Review

Something old, Something New: Radiation and Immunotherapy in Soft Tissue Sarcoma

Adrienne Victor[1], Deepak Sahasrabudhe[1]

1. University of Rochester Wilmot Cancer Institute, USA

Corresponding author: Adrienne Victor, M.D., M.S. University of Rochester Wilmot Cancer Institute, USA

Introduction

The "father of immunotherapy," William Coley, began his research on provoking the immune system to fight cancer in sarcoma patients. He was profoundly affected by the death of a young patient with what is now termed Ewing sarcoma, and investigated reports of another patient with "round cell sarcoma of the neck" who experienced regression following an erysipelas infection [1]. This phenomenon was first reported by Busch in 1868 when he intentionally inoculated a cancer patient with erysipelas and saw shrinkage of the malignancy.Fehleisen repeated this treatment in 1882 and eventually identified Streptococcus pyogenes as the causative agent of erysipelas [2] .William Coley systematically treated bone and soft tissue sarcoma patients, first with live Streptococcus and, after the death of 2 out of 3 patients from the resultant infection, with heat-killed streptococcal organisms combined with Serratia marcescens. This concoction became known as Coley's Toxins [2]. The toxins were injected until the patient became febrile, and Dr. Coley reported incredible responses [3,4]. Further publications showed that using the toxins in conjunction with surgery and/or radiation for patients with "reticulum cell sarcoma of bone" resulted in survival rates far higher than with surgery alone [5].

As understanding of neoplasia and cell of origin evolved, "primary reticulum-cell sarcoma of bone" was subsequently re-categorized as a non-Hodgkin lymphoma. It is worth noting that Coley's results, particularly remarkable in sarcomas, may have actually comprised a variety of other malignant tumors.2 The first cancer registry in the United States, the Bone Sarcoma Registry, collected and reviewed 560 cases of bone tumors from various surgeons and by 1925, and found that approximately half were likely metastatic carcinomas or non-neoplastic diseases rather than true sarcomas [6]. In the intervening years, chemotherapy superseded and eventually replaced immunotherapy in the treatment of sarcoma. Much has been learned about cancer biology and the immune system since Coley first injected a patient in 1891 [7], and with new discoveries there is resurgence in novel combinations of immunomodulation and radiation to improve survival in soft tissue sarcoma [8].

Soft Tissue Sarcoma

Sarcomas are malignancies of mesenchymal origin, arising from bone (osteosarcoma) or soft tissues. Sarcomas are uncommon, accounting for less than 1% of all malignancies [9,10], and soft tissue sarcomas are over five times more common than osteosarcomas [11]. Despite their relative rarity, sarcomas have been well-described in medical history; a fossilized osteosarcoma in the toe of a 1.8 million year old hominid is the earliest evidence of cancer in our ancestors [12]. The first evidence of oncogenic viruses was found in sarcoma when Peyton Rous described transmission of sarcoma in chickens using a cell free extract in 1911 [13]. Further research led to the discovery of a retrovirus, now called Rous sarcoma virus, which had acquired a highly conserved "proto-oncogene" src. This work eventually resulted in a Nobel Prize for the investigator Dr. Rous in 1966, as well Bishop and Varmus in 1989. Subsequent understanding of molecular drivers has led to the development of a new treatment modality, small molecules that specifically target/inhibit oncogenic mutations. Imatinib, the first successful targeted drug, remains the first line treatment for one of the most common types of sarcoma, gastrointestinal stromal tumor (GIST) [14].

There are over 50 different histologic subtypes of sarcomas, many with unique clinical and molecular characteristics [15]. Sarcomas tend to have low tumor mutational burden (TMB) [16], and a bimodal age distribution, although different subtypes predominate the pediatric vs. the older adult population. Given the relative rarity of sarcoma, clinical trials and treatment advances have often lagged behind those in other cancers. Still, studies in rare tumors are feasible, with several large trials investigating novel antibodies for sarcoma recently published, and new therapeutic modalities are in development. These include negative studies of ganitumab (antibody against type 1 insulin-like growth factor receptor) in AEWS1221, which enrolled 299 patients with metastatic Ewing sarcoma in 5 years [17], and the double blind phase III ANNOUNCE trial which showed a lack of benefit in adding olaratumab (antibody against PDGF-R α) to doxorubicin for soft tissue sarcoma [18], despite promising phase II data [19]. Although the result was negative, it is worth noting that the trial was successful in enrolling 509 patients and reporting conclusive results within 5 years of opening.

