# 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 2–3, randomised, controlled trial

*Sylvie Bonvalot, Piotr L Rutkowski, Juliette Thariat, Sébastien Carrère, Anne Ducassou, Marie-Pierre Sunyach, Peter Agoston, Angela Hong, Augustin Mervoyer, Marco Rastrelli, Victor Moreno, Rubi K Li, Béatrice Tiangco, Antonio Casado Herraez, Alessandro Gronchi, László Mangel, Teresa Sy-Ortin, Peter Hohenberger, Thierry de Baère, Axel Le Cesne, Sylvie Helfre, Esma Saada-Bouzid, Aneta Borkowska, Rodica Anghel, Ann Co, Michael Gebhart, Guy Kantor, Angel Montero, Herbert H Loong, Ramona Vergés, Lore Lapeire, Sorin Dema, Gabriel Kacso, Lyn Austen, Laurence Moureau-Zabotto, Vincent Servois, Eva Wardelmann, Philippe Terrier, Alexander J Lazar, Judith V M G Bovée, Cécile Le Péchoux, Zsusanna Papai*

*Lancet Oncol* 2019; 20: 1148–59

Published Online July 8, 2019 http://dx.doi.org/10.1016/S1470-2045(19)30326-2

This online publication has been corrected. The corrected version first appeared at thelancet.com/oncology on September 2, 2019

See Comment page 1046

Department of Surgery (S Bonvalot PhD), Department of Radiation Therapy (S Helfre MD), and Department of Radiology (V Servois MD), Institut Curie, PSL Research University, Paris, France; Department of Soft Tissue/Bone Sarcoma and Melanoma (Prof P L Rutkowski MD) and Department of Radiotherapy (A Borkowska MD), Maria Sklodowska-Curie Institute—Oncology Center, Institute of Oncology, Warsaw, Poland; Department of Radiation Oncology, Centre François Baclesse, Caen, France (Prof J Thariat MD); Department of Radiation Oncology, Centre Lacassagne, Nice, France (Prof J Thariat); Department of Surgical Oncology, Centre Regional De Lutte Contre Le Cancer Paul Lamarque, Montpellier, France (S Carrère MD); Department of Radiation Oncology, Institut Claudius Regaud (ICR), Institut Universitaire du Cancer de Toulouse-Oncopole (IUCT-O), Toulouse, France (A Ducassou MD); Department of Radiotherapy, Léon Bérard

## Summary

**Background** Pathological complete response to preoperative treatment in adults with soft-tissue sarcoma can be achieved in only a few patients receiving radiotherapy. This phase 2–3 trial evaluated the safety and efficacy of the hafnium oxide ($HfO_2$) nanoparticle NBTXR3 activated by radiotherapy versus radiotherapy alone as a pre-operative treatment in patients with locally advanced soft-tissue sarcoma.

**Methods** Act.In.Sarc is a phase 2–3 randomised, multicentre, international trial. Adults (aged ≥18 years) with locally advanced soft-tissue sarcoma of the extremity or trunk wall, of any histological grade, and requiring preoperative radiotherapy were included. Patients had to have a WHO performance status of 0–2 and a life expectancy of at least 6 months. Patients were randomly assigned (1:1) by an interactive web response system to receive either NBTXR3 (volume corresponding to 10% of baseline tumour volume at a fixed concentration of 53·3 g/L) as a single intratumoural administration before preoperative external-beam radiotherapy (50 Gy in 25 fractions) or radiotherapy alone, followed by surgery. Randomisation was stratified by histological subtype (myxoid liposarcoma *vs* others). This was an open-label study. The primary endpoint was the proportion of patients with a pathological complete response, assessed by a central pathology review board following European Organisation for Research and Treatment of Cancer guidelines in the intention-to-treat population full analysis set. Safety analyses were done in all patients who received at least one puncture and injection of NBTXR3 or at least one dose of radiotherapy. This study is registered with ClinicalTrials.gov, number NCT02379845, and is ongoing for long-term follow-up, but recruitment is complete.

**Findings** Between March 3, 2015, and Nov 21, 2017, 180 eligible patients were enrolled and randomly assigned and 179 started treatment: 89 in the NBTXR3 plus radiotherapy group and 90 in the radiotherapy alone group.Two patients in the NBTXR3 group and one patient in the radiotherapy group were excluded from the efficacy analysis because they were subsequently discovered to be ineligible; thus, a total of 176 patients were analysed for the primary endpoint in the intention-to-treat full analysis set (87 in the NBTXR3 group and 89 in the radiotherapy alone group). A pathological complete response was noted in 14 (16%) of 87 patients in the NBTXR3 group and seven (8%) of 89 in the radiotherapy alone group (p=0·044). In both treatment groups, the most common grade 3–4 treatment-emergent adverse event was postoperative wound complication (eight [9%] of 89 patients in the NBTXR3 group and eight [9%] of 90 in the radiotherapy alone group). The most common grade 3–4 adverse events related to NBTXR3 administration were injection site pain (four [4%] of 89) and hypotension (four [4%]) and the most common grade 3–4 radiotherapy-related adverse event was radiation skin injury in both groups (five [6%] of 89 in the NBTXR3 group and four [4%] of 90 in the radiotherapy alone group). The most common treatment-emergent grade 3–4 adverse event related to NBTXR3 was hypotension (six [7%] of 89 patients). Serious adverse events were observed in 35 (39%) of 89 patients in the NBTXR3 group and 27 (30%) of 90 patients in the radiotherapy alone group. No treatment-related deaths occurred.

**Interpretation** This trial validates the mode of action of this new class of radioenhancer, which potentially opens a large field of clinical applications in soft-tissue sarcoma and possibly other cancers.

**Funding** Nanobiotix SA and PharmaEngine, Inc.

## Research in context

### Evidence before this study

So far, most of the evidence for the use of nanoparticles to enhance the efficacy of radiotherapy comes from preclinical studies, with no randomised data available. For our search of the existing published literature, we considered all Medline-listed journal articles written in English from any year until Nov 23, 2018, reporting the use of nanoparticles with radiotherapy. Search terms included "nanoparticle" AND "radiotherapy" and yielded more than 1100 results. However, our previous phase 1 study, NBTXR3-101, constitutes the only trial so far of nanoparticles as radiation enhancers in humans. Soft-tissue sarcomas are good candidates to show the validity of the use of radiation-enhancing nanoparticles for the local treatment of solid tumours that are treated with radiotherapy. The use of radiotherapy in this setting is validated by two randomised trials in high-risk sarcoma, and a third trial has shown that preoperative and postoperative radiotherapy yielded similar results. However, a pathological complete response is rarely achieved in patients with soft-tissue sarcoma when radiotherapy is applied preoperatively. In our phase 1 study, we examined the safety of preoperative 50 nm crystalline hafnium oxide ($HfO_2$) nanoparticles (NBTXR3) plus radiotherapy in soft-tissue sarcoma and concluded that a single intratumoural administration of NBTXR3 at the recommended dose (10% of baseline tumour volume of a suspension at the fixed concentration of 53·3 g/L), before radiotherapy, was technically feasible with acceptable toxicity. At this dose, the median percentage of residual viable tumour cells was 26% (range 10–90%) and all patients had clear resection margins. The promising results of this study constituted the basis for the randomised phase 2–3 Act.In. Sarc trial.

### Added value of this study

To our knowledge, this study is the first large randomised trial evaluating the potential benefit of the intratumoural injection of radiation-enhancing nanoparticles. The results of this phase 2–3 trial comparing NBTXR3 activated by radiotherapy with radiotherapy alone in soft-tissue sarcomas of the extremity and trunk wall, as preoperative treatment, show that a significantly higher proportion of patients (twice as many) given NBTXR3 plus radiotherapy achieved a pathological complete response compared with patients who received radiotherapy alone. No additional or new radiation-related adverse events were identified in the NBTXR3 group compared with the control group, and the safety profile was manageable with acute and transient immune reactions attributed to NBTXR3.

### Implications of all the available evidence

This study validates the mode of action of NBTXR3 since an increased proportion of patients with a pathological complete response—indicating increased tumour cell death, through increased production of free radicals—is a direct sign of efficacy. These results open the possibility of using this treatment method in other soft-tissue sarcoma settings and other cancers where radiotherapy is used, when surgery might be unfeasible, or as a basis for evaluating radiotherapy dose reduction.

