Intratumoral holmium-166 microsphere injection in pancreatic cancer

Starting to treat from the inside out

C. Ysbrand Willink

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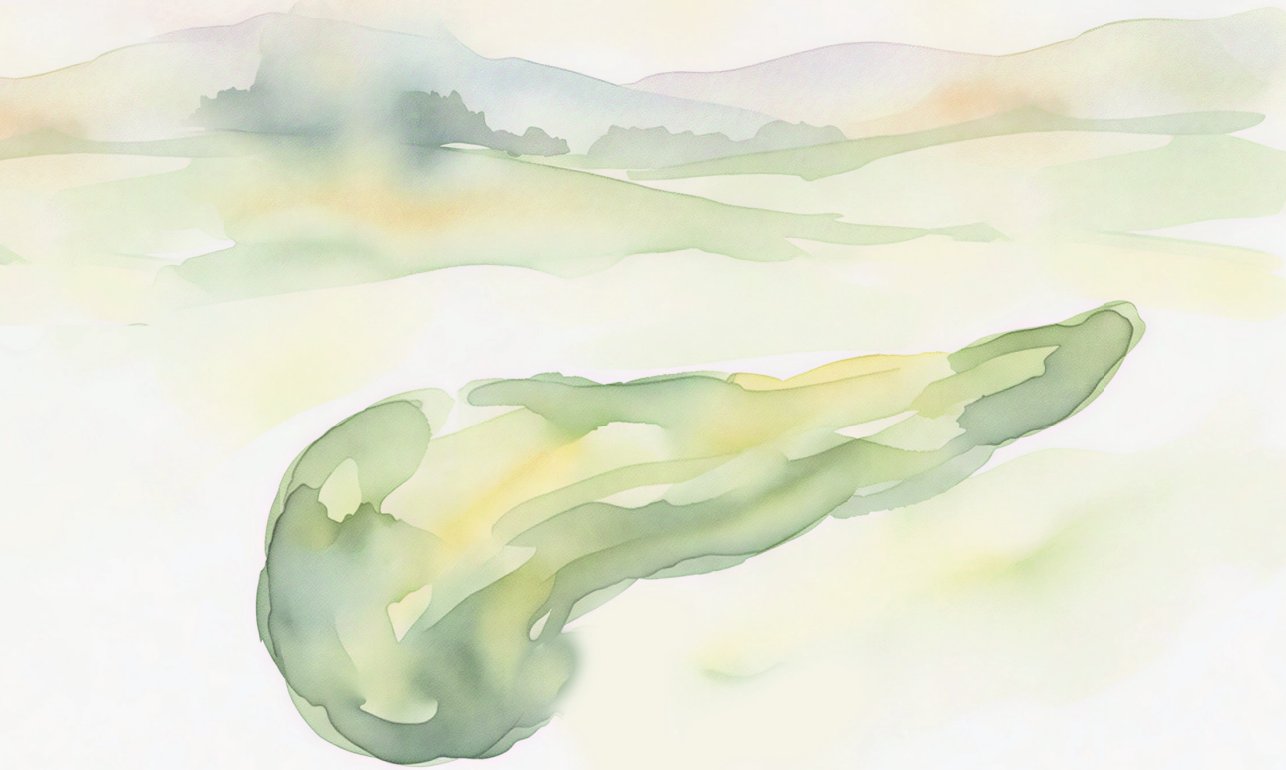
Chapter 1

GENERAL INTRODUCTION AND THESIS OUTLINE



An introduction to pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) accounts for 90% of all pancreatic cancers and is the most aggressive and lethal form of the disease.[1] PDAC typically presents later in life, with a median age of 71 years old at diagnosis.[2] Risk factors related to developing PDAC are type 2 diabetes, obesity, smoking, malnutrition, chronic pancreatitis, and excessive consumption of alcohol and red meat.[2,3] While incidence rates are slightly higher in men, gender discrepancies are minimal. Despite its lower prevalence, ranked as the 10th most commonly diagnosed cancer in 2024 (1 out of 5000 people develop PDAC), PDAC remains the third leading cause of cancer-related mortality for men and women combined in the USA and the Netherlands, after colorectal and lung cancer.[4,5] The prognosis is dismal, with a 1-year survival rate between 21.1–30.9% and a 5-year survival rate between 6.6–13%.[4,6]

The high mortality rate is primarily caused by late diagnosis and the limited number of patients eligible for curative treatment. Currently, curative treatment is only possible through radical resection such as pancreatectomy (including pancreatoduodenectomy, or distal pancreatectomy) with clear margins (R0, ≥1mm). In addition to surgery, the treatment regimen includes systemic or locoregional therapy and can be tailored based on tumor stage, which also significantly influences the patient's prognosis. The Dutch Pancreatic Cancer Group (DPCG) developed an internationally recognized staging system that aligns with clinical practices.[7] Similarly, other commonly used staging systems, such as the National Comprehensive Cancer Network (NCCN)[8] and American Joint Committee on Cancer (AJCC)[9], are primarily based on the extent of tumor involvement in major local vasculature and the presence or absence of metastatic disease, much like the DPCG as described below:

- **Resectable pancreatic cancer:** The tumor is confined to the pancreas without arterial involvement and no or minimal (≤90 degrees) involvement of the superior mesenteric vein (SMV) or portal vein (PV), without distant metastasis. Surgical resection is the primary treatment option, often complemented with adjuvant chemotherapy.
- **Borderline resectable:** The tumor exhibits partial involvement of surrounding arterial blood vessels (≤90 degrees), such as the superior mesenteric artery (SMA), celiac artery (CA) or the common hepatic artery (CHA) and/or moderate involvement (≤90–270 degrees) of the SMV-PV without occlusion and without distant metastasis (Figure 1). Surgical resection is more challenging, sometimes requiring vascular reconstruction, but potentially feasible following neoadjuvant chemotherapy and often followed by adjuvant chemotherapy.
- **Locally advanced pancreatic cancer (LAPC):** The tumor extensively involves major arterial blood vessels such as the SMA, CA or CHA, or occludes or strongly involves (>270 degrees) the SMV-PV, precluding surgical resection. Induction or palliative chemotherapy or radiotherapy is employed, aiming to control symptoms and prolong survival. Exploratory

surgery with intent of surgical resection is considered for 10–25% of patients who experience sufficient tumor downstaging.[10-12]

- **Metastatic disease:** Tumors with distant spread, typically to the liver, peritoneum, or lungs, for which systemic chemotherapy is the primary mode of treatment, focusing on palliation and quality of life.

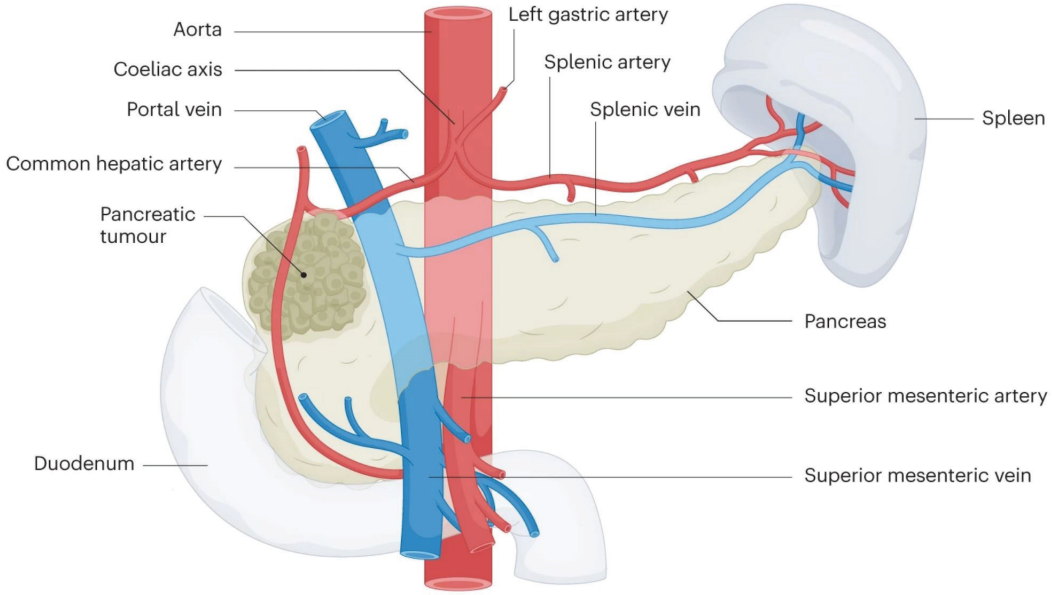


Figure 1. *Schematic overview of the pancreas and vital nearby vasculature and organs. This example shows a borderline resectable pancreatic tumor caused by the partial involvement of the common hepatic artery. This figure was reused with permission from T.F. Stoop.[13]*

Overall, 20% of patients present with resectable and borderline resectable PDAC, while 30% have LAPC and 50% have metastatic disease at diagnosis.[14,15] Across all stages, systemic chemotherapy, primarily FOLFIRINOX (folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin) or gemcitabine nab-paclitaxel, plays a central role in neoadjuvant, adjuvant, induction or palliative treatment regimens.[16] A treatment flowchart for local and locoregional PDAC is presented in Figure 2.[16] It is the preferred treatment when upfront resection is not possible. However, it is often perceived as highly invasive and is associated with high toxicity, with 66.4–76.2% of patients experiencing severe grade 3–4 toxicities.[10,17] The limited effectiveness of chemotherapy for PDAC can be partly attributed by the tumor's challenging microenvironment (TME). The TME is characterized by dense desmoplasia, cancer-associated fibroblasts, hyaluronic acid, high interstitial pressure, which suppresses vasculature and creates hypoxia. The TME creates both physical and biological barriers to impair therapy delivery and suppress immune responses.[18] Locoregional therapies may help overcome these barriers and improve oncological outcomes.

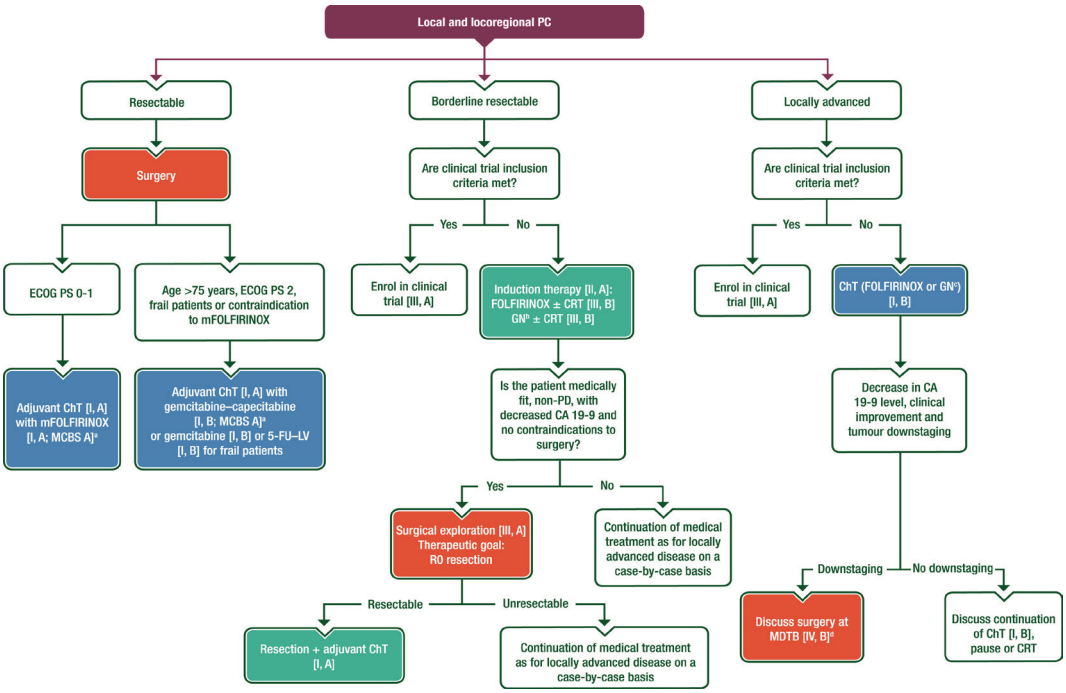


Figure 2. *Treatment flowchart for local and locoregional pancreatic cancer (PC). Red: surgery; blue: systemic anticancer treatment; green: combination therapies; white: other strategies. ECOG PS, Eastern Cooperative Oncology Group Performance Score; ChT, chemotherapy; FOLFIRINOX, leucovorin-5-fluorouracil-irinotecan-oxaliplatin; MCBS, magnitude of clinical benefit scale; 5-FU-LV, 5-fluorouracil-leucovorin; CRT, chemoradiotherapy; GN, gemcitabine-nab-paclitaxel; PD, progressive disease, CA 19-9, carbohydrate antigen 19-9; R0, no tumor at the resection margin; MDTB, multidisciplinary tumor board. [a]ESMO-MCBS v1.1. [b]Not approved as induction therapy by the European Medicines Agency (EMA) or the Food and Drug Administration (FDA). [c]Not EMA or FDA approved for locally advanced disease. [d]To be discussed if significant decrease in CA 19-9 level, clinical improvement, and tumor downstaging. This figure was reused with permission from CCC (501960653).*[16]

In the Netherlands, locoregional therapies for PDAC are not standardized, except for the possible use of neoadjuvant, induction, or palliative chemoradiotherapy as a second-line treatment option for borderline resectable and LAPC patients, a practice that is more commonly adopted outside the Netherlands.[16] However, due to the poor outcomes and high toxicity risks associated the chemotherapy, and technological advancements in locoregional therapies, they have gained increasing attention in medical research. The most studied locoregional treatment modalities include radiofrequency ablation (RFA), irreversible electroporation (IRE), and particularly radiotherapy, which can be delivered through conventional methods, stereotactic body radiation therapy (SBRT), or by magnetic resonance-guided linear accelerator (MR-Linac).[19] Radiotherapy can be utilized for palliative pain relief when medication is insufficient.[20] For borderline resectable and LAPC, complementary radiotherapy may be administered as neoadjuvant or induction therapy. However, there is limited evidence for an increased overall survival for local therapy following chemoradiotherapy, with a median overall survival of 15.2–17 months (95% CI 13.9–17.3) compared to 16.5 months (95% CI 14.5–18.5) following chemotherapy alone.[21,22] Nevertheless, in a pooled analysis to SBRT for LAPC a higher total

dose and more radiation fractions were significantly associated with a higher 1-year local control rate (*p=.03 and p=.019, respectively)*, although both factors were constrained by nearby vital structures and organs to a maximum dose of 50 Gy.[22]

Despite advancements in diagnostics, surgery, systemic therapy, and locoregional therapy, survival outcomes for PDAC remain poor. Between 2012 and 2021, the mortality rate decreased with only 0.2% per year.[4] This underscores the limited progress made for this patient population. Furthermore, patient comorbidities and the invasiveness of chemotherapy and surgery limit potential for further treatment intensification. There is a clear unmet clinical need for minimally invasive therapies that can improve tumor control, extend survival, and enhance quality of life.

