Clinical Cancer Research

First-in-Human Study Testing a New Radioenhancer Using Nanoparticles (NBTXR3) Activated by Radiation Therapy in Patients with Locally Advanced Soft Tissue Sarcomas

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Abstract

Purpose: This phase I study aimed to determine the recommended dose (RD), safety profile, and feasibility of a procedure combining intratumoral injection of hafnium oxide nanoparticles (NBTXR3; a radioenhancer) and external beam radiotherapy (EBRT) for preoperative treatment of adults with locally advanced soft tissue sarcoma (STS).

Experimental Design: Patients had a preoperative indication of EBRT for STS of the extremity or trunk. Baseline tumor volume (TV) was calculated by MRI. NBTXR3 was injected percutaneously into tumors at 53.3 g/L. Dose escalation was based on four levels equivalent to 2.5%, 5%, 10%, and 20% of baseline TV. NBTXR3 was visualized in the tumor 24 hours postinjection, and EBRT was initiated (50 Gy over 5 weeks). Surgery was performed 6 to 8 weeks after EBRT completion.

Results: Twenty-two patients completed NBTXR3 injection, EBRT, and surgery and were followed for a median 22 months (range, 6–40). At NBTXR3 20% of TV, two dose-limiting toxicities occurred: injection-site pain and postoperative scar necrosis. The RD was defined as 10%. No leakage of NBTXR3 into surrounding tissues occurred; intratumor NBTXR3 levels were maintained during radiotherapy. At the RD, median tumor shrinkage was 40% (range 71% shrinkage, 22% increase); median percentage of residual viable tumor cells was 26% (range, 10%–90%). Patients receiving 20% of TV demonstrated pathologic complete responses. Seven grade 3 adverse events occurred, which were reversible.

Conclusion: A single intratumoral injection of NBTXR3 at 10% of TV with preoperative EBRT was technically feasible with manageable toxicity; clinical activity was observed. *Clin Cancer Res; 1–10.*

Introduction

Surgery is the standard treatment for localized extremity soft tissue sarcomas (STS). The benefit of adjuvant radiotherapy in extremity STS therapy has been demonstrated in three randomized studies (1–3). Preoperative radiotherapy has been associated with an increase in surgery-related wound complications compared with postoperative radiotherapy (4), whereas long-term morbidity was improved with pre- versus postoperative radiotherapy (4, 5). To minimize the toxicity of preoperative radiotherapy, image-guided intensity-modulated radiotherapy has been reported to reduce wound complications (6). However, tumor shrinkage and pathologic response rates with preoperative radiotherapy still need to be improved, particularly when a tumor is deemed to be unresectable or when local control could be suboptimal. Various radio-potentiation methods have been evaluated with encouraging efficacy, but they are hampered by significant systemic toxicity (7–9).

Nanomedicine has made tremendous progress and provided new treatment concepts. Drug delivery systems that change the biodistribution of drugs have been extensively used (10). Inorganic crystalline nanoparticles with new and specific physical properties have been developed. These include a new class of man-made radiation enhancers composed of functionalized nanoparticles containing high Z material (where Z is atomic number) with a high electron density (transition metals and their compounds, e.g., hafnium oxide, gold, and platinum) and a well-defined size and shape. These NPs are highly efficient at absorbing radiation because of their physicochemical properties and, therefore, can augment the radiation applied to a tumor. They can enter tumor cells and deposit high levels of energy into the cells, leading to DNA damage and cell destruction, but only when exposed to

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Translational Relevance

Evidence before this study

The standard of care for high-risk soft tissue sarcoma is surgery and external beam radiotherapy; however, improvements are needed. A new class of radiation enhancers, composed of nanoparticles, is highly efficient in absorbing radiation via their physicochemical properties and therefore delivers a higher dose within the tumor. These inorganic nanoparticles may represent a breakthrough approach for the local treatment of solid tumors. Among them, NBTXR3 first in clinical trial (hafnium oxide) has been designed for optimal uptake into cancer cells, with a highly favorable benefit/risk ratio.

Value added by this study

This first-in-human phase I trial reports the safety of a procedure combining intratumoral injection of NBTXR3 and EBRT.

Implications of the evidence

This study constitutes the basis for the current development of NBTXR3 in a phase III trial in STS and in four phase I trials in others cancers.

ionizing radiation (on/off activity; ref. 11). These nanoparticles may represent a breakthrough approach for the local treatment of solid tumors. Among them, NBTXR3 NPs contain hafnium oxide, as the moiety of high electron density, and have been engineered with an average diameter of 50 nm and a functionalized negatively charged polymer comprising phosphate groups on their surface. Their chemistry, size, shape, and surface charge have been designed for optimal cancer cell uptake and the best benefit:risk ratio. Hafnium oxide is a compound made of hafnium (atomic number, 72), with a high density of 9, making it an efficient radioenhancer. This compound is physically and chemically inert (insulator, nondegradable, no redox activities), thereby providing beneficial characteristics from a safety perspective. The interaction of ionizing radiation with hafnium allows for a higher energy deposit than radiotherapy alone, thereby generating many more electrons at the same dose of radiation and increasing subsequent cell death (11–13). Preclinical studies have demonstrated that NBTXR3 nanoparticles have a strong impact of on cell replication, tumor control, and survival in animals (12).

