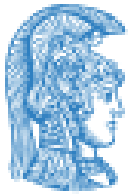

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SCHOOL OF HEALTH SCIENCES
DEPARTMENT OF MEDICINE

Interdisciplinary M.Sc. course in Nanomedicine

Master Thesis

Title: “The role of nanoparticles in radiation treatment of soft tissue sarcomas”

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Abstract

Soft tissue sarcomas (STS) are a diverse group of malignant tumors arising from mesenchymal tissue, presenting significant challenges in treatment due to their complex anatomical locations and resistance to conventional therapies. In recent years, the integration of nanoparticles into radiotherapy has emerged as a promising strategy to improve the therapeutic outcomes of STS. Nanoparticles possess unique physicochemical properties that can enhance the efficacy of radiotherapy by selectively targeting tumor cells, enhancing radiation absorption, and mitigating damage to healthy tissues. In this thesis the literature was searched for the use of nanoparticles in the management of STS. A phase 1 and a phase 2/3 trials were retrieved and analyzed. These trials are focused on the use of NBTXR3 hafnium-based nanoparticles and radiotherapy followed by surgical removal of the sarcoma. These reviews showed that using NBTXR3 nanoparticles the achieved pathological complete response-pCR (measured as < 5% of viable cancer cells found in the surgically removed mass) of the preoperative radiotherapy was doubled. Considering that pCR has been found to be associated with a benefit in the overall survival of these patients, the use of NBTXR3 was suggested for the management of STS.

1. Introduction

Soft tissue sarcoma (STS) is a heterogeneous group of rare tumors that originate from mesenchymal tissue.[1,2] Primary STS locations are the trunk, head and neck and abdomen, but the extremities are the most frequently affected site. The management of localized STSs in the extremities typically involves a multimodal approach, aiming to preserve limb function and is usually carried out in specialized referral centers.[3–9] Radiotherapy (RT) is an integral part of sarcoma treatment and can be administered in the preoperative or postoperative setting, with similar outcomes in terms of local control and survival rates.[10–14] However, the toxicity profiles differ between preoperative and postoperative radiotherapy. Preoperative RT increases the risk of wound complications, while postoperative RT can have long-term effects on functional outcomes.[11] There is now a changing therapeutic landscape with a growing trend towards the use of preoperative RT in the management of STSs.[3,4] This shift is driven by technical advancements in RT, as well as improved wound management techniques postoperatively. Retrospective studies have shown that pathological complete response-pCR (defined as death of more than 95% of cancer cells) after radiotherapy is associated with improved survival.[15–19] It must be noted however, that using the current RT protocols a pCR of less than 10% is achieved, suggesting that effort should be undertaken to increase pCR rates.[15–19]

In recent years, there has been growing interest in exploring the potential of nanoparticles in the field of RT.[20–22] These particles, typically ranging in size from a few to hundreds of nanometers, can be designed to possess specific characteristics that allow them to interact with radiation and tumor cells in targeted ways. The use of nanoparticles (NPs) in RT holds promise for increasing tumor cell killing and improving tumor control while minimizing damage to surrounding healthy tissues. By leveraging their ability to enhance radiation effects, NPs have the potential to revolutionize the therapeutic landscape and pave the way for more personalized and effective treatments for STSs and other malignancies.[20–23]

In the current thesis the use of NPs in the treatment of STSs in the extremities is studied. The manuscript is structured as follows: The first two sections provide clinical details on STSs and their management using radiotherapy. Sections four and five briefly describe the characteristics of NPs used in clinical practice. Sections six and seven delve into the phenomena of the interaction of ionizing radiation with matter and the radiobiology aspects, aiming to enhance the reader's understanding of the underlying principles behind the use of NPs in RT. Section eight outlines the methodology employed to search the literature for the use of NPs in STS treatment. The findings are presented in section nine and subsequently discussed in section ten. Sections eleven and twelve address the limitations of NP utilization and present the conclusions drawn from the study.

2. Soft Tissue Sarcoma

Sarcomas are a rare and heterogeneous group of solid tumors of mesenchymal origin accounting for only 1% of all adult malignancies and 15% of childhood malignancies.[24] They can be broadly divided into:

- Sarcomas of soft tissues (including fat, muscle, nerve and nerve sheath, blood vessels, and other connective tissues) (see Figure 1) and
- Sarcomas of bone.

The anatomic site of the primary disease represents an important variable that influences treatment and outcome. Extremities (43%), the trunk (10%), visceral (19%), retroperitoneum (15%), or head & neck (9%) are the most common primary sites.[25]

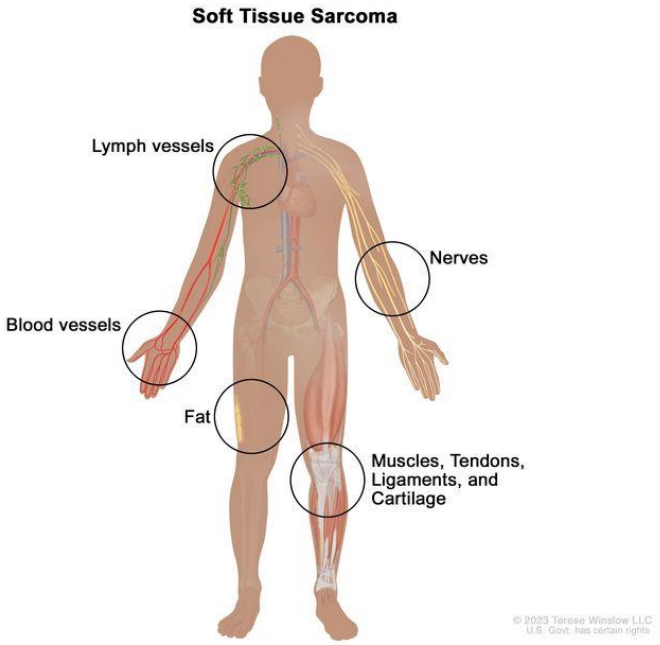

Figure 1. Soft tissue sarcoma forms in the soft tissues of the body, including the muscles, tendons, ligaments, cartilage, fat, blood vessels, lymph vessels, nerves, and tissues around joints. Image taken from : Soft Tissue Sarcoma Treatment - NCI (cancer.gov)

More than 50 different histologic subtypes of STS have been identified based on the specific type of soft tissue cell they originate from.[26] Some of the most common subtypes include:

- Liposarcoma: Arising from fat cells, liposarcoma is one of the most common STS. It usually occurs in the limbs or abdomen.
- Leiomyosarcoma: Originating from smooth muscle cells, leiomyosarcoma can occur in various locations, including the uterus, gastrointestinal tract, and blood vessels.

- Rhabdomyosarcoma: Most often seen in children and adolescents, rhabdomyosarcoma develops from skeletal muscle cells. It typically affects the head & neck area, urinary and reproductive organs, or extremities.
- Synovial sarcoma: Typically found near joints in the extremities, synovial sarcoma arises from the cells surrounding the synovial membrane of joints.
- Malignant peripheral nerve sheath tumor (MPNST): Originating from cells that form the protective lining around nerves, MPNST can occur in any nerve in the body.

Soft tissue sarcomas can cause various symptoms, depending on their location and size. Some common signs and symptoms include a painless lump or swelling, pain or soreness, difficulty moving or using muscles, and a feeling of fullness or pressure. However, it's important to note that these symptoms can also be caused by other conditions. The most frequent site of metastasis for STSs is the lungs, whereas tumors originating in the abdominal cavity tend to metastasize more commonly to the liver and peritoneum.[27] The exact cause of STS is often unknown, but certain factors may increase the risk of developing these tumors. These factors include exposure to high doses of radiation, certain genetic conditions (e.g., Li-Fraumeni syndrome), inherited mutations (e.g., neurofibromatosis type 1), and various chemicals (e.g., herbicides, such as agent orange).[28]

The diagnosis of STS usually involves a combination of imaging examinations such as Magnetic Resonance Imaging (MRI) and/or Computed Tomography (CT) scans, biopsy to obtain a tissue sample for examination, and pathological analysis.[29] The pathology report should include specific details about the primary diagnosis (using standardized nomenclature according to the WHO Classification of STS tumor); the organ and site of sarcoma; depth, size, and histologic grade of the tumor; presence or absence of necrosis; status of excision margins and lymph nodes; tumor, node, and metastasis (TNM) stage; and additional features such as mitotic rate, presence or absence of vascular invasion, and the type and extent of inflammatory infiltration.[26]

Treatment options for STS typically include surgery, radiotherapy, and chemotherapy (in the case of chemo-sensitive histologies).[6,30,31] The choice of treatment depends on factors such as the subtype, location, stage, and grade of the tumor, as well as the overall health of the individual, however surgical resection is the standard primary treatment for most patients with STS.

3. Radiotherapy

3.1. General aspects

Radiotherapy is a widely used and effective treatment modality for cancer. It involves the controlled use of high-energy radiation to target and destroy cancer cells while minimizing damage to surrounding healthy tissues.[32,33] RT can be used as a primary treatment or in combination with other therapies such as surgery and chemotherapy, depending on the

specific characteristics of the cancer. The goal of RT is to deliver a precise dose of radiation to the tumor, damaging the DNA within cancer cells and preventing them from dividing and growing. This can lead to shrinkage or elimination of the tumor.