Radiation for soft tissue sarcoma

The variation in a slurry of heat killed bacteria and difficulty in gaining approvals after the advent of the FDA caused Coley's

toxins to fall out of favor in the face of predictable results of radiation and chemotherapy for management of sarcoma [20]. Sarcoma subtypes have differing sensitivity to radiation; external beam radiotherapy alone can produce responses in 50% of liposarcomas, fibrosarcomas, leiomyosarcomas, or chondrosarcomas. The response rate is lower in malignant fibrous histiocytomas, synovial, and other rare soft tissue sarcomas [21]. Generally, doses of 60 Gy are used. Development of stereotactic body RT in the 1990s allowed for higher doses to be safely delivered, with over 80% local control rates reported in treated metastasis on retrospective review [22].

In non-metastatic soft-tissue sarcoma, radiation is used to achieve improved local control and facilitate limb-sparing wide excision, avoiding the morbidity of amputation. Radiation can be given neo-adjuvantly or adjuvantly, and in unresectable cases, definitively. Preoperative therapy may allow for a lower dose (50 Gy) and a smaller volume of radiation to normal tissue margin, whereas the entire surgical bed plus a margin must be irradiated postoperatively [23], and at higher doses (60-66Gy). Only one randomized phase III clinical trial has evaluated perioperative timing of radiation; the SR2 study assigned 190 patients with extremity STS preoperative or postoperative treatment [24]. This revealed a greater incidence of wound complications in the preoperative group (35%) than in the postoperative (17%) group. Late complications, however, were greater in the postoperative group, including fibrosis (48.2% in the postoperative group vs. 31.5% in the preoperative group), edema (23.2% vs. 15.1%), and joint stiffness (23.2% vs. 17.8%) [25]. In both long term follow up of SR2 as well as a large retrospective study of 517 patients, no differences were seen in local control, metastatic disease, or disease-specific survival between pre- and postoperative radiation [26,27].

Preoperative RT may have the added benefit of providing prognostic information; analysis of RTOG (Radiation Therapy Oncology Group) 9514 (neoadjuvant chemoradiotherapy) and 0630 (neoadjuvant radiation) showed that, among the 22 evaluable patients with a pathologic complete response, 5 year survival was 100% and there were no local failures. In total, 123 patients were evaluable for response: 14/51 (27.5%) on 9514 and 14/72 (19.4%) on 0630 had a complete response. Both overall survival and disease free survival were significantly improved compared to those without a complete pathologic response [28].

In patients with unresectable localized disease, definitive RT is also an option. A review of 112 patients treated with radiation alone showed 5-year local control, disease free survival, and overall survival rates of 60%, 36%, and 52%, respectively, for patients who received doses of 63 Gy or more. Major radiotherapy complications were increased in patients treated with more than 68 Gy, however; 27% of patients who received doses of 68 Gy or more experienced complications compared with 8% of patients treated with doses less than 68 Gy [29].

For sarcomas in non-extremity locations, such as retroperitoneal, the role of radiation in resectable disease is less clear. A retrospective review of 607 patients with localized retroperitoneal well - differentiated liposarcomas and dedifferentiated liposarcomas who underwent surgical resection with or without RT found no benefit to overall survival or distant metastasis free survival with radiation, but did show improvement in local control [30].

Prior radiation therapy can also be a risk factor for development of sarcoma. Radiation-associated sarcomas have been reported to occur as early as a few months following completion of radiation therapy to as long as 54 years later. Median latency period for angiosarcoma following breast cancer was 7 years [31], and for all sarcomas two large reviews showed a median latency of 8.5 years [32], to a mean of 17 years [33]. Radiation induced sarcomas make up around 3% of all cases of sarcoma; the incidence in adults is lower than children, around 0.11% in one review [34], versus slightly over 1% in children [35,36], although this varies on indication, dose, and modality. Radiation induced sarcomas are usually high grade and prognosis tends to be worse than for de novo sarcomas [37]. Radiation induced sarcomas are associated with a gene signature indicative of chronic oxidative stress particularly in mitochondria [38].

