# Supplemental Data 1 Full Materials and Methods

This single-center, nonrandomized, single-arm, open-label, feasibility, and safety study with a medical device (radioactive implant) was registered on clinicaltrials.gov (ID: NCT05191498). The study was designed and conducted in accordance with the Declaration of Helsinki and reviewed and approved by the Medical Research Ethics Committee Oost-Nederland in March 2021 (NL76311.091.20). The expected enrollment was 3–6 patients in the 166HoMS intervention group, without a control arm, because of the early research phase of this study. The sample size was determined on the number of patients deemed necessary for assessment of the feasibility. If technical feasibility was achieved within 6 patients, but after a minimal of 3 patients, the study could be preliminary advanced to a follow-up trial. All participants voluntarily provided written informed consent before enrollment. Patients were specifically informed before enrollment that if surgery should fail; the study intervention was performed only as a palliative intervention, and no benefits regarding survival or decreased tumor burden could be expected in this experimental setting. Additionally, only local intervention in the primary tumor was performed without intervention of any metastatic disease diagnosed during open exploratory surgery.

## Patient selection and planning

Eligible participants were both females and males 18 years and older who were diagnosed with pathologically proven PDAC with evidence of no metastatic disease on contrast-enhanced CT (CECT) and/or MRI and were eligible for surgical exploration with the purpose of surgical resection of the primary tumor by either pancreatoduodenectomy or pancreatic tail and/or body resection. Patients had to have a World Health Organization (WHO) performance score of 0–1, a tumor >10 mm in longest diameter and a life expectancy of at least 12 weeks. Patients had no prior radiation therapy, no major tumor calcifications, no unresolved toxicity or adverse events (AEs) of grade 3 or higher (Common Terminology Criteria for Adverse Events (CTCAE) v4.0), no significant cardiac events within 3 months before entry, no immune or blood coagulation deficiency (leukocytes <4.0\*109 and platelet count <100\*109), were not breastfeeding, had no childbearing potential or underwent a pregnancy test, and were not declared incompetent or suffered from psychic disorders that impair comprehensive judgment. Patients who were considered ineligible for intratumoral 166HoMS injection by an expert panel including a surgeon, nuclear medicine physician, interventional/abdominal radiologist and researcher were excluded from enrollment. Demographic data, including age, sex, WHO performance score, body mass index (BMI), tumor characteristics (location and resectability status according to the Dutch Pancreatic Cancer Group (DPCG) criteria[1], largest diameter, and volume), and neoadjuvant chemotherapy, were collected.

Patients underwent preoperative CECT not more than four weeks before surgery for the purpose of patient-specific intratumoral 166HoMS injection planning. The tumor volume was assessed via 3D segmentation of the arterial and/or venous phase via 3D Slicer (version 4.11–5.4), which was supervised by an expert radiologist (J.J.H.). Vital structures near the tumor, including nearby major arteries and vessels, the pancreatic duct, and the common bile duct, were also segmented for preoperative planning. The CT image acquisition parameters and reconstruction parameters are shown in Table S1.