There are many techniques for delivering RT with the main one being External Beam Radiation Therapy (EBRT). EBRT involves directing ionizing radiation beams from an external radiation generator, such as a linear accelerator, towards the tumor site.[32,33] The radiation beams are carefully shaped and delivered to the tumor from various angles to maximize the dose to the tumor while minimizing exposure to nearby healthy tissues. This technique is commonly referred to as 3D-conformal radiotherapy-3DCRT. RT technological advancements include intensity modulated RT (IMRT), image guided RT (IGRT), brachytherapy (BRT) and intraoperative RT (IORT).[33] In IMRT, the intensity of the emitted radiation beam is modulated, along with its shape, to enhance the sparing of healthy structures from excessive dosage. In IGRT imaging systems are incorporated within the treatment room to ensure greater spatial accuracy in delivering the dose distribution. Brachytherapy involves the direct placement of radioactive seeds into the tumor bed through catheters inserted during surgery. Different approaches can be employed, including low dose-rate (LDR) brachytherapy, fractionated high dose-rate (HDR) brachytherapy, or intraoperative HDR brachytherapy. IORT is a technique that administers radiation during surgery, utilizing methods such as electron beam radiation therapy or brachytherapy. IORT allows for precise radiation delivery to the tumor bed while minimizing exposure to critical nearby structures. All these RT techniques aim to enhance the precision and effectiveness of radiation delivery, resulting in reduced side effects and preservation of surrounding healthy tissues.

Radiotherapy is typically delivered in multiple sessions called fractions over a period of several weeks. The treatment schedule is determined by the radiation oncologist based on the type and stage of cancer, as well as the overall health condition of the patient. It must be noted that while RT is targeted to the tumor, some healthy cells in the treatment area may also be affected from the absorbed radiation. This can result in side effects, which vary depending on the site being treated and the tolerance of the normal tissues. Common side effects include fatigue, skin changes, hair loss, and temporary or long-term effects on normal tissues. The treatment team closely monitors the patient throughout the RT course to ensure the effectiveness of treatment and manage any potential side effects.

3.2. Radiotherapy for STS

Radiotherapy plays a significant role in the treatment of STS. In a landmark randomized controlled study conducted in 1982, the effectiveness of limb-sparing surgery with RT as a treatment option for patients with high-grade STS of the extremities was demonstrated.[34] This study involved 43 patients and aimed to compare the outcomes of limb-sparing surgery with RT to those of amputation. The study reported a local recurrence (LR) rate of 15% in the limb-sparing surgery with RT group. Importantly, there was no significant difference observed in overall survival (OS) and disease-free survival (DFS) between the limb-sparing surgery with

RT group and the amputation group.[34] These findings provided evidence that limb-sparing surgery with RT could achieve comparable outcomes in terms of OS and DFS as compared to amputation, while preserving the affected limb.

A recent systematic review and meta-analysis aimed to explore the impact of EBRT compared to no EBRT on LR and OS in patients with soft tissue sarcoma.[35] The analysis included data from 16 studies, comprising a total of 3958 participants. The results indicated that EBRT was associated with a reduction in LR and improved OS specifically for retroperitoneal STS. Furthermore, it demonstrated a decrease in LR for STS located in the extremity, head & neck, or trunk wall, with an odds ratio (OR) of 0.49 (95% confidence interval, 0.31-0.77; P = 0.002).[35]

RT can be administered either as primary, preoperative, or postoperative treatment. The total radiation dose is determined based on the tolerance of the surrounding healthy tissues. Various RT techniques, such as brachytherapy, IORT and IMRT, have contributed to improved treatment outcomes in STS. In a retrospective analysis of 41 patients with STS of extremity treated with limb-sparing surgery, postoperative IMRT resulted in a 5-year local control rate of 94% in patients with negative as well as positive or close margins, in selected patients with high-risk features.[36] The risk of complications such as edema and joint stiffness were also favorable when compared with conventional RT. In a more recent phase II study, O'Sullivan and colleagues reported that preoperative IMRT resulted in lower wound complication rate in patients with high-grade lesions (30.5% vs. 43% reported in earlier study using conventional EBRT).[37] In a nonrandomized comparison of IMRT and brachytherapy in patients with high-grade, primary, nonmetastatic STS of extremity, local control was significantly better with IMRT than brachytherapy (5-year local control rates were 92% and 81%, respectively; P = 0.04) despite higher rates of adverse features for IMRT.[38] Additionally, image-guided techniques may allow for reduced target volumes, further minimizing toxicity. In a recent phase II trial (RTOG-0630; n = 86), the use of preoperative IGRT to a reduced target volume resulted in significantly reduced late toxicity without any marginal field recurrences.[39]

Preoperative RT

Preoperative EBRT offers several key benefits. Firstly, compared to post-operative radiation, the total radiation dose is lower (50 Gy versus 60-66 Gy), resulting in reduced late toxicity and improved long-term functional outcomes. Additionally, treatment volumes are often smaller, thanks to the clear visibility of the disease in MRI, further reducing potential side effects. Another advantage is the enhanced radiobiological efficacy stemming from improved tumor oxygenation and vascularization. Moreover, preoperative EBRT can help minimize tumor seeding during surgical manipulation. While tumor regression may or may not occur with preoperative RT, the pseudocapsule tends to thicken and become acellular, facilitating resection, and lowering the risk of recurrence. Patients with deep, high-grade disease exceeding 5 cm, or cases where surgery is complicated due to the proximity of the STS to neurovascular bundles or bones, stand to benefit the most from neoadjuvant EBRT. Notably,

preoperative radiation also proves advantageous for both surgical procedures and local control in patients with myxoid liposarcoma, considering the tumor's high potential for shrinkage and size reduction.

A significant drawback of neoadjuvant EBRT is the notable incidence of surgical wound complications. In a randomized trial conducted by O'Sullivan et al., it was found that the preoperative group had a 35% occurrence of wound complications, compared to 17% in the postoperative group.[11] Another potential disadvantage of preoperative RT is the risk of disease progression in patients who are unresponsive to radiation treatment, rendering them ineligible for definitive surgery. Following preoperative RT, a waiting period of 3 to 6 weeks is typically required before resection to allow for the subsiding of acute reactions and minimize the risk of wound complications. In cases where preoperative RT is considered, involving a plastic surgeon on the medical team may be necessary to mitigate the occurrence of wound complications. However, it is important not to delay surgery excessively, as this could increase the likelihood of developing late fibrosis, which could impede surgical interventions.

The current standard dose for neoadjuvant EBRT is 50 Gy administered in 25 fractions over a period of 5 weeks. International guidelines suggest the possibility of an adjuvant boost (14-20 Gy) using either EBRT or brachytherapy in cases of positive surgical margins. Postoperative RT boost of 16 Gy has been utilized in patients with positive surgical margins once the wound has healed. However, the findings of a retrospective analysis indicated that postoperative RT boost did not confer any advantage in preventing local recurrence (LR) in certain patients with positive surgical margins, such as those with low-grade, well-differentiated liposarcoma (WDLS) and a focally “planned” positive margin on an anatomically fixed critical structure. Similarly, another retrospective matched cohort study involving patients with extremity STS revealed no additional benefit of a postoperative RT boost in terms of LR, distant metastasis, and mortality. Furthermore, the potential advantages of incorporating a postoperative RT boost have not been evaluated through randomized clinical trials. The topic remains highly debated, and the risk of local failure versus potential toxicity should be carefully assessed for each individual patient.

The Radiation Therapy Oncology Group (RTOG) and Haas, along with their European, American, and Canadian colleagues, have reached a consensus on the definition of gross target volume (GTV) and clinical target volume (CTV).[40,41] The GTV is determined based on T1 contrast-weighted MRI images, and it is recommended to fuse CT and MRI images for treatment planning purposes. The CTV is defined as the GTV plus microscopic clinical extension, and currently, it is achieved by expanding 3 to 4 cm longitudinally and 1.5 cm radially from the GTV. The extension of the CTV may be limited to the boundaries of the anatomical compartment. The planning target volume (PTV) is created by expanding the CTV isotropically by 5 to 10 mm. The specific expansion may vary depending on the immobilization systems, techniques, and IGRT used at each individual center.

Postoperative RT

Postoperative RT offers certain advantages, such as allowing for a definitive pathologic assessment and being associated with a lower incidence of scar formation and postoperative wound healing complications. It is particularly recommended for patients with significant comorbidities who are at a higher risk of developing wound complications. However, postoperative RT also comes with several drawbacks. This includes the need to irradiate larger target volumes compared to preoperative RT and the requirement for higher total radiation doses. As a result, postoperative RT is associated with a higher rate of late toxicity compared to preoperative RT. Common long-term side effects of postoperative RT, such as fibrosis, joint weakness, bone fracture, and edema, often become permanent and can significantly impact the patient's quality of life. In a retrospective analysis, although there was no evidence of differences in disease outcomes between preoperative and postoperative RT, there was a slight increase in late treatment-related side effects with postoperative RT, primarily due to the use of higher radiation doses.

The current standard dose for adjuvant EBRT is 50 Gy using standard fractionation, covering a larger volume that includes the surgical bed with appropriate safety margins. If the surgical margins are not adequate, a boost of 10-20 Gy is administered to the tumor bed, resulting in a total dose of 60-70 Gy. The CTV should encompass the tumor bed, all surgically manipulated tissues, visible metal clips, the entire surgical scar, extent of the operative field, and drain sites. It is recommended to include a radio-protected drainage area to minimize distal edema and reduce the risk of severe complications in the long term. The target boost volume should accurately correspond to the original tumor extension, requiring pre-operative CT or MRI data sets for precise definition. The proper positioning of metal surgical clips during excision by the surgeon is crucial to enable the radiation oncologist to delineate the target volume accurately. For the PTV, an isotropic expansion of 5-10 mm from the CTV is applied. It is not recommended to exceed an interval of 8 weeks between resection and postoperative RT to avoid the development of late fibrosis and proliferation of malignant cells.