This report summarizes findings from the first-in-human study testing NBTXR3 activated by fractionated external beam radiotherapy (EBRT) in patients with locally advanced STS. The study objectives were to assess safety and feasibility of intratumoral injections of NBTXR3 at increasing volumes to determine the MDT and recommended dose (RD).

Materials and Methods

Patient population

This was a phase I, open-label, nonrandomized, dose–escalation trial, conducted in two centers in France; patients were enrolled between November 2011 and April 2014.

Primary eligibility criteria were: patients over 18 years of age with histologically confirmed STS of the extremity or trunk wall (primary or recurrent), World Health Organization (WHO) performance scores of 0–1, and adequate bone marrow, kidney, and liver function. Patients with metastatic disease were included if their life expectancy was more than 6 months.

Key exclusion criteria were: prior radiotherapy on the anatomic area to be treated and the following histological subtypes: embryonal or alveolar rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, Kaposi sarcoma, primitive neuroectodermal tumor, angiosarcoma, aggressive fibromatosis, or dermatofibrosarcoma protuberans. The tumor types were characterized following the WHO criteria (14) and graded according to the French Federation of Cancer Centers system (15). Other investigational drugs were not allowed. Approval was obtained from the ethics committees of the participating institutions and the regulatory authorities. All patients provided informed consent. The study was performed in accordance with the Declaration of Helsinki, good clinical practice guidelines, and local regulations. The study was registered on ClinicalTrials.gov (registration number, NCT01433068).

Study characteristics and objectives

Patients were sequentially assigned to escalating volume levels (doses) of NBTXR3 at a fixed concentration of 53.3 g/L, following a traditional 3 + 3 design that was modified for the first two levels, in which 6 patients were enrolled per level. Primary objectives were to determine the MTD, RD, and early dose-limiting toxicities (DLT), in addition to safety and feasibility of the intratumoral injection of NBTXR3 activated by EBRT. Early DLTs were defined as any grade 3–4 adverse event (AE) that could reasonably be related to NBTXR3 and/or radiotherapy, including hematologic and biochemistry toxicity, according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE, version 4.0). All AEs, regardless of the causality and onset time, were followed until the end of the study. An independent data monitoring committee was implemented for this first-in-human study.

NBTXR3 product

NBTXR3 was provided by NANOBIOTIX (Nanobiotix, a nanomedicine company; www.nanobiotix.com). It is a suspension of nanoparticles composed of hafnium oxide crystallites and phosphate groups in an aqueous medium. The NBTXR3 product was supplied for local injection in the form of a nonpyrogenic, sterile, white suspension processed by terminal sterilization (γ irradiation), to be administered at a fixed concentration of 53.3 g/L.

Tumor volume measurement

For measurement of tumor volume, the baseline gross tumor volume (GTV) was defined up to 1 week before study treatment using MRI and a CT scan and calculated as length × width × depth. The area surrounding the GTV was included to take into account adjacent tissue at risk for microscopic extension of disease (termed the clinical tumor volume, or CTV); this area was further expanded to account for set-up errors or possible uncertainties in patient positioning or motion (termed the planning tumor volume, or PTV).

NBTXR3 dose escalation

Four dose levels of NBTXR3 were used: levels 1 to 4 were volumes of NBTXR3 equivalent to 2.5%, 5%, 10%, and 20%, respectively, of the baseline tumor volume. For levels 1 and 2, 6 patients per level were treated. For levels 3 and 4, 3 to 6 patients