Intratumoral injection therapy

Combining systemic therapy with minimally invasive intratumoral injection therapy offers a promising approach to improve local tumor control. For LAPC, this strategy could increase downstaging rates and increase R0 resection rates. In palliative care, it may reduce tumor burden, enhance quality of life, and extend survival. Intratumoral injections bypass systemic pathways, delivering therapeutics directly into the primary tumor. This approach aims to maximize tumor damage while minimizing harm to adjacent healthy tissues. Ideally, intratumoral injection therapies are administered minimally invasively, by carefully placing a needle directly into the tumor, either percutaneously or by endoscopic ultrasound (EUS). Therapeutics may include chemotherapeutic agents, immunotherapies, radioactive particles, or radioactive microparticles. One key focus of this dissertation is the last, also called microbrachytherapy**,** involving the intratumoral injection of radioactive microparticles.

A notable example of microbrachytherapy involves intratumoral injection of phosphorus-32 (^{32}P) microparticles. As a pure β^- particle emitter with a half-life of 14.3 days, ^{32}P delivers a localized therapeutic effect with a mean tissue penetration range of 3–4 mm and a maximum range of 8.2 mm.[23,24] In a pilot study by Ross et al. (2021), 43 patients with LAPC received EUS-guided ^{32}P microparticle injection aimed at a mean tumor dose of 100 Gray (Gy), achieving a local disease control rate of 82% (95% CI 68.6–90.9%) and a median overall survival of 15.5 months.[25] A comparative analyses with conventional treatment indicates a significant impact on overall survival, surgical resection rates and progression-free survival (p<.001) when ^{32}P intratumoral injection is combined with chemotherapy.[26] However, ^{32}P is a pure β^- emitter and lacks robust imaging capabilities for therapy delivery confirmation. In the study by Ross, et al. (2021) ^{32}P delivery confirmation was restricted to the presence or absence of radiation at the injection site on Bremsstrahlung SPECT, without any further dosimetry, which was present in 40 of 42 patients 4 hours post-procedure, and in 36 out of 42 patients 7 days post-procedure.[25] A more detailed description of the Bremsstrahlung SPECT of the same study

describes no radiation found in the tumor in 6 out of 42 patients (14.3%) radiation in other parts of the pancreas in 10 out of 42 patients (23.8%) and radiation in the gastrointestinal tract in 19 out of 42 patients (45.2%), 4 hours post-injection.[27]

Holmium-166 microspheres

Another promising therapeutic agent for microbrachytherapy, and a key focus of this dissertation, is holmium-166 microspheres (^{166}HoMS). Like ^{32}P, holmium-166 (^{166}Ho) is a radioactive isotope that emits β^- particles (1.7 MeV vs. 1.85 MeV, respectively), but with a significantly shorter half-life (26.83 hours vs. 14.3 days). This yields a higher dose rate and a faster therapeutic delivery for an equivalent total dose. Holmium-166 microspheres have been studied in various preclinical and clinical settings for intratumoral injection. First in 2011, in subcutaneous tumor-bearing rabbits[28], thereafter in orthotopic renal tumors in mice[29], in spontaneous liver and oral tumors in felines[30,31], in a spontaneous pituitary tumor and various soft tissue sarcomas in canines[32,33], and in human head and neck carcinomas.[34] From these studies, three overarching conclusions could be drawn: intratumoral injection with ^{166}HoMS appears to be minimally invasive and safe[28,30-34]; achieving a high tumor dose correlates with a pronounced tumor response[28-30,32,33]; and improving intratumoral distribution while minimizing extratumoral leakage remains critical.[31,34]

A key attribute of ^{166}HoMS, and a distinction from most other therapeutics, is its multimodality imaging capability, enabling conventional SPECT, computed tomography (CT) and magnetic resonance imaging (MRI), as presented in Figure 3. These properties facilitate enhanced analysis of periprocedural and postprocedural therapy delivery.

- **SPECT:** In addition to β^- particle emission, ^{166}Ho emits low-energy gamma (γ) rays with an energy of 80.6 KeV and a yield of 6.7%.[35] Contrary to the β^- particles, γ-rays penetrate out of the body with minimal cell damage and can be detected by γ-sensitive cameras. The γ-rays allow for conventional SPECT imaging.[36] Simplified, SPECT quantification is performed by assessing the spatial location and distribution of the ^{166}HoMS by the distribution of the detected γ-rays.
- **CT:** due to the high atomic mass (165.9 u) of ^{166}Ho and the fact that it constitutes between 17–20% of the microsphere mass, the microspheres attenuate X-rays, making it appear hyperdense on CT and enabling visualization and quantification.[34,37-39] CT quantification is based on the linear relationship between the holmium concentration in a voxel, and its hyperdensity, measured in Hounsfield Units (HU).[37,40]
- **MRI:** Holmium's paramagnetic properties affect the magnetic field utilized in MRI, causing fast signal decay in the voxels that contain holmium and enabling holmium visualization and

quantification.[39,41-43] Simplified, MRI quantification is based on the relationship between the holmium concentration in a voxel and the speed of the signal decay in that voxel

Within the ^{166}HoMS microspheres, only a minimal fraction (<0.001%) of the incorporated holmium-165 (stable isotope) is converted to holmium-166 (unstable isotope) through neutron bombardment in a nuclear reactor. Consequently, the CT and MRI imaging capabilities of the microspheres rely on holmium-165, which is also present in stable, non-radioactive, holmium-165 microspheres (^{165}HoMS). These non-radioactive but imageable ^{165}HoMS are particularly valuable for preclinical research (Figure 3). Multimodality imaging techniques utilizing both ^{165}HoMS and ^{166}HoMS provide critical insights into intratumoral injection therapies in PDAC. They could facilitate patient-specific treatment planning and execution, ultimately improving the safety and efficacy of therapeutic approach.

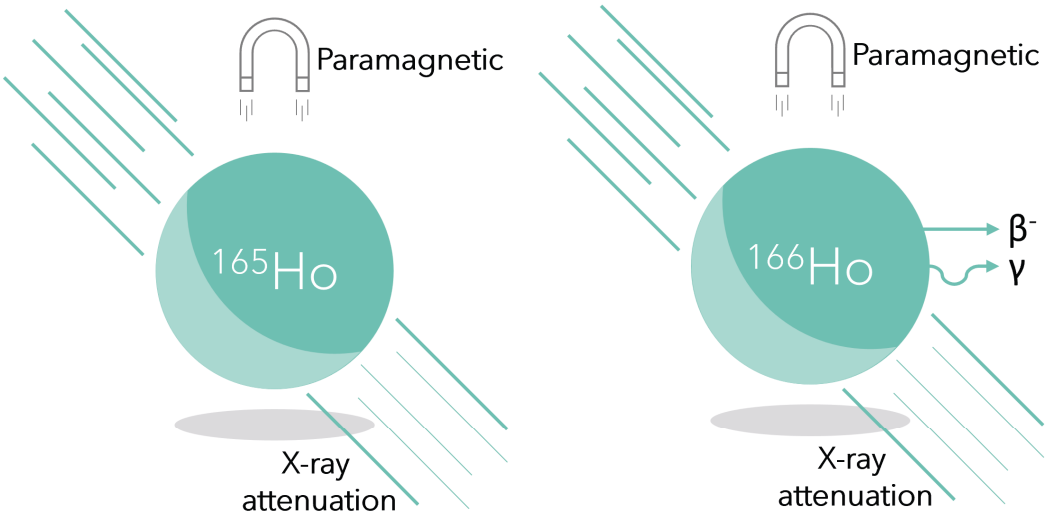


Figure 3. *Schematic overview of the multimodality imaging parameters (paramagnetic, X-ray attenuation, and γ-rays) of stable (non-radioactive) holmium-165 microspheres (^{165}Ho) and unstable (radioactive) holmium-166 microspheres (^{166}Ho). The β- particle emission causes the therapeutic effect of ^{166}Ho.*

Thesis outline

The primary aim of this thesis was to develop and evaluate the clinical application of holmium-166 microspheres for intratumoral injection in patients with pancreatic ductal adenocarcinoma, with the focus on safety and feasibility and the additional use of image guidance for periprocedural and post-procedural therapy delivery confirmation. An overview of all Chapters and their connections is presented in Figure 4.

Over the past decades, intratumoral injection therapies have been explored as complementary treatment options to improve local tumor control in patients with locally advanced pancreatic cancer. **Chapter 2** presents a systematic review of intratumoral injection therapies for locally advanced pancreatic cancer, aiming to provide a comprehensive understanding of intratumoral injection and evaluate its safety and oncological outcomes.

In **Chapter 3**, the insights gained from the review were applied in a preclinical study were the intratumoral holmium microsphere injections method was developed in surgically removed pancreatic cancer samples. This foundational research addresses key components, such as equipment, methodologies, imaging modalities, safety considerations, and risks, required before implementing this intervention in clinical practice.

The first in-human pilot study assessing the safety and feasibility of intratumoral holmium microsphere injection in patients with pancreatic cancer is presented in **Chapter 4**. This study translates and continues on the findings from Chapter 3 to a clinical setting, providing the first safety and feasibility results of this intervention. A novel and patient-friendly research method was applied to perform ultrasound-guided intratumoral injections as a backup treatment option for patients diagnosed with unresectable tumors during open exploratory surgery.

Chapter 5 presents the feasibility and safety results of minimally invasive and CT-guided intratumoral injections of holmium-166 microsphere in palliative patients with unresectable pancreatic ductal adenocarcinoma.

Finally, **Chapter 6** offers a general discussion about the main findings of this thesis and an extensive section on future perspectives. **Chapter 7** provides an overview in the form of summaries in both English and Dutch.

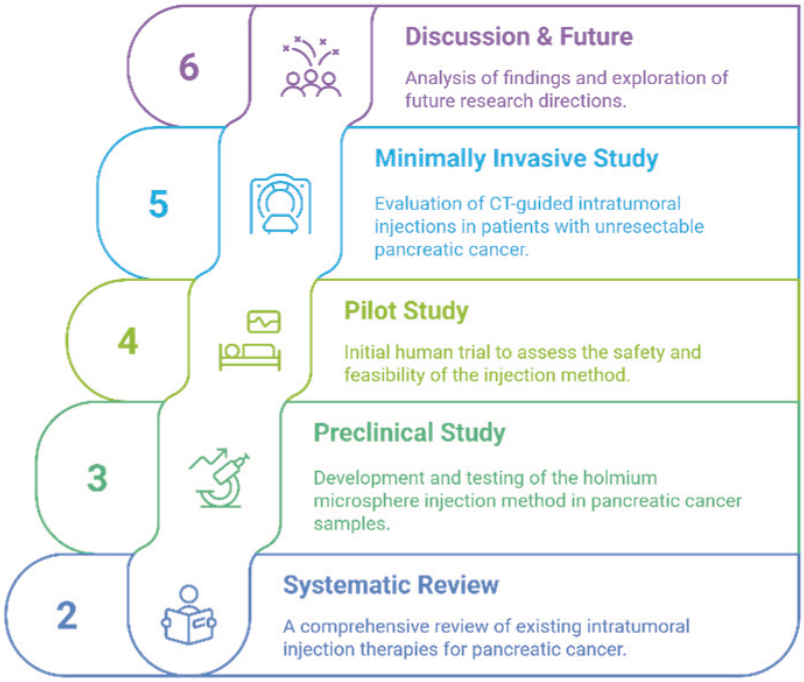


Figure 4. *The progression of intratumoral holmium-166 microsphere injection therapy in pancreatic cancer investigated in this thesis.*

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Chapter 2

INTRATUMORAL INJECTION THERAPIES FOR LOCALLY ADVANCED PANCREATIC CANCER: SYSTEMATIC REVIEW

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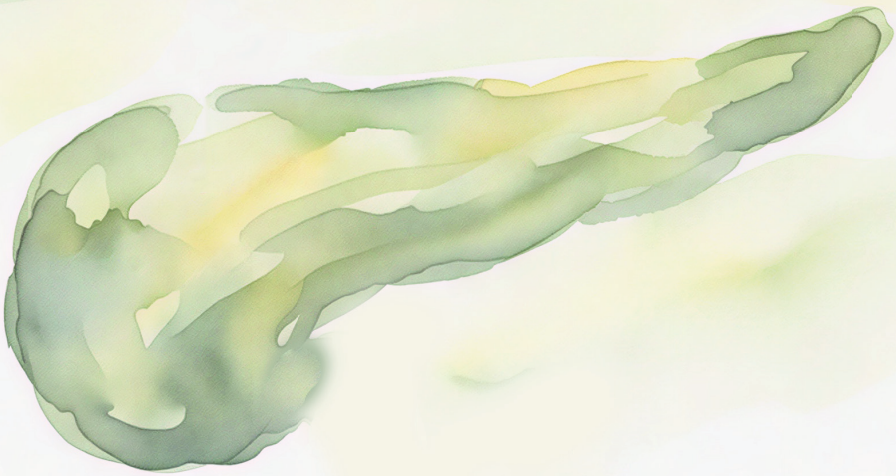

Abstract

Introduction: Pancreatic cancer has one of the worst prognoses of all cancers. Patients with locally advanced pancreatic cancer have a 12.7–20.2 per cent chance of receiving curative surgery after induction systemic chemotherapy. Intratumoral injection therapies have been studied as complementary treatment options for improved local tumor control. The aim of this systematic review was to provide an overview of intratumoral injection therapies, their safety, and oncological outcome in patients with locally advanced pancreatic cancer.