4. Nanoparticles

According to the ISO International Standards, NPs are defined as particles with dimensions from 1 nm to 100 nm.[42] This nanoscale size range provides distinct advantages in terms of their behavior and interactions with biological systems. In the context of oncology, NPs exhibit unique physicochemical properties that enable them to be utilized in three main areas: medical imaging, drug delivery, and radiation sensitization.[43] NPs have demonstrated remarkable potential in medical imaging, enabling enhanced visualization of tumors and metastatic lesions.[44] By incorporating imaging agents or contrast agents into NPs, such as fluorescent dyes or magnetic nanoparticles, they can specifically accumulate in cancerous tissues and produce detectable signals for precise imaging modalities like magnetic resonance imaging (MRI), computed tomography (CT), or optical imaging. This improved imaging capability facilitates early detection, accurate staging, and monitoring of cancer progression,

leading to more effective treatment planning and evaluation. As far as treatment is concerned, NPs serve as efficient carriers for delivering anticancer drugs directly to the tumor site. Through surface modifications or encapsulation within the NP structure, drugs can be protected from degradation and delivered selectively to cancer cells, minimizing systemic toxicity, and enhancing therapeutic efficacy. NPs can be designed to release the drugs in a controlled manner, ensuring sustained drug exposure and potentially overcoming drug resistance mechanisms. This targeted drug delivery approach holds great promise in optimizing cancer treatment outcomes and minimizing adverse effects. Moreover, NPs have emerged as promising agents for radiation sensitization, enhancing the effectiveness of radiation therapy in eradicating cancer cells. By selectively accumulating in tumor tissues, NPs can increase the local dose of radiation and improve the precision of treatment. They can act as radiosensitizers by enhancing the energy deposition within tumor cells, leading to increased DNA damage and cell death. Additionally, NPs can generate reactive oxygen species (ROS) upon exposure to radiation, further amplifying the cytotoxic effects. This combination of radiation and NP therapy has the potential to improve tumor control while reducing the radiation dose to healthy surrounding tissues.

Hafnium nanoparticles

Hafnium presents a high atomic number (Z = 72), which is crucial for efficient hafnium oxide nanoparticle–ionizing radiation interactions. They have inert behavior in biological media, a very low solubility throughout a large pH range, which is inherent to oxide nanoparticles from metal cations with a +4 oxidation state. Additionally, hafnium oxide is an electrical insulator with a band gap close to 6 eV. Thus, hafnium oxide NPs are unlikely to be involved in redox phenomena or electron transfer mechanisms in biological media. Hafnium oxide particles in aqueous media in the presence of sodium perchlorate have been reported to have a point of zero charge of 7.4. The point of zero charge is defined as the pH of the medium for which the surface charge becomes zero. Thus, no marked surface acidobasicity is expected at neutral pH for hafnium oxide nanoparticles.

Coating of nanoparticles with protective shells (surface functionalization) appears to be an effective means of preventing their dissolution and release of toxic ions. Surface functionalization may inhibit physicochemical mechanisms that can occur at the surface of inorganic nanoparticles (e.g., redox properties), which are responsible for nanoparticle toxicity. However, many coatings are labile and/or biodegradable in biological environments, and an initially nontoxic nanoparticle may ultimately display deleterious properties after shedding its shell. Furthermore, nonspecific coating (i.e., exclusion of specific biological molecular targets) may be an important factor in controlling nanoparticle–cell interactions and consequently nanoparticle efficacy.

Gold nanoparticles (GNPs)

Gold nanoparticles (GNPs) have garnered significant attention in research due to their numerous advantages in the field of oncology. Their high biocompatibility, ability to penetrate tumors effectively, and low clearance make them a preferred choice for various applications. GNPs tend to accumulate preferentially in tumor tissues, allowing for easy quantification and analysis of their pharmacokinetics. Studies have shown that GNPs can enhance the effects of ionizing radiation by increasing DNA damage within cancer cells, thereby exerting anti-tumor effects. To predict the radio-sensitizing effect of GNPs, a Monte Carlo-based model has been developed. Traditional dosimetry methods are not applicable to NP-enhanced radiation therapy, leading to the development of new techniques. An interesting study conducted on a murine model of glioblastoma xenografts suggested that radiation exposure can modulate the blood-brain barrier, resulting in increased uptake of GNPs. Consequently, the accumulation of GNPs at the site of cerebral tumors could potentially enhance the therapeutic benefits of radiation and improve overall survival rates.

Gadolinium nanoparticles (GBN)

Gadolinium nanoparticles (GBNs) have gained attention in recent years as a noteworthy type of nanoparticle. One significant advantage of GBNs is their ability to be easily visualized using MRI. This unique property makes them highly valuable for diagnostic purposes. Furthermore, in-vitro and in-vivo studies have demonstrated the radio-sensitizing effect of GBNs. This suggests that GBNs have the potential to enhance the therapeutic response in the treatment of various conditions. For instance, in mice with melanoma, the administration of GBNs has been shown to lead to improvements in therapeutic outcomes. These findings highlight the promising role of gadolinium nanoparticles in advancing cancer treatment strategies.

Other nanoparticles

Other types of nanoparticles also exhibit radio-sensitizing properties and have been investigated for their potential in radiation therapy.

- Silver nanoparticles (AgNPs) possess radio-sensitizing properties similar to other high atomic number atoms. Although they are less expensive than gold nanoparticles (GNPs), their biocompatibility is relatively lower. In murine glioblastoma, intra-tumor injection of silver nanoparticles has been shown to induce a higher anti-tumor effect without causing increased systemic toxicity following irradiation with 10 Gy of 6 MeV photons.
- Platinum nanoparticles (PtNPs) have a high atomic number similar to gold and have been used in combination with radiochemotherapy. When combined with hadron radiation, platinum nanoparticles have demonstrated a 2-fold increase in DNA damage, likely due to enhanced energy deposition in situ.
- Quantum dots are nanoparticles made of semiconductor materials such as calcium fluoride (CaF), lanthanum fluoride (LaF), zinc sulfide (ZnS), or zinc oxide (ZnO). Their radio-

sensitizing activities have been reported, but they are more suitable for treating superficial cancers.

- Carbon nanotubes (CNTs) have garnered significant interest in the scientific community. They have been studied for drug delivery and biomedical imaging purposes and are actively being explored for their theranostic effects. CNTs exhibit high endocytosis ability and demonstrate minimal side effects. Although their application in radiation therapy is not extensively investigated at present, their drug delivery and imaging functions could potentially be harnessed in this field as well. CNTs have low solubility in solvents, but the attachment of hydrophilic segments on polymer functionalized CNTs allows for their application in the medical field.

5. THE NBTXR3 radio-enhancer

The NBTXR3 (Nanobiotix SA, Paris, France) is a first-in-class radio-enhancer comprising of functionalized hafnium oxide NPs (see Figure 2).[45] NBTXR3 nanoparticles are coated with a simple chemical envelope that provides them with a negative surface charge at neutral pH. NBTXR3 nanoparticle interaction with the cytoplasmic membrane is independent from specific processes of internalization. Colloidal substances such as NBTXR3 nanoparticles underlying no degradation (or poor degradation) give rise to marked uptake by the mononuclear phagocytic cells (reticuloendothelial system). Unlike small molecules, nanoparticle passage into tissues relies on the structure of endothelial fenestrae. These openings in the endothelial structure and their consequent density determine the bioavailability (passage of nanoparticles) into tumor and organ tissues. Nanoparticle shape and size are physical characteristics known to affect biological functions such as phagocytosis, body circulation and adhesion. NBTXR3 hafnium oxide nanoparticles are 50 nm sized spheres. Consequently, NBTXR3 nanoparticle characteristics – size, shape and surface charge – are designed for efficient nanoparticle trafficking at the cellular level: cell membrane binding and cellular uptake.

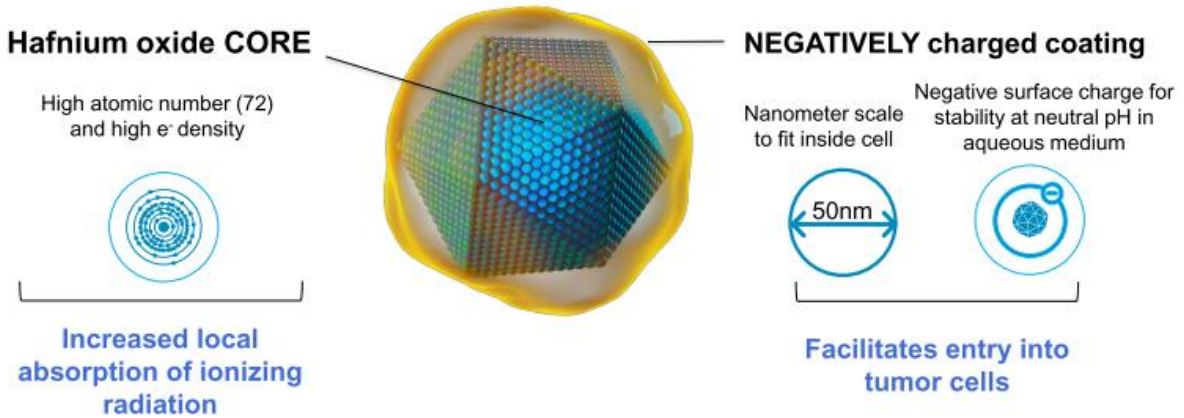

Figure 2. Graphic representation of the NBTXR3 hafnium oxide nanoparticle. The main characteristics of the NBTXR3 are noted.

Radioresistant and radiosensitive human tumor xenograft models from mesenchymal and epithelial cell lines were investigated for NBTXR3 tolerance and antitumor efficacy in activated state by different energies of ionizing radiation. Several experiments evaluated the IT injection of NBTXR3 as well as nanoparticle dispersion, potential leak, and permanency within the tumor volume. A very good benefit to risk ratio was observed in all explored tumors. Quantitative assessment of NBTXR3 by inductively coupled plasma mass spectrometry within tumor structure, surrounding skin and muscle at different time points, and concomitant dosage of NBTXR3 in plasma and organs, was performed to obtain more accurate information about the permanence of NBTXR3 within the tumor and its potential leak within the healthy surrounding tissues. The experiments demonstrated low hafnium content in all organs when compared with the IT bioavailability. Tolerance of the IT injection of NBTXR3 not activated or activated by ionizing radiation was evaluated in comparison with control cohorts in all in vivo xenograft models. No toxicity difference was observed between tested and control groups, suggesting absence of toxicity related to this treatment modality. The antitumor effect of NBTXR3 activated by ionizing radiation was assessed in mesenchymal tumors (HT1080 human fibrosarcoma cell line and A673 human Ewing's sarcoma cell line) and epithelial tumors (HCT 116 human colon cancer cell line) xenografted in nude mice. Cobalt 60 activation of NBTXR3 leads to a major radio enhancement effect in in-vivo/in-vitro study. This methodology allows in vivo NBTXR3 activation by local irradiation of the tumor and in vitro exact quantification of radiation response of cells from the 3D tumor environment. NBTXR3 showed marked advantages in terms of survival, tumor specific growth delay and local control when compared with radiation therapy alone.

