBMCs were thawed, and DCs were generated using serum-free X-VIVO-15 medium, interleukin-4, and GM-CSF. In preparation for this trial, we optimized the conditions for DC generation using serum-free medium and clinical-grade growth factor and cytokines. The radiation treatment was started on Monday; DCs were injected on Fridays 2–4 hours after that day's radiation fraction. The 3-day interval between the DC injection and the following Monday's dose of radiation allowed DCs to engulf apoptotic tumor cells and migrate out of the tumor site into draining lymph nodes. Injections of DCs were performed after the 10th, 15th, and 20th doses of radiation. Biopsies of the tumor area were obtained before EBRT, after the second DC injection, and at completion of surgical resection. Blood was collected for immunological testing initially, after the second DC injection, and in follow-up at 2-month intervals to monitor the longevity of the response [42–45].

To date, all patients have completed treatment and full immunologic analysis. The treatment was well tolerated with no unexpected, serious adverse events occurring. No patient had assay-detected tumor-specific immune responses before combined EBRT/DC therapy; 9 patients (52.9%) developed tumor-specific immune responses, which lasted from 11 to 42 weeks. Interestingly, this response became progressively stronger throughout the course of treatment (in some cases a 4-fold increase over control) after the last DC injection. Furthermore, these responses persisted even 30 weeks after the treatment start. Twelve of 17 patients were progression free after 1 year, (71%, 95% CI 47–87%). Histologic evaluation demonstrated that treatment caused a dramatic accumulation of T cells in the tumor. The presence of CD4+ T cells in the tumor positively correlated with tumor-specific immune responses that developed following combined therapy. Accumulation of myeloid-derived suppressor cells negatively correlated with the development of tumor-specific immune responses, but for regulatory T cells, it was not the case. Experiments with ¹¹¹I-labeled DCs demonstrated that these APCs need at least 48 hours to start migrating from tumor site. Thus, we concluded that the combination of intratumoral DC administration and EBRT is safe and results in induction of antitumor immune responses. Indeed, this therapy is promising and needs further testing in clinical trials designed to assess clinical efficacy [42–45].

Initial results of pilot clinical trial in synovial cell sarcoma patients

A separate clinical trial was performed using genetically engineered lymphocytes reactive with antigen for sarcoma in patients with metastatic synovial cell sarcoma refractory to all standard treatments [2]. Patients with tumors positive for the NY-ESO-1 antigen were treated with autologous T cells that had been transduced with the corresponding TCR specific for peptide of that antigen, and also treated with interleukin-2 at a dose of 720,000 IU/kg of interleukin-2, up to tolerance, with the immunotherapy given following after preparative chemotherapy. Objective clinical responses were evaluated using Response Evaluation Criteria in Solid Tumors (RECIST). Objective clinical responses were observed in 4 of 6 patients with synovial cell sarcoma and in 5 of 11 patients with melanoma bearing tumors expressing NY-ESO-1. Two of 11 patients with melanoma demonstrated complete regression that persisted after 1 year. A partial response lasting 18 months was observed in one patient with synovial cell sarcoma. These observations indicate that TCR-based gene

therapy directed against NY-ESO-1 is a viable therapeutic approach to develop for patients with synovial cell sarcoma [2].

Future perspective

Cellular immunotherapy is a novel in treatment for sarcoma. The intratumoral placement of ex vivo-generated, autologous DCs in concert with apoptosis induction can generate systemic immunity in murine systems and is feasible and promising in an early clinical trial. In addition, TCR-based gene therapy directed against a known sarcoma antigen bears interest for treating a nonmelanoma tumor using TCR-transduced T cells. In the clinical setting a successful induction of systemic antitumor immunity may provide substantial clinical benefit. Our approach, with autologous dendritic cells and in situ tumor apoptosis as the antigen source presents an advantage of eliminating the cumbersome requirement for patient selection based on MHC class I type in future clinical development. Similarly, this approach eliminates patient selection based on expression of defined TAAs and reduces the problem of TAA loss during tumor progression. Thus, we believe our approach could dramatically accelerate developing immune-based treatments, as compared to those immunotherapies directed at specific TAAs.

Our group has already demonstrated that the combination of intratumoral administration of DCs with local radiation can generate antitumor immune responses in a pilot study involving soft tissue sarcoma patients possessing localized disease with high-risk features. We have embarked in confirming these observations in a larger, multi-institutional phase II trial with a 10-Gy boost of radiation, which is currently enrolling patients (NCT01347034). Use of TCR-based gene therapies directed against sarcoma antigens continues as well. Future work, and potential integration with acellular immunotherapeutics such as immune checkpoint inhibitors [46] and modulators of dendritic cell phenotype [47] will more importantly define the best ways that this therapy can be translated into impact most meaningfully on the prognosis of soft tissue sarcoma.

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EXECUTIVE SUMMARY

Clinical challenges in the treatment of soft-tissue sarcomas

- Little progress has been made in the treatment of metastatic soft tissue sarcomas; development of new options is urgent.

Biology and challenges of cancer immunotherapy

- Tumor-associated antigens and induction of an antitumor immune response by immunization with these antigens have been demonstrated
- Dendritic cells (DCs) are the most potent antigen presenting cells, capable of taking-up apoptotic cells and processing and presenting their antigens.
- Gamma-radiation provides a good method of producing tumor-specific antigens, as apoptotic bodies, for induction of antitumor immune responses using DCs.

Novel immunotherapy combined with standard therapy

- In animal tumor models, we have shown that combining apoptosis-inducing therapy with intratumoral administration of DCs results in a potent antitumor immune response.
- DCs play a crucial role in antitumor immune responses. Their use in combination with apoptosis-inducing radiotherapy has been demonstrated pre-clinically. Thus, translational clinical testing of this immunotherapeutic strategy is warranted.

Initial results of pilot clinical trial in soft tissue sarcoma patients

- We have conducted a phase I/II clinical trial in large, high-grade, soft tissue sarcomas, known to have a 50% risk of developing metastatic disease and to benefit from neoadjuvant external beam radiation therapy [EBRT]. The goal of this trial was to evaluate the safety of combined administration of EBRT and intratumoral placement of DCs in the neoadjuvant setting
- Seventeen patients were enrolled. The treatment was well tolerated. Nine patients (52.9%) developed tumor-specific immune responses which positively correlated with the dramatic accumulation of T cells observed in the tumor. Accumulation of myeloid-derived suppressor cells negatively correlated with the development of tumor-specific immune responses, but accumulation of regulatory T cells did not.
- The combination of intratumoral DC administration and EBRT is safe, results in the induction of strong antitumor immune response that are promising and warrants further testing in clinical trials designed to assess clinical efficacy

Future perspective

- Other strategies to elicit an enhanced antitumor immune responses such as utilizing a local radiotherapy boost or genetically engineered lymphocytes reactive to specific TAAs, are also being evaluated, with preliminary clinical

success. Inhibitors of immunologic checkpoints are of contemporary interest in cancer as well.

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Figure 1. MCC 14497 Trial Design

General treatment scheme for cellular dendritic cell (DC) immunotherapy of sarcoma.