Particle beam therapy is under exploration, particularly proton beam radiotherapy, which may reduce the risk of secondary malignancies. Other particles such as carbon ion are also in development. Thus far there are very limited data comparing proton to photon therapy, but modeling of proton therapy vs. photon fields indicates substantial sparing of normal tissues and resultant decreased risk of secondary malignancies in pediatric brain tumor cases [39]. Proton beam is also favored for eye lesions, skull base lesions, and chordoma. Chordomas arise from the notochord and are of ectodermal rather than mesenchymal origin, but are categorized as a bone malignancy. The local control rate in chordoma with conventional photon radiation was only 21 percent, although 85 percent of patients achieved palliation of pain [40]. In contrast, one series of 48 chordoma patients treated definitively with proton and carbon ion therapy, the 2-year local control was 86%, progression free survival 83%, and overall survival of 93% [41].

Radioactive particles can also be used to deliver high doses of radiation to target organs. Radioembolization with yttrium-90 (90Y) was developed for the purpose of treating liver cancer, and has been used in treating liver metastases. With this procedure, small radioactive particles are administered via a catheter into the hepatic artery. They accumulate in the neoplastic capillaries, which disproportionately rely on the hepatic artery for blood supply [42]. In contrast to external beam radiation, radiation doses as high as 200 Gy can be applied to the area treated by using radioembolization while largely sparing normal liver parenchyma [43]. Although most sarcomas have a proclivity to spread to the lungs, GIST is unusual in metastasizing almost exclusively within the abdomen and liver. GIST are relatively radioresistant, one study of 25 patients with GISTs treated with 40 Gy to abdominal sites found two (8%) patients achieved partial remission, 20 (80%) had stable target lesion size for $\geq$ 3 months after radiotherapy with a median duration of stabilization of 16 months, and 3 (12%) progressed [44]. A series evaluating the role of 90Y in GIST found that among 10 patients treated, 3 patients showed complete response, 5 showed partial response, and 1 showed stable disease on initial imaging [45]. Ultimately, 8 of 9 patients did progress on follow up, with a median progression free interval of 15.9 months (range, 4–29 months).

Immunotherapy for soft tissue sarcoma

Coley's toxins were tantalizing but ultimately unsatisfactory as they were variable mixtures of heat-killed bacteria. They could not meet the FDA's stringent guidelines to ensure consistency of a given drug product [46].

In the 1980s, cytokines took the cancer world by storm. Identification of these molecules allowed for synthesis of pure and consistent drug that induced responses, even cures in some solid tumors. This lead to approvals of IL-2 for renal cell and melanoma, although these agents showed little activity in sarcoma.[47, 48]. Cytokines have now been largely superseded by checkpoint inhibitors; the anti-CLTA4 antibody Ipilimumab received its first FDA approval in 2011, then the anti-PD1 antibodies nivolumab and pembrolizumab were approved in 2014.

Predictive biomarkers for checkpoint inhibition are emerging though still imperfect. Microsatellite instability has been approved as an indication for pembrolizumab across all tumor types; high TMB is clearly correlated with responses [49], but cutoffs are uncertain. Both of these are relatively rare in sarcoma with only undifferentiated soft tissue sarcoma and angiosarcoma showing over 5% incidence of high TMB16 and under 2% of sarcomas MSI-H [50]. And yet, there are subtypes that defy the paradigm; alveolar soft part sarcoma has a nearly 40% response rate to the anti PD-L1 antibody atezolizumab [51], and comparable results with pembrolizumab + axitinib [52]. despite being a low TMB, translocation driven malignancy.

Other markers include PD1 expression, PD-L1 expression and tumor infiltrating lymphocytes. In a study of 105 cases of soft tissue sarcoma Kim et al. have reported that 58% had intratumoral infiltration of PD1-positive lymphocytes and 65% of tumors expressed PD-L1. Both PD1-positivity and PD-L1 positivity were independent and negative prognostic indicators for overall and event-free survival by multivariate analysis, with 13% 5-year overall survival in the group of PD-1+/PD-L1+ tumors vs. 90% for PD-1-/PDL1-[53]. Frequency of PD-L1 expression on tumor cells varied widely in different histologic subtypes, with highest rates of positivity (40%) seen in undifferentiated pleomorphic sarcomas (UPS) and rare positivity detected in synovial sarcomas (6%) [54]. Note, this prognostic impact of PD1 expression was reported prior to widespread use of checkpoint inhibitors. These agents are now being evaluated in sarcoma. Alliance A091401 and SARC028 trials examined checkpoint inhibitors in a variety of soft tissue sarcomas, and responses to pembrolizumab (anti-PD-1), nivolumab (anti-PD-1), and nivolumab + ipilimumab (anti-CTLA-4) were seen in several subtypes of metastatic soft tissue sarcomas, most notably in UPS [55]. In the Alliance trial, response rates were more frequent (although not statistically significant) in the combination group ipilimumab 1mg/kg + nivolumab 3mg/kg q3 weeks x4 doses than nivolumab alone, 16% vs. 5%, respectively [56]. Correlative analysis on the association with PD-L1 expression and response from Alliance is pending. The tumors that responded to pembrolizumab in SARC028, were more likely to have higher densities of activated T cells (CD8+ CD3+ PD-1+) and increased percentage of tumor associated macrophages expressing PD-L1 pre-treatment compared to non-responders [57]. PD-L1 expression was observed in only 2 (5%) of 40 tumors with evaluable biopsies. Although both tumors expressing PD-L1 were UPS and responded to therapy, responses to pembrolizumab were also