Integrity of the hafnium oxide nanoparticle crystal structure was demonstrated in Kupffer's cells after chronic exposure in rodents. This is a remarkable property. Unchanged crystal structure of hafnium oxide is the key feature supporting the quality and the outcome of the interaction between these solid nanoparticles and ionizing radiation, allowing the on/off mode of action through successive fractions of radiotherapy. In the meantime, specific immunology safety explorations suggest that NBTXR3 taken up by phagocytic cells does not have any significant toxicity on their viability. Local tolerance studies after exposure for 13 and 26 weeks showed no evidence of local intolerance and no irritant potential. Otherwise, NBTXR3 is not a mutagenic product (absence of genotoxicity), as demonstrated in in vitro and in vivo studies.

6. Interaction of ionizing radiation with matter

The presence of high Z material near a localized irradiation has been reported to enhance the biological effects.[23,46] Densely packed metal particles have the ability to selectively scatter and/or absorb high energy radiation. High-Z atoms interact with ionizing radiation by generating secondary particles such as diffused photons, photoelectrons, Auger electrons, Compton electrons, and fluorescence photons.[33] In the following paragraphs the physics of interaction of ionizing radiation with nanoparticles are briefly described.

6.1. High energy photons

Photons possess characteristics of both waves and particles, serving as quanta of electromagnetic energy. They possess properties such as wavelength and frequency (similar to waves), as well as momentum and energy (similar to particles). However, despite carrying electromagnetic energy, photons lack a charge and have a significantly lower probability of interacting with matter compared to charged particles like electrons and protons. When photons do interact with matter, they transfer their energy to electrons and/or positrons within that material (see Figure 3). The main interaction types of photons with matter are:

Photoelectric effect

In this phenomenon, the atom absorbs the photon, transferring its energy to an atomic electron. This electron is then expelled from the atom with kinetic energy equal to the energy of the photon minus the electron's binding energy. Another orbital electron fills the vacancy left by the departing electron, resulting in the emission of characteristic x-rays or Auger electrons (when a characteristic x-ray ejects another orbital electron within the same atom). Fluorescence photons experience low attenuation, while Auger electrons cause localized ionization in the surrounding tissue up to a distance of approximately 10 nm. The probability of the photoelectric effect taking place is inversely proportional to the cube of the photon energy ($1/E^3$) and directly proportional to the cube of the atomic number (Z^3). Therefore, as the energy of the photon increases, the likelihood of the photoelectric effect decreases significantly. Conversely, as the atomic number of the material increases, the probability of the photoelectric effect happening rises rapidly.

Incoherent scattering

In radiotherapy, incoherent scattering or Compton scattering is the primary interaction of significance. It occurs when a photon interacts with an electron in an outer orbital, resulting in the photon relinquishing some of its energy and undergoing scattering. The electron, in turn, absorbs this energy and changes its direction of motion. While photons can be scattered in any direction, electrons are only scattered in a forward direction, with a maximum angle of 90 degrees from the original photon direction. The amount of energy transferred to the electron depends on the initial energy of the photon (lower energies tend to transfer minimal energy to the electron) and the angle at which the interaction occurs. It is important to note that incoherent scattering is not directly influenced by the atomic number of the material, but rather by the concentration of electrons present in the tissue.

Coherent scattering

Coherent (or Rayleigh) scattering occurs at low photon energy and high atomic number materials. In this type of scattering, the photon interacts with the bound electrons of the atom without causing any excitation or ionization of the atom. The energy of the photon remains unchanged, and no other significant effects occur. In the context of radiotherapy, coherent

scattering has minimal impact on attenuation, meaning it has little influence on the absorption or weakening of the radiation beam.

Pair production.

During pair production, a photon interacts with the electric field of the nucleus. The photon is absorbed, resulting in the creation of an electron and a positron. These two particles share the energy of the original photon, subtracting the energy required to produce them ($2mec^2$ = 1.022 MeV). Pair production can only occur when the photon's energy exceeds the threshold of 1.022 MeV. As the energy of the photon increases beyond this threshold, the likelihood of pair production also increases. The occurrence of pair production is influenced by the atomic number of the material, which is related to it through Z^2.

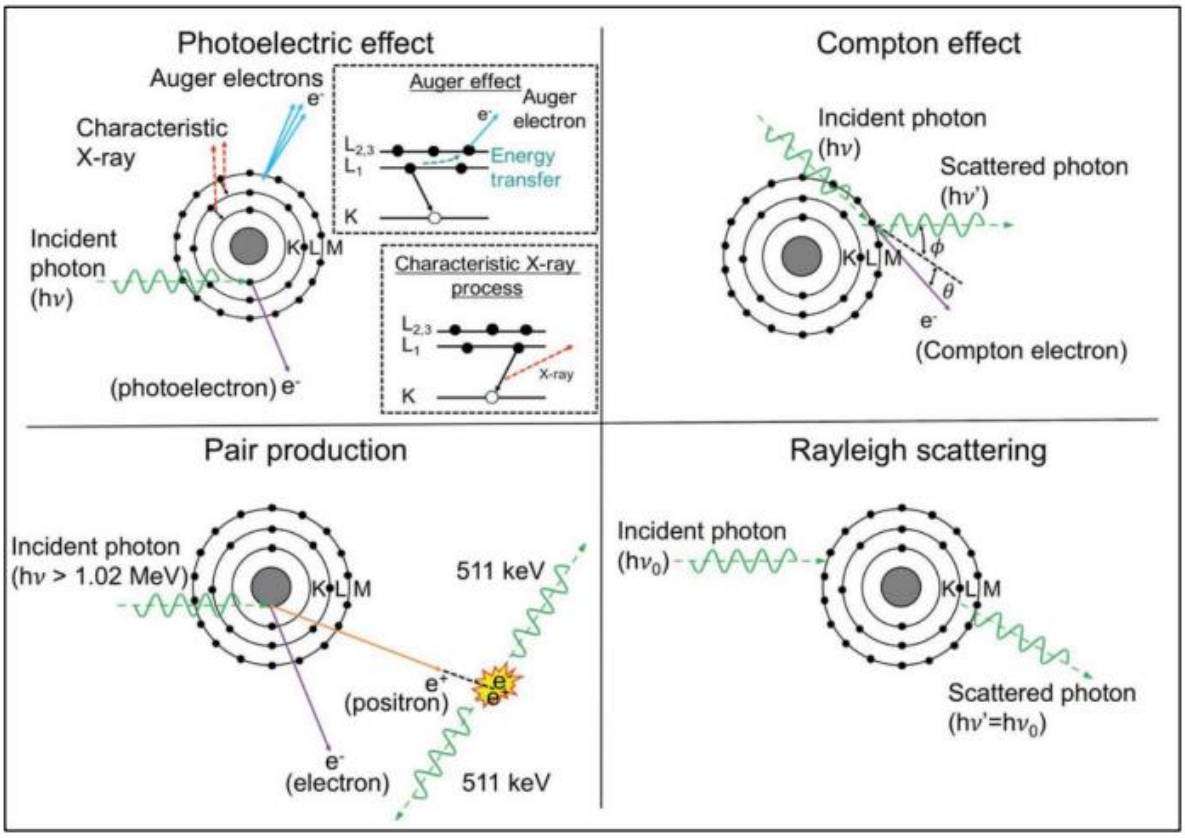

Figure 3. The different phenomena of interaction of photons with matter.[22]

6.2. Charge particles

The interaction mechanisms of ionizing radiation with matter depend on the radiation type (charged particles, photons), its energy and the properties of the medium its passes.[33] Charge particles release their energy directly in the medium with electromagnetic interactions. The main interaction processes of charged particles with matter in the energy range used in radiotherapy are:

Inelastic collision with atomic electrons.

It is the primary mechanism through which charged particles dissipate their energy. In this process, the energy of the charged particle is transferred to one or more electrons within an atom. If the transferred energy is inadequate, the electron may transition to a higher energy state, resulting in the excitation of the atom. However, if the energy transferred is sufficient, the electron can break free from the atom, causing ionization. The released electron then transfers its energy to other electrons in the material, leading to further ionizations and excitations.

Inelastic collision with a nucleus

When a charged particle comes close to a nucleus, it experiences deflection or deviation from its original path. This deflection causes the emission of electromagnetic radiation known as bremsstrahlung. In the context of medical physics, this interaction process is particularly relevant for electrons and positrons in the energy range used.

Elastic scattering with a nucleus

When the particle is deflected without exciting the nucleus or emitting radiation, there will be elastic scattering.

7. Radiation Chemistry/ Biology

The presence of nanoparticles in the irradiated tumor tissue contributes to the local enhancement of dose due to the increased production of secondary photons and electrons. A new cycle of interactions is initiated by the products of the initial interactions, resulting in a plethora of particles (secondary photons, photoelectrons, Compton electrons, Auger electrons) surrounding the region where the nanoparticles have been deposited. This, in turn, leads to more damage to crucial cellular structures and promotes the production of free radicals (e.g., hydroxyl radical •OH) and other reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), etc. (see Figure 4). The free radicals and ROS, upon contact with surrounding biomolecules (DNA, mitochondria, etc.), cause damage to these components, resulting in increased oxidative stress and, consequently, more cancer cell deaths. Free radicals can also interact with cell membranes, leading to cellular apoptosis. Hydroxyl radicals have been identified as a key factor in inducing cell death through lipid peroxidation. Additionally, ROS can lead to increased oxidation of mitochondrial membranes, resulting in the release of superoxide into the cytoplasm, which can then be converted to H_2O_2 molecules. It appears that the presence of nanoparticles alone in the irradiated tumor volume can enhance the production of oxidative stress and free radicals in cells, while the contact between the surface of the nanoparticles and O_2 promotes the generation of superoxide and ROS. As a result, there is an increased diffusion of these reactive molecules, stemming from the presence of nanoparticles in the tumor volume and the higher deposited dose in the soft tissue. The increase in reactive molecules, in turn, leads to enhanced damage

to the DNA of cancer cells where nanoparticles have been deposited, ultimately improving disease control.