## Introduction

The use of a new class of radiation-enhancing nanoparticles could be a breakthrough approach for the local treatment of solid tumours that are treated with radiotherapy. The radiobiological effects of ionising radiation used in cancer treatment are dependent on the excitation and ionisation of atoms and molecules of the irradiated tissue, and the occurrence of these effects increases with the atomic number ($Z$) of the target. Consequently, accumulation of high-$Z$ atoms (such as hafnium oxide [$HfO_2$], $Z$=72) within the target tissue, acting as radioenhancers, amplifies energy deposit and radiobiological effects, leading to direct or indirect DNA damage and cell death. When injected directly into the tumour and exposed to ionising radiation (on–off activity) nanoparticles can augment cell damage applied to the tumour only, without adding toxicity to adjacent normal tissue.[1]

Soft-tissue sarcoma is a good candidate tumour in which to assess the validity of this new therapeutic approach. Although surgery done at specialised centres is the mainstay of treatment,[2] for most patients preoperative or postoperative radiotherapy is needed to maximise local tumour control[3,4] and is part of the standard of care in high-risk soft-tissue sarcomas of the extremities, as validated in two randomised trials.[5,6] In some clinical situations, patients might be treated with radiotherapy alone with the aim of limb sparing.[7] However, pathological complete response, which might also be prognostic in soft-tissue sarcoma for better outcomes,[8] is rarely achieved when radiotherapy is delivered preoperatively.[9–11]

Although preoperative radiotherapy has been associated with an increase in surgery-related wound complications compared with postoperative radiotherapy, the risk of long-term morbidity is lower in the preoperative than in the postoperative setting,[12] explaining its increased use.[13] Various neoadjuvant combined modality treatments have been evaluated and have shown encouraging efficacy, but they are hampered by substantial systemic toxicity (eg, Chemoradiation therapy leading to acute toxicity (haematological toxicity, etc).[14,15] NBTXR3 is a first-in-class 50 nm nanoparticle composed of crystalline hafnium oxide ($HfO_2$) functionalised by a negatively charged phosphate coating.[16] These physicochemical properties are fundamental to its intratumour bioavailability and persistence in cancer cells.[16] $HfO_2$ nanoparticles were chosen for clinical development because of their excellent

**Cancer Center, Lyon, France** (M-P Sunyach MD); **Department of Radiation Oncology, Országos Onkologiai Intézet, Budapest, Hungary** (P Agoston MD); **Department of Radiation Oncology, Chris O'Brien Lifehouse and The University of Sydney, Camperdown, NSW, Australia** (A Hong MBBS); **Department of Radiation Oncology, Institut de Cancerologie de l'Ouest- Rene Gaudducheau, Saint-Herblain, France** (A Mervoyer MD); **Department of Surgical Oncology, Istituto Oncologico Veneto IRCCS, Padova, Italy** (M Rastrelli MD); **Department of Medical Oncology, Hospital Fundación Jimenez Diaz, Madrid, Spain** (V Moreno MD); **Department of Medical Oncology, St Luke's Medical Center, Quezon City, Philippines** (R K Li MD); **Department of Medical Oncology, The Medical City Cancer Center, Pasay City, Philippines** (B Tiangco MD); **Department of Medical Oncology, Hospital Clinico Universitario San Carlos, Madrid, Spain** (A C Herraez MD); **Department of Surgery, Fondazione IRCCS Istituto Nazionale Dei Tumori, Milan, Italy** (A Gronchi MD); **Department of Oncotherapy, University of Pecs, Pecs, Hungary** (L Mangel MD); **Department of Radiation Oncology, Benavides Cancer Institute, University of Santo Tomas Hospital, Manila, Philippines** (T Sy-Ortin MD); **Department of Surgery, Division of Surgical Oncology and Thoracic Surgery, University Hospital Mannheim, University of Heidelberg, Mannheim, Germany** (Prof P Hohenberger MD); **Department of Interventional Radiology** (Prof T de Baère MD), **Department of Medical Oncology** (A Le Cesne MD), **and Department of Radiation Oncology** (C Le Péchoux MD), **Gustave Roussy—Cancer Campus, Villejuif, France; Department of Medical Oncology, Centre Anticancer Antoine Lacassagne, Nice, France** (E Saada-Bouzid MD); **Institutul Oncologic Bucuresti Prof Dr Alexandru Trestioreanu, Bucharest, Romania** (R Anghel MD); **Cebu Cancer Institute, Perpetual Succour Hospital, Cebu City, Philippines**

(A Co MD); **Department of Orthopedic Surgery, ULB, Institut Jules Bordet, Brussels, Belgium** (Prof M Gebhart MD); **Department of Radiotherapy, Institut Bergonié, Comprehensive Cancer Center, Bordeaux, France** (Prof G Kantor MD); **Radiation Oncology Department, Hospital HM Universitario Sanchinarro, Madrid, Spain** (A Montero MD); **Department of Clinical Oncology, Prince of Wales Hospital, Sha Tin, Hong Kong** (H H Loong MD); **Radiation Oncology Department, Vall d'Hebron University Hospital, Barcelona, Spain** (R Vergés MD); **Department of Medical Oncology, Ghent University Hospital, Gent, Belgium** (L Lapeire MD); **Municipal Emergency Hospital Timisoara, Timisoara, Romania** (S Dema MD); **Iuliu Hatieganu Medical University, RTC Amethyst, Cluj, Romania** (G Kacso MD); **Department of Radiation Oncology, Canberra Region Cancer Centre, Garran, Australia** (L Austen MD); **Department of Radiation-Oncology, Institut Paoli Calmettes, Marseille Cedex, France** (L Moureau-Zabotto MD); **Gerhard Domagk Institute of Pathology, University Hospital Münster, Münster, Germany** (Prof E Wardelmann MD); **Gustave Roussy, Cancer Campus, Paris-Sud University, Villejuif, France** (P Terrier MD); **Departments of Pathology & Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA** (A J Lazar MD); **Department of Pathology, Leiden University Medical Center, Leiden, Netherlands** (Prof J V M G Bovée MD); **and Medical Centre, Hungarian Defence Forces, Budapest, Hungary** (Prof Z Papai MD)

Correspondence to:
Dr Sylvie Bonvalot, Institut Curie, 75005 Paris, France
**sylvie.bonvalot@curie.fr**

ratio for x-ray absorption and acceptable safety.[16,17] Preclinical studies have shown that NBTXR3 has a physical mode of action that does not target specific biological pathways, and might provide an opportunity to improve patient outcomes in many types of cancer.[17,18] Once activated by ionising radiation, NBTXR3 administered intratumourally yields a cell-localised high energy deposit and increased cell death compared with the same dose of radiation alone, without adding toxicity to the surrounding tissues.[17] The first-in-human study of NBTXR3 (NCT01433068; n=22) showed that one intratumoural administration of NBTXR3 before external-beam radiotherapy could yield remarkable local tolerance, homogeneous dispersion in the tumour, no leakage, and promising signs of antitumour activity in terms of pathological responses.[19]

On the basis of these results, the phase 2–3 Act.In.Sarc trial was designed with the aim to compare NBTXR3 given at 10% of baseline tumour volume (at a fixed concentration of 53·3 g/L) plus external-beam radiotherapy versus external-beam radiotherapy alone in the preoperative treatment of adults with locally advanced soft-tissue sarcoma of the extremity or trunk wall.

## Methods

### Study design and participants

For this randomised, open-label, active-controlled, phase 2–3 trial, eligible patients were aged 18 years or older with documented locally advanced soft-tissue sarcoma of the extremity or trunk wall according to WHO classification[20] (primary tumour or relapsed tumour situated outside a previously irradiated area); a WHO performance score of 0–2; a life expectancy of at least 6 months; and were candidates for radiotherapy plus surgery as decided in a multidisciplinary tumour board.[3] All histological grades[21] of soft-tissue sarcoma were eligible. Additionally, patients had to have adequate bone marrow, renal, and hepatic function (white blood cell count ≥3×10⁹ cells/L, platelet count ≥75×10⁹/L, haemoglobin ≥8 g/dL, creatinine ≤1·5×upper limit of normal [ULN], aspartate aminotransferase ≤3·0×ULN, alanine aminotransferase ≤3·0×ULN, and bilirubin ≤1·5 mg/dL) and adequate pulmonary function. Female patients had to have a negative serum pregnancy test within 7 days of randomisation or be postmenopausal, surgically sterile, or using effective contraception as established by the investigator.