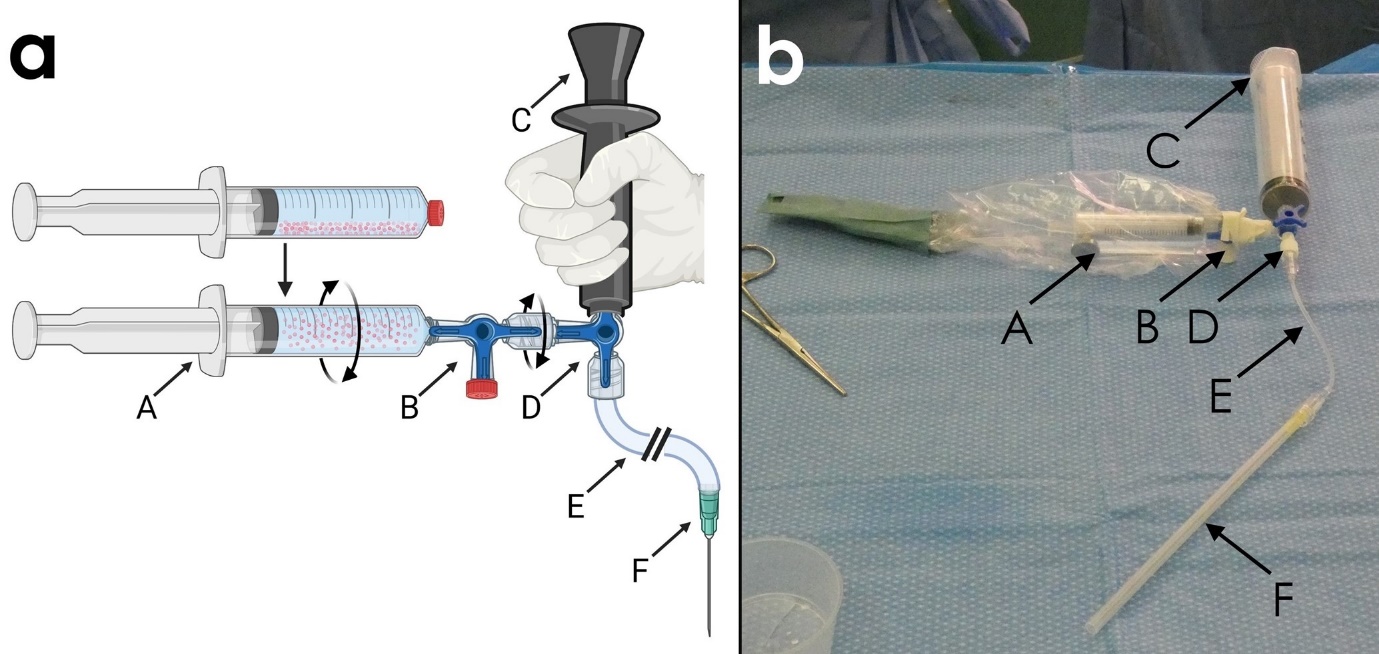
## Holmium microsphere preparation

Radioactive holmium-166 poly-L-lactic acid microspheres in a 0.1% Pluronic phosphate-buffer suspension (Quirem Medical, Deventer, The Netherlands) were delivered to the hospital in a glass V-vial. A detailed description of the 166HoMS characteristics is given in Supplementary 1. 166HoMS in suspension were loaded in 3 mL syringes with a Luer-lock connection (Plastipak, BD Medical, New Jersey, USA) in the onsite hotlab with a by planning defined activity (MBq) measured in a dose calibrator (VIK-202, Comecer, Joure, the Netherlands; lower detection limit 0.01 MBq) and a by planning defined suspension volume (mL). At the planned moment of treatment, the specific activity of the 166HoMS was between 3.0 and 10.0 MBq/mg. The activity required per patient was based on a dose coefficient of 15.87 mJ/MBq/kg tissue[2], which resulted in equation (1).

The required injection activity (*A*) in MBq was calculated for a maximum achievable mean tumor dose (*D*)in Gray (Gy) of 125 Gy in tumors with a mass *M* in grams (g) and an assumed tumor density of 1,0 g/cm3. The activity loaded into the syringe (Asyr) compensated for 21% of the expected loss of microspheres in the injection device because of agglomeration and the addition of a flush for the injection device [C.Y. Willink, et al. (2024) - unpublished]. The maximum injection volume was calculated as a fraction of 10–20% of the total tumor volume plus a flush of the injection system of 0.5–1.0 mL. One or more 3 mL syringes were prepared per patient depending on the required injection volume, with the use of one injection system and one flush per patient.

## Treatment

All patients received piperacillin/tazobactam as antibiotic prophylaxis before surgery. All patients received general anesthesia, with locoregional anesthesia using an epidural if possible. After open exploratory surgery, patients either underwent surgical resection of the tumor or received intratumoral 166HoMS injection if surgical resection was determined to be not feasible by the surgeon. In the case of surgical resection, no study intervention was performed, and these patients were excluded from further follow-up. In the case of no surgical resection, either caused by local blood vessel involvement or by the perioperative observation of distant metastatic disease, intratumoral 166HoMS injection was performed immediately in an open abdomen surgical setting. Using intraoperative ultrasound (US, Canon Medical Systems Corporation, Amstelveen, the Netherlands) with the probe in a sterile sleeve, the tumor location, tumor volume, injection volume, nearby vital structures, needle insertion location(s) and deposit location(s) were reassessed. For 166HoMS injection, a manual rotation device (Figure S1.a), described in detail in previous research [C.Y. Willink, et al. (2024) - unpublished], was used to sustain and inject a homogeneous suspension. To limit radiation exposure to the operators and patients, the syringe was fixed in a 9 mm thick acrylic glass syringe holder. The syringe with acrylic glass was wrapped in a sterile plastic sleeve (Figure S1.b). The used injection system parameters were in line with the recommended parameters previously studied [C.Y. Willink, et al. (2024) - unpublished] and are further described in the results section. Intratumoral insertion of 110–150 mm 20G needles (MediPlast Special Cannula, MediPlast, Malmö, Sweden) was guided with US. Intratumoral 166HoMS injection was performed in line with the advised intratumoral injection parameters as described in earlier research [C.Y. Willink, et al. (2024) - unpublished] and is further described in the results section.