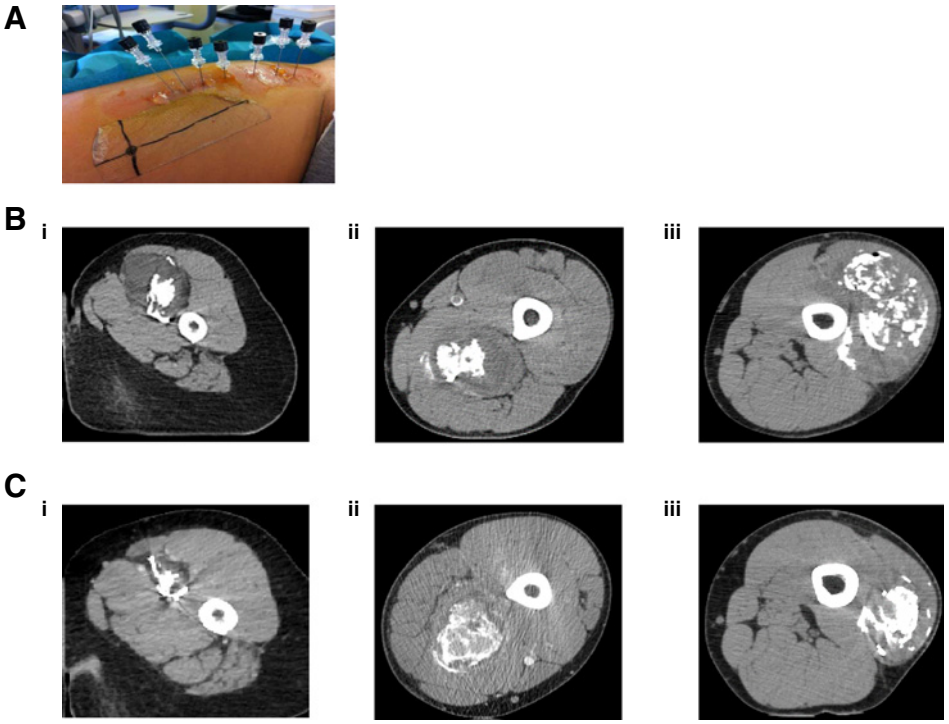

Figure 1.
A, The NBTRX3 injection procedure, showing needle positioning. **B,** CT scans showing intratumoral localization of NBTRX3 24 hours postinjection (i, level 2; ii, level 3; iii, level 4). **C,** CT scans showing intratumoral localization of NBTRX3 immediately prior to surgery, in the same patients as **B** (i, level 2; ii, level 3; iii, level 4).

per level were added sequentially, based on the occurrence of early DLTs. If 2 of 6 patients experienced early DLTs at a given level, the dose escalation was stopped. The RD was defined as the level immediately below that associated with an early DLT. If only 3 patients had been treated at this RD, an additional 6 patients were included for further safety evaluation.

Injection procedure

A single intratumoral injection of NBTXR3 53.3 g/L was administered with ultrasound guidance and visualized 24 hours later by a CT scan (NBTXR3 is easily visualized as a hyperdense particle). Patients received local anesthesia and anxiolytic treatment with the injection. Skin access was defined by the surgeon, according to the planned surgical incision line. Standard syringes and 22 G needles were selected for insertion every 1.5 to 2.0 cm, using different angulations, to cover as much of the tumor as possible (Fig. 1A). After the needles were positioned, NBTXR3 was injected through each needle, using slow hand pressure to limit pain. The mandrel was placed in the needle at least 1 minute before retrieving the needle, to minimize the risk of NBTXR3 dripping along the needle pathway. Needles were positioned to avoid the peripheral region of the tumor (1.0 cm) to protect the pseudocapsule from trauma.

Local nanoparticle dispersion and evaluation of NBTXR3 potential leakage

In addition to the CT scan performed 24 hours after the intratumoral injection, a second CT scan was performed 10 weeks after to evaluate the presence and dispersion of NBTXR3 inside the tumor, potential leakage into peritumoral tissues, and persistence of NBTXR3 in the tumor during the entire radiotherapy course. A chest CT scan was performed to evaluate the potential presence of NBTXR3 in the lungs. NBTXR3 is an intracellular device, macroscopically and microscopically occult. Accordingly, it was not possible for the pathologist to determine the distribution.

Quantification of NBTXR3 in the blood and urine was performed to evaluate passage into the systemic circulation. The hafnium component of NBTXR3 was quantified through inductively coupled plasma mass spectrometry. Whole blood samples were collected on day 1: before injection, immediately after the injection start, at the end of the injection, 5, 10, 15, 60, 120, and 240 minutes after completion of the injection; and on day 2. Three additional blood samples were collected during radiotherapy, between the eleventh and twentieth radiotherapy sessions. One blood sample was also collected prior to surgery. Urine samples were collected after the NBTXR3 injection (day 1) and before surgery.

EBRT and surgery

Conformal 3D radiotherapy was initiated on day 2 (24 hours postinjection). A dose of 50 Gy (in 25 daily fractions of 2 Gy over a 5-week period) was delivered to the PTV, following the recommendations of the International Commission on Radiation Units and Measurements (ICRU reports 50 and 62; refs. 16, 17). The radiotherapy dose distribution within the PTV was ideally 95% to 107% of the prescribed dose; the ICRU reference point was located at the center (or in a central part) of the PTV and positioned at the intersection of the treatment beam axes. Beam energies of 6 to 18 MV were used. Tumor resection was planned 5 to 7 weeks after the completion of radiotherapy according to surgical rules.

Skin access was defined by the surgeon, according to the planned surgical incision line, to resect the injection sites and tracts as well.

Safety

Clinical and laboratory safety parameters, and concomitant medications, were assessed at baseline, day 1 post-NBTXR3

injection, day 2 before the onset of radiotherapy, once a week during radiotherapy, at the preoperative visit, during the 14 days post-tumorectomy, and at follow-up visits every 8 weeks until the study cut-off date of February 26, 2015.

A serious AE (SAE) was defined as that resulting in death, was life threatening, required hospitalization or prolongation of existing hospitalization, resulted in persistent or significant disability or incapacity, or was a congenital abnormality or birth defect.