Patients with embryonal or alveolar rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, angiosarcoma, primitive neuroectodermal tumour, desmoid-type fibromatosis, or dermatofibrosarcoma protuberans were not eligible. Patients with soft-tissue sarcoma of the trunk wall localised in the anterior abdominal region and those with a tumour volume (longest dimensions: length×width×depth) larger than 3000 mL at baseline, evaluated by the central imaging review board, were also excluded (as of a protocol amendment on Oct 15, 2015, because the required volume of NBTXR3 was >300 mL, which had a high probability of unfeasibility of injection). Patients with metastatic disease, other concomitant cancer or history of cancer treated and controlled within the previous 3 years, planning to or receiving concurrent treatment with any other anticancer therapy at baseline, previous neoadjuvant chemotherapy given as upfront of the current treatment line, radiotherapy in a relapse site of soft-tissue sarcoma, or unable to comply with scheduled visits, treatment plans, laboratory tests, and other study procedures were excluded. Because of the innovative nature of NBTXR3 and the unknown potential effect on some systems, patients with active autoimmune diseases and haemolytic anaemia were also excluded.

Written informed consent was obtained from all patients before study entry. The study protocol was approved by the institutional review boards or ethics committees of all participating institutions, in the coordinator's centre it was approved by the Paris Ile-de-France VII Ethics Committee of Paris (CPP IDF VII). An independent data monitoring committee monitored the study and reviewed the primary efficacy endpoint and safety data at the interim stage of analysis. Amendments to the protocol, exclusion criteria, efficacy and safety follow-up procedures, and patient withdrawal information are provided in the protocol.

### Randomisation and masking

Participants were recruited by study investigators. Eligible patients were randomly assigned 1:1 to receive either preoperative NBTXR3 activated by external-beam radiotherapy or external-beam radiotherapy alone followed by surgical resection of the tumour. Patients were registered by an interactive web response system which assigned a unique identification number and randomly allocated patients to one of the treatment groups. Randomisation was stratified by histological type (myxoid liposarcoma *vs* others) and was done through a biased coin dynamic method to avoid extreme imbalance of treatment assignment within the histological stratum. Treatment assignment could not be masked owing to the radio-opaque nature of NBTXR3; thus, no equivalent implantation was done in the radiotherapy-alone group because blinding was not feasible.

### Procedures

The study design is shown in figure 1. Patients in the NBTXR3 group received NBTXR3 as a single intratumoural administration of a volume equivalent to 10% of baseline tumour volume, calculated by the central imaging review board as the product of the three longest dimensions of the tumour (length×width×depth) assessed by MRI up to 1 week before treatment. The image-guided injection procedure and evaluation of nanoparticle dispersion and stability were done as previously reported.[19] NBTXR3 (Nanobiotix SA, Paris, France) was supplied as a suspension of nanoparticles composed of $HfO_2$ crystallites

and phosphate groups in an aqueous medium at a concentration of 53·3 g/L.

In both groups, all patients received radiotherapy (intensity-modulated radiotherapy or 3D conformal radiotherapy according to radio-oncologist discretion) to a total dose of 50 Gy in 25 fractions of 2 Gy over 5 weeks (once daily on 5 days per week) as per standard-of-care recommendations for preoperative radiotherapy in soft-tissue sarcomas of the extremity and trunk wall.[3] Premedication with steroids was adopted as a protocol amendment as of June 2, 2016 to reduce the risk of acute immune reaction. In the NBTXR3 group, radiotherapy was started within 1–5 days post-NBTXR3 injection, in the control group, radiotherapy started within 7 days after randomisation. 4–8 weeks after completion of radiotherapy, all patients were planned for wide resection as recommended by guidelines.[3,4] NBTXR3 injection points were defined, according to the planned surgical incision line, to anticipate the resection of all NBTXR3 injection sites and tracts.

The evaluation of patients for pathological complete response was based on the most recent European Organization for Research and Treatment of Cancer (EORTC) recommendations for histological evaluation of response to preoperative treatment in soft-tissue sarcoma.[22] Tumour samples were anonymised for treatment allocation and microscopically analysed for pathological response by a central review board composed of four pathologists (EW, PT, AJL, and JVMGB) who were authors of the EORTC guidelines.[22] Each of the four pathologists analysed a subset of slides; each slide was analysed by only one pathologist.

Clinical and laboratory safety parameters were evaluated at all visits: during the 21-day screening period before the initiation of treatment, on day 1 (the day of NBTXR3 administration), during the 5 weeks of radiotherapy, and upon surgery. Furthermore, patients were assessed during the 15 days after surgery, at the end-of-treatment visit at week 12–13 (day 86–93), and during the subsequent 2-year follow-up period. MRI was done at screening, before surgery, and during the follow-up period every 3 months for the first year and then every 6 months thereafter. A CT scan of the thorax, abdomen, and pelvis was done at screening, and during the follow-up period every 3 months for the first year and then every 6 months thereafter. A tumour CT scan was done on day 1 (the day of NBTXR3 injection) and within 1 week before surgery for NBTXR3 visualisation.

Patients were to be withdrawn from the study treatment if in the investigator's opinion, continuation of the study treatment would be detrimental to the patient's wellbeing, at the specific request of the sponsor, or in the case of pregnancy, locoregional progressive disease, or unacceptable toxicity. In case of treatment discontinuation, follow-up was to be completed unless the patient withdrew consent. Radiotherapy was to be delayed or interrupted if any adverse events occurred. Radiotherapy did not begin or resume until toxicity resolved to grade 1, as per National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

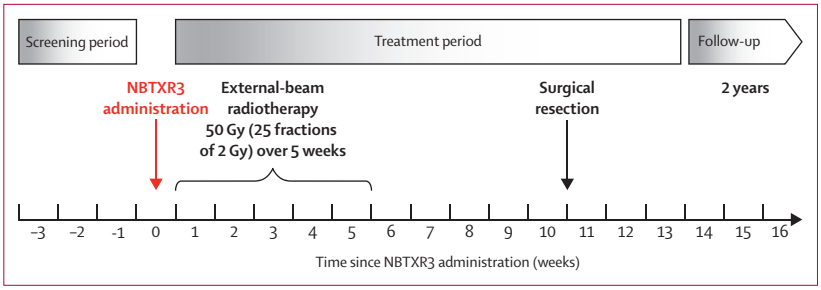


*Figure 1:* **Study design**

The study cutoff date was defined as the date when primary and secondary endpoint assessments and a follow-up period of 2 years have been achieved for all patients.

## Outcomes

The primary endpoint was the proportion of patients achieving a pathological complete response, defined as the presence of less than 5% residual malignant viable cells and assessed as described by Wardelmann and colleagues.[22] A prespecified exploratory analysis of pathological complete response response with a cutoff of 0% stainable cells and the proportion of tumour necrosis or fibrosis was also done, as described by Schaefer and colleagues.[23]

Secondary endpoints were the proportion of patients achieving an objective response (complete or partial response according to Response Evaluation Criteria in Solid Tumors version 1.1 [RECIST 1.1]); tumour volume changes; the histological appearance of the tumour in terms of hyalinisation, fibrosis, necrosis, and tumour infarction; the proportion of patients with R0 resection (the resection margin was judged to be negative if no ink was noted on the margins); the proportion of patients requiring limb amputation; and safety. Of the secondary endpoints, only hyalinisation is not presented in this article. Quality of resection was assessed according to the R classification.[24] Long-term efficacy analyses (prespecified), including the proportion of patients with local recurrence and time to distant recurrence at 12 and 24 months, will be evaluated and reported when follow-up is complete.

Safety was assessed as the incidence of early and late treatment-emergent adverse events, and laboratory abnormalities according to CTCAE version 4.0.[25] Because NBTXR3 increases the deposited energy dose within the tumour with radiation, the parameters of the radiotherapy schedule and its relative dose intensity were evaluated as the main indirect parameters of radiotherapy toxicity.

## Statistical analysis

The primary efficacy hypothesis was that NBTXR3 activated by radiotherapy would be superior to radiotherapy

alone for the endpoint of pathological complete response. A total of 180 patients were planned to be enrolled and randomly assigned to ensure a sample size of 156 evaluable patients (78 per treatment group). This number was calculated to detect a significant improvement in pathological complete responses between the control and investigational groups (ie, from 5% to 17·5%), with 80% power for a one-sided test at the 5% level and assuming a 15% screening failure prevalence. This calculation, which was based on a group sequential approach, included a planned interim analysis (first stage) that was done when two-thirds of patients (52 per group) had undergone tumour resection and for whom pathological response data were available. This interim analysis of pathological complete response was planned to evaluate early the efficacy of NBTXR3 activated by radiotherapy versus radiotherapy alone. Calculations were based on an O'Brien-Fleming α-spending function for efficacy boundaries in a group sequential design. No futility analysis was planned. The interim analysis review was done under the supervision of an independent data monitoring committee on March 22, 2019, which recommended continuation of the study. The final statistical analysis (second stage) comparing the proportion of patients with a pathological complete response between groups was done when all treated patients had completed their treatment period and considered the effect of the interim analysis on the overall α value.