seen in 5 patients in the absence of tumor PD-L1 expression at baseline. Overall, PD-1 and PD-L1 expression do not have clear predictive value for immunotherapy in sarcoma.

Other promising agents in development include drug-antibody conjugates and cellular therapies. The phase 1 trial of ABBV-085 (an antibody against leucine-rich repeat containing 15 (LRRC15) conjugated to 2 monomethyl auristatin E molecules) in 27 advanced sarcoma patients reported 4 (14.8%) had a confirmed partial response, 8 (29.6%) had stable disease, 11 (40.7%) had progressive disease; 2 (7.4%) were not evaluable. This came at the cost of 71.8% grade 3 or greater treatment related adverse events, however [58].

A study evaluating genetically-engineered ADP-A2M4 (anti MAGE-A4 SPEAR T-cell therapy) in subjects with metastatic or inoperable synovial sarcoma expressing the MAGE-A4 antigen reported 4 partial responses among the first 10 patients treated, stable disease in 5, and progression in 1 patient [59]. An ongoing trial of genetically engineered NY-ESO-1 specific T-cells (NY-ESO-1 TCR T Cells; GSK3377794) reported results on 28 patients with synovial sarcoma; 59% stable disease, with a median duration of stable disease of 17 weeks [60]. Engineered cellular therapies are generally restricted to patients with a matching HLA type, most often HLA-A*02:01, which ranges in frequency by population from over 60% to under 5%, in addition to target protein expression on the tumor [61].

Combinations

Combining immunotherapy with other local control measures may further boost response. In a study of 20 patients with locally advanced or metastatic sarcoma given pembrolizumab and intratumoral T-VEC injections, the best ORR was 30% [62]. Multiple clinical trials are currently evaluating combining checkpoints with potentially curative radiation and resection in soft tissue sarcoma.

Conclusions

Sarcoma therapy has come a long way since Coley injected his first patient with streptococcal organisms in 1891. Advances in physics made it possible to use radiation therapy effectively as an adjunct, and sometimes even replacement, to surgery. Refinement in radiation delivery methods allow for higher doses to be delivered to tumors with greater sparing of normal tissues, rendering even previously radioresistant sarcomas sensitive. Chemotherapy is still the mainstay in most sarcoma systemic therapy, however, improved understanding of the mechanism of action of immunotherapy and the influence of tumor microenvironment is starting to reveal a subset of sarcomas that are sensitive to this treatment modality. Further advances in bioengineering hold promise to target even the immunologically inert, checkpoint resistant sarcomas by utilizing tumor specific antigens to guide drug payloads and immune cells. Despite their rarity, sarcomas remain a source of innovation and discovery.

References

1. Coley WB. The treatment of malignant tumors by repeated inoculations of erysipelas. With a report of ten original cases. 1893. Clin Orthop Relat Res.1991: 3-11.
2. McCarthy EF. The toxins of William B. Coley and the treatment of

bone and soft-tissue sarcomas. The Iowa orthopaedic journal.2006; 26: 154-158.

3. Coley WB. IX. Contribution to the Study of Sarcoma of the Femur: Periosteal Round-Celled Sarcoma of the Femur, Involving Two-Thirds of the Shaft, with Very Extensive Multiple Metastases-Apparent Cure by the Mixed Toxins of Erysipelas and Bacillus Prodigiosus. Well 10(1/2) Years, when a Malignant Tumor (Sarcoma and Epithelioma) Developed in the Thigh at the Site of an Old X-Ray Dermatitis. Annals of surgery.1913; 58: 97-108.