Efficacy

Dynamic contrast-enhanced ultrasonography was performed to examine treatment-related changes to the tumor. Tumor response was evaluated based on RECIST criteria v.1.1 (18) and tumor volume changes, both of which were measured by MRI during the week preceding surgery. Response evaluation was performed according to the European Organisation for Research and Treatment of Cancer (EORTC) Soft Tissue and Bone Sarcoma Group (STBSG) recommendations for pathologic examination and reporting (19).

Antitumor efficacy was evaluated in terms of pathologic response (pR), and tumor size and volume, according to RECIST criteria, where pR was expressed as the percentage of residual malignant viable cells, and pathologic complete response (pCR) was defined as less than 5% of residual malignant viable cells. Complete response (CR) was defined as the disappearance of all target lesions; any pathologic lymph nodes (whether target or nontarget) were required to have reductions in their short axis to less than 10 mm. Partial response (PR) was defined as at least a 30% decrease in the sum of target lesion diameters, using the sum of the baseline diameters as reference. Progressive disease (PD) was at least a 20% increase in the sum of target lesion diameters, taking the smallest sum measured during the study as the reference (including the baseline sum if it was the smallest); the sum also had to be an absolute increase of at least 5 mm. Stable disease (SD) was defined as neither sufficient shrinkage to qualify for PR, nor sufficient increase to qualify for PD, taking the smallest sum diameters measured during the study as reference. Margin status was also assessed.

Statistical analysis

All analyses were descriptive. A preliminary efficacy analysis was performed in the "All treated population" (i.e., patients who received a single injection of NBTXR3, even if the full dose was not administered). Continuous data were summarized for each initial planned level and cohort, using the number of nonmissing observations. Qualitative data were summarized using the number and frequency of nonmissing observations. All analyses were performed using SAS version 9.2.

Results

Patient characteristics

Patient characteristics are summarized in Table 1. Twenty-two patients were enrolled and treated as follows: 6 patients at level 1 (2.5%) and 6 patients at level 2 (5%). Three patients were initially assigned to level 3 (10%), with no early DLTs. Two patients were assigned level 4 (20%); one of these patients experienced two grade 3 AEs related to NBTXR3 and radiotherapy (injection-site pain during the injection procedure and postoperative wound complication), which were considered to be early DLTs. As a result, no more patients were assigned to level 4. Therefore, 5 additional patients were assigned to level 3 for further safety exploration.

Treatments

All 22 patients received a single injection of NBTXR3, completed EBRT, and underwent tumorectomy. The tumor volume change between baseline and day 1 postinjection of NBTXR3 was minimal; nanoparticles were retained in the tumor, while the aqueous solution that the nanoparticles were suspended in showed rapid clearance. Table 2 shows the NBTXR3 intratumoral injection characteristics. The duration of the injection procedure

Table 1. Baseline patient demographics and disease characteristics

	Level (% of tumor volume)				
	1 (2.5%) ***n*** **= 6**	**2 (5%)** ***n*** **= 6**	**3 (10%)** ***n*** **= 8**	**4 (20%)** ***n*** **= 2**	**Total** ***n*** **= 22**
Female/male, *n* (%)	3 (50)/3 (50)	3 (50)/3 (50)	3 (37.5)/5 (62.5)	2 (100)/0 (0)	11 (50)/11 (50)
Median age, years (range)	48.5 (42–78)	46.0 (31–82)	54.5 (28–57)	66.0 (65–67)	53.5 (28–82)
WHO performance status 0/1, *n* (%)	5 (83.3)/1 (16.7)	5 (83.3)/1 (16.7)	6 (75.0)/2 (25.0)	2 (100)/0	18 (82)/4 (18)
Tumor localization, *n* (%)					
Limb	6 (100)	6 (100)	7 (87.5)	2 (100)	21 (95.5)
Trunk wall	0	0	1 (12.5)	0	1 (4.5)
FNCLCC tumor grade classification, *n* (%)					
Grade 1	1 (16.7)	4 (66.7)	2 (25.0)	0	7 (31.8)
Grade 2	3 (50.0)	2 (33.3)	4 (50.0)	1 (50.0)	10 (45.5)
Grade 3	2 (33.3)	0	1 (12.5)	1 (50.0)	4 (18.1)
Unknown	0	0	1 (12.5)	0	1 (4.5)
Histology subtype, *n* (%)					
Myxoid liposarcoma					5 (22.7)
Undifferentiated pleomorphic sarcoma					4 (18.5)
Well-differentiated liposarcoma					3 (13.6)
Fibromyxoid sarcoma					2 (9.1)
Synovial sarcoma					2 (9.1)
Myxoid chondrosarcoma					2 (9.1)
Dedifferentiated liposarcoma					1 (4.5)
Pleomorphic rhabdomyosarcoma					1 (4.5)
Clear cell sarcoma					1 (4.5)
Leiomyosarcoma					1 (4.5)

Abbreviation: FNCLCC, Federation Nationale de Centres de Lutte Contre le Cancer.