The primary and key secondary efficacy endpoints (pathological complete response and R0 resection margin) were assessed in both the intention-to-treat full analysis set and in the evaluable patient population.[27] The intention-to-treat full analysis set population describes an analysis set which is as complete as possible, while also remaining as close as possible to the intention-to-treat ideals. Specifically, it includes all patients who signed informed consent, underwent randomisation, received treatment, had data post-randomisation, and did not have a major eligibility violation or randomisation issue. Major deviations from the protocol for the primary endpoint were absence of surgery and absence of centralised evaluation of pathological response. Minor deviations included absence of haematology or biochemistry evaluations at some visits, but this did not preclude evaluation for the primary endpoint. Patients without a pathological evaluation, whatever the reason, were considered non-responders and were included in the denominator when calculating the proportion of patients with a complete response.

The evaluable patient population for efficacy included all patients who received at least 80% of the intended volume of NBTXR3 dose or at least 44 Gy of radiotherapy, (or both in the combined treatment group), and for whom efficacy response data (either pathological response or resection margin data) could be obtained upon surgical resection. Patients who did not have surgery or had limb amputations were not included in the analysis of R0 resection margins in the evaluable population. Safety outcomes were assessed in the all-treated patient population, which included all randomly assigned patients who received any amount of investigational agent or at least one fraction of radiotherapy.

The proportion of patients with a pathological complete response, the proportion of patients with R0 resection margins, histological appearance in terms of tumour necrosis and percentage of tumor fibrosis or necrosis were compared between treatment groups with a two-sample Z test. The proportion of patients achieving an overall response as per RECIST 1.1 was compared between treatment groups with a one-sided Cochran–Mantel–Haenszel test adjusted for histological subtype. Tumour volume change at surgical visit (before tumourectomy) was analysed using a Wilcoxon rank sum test. A Hodges-Lehman estimate of the shift between the two treatment groups was obtained with its 95% CI according to Moses.[27,28] No comparison was performed between treatment groups for limb amputation.

No adjustment for covariates was planned for the primary endpoint analysis. Mean, standard deviation, median, and range were used to describe quantitative data, and for qualitative data, number of observations and frequency were used. SAS version 9.4 was used for all statistical analyses.

This study is registered with ClinicalTrials.gov, number NCT02379845.

### Role of the funding source

The funders of the study had a role in study design, data collection, data analysis, and data interpretation, and writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

## Results

Between March 3, 2015, and Nov 21, 2017, 223 patients with locally advanced soft-tissue sarcoma of the extremity or trunk wall were screened from 32 sites (sarcoma referral centre or national cancer centres) in 11 countries in Europe and Asia-Pacific. After exclusion of 43 patients, 180 eligible patients were enrolled and randomly assigned to receive NBTXR3 activated by external-beam radiotherapy (NBTXR3 group; n=90) or external-beam radiotherapy alone (radiotherapy alone group; n=90). One patient in the NBTXR3 group was not treated as ultimately the patient did not fulfil the inclusion criteria; thus, 179 patients actually started treatment (89 in the NBTXR3 group and 90 in the radiotherapy alone group). After randomisation, one patient in the NBTXR3 group was found to have a non-Hodgkin's lymphoma and was excluded from the efficacy analysis. Two other patients were excluded from the efficacy analysis as histological analysis of tumour specimen following tumourectomy demonstrated that it was not a sarcoma: one patient with myxoma (benign tumour) in the

NBTXR3 group and one patient with melanoma in the radiotherapy alone group (appendix p 3). Therefore, a total of 176 patients were analysed for the primary endpoint in the intention-to-treat full analysis set (87 in the NBTXR3 group and 89 in the radiotherapy alone group; figure 2). The evaluable patient population for pathological response evaluation included patients for whom the pathological response, as per central assessment was available and included 154 patients (73 in the NBTXR3 group and 81 in the radiotherapy alone group). The evaluable population for cancer resection margins evaluation included patients for whom the margins evaluation was available, as per local assessment and excluded patients with limb amputation (155 patients, 73 in the NBTXR3 group and 82 in the radiotherapy group).

Baseline characteristics were generally well balanced between the two groups, except there were more male patients in the NBTXR3 group than in the radiotherapy alone group (table 1). Tumour characteristics were also well balanced between the groups (table 1). The median follow-up of the patients at the time of analysis was 9·7 months (range 0·2–28·9).

72 (81%) of 89 patients in the NBTXR3 group received between 80% and 100% of the planned NBTXR3 dose, whereas ten (11%) received less than 80%, and seven (8%) received more than 100% of the planned dose (where >100% represents the situation in which the calculated volume to be injected was rounded for practical reasons). Pain was the most common reason for not receiving the entire planned dose of NBTXR3. NBTXR3 administration was not complete in 18 (21%) of 87 patients (appendix p 2). In the injection procedure, the median number of needle punctures per patient was 8 (range 2–40) with a median time for administration of 28·5 min (range 3–331) in a single session. In the intention-to-treat full analysis set, all 87 patients in the NBTXR3 group and 89 in the radiotherapy alone group received the planned radiotherapy group dose of 50 Gy. The two patients in the radiotherapy alone group who did not receive the full radiotherapy dose discontinued treatment because they withdrew consent. No radiotherapy dose reductions occurred in either group. Two (2%) of 87 patients in the NBTXR3 group and five (6%) of 89 in the radiotherapy group had at least one fraction of radiotherapy delayed owing to an adverse event, radiotherapy treatment was delivered over a median time of 36 days in both treatment groups (range 32–45 days in the NBTXR3 group and 9–69 days in the radiotherapy alone group). In the intention-to-treat full analysis set efficacy population four (5%)of 87 patients did not have surgery and three (3%) patients in the radiotherapy group did not undergo their planned surgical resection because of progressive disease (two patients in the NBTXR3 group and one in the radiotherapy group), withdrawal of consent (two and one patients, respectively), or adverse events (one patient in the radiotherapy group; appendix p 2). Of the patients who underwent tumourectomy, two (2%) of 83 in the NBTXR3 group and four (5%) of 86 in the radiotherapy group had an upfront amputation of the limb instead of the planned resection per protocol, owing to the estimated high likelihood of an R2 resection in these patients if limb-conserving surgery was attempted.

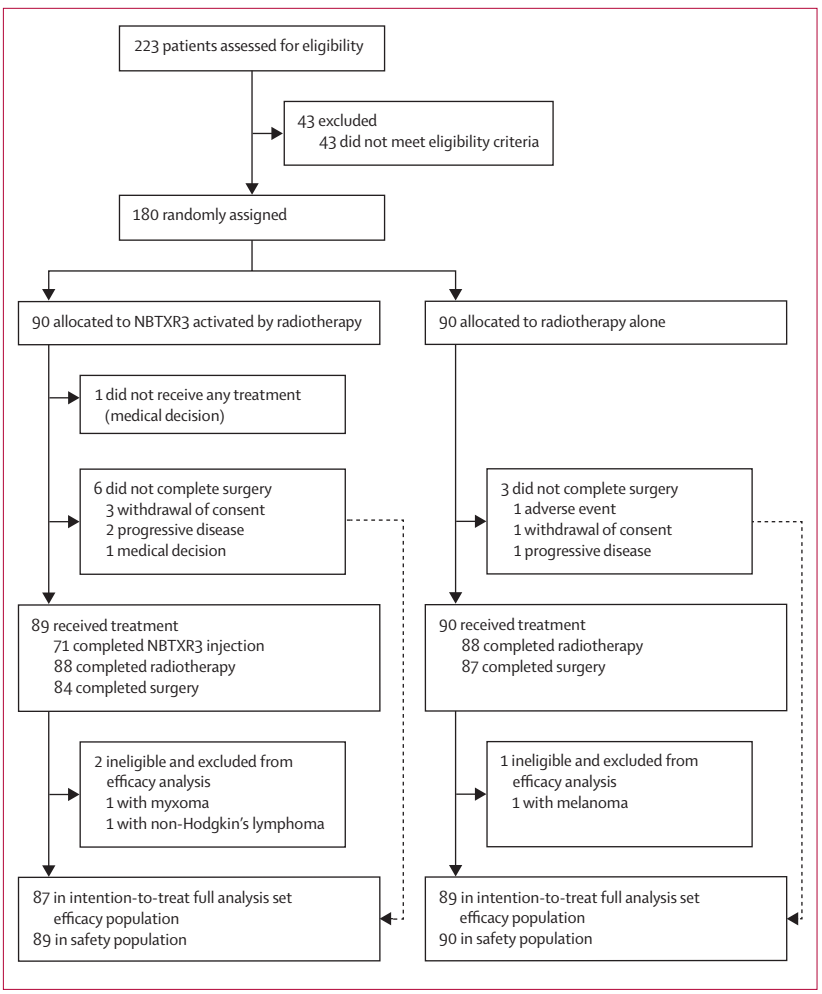


*Figure 2:* **Trial profile**

The proportion of patients with a pathological complete response (<5% of residual viable tumour cells) in the intention-to-treat full analysis set was 14 (16%) of 87 patients in the NBTXR3 group versus seven (8%) of 89 in the radiotherapy-alone group (p=0·044; table 2). Similarly, in the evaluable patient population for pathological response, the proportion of patients with pathological complete response was significantly higher in the NBTXR3 group (14 [19%] of 73) than in the radiotherapy-alone group (7 [9%] of 81]; p=0·047).