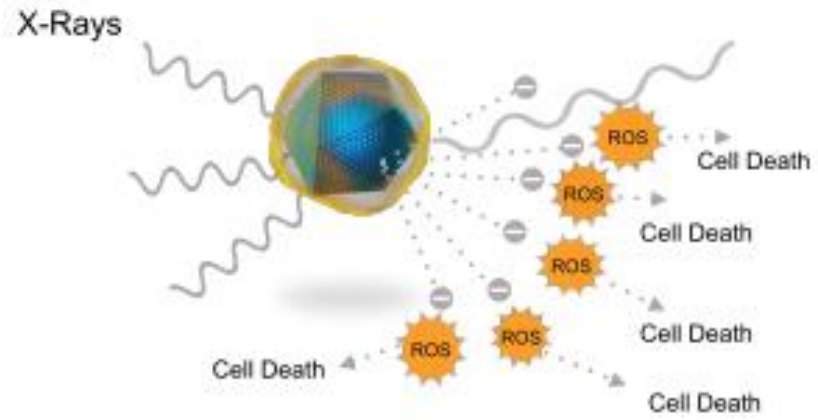

Figure 4. Graphical representation of the enhanced interaction of x-rays with the hafnium oxide nanoparticle which result to an increase of the number of secondary electrons and reactive oxygen species (ROS) produced and subsequently cell deaths.

8. Literature search methodology

An electronic search of the PubMed database was performed to obtain key literature in the management of STS, using the following search terms: “Soft tissue sarcoma” AND “nanoparticles” AND (radiotherapy OR “radiation therapy”). The PubMed database was chosen as it remains the most widely used resource for medical literature and indexes only peer-reviewed biomedical literature. The search results were narrowed by selecting studies in humans published in English. Results were confined to the following article types: Clinical Study; Clinical Trial; Guideline; Randomized Controlled Trial; Meta-Analysis; Systematic Reviews; and Validation Studies. The PubMed search resulted in six relevant citations.[47–52]

9. Review results

The studies found in the literature reported results from a phase-1[47,48] and a phase-2/3 trial[49–52] both investigating the combination of NBTXR3 hafnium oxide NPs and RT in adult patients with locally advanced STS. The first was a first-in-human pilot study and has a registration ID of NCT01433068 in the ww.clinicaltrials.gov electronic database. It was contacted in two French institutes: the Institut Bergonie and the Institut Gustave Roussy between 2011 and 2014 and enrolled 22 patients. The second was a phase-2/3 clinical trial and has a registration ID of NCT02379845 in the ww.clinicaltrials.gov electronic database. This was a randomized open label multicentric trial including 42 international institutes and enrolled 180 patients in total from 2015 to 2017.

The results of the phase 1 study were presented by Bonvalot *et al*[47] at the 2014 ASCO congress. The study aimed to determine the recommended dose, assess the safety profile,

and evaluate the feasibility of using NBTXR3 nanoparticles in combination with EBRT as a preoperative treatment for adults with locally advanced STS. Its main finding was that a single intratumoral injection of NBTXR3, equivalent to 10% of the initial tumor volume, was technically feasible and well-tolerated, with manageable toxicity. The NBTXR3 injections remained stable within the tumor volume and did not leak into the surrounding tissue or bloodstream after injection. Encouraging signs of antitumor activity were observed across various subtypes of sarcoma. Following the promising results of the phase-1 study, a randomized, multicenter, international phase 2/3 trial was conducted between 2015 and 2017. The trial compared preoperative RT alone with an investigational arm involving intratumoral NBTXR3 injection prior to RT. The protocol and findings of this phase 2/3 trial are summarized in the following subsections of the thesis.

9.1. The NBTXR3 treatment protocol

The phase 2/3 study design is shown in Figure 5.[50] The study enrolled a total of 180 patients with STS of the extremity or trunk wall who required preoperative RT. The patients were randomly assigned in a 1:1 ratio.

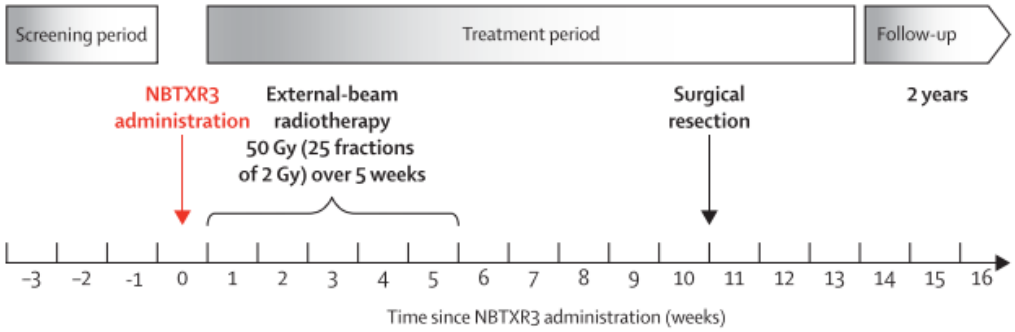

Figure 5. The study design of the NCT02379845 phase 2/3 randomized multicentric trial investigating the pathologic complete response rate when NBTXR3 hafnium oxide nanoparticles are added in the management of locally advanced soft tissue sarcomas.[50]

In the NBTXR3 group, patients received a single intratumoral image-guided injection of NBTXR3, with the volume being equivalent to 10% of the baseline tumor volume. The baseline tumor volume was calculated by the central imaging review board using the product of the three longest dimensions of the tumor (length × width × depth) assessed by MRI up to 1 week before treatment. NBTXR3, supplied by Nanobiotix SA (Paris, France), consisted of nanoparticles composed of HfO_2 crystallite and phosphate groups suspended in an aqueous medium at a concentration of 53.3 g/L.

Both groups, NBTXR3 and control, received 3DCRT or IMRT, as determined by the discretion of the radio-oncologist. The total RT dose was 50 Gy, delivered in 25 fractions of 2 Gy over a period of 5 weeks, following the standard-of-care recommendations for preoperative RT in STS of the extremity and trunk wall. Premedication with steroids was introduced to reduce the risk of acute immune reaction. In the NBTXR3 group, RT began

within 1-5 days after NBTXR3 injection, while in the control group, RT commenced within 7 days after randomization. Following RT completion, all patients were scheduled for wide resection, adhering to the guidelines. The injection points of NBTXR3 were defined based on the planned surgical incision line to ensure the removal of all NBTXR3 injection sites and tracts.

The evaluation of patients for pathological complete response followed the European Organization for Research and Treatment of Cancer (EORTC) recommendations for histological evaluation of response to preoperative treatment in soft-tissue sarcoma. Pathological response was assessed by a central review board composed of four pathologists who were authors of the EORTC guidelines. Clinical and laboratory safety parameters were monitored at various timepoints throughout the treatment and follow-up period, including visits during the screening period, NBTXR3 administration, radiotherapy, surgery, post-surgery, end-of-treatment visit, and the subsequent 2-year follow-up. Imaging studies, such as MRI and CT scans, were conducted at specific intervals to assess tumor response and monitor the patients' condition. Patients could be withdrawn from the study treatment under certain circumstances, such as upon request, pregnancy, locoregional progressive disease, or unacceptable toxicity. Any adverse events could lead to delays or interruptions in radiotherapy. Radiotherapy was initiated or resumed only after the resolution of toxicity to grade 1, in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The cutoff date for the study was determined as the point at which all patients had completed primary and secondary endpoint assessments and a follow-up period of 2 years. This ensured that all necessary data for analysis and evaluation had been obtained for the entire patient cohort.

After completing RT, 176 patients (89 in the NBTXR3 group and 87 in the RT alone group) underwent surgery. Out of the 89 patients in the NBTXR3 group, 72 (81%) received between 80% and 100% of the planned NBTXR3 dose, while ten (11%) received less than 80%, and seven (8%) received more than 100% of the planned dose (where >100% indicated rounding of the calculated volume for practical reasons). The most common reason for not receiving the entire planned dose of NBTXR3 was pain. NBTXR3 administration was incomplete in 18 (21%) of the 87 patients. During the injection procedure, the median number of needle punctures per patient was 8 (range 2-40), and the median time for administration was 28.5 minutes (range 3-331) in a single session.

The primary endpoint of the study was the proportion of pCR, as determined by central blind review, which was defined as the presence of less than 5% residual malignant viable cells. The results of the study were presented at ASTRO in October 2018[49] and were published in the peer reviewed literature.[51–53] The outcomes of this trial are further analyzed in the following subsections.

9.2. Pathological Complete Response Rate – pCRR

In the intention-to-treat full analysis set, the proportion of patients achieving a pCR was 16% (14 out of 87) in the NBTXR3 group compared to 8% (7 out of 89) in the RT-alone group (p = 0.044) (see Table 1). Similarly, within the evaluable patient population for pathological response, the NBTXR3 group demonstrated a significantly higher proportion of patients with a pCR of 19% (14 patients out of 73) compared to the RT-alone group were pCR was 9% (7 patients out of 81) (p = 0.047).

Exploratory analysis of pCR, using a threshold of 0% stainable cancer cells and evaluating pathological response based on the percentage of tumor necrosis or infarction as described by Schaefer *et al*,[19] yielded similar results to the primary analysis. In a planned exploratory examination of the proportion of patients achieving pCR, categorized by histological grade, it was observed that the difference between the treatment groups was more pronounced among patients with grade 2 and 3 tumors in comparison to those with grade 1 tumors.