4. Coley WB. The Treatment of Inoperable Sarcoma by Bacterial Toxins (the Mixed Toxins of the Streptococcus erysipelas and the Bacillus prodigiosus). Proceedings of the Royal Society of Medicine.1910; 3: 1-48.

5. Miller TR, JT Nicholson, End results in reticulum cell sarcoma of bone treated by bacterial toxin therapy alone or combined with surgery and-or radiotherapy (47 cases) or with concurrent infection (5 cases). Cancer.1971; 27: 524-48.

6. McCarthy EF. The Registry of Bone Sarcoma. A history. Iowa Orthop J.1995; 15: 74-8.

7. Coley WB. II. Contribution to the Knowledge of Sarcoma. Annals of surgery.1891; 14: 199-220.

8. Que Y, W Xiao, BS Xu, et al. The changing landscape of phase II/III metastatic sarcoma clinical trials-analysis of ClinicalTrials.gov. BMC cancer.2018; 18: 1251-1251.

9. Division of Cancer Prevention and Control, Centers for Disease Control and Prevention [Web site]. Expected New Cancer Cases and Deaths in 2020

10. team TACSmaec, Cancer.org [Web site]. Key Statistics for Soft Tissue Sarcomas,

11. Burningham Z, M Hashibe, L Spector, et al. The epidemiology of sarcoma. Clinical sarcoma research. 2012; 2: 14-14.

12. Edward JO, SR-Q Patrick, S Maryna, et al. Earliest hominin cancer: 1.7-million-year-old osteosarcoma from Swartkrans Cave, South Africa. South African Journal of Science. 2016; 112.

13. Rous P. Landmark article (JAMA 1911;56:198). Transmission of a malignant new growth by means of a cell-free filtrate. By Peyton Rous. Jama. 1983; 250: 1445-9.

14. Iqbal N and N Iqbal, Imatinib: a breakthrough of targeted therapy in cancer. Chemotherapy research and practice. 2014; 2014: 357027-357027.

15. Javier A,Laurini MKC, MBChB, Christopher D.M, et al. College of American Pathologists [Web site]. Protocol for the Examination of Resection Specimens From Patients With Soft Tissue Tumors,

16. Chalmers ZR, CF Connelly, D Fabrizio, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Medicine.2017; 9: 34.

17. DuBois S, JG Bender, A Buxton, et al. Randomized Phase 3 trial of Ganitumab added to interval compressed chemotherapy for patients with newly diagnosed metastatic ewing sarcoma: a report from the Children's oncology Group (COG). Presented at the CTOS Annual Meeting, Tokyo, Japan, Nov 16, 2019

18. Tap WD, AJ Wagner, Z Papai, et al. ANNOUNCE: A randomized, placebo (PBO)-controlled, double-blind, phase (Ph) III trial of doxorubicin (dox) + olaratumab versus dox + PBO in patients (pts) with advanced soft tissue sarcomas (STS). Journal of Clinical Oncology.2019; 37: LBA3-LBA3.

19. Tap WD, RL Jones, BA Van Tine, et al. Olaratumab and doxorubicin versus doxorubicin alone for treatment of soft-tissue sarcoma:

an open-label phase 1b and randomised phase 2 trial. Lancet. 2016; 388: 488-97.

20. Kramer MG, M Masner, FA Ferreira, et al. Bacterial Therapy of Cancer: Promises, Limitations, and Insights for Future Directions. Frontiers in microbiology.2018; 9: 16-16.

21. Rhomberg W. The radiation response of sarcomas by histologic subtypes: a review with special emphasis given to results achieved with razoxane. Sarcoma.2006; 2006: 87367-87367.

22. Stragliotto CL, K Karlsson, I Lax, et al. A retrospective study of SBRT of metastases in patients with primary sarcoma. Medical Oncology.2012; 29: 3431-3439.

23. Wortman JR, SH Tirumani, JP Jagannathan, et al. Radiation Therapy for Soft-Tissue Sarcomas: A Primer for Radiologists. Radiographics. 2016; 36: 554-72.

24. O'Sullivan B, AM Davis, R Turcotte, et al. Preoperative versus postoperative radiotherapy in soft-tissue sarcoma of the limbs: a randomised trial. Lancet. 2002; 359: 2235-41.