Table 2. NBTXR3 (53.3 g/L) intratumoral injection characteristics; values shown are median (range)

	Dose level (% of tumor volume)			
	1 (2.5%) $n = 6$	2 (5%) $n = 6$	3 (10%) $n = 8$	4 (20%) $n = 2$
Tumor volume, mL	185 (55–1,814)	567 (85–3,682)	305 (130–1,001)	725 (490–960)
Volume of NBTXR3 injected, mL	5 (1–45)	27 (4–184)	30 (13–101)	138 (84–192)
Number of punctures	4 (2–10)	6 (2–11)	8 (5–33)	13 (12–13)
Duration of injection procedure, min	5 (2–15)	6 (2–16)	11 (6–55)	34 (19–48)

was dependent upon the number of punctures, with a median time of 8.5 minutes (range, 2–55 minutes).

Local nanoparticle dispersion and evaluation of NBTXR3 potential leakage

Tumor CT scans showed appropriate diffusion of NBTXR3 throughout the tumor in different sizes and histology types, without leakage into the surrounding healthy tissues, as well as persistence of NBTXR3 during the entire duration of radiotherapy (Fig. 1B and C). Chest CT scans showed no presence of NBTXR3 in the lungs. The maximum concentration of hafnium (Hf_{max}) in whole blood is shown in Fig. 2 (and in Supplementary Table S1). In most patients, Hf_{max} was observed at the end of the NBTXR3 injection. No hafnium was found in urine, which confirmed that NBTXR3 was not excreted renally.

Safety: AEs, early DLTs, and RD

All AEs and laboratory test abnormalities are presented in Table 3. The worst (grade 3) biological or hematologic abnormalities were reversible in all cases: one case each of elevated alkaline phosphatase and alanine aminotransferase levels at level 3, one case of lymphopenia at level 1, and one case of anemia at level 2. Biochemical and hematologic changes were not considered clinically significant.

The most frequently occurring AEs related to radiotherapy were erythema and radiation skin injury (grade 1–2), with three occurrences each (Table 3). AEs related to both NBTXR3 and radiotherapy were grade 1 injection-site reaction, grade 1 pyrexia, and grade 3 postoperative wound complication, with one occurrence of each. All patients received the planned volume of NBTXR3 except two patients: one patient experienced a vasovagal reaction during the injection, which led to injection interruption (42.5 mL instead of 52.5 mL). Grade 3 injection-site pain in the second patient led to a dose reduction of NBTXR3 (84.5 mL instead of 98 mL).

A total of 16 SAEs in 11 patients were observed: 5 patients at level 1, 2 patients at level 2, 2 patients at level 3, and 2 patients at level 4. Eight SAEs were grade 3, four were grade 2, and four were grade 1 (Table 4). During the posttumorectomy follow-up period, no postoperative wound dehiscence or local infection was observed.

No early DLTs were observed at levels 1 to 3. At level 4, one patient experienced two AEs that were considered to be early DLTs: grade 3 injection-site pain relating to high NBTXR3 volume occurred during NBTXR3 injection, and a grade 3 postoperative wound complication (postsurgical scar necrosis related to severe local inflammation), which required remedial surgery with a skin flap. Level 4 was therefore considered to be nonfeasible due to the high volume of NBTXR3 injected; enrollment at this level was stopped, and level 4 was defined as the MTD. Consequently, 5 additional patients were assigned to level 3 (10%), with no occurrence of early DLTs. Hence, NBTXR3 53.3 g/L, at a volume equivalent to 10% of the calculated baseline tumor volume, was defined as the RD for further development.