*FIGURE S1. Overview of the holmium-166 microsphere (166HoMS) manual rotation and injection device. (a) Schematic overview. Created with BioRender.com (b) Device during the injection procedure with the therapy syringe with acrylic glass shielding in a sterile plastic sleeve. Components: A) therapy syringe; B) 360o rotating three-way stopcock; C) handheld; D) three-way stopcock; E) extension tube; F) injection needle.*

Intratumoral 166HoMS injection was performed by operators, including an interventional radiologist, assisted by a surgeon and researcher with sufficient radiation safety experience, and supervised by a nuclear medicine physician. All operators received training in the procedure’s protocol. The interventional radiologist performed US-guided placement of the needle, while the researcher homogenized the 166HoMS suspension by rotating the 166HoMS-filled syringe. The surgeon supervised surgery-related safety, whereas a nuclear medicine physician supervised overall radiation safety. The radiation exposure to the body and hands of the interventional radiologist and the researcher, who were most exposed to radiation from the device, were monitored by wearing personal dose badges (Mirion Technologies, Atlanta, Georgia, USA) and hand dosimeters (SCK CEN, Mol, Belgium).

After injection, the patient’s abdomen was closed with sutures. Wipe tests were performed on the abdominal wound to check for radioactive residue (contamination) using a contamination monitor (CoMo 170, PI Medical, Raamsdonksveer, the Netherlands; Mini 900 type 44A, Mini-Instruments, Essex, U.K.) and cleaned with moist gauze if contamination was present. When declared free of contamination (less than 2x background levels), the wound was bandaged with clean gauze. The operators, equipment, and room were also checked for contamination by a nuclear medicine physician. The procedure duration was logged as the time in which an operator held the injection device, from needle placement until the final injection was completed. If contamination was present, it was cleaned or disposed of in nuclear waste bins and transported to the Department of Nuclear Medicine.

## Follow-up

Patients were hospitalized for at least 48 hours after surgery for contamination monitoring. After surgery, dalteparin was prescribed for thromboembolic prophylaxis. Any excretion of holmium-166 was analyzed by measuring wound gauze, feces, and urine for at least 48 hours after intervention with a contamination monitor. Samples that were positively contaminated on the contamination monitor were taken to the Department of Nuclear Medicine and measured in a dose calibrator (VIK-202, Comecer, Joure, the Netherlands; lower detection limit 0.01 MBq). Extracorporeal contamination was also registered during hospitalization with a contamination monitor in the patient’s room and by collecting and measuring all medical waste from the patient’s room. The monitoring of complications by CTCAE v4.0 was performed continuously during hospitalization. Blood tests were performed according to hospital guidelines after surgery and were also analyzed for complication monitoring. CECT, planar thorax-abdomen gamma camera imaging, and single-photon emission computed tomography (SPECT)/CT were acquired within 72 hours after intervention. The planar gamma camera and SPECT image acquisition and reconstruction parameters are shown in Table S2. Contamination monitoring was released after minimal 48 hours post-intervention, if no (major) contaminations were present and in consultation with a nuclear medicine physician after evaluation of the SPECT/CT. Outpatient clinics were used for complication registration at one, eight and twelve weeks after intervention. CECT was repeated twelve weeks after the intervention, after which follow-up was completed.

## Data analysis

Laboratory measurements

To determine the total injection activity (Ainj), all contaminated disposables from the procedure, such as gauzes, gloves, needles, syringes, and the injection device, were individually measured in a dose calibrator (VIK-202, Comecer, Joure, the Netherlands; lower detection limit 0.01 MBq) at the Department of Nuclear Medicine after intervention. The measured activities from the disposables (Adisp) were converted to the activity at the time of treatment (t = 0) and cumulatively subtracted from the prepared syringe activity at t = 0 (Asyr), as shown in equation 2.

Equation 2:

Where *Ainj* is the total activity injected (MBq), *Asyr*is the initially prepared syringe activity at t = 0 (MBq), *Adisp*is the cumulative activity from all measured disposables (MBq), *∆t* is the amount of time between the time of measurement and the time of treatment (hours), and *t1/2*is the half-life of holmium-166 at 26.83 hours.[3]

Planar thorax-abdomen gamma camera imaging analysis

The two-dimensional planar images were analyzed with Hermia Gold Smart (version: 2.17.0.87). Regions of interest (RoIs) on the anterior windows of the planar images were manually drawn and then mirrored to the posterior window. Background regions (BR) were drawn in the same manner, adjacent to the patient’s body. Each RoI and BR contained a surface area (cm2), and the detected counts (C) which was related to the amount of radioactive 166HoMS present. To determine in which RoI clinically relevant amounts of activity were present, compared with scatter, RoIs with over twice the count concentration ([C] in counts/cm2)in comparison with an adjacent BR were selected. These RoIs were then background corrected (RoIBC) (equation 3).