Table 1. Pathological complete response rates of the phase 2/3 study evaluating the use of NBTXR3 in the treatment of locally advanced soft tissue sarcomas.[50]

Endpoint	NPTXR3 and RT (n = 87)	RT alone (n=89)	P value
Pathologic complete response-pCR, n(%)	14 (16%)	7 (8%)	0.044
Tumor necrosis or infarction (%)	-	-	0.014
Mean (SD)	29 (31%)	19 (24%)	
Median; range	20; 0-95	10; 0-95	
Objective response evaluated per RECIST 1.1	6 (7%)	9 (10%)	0.86
Pathological complete response (<5% viable tumor cells) by histological grade*			
Grade 1	1/76 (1%)	3/77 (4%)	-
Grade 2	6/76 (8%)	2/77 (3%)	-
Grade 3	7/76 (9%)	1/77 (1%)	-
Pathological complete response (0% viable cells), %	12 (14%)	7 (8%)	-

*only assessed in patients with pathological response and known histological grade (n = 76 in the NBTXR3 group and n = 77 in the RT alone group)

9.3. Objective Response

There was no significant disparity observed in the proportion of patients who achieved an objective response, as evaluated according to RECIST 1.1 criteria, between the treatment

groups (see Table 1). In arm A, the objective response rate was 7% (6 out of 87), whereas in arm B, it was 10% (9 out of 89) (p = 0.863).

9.4. Status of resection margins

The important secondary outcome, evaluating the resection margin after neoadjuvant treatment, demonstrated that a larger proportion of patients in the NBTXR3 group achieved R0 margins compared to the RT-alone group (p = 0.042) (see Table 2). Furthermore, among the population eligible for resection margin assessment, the NBTXR3 group exhibited a higher percentage of patients with R0 margins (67 out of 83, or 81%) compared to the RT-alone group (57 out of 86, or 66%; p = 0.030).

Table 2. Resection margin results of the phase 2/3 study evaluating the use of NBTXR3 in the treatment of locally advanced soft tissue sarcomas.[50]

Resection Margin	NPTXR3 and RT (n = 87)	RT alone (n=89)	P value
R0 resections	67 (77%)	57 (64%)	0.042
Resection margin*			
R0	67 (81%)	57 (66%)	0.03
R1	9 (11%)	19 (22%)	
R2	5 (6%)	5 (6%)	
NA	2 (2%)	4 (5%)	
* Only assessed in patients with evaluable resection margins (n=83 in the NBTXR3 group; n=86 in the radiotherapy alone group; unknown for one patient in the radiotherapy alone group)			

9.5. Toxicity

Toxicity has been described for both the on-treatment period and the follow-up period oa at least 2 years in the all-treated population.[52]

During the on-treatment phase 36.0% (32 out of 89) of patients experienced at least one adverse event associated with NBTXR3. The most common adverse event was hypotension, occurring in 11.2% (10 out of 89) of patients at any grade. Among these adverse events, there were two grade 4 events, including one case of hypotension and one case of anaphylactic shock. Additionally, in the NBTXR3 group, 46.1% (41 out of 89) of patients had at least one adverse event related to the injection procedure, with injection site pain being the most common (all grades: 12.4% [11 out of 89]). One patient experienced a grade 4 pulmonary embolism. There were no grade 4 treatment-emergent adverse events (TEAEs) related to RT during the on-treatment period. Adverse events related to RT were observed in 78.7% (70 out of 89) of patients in the NBTXR3 group and 80.0% (72 out of 90) of patients in the RT-alone group. The most frequent adverse event associated with RT was radiation skin injury,

occurring in 52.8% (47 out of 89) of patients in the NBTXR3 group and 63.3% (57 out of 90) of patients in the RT alone group, at any grade.

In terms of postsurgical toxicity, the incidence of grades 3 to 4 TEAEs (occurring as wound complications after surgical resection) was 21.3% (19 out of 89) in the NBTXR3 group and 22.2% (20 out of 90) in the RT-alone group. During a follow-up period of at least 2 years, 4.5% (4 out of 89) of patients in the NBTXR3 group experienced at least one adverse event related to NBTXR3 and RT. This included one case of grade 3 postoperative wound complication and one case of grade 3 osteonecrosis. Posttreatment RT-related adverse events were observed in 24.7% (22 out of 89) versus 25.6% (23 out of 90) of patients, in the NBTXR3 and RT-alone groups, respectively. The most common adverse event was radiation skin injury, occurring in 9.0% (8 out of 89) of patients in the NBTXR3 group and 5.6% (5 out of 90) of patients in the RT-alone group, at any grade. The most frequent grade 3 event was postoperative wound complication, reported in 3.4% (3 out of 89) of patients in the NBTXR3 group and 4.4% (4 out of 90) of patients in the RT-alone group, with no grade 4 events observed. Late-onset radiation toxicities in the NBTXR3 versus RT-alone group included fibrosis (4.5% [4 out of 89] vs. 7.8% [7 out of 90]) and edema (6.7% [6 out of 89] vs. 2.2% [2 out of 90]).

Regarding serious adverse events (SAEs), during the on-treatment period, 10.1% (9 out of 89) of patients in the NBTXR3 group experienced SAEs related to NBTXR3, 9.0% (8 out of 89) experienced SAEs related to the injection procedure, and 5.6% (5 out of 89) experienced SAEs related to RT. In the RT-alone group, the occurrence of SAEs related to RT was 5.6% (5 out of 90) of patients. It must be noted that two patients of the NBTXR3 group had a total of three serious treatment-emergent adverse events (TEAEs) related to NBTXR3 and RT. Post surgery hospitalization due to SAEs was observed in 15.7% (14 out of 89) of patients in the NBTXR3 plus RT group and 24.4% (22 out of 90) of patients in the RT-alone group. During the follow-up period, regardless of cause, posttreatment SAEs of any grade occurred in 13.5% (12 out of 89) of patients and 24.4% (22 out of 90) of patients in the NBTXR3 and RT-alone groups, respectively. The proportion of patients experiencing a change from grade 0 or 1 to grade 3 for most hematology and biochemistry parameters was similar between the treatment groups during the on-treatment period, as well as the proportion of patients with a shift from grade 0 to 1 to grade 1 to 2 during the follow-up period.

9.6. Recurrence

The cumulative rate of local recurrence at 24 months was 12.0% (9 out of 75; 95% CI, 5.6%-21.6%) and 7.1% (6 out of 84; 95% CI, 2.7%-14.9%) in the NBTXR3 plus RT and RT-alone group, respectively. The cumulative rate of distant recurrence at 24 months was 33.3% (25 out of 75; 95% CI, 22.9%-45.2%) in the NBTXR3 group and 26.2% (22 out of 84; 95% CI, 17.2%-36.9%) in the RT-alone group, based on the evaluable patient population. Throughout the entire study, a total of 46 patients died, with 24 patients of the NBTXR3 and 22 patients of the RT-alone group. None of the deaths were related to the treatment, and the primary cause of death was progressive disease.

9.7. HRQoL

The administration of NBTXR3 did not have a negative impact on the patient's Health-Related Quality of Life (HRQoL). At the 2-year follow-up, when comparing the NPTXR3 plus RT (Arm A) and RT-alone (Arm B) groups, the mean change from baseline in various HRQoL assessments was as follows:

- TESS (Treatment Emergent Symptom Scale): Arm A: -3.4 vs Arm B: -6.1
- EQ-5D-5L: Arm A: -0.093 vs Arm B: -0.038
- EQ5D02-EQ VAS: Arm A: 6.5 vs Arm B: 2.3
- RNLI (Rotterdam Neck Discomfort and Disability Scale): Arm A: 2.0 vs Arm B: 0.0
- MSTS (Musculoskeletal Tumor Society): Arm A: 1.7 vs Arm B: 1.2

During the follow-up period, there was a gradual improvement observed in most HRQoL evaluations, as indicated by the mean scores of TESS, EQ-5D-5L, EQ5D02-EQ VAS, RNLI, and MSTS. Comparison of the baseline scores with the end-of-treatment scores, showed an increase in mean scores for TESS, EQ-5D-5L, EQ5D02-EQ VAS, RNLI, and MSTS over the follow-up period in both arm A and arm B.

10. Discussion

In this thesis the use of nanoparticles in the treatment of soft-tissue sarcoma was studied by analyzing the corresponding publications found in the peer reviewed literature. Six studies were found reporting results of a pilot phase 1 study and a phase 2/3 multicentric randomized trial investigating the effectiveness of NBTXR3 hafnium-based nanoparticles activated by RT delivered in the preoperative setting.

NBTXR3 was chosen among other NPs due to its unique physical properties and mechanisms of action. Its high electron density, achieved through the compact arrangement of Hf atoms in crystalline HfO_2, allows for enhanced energy deposition when exposed to ionizing radiation. Importantly, NBTXR3's primary mode of action is physical rather than biological, making it universally applicable across various tumor types. Additionally, in the current setting NBTXR3 was injected as a single dose directly inside the tumor which offered better control of localization, maximizing its availability, and limiting exposure to healthy tissues.

Retrospective studies and a meta-analysis have provided evidence that achieving pathological complete responses after preoperative treatment is associated with long-term benefits in patients with locally advanced STS.[15–18] In a meta-analysis conducted by Salah *et al*,[16] 21 studies involving a total of 1663 patients were included. The analysis revealed that tumor necrosis below 90% following neoadjuvant therapy is linked to an increased risk of recurrence and inferior overall survival compared to patients with tumor necrosis of 90% or higher. Furthermore, the presence of hyalinization or fibrosis was found to have prognostic significance for patient outcomes within the same population. These findings highlight the importance of achieving high levels of tumor necrosis and the potential role of hyalinization

or fibrosis as prognostic indicators in locally advanced STS patients undergoing preoperative treatment. The ability of NBTXR3 to double the proportion of patients achieving a pathological complete response, which is a major outcome measure in this context, suggests its potential for use in sarcoma and other solid tumors, particularly when radiotherapy is the primary treatment modality or when surgical resection is challenging. Moreover, there is potential to explore dose de-escalation in radiotherapy by utilizing NBTXR3 to minimize morbidity, as well as investigating its effectiveness in cases requiring re-irradiation.