25. Davis AM, B O'Sullivan, R Turcotte, et al. Late radiation morbidity following randomization to preoperative versus postoperative radiotherapy in extremity soft tissue sarcoma. Radiother Oncol. 2005; 75: 48-53.

26. Zagars GK, MT Ballo, PW Pisters, et al. Preoperative vs. postoperative radiation therapy for soft tissue sarcoma: a retrospective comparative evaluation of disease outcome. Int J Radiat Oncol Biol Phys. 2003; 56: 482-8.

27. O'Sullivan B, A Davis, R Turcotte, et al. Five-year results of a randomized phase III trial of pre-operative vs post-operative radiotherapy in extremity soft tissue sarcoma. Journal of Clinical Oncology.2004; 22: 9007-9007.

28. Wang D, J Harris, WG Kraybill, et al. Pathologic complete response and survival outcomes in patients with localized soft tissue sarcoma treated with neoadjuvant chemoradiotherapy or radiotherapy: Long-term update of NRG Oncology RTOG 9514 and 0630. Journal of Clinical Oncology. 2017; 35: 11012-11012.

29. Kepka L, TF DeLaney, HD Suit, et al. Results of radiation therapy for unresected soft-tissue sarcomas. Int J Radiat Oncol Biol Phys. 2005; 63: 852-9.

30. Haas RLM, S Bonvalot, R Miceli, et al. Radiotherapy for retroperitoneal liposarcoma: A report from the Transatlantic Retroperitoneal Sarcoma Working Group. Cancer.2019; 125: 1290-1300.

31. Torres KE, V Ravi, K Kin, et al. Long-term outcomes in patients with radiation-associated angiosarcomas of the breast following surgery and radiotherapy for breast cancer. Ann Surg Oncol. 2013; 20: 1267-74.

32. Cha C, CR Antonescu, ML Quan, et al. Long-term results with resection of radiation-induced soft tissue sarcomas. Ann Surg, 2004; 239: 903-9; discussion 909-10.

33. Inoue YZ, FJ Frassica, FH Sim, et al. Clinicopathologic features and treatment of postirradiation sarcoma of bone and soft tissue. J Surg Oncol. 2000; 75: 42-50.

34. Amendola BE, MA Amendola, KD McClatchey, et al. Radiation-associated sarcoma: a review of 23 patients with postradiation sarcoma over a 50-year period. Am J Clin Oncol.1989; 12: 411-5.

35. Nguyen F, C Rubino, S Guerin, et al. Risk of a second malignant neoplasm after cancer in childhood treated with radiotherapy: correlation with the integral dose restricted to the irradiated fields. Int J Radiat Oncol Biol Phys.2008; 70: 908-15.

36. Guérin S, M Hawkins, A Shamsaldin, et al. Treatment-adjusted

predisposition to second malignant neoplasms after a solid cancer in childhood: a case-control study. J Clin Oncol.2007; 25: 2833-9.

37. Gladdy RA, LX Qin, N Moraco, et al. Do radiation-associated soft tissue sarcomas have the same prognosis as sporadic soft tissue sarcomas? J Clin Oncol.2010; 28: 2064-9.

38. Hadj-Hamou NS, N Ugolin, C Ory, et al. A transcriptome signature distinguished sporadic from postradiotherapy radiation-induced sarcomas. Carcinogenesis.2011; 32: 929-34.

39. Miralbell R, A Lomax, L Cella, et al. Potential reduction of the incidence of radiation-induced second cancers by using proton beams in the treatment of pediatric tumors. Int J Radiat Oncol Biol Phys.2002; 54: 824-9.

40. Catton C, B O'Sullivan, R Bell, et al. Chordoma: long-term follow-up after radical photon irradiation. Radiotherapy and Oncology.1996; 41: 67-72.

41. Guan X, J Gao, J Hu, et al. The preliminary results of proton and carbon ion therapy for chordoma and chondrosarcoma of the skull base and cervical spine. Radiation Oncology.2019; 14: 206.

42. Archer SG, BN Gray, Vascularization of small liver metastases. Br J Surg.1989; 76: 545-8.

43. Srinivas SM, N Natarajan, J Kuroiwa, et al. Determination of Radiation Absorbed Dose to Primary Liver Tumors and Normal Liver Tissue Using Post-Radioembolization (90)Y PET. Frontiers in oncology.2014; 4: 255-255.