Exploratory analysis of pathological complete response using a threshold of 0% stainable cancer cells and evaluation of the pathological response by means of percentage of tumour necrosis or infarction as described by Schaefer and colleagues[23] yielded similar results to the primary analysis (table 2).

See **Online** for appendix

A planned exploratory analysis of the proportion of patients with a pathological complete response by histological grade showed that the difference between groups was greater for patients with grade 2 and 3 tumours than for those with grade 1 tumours (table 2).

The key secondary endpoint evaluating the resection margin after neoadjuvant treatment showed that more patients in the NBTXR3 group achieved R0 margins than in the radiotherapy alone group (p=0·042; table 2). Similarly, in the evaluable population for resection margin evaluation, the proportion of patients with R0 margin was higher in the NBTXR3 group (61 [84%] of 73) than in the radiotherapy alone group (57 [70%] of 82; p=0·030).

There was no difference in the proportion of patients who achieved an objective response, evaluated per RECIST 1.1, between treatment groups (table 2). Of the secondary endpoints, only hyalinisation is not presented in this article.

Serious adverse events were recorded in 35 (39%) of 89 patients in the NBTXR3 group and 27 (30%) of 90 patients in the radiotherapy alone group. Serious treatment-emergent adverse events (adverse events that occurred during the on-treatment period but which are not necessarily related to treatment; ) were reported in 28 (31%) of 89 of patients in the NBTXR3 group and 14 (16%) of 90 of patients in the radiotherapy alone group). In the NBTXR3 group, serious adverse events related to NBTXR3 were reported in nine (10%) of 89 patients, and the most frequent was hypotension in three (3%) of 89 patients (two grade 3 and one grade 4). Serious adverse events related to radiotherapy were reported in five (6%) of 89 patients in the NBTXR3 group and five (6%) of 90 patients in the radiotherapy alone group (appendix p 1). The most frequent serious treatment-emergent adverse event related to radiotherapy was postoperative wound complication and postprocedural infection in the NBTXR3 group, and postoperative wound complication in the radiotherapy alone group (appendix p 1).

Adverse events related to NBTXR3 were reported in 31 (35%) of 89 patients in the NBTXR3 group (table 3). Intratumoural injection of NBTXR3 led to injection-site pain in 12 (13%), haematoma or ecchymosis in six (7%), and tumour pain in five (6%) of 89 patients. The most common grade 3–4 adverse events related to NBTXR3 intratumoural injection were injection site pain (four [4%] of 89) and hypotension (four [4%]). NBTXR3 administration was associated with grade 3–4 acute immune reactions in seven (8%) of 89 patients (data not shown). These immune reactions were of short duration, manageable, and resolved spontaneously in most cases (data not shown). Premedication with steroids was adopted as a protocol amendment on June 2, 2016, to reduce these signs and symptoms.

Adverse events related to radiotherapy were reported in 65 (73%) of 89 patients in the NBTXR3 group and 72 (80%) of 90 patients in the radiotherapy alone group. The most frequently reported were radiation skin injury (any grade: 51 [57%] of 89 in the NBTXR3 group *vs* 62 [69%] of 90 in the radiotherapy alone group; grade 3: five [6%] *vs* four [4%]); and no grade 4, postprocedural wound complications including oozing and infection (eight [9%] *vs* and seven [8%] (grades 1–3), and peripheral oedema (eight [9%] *vs* eight [9%] grades 1–2).

Localised grade 1–2 peripheral oedema was observed in eight (9%) of 89 patients in the NBTXR3 group and one (1%) of 90 patients in the radiotherapy alone

| | NBTXR3 and radiotherapy group (n=87) | Radiotherapy alone group (n=89) |
|---|---|---|
| **Patient characteristics** | | |
| Sex | | |
| Female | 34 (39%) | 42 (47%) |
| Male | 53 (61%) | 47 (53%) |
| Age, years* | | |
| Mean (SD) | 60·5 (14·1) | 60·0 (14·7) |
| Median; range | 63·5; 22–83 | 64·0; 21–86 |
| Weight, kg† | | |
| Mean (SD) | 77·0 (18·0) | 75·4 (17·6) |
| Median; range | 76·5; 46–125 | 72·0; 43–117 |
| WHO performance score | | |
| 0 | 57 (66%) | 56 (64%) |
| 1 | 27 (31%) | 29 (33%) |
| 2 | 3 (3%) | 3 (3%) |
| **Disease status** | | |
| Primary locally advanced | 79 (91%) | 83 (93%) |
| Locally relapsed | 8 (9%) | 6 (7%) |
| Synchronous metastasis | 2 (2%) | 2 (2%) |
| **Tumour characteristics** | | |
| Tumour site | | |
| Upper limb | 9 (10%) | 9 (10%) |
| Lower limb | 64 (74%) | 66 (74%) |
| Trunk | 14 (16%) | 14 (16%) |
| Tumour histological type | | |
| Myxoid liposarcoma | 14 (16%) | 15 (17%) |
| Other | 73 (84%) | 74 (83%) |
| Undifferentiated or unclassified sarcoma | 30 (41%) | 28 (38%) |
| Liposarcoma | 13 (18%) | 15 (20%) |
| Adult fibrosarcoma | 10 (14%) | 9 (12%) |
| Leiomyosarcoma | 8 (11%) | 10 (14%) |
| Myxofibrosarcoma | 4 (5%) | 5 (7%) |
| Malignant peripheral nerve sheath tumour | 4 (5%) | 4 (5%) |
| Rhabdomyosarcoma | 1 (1%) | 1 (1%) |
| Extraskeletal myxoid chondrosarcoma | 0 (0%) | 1 (1%) |
| Fibroblastic–myofibroblastic tumours | 2 (3%) | 0 (0%) |
| Fibromyxoid sarcoma | 1 (1%) | 0 (0%) |
| Synovial sarcoma | 0 | 1 (1%) |
| Histological grade | | |
| 1 | 15 (17%) | 16 (18%) |
| 2 | 36 (41%) | 44 (49%) |
| 3 | 30 (34%) | 23 (26%) |
| Undetermined | 6 (7%) | 6 (7%) |

(Table 1 continues on next page)

group, although musculoskeletal and connective tissue disorders were reported in ten (11%) patients in the NBTXR3 group and nine (10%) in the radiotherapy alone group.

After all treatment and surgical resection, in both treatment groups, the most common grade 3–4 treatment-emergent adverse event was postoperative wound complication, with the same prevalence in both groups (eight [9%] of 89 in the NBTXR3 group and eight [9%] of 90 in the radiotherapy alone group; table 4).

As of May 22, 2018, the proportion of patients with any-grade post-treatment adverse events is 41 (46%) of 89 in the NBTXR3 group compared with 45 (50%) of 90 in the radiotherapy alone group. The long-term evaluation of the study treatment adverse events is ongoing with a planned cutoff date in April, 2020, to ensure complete data collection for late-onset adverse events.

As of data cutoff for the present analysis (May 22, 2018), 18 patient deaths have been reported: ten in the NBTXR3 group and eight in the radiotherapy alone group. The main cause of death in the NBTXR3 group was progressive disease (n=8); and one patient died of multi-organ failure and septic shock attributed to the surgery; and one died owing to clinical deterioration. Similarly, six patients treated in the radiotherapy alone group died from progressive disease, one of cardiac arrest, and one owing to multi-organ failure with bilateral bronchopneumonia. No treatment-related deaths occurred.