Equation 3:

Equation (3) is used for each RoI individually, as shown in equations (3), (4) and (5) by *(x)*. In equation 3, *BCRoI*is the background-corrected number of counts in a RoI (counts), *CRoI*is the total number of counts in a RoI not compensated for background (counts), *[CBR]* is the count concentration in the BR adjacent to RoI(x) (counts/cm2), and *ARoI* is the surface area of a RoI (cm2). Next, the anterior and posterior background-corrected RoIs were fused using the geometric mean method (equation (4)) to compensate for the difference in attenuation over the path lengths from the source to the anterior or posterior gamma camera.[4]

Equation 4:

In which *TRoI* is the total number of counts in a RoI compensated for background (performed in equation 3) and anterior‒posterior windows fused (counts), *BCant* is the background-corrected number of counts in the anterior window of an RoI (counts), and *BCpost* is the background-corrected number of counts in the posterior window of an RoI (counts).

The sum of all resulting counts (TRoI’s)in one patient represents the total activity injected (Ainj); thus, the activity per RoI (ARoI)in MBq was determined as shown in equation 5.

Equation 5:

SPECT/CT gamma camera imaging analysis

The calculated activities found via planar gamma camera imaging (ARoI(x)) were converted to the corresponding 3-dimensional areas of interest (AoIs) on SPECT/CT images with Qsuite (version 2.2, build 2.2.0.6237, Quirem Medical, Deventer, The Netherlands). With manual segmentation of the AoI on the SPECT and next the on-target (tumor) and off-target (organs) on the CT of the SPECT/CT, the mean dose (Gy) and maximum dose (Gy) were calculated per target with a Ho166 dedicated dose point kernel built into the software. If structures/organs were not completely visible on SPECT/CT, the mean dose was calculated using equation (1), with the calculated activity from planar gamma camera imaging in MBq (*A*) and the reference organ mass from IRCP-089 in grams (*D*).[5] For the tumor, the original tumor volume as planned in the preoperative CECT was used.

## Study outcomes

The primary outcome of feasibility was assessed by injection success, which was defined as the ability to inject 166HoMS into the tumor, as a percentage of the planned volume and activity, and by injection accuracy, which was defined by on-target and off-target radiation doses in Gy defined by SPECT dosimetry. The primary outcome of safety was assessed by AE monitoring (CTCAE 4.0) from the time of inclusion to the end of follow-up at 12 weeks. AEs were categorized by severity (grade 1, least severe; grade 5, most severe). If an AE developed over multiple grades, only the highest-grade AE was reported. AEs were also categorized by likelihood of study attribution, which indicates an AE to be caused by the needle insertion(s) or the radioactive implant (*unrelated, unlikely, possible, probable, definite*). An AE was only described as *unrelated* to study attribution if the AE was present before the intervention and did not worsen in grade after the intervention. If an AE developed toward a higher or lower affinity to study attribution, the highest study attribution was reported. Study attribution was further categorized as *high study attribution* (*possible, probable, definite*) or *low study attribution* (*unrelated, unlikely*)*.* All adverse events were screened by an investigator and a hepatobiliary surgeon (CYW; MWJS) on grade and study attribution.

As secondary outcomes, the radiation exposure of the operators was measured in millisievert (mSv) for the skin dose equivalent (Hp 0.07) hands and body and the deep dose equivalent (Hp 10) of the body, with a lower detection limit of 0.15 mSv for the hands and 0.04 mSv for the body. The maximum radiation exposure was set at 500 mSv/year for the hand skin dose and body skin dose equivalent and 20 mSv/year for the body deep dose equivalent, in line with the ICRP recommended guidelines for exposed workers.[6] As an exploratory outcome, the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) was estimated after twelve weeks or after the last available clinical CT within the follow-up period of 12 weeks. Moreover, since CT has been used to visualize and quantify HoMS in previous studies[7-9], the feasibility of CT application for intratumoral 166HoMS injection in patients with PDAC was also explored.

Due to the study's focus on feasibility and safety, the absence of comparative groups, and the small sample size, statistical analysis could not be performed.

# References

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