Achieving negative surgical margins (R0) through surgical resection is widely recognized as a crucial factor for local control and overall survival in limb sarcoma and truncal or girdle sarcoma.[54–56] Surgeons face the challenge of balancing the benefits of extensive surgical excision against potential impacts on limb function or preservation, while considering the increased risk of recurrence associated with positive surgical margins. In the preoperative setting, the use of multi-modal treatment with NBTXR3 has demonstrated superiority by increasing the proportion of patients achieving R0 resection compared to those treated with radiotherapy alone. This improvement could potentially be attributed to enhanced capsular integrity, as suggested by a retrospective study indicating that neoadjuvant treatment, including preoperative radiotherapy, induces fibrosis and stabilizes the tumor capsule in high-grade soft-tissue sarcoma. Notably, the observed effect is not solely related to tumor size reduction but rather signifies a tissue response characterized by a decrease in viable cell count.

Overall similar toxicity profiles were found for both the NBTXR3 and the RT-alone groups. In more detail, in the NBTXR3 group SAEs occurred in 39% of patients, while in the RT alone group, SAEs were reported in 30% of patients. Serious treatment-emergent adverse events, which may not be directly related to the treatment, were observed in 31% of patients in the NBTXR3 group and 16% of patients in the RT alone group. Within the NBTXR3 group, 10% of patients experienced serious adverse events related to NBTXR3, with hypotension being the most frequent event. Serious adverse events related to radiotherapy were reported in both groups, with postoperative wound complication being the most common. A long-term follow-up of 2 years showed that NBTXR3 did not have a negative impact on postsurgical wound complications or late radiation toxicities such as fibrosis and oedema. Additionally, NBTXR3 did not adversely affect patients' health-related quality of life (HRQoL) in terms of late-onset adverse effects or sequelae in patients with STS in the extremity.

11. Limitations

The two trials presented in this thesis have several recognized limitations. One limitation is the inability to use a placebo injection as a control, which could have provided a better comparison. Additionally, patients in the control group had slightly larger tumors compared to the NBTXR3 group. However, a multivariate analysis showed that tumor size does not significantly impact survival outcome or local control, which is directly linked to the margin. Therefore, the larger tumor size in the control group may be justified by the need for a larger

surgical margin. There was also a higher proportion of men in the investigational group compared to the RT-alone group. However, when studies find a prognostic influence of gender, male gender is generally unfavorable, which would benefit the control group. Moreover, the phase 2/3 study was unblinded for surgery, but the surgical approach was based on tumor characteristics rather than the preoperative treatment received. Finally, the surgical capacity of the center could have affected the quality of resection margin. However, all participating centers in this study were either high-volume or National Cancer Institute-designated centers, ensuring a certain level of surgical expertise. No stratification by center was performed in the study.

12. Conclusion and future perspectives

The findings analyzed in this context indicate that the use of NBTXR3 activated by radiotherapy shows promise as a treatment approach for patients diagnosed with locally advanced soft tissue sarcomas (STS) in the extremities or trunk wall. The results suggest that NBTXR3 has the potential to improve the outcomes and therapeutic options for individuals with this particular form of malignancy. Additionally, it is reasonable to consider exploring the utilization of NBTXR3 in eligible patients with other cancer types who undergo preoperative radiotherapy, as supported by previous randomized studies. Furthermore, ongoing research indicates that the effects of radiation activated NBTXR3 may extend beyond its ability to enhance the efficacy of radiotherapy. Emerging evidence suggests that NBTXR3, when activated by radiation, acts as a potent immunomodulator at the cellular level of cancer cells. This activation induces strong immunogenic cell stress, resulting in the generation of an effective antitumor immune response. These findings highlight the significant potential of high-Z radio-enhancer nanoparticles in modulating cancer cell immunogenicity, thus offering new therapeutic opportunities for patients.

The wide range of potential applications for NBTXR3 underscores the significance of continued research and exploration in this field. Further investigation is crucial to uncovering its full therapeutic potential and expanding its applications in the field of radio-immuno-oncology.

13. References

1. Gamboa AC, Gronchi A, Cardona K. Soft-tissue sarcoma in adults: An update on the current state of histiotype-specific management in an era of personalized medicine. *CA Cancer J Clin*. 2020;70(3):200-229. doi:10.3322/caac.21605
2. Clark MA, Fisher C, Judson I, Thomas JM. Soft-Tissue Sarcomas in Adults. *N Engl J Med*. 2005;353(7):701-711. doi:10.1056/nejmra041866
3. Dangoor A, Seddon B, Gerrand C, Grimer R, Whelan J, Judson I. UK guidelines for the management of soft tissue sarcomas. *Clin Sarcoma Res*. 2016;6(1):1-26. doi:10.1186/s13569-016-0060-4
4. Mehren M Von, Randall RL, Benjamin RS, et al. Soft tissue sarcoma, version 2.2018: Clinical practice guidelines in oncology. *JNCCN J Natl Compr Cancer Netw*.

2018;16(5):536-563. doi:10.6004/JNCCN.2018.0025
5. Haas RLM, Miah AB, LePechoux C, et al. Preoperative radiotherapy for extremity soft tissue sarcoma; Past, present and future perspectives on dose fractionation regimens and combined modality strategies. *Radiother Oncol*. 2016;119(1):14-21. doi:10.1016/j.radonc.2015.12.002
6. Dei Tos AP, Bonvalot S, Haas R. Evolution in the management of soft tissue sarcoma: classification, surgery and use of radiotherapy. *Expert Rev Anticancer Ther*. 2020;20(sup1):3-13. doi:10.1080/14737140.2020.1753509
7. Gronchi A, Palmerini E, Quagliuolo V, et al. Neoadjuvant chemotherapy in high-risk soft tissue sarcomas: Final results of a randomized trial from Italian (ISG), Spanish (GEIS), French (FSG), and Polish (PSG) sarcoma groups. *J Clin Oncol*. 2020;38(19):2178-2186. doi:10.1200/JCO.19.03289
8. Blay JY, Soibinet P, Penel N, et al. Improved survival using specialized multidisciplinary board in sarcoma patients. *Ann Oncol*. 2017;28(11):2852-2859. doi:10.1093/annonc/mdx484
9. Blay JY, Honoré C, Stoeckle E, et al. Surgery in reference centers improves survival of sarcoma patients: a nationwide study. *Ann Oncol*. 2019;30(7):1143-1153. doi:10.1093/annonc/mdz124
10. Yang JC, Chang AE, Baker AR, et al. Randomized prospective study of the benefit of adjuvant radiation therapy in the treatment of soft tissue sarcomas of the extremity. *J Clin Oncol*. 1998;16(1):197-203. doi:10.1200/JCO.1998.16.1.197
11. O'Sullivan B, Davis AM, Turcotte R, et al. Preoperative versus postoperative radiotherapy in soft-tissue sarcoma of the limbs: a randomised trial. *Lancet*. 2002;359(9325):2235-2241. doi:10.1016/S0140-6736(02)09292-9
12. Wang D, Abrams RA. Radiotherapy for Soft Tissue Sarcoma: 50 Years of Change and Improvement. *Am Soc Clin Oncol Educ B*. 2014;(34):244-251. doi:10.14694/edbook_am.2014.34.244
13. Cammelli S, Cortesi A, Buwenge M, et al. The role of radiotherapy in adult soft tissues sarcoma of the extremities. *Eur J Orthop Surg Traumatol*. 2021;31(8):1583-1596. doi:10.1007/s00590-021-02990-6
14. Kungwengwe G, Clancy R, Vass J, et al. Preoperative versus Post-operative Radiotherapy for Extremity Soft tissue Sarcoma: a Systematic Review and Meta-analysis of Long-term Survival. *J Plast Reconstr Aesthetic Surg*. 2021;74(10):2443-2457. doi:10.1016/j.bjps.2021.05.043
15. Shah D, Borys D, Martinez SR, et al. Complete pathologic response to neoadjuvant radiotherapy is predictive of oncological outcome in patients with soft tissue sarcoma. *Anticancer Res*. 2012;32(9):3911-3916.
16. Salah S, Lewin J, Amir E, Abdul Razak A. Tumor necrosis and clinical outcomes following neoadjuvant therapy in soft tissue sarcoma: A systematic review and meta-analysis. *Cancer Treat Rev*. 2018;69(May):1-10. doi:10.1016/j.ctrv.2018.05.007
17. Bonvalot S, Wunder J, Gronchi A, et al. Complete pathological response to neoadjuvant treatment is associated with better survival outcomes in patients with soft tissue sarcoma: Results of a retrospective multicenter study. *Eur J Surg Oncol*. 2021;47(8):2166-2172. doi:10.1016/j.ejso.2021.02.024
18. Eilber FC, Rosen G, Eckardt J, et al. Treatment-induced pathologic necrosis: A predictor of local recurrence and survival in patients receiving neoadjuvant therapy for high-grade extremity soft tissue sarcomas. *J Clin Oncol*. 2001;19(13):3203-3209.