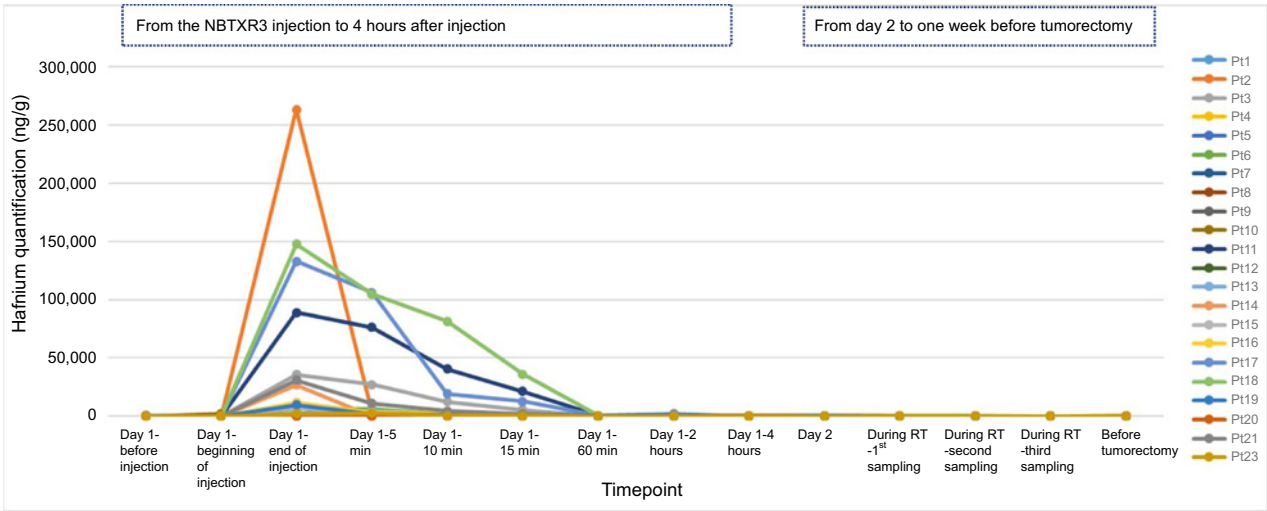

Figure 2.
Whole blood hafnium concentrations following NBTXR3 injection shown for each patient. The minutes for day 1 correspond to the time after completion of the injection procedure. Pt, patient; RT, radiotherapy.

Table 3. Treatment-emergent AEs and clinical laboratory evaluations by NBTR3 (53.3 g/L)

	Dose level (% of tumor volume)			
	1 (2.5%) ***n*** **= 6**	**2 (5%)** ***n*** **= 6**	**3 (10%)** ***n*** **= 8**	**4 (20%)** ***n*** **= 2**
Grade 1/2/3 AEs related to NBTXR3, *n*				
Injection-site pain	0	2/0/0	0/1/0	0/0/1
Pyrexia	1/0/0	1/0/0	0	0
Abdominal pain	0/1/0	0	0	0
Headache	0	0/1/0	0	0
Hypotension	1/0/0	0	0	0
Injection-site reaction	1/0/0	0	0	0
Paresthesia	1/0/0	0	0	0
Peripheral edema	0	0/1/0	0	0
Postoperative wound complication	0	0	0	0/0/1
Total grade ≥3 AEs	0	0	0	2
Grade 1/2/3 AEs related to radiotherapy, *n*				
Erythema	1/1/0	2/0/0	1/0/0	0
Radiation skin injury	0	2/1/0	1/0/0	1/0/0
Asthenia	0	2/0/0	2/0/0	0
Pain in extremity	0/1/0	0/1/0	0	0
Postoperative wound complication	1/0/0	0	0	0/0/1
Dysesthesia	0	0	1/0/0	0
Injection-site reaction	1/0/0	0	0	0
Joint range of motion decreased	0	0/1/0	0	0
Neuralgia	0/1/0	0	0	0
Peripheral edema	0/1/0	0/1/0	0	0
Pyrexia	1/0/0	0	0	0
Wound secretion	0	1/0/0	0	0
Total grade ≥3 AEs	0	0	0	1
Grade 2/3 clinical laboratory evaluations, *n*				
Anemia	1/0	1/1	2/0	1/0
Lymphocytes decreased	2/1	2/0	0	1/0
ALAT increased	0	1/0	0/1	0
Total bilirubin increased	0	0	2/0	0
Albumin decreased	0	1/0	0	0
Alkaline phosphatase increased	0	0	0/1	0
ASAT increased	0	0	1/0	0
Blood creatinine increased	0	0	1/0	0
White blood cell decreased	0	1/0	0	0
Total grade ≥3 AEs	1	1	2	0

NOTE: AEs graded according to NCI-CTCAE version 4; no grade 4 or 5 AEs were reported.
Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events.

Efficacy

Twenty-two patients were evaluated for pR (Fig. 3C), and 21 patients were evaluated for changes in tumor diameter and volume (Fig. 3A and B); one patient at level 3 was considered nonevaluable according to RECIST criteria due to an inconsistency in their MRI evaluation. Five patients achieved a PR: one at level 2, three at level 3 (the RD), and one at level 4. Of the patients achieving PR, three had undifferentiated pleomorphic sarcoma, one had myxoid liposarcoma, and one had myxoid chondrosarcoma. Fifteen patients had SD, and one patient had PD. Overall, 5 of the 22 patients had 10% or less residual malignant cells posttreatment. Both patients at level 4 achieved pCR.

At the RD (level 3), the median percentage of residual malignant viable cells was 26% (range, 10%–90%), and 3 of the 7 evaluable patients (43%) achieved PR with a median maximal tumor diameter change of −29% (range, −34% to +32%) and a median tumor volume change of −40% (−71% to +22%). The median minimal margin was 1 mm (range, 0–4 mm). With a median follow-up of 22 months (range, 6–40), no patient experienced a local recurrence (LR), and 5 patients exhibited a distant recurrence, which included nodules in the right and left lungs (in a patient who was not treated for a primary lesion, but for a local relapse), bone metastasis in the vertebrae, a paracardiac nodule, muscular lesions in the left psoas, and a bone nodule.