## Discussion

This Act.In.Sarc randomised phase 2–3 trial met its primary endpoint, with a greater proportion of patients with a pathological complete response in the NBTXR3 plus external-beam radiotherapy group than in the control group of patients who received external-beam radiotherapy alone, validating the mode of action of the NBTXR3 product in enhancing the efficacy of radiotherapy. Importantly, the proportion of patients with a pathological complete response in the control group was similar to that reported previously in soft-tissue sarcoma.[8–11]

Until now, no other similar product has been clinically tested, which renders NBTXR3 a first-in-class radioenhancer. The unique physical properties and mechanism of action of NBTXR3 showed a favourable safety profile when compared with other products aiming to potentiate radiotherapy.[14,15,19] The high electron density provided by the tight packing of Hf atoms in crystalline $HfO_2$ maximises the local high energy deposit on exposure to ionising radiation. This radioenhancer does not involve biological pathways in its physical primary mode of action and is therefore universal, enabling its use in all tumour types. Furthermore, the administration of NBTXR3 as one dose inside the tumour also allows for a better control of localisation, maximises bioavailability, and restricts exposure of healthy tissues, as shown in a phase 1 study.[19] Thus, NBTXR3 has the potential to be used in several solid tumours, including metastases.

We chose pathological complete response as our primary endpoint because it is the most objective

| | NBTXR3 and radiotherapy group (n=87) | Radiotherapy alone group (n=89) |
|---|---|---|
| (Continued from previous page) | | |
| Tumour longest diameter by MRI, mm | | |
| Mean (SD) | 83·1 (34·5) | 86·9 (28·3) |
| Median; range | 80·0; 25–191 | 91·0; 33–152 |
| Target theoretical tumour volume, mL (centralised reading)‡§ | | |
| Mean (SD) | 904·4 (1127·9) | 879·0 (783·0) |
| Median; range | 525·0; 16–6326 | 717·8; 29–4117 |

*Age was recorded for 84 patients in the NBTXR3 group and 85 patients in the radiotherapy group. †Weight was recorded for 87 patients in the NBTXR3 group and 88 patients in the radiotherapy group. ‡Tumour volume at baseline is the theoretical tumour volume estimated by the product of the three longest dimensions (length × width × depth), measured by MRI. §For one patient in the radiotherapy alone group, the tumour volume calculation was based on TAP CT scan assessments (length × width × depth), at baseline and before tumour resection.

***Table 1:*** **Demographics and baseline characteristics of the intention-to-treat full analysis set**

| | NBTXR3 and radiotherapy group (n=87) | Radiotherapy alone group (n=89) | p value |
|---|---|---|---|
| **Primary endpoint** | | | |
| Pathological complete responses, n (%)* | 14 (16%) | 7 (8%) | 0·044 |
| **Secondary endpoints** | | | |
| R0 resections† | 67 (77%) | 57 (64%) | 0·042 |
| Resection margin‡ | | | .. |
| NA | 2/83 (2%) | 4/86 (5%) | |
| R0 | 67/83 (81%) | 57/86 (66%) | .. |
| R1 | 9/83 (11%) | 19/86 (22%) | .. |
| R2 | 5/83 (6%) | 5/86 (6%) | .. |
| Tumour necrosis or infarction (%)† | .. | .. | 0·014 |
| Mean (SD) | 28·8 (30·8%) | 19·2 (23·9%) | .. |
| Median; range | 20·0; 0–95 | 10·0; 0–95 | .. |
| Objective response | 6 (7%) | 9 (10%) | 0·86 |
| Type of radiological response (RECIST 1.1) | | | |
| Complete response | 0 | 0 | .. |
| Partial response | 6 (7%) | 9 (10%) | .. |
| Stable disease | 72 (83%) | 71 (80%) | .. |
| Progressive disease | 6 (7%) | 3 (3%) | .. |
| **Exploratory analysis§** | | | |
| Pathological complete responses (<5% viable tumour 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 responses (0% viable tumour cells), % | 12/87 (14%) | 7/89 (8%) | .. |

Data are n (%) or n/N (%). NA=not applicable. *Statistically significant at α=0·4575. †Statistically significant at α=0·05. ‡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). §These exploratory analyses were not defined in the statistical plan; therefore no p-values have been calculated. ¶Only assessed in patients with a pathological response and known histological grade (n=76 in the NBTXR3 group; n=77 in the radiotherapy alone group).

***Table 2:*** **Efficacy results (intention-to-treat full analysis set)**

| | All grade | Grade 1–2 | Grade 3 | Grade 4 |
|---|---|---|---|---|
| Hypotension | 10 (11%) | 4 (5%) | 5 (6%) | 1 (1%) |
| Injection site pain | 7 (8%) | 6 (7%) | 1 (1%) | 0 |
| Tumour pain | 5 (6%) | 5 (6%) | 0 | 0 |
| Feeling hot | 3 (3%) | 2 (2%) | 1 (1%) | 0 |
| Oedema peripheral | 3 (3%) | 3 (3%) | 0 | 0 |
| Radiation skin injury | 2 (2%) | 1 (1%) | 1 (1%) | 0 |
| Pain in extremity | 2 (2%) | 2 (2%) | 0 | 0 |
| C-reactive protein increased | 2 (2%) | 2 (2%) | 0 | 0 |
| Erythema | 2 (2%) | 2 (2%) | 0 | 0 |
| Anaphylactic shock | 1 (1%) | 0 | 0 | 1 (1%) |
| Postprocedural infection | 1 (1%) | 0 | 1 (1%) | 0 |
| Postoperative wound complication | 1 (1%) | 0 | 1 (1%) | 0 |
| Apnoea | 1 (1%) | 0 | 1 (1%) | 0 |
| Hyperhidrosis | 1 (1%) | 0 | 1 (1%) | 0 |
| All | 46 (52%) | 35 (39%) | 9 (10%) | 2 (2%) |

Data are number of patients n (%) for a given category (preferred term grade). Treatment-emergent adverse events related to NBTXR3 which occurred in at least two patients for grade 1–2 and at least one patient for grades 3 and 4.

***Table 3:*** **All grade NBTXR3-related treatment emergent adverse events—all treated population**

| | NBTXR3 and radiotherapy group (n=89) | | Radiotherapy alone group (n=90) | |
|---|---|---|---|---|
| | Grade 3 | Grade 4 | Grade 3 | Grade 4 |
| Postoperative wound complication | 8 (9%) | 0 | 8 (9%) | 0 |
| Postoperative wound infection | 5 (6%) | 0 | 7 (8%) | 1 (1%) |
| Postprocedural infection | 3 (3%) | 0 | 2 (2%) | 0 |
| Postprocedural haemorrhage | 2 (2%) | 0 | 1 (1%) | 1 (1%) |
| Seroma | 1 (1%) | 0 | 0 | 2 (2%) |
| Postoperative abscess | 0 | 1 (1%) | 0 | 0 |
| Postprocedural complication | 1 (1%) | 0 | 0 | 0 |
| Skin flap necrosis | 1 (1%) | 0 | 1 (1%) | 0 |
| All | 19 (21%) | 1 (1%) | 18 (20%) | 2 (2%) |

Data are number of patients n (%). One patient may experience more than one adverse event in the same category.

***Table 4:*** **Grade 3–4 wound complications following surgical resection in the all-treated population**

parameter to evaluate biological efficacy and can provide a more rapid answer about the benefit of this new class of radioenhancer compared with survival endpoints. Furthermore, pathological complete response is a relevant endpoint for the evaluation of response to preoperative treatment, as is already used in other settings such as high-risk breast cancer and locally advanced rectal cancer.[36]

Regardless of the method used to assess pathological response (EORTC guidelines[22] or the Fletcher group method[23]), we recorded better results with NBTXR3 plus radiotherapy compared with radiotherapy alone. The difference between treatment groups was particularly notable for patients with high-grade tumours (grade 3) which are more aggressive and benefit the most from radiotherapy. Achievement of pathological complete responses after preoperative treatment was associated with long-term benefit in retrospective studies and in a meta-analysis.[8,11,30] Notably, a meta-analysis of 21 studies (n=1663) of patients with locally advanced soft-tissue sarcoma showed that tumour necrosis below 90% following neoadjuvant therapy is associated with increased risk of recurrence and inferior overall survival as compared with patients with tumor necrosis of 90% or higher.[11] In the same population, hyalinisation or fibrosis was also found to have a prognostic significance for patient outcomes. The ability of NBTXR3 to double the proportion of patients with a pathological complete response, which is a major outcome parameter in this setting, opens a large field of potential use in sarcoma or in other solid tumours, especially when these tumours are being treated exclusively with radiotherapy or if there is a high risk of surgical resection being unfeasible. Moreover, a de-escalation of the radiotherapy dose with the use of NBTXR3 to decrease morbidity could be investigated, and it could potentially be used in case of re-irradiation when necessary.