Methods: A literature search was conducted in PubMed, Embase and the Cochrane Library for articles written in English up to 28 November 2022. All study designs involving at least five patients with locally advanced pancreatic cancer who were treated with an intratumoral injection therapy were included. Critical appraisal of the included studies was performed using the Newcastle– Ottawa scale.

Results: After evaluation of the 1680 articles yielded by the systematic search, 52 studies treating 1843 patients were included. Included intratumoral injection treatment modalities comprised iodine-125 (^{125}I) seed brachytherapy (32 studies, 1283 patients), phosphorus-32 (^{32}P) microbrachytherapy (5 studies, 133 patients), palladium-103 (^{103}Pd) seed brachytherapy (2 studies, 26 patients), immunotherapy (9 studies, 330 patients), and chemotherapy (4 studies, 71 patients). Overall survival ranged between 7.0 and 16.0 months for ^{125}I, 5.2 and 15.5 months for ^{32}P, 6.9 and 10.0 months for ^{103}Pd, 5.8 and 13.8 months for immunotherapy, and 9.0 and 16.2 months for chemotherapy. Severe complication (greater than or equal to grade III complications using Clavien–Dindo classification) rates were 6.2 per cent for ^{125}I, 49.2 per cent for ^{32}P, 15 per cent for ^{103}Pd, 57.9 per cent for immunotherapy, and 0 per cent for chemotherapy.

Conclusion: Five intratumoral injection therapies are described and an overview is reported. Some intratumoral injection therapies for patients with locally advanced pancreatic cancer seem safe, although ^{32}P microbrachytherapy and immunotherapy require additional evidence. Currently available data are insufficient to provide firm conclusions regarding the added value to survival. The potential advantage of intratumoral injection therapies complementary to conventional care should be studied in well designed RCTs.

Introduction

Pancreatic cancer is diagnosed in over 440 000 people worldwide every year and the incidence has increased by 55% over the past 25 years.[1] The mortality is similar to the incidence due to the poor prognosis of this malignancy. With a 1-year overall survival (OS) of just 20% and 5-year survival of 9%, pancreatic cancer is one of the most aggressive forms of all common cancers.[2] When untreated, 5-year survival decreases to 3%.[3] Resection can be performed in just 20% of all patients and is the only potentially curative treatment option. At the time of diagnosis, around 50% of all patients with pancreatic cancer are affected by distant metastases and the remaining 30% have locally advanced pancreatic cancer (LAPC), making resection futile.[4,5] The most commonly used criteria for LAPC are those from the National Comprehensive Cancer Network (NCCN) guidelines, defining LAPC as greater than 180° arterial encasement or unreconstructible venous involvement without evidence of distant metastases.[6] Commonly, tumor involvement in the superior mesenteric artery, celiac axis or common hepatic artery or definite occlusion of the superior mesenteric vein or portal vein make pancreatic cancer unresectable.[7]

The current therapy of choice for LAPC is induction/palliative chemotherapy with FOLFIRINOX (a combination of 5-fluorouracil, irinotecan, leucovorin, and oxaliplatin) or gemcitabine with nab-paclitaxel, and response evaluation after 8 weeks.[8] During re-evaluation, if metastases remain absent and no tumor progression is observed, approximately 28–31% become eligible for exploration surgery, 13–20% receive surgical resection, and 16% have an R0 (greater than 1 mm) outcome.[9,10] Of the 80–87% of patients that do not receive surgical resection, approximately 25% have local tumor progression without metastatic disease and may benefit from local therapies.[9,10] Gemcitabine has been the recommended induction therapy for LAPC for over a decade and is still used in patients with a WHO performance score of 2 and higher.[8] OS for LAPC is approximately 14.8–24.2 months for FOLFIRINOX chemotherapy[11,12] and 9.0–16.0 months for gemcitabine-based chemotherapy[9,13]; however, most patients also undergo radiotherapy, ablation therapies, second-line chemotherapy, or resection before, during, or after the first-line chemotherapy treatment. For patients with severe comorbidities these extensive combination treatments are often considered impossible.[14] Some studies suggest that almost half of elderly patients (greater than 65 years) with no metastatic pancreatic cancer do not undergo chemotherapy or surgery, possibly due to comorbidities.[15]

For patients with stable unresectable disease after chemotherapy, local ablation is occasionally applied in clinical trials aiming to control local progression and to prolong survival.[16,17] Although ablation is considered feasible, it is also associated with substantial morbidity and mortality.[16] The effectiveness of additional local ablation is disputable because of the paucity of high-level evidence. Overall, small non-comparing case studies, hampered by selection bias, show a wide variation in OS from

5.0 up to 25.6 months.[16] Although some survival outcomes after ablation seem promising, the clinical demand for a minimally invasive therapy to improve local tumor control is still unmet.

Optimally, a local therapy for pancreatic cancer is minimally invasive, offers accurate treatment delivery with complete tumor coverage, spares healthy surrounding tissue, and has accurate therapy prediction and control. To meet these demands, over the past decades, novel intratumoral injection therapies for pancreatic cancer have been studied worldwide. Advancements in therapy control, advanced image acquisition, and processing, personalized treatment planning and immunological pathways have changed the perspective to achieve optimal local tumor control. With less invasive therapies, hospital stay and healthcare costs may decrease.[18] Safe treatment delivery reduces complication rates and benefits the quality of life. The aim of this systematic review was to provide an overview of intratumoral injection therapies, their safety, and oncological outcome in patients with LAPC.

Methods

This systematic review was conducted and reported conforming to PRISMA guidelines.[19] The methodology and inclusion/exclusion criteria were defined in advance by a Biomedical Information Specialist and the authors. This study was registered on PROSPERO, the international prospective register of systematic reviews (registration ID: CRD42020212862).

Search strategy

A literature search was conducted in PubMed, Embase, and the Cochrane Library for articles written in English from dates of inception up to 28 November 2022. The literature search was performed using medical domains combined by 'AND' between domains and within the domain by 'OR'. The first domain contained terms regarding pancreatic cancer, the second regarding intratumoral therapy, and the third regarding LAPC. Search terms were restricted to Medical Subject Headings, title, abstract, and keywords. Study selection and organization were performed using EndNote X9.2. The complete search strategy for each library is presented in Appendix S1. After a first scan to remove duplicate publications, the titles and abstracts were scanned for inclusion and exclusion criteria. Publications limited to an abstract were not excluded if the information was adequate, as described below. If multiple studies contained the same patient cohort, only the latest published article was included. If there was uncertainty regarding inclusion, a second author was consulted.

Definitions

LAPC was defined as an unresectable tumor due to vascular involvement without distant metastasis. Patients with vascular involvement resected at diagnosis or at any time during follow-up (for example

after induction chemotherapy) were not considered. A more detailed definition of LAPC (for example type and extent of vascular involvement)[20] could not be applied due to the time span and heterogeneity of the included studies.

Intratumoral injection therapy was defined as the injection of an active substance in the pancreatic tumor mass with the intention to treat or control the primary pancreatic cancer. Angiographically delivered therapy, infusion therapy, stenting, ablation, or post-resection treatments were not defined as intratumoral injection therapy. Studies performing non-resection surgical procedures, including cholangiojejunostomy, gastrojejunostomy, biliary/gastric bypass, and stent placement, were included if performed complementary or secondary to intratumoral injection therapy.

Study selection

Studies were included if they treated human patients suffering from LAPC with a single intratumoral injection therapy and presented outcomes regarding survival and/or safety. Articles had to be published in a registered journal defined by the SCImago Journal & Country Rank.[21] Studies were excluded if one or more of the following criteria was met: reviews, non-English articles, animal studies, case studies (or less than five patients in a single treatment population), minority LAPC in a treatment population, and resection immediately after intratumoral injection therapy.

Quality assessment

All studies passing the full-text assessment were critically appraised according to the Newcastle-Ottawa scale (NOS) for assessing the quality of non-randomized studies. The NOS is a validated scoring system with appraisals for case-control and cohort studies. RCTs were assessed using the cohort evaluation. A total of nine points could be appraised per study; four by selection, two by comparability, and the last three by either exposure or outcome of interest for case-control and cohort studies respectively. The complete scoring criteria are presented in Appendix S2. Studies with five stars or more were considered of good quality. Studies with less than five stars were not excluded.

Data extraction

Data on intratumoral injection therapy, dose, approach, cancer stage, metastases, combination therapies, median OS, and complications by the Clavien-Dindo classification[22], were extracted when available.[23] Furthermore, study characteristics, such as design, country, population characteristics, and sample size, were extracted from the included studies. Data extraction and organization was performed using Microsoft Excel for Microsoft 365.

Statistical analysis

Most outcomes were descriptive and due to the heterogeneity of the included studies, no meta-analysis or statistical analysis was performed.

Results

Starting with 1680 articles, after title and abstract screening for duplicates and exclusion criteria, 1600 studies were excluded. Eighty studies entered full-text assessment. Of these, 28 studies were excluded because of small sample size (12) and/or intervention not meeting the inclusion criteria (16). Some 52 clinical studies with 1843 patients were included for quality assessment. The complete results of the quality assessment are reported in Appendix S3. A detailed selection flow chart is shown in Figure 1.

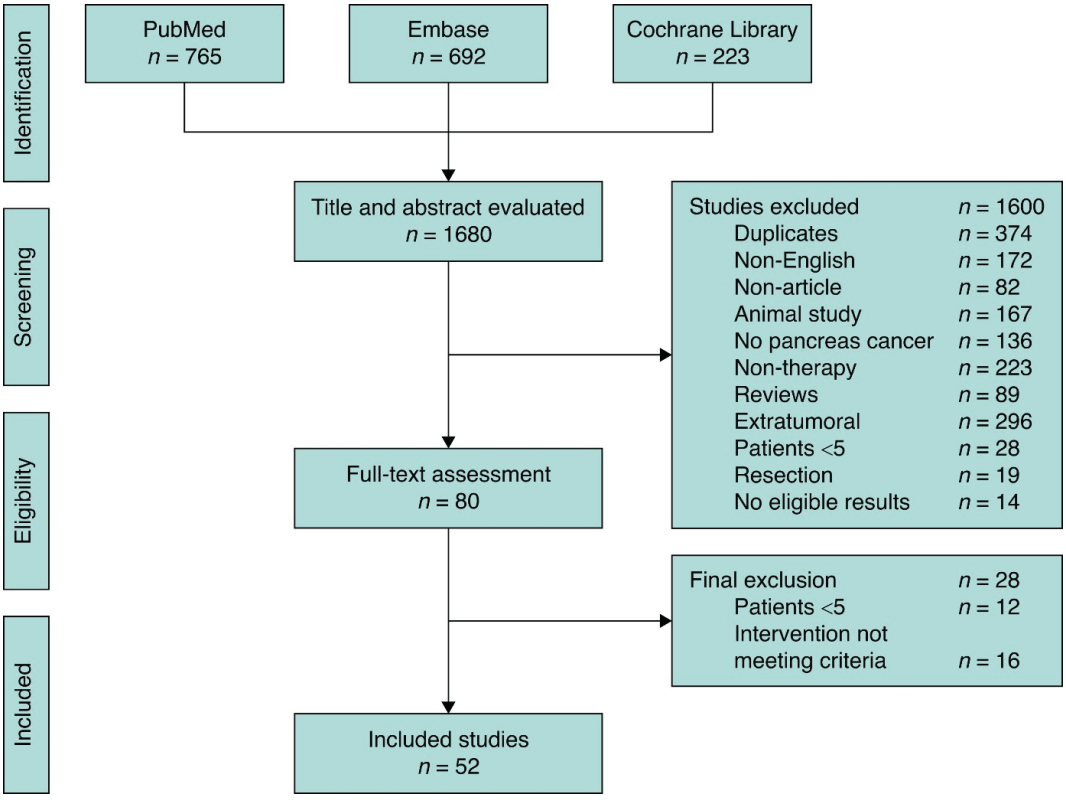

Figure 1. *PRISMA flow chart of the study selection*

The included studies comprised five different intratumoral injection treatment modalities: iodine-125 (^{125}I) seed brachytherapy (32 studies, 1283 patients), phosphorus-32 (^{32}P) microbrachytherapy (5 studies, 133 patients), palladium-103 (^{103}Pd) seed brachytherapy (2 studies, 26 patients), immunotherapy (9 studies, 330 patients), and chemotherapy (4 studies, 71 patients). Most of the included

studies had the following inclusion criteria in common: age greater than or equal to 18 years, adequate performance status (WHO/Eastern Cooperative Oncology Group (ECOG), Karnofsky), and an adequate hepatic, haematological, immune, and/or renal function. Gemcitabine-based chemotherapy was the most frequently used form of induction/palliative chemotherapy. One study combined intratumoral injection therapy with FOLFIRINOX chemotherapy.[24] All results are presented per modality and an overview of all intratumoral injection therapies is presented in Table 1.