44. Joensuu H, M Eriksson, J Collan, et al. Radiotherapy for GIST progressing during or after tyrosine kinase inhibitor therapy: A prospective study. Radiother Oncol.2015; 116: 233-8.

45. Rathmann N, SJ Diehl, D Dinter, et al. Radioembolization in patients with progressive gastrointestinal stromal tumor liver metastases undergoing treatment with tyrosine kinase inhibitors. J Vac Interv Radiol.2015; 26: 231-8.

46. Tontonoz M. What Ever Happened to Coley's Toxins? 2015, Cancer Research Institute.

47. Rosenberg SA, MT Lotze, JC Yang, et al. Experience with the use of high-dose interleukin-2 in the treatment of 652 cancer patients. Annals of surgery.1989; 210: 474-485.

48. Schwinger W, V Klass, M Benesch, et al. Feasibility of high-dose interleukin-2 in heavily pretreated pediatric cancer patients. Ann Oncol.2005; 16: 1199-206.

49. Goodman AM, S Kato, L Bazhenova, et al. Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. Mol Cancer Ther, 2017; 16: 2598-2608.

50. Bonneville R, MA Krook, EA Kautto, et al. Landscape of Microsatellite Instability Across 39 Cancer Types. JCO Precision Oncology.2017: 1-15.

51. Coyne GO SE, Moore N, et al. Phase 2 study of atezolizumab in patients with alveolar soft part sarcoma. Presented at the CTOS Annual Meeting, Rome, Italy

52. Wilky BA, MM Trucco, TK Subhawong, et al. Axitinib plus pembrolizumab in patients with advanced sarcomas including alveolar soft-part sarcoma: a single-centre, single-arm, phase 2 trial. The Lancet Oncology.2019; 20: 837-848.

53. Kim JR, YJ Moon, KS Kwon, et al. Tumor infiltrating PD1-positive lymphocytes and the expression of PD-L1 predict poor prognosis of soft tissue sarcomas. PLoS One.2013; 8: e82870.

54. Boxberg M, K Steiger, U Lenze, et al. PD-L1 and PD-1 and characterization of tumor-infiltrating lymphocytes in high grade sarcomas of soft tissue - prognostic implications and rationale for immunotherapy. Oncoimmunology.2018. 7, e1389366 DOI: 10.1080/2162402x.2017.1389366.

55. Tawbi HA, M Burgess, V Bolejack, et al. Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial. The Lancet Oncology.2017; 18: 1493-1501.

56. D'Angelo SP, MR Mahoney, BA Van Tine, et al. Nivolumab with or without ipilimumab treatment for metastatic sarcoma (Alliance A091401): two open-label, non-comparative, randomised, phase 2 trials. The Lancet Oncology, 2018; 19: 416-426.

57. Keung EZ, M Burgess, R Salazar, et al. Correlative Analyses of the SARC028 Trial Reveal an Association Between Sarcoma-Associated Immune Infiltrate and Response to Pembrolizumab. Clinical Cancer Research, 2020: clincanres.1824.2019.

58. Demetri GD, JJ Luke, A Hollebecque, et al. First-in-human phase 1 study of ABBV-085, an antibody-drug conjugate (ADC) targeting LRRC15, in sarcomas and other advanced solid tumors. Journal of Clinical Oncology.2019; 37: 3004-3004.

59. Tine BAV, DS Hong, D Araujo, et al. The Synovial Sarcoma subset analysis of the multi-histology Phase I trial of ADP-A2M4 (MAGE-A4). Presented at the CTOS Annual Meeting, Tokyo, Japan

60. D'Angelo SP, GD Demetri, BAV Tine, et al. Phase 1 trial of NY-ESO-1-Specific adoptive T-cell therapy with GSK3377794 in patients with advanced Synovial Sarcoma, in CTOS Annual Meeting. 2019, Connective Tissue Oncology Society: Tokyo, Japan.

61. Gonzalez-Galarza F, A McCabe, E Santos, et al. Allele Frequencies Website [Web site]. Allele*Frequencies in Worldwide Populations,

62. Kelly CM, CR Antonescu, T Bowler, et al. Objective Response Rate Among Patients With Locally Advanced or Metastatic Sarcoma Treated With Talimogene Laherparepvec in Combination With Pembrolizumab: A Phase 2 Clinical Trial. JAMA Oncol. 2020; 6: 402-8.