Surgical resection with negative margins (R0) is a validated surrogate for local control in limb sarcoma and for overall survival for truncal or girdle sarcoma.[31] With the knowledge that positive surgical margins have an increased risk for recurrence, surgeons must weigh up the benefits of a wide surgical excision with a possible effect on limb function or preservation.[3,4,9] In this clinical evaluation, multi-modal treatment with NBTXR3 showed superiority in the preoperative setting, with a higher proportion of patients treated with NBTXR3 having R0 resection than those treated with radiotherapy alone. A possible explanation for this observation could be improved capsular integrity, as described in a retrospective study showing that neoadjuvant treatment, including preoperative radiotherapy, stabilises the tumour capsule by fibrosis in high-grade soft-tissue sarcoma.[32,33] The effect observed is not a tumour size response but rather a tissue response (decrease in number of viable cells). The proportion of patients with R0 resection margins in the control group (66%) was only slightly higher than previously observed in a retrospective study of patients with soft-tissue sarcoma treated at various French centres (57·5%),[2] which indicates that our study was well controlled. A higher proportion of patients with R0 has been reported with chemotherapy and radiotherapy by a few high-volume centres, but with higher morbidity.[33,34] Clearly, our results are representative of the large number of participating centres with different volume of patients with sarcoma treated.

The safety findings related to the NBTXR3 injection were consistent with observations found in our pilot study, NBTXR3-101.[19] The most common adverse event related to NBTXR3 administration was pain, the

incidence of which decreased when more analgesia was used both before and during the injection, as well as with patient's anxiety management, delivery of more explanation on the injection procedure, and investigator product experience. Grade 3–4 acute immune reactions were not reported in previous or other ongoing studies (there are five phase 1 trials in progress). They were manageable and of short duration. Since NBTXR3 remains in the tumour and does not leak into the surrounding tissues,[19] its use does not increase radiotherapy-related toxicities. Wound complication in the preoperative external-beam radiotherapy setting (grades 3–4, 22% in each group, table 4) was similar to that observed in the randomised trial of preoperative versus postoperative radiotherapy in soft- tissue sarcoma by O'Sullivan and colleagues[12] (35% of patients had wound complications in the preoperative external-beam radiotherapy group) and in a recent study[35] of neoadjuvant intensity-modulated radiotherapy (36·8% of patients had wound complications). Furthermore, the safety profile observed during the postradiotherapy follow-up, including wound complications, revealed the same type and severity of adverse events in both treatment groups (Table 4). In a highly selective study using image-guided intensity-modulated radiotherapy, the prevalence of wound complications was 30·5%,[36] but was not substantially different from the 43% risk derived from the National Cancer Institute of Canada SR2 trial[36] The reduction of morbidity observed with image-guided intensity-modulated radiotherapy concerns mostly late complications.[37]

The use of pathological complete response as a surrogate measurement for progression-free survival or overall survival has some limitations, since a correlation between these parameters has been shown in various solid tumours including soft-tissue sarcoma but only for chemotherapy.[29,38] Since soft-tissue sarcoma is a rare cancer in adults and preoperative treatment is indicated in only a subset of this population, it is difficult to show a robust association between these outcome parameters. Interobserver variability in assessing pathological complete response is also a potential source of bias. In our study, we used central review by four pathology experts—authors of the EORTC guidelines—to limit this variability when considering the technical aspects of the evaluation. Furthermore, the use of pathological complete response as an endpoint does not allow the capture of long-term outcomes, including toxicity parameters, which are being evaluated in this ongoing study.

Another limitation of the study is the fact that no placebo injection could be used as a control. Patients in the control group had slightly larger tumours than those in the NBTXR3 group; however, a multivariate analysis showed that tumour size is not a prognostic factor for survival outcome and does not affect local control, which is directly linked to the margin, possibly justifying a larger surgical margin.[2] There were also more men than women in the investigational group than in the radiotherapy alone group; however, when studies find a prognostic influence of gender, male gender is generally unfavourable, which would benefit the control group.[2] The study was unblinded for surgery, but surgery was planned and done according to the tumour characteristics and not to the preoperative treatment received. Finally, the surgical capacity of the centre could affect the quality of resection margin. However, in this study, all centres were either high-volume or National Cancer Institute-designated centres, and therefore no stratification by centre was done.

Neoadjuvant or adjuvant chemotherapy remains an option for some soft-tissue sarcoma subtypes[3,4] but is not the standard of care.[31] Although a phase 3 study comparing histological subtype-tailored neoadjuvant chemotherapy versus a standard neoadjuvant chemotherapy regimen in patients with high-risk soft- tissue sarcoma showed an improved progression-free survival in the standard neoadjuvant chemotherapy group,[39] the data need consolidation.

In conclusion, the results of the Act.In.Sarc study suggest that NBTXR3 activated by radiotherapy could represent a new treatment option in patients with locally advanced soft-tissue sarcoma of the extremities or trunk wall. Moreover, these data open a large field of applications and justify ongoing studies evaluating NBTXR3, including phase 1–2 trials in head and neck squamous cell carcinoma (NCT01946867; NCT02901483), liver cancer (NCT02721056), prostate cancer (NCT02805894), rectal cancer (NCT02465593), and recurrent or metastatic head and neck squamous cell carcinoma or metastatic non-small-cell lung cancer (NCT03589339). Since NBTXR3 improves the efficacy of radiotherapy, all patients with resectable tumours eligible for preoperative radiotherapy treatment as defined by previous randomised studies could benefit from its use.

#### Contributors

SB, CLP, TdB, and ALC participated in the design of the study. All authors recruited patients, collected data, or both. SB wrote the manuscript and all authors reviewed and approved the final version of the manuscript.

#### Declaration of interests

SB declares personal fees and non-financial support from Nanobiotix during the conduct of the study, and non-financial support from Lilly, Novartis, Pfizer, and Pharmamar, outside the submitted work. JT, AD, PT, AJL, and JVMGB declare personal fees from Nanobiotix. PLR reports personal fees from Novartis, BMS, MSD, Roche, Amgen, Eli Lilly, Pfizer, and Blueprint Medicines, outside the submitted work. ALC declares personal fees from Pharmamar, Eli Lilly, Pfizer, and Amgen, outside the submitted work. HHL declares personal fees from Boehringer-Ingelheim, Takeda, Eisai, and Guardant Health; grants and personal fees from Novartis; and grants from MSD and Mundipharma, outside the submitted work. EW declares personal fees from Novartis, Lilly, Bayer, PharmaMar, Milestone, Menarini, and New Oncology, outside the submitted work. CLP declares personal fees from Nanobiotix, during the conduct of the study; and personal fees from Amgen, Roche, PrimeOncology, Medscape Lilly, and Astra Zeneca, outside the submitted work. SC, M-PS, PA, AH, AMe, MR, VM, RKL, BT, ACH, AG, LM, TS-O, PH, TdB, SH, ES-B, AB, RA, AC, MG, GKan, AMo, RV, LL, SD, GKac, LA, LM-Z, VS, and ZP declare no competing interests.

### Data sharing

Nanobiotix has committed to sharing the data underlying this publication, on request from qualified scientific and medical researchers, patient-level clinical trial data, study-level clinical trial data, and protocols from clinical trials in patients for NBTXR3 and indications approved in the USA and EU by regulatory agencies as necessary for legitimate research. Interested researchers can request access to anonymised patient-level data and supporting documents from clinical studies to do further research that could help to advance medical science or improve patient care. Data access will be granted to anonymised patient-level data, protocols, and clinical study reports after approval by an independent scientific review panel which will ensure thorough scrutiny on the application of the global data protection regulation. Nanobiotix is not involved in the decisions made by the independent review panel. Nanobiotix takes all necessary measures to ensure that patient privacy is safeguarded. Contact: dataprivacy@nanobiotix.com.