There was no tumor size effect for the distribution of particles, as the NBTXR3 quantity to be implanted was a percentage of the tumor volume at fixed concentration.

Discussion

This phase 1 study is the first human trial to report on a new concept of radioenhancement with functionalized hafnium oxide nanoparticles activated by fractionated radiotherapy. It showed that preoperative NBTXR3 with radiotherapy is a feasible therapeutic approach that yields encouraging radiological and pathologic responses in patients with locally advanced STS of the extremity and trunk wall. At a concentration of 53.3 g/L, the RD for further development of NBTXR3 is equivalent to 10% of the tumor volume, as measured by MRI at baseline.

The worst (grade 3) abnormalities in biology or hematology were reversible in all cases. At level 4 (20%), one patient experienced two grade 3 AEs: injection-site pain and postoperative wound complication that required a flap. This AE may be due to radiotherapy itself or to the association (4). Hence,

Table 4. SAEs - All treated population

Level	System Organ Class/ Preferred term	NCI-CTCAE grade	Serious	Duration[a] (days)	Causality	Radiation dose received at onset of the SAE (Gy)	Total radiation dose received (Gy)	Action taken regarding NBTXR3	Action taken regarding radiotherapy	Period
Level 1	Abdominal pain	2	Y	2	NBTXR3	36	50	Not applicable	None	On treatment
	Postoperative wound infection	2	Y	11	Radiotherapy & other: surgery	50	50	Not applicable	None	Posttreatment
	Postoperative wound infection	3	Y	7	Radiotherapy & other: surgery	50	50	Not applicable	None	Posttreatment
	Postoperative wound complication	2	Y	15	Radiotherapy	50	50	Not applicable	None	Posttreatment
	Postoperative wound complication	3	Y	1	Other: surgery	50	50	Not applicable	None	On treatment
	Injection-site reaction	1	Y	2	NBTXR3 & radiotherapy	4	50	Not applicable	None	On treatment
	Spinal cord compression	3	Y	30	Disease related	50	50	Not applicable	None	Posttreatment
Level 2	Traumatic hematoma	1	Y	22	Other: falling	50	50	Not applicable	None	Posttreatment
	Hematoma infection	2	Y	9	Other: falling	50	50	Not applicable	None	Posttreatment
	Pyrexia	1	Y	3	NBTXR3	2	50	Not applicable	None	On treatment
Level 3	Presyncope	3	Y	1	Other: morphine related	0	50	Not applicable	None	On treatment
	Postoperative wound infection	3	Y	13	Other: postsurgery	50	50	Not applicable	None	On treatment
	Pyrexia	1	Y	3	Other: postsurgery	50	50	Not applicable	None	On treatment
Level 4	Injection-site pain	3	Y	3	Procedure implantation injection	0	50	Dose reduced	None	On treatment
	Hypoesthesia	3	Y		Procedure implantation injection & other: volume of injection	12	50	Not applicable	None	On treatment
	Postoperative wound complication	3	Y	16	NBTXR3 & radiotherapy	50	50	Not applicable	None	On treatment

Abbreviation: Y, yes.

[a]Duration of SAE = [(stop date − start date) + 1].