Table 1. *Results of all intratumoral injection therapies for locally advanced pancreatic cancer*

Intratumoral injection therapy	No. of studies	No. of patients	Metastasis	Chemo-therapy	Chemo-radio-therapy	Radio-therapy	Overall complications, *n*	Complications ≥grade III	OS range (months)
Iodine-125	32	1283	212 of 1026 (20.6)	538 (47.5)	161 (14.2)	137 (12.1)	324 in 771	37 (6.2)	7–16
Phosphorus-32	5	133	19 of 133 (14.3)	53 (39.8)	65 (48.9)	0	1122 in 89	61 (49.2)	5.2–15.5
Palladium-103	2	26	1 of 26 (4)	2 (8)	20 (77)	0	6 in 26	4 (15)	5–7
Immunotherapy	9	330	147 of 294 (50.0)	72 (21.8)	252 (76.4)	0	316 in 223	33 (57.9)	5.8–13.8
Chemotherapy	4	71	15 of 53 (28)	18 (33)	36 (67)	0	3 in 41	0 (0)	9–16.2

Values are n (%) unless otherwise stated. OS, overall survival.

Iodine-125 seed brachytherapy

An overview of the results of intratumoral injection ^{125}I brachytherapy is presented in Table 2. Of the 32 studies applying ^{125}I brachytherapy in 1283 patients suffering from pancreatic adenocarcinoma, 15 had a retrospective design[25-39], 16 had an open-label prospective design[40-55], and 1 compared ^{125}I combined with chemotherapy versus chemotherapy alone in an RCT.[56] An overview of the characteristics of all applied radioactive isotopes is presented in Appendix S4.

In 27 studies reporting metastases, 212 of 1026 patients (21%) had or developed stage IV pancreatic cancer. The included studies utilized ^{125}I seeds with a length of 4.4 to 4.6 mm with a diameter of less than 1 mm.[42,57] Each patient received between 10 and 150 seeds in one or multiple operations depending on tumor volume, characteristics, and response. The median tumor dose ranged from 52 Gy[55] to 167 Gy[53], with most of the studies ranging between 100 and 150 Gy. For the application method, 14 studies (542 patients) implanted the seeds intraoperatively in an open approach using X-ray, CT, or ultrasound guidance.[27,28,30,34-36,38,40,44,46,48,50,51,54] Twelve studies (401 patients) used percutaneous implantation guided by ultrasound or CT.[25,26,31,32,39,43,45,49,52,53,55,56] Since 2006, four studies (179 patients) implemented endoscopic ultrasound (EUS) to deliver the radioactive seeds to the tumor with a transgastric or transduodenal injection.[37,41,42,47] In 28 studies with 1132 patients, 538 patients (48%), 161 patients (14%) and 137 patients (12%) received chemotherapy, chemoradiotherapy, or radiotherapy respectively.

Out of 600 patients in 15 studies reporting complications, 37 (6.2%) suffered from greater than or equal to grade III complications (using Clavien-Dindo classification). Three studies reported postprocedural mortality.[34,38,44] Four deaths were caused by abscesses or anastomotic leakage[38], three were caused by a pulmonary embolism[38,44], two were caused by duodenal ulcers[38], and one cause was not reported.[34] The most common complications reported were gastrointestinal haemorrhages[35,38,46,54], pancreatic fistula[34,35,44,54], leucocytopenia[47] and different intra-abdominal infections like pancreatitis and cholangitis[35,38,42,47,48,54]. The median OS ranged from 7.0 months[35,41] to 16 months.[31] In the RCT, no significant difference ($P > 0.05$) was found in adverse events between the ^{125}I combined with chemotherapy, versus chemotherapy alone.[56] However, a statistically significant difference ($P < 0.05$) was established between the OS of ^{125}I combined with chemotherapy (11.84 months) versus chemotherapy alone (10.40 months).[56]

Table 2. *Results of intratumoral iodine-125 seed brachytherapy for locally advanced pancreatic cancer*

Reference	*n*	Metastasis	Tumor dose (Gy), median* (range)	Combination therapy	Overall complications, *n*	Complications ≥grade III	Median* OS (months); OS initiation	NOS 1–9
Intraoperative								
Dobelbower *et al.*, 1986[40]	12	2 (17)	140 (120–210)	CRT 6 (50)	5	NR	15; D	8
Goertz *et al.*, 1990[27]	11	2 (18)	160	RT 11 (100)	11	1 (9)	8	7
Li *et al.*, 2020[28]	50	0 (0)	(110–160)	CHT 26 (52)	4	NR	12	9
Li *et al.*, 2016[30]	137	NR	NR	CHT 137 (100.0)	21	0 (0.0)	9.4; T	9
Montemaggi *et al.*, 1991[44]	7	0 (0)	82 (60–100)	RT 4 (57)	NR	3 (43)	7; T	5
Morrow *et al.*, 1984[34]	33	9 (27)	NR	NR	NR	8 (24)	8; T	4
Peretz *et al.*, 1989[35]	98	0 (0)	136	CHT 27 (28), RT 27 (28)	9	NR	7	4
Schuricht *et al.*, 1991[36]	42	0 (0)	(120–150)	CRT 42 (100)	24	NR	12.8	8
Shipley *et al.*, 1980[46]	12	6 (50)	160	RT 12 (100)	5	NR	11	6
Syed *et al.*, 1983[48]	18	1 (6)	(100–150)	RT 18 (100)	NR	4 (22)	14	7
Wang *et al.*, 2013[50]	28	0 (0)	120	CHT 10 (36), RT 7 (25)	NR	NR	10.1	4
Wang *et al.*, 2011[51]	27	15 (56)	(110–160)	CRT 6 (22), RT 1 (4)	NR	NR	8	4
Whittington *et al.*, 1984[38]	33	0 (0)	120	CRT 20 (61), RT 13 (39)	37	NR	9; D	8
Zheng *et al.*, 2017[54]	34	0 (0)	NR	CHT 8 (24)	6	NR	11; T	7
Total intraoperative	542	70 of 405 (17.3)	NA	CHT 208 (40.8), CRT 74 (14.5), RT 93 (18.3)	122 of 429	16 of 206 (7.8)	NA	NA
Percutaneous								
Chen *et al.*, 2021[25]	22	4 (18)	130	CHT 22 (100)	22	NR	11.7; T	9
Chi *et al.*, 2021[26]	21	0 (0)	130	CHT 21 (100)	24	0 (0)	13.2; T	4
Joyce *et al.*, 1990[43]	19	NR	160	RT 12 (63)	22	NR	8.1; T	8
Liu *et al.*, 2014[31]	30	0 (0)	NR	NR	6	NR	16	8
Lun *et al.*, 2015[56]	38	NR	NR	CHT 38 (100)	NR	NR	11.8	4†
Luo *et al.*, 2019[32]	35	NR	NR	CHT 35 (100)	NR	NR	9.5	7
Niu *et al.*, 2016[45]	60	0 (0)	115 (110–130)	NR	NR	0 (0)	10.4	6
Wang *et al.*, 2021[49]	28	NR	NR	NR	2	0 (0)	11.6	5
Wang *et al.*, 2017[52]	32	25 (78)	120	CHT 16 (50)	NR	0 (0)	14	6
Yang *et al.*, 2016[53]	18	0 (0)	167 (164–170)	CHT 18 (100), RT 1 (6)	10	3 (17)	7.3; T	5
Zhongmin *et al.*, 2010[55]	31	12 (39)	52	CHT 10 (32)	NR	NR	10.3; T	7
Zhou *et al.*, 2021[39]	67	0 (0)	NR	CHT 6 (9)	20	0 (0)	11; T	8
Total percutaneous	401	41 of 281 (14.6)	NA	CHT 166 (58.7), RT 13 (4.6)	106 of 205	3 of 226 (1.3)	NA	NA
EUS								
Du *et al.*, 2013[41]	100	40 (40.0)	140 (120–210)	CHT 100 (100.0)	52	0 (0.0)	7; T	8
Jin *et al.*, 2008[42]	22	8 (36)	NR	CHT 22 (100)	13	NR	9	6
Sun *et al.*, 2006[47]	15	7 (47)	140	RT 1 (7)	31	4 (27)	10.6; T	8
Sun *et al.*, 2017[37]	42	24 (57)	95	CHT 42 (100)	NR	0 (0)	9; T	2
Total EUS	179	79 of 179 (44.1)	NA	CHT 164 (91.6), RT 1 (0.6)	96 of 137	4 of 157 (2.5)	NA	NA
Other								
Li *et al.*, 2020[29]	50	22 (44)	NR	CRT 6 (12)	17	0 (0)	8.8	8
Mohiuddin *et al.*, 1994[33]	111	0 (0.0)	NR	CRT 81 (73.0), RT 30 (27.0)	NR	NR	11.4	3
Total (intraoperative, percutaneous, EUS, and other)	1283	212 of 1026 (20.7)	NA	CHT 538 (47.5), CRT 161 (14.2), RT 137 (12.1)	324 of 771	37 out of 600 (6.2)	NA	NA

*Values are n (%) unless otherwise stated. *If the median was unavailable the mean is presented. †RCT. OS, overall survival; NOS, Newcastle–Ottawa scale; CRT, chemoradiotherapy; NR, not reported; D, diagnosis; RT, radiotherapy; CHT, chemotherapy; T, treatment; NA, not applicable; EUS, endoscopic ultrasonography.*

Phosphorus-32 microbrachytherapy

An overview of the results of intratumoral injection ^{32}P microbrachytherapy is presented in Table 3. All five included studies (133 patients) using ^{32}P had a prospective design. One study compared ^{32}P combined with prior 5-fluorouracil and gemcitabine chemotherapy after injection versus chemotherapy alone in an RCT.[58]

One study included 19 patients (40%) with stage IV pancreatic cancer[59]. The remaining studies had no patients with metastases (total 14%).[24,58,60,61] ^{32}P was only injected percutaneously with CT guidance and achieved a tumor dose between 1255 and 19 000 Gy.[58,59,61] This was a notably higher dose than the dose of 100 Gy achieved by the more recent microparticle brachytherapy utilizing EUS application.[24,60]

Four studies (124 patients) reported complications. Some 61 patients (49%) patients suffered from greater than or equal to grade III complications. The most frequently reported complications included haematological toxicities[24,58-60], gastrointestinal haemorrhage[58,61], fatigue[24,58], and nausea[24,58]. The median OS of all five studies ranged from 5.2 months[58] to 15.5 months after inclusion.[24] The RCT by Rosemurgy et al. (2008) was abandoned at a preliminary stage after treating 18 out of 40 intended patients with ^{32}P, due to a statistically significant higher complication rate ($P = 0.03$) and lower survival ($P = 0.18$) in the ^{32}P group.[58] The authors also found the highest complication rate, with 75 greater than or equal to grade III complications in 16 out of 18 patients (89%)[58], followed closely by Ross et al. (2021) with 139 greater than or equal to grade III complications in 34 out of 42 patients (81%).[24] In contrast, Ross et al. (2021) did find the highest survival of 15.5 months in the intratumoral injection ^{32}P microbrachytherapy treatment group.[24]

Table 3. *Results of intratumoral phosphorus-32 microbrachytherapy for locally advanced pancreatic cancer*

Reference	*n*	Metastasis	Type of ^{32}P therapy	Tumor dose (Gy), median* (range)	Combination therapy	Overall complications, *n*	Complications ≥grade III	Median* survival (months); OS initiation	NOS 1–9
Percutaneous									
Order *et al.*, 1996[59]	47	19 (40)	MAA and colloidal chromic 32P	(9000–17 000)	CRT 47 (100)	27	10 (53)	9.9; T	8
Rosemurgy *et al.*, 2008[58]	18	0 (0)	Colloidal chromic 32P	1255	CRT 18 (100)	NR	16 (89)	5.2	8†
Westlin *et al.*, 1997[61]	17	0 (0)	MAA and colloidal chromic 32P	5000 (1390–19 000)	CHT 2 (12)	NR	1 (6)	7.6; T	6
Total percutaneous	82	19 of 82 (23)	MAA and/or colloidal chromic 32P	NA	CHT 2 (2), CRT 65 (79)	27 of 47	27 of 82 (33)	NA	NA
EUS									
Bhutani *et al.*, 2019[60]	9	0 (0)	Microparticle	100	CHT 9 (100)	NR	24 (NR)‡	NR	4
Ross *et al.*, 2021[24]	42	0 (0)	Microparticle	100	CHT 42 (100)	1095	34 (81)	15.5; Inc	5
Total EUS	51	0 of 51 (0)	Microparticle	NA	CHT 51 (100)	1095 of 42	34 of 42 (81)	NA	NA
Total	133	19 of 133 (14.3)	MAA and/or colloidal chromic 32P or Microparticle	NA	CHT 53 (39.8), CRT 65 (48.9)	1122 of 89	61 of 124 (49.2)	NA	NA