doi:10.1200/JCO.2001.19.13.3203
19. Schaefer IM, Hornick JL, Barysauskas CM, et al. Histologic Appearance After Preoperative Radiation Therapy for Soft Tissue Sarcoma: Assessment of the European Organization for Research and Treatment of Cancer–Soft Tissue and Bone Sarcoma Group Response Score. *Int J Radiat Oncol Biol Phys*. 2017;98(2):375-383. doi:10.1016/j.ijrobp.2017.02.087
20. Haque M, Shakil MS, Mahmud KM. The Promise of Nanoparticles-Based Radiotherapy in Cancer Treatment. *Cancers (Basel)*. 2023;15(6). doi:10.3390/cancers15061892
21. Scher N, Bonvalot S, Le Tourneau C, et al. Review of clinical applications of radiation-enhancing nanoparticles. *Biotechnol Reports*. 2020;28:e00548. doi:10.1016/j.btre.2020.e00548
22. Laprise-Pelletier M, Simão T, Fortin MA, Laprise-Pelletier M, Simão T, Fortin MA. Gold Nanoparticles in Radiotherapy and Recent Progress in Nanobrachytherapy. *Adv Healthc Mater*. 2018;7(16):1701460. doi:10.1002/adhm.201701460
23. Rancoule C, Magné N, Vallard A, et al. Nanoparticles in radiation oncology: From bench-side to bedside. *Cancer Lett*. 2016;375(2):256-262. doi:10.1016/j.canlet.2016.03.011
24. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72(1):7-33. doi:10.3322/caac.21708
25. Birch JM. Epidemiology of sarcomas. *Curr Opin Oncol*. 1990;2(3):462-466. doi:10.1097/00001622-199006000-00002
26. Sbaraglia M, Bellan E, Dei Tos AP. The 2020 WHO Classification of Soft Tissue Tumours: News and perspectives. *Pathologica*. 2021;113(2):70-84. doi:10.32074/1591-951X-213
27. Skafida E, Kokkali S, Nikolaou M, Digklia A. Metastatic soft tissue sarcoma: current treatment landscape and future perspectives. *Expert Rev Anticancer Ther*. 2017;17(6):537-543. doi:10.1080/14737140.2017.1321989
28. Penel N, Grosjean J, Robin YM, Vanseymortier L, Clisant S, Adenis A. Frequency of certain established risk factors in soft tissue sarcomas in adults: A prospective descriptive study of 658 cases. *Sarcoma*. 2008;2008(September 2005). doi:10.1155/2008/459386
29. Gronchi A, Miah AB, Dei Tos AP, et al. Soft tissue and visceral sarcomas: ESMO–EURACAN–GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up☆. *Ann Oncol*. 2021;32(11):1348-1365. doi:10.1016/j.annonc.2021.07.006
30. Mendenhall WM, Indelicato DJ, Scarborough MT, et al. The management of adult soft tissue sarcomas. *Am J Clin Oncol Cancer Clin Trials*. 2009;32(4):436-442. doi:10.1097/COC.0b013e318173a54f
31. Esmo T, Sarcoma E, Working N. Soft tissue and visceral sarcomas: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2014;25(Supplement 3):iii102-iii112. doi:10.1093/annonc/mdu254
32. Mayles P, Nahum A, Rosenwald JC. *Handbook of Radiotherapy Physics: Theory and Practice*. (Mayles P, Nahum A, Rosenwald JC, eds.). Taylor and Francis Ltd; 2007. doi:10.1118/1.2969650
33. Khan FM, Gibbons JP. *KHAN's Physics of Radiation Therapy*. 5th ed. (Khan FM, Gibbons JP, eds.). Wolters Kluwer; 2014.
34. Gerrand CH, Rankin K. The treatment of soft-tissue sarcomas of the extremities. Prospective randomized evaluations of (1) limb-sparing surgery plus radiation therapy compared with amputation and (2) the role of adjuvant chemotherapy. *Class Pap Orthop*. 2014;(1):483-484. doi:10.1007/978-1-4471-5451-8_125
35. Albertsmeier M, Rauch A, Roeder F, et al. External Beam Radiation Therapy for Resectable Soft Tissue Sarcoma: A Systematic Review and Meta-Analysis. *Ann Surg Oncol*.

2018;25(3):754-767. doi:10.1245/s10434-017-6081-2
36. X. Allen Li. Margin Reduction from IGRT for Soft-Tissue Sarcoma: Secondary Analysis of RTOG 0630 Results. *Pr Radiat Oncol*. 2016. doi:10.1016/j.prro.2015.11.012
37. O'Sullivan B, Griffin AM, Dickie CI, et al. Phase 2 study of preoperative image-guided intensity-modulated radiation therapy to reduce wound and combined modality morbidities in lower extremity soft tissue sarcoma. *Cancer*. 2013;119(10):1878-1884. doi:10.1002/CNCR.27951
38. Alektiar KM, Brennan MF, Singer S. Local control comparison of adjuvant brachytherapy to intensity-modulated radiotherapy in primary high-grade sarcoma of the extremity. *Cancer*. 2011;117(14):3229-3234. doi:10.1002/cncr.25882
39. RTOG-0630. *Radiation Therapy Oncology Group Rtog 0630 a Phase Ii Trial of Image Guided Preoperative Radiotherapy*.; 2014.
40. Wang D, Bosch W, Roberge D, et al. RTOG sarcoma radiation oncologists reach consensus on gross tumor volume and clinical target volume on computed tomographic images for preoperative radiotherapy of primary soft tissue sarcoma of extremity in radiation therapy oncology group studies. *Int J Radiat Oncol Biol Phys*. 2011;81(4):525-528. doi:10.1016/j.ijrobp.2011.04.038
41. Salerno KE, Alektiar KM, Baldini EH, et al. Radiation Therapy for Treatment of Soft Tissue Sarcoma in Adults: An ASTRO Clinical Practice Guideline.
42. *ISO/TR 18401:2017 - Nanotechnologies — Plain Language Explanation of Selected Terms from the ISO/IEC 80004 Series*. https://www.iso.org/standard/62384.html. Accessed June 11, 2023.
43. Kulkarni S, Kumar S, Acharya S. Gold Nanoparticles in Cancer Therapeutics and Diagnostics. *Cureus*. 2022;14(10). doi:10.7759/cureus.30096
44. Baetke SC, Lammers T, Kiessling F. Applications of nanoparticles for diagnosis and therapy of cancer. *Br J Radiol*. 2015;88(1054). doi:10.1259/bjr.20150207
45. Maggiorella L, Barouch G, Devaux C, et al. Nanoscale radiotherapy with hafnium oxide nanoparticles. *Futur Oncol*. 2012;8(9):1167-1181. doi:10.2217/fon.12.96
46. Pottier A, Borghi E, Levy L. Metals as radio-enhancers in oncology: The industry perspective. *Biochem Biophys Res Commun*. 2015;468(3):471-475. doi:10.1016/j.bbrc.2015.09.027
47. Bonvalot S, Le Pechoux C, De Baere T, et al. Phase I study of NBTXR3 nanoparticles, in patients with advanced soft tissue sarcoma (STS). In: *Journal of Clinical Oncology*. Vol 32. ; 2014:10563-10563. doi:10.1200/jco.2014.32.15_suppl.10563
48. Bonvalot S, Ecile Le Pechoux C, De Baere T, et al. Cancer Therapy: Clinical First-in-Human Study Testing a New Radioenhancer Using Nanoparticles (NBTXR3) Activated by Radiation Therapy in Patients with Locally Advanced Soft Tissue Sarcomas. *Clin Cancer Res*. 2017;23(4). doi:10.1158/1078-0432.CCR-16-1297
49. Bonvalot S, Rutkowski P, Thariat JO, et al. Act.in.Sarc: An International Randomized Phase III Trial Evaluating Efficacy and Safety of First-in-Class NBTXR3 Hafnium Oxide Nanoparticles Activated By Preoperative Radiotherapy in Locally Advanced Soft Tissue Sarcoma. *Int J Radiat Oncol*. 2018;102(5):1606. doi:10.1016/j.ijrobp.2018.08.054
50. Bonvalot S, Rutkowski PL, Thariat J, et al. NBTXR3, a first-in-class radioenhancer hafnium oxide nanoparticle, plus radiotherapy versus radiotherapy alone in patients with locally advanced soft-tissue sarcoma (Act.In.Sarc): a multicentre, phase 2â€“3, randomised, controlled trial. *Lancet Oncol*. 2019. doi:10.1016/S1470-2045(19)30326-2
51. Bonvalot S, Rutkowski P, Thariat JO, et al. Study of Novel Radioenhancer NBTXR3 Plus

Radiotherapy in Patients With Locally Advanced Soft Tissue Sarcoma: Results of the Long-Term Evaluation in the Phase II/III Act.In.Sarc Trial. *Int J Radiat Oncol*. 2021;111(3):S40-S41. doi:10.1016/j.ijrobp.2021.07.115

52. Bonvalot S, Rutkowski PL, Thariat J, et al. Final Safety and HRQoL Results of the Phase 2/3 Act.In.Sarc Study With Preoperative NBTXR3 Plus Radiation Therapy Versus Radiation Therapy in Locally Advanced Soft-Tissue Sarcoma. *Int J Radiat Oncol Biol Phys*. 2022. doi:10.1016/J.IJROBP.2022.07.001
53. Scher N, Bonvalot S, Le Tourneau C, et al. Review of clinical applications of radiation-enhancing nanoparticles. *Biotechnol Reports*. 2020;28(March):e00548. doi:10.1016/j.btre.2020.e00548
54. O'Donnell PW, Griffin AM, Eward WC, et al. The effect of the setting of a positive surgical margin in soft tissue sarcoma. *Cancer*. 2014;120(18):2866-2875. doi:10.1002/cncr.28793
55. Liu CY, Yen CC, Chen WM, et al. Soft tissue sarcoma of extremities: The prognostic significance of adequate surgical margins in primary operation and reoperation after recurrence. *Ann Surg Oncol*. 2010;17(8):2102-2111. doi:10.1245/s10434-010-0997-0
56. Tanabe KK, Pollock RE, Ellis LM, Murphy A, Sherman N, Romsdahl MM. Influence of surgical margins on outcome in patients with preoperatively irradiated extremity soft tissue sarcomas. *Cancer*. 1994;73(6):1652-1659. doi:10.1002/1097-0142(19940315)73:6<1652::AID-CNCR2820730617>3.0.CO;2-X