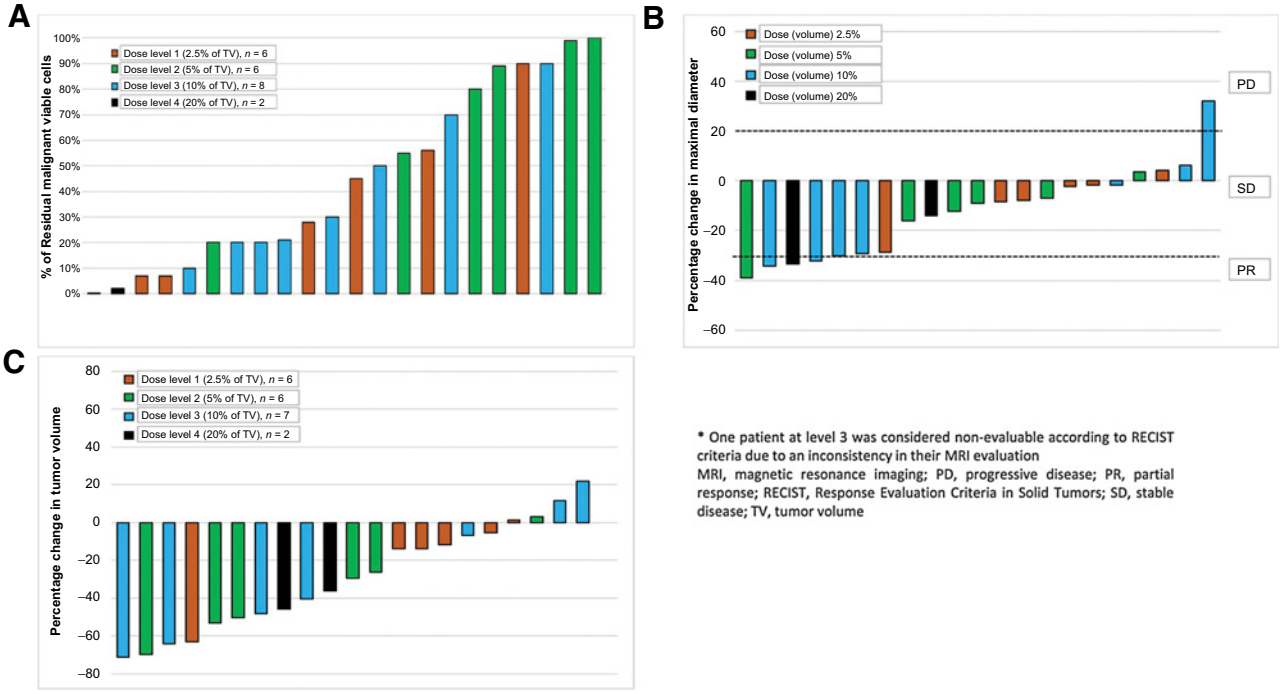

Figure 3.

Changes to the tumor for each patient following injection of NBTRX3 from baseline, measured in the week preceding tumorectomy. **A,** residual viable malignant cells ($n = 22$). **B,** percentage change in MTD ($n = 21^*$), with pathologic response thresholds (according to RECIST v1.1) indicated by the dotted lines. **C,** percentage change in tumor volume ($n = 21^*$). Tumor dimensions were measured using MRI.

this level was considered to be nonfeasible in this cancer population.

Concerning the intratumoral injection, its main practical parameter is the injection technique, which should apparently fulfil opposing goals: optimal dispersion within the tumor, no risk for tumor cell seeding through needle pathways, and minimum discomfort for the patient. Ultrasound images during injection showed that the NBTXR3 was diffused in the tumor volume, meaning that the positioning of the needle inside the tumor is not fundamental. According to the authors who are radiologists (T. De Baere and X. Buy), the learning curve of the technique is fast and reproducible. The injection quality was demonstrated by the optimal intratumoral localization of NBTXR3 and the absence of leakage to healthy tissues.

Nanoparticles have localized action (less than 10 μm), which provides a good safety basis, but they are not designed to be immovable in tumors with changing shapes during radiotherapy, and they could affect the whole tumor volume. No patient required radiotherapy replanning due to changes. The mainly local toxicity profile significantly differentiates this radioenhancer from radiosensitizers that have their own cytotoxicity and lead to various systemic side effects.

The ultimate objective, which is being evaluated in a current phase II/III trial in STS (ClinicalTrials.gov; NCT02379845), is to increase the efficiency of an already validated treatment with an additional physical mechanism and to demonstrate large volume feasibility. With multimodal treatments, LR rates have been decreasing over time, dropping from more than 20% (20) to approximately 10% in modern series (21). The current focus is devoted to further extend this progress to advanced localized diseases through the reduction of tumor volume. When radiotherapy is indicated (22, 23), its preoperative use achieves lower long-term morbidity, but it will not extend the possibilities of surgery because the median change in maximal tumor diameter (MTDia) is moderate in the majority of cases (24–26), and the median change in volume in high-grade tumors is nearly null (24). In the current study, the median change in MTDia at the RD was −29%, with a median decrease in volume of −40%. This favorable tumor shrinkage could promote better margins in locally advanced sarcomas because they are closely related to tumor volume, which could translate into more functional surgery.

In other cancers (27), pCR could be a surrogate marker of efficacy and possibly of survival (28). In STS, the prognostic impact of histologic response to chemotherapy is less clear, with contradictive results, and it needs further evaluation (29–31). A caveat in STS is that necrosis is only one type of treatment-related tumor change. Moreover, posttreatment necrosis cannot be reliably distinguished from preexisting necrosis. In this context, an effort was recently made by the STBSG team (from EORTC) to harmonize the interpretation of pathologic responses (19). At the RD (10%), the median percentage of residual malignant cells was 26%.

The choice of limb sarcoma in this first phase I study was selected because it is an easily accessible tumor to evaluate the feasibility and safety of this product and the potential improvement of radiotherapy efficacy. However, further development

includes tumors where radiotherapy is (or could be) the main treatment option. With these promising results, this new treatment strategy opens a new therapeutic landscape of radioenhancement for solid tumors. This study provides the basis for the current development of NBTXR3 in the phase II/III trial in STS and in other phase I head and neck cancer (NCT01946867), liver cancer (NCT02721056), and rectal cancer (NCT02465593) trials.

Disclosure of Potential Conflicts of Interest

S. Bonvalot reports receiving speakers bureau honoraria and commercial research grants from Nanobiotix. E. Borghi has ownership interests (including patents) at Nanobiotix. L. Levy has ownership interests (including patents) at and is a consultant/advisory board member for Nanobiotix. E. Deutsch reports receiving commercial research support from Nanobiotix. J.C. Soria is a consultant/advisory board member for Nanobiotix. No potential conflicts of interest were disclosed by the other authors.

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