*Values are n (%) unless otherwise stated. *If the median was unavailable the mean is presented. †RCT. ‡n = number of complications. OS, overall survival; NOS, Newcastle–Ottawa scale; MAA, macroaggregated albumin; CRT, chemoradiotherapy; T, treatment; NR, not reported; CHT, chemotherapy; NA, not applicable; EUS, endoscopic ultrasonography; Inc, inclusion.*

Palladium-103 seed brachytherapy

An overview of the results of ^{103}Pd seed brachytherapy is presented in Table 4. In 1996, two prospective studies applied ^{103}Pd seed brachytherapy in 26 patients.[62,63]

From the included 26 patients, 1 patient suffered stage IV pancreatic cancer.[63] On average, all patients were submitted to a dose of 110–124.2 Gy after intraoperative implantation. Two patients also underwent complementary chemotherapy[63] and 20 underwent chemoradiotherapy.[62,63] Some four patients suffered greater than or equal to grade III complications, including duodenal perforation, sepsis, cerebral vascular accident, and radiation enteritis.[63] An OS was found of 6.9 months[63] and 10 months.[62]

Table 4. *Results of intratumoral palladium-103 seed brachytherapy for locally advanced pancreatic cancer*

Reference	*n*	Metastasis	Median* tumor dose (Gy)	Combination therapy	Overall complications, *n*	Complications ≥grade III	Median* survival (months); OS initiation	NOS 1–9
Nori *et al.*, 1996[62]	15	0 (0)	110	CRT 15 (100)	NR	0 (0)	10; T	5
Raben *et al.*, 1996[63]	11	1 (9)	124.4	CHT 2 (18), CRT 5 (45)	6	4 (36)	6.9	7
Total	26	1 of 26 (4)	NA	CHT 2 (8), CRT 20 (77)	6 of 26	4 of 26 (15)	NA	NA

*Values are n (%) unless otherwise stated. *If the median was unavailable the mean is presented. OS, overall survival; NOS, Newcastle–Ottawa scale; CRT, chemoradiotherapy; NR, not reported; T, treatment; CHT, chemotherapy; NA, not applicable.*

Immunotherapy

An overview of the results of intratumoral injection immunotherapy is presented in Table 5. Nine studies applied immunotherapy to 330 patients within a prospective design[64-72], of which two were RCTs.[67,71] The two RCTs compared chemotherapy alone versus chemotherapy with an oncolytic virus.[67,71] The first RCT used TNFerade adenovirus with 5-fluorouracil chemoradiotherapy[67] and the other one an H101 adenovirus for p53 activation with gemcitabine[71].

Six studies reported metastases and half of the patients (n= 147) had metastatic disease.[65-70] Five studies injected adenoviruses to increase p53 activation.[64,65,69,71,72] Two studies implanted TNFerade biologic, which enables tumor-specific delivery of tumor necrosis factor (TNF)-α by radiation-inducible gene transfer.[66,67] One study injected zoledronate-pulsed dendritic cells combined with intravenous adoptive activated T lymphocytes to induce a CD8+ response.[68] Another study injected a double-stranded RNA oligonucleotide, called STNM01, to suppress a specific tumor growth factor (CHST15).[70] In six studies the injection was guided by EUS[64,65,68,70-72], in one study the injection was percutaneous[69], and two studies used both methods.[66,67] From the 330 patients receiving immunotherapy, 72 patients (22%) also underwent chemotherapy[64,65,68,71,72] and 252 (76%) underwent chemoradiotherapy.[66,67,69]

Four studies reported complication rates; 9 out of 36 patients (81%) after p53 adenovirus therapy[65,69], 4 out of 15 patients (27%) after zoledronate-pulsed dendritic cell injection[68], and 0 out of 6 patients (0%) after STNM01 injection suffered greater than or equal to grade III complications.[70] One study reported two cases of postprocedural mortality. One was caused by progressive disease and one was caused by a splenic artery thrombosis within 30 days post-intervention.[66] The most frequently presented complications included leucocytopenia[65,67-69], severe pain[66,67], fever[64,65,67-69,71,72], gastrointestinal bleeding[66,67], and intra-abdominal infection.[66,67] The median OS ranged between 5.8 months[70] and 13.8 months.[69] In the first RCT, no significant difference in greater than or equal to grade II complications ($P = 0.08$) or OS ($P = 0.26$) was found between the TNFerade adenovirus injection combined with 5-fluorouracil chemoradiotherapy (greater than or equal to grade II complications 75.9%, OS 10 months) and the chemoradiotherapy alone (greater than or equal to grade II complications 65.6%, OS 10 months).[67] The RCT applying H101 adenovirus for p53 activation did not report complications; this RCT found a significant difference ($P = 0.004$) in OS between the H101 adenovirus injection combined with gemcitabine (9 months) versus gemcitabine alone (6 months).[71]

Table 5. *Results of intratumoral immunotherapy for locally advanced pancreatic cancer*

Reference	*n*	Metastasis	Type of immunotherapy	Imaging	Combination therapy	Overall complications, *n*	Complications ≥grade III	Median* survival (months); OS initiation	NOS 1–9
p53 activation pathway									
Gong *et al.*, 2011[64]	9	NR	H101 adenovirus	EUS (100)	CHT 9 (100)	NR	NR	7	7
Xiao *et al.*, 2011[71]	19	NR	H101 adenovirus	EUS (100)	CHT 19 (100)	NR	NR	9	6†
Yunwei *et al.*, 2010[72]	8	NR	H101 adenovirus	EUS (100)	CHT 8 (100)	NR	NR	6	3
Hecht *et al.*, 2003[65]	21	12 (57)	ONYX-015 adenovirus	EUS (100)	CHT 21 (100)	NR	21 (100)	7.5; T	4
Li *et al.*, 2011[69]	15	8 (53)	p53 adenovirus	Percutaneous ultrasonography (100)	CRT 15 (100)	64	8 (53)	13.8	7
Total p53 activation pathway	72	20 of 36 (56)	NA	EUS (79), Percutaneous ultrasonography (21)	CHT 57 (79), CRT 15 (21)	64 of 15	29 of 36 (81)	NA	NA
Tumor necrosis factor-α pathway									
Hecht *et al.*, 2012[66]	50	0 (0)	TNFerade biologic	EUS (54), percutaneous (46)	CRT 50 (100)	NR	65 (NR)‡	9.9; Inc	8
Herman *et al.*, 2013[67]	187	132 (70.6)	TNFerade biologic	EUS (50.8), Percutaneous, ultrasonography/ CT (49.2)	CRT 187 (100.0)	219	116 (NR)‡	10; R	8†
Total tumor necrosis factor-α pathway	237	132 of 237 (55.7)	NA	EUS (51.5), percutaneous ultrasonography/ CT (48.5)	CRT 237 (100.0)	219 of 187	NR	NA	NA
Other immunotherapy									
Hirooka *et al.*, 2018[68]	15	0 (0)	Zoledronate-pulsed dendritic cells	EUS	CHT 15 (100)	33	4 (27)	11.5	8
Nishimura *et al.*, 2018[70]	6	5 (83)	STNM01 oligonucleotide	EUS	None	0	0 (0)	5.8	5
Total	330	147 of 294 (50.0)	NA	NA	CHT 72 (21.8), CRT 252 (76.4)	316 of 223	33 of 57 (57.9)	NA	NA

*Values are n (%) unless otherwise stated. *If the median was unavailable the mean is presented. †RCT. ‡n = number of complications. OS, overall survival; NOS, Newcastle–Ottawa scale; NR, not reported; EUS, endoscopic ultrasonography; CHT, chemotherapy; T, treatment; CRT, chemoradiotherapy; NA, not applicable; Inc, Inclusion; R, randomization.*

Intratumoral chemotherapy

An overview of the results of intratumoral injection chemotherapy is presented in Table 6. Four prospective studies performed intratumoral chemotherapy in 71 patients.[73-76] One RCT studied a chemotherapy capsule implant (5-fluorouracil) combined with systemic chemotherapy of gemcitabine versus systemic gemcitabine alone.[74]

Three studies reported patients with metastases (15 out of 53 patients (28%)).[73,75,76] Gemcitabine injection was guided by EUS.[73,75] One study analyzed the intratumoral distribution of percutaneous injection by injecting 1–2 ml of radiopaque agent before injecting gemcitabine and cisplatin with fibrin glue.[76] Capsules incorporating 5-fluorouracil were implanted intraoperatively followed by fibrin gel to prevent

pancreatic fistula.[74] Two studies, including 54 patients, reported 18 patients (33%) with combined systemic chemotherapy and 36 (67%) with chemoradiotherapy.[73,74]

Three studies reported no occurrence of greater than or equal to grade III complications.[73,75,76] OS ranged from 9 months[75] to 16.2 months.[76] The RCT did not report complication rates and no significant difference ($P = 0.07$) was found in the survival between treatment with implanted 5-fluorouracil capsules combined with systemic gemcitabine (10.3 months) versus systemic gemcitabine alone (8.1 months).[74]

Table 6. *Results of intratumoral chemotherapy for locally advanced pancreatic cancer*

Reference	*n*	Metastasis	Type of intratumoral CHT	Intratumoral CHT dose (mg), median* (range)	Combination therapy	Overall complications, *n*	Complications ≥grade III	Median* survival (months); OS initiation	NOS 1–9
Levy *et al.*, 2011[73]	36	11 (30)	Gemcitabine	90 (28–280)	CRT 36 (100)	0	0 (0)	9.3; T	5
Li *et al.*, 2016[74]	18	NR	5-Fluorouracil capsule	(800–1500)	CHT 18 (100)	NR	NR	10.3	7†
Mohamadnejad *et al.*, 2015[75]	12	0 (0)	Gemcitabine	168 (80–200)	CHT (NR), CRT (NR)	NR	0 (0)	9	8
Yang *et al.*, 2017[76]	5	4 (80)	Gemcitabine	(400–600)	NR	3	0 (0)	16.2	7
			Cisplatin	(11.25–22.5)					
Total	71	15 of 53 (28)	NA	NA	CHT 18 (33), CRT 36 (67)	3 of 41	0 of 53 (0)	NA	NA

*Values are n (%) unless otherwise stated. *If the median was unavailable the mean is presented. †RCT. CHT, chemotherapy; OS, overall survival; NOS, Newcastle–Ottawa scale; CRT, chemoradiotherapy; T, treatment; NR, not reported; NA, not applicable.*

Discussion

This systematic review reveals data on five types of intratumoral injection therapy with widely heterogeneous safety and survival outcomes in patients with LAPC.

^{125}I brachytherapy, intratumoral chemotherapy, and ^{103}Pd brachytherapy are associated with low rates of greater than or equal to grade III complications in the current literature review. In contrast, the complication rates of ^{32}P brachytherapy and intratumoral immunotherapy were at least three-fold higher. Within the ^{32}P and immunotherapy intervention groups, less complications seemed to be related to the injection procedure and more to the injected agents.[24,60,61,64,68,69] A common procedure-related complication was bacterial infection from the gastrointestinal tract into the pancreas, which was easily treated with antibiotics.[65] For immunotherapy the method of injection (EUS or percutaneous) did not seem to influence complication rates.[65,66] An explanation for the high severe complication rate after immunotherapy is the triggered autoimmune response. After immunotherapy intra-abdominal infection[66,67] and fever were often observed.[64,65,67-69,71,72] These are clear signs of an autoimmune response.[77] Current research into upcoming immunotherapies also attempts to identify and control these side

effects.[78] The high complication rate after ^{32}P microbrachytherapy is possibly due to a radiation overdose and therapy diffusion into healthy tissues.[58] Rosemurgy et al.[58] (2008), reported therapy diffusion of ^{32}P into nearby tissues and found a high complication rate (89%), whereas Westlin et al.[61] (1997) did not report therapy diffusion and found much lower complication rate (6%) with an exceptionally higher median tumor dose (1227 versus 11050 Gy, respectively). Ross et al.[24] (2021) also found a high complication rate (81%) after a median tumor dose of only 100 Gy (±20%); however, they also claimed that only 8 out of 139 (5.8%) severe complications were ^{32}P or procedure related, and reported that almost no therapy diffusion occurred outside the tumor. These heterogeneous results might suggest that, with therapy deposition central within the tumor, possibly with image guidance for improved treatment control and clear safety margins, microbrachytherapy could still prove to be a safe treatment method for LAPC.

The rate of severe complications after ^{125}I brachytherapy, intratumoral chemotherapy, and ^{103}Pd brachytherapy was below the complication rate of the most common ablative treatment for LAPC (radiofrequency ablation (RFA)). Rombouts et al.[16] (2015) published a systematic review concerning ablative treatment methods for LAPC.[16] In the RFA group an overall complication rate of 24.2% was found, with a 13.6% RFA-procedure-related complication rate.[16]

LAPC patients undergoing intratumoral injection therapy are generally also treated with systemic chemotherapy. Systemic chemotherapy is associated with side effects, such as leucocytopenia and thrombocytopenia.[24,47,58-60,65,67-69,79] Even after modern chemotherapy regimens, such as FOLFIRINOX, complication rates range from 19–23%[80] up to 50%.[11] Whether complications are related to intratumoral injection therapy or systemic chemotherapy can be difficult to identify. Overall, 11 cases of short-term postprocedural mortality were reported. Three RCTs compared complication rates of systemic chemotherapy combined with intratumoral injection therapy versus systemic chemotherapy alone. Two found no significant difference (^{125}I and TNFerade)[56,67] and one did find a significant difference with disadvantage towards ^{32}P.[58] An RCT with modern chemotherapy regimens, with and without intratumoral injection therapy, should be the cornerstone to assess safety in this patient population.