### Acknowledgments

This study was funded by Nanobiotix SA (Paris, France) and PharmaEngine, Inc (Taipei, Taiwan, Republic of China). We thank all patients, investigators, and staff at participating centres, and the data managers. We also thank the following study investigators: Claire Alapetite (Paris, France), Marinela Augustin (Nürnberg, Germany), Raymond Barba (Manila, Philippines), Jean-Yves Blay (Lyon, France), Hervé Brisse (Paris, France), Kenneth Cortez Sy (Quezon City, Philippines), Samuel Fourie (Petroria, South Africa), François Gouin (Saint Herblain, France), Jozsef Kuzsner (Pècs, Hungary), Carmen Llacer-Moscardo (Montpellier, France), Marick Laé (Paris, France), Carlo Morosi (Milan, Italy), Jan Peter Poulsen (Oslo, Norway), Giovanni Scarzello (Padova, Italy), Anna Simeonova-Chergou (Mannheim, Germany), Mateusz Spalek (Warsaw, Poland), Paul Stalley (Sydney, Australia), Vlad Stefanel (Bucharest, Romania), Eberhard Stoeckle (Bordeaux, France), Raphaël Tetreau (Montpellier, France), Cornelia Toganel (Targu Mures, Romania), Dirk Van Gestel (Brussels, Belgium), Gualter Vaz (Lyon, France), and Roberto Velez (Barcelona, Spain). Finally, we acknowledge editorial assistance from Joëlle Morvan and Angelo Cho (Nanobiotix SA, Global Medical Affairs department).

### References

1 Pottier A, Borghi E, Levy L. The future of nanosized radiation enhancers. *Br J Radiol* 2015; **88:** 20150171.

2 Blay JY, Soibinet P, Penel N, et al. Improved survival using specialized multidisciplinary board in sarcoma patients. *Ann Oncol* 2017; **28:** 2852–59.

3 Casali PG, Abecassis N, Bauer S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2018; **29** (suppl 4)**:** iv51–iv67.

4 von Mehren M, Randall RL, Benjamin RS, et al. Soft Tissue Sarcoma, Version 2.2018, NCCN clinical practice guidelines in Oncology. *J Natl Compr Canc Netw* 2018; **16:** 536–63.

5 Pisters PW, Harrison LB, Leung DH, Woodruff JM, Casper ES, Brennan MF. Long-term results of a prospective randomized trial of adjuvant brachytherapy in soft tissue sarcoma. *J Clin Oncol* 1996; **14:** 859–68.

6 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:** 197–203.

7 Kepka L, DeLaney TF, Suit HD, Goldberg SI. Results of radiation therapy for unresected soft-tissue sarcomas. *Int J Radiat Oncol Biol Phys* 2005; **63:** 852–59.

8 Mullen JT, Hornicek FJ, Harmon DC, et al. Prognostic significance of treatment-induced pathologic necrosis in extremity and truncal soft tissue sarcoma after neoadjuvant chemoradiotherapy. *Cancer* 2014; **120:** 3676–82.

9 Canter RJ, Martinez SR, Tamurian RM, et al. Radiographic and histologic response to neoadjuvant radiotherapy in patients with soft tissue sarcoma. *Ann Surg Oncol* 2010; **17:** 2578–84.

10 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:** 3911–15.

11 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:** 1–10.

12 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:** 2235–41.

13 Haas RL, Delaney TF, O'Sullivan B, et al. Radiotherapy for management of extremity soft tissue sarcomas: why, when, and where? *Int J Radiat Oncol Biol Phys* 2012; **84:** 572–80.

14 Kraybill WG, Harris J, Spiro IJ, et al. Phase II study of neoadjuvant chemotherapy and radiation therapy in the management of high-risk, high-grade, soft tissue sarcomas of the extremities and body wall: Radiation Therapy Oncology Group Trial 9514. *J Clin Oncol* 2006; **24:** 619–25.

15 Bristow RG, Alexander B, Baumann M, et al. Combining precision radiotherapy with molecular targeting and immunomodulatory agents: a guideline by the American Society for Radiation Oncology. *Lancet Oncol* 2018; **19:** e240–e51.

16 Marill J, Anesary NM, Zhang P, et al. Hafnium oxide nanoparticles: toward an in vitro predictive biological effect? *Rad Oncol* 2014; **9:** 150.

17 Maggiorella L, Barouch G, Devaux C, et al. Nanoscale radiotherapy with hafnium oxide nanoparticles. *Future Oncol* 2012; **8:** 1167–81.

18 Pottier A, Borghi E, Levy L. Metals as radio-enhancers in oncology: the industry perspective. *Biochem Biophys Res Commun* 2015; **468:** 471–75.

19 Bonvalot S, Le Pechoux C, De Baere T, et al. 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:** 908–17.

20 Fletcher CDM, Bridge JA, Hogendoorn P, Mertens F. WHO classification of tumours of soft tissue and bone, 4th edn. Lyon, France: IARC Press, 2013.

21 Trojani M, Contesso G, Coindre JM, et al. Soft-tissue sarcomas of adults; study of pathological prognostic variables and definition of a histopathological grading system. *Int J Cancer* 1984; **33:** 37–42.

22 Wardelmann E, Haas RL, Bovee JV, et al. Evaluation of response after neoadjuvant treatment in soft tissue sarcomas; the European Organization for Research and Treatment of Cancer-Soft Tissue and Bone Sarcoma Group (EORTC-STBSG) recommendations for pathological examination and reporting. *Eur J Cancer* 2016; **53:** 84–95.

23 Schaefer I-M, 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:** 375–83.

24 Gundle KR, Kafchinski L, Gupta S, et al. Analysis of margin classification systems for assessing the risk of local recurrence after soft tissue sarcoma resection. *J Clin Oncol* 2018; **36:** 704–09.

25 US Department of Health and Human Services. National Institutes of Health. National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. May 28, 2009 (v4.03: June 14, 2010). https://www.eortc.be/services/doc/ctc/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf (accessed May 22, 2019)

26 Hellmann MD, Chaft JE, William WN Jr, et al. Pathological response after neoadjuvant chemotherapy in resectable non-small-cell lung cancers: proposal for the use of major pathological response as a surrogate endpoint. *Lancet Oncol* 2014; **15:** e42–50.

27 European Medicines Agency. ICH Topic E9. Step 5. Note for guidance on Statistical Principles for Clinical Trials. September, 1998. CPMP/ICH/363/96. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e-9-statistical-principles-clinical-trials-step-5_en.pdf (accessed July 10, 2018).

28 Moses L Nonparametric methods. In: Walker HM and Lev J, eds. Statistical inference. New York: Henry Holt, 1953: 426–50.

29 Moses L Confidence limits from rank tests, *Technometrics* 1965; **7:** 257–60.

30 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:** 3203–09.

31 Bonvalot S, Levy A, Terrier P, et al. Primary extremity soft tissue sarcomas: does local control impact survival? *Ann Surg Oncol* 2017; **24:** 194–201.

32 King DM, Hackbarth DA, Kirkpatrick A. Extremity soft tissue sarcoma resections: how wide do you need to be? *Clin Orthop Relat Res* 2012; **470:** 692–99.

33 Grabellus F, Podleska LE, Sheu SY, et al. Neoadjuvant treatment improves capsular integrity and the width of the fibrous capsule of high-grade soft-tissue sarcomas. *Eur J Surg Oncol* 2013; **39:** 61–67.

34 Callegaro D, Miceli R, Bonvalot S, et al. Impact of perioperative chemotherapy and radiotherapy in patients with primary extremity soft tissue sarcoma: retrospective analysis across major histological subtypes and major reference centres. *Eur J Cancer* 2018; **105:** 19–27.

35 Peeken JC, Knie C, Kessel KA, et al. Neoadjuvant image-guided helical intensity modulated radiotherapy of extremity sarcomas—a single center experience. *Radiat Oncol* 2019; **14:** 2.

36 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:** 1878–84.

37 Davis A, Osullivan B, Turcotte R, et al. Late radiation morbidity following randomization to preoperative versus postoperative radiotherapy in extremity soft tissue sarcoma. *Radiother Oncol* 2005; **75:** 48–53.

38 Wang D, Zhang Q, Eisenberg BL, et al. Significant reduction of late toxicities in patients with extremity sarcoma treated with image-guided radiation therapy to a reduced target volume: results of Radiation Therapy Oncology Group RTOG-0630 Trial. *J Clin Oncol* 2015; **33:** 2231–38.

39 Gronchi A, Ferrari S, Quagliuolo V, et al. Histotype-tailored neoadjuvant chemotherapy versus standard chemotherapy in patients with high-risk soft-tissue sarcomas (ISG-STS 1001): an international, open-label, randomised, controlled, phase 3, multicentre trial. *Lancet Oncol* 2017; **18:** 812–22.