The survival outcomes of the intratumoral injection modalities varied considerably between 5.0 and 16.2 months. No single intratumoral injection modality showed consistent high survival outcomes. Regarding survival outcome, Ross et al.[24] (2021) showed the most promising results with a median OS of 15.5 months in 42 patients after receiving ^{32}P microbrachytherapy. Considering, the absence of a control group and, therefore, the high chance of selection bias, the benefit of ^{32}P is still questionable.[24]

With regards to ablative treatment, Rombouts et al.[16] (2015) found an OS between 5.0 and 25.6 months in the RFA group. The highest survival of 25.6 months was found when RFA was combined with several different therapies, including intra-arterial plus systemic chemotherapy.[81] When RFA was applied as monotherapy, the median survival was 14.7 months. Still, evident selection bias was present.[81] More recent studies applying RFA for LAPC patients found a survival between 5.0 and 9.0 months with and without a combination of chemotherapy.[82,83] Overall, similar survival results are shown for most intratumoral therapies in this review.

Whether intratumoral injection therapy contributes to the survival of patients with LAPC remains questionable with the currently available literature. Due to the insidious onset and probable microscopic spread at the time of diagnosis, pancreatic cancer is essentially a systemic disease and local therapies may not contribute to survival.[36] Even if no metastases are found at the time of diagnosis, the disease may have already spread to the pancreatic surroundings. The OS results of the current review substantiate this theory by showing slightly improved survival after chemotherapy, compared with no chemotherapy in the ^{125}I group and similar survival between studies with and without metastatic disease.[69,76] The potential clinical benefit of local tumor therapy in patients with pancreatic cancer is not limited to survival. Local tumor response and local progression-free survival can be of great value for patients, especially when providing pain relief and improving and prolonging the performance score and quality of life.

The included studies have several limitations. Most studies were case series and cohort studies with small sample sizes. Selection bias in several forms hampers the quality of the studies, such as the type of LAPC classification guideline (NCCN OR AJCC)[17], local diagnostic and treatment protocols, additional diagnostic research, and prior treatment completion. To take selection bias into consideration, the NOS was applied.

To present a clear overview, many results had to be filtered or adjusted to fit certain classifications. Therefore, undetailed data were often excluded from the analysis.

Combination therapies have been categorized by the type of therapy (for example chemotherapy, chemoradiotherapy, or radiotherapy) and not by the technical aspects, start, duration, dose, and iteration of the cycles. Even though all studies, except one, used gemcitabine-based chemotherapy, the current movement towards FOLFIRINOX-based chemotherapy might have a radical impact on oncological outcomes soon. Metastases were present at different rates, locations, and quantities within each study. Additionally, studies were not excluded if metastatic disease was present or occurred in a minority of the included patients. A large variation in survival was seen, which could partly be

explained by the moment from which survival was measured (for example the initial diagnosis, inclusion in the study, or the intervention); however, this was not consistently reported. Potential differences in lead time of several months may have had a great impact on OS differences.

Conclusion

Five intratumoral injection therapies are described and an overview is reported. Some intratumoral injection therapies for patients with LAPC seem safe, although ^{32}P microbrachytherapy and immunotherapy require additional evidence. Currently available data on all modalities are insufficient to provide firm conclusions regarding the added value to survival. Clinical benefits of these procedures are potentially not limited to survival, but control of local tumor growth could be of great value for patients, especially when providing pain relief and improving quality of life. The potential advantage of intratumoral injection therapies complementary to conventional care should therefore be studied in well designed RCT.

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Appendices

Appendix S1 – Search strategy

PubMed

"Pancreatic Neoplasms"[Mesh] OR
Pancreatic Neoplasm*[tiab] OR
Pancreatic adeno*[tiab] OR
Pancreas adeno*[tiab] OR
Pancreas Neoplasm*[tiab] OR
Pancreatic carcino*[tiab] OR
Pancreas carcino*[tiab] OR
Pancreas tum*[tiab] OR
Pancreatic tum*[tiab] OR
Cancer of Pancreas[tiab] OR
Tumour of the pancreas[tiab] OR
Pancreas Cancer*[tiab] OR
Pancreatic Cancer*[tiab] OR
Cancer of the Pancreas[tiab] OR
Carcinoma of the pancreas[tiab] OR
Islet Cell Adenoma* [tiab] OR
Insulinoma*[tiab] OR
Islet Cell Carcinoma* [tiab] OR
Gastrinoma*[tiab] OR
Glucagonoma*[tiab] OR
Somatostatinoma*[tiab] OR
Vipoma*[tiab] OR
Pancreatic Ductal Carcino* [tiab] OR
Pancreatic Ductal Adeno*[tiab] OR
Pancreatic Ductal Neoplasm*[tiab] OR
Pancreatic Ductal Cancer*[tiab] OR
Pancreatic Intraductal Carcino*[tiab] OR
Pancreatic Intraductal Adeno*[tiab] OR
Pancreatic Intraductal Neoplasm*[tiab] OR
Pancreatic Intraductal Cancer*[tiab] OR
Pancreas Ductal Carcino* [tiab] OR
Pancreas Ductal Adeno*[tiab] OR
Pancreas Ductal Neoplasm*[tiab] OR
Pancreas Ductal Cancer*[tiab] OR
Pancreas Intraductal Carcino*[tiab] OR
Pancreas Intraductal Adeno*[tiab] OR
Pancreas Intraductal Neoplasm*[tiab] OR
Pancreas Intraductal Cancer*[tiab]

AND

"Brachytherapy"[Mesh] OR
Brachytherap*[tiab] OR
Curietherap*[tiab] OR
Intracavit*[tiab] OR
Interstitial*[tiab] OR
intratumo*[tiab] OR
intra-tumo*[tiab] OR
tumour-infiltrat*[tiab] OR
tumourinfiltrat*[tiab] OR
tumour-infiltrat*[tiab] OR
tumourinfiltrat*[tiab] OR
intralesion*[tiab] OR
intra-lesion*[tiab] OR
interstitial*[tiab] OR
implant*[tiab] OR
Local therap*[tiab]

AND

unresect*[tiab] OR
non-resect*[tiab] OR
nonresect*[tiab] OR
not-resect*[tiab] OR
unremov*[tiab] OR
LAPC[tiab] OR
Locally advanced pancreatic[tiab] OR
Incompletely resectable*[tiab] OR
incomplete resection*[tiab] OR
Partial-resect*[tiab] OR
Inoper*[tiab] OR
Non-opera*[tiab] OR
Nonopera*[tiab] OR
Not-opera*[tiab] OR
Not-surgic*[tiab] OR
Non-surgic*[tiab] OR
Nonsurgic*[tiab]

EMBASE

exp pancreas islet cell carcinoma/ or exp pancreas adenoma/ or exp pancreas malformation/ or exp pancreas cancer/ or exp pancreas islet cell tumour/ or exp pancreas adenocarcinoma/ or exp pancreas tumour/ or exp pancreas carcinoma/ or exp pancreas metastasis/ or exp pancreas islet cell hyperplasia/ or exp insulinoma/ or exp gastrinoma/ or exp glucagonoma/ or exp somatostatinoma/ or exp vipoma/ or exp pancreatic cancer cell line/ or exp pancreatic ductal carcinoma cell line/ or

Pancreatic Neoplasm*.ti,ab,kw. OR
Pancreatic adeno*.ti,ab,kw. OR
Pancreas adeno*.ti,ab,kw. OR
Pancreas Neoplasm*.ti,ab,kw. OR
Pancreatic carcino*.ti,ab,kw. OR
Pancreas carcino*.ti,ab,kw. OR
Pancreas tum*.ti,ab,kw. OR
Pancreatic tum*.ti,ab,kw. OR
Cancer of Pancreas.ti,ab,kw. OR
Tumour of the pancreas.ti,ab,kw. OR
Pancreas Cancer*.ti,ab,kw. OR
Pancreatic Cancer*.ti,ab,kw. OR
Cancer of the Pancreas.ti,ab,kw. OR
Carcinoma of the pancreas.ti,ab,kw. OR
Islet Cell Adenoma*.ti,ab,kw. OR
Insulinoma*.ti,ab,kw. OR
Islet Cell Carcinoma*.ti,ab,kw. OR
Gastrinoma*.ti,ab,kw. OR
Glucagonoma*.ti,ab,kw. OR
Somatostatinoma*.ti,ab,kw. OR
Vipoma*.ti,ab,kw. OR
Pancreatic Ductal Carcino*.ti,ab,kw.OR
Pancreatic Ductal Adeno*.ti,ab,kw. OR
Pancreatic Ductal Neoplasm*.ti,ab,kw.OR
Pancreatic Ductal Cancer*.ti,ab,kw. OR
Pancreatic Intraductal Carcino*.ti,ab,kw. OR
Pancreatic Intraductal Adeno*.ti,ab,kw.OR
Pancreatic Intraductal Neoplasm*.ti,ab,kw. OR
Pancreatic Intraductal Cancer*.ti,ab,kw. OR
Pancreas Ductal Carcino*.ti,ab,kw. OR
Pancreas Ductal Adeno*.ti,ab,kw. OR
Pancreas Ductal Neoplasm*.ti,ab,kw. OR
Pancreas Ductal Cancer*.ti,ab,kw. OR
Pancreas Intraductal Carcino*.ti,ab,kw. OR
Pancreas Intraductal Adeno*.ti,ab,kw. OR
Pancreas Intraductal Neoplasm*.ti,ab,kw. OR
Pancreas Intraductal Cancer*:ti,ab,kw

AND

exp intratumoral drug administration/ or exp brachytherapy implant/ or exp brachytherapy/ or

Brachytherap*.ti,ab,kw. OR
Curietherap*.ti,ab,kw. OR
Intracavit*.ti,ab,kw. OR
Interstitial*.ti,ab,kw. OR
intratumo*.ti,ab,kw. OR
intra-tumo*.ti,ab,kw. OR
tumour-infiltrat*.ti,ab,kw. OR
tumourinfiltrat*.ti,ab,kw. OR
tumour-infiltrat*.ti,ab,kw. OR
tumourinfiltrat*.ti,ab,kw. OR
intralesion*.ti,ab,kw. OR
intra-lesion*.ti,ab,kw. OR
interstitial*.ti,ab,kw. OR
implant*.ti,ab,kw. OR
Local therap*:ti,ab,kw

AND

unresect*.ti,ab,kw. OR
non-resect*.ti,ab,kw. OR
nonresect*.ti,ab,kw. OR
not-resect*.ti,ab,kw. OR
unremov*.ti,ab,kw. OR
LAPC.ti,ab,kw. OR
Locally advanced pancreatic.ti,ab,kw.OR
Incompletely resectable*.ti,ab,kw. OR
incomplete resection*.ti,ab,kw. OR
Partial-resect*.ti,ab,kw. OR
Inoper*.ti,ab,kw. OR
Non-opera*.ti,ab,kw. OR
Nonopera*.ti,ab,kw. OR
Not-opera*.ti,ab,kw. OR
Not-surgic*.ti,ab,kw. OR
Non-surgic*.ti,ab,kw. OR
Nonsurgic*:ti,ab,kw

Cochrane Library

#1 MeSH descriptor: [Pancreatic Neoplasms] explode all trees

#2

Pancreatic Neoplasm*:ti,ab,kw or
Pancreatic adeno*:ti,ab,kw or
Pancreas adeno*:ti,ab,kw or
Pancreas Neoplasm*:ti,ab,kw or
Pancreatic carcino*:ti,ab,kw or
Pancreas carcino*:ti,ab,kw or
Pancreas tum*:ti,ab,kw or
Pancreatic tum*:ti,ab,kw or
Cancer of Pancreas:ti,ab,kw or
Tumour of the pancreas:ti,ab,kw or
Pancreas Cancer*:ti,ab,kw or
Pancreatic Cancer*:ti,ab,kw or
Cancer of the Pancreas:ti,ab,kw or
Carcinoma of the pancreas:ti,ab,kw or
Islet Cell Adenoma*:ti,ab,kw or
Insulinoma*:ti,ab,kw or
Islet Cell Carcinoma*:ti,ab,kw or
Gastrinoma*:ti,ab,kw or
Glucagonoma*:ti,ab,kw or
Somatostatinoma*:ti,ab,kw or
Vipoma*:ti,ab,kw or
Pancreatic Ductal Carcino*:ti,ab,kw or
Pancreatic Ductal Adeno*:ti,ab,kw or
Pancreatic Ductal Neoplasm*:ti,ab,kw or
Pancreatic Ductal Cancer*:ti,ab,kw or
Pancreatic Intraductal Carcino*:ti,ab,kw or
Pancreatic Intraductal Adeno*:ti,ab,kw or
Pancreatic Intraductal Neoplasm*:ti,ab,kw or
Pancreatic Intraductal Cancer*:ti,ab,kw or
Pancreas Ductal Carcino*:ti,ab,kw or
Pancreas Ductal Adeno*:ti,ab,kw or
Pancreas Ductal Neoplasm*:ti,ab,kw or
Pancreas Ductal Cancer*:ti,ab,kw or
Pancreas Intraductal Carcino*:ti,ab,kw or
Pancreas Intraductal Adeno*:ti,ab,kw or
Pancreas Intraductal Neoplasm*:ti,ab,kw or
Pancreas Intraductal Cancer*:ti,ab,kw

#3 MeSH descriptor: [Brachytherapy] explode all trees

#4

Brachytherap*:ti,ab,kw or
Curietherap*:ti,ab,kw or
Intracavit*:ti,ab,kw or
Interstitial*:ti,ab,kw or
intratumo*:ti,ab,kw or
intra-tumo*:ti,ab,kw or
tumour-infiltrat*:ti,ab,kw or
tumourinfiltrat*:ti,ab,kw or
tumour-infiltrat*:ti,ab,kw or
tumourinfiltrat*:ti,ab,kw or
intralesion*:ti,ab,kw or
intra-lesion*:ti,ab,kw or
interstitial*:ti,ab,kw or
implant*:ti,ab,kw or
Local therap*:ti,ab,kw

#5

unresect*:ti,ab,kw or
non-resect*:ti,ab,kw or
nonresect*:ti,ab,kw or
not-resect*:ti,ab,kw or
unremov*:ti,ab,kw or
LAPC:ti,ab,kw or
Locally advanced pancreatic:ti,ab,kw or
Incompletely resectable*:ti,ab,kw or
incomplete resection*:ti,ab,kw or
Partial-resect*:ti,ab,kw or
Inoper*:ti,ab,kw or
Non-opera*:ti,ab,kw or
Nonopera*:ti,ab,kw or
Not-opera*:ti,ab,kw or
Not-surgic*:ti,ab,kw or
Non-surgic*:ti,ab,kw or
Nonsurgic*:ti,ab,kw

#6 (#1 or #2) and (#3 or #4) and #5

Appendix S2 – Newcastle Ottawa Quality Assessment Scale

CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?

a) yes, with independent validation ☆
b) yes, e.g. record linkage or based on self-reports
c) no description

2) Representativeness of the cases

a) consecutive or obviously representative series of cases ☆
b) potential for selection biases or not stated

3) Selection of Controls

a) community controls ☆
b) hospital controls
c) no description

4) Definition of Controls
a) no history of disease (endpoint) ☆
b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis

a) study controls for cancer staging ☆
b) study controls for (neo)adjuvant therapy ☆

Exposure

1) Ascertainment of exposure

a) secure record (e.g. surgical records) ☆
b) structured interview where blind to case/control status ☆
c) i nterview not blinded to case/control status
d) written self-report or medical record only
e) no description

2) Same method of ascertainment for cases and controls

a) yes ☆
b) no

3) Non-Response rate

a) same rate for both groups ☆
b) non respondents described
c) rate different and no designation

COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort

a) truly representative of the average patient with locally advanced pancreatic cancer in the community ☆
b) somewhat representative of the average patient with locally advanced pancreatic cancer in the community ☆
c) selected group of users e.g. nurses, volunteers
d) no description of the derivation of the cohort

2) Selection of the non-exposed cohort

a) drawn from the same community as the exposed cohort ☆
b) drawn from a different source
c) no description of the derivation of the non-exposed cohort

3) Ascertainment of exposure

a) secure record (e.g. surgical records) ☆
b) structured interview ☆
c) written self-report
d) no description

4) Demonstration that outcome of interest was not present at start of study

a) yes ☆
b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis

a) study controls for cancer staging ☆
b) study controls for (neo)adjuvant therapy ☆

Outcome

1) Assessment of outcome

a) independent blind assessment ☆
b) record linkage ☆
c) self-report
d) no description

2) Was follow-up long enough for outcomes to occur

a) yes (select an adequate follow up period for outcome of interest) ☆
b) no

3) Adequacy of follow up of cohorts

a) complete follow up - all subjects accounted for ☆
b) subjects lost to follow up unlikely to introduce bias - small number lost - >90 % follow up, or description provided of those lost ☆
c) follow up rate < 90% and no description of those lost
d) no statement

Appendix S3 – Newcastle Ottawa Quality Assessment Scale Results

Quality assessment of the cohort studies by the Newcastle-Ottawa Scale

Study	Selection	Comparability	Outcome of Interest
Bhutani, M.S. et al.	☆	☆☆	☆
Dobelbower, R.R. Jr. et al.	☆☆☆	☆☆	☆☆☆
Du, Y. et al.	☆☆☆	☆☆	☆☆☆
Gong, T. et al.	☆☆☆	☆	☆☆☆
Hecht, J.R. et al.	☆☆	-	☆☆
Hecht, J.R. et al.	☆☆☆	☆☆	☆☆☆
Herman, J.M. et al.*	☆☆☆☆	☆	☆☆☆
Hirooka, Y. et al.	☆☆☆	☆☆	☆☆☆
Jin, Z. et al.	☆☆☆	-	☆☆☆
Joyce, F. et al.	☆☆☆	☆☆	☆☆☆
Levy, M.J. et al.	☆☆	-	☆☆☆
Li, J.L. et al.	☆☆☆	☆	☆☆☆
Li, J.Q. et al.*	☆☆☆	☆	☆☆☆
Lun, J.J. et al.	☆☆	-	☆☆
Mohamadnejad, M. et al.	☆☆☆	☆☆	☆☆☆
Montemaggi, P. et al.	☆☆	-	☆☆☆
Mutignani, M. et al.	☆☆☆	-	☆☆☆
Nishimura, M. et al.	☆☆	-	☆☆☆
Niu, L. et al.	☆☆☆	☆	☆☆
Nori D, et al.	☆☆	☆	☆☆
Order, S.E. et al.	☆☆☆	☆☆	☆☆☆
Raben, A. et al.	☆☆☆	☆	☆☆☆
Rosemurgy, A. et al.*	☆☆☆☆	☆	☆☆☆
Ross, P.J. et al.	☆☆☆	-	☆☆
Shipley, W.U. et al.	☆☆☆	☆	☆☆
Sun, S. et al.	☆☆☆	☆☆	☆☆☆
Syed, A.M. et al.	☆☆☆	☆	☆☆☆
Wang, B.	☆☆☆	-	☆☆☆
Wang, H. et al.	☆☆	-	☆☆
Wang, J. et al.	☆	☆	☆☆
Wang, W. et al.	☆☆☆	☆	☆☆
Westlin, J.E. et al.	☆☆☆	-	☆☆☆
Xiao, B. et al.*	☆☆☆	☆	☆☆
Xu, K.C. et al.	☆☆☆	-	☆☆☆
Yang, B. et al.	☆☆☆	☆	☆☆☆
Yang, M. et al.	☆☆	-	☆☆☆
Yunwei, S. et al.	-	-	☆☆☆
Zheng, Z. et al.	☆☆☆☆	-	☆☆☆
Zhongmin, W. et al.	☆☆☆	☆	☆☆☆
Zou, Y.P. et al.	☆☆☆	☆☆	☆☆

Quality assessment of the case-control studies by the Newcastle-Ottawa Scale

Study	Selection	Comparability	Exposure
Goertz, S.R. et al.	☆☆☆☆	☆	☆☆
Li, W. et al.	☆☆☆☆	☆	☆☆☆
Li, Y.F. et al.	☆☆☆☆	☆☆	☆☆☆
Liu, K. et al.	☆☆☆	☆☆	☆☆☆
Luo, M. et al.	☆☆☆☆	-	☆☆☆
Mohiuddin, M. et al.	-	☆	☆☆
Morrow, M. et al.	☆☆	-	☆☆
Peretz, T. et al.	☆☆	☆	☆
Schad, F. et al.	-	☆☆	☆
Schuricht, A. L. et al.	☆☆☆	☆☆	☆☆☆
Sun, X. et al.	-	☆	☆
Whittington, R. et al.	☆☆☆☆	☆	☆☆☆

Appendix S4 – An overview of applied radioactive isotopes for intratumoral injection therapies in pancreatic cancer

Isotope	Decay	T1/2 (days)	Mean energy (KeV)	Mean tissue penetration (mm)	Mean particle size
Iodine-125	Electron capture	59.49	27.5	17	4.4 - 4.6 mm
Phosphorus-32	Beta-minus	14.29	695	2 - 3	20 nm - 30 μm
Palladium-103	Electron capture	16.99	20.2	<17	4.5 - 4.7 mm

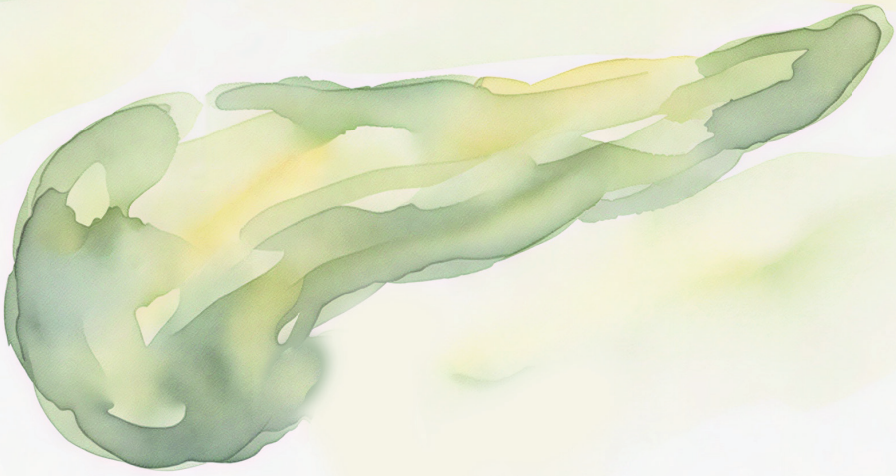
Chapter 3

DEVELOPMENT OF AN INTRATUMORAL HOLMIUM MICROSPERE INJECTION METHOD IN EX VIVO HUMAN PANCREATIC DUCTAL ADENOCARCINOMA: A PRECLINICAL FEASIBILITY STUDY

C. Ysbrand Willink[1], Sjoerd F. M. Jenniskens[1], Nienke J. M. Klaassen[1], Martijn W. J. Stommel[2], Kees J. H. M. van Laarhoven[2], Jurgen J. Fütterer[1], J. Frank W. Nijsen[1]

[1] Department of Medical Imaging, Radboud University Medical Center, Nijmegen, The Netherlands
[2] Department of Surgery, Radboud University Medical Center, Nijmegen, The Netherlands

Cancers, 2025, 17 (6)



Abstract

Introduction: Patients diagnosed with pancreatic ductal adenocarcinoma (PDAC) have a poor prognosis. Local therapy may enhance tumor control and increase resectability. Intratumoral injection of radioactive holmium-166 microspheres presents a promising and minimally invasive treatment with multimodality imaging capabilities (SPECT, CT, MRI). However, holmium-166 microspheres are not commonly used for intratumoral injections, and PDAC is notorious for its high intratumoral pressure. This study developed an intratumoral injection method with nonradioactive holmium-165 microspheres in ex vivo human PDAC specimens using a novel injection system for suspension homogenization.

Materials and methods: An injection system was developed and validated in a laboratory setting. Thereafter, intratumoral injections in surgically removed ex vivo PDACs were performed, and parameters were established to optimize feasibility, defined by the ability to inject and control the microsphere distribution. Also, injection limitations and cutoff values were determined. The distribution was assessed by visual confirmation, CT, MRI, ultrasound, and histopathology.

Results: With a validated injection system, intratumoral injections were performed in ten ex vivo PDAC samples. Feasible injection guidelines include but are not limited to ultrasound or CT needle guidance, a maximum injection volume of <20.0% from the tumor volume, ≤3 needle positions, and an injection volume of 0.3–1.0 mL per needle position.

Conclusion: Intratumoral injection of holmium165 microspheres in ex vivo pancreatic ductal adenocarcinoma was feasible with adherence to injection parameters necessary for effective intratumoral deposition and minimal leakage. The injection system and parameters developed here provide a foundation for future studies on holmium-166 microsphere injections in pancreatic cancer patients, with the aim to improve local tumor control as a part of a multimodal therapy.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer. With an overall 5-year survival of 4.2–11% in the past decade, the outcome of patients with PDAC remains dismal.[1,2] Approximately 13–20% of all patients diagnosed with PDAC are eligible for surgical resection, which increases the 5-year survival rate to 17%.[3-6] The remaining patients have either metastatic disease (50%) or locally advanced pancreatic cancer (LAPC, 30%).[3,4] Curative treatment for patients with LAPC is only available after a significant tumor response and downstaging, most commonly attempted by induction chemotherapy, which is sufficiently effective in approximately one out of five LAPC patients.[7] Obvious challenges when subjecting (near) terminal patients to extensive treatment are comorbidities, toxicity, and recovery time.[8,9] Although several new minimally invasive therapies have been developed and studied, oncological outcomes have hardly improved.[10,11]

Minimally invasive delivery of an anticancer substance by direct intratumoral injection may contribute to local tumor control of LAPC.[11] Intratumoral injection, also called interstitial injection, involves the placement of a needle not in a blood vessel or duct, but directly in the tumorous tissue, and the needle is then used to deliver a substance between the tumor cells.[11] When radioactive microparticles are injected, this therapy is called (micro)brachytherapy. The microparticles act as an implant and are fixated within the tissue after the radiation has decayed.[12] Local irradiation causes a high local tumor dose without damaging nearby vital structures. Additionally, the cytokines induced by the local apoptosis and necrosis might drain towards the tumor-draining lymph nodes and activate a broader immunogenic antitumor effect.[13] Minimally invasive microbrachytherapy can potentially reduce the tumor burden, prolong survival, and cause tumor downstaging, and therefore may increase surgical resection rates.[14]

The potential of microbrachytherapy was first seen in the OncoPaC-1 trial, showing successful endoscopic ultrasound (EUS)-guided intratumoral injection of microparticles incorporating radioactive phosphorus-32 (^{32}P) in 42 patients with LAPC.[14] A local disease control rate (RECIST 1.1) of 82% (95% confidence interval 68.6–90.9%) after 16 weeks and a median overall survival of 15.5 months was achieved.[14] A disadvantage of ^{32}P is that it is a pure beta (β^-) emitter (characteristics are described in Table 1), which limits imaging of the administered microparticles to low-resolution Bremsstrahlung single-photon emission computed tomography (bSPECT). This hinders accurate assessment of therapy distribution after injection and makes it impossible to assess or adjust therapy distribution during intervention.

Table 1. *Microbrachytherapy agents studied for injection in pancreatic ductal adenocarcinoma.*

Characteristic	Holmium-166 Microsphere	Phosphorus-32 Microparticle
Material	Poly L-lactic acid	Silicon
Diameter	30 μm (15–60 μm)	30 μm (15–50 μm)
Isotope	Holmium-166	Phosphorus-32
Half-life	26.8 h	14.27 days
Emission type (yield)	Beta (93.3%), gamma (6.7%)	Beta (100%)
Maximum beta energy	1.85 MeV	1.711 MeV
Mean β^- penetration	2.5 mm	2.76 mm
Maximum β^- penetration	8.7 mm	8.2 mm
Imaging modalities	SPECT, CT, MRI	Bremsstrahlung SPECT

Other microparticles eligible for intratumoral injection in LAPC are holmium-166 (^{166}Ho) incorporated poly L-lactic acid microspheres (^{166}HoMS). Holmium-166 is a radioactive β^- emitting isotope similar to ^{32}P, as shown in Table 1. However, ^{166}Ho has a shorter half-life, and besides β^- also emits low-energetic gamma radiation (γ) with an energy of 80.6 KeV. The γ radiation makes ^{166}Ho eligible for conventional SPECT imaging with a higher resolution than bSPECT. Moreover, since ^{166}Ho has a high atomic mass (165.9 u) and makes up approximately 19% of the mass of the microspheres, it causes attenuation of X-rays, which makes the microspheres hyperdense on computerized tomography (CT).[15] Owing to the paramagnetic properties of holmium, the microspheres are also imageable by magnetic resonance imaging (MRI).[16] For research purposes, holmium-165 microspheres (^{165}HoMS) are often utilized. These microspheres are identical to ^{166}HoMS with the exception of containing a stable isotope and therefore not emitting any β^- or γ radiation. Although ^{165}HoMS may not serve a therapeutic purpose, they can still be analyzed using CT or MRI imaging.

^{166}HoMS are commonly used for transarterial radioembolization (TARE) of primary and secondary hepatic malignancies.[17,18] For TARE, it serves as a feasible and safe treatment with beneficial clinical outcomes and can achieve treatment-free intervals or bridging-to-transplant.[19,20] TARE can only be applied in the liver because of the unique dual blood supply, with the tumor receiving primarily arterial blood and the liver parenchyma portal-venous blood.[21] However, intratumoral ^{166}HoMS injection has also been previously studied, for example, in feline patients with oral squamous cell carcinoma.[22] Intratumoral injection of ^{166}HoMS resulted in minimal side effects, a complete response or partial response with subsequent marginal resection in 6 out of 11 (55%) feline patients, and more than doubled the survival time in responders vs. nonresponders.[22] Moreover, intratumoral ^{166}HoMS injection was performed in three human patients suffering from head and neck cancer.[23] Even though no adverse events were observed, the therapeutic effect was minimal due to the low tumor dose and technical obstacles that further limited the delivered tumor dose. The microspheres are known to settle quickly in a suspension because the density is greater than that of the injection fluid. This caused heterogeneous suspensions in the syringe during injection,

resulting in needle blockage and a varying injected ^{166}HoMS concentration between 17.8 and 84.3% of the initial concentration.[23]

This study aimed to develop an intratumoral injection method in ex vivo human pancreatic ductal adenocarcinomas using nonradioactive ^{165}HoMS. First, an administration system that enables continuous motion of the microsphere suspension was developed to gain more control of the administered microsphere concentration. This system was tested to establish injection system parameters (ISPs), which were then validated in a laboratory setting. Next, the validated ISPs were translated to intratumoral injections in ten ex vivo pancreatic ductal adenocarcinoma samples obtained from patients after surgical resection to establish intratumoral injection parameters (IIPs) and determine the feasibility of the developed method.

Materials and methods

In this study, a new intratumoral injection system was designed, and ISPs were validated for homogeneous ^{165}HoMS injection and thereafter applied to ten surgically removed human pancreatic ductal adenocarcinoma samples. IIPs were established to determine the optimal intratumoral injection control and distribution and to find the cutoff values and limitations.

Injection system design

The developed injection system is schematically presented in Figure 1. The ^{165}HoMS syringe (Figure 1A) was connected to a 3-way stopcock with a single 360° rotating male Luer-lock connection (Figure 1B: CODAN, Lensahn, Germany), enabling the 3-way stopcock and ^{165}HoMS syringe to be manually rotated over its long axis. Opposite the ^{165}HoMS syringe, the rotatable 3-way stopcock was connected to a second 3-way stopcock (Figure 1D) with a handgrip (Figure 1C) and needle (Figure 1E) attached perpendicular to the long axis of the ^{165}HoMS syringe. Needles of 18–21 Gauge (G) and 50–150 mm were used. Manual syringe rotation was performed with the aim of creating a homogeneous ^{165}HoMS suspension up to the moment of injection to enable the injection of constant ^{165}HoMS concentrations. A plastic extension tube (21 G, 10–30 cm; CODAN, Lensahn, Germany) could be attached between the second 3-way stopcock (Figure 1D) and the needle (Figure 1E) to enable more freedom for needle placement. Air was removed from the injection system by priming the system with either demi-water for validation testing or saline for ex vivo intratumoral injection before the ^{165}HoMS syringe was connected. All the components were connected by a Luer-lock to withstand internal pressures and prevent suspension leakage.

Initial laboratory testing was performed to assess the feasibility of the injection system and visual effectiveness in suspension homogenization with multiple ISPs, including the syringe volume (1.0–10.0 mL),

rotation time (~1–120 s), rotation speed (30–120 rotations per minute; RPM), injection speed (0.05–1.0 mL/s), minimal injection volume (0.1–1.0 mL), and injection system components. The most promising ISPs from the initial laboratory tests were then validated.

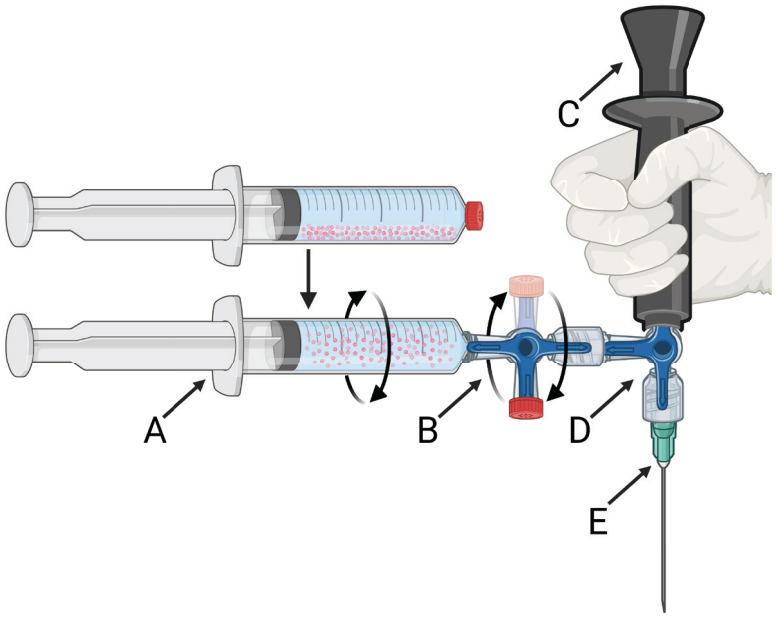


Figure 1. *A schematic overview of the 360° syringe-rotating injection system that was used to create a homogeneous ^{165}HoMS suspension and to inject constant concentrations. The injection system consists of a ^{165}HoMS syringe (**A**) connected to a 360° rotating 3-way stopcock (**B**), enabling the syringe to be manually rotated over its long axis. The rotatable 3-way stopcock was connected to a second 3-way stopcock (**D**) with a handgrip (**C**) and needle attached (**E**) perpendicular to the ^{165}HoMS syringe. Created with BioRender.*

Injection system validation testing

During injection system validation, a set of ISPs defined during initial laboratory testing was used to validate a homogeneous and constant ^{165}HoMS concentration injection. Injection system validation was performed by injecting ^{165}HoMS suspensions in glass validation vials, evaporating the injection fluid, and weighing the residual ^{165}HoMS to establish injection recoveries (R_{HoMS} in percentages) and consistency between injections, as described below.

Microsphere preparation

In this study, dry nonradioactive holmium-165 poly L-lactic acid microspheres (^{165}HoMS) were used (Quirem Medical, Deventer, The Netherlands). The ^{165}HoMS values were within the specifications of radioactive microspheres (QuiremSpheres®), with a diameter of 30 µm (±5 µm) and 19.0–20.0 *w/w*% ^{165}Ho. The ^{165}HoMS were suspended in a water-based injection fluid with an isotonic phosphate buffer (116 mM, pH 7.4) and 0.1–2.0% Pluronic (Quirem Medical, Deventer, The Netherlands), unless stated otherwise.

Laboratory validation testing

For validation assessment, a predetermined concentration aimed at 20 milligrams of ^{165}HoMS per milligram of injection fluid (mg/mg) in 3.0 mL syringes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was prepared and weighed to determine the prepared ^{165}HoMS concentration in the injection fluid ($C_{syringe}$ in mg/mg). Each syringe was connected to its own primed injection system. After manual rotation, the suspension from each syringe was manually injected in 0.5 mL fractions into six separate 20.0 mL glass validation vials (PerkinElmer, Drachten, The Netherlands). After a syringe was emptied, the injection system was flushed repeatedly with 6.0 mL of demi-water to remove agglomerated microspheres. The agglomerated microspheres were collected in glass flush vials. Control vials were prepared in triplicate with 1.0 mL of injection fluid and 1.0 mL of demi-water. After all the injections were performed, the injection vials and control vials were placed in a 50 °C stove for at least 48 h to evaporate all the liquid. All vials were weighed in mg on a microbalance (XPE105, d = 0.01 mg; Mettler Toledo, Tiel, The Netherlands) when empty (M_{empty}), full (M_{full}), and after evaporation ($M_{evaporation}$). With these measurements, the mass of the vial's contents (indicated by M_c) and the residue after evaporation (indicated by M_r) were also determined in mg (Equations (1) and (2)).

(1) $$M_c = M_{full} - M_{empty}$$

(2) $$M_r = M_{evaporation} - M_{empty}$$

The concentration of the residue (C_r in mg/mg) per vial was determined as shown in Equation (3).

(3) $$C_r = \frac{M_r}{M_c}$$

The concentration of ^{165}HoMS (C_{HoMS} in mg/mg) in the flush and validation vials was determined by extracting the mean concentration residue ($C_{r-control}$) from the demi-water or injection fluid control vials from the concentration residue (C_r) of the flush or validation vial, respectively, as shown in Equation (4).

(4) $$C_{HoMS} = C_r - \bar{x}C_{r-control}$$

The recovered ^{165}HoMS concentration (R_{HoMS}, percentages) from the flush ($R_{HoMS-flush}$) and validation vials ($R_{HoMS-injection}$) was determined by dividing the concentration of ^{165}HoMS (C_{HoMS}) in the vials by the concentration of ^{165}HoMS in the syringe ($C_{syringe}$), as shown in Equation (5).

(5) $$R_{HoMS} = \frac{C_{HoMS}}{C_{syringe}} * 100\%$$

A maximum range of 20% for the $R_{HoMS-injection}$ (median ±20%) was considered an improvement over previous intratumoral injection results, where injection ranges of up to 33.3% were observed.[23]

Ex vivo human pancreatic ductal adenocarcinoma

This study was approved by the Medical Ethics Assessment Committee (METC) Oost-Nederland (CMO 2019-5578) and was not subjected to the Law for Medical Research involving Human Subjects. Patients were required to provide informed consent before undergoing surgery to approve the use of medical information, provide consent for publication, and donate resected tissue for research.

Patients older than 18 years with pathologically proven PDAC and a single lesion eligible for surgical resection via pancreatoduodenectomy or distal or total pancreatectomy were eligible for tissue donation. No distinction was made between the presence or type of neoadjuvant therapy. Patients with PDAC < 10 mm in largest diameter on diagnostic contrast-enhanced CT (CECT) were excluded. Tumor volumes were assessed via manual 3D segmentation of diagnostic CECT.

Immediately following surgical resection, the sample was registered and examined for study approval by the Department of Pathology. Metal clips, staples, or stents that could intervene with CT or MRI were removed. Clinical imaging was introduced before, during, and/or after injection with CT (Aquilion, Canon Medical Systems Europe B.V., Zoetermeer, The Netherlands), MRI (Skyra 3T, Siemens Healthineers, Den Hague, The Netherlands), and/or ultrasound (US; Xario 200, Canon Medical Systems Europe B.V.; Zoetermeer, The Netherlands). The imaging parameters used are presented in Table S1. After surgery, no study-related follow-up was performed on the patients.

The evaluated IIPs during ex vivo intratumoral injection included the total injection volume (0.2–4.5 mL), total injection volume with respect to the tumor volume (8–83%), injection volume per deposit (0.1–1.0 mL), number of needle passes (1–13), number of ^{165}HoMS deposits (1–45), ^{165}HoMS concentration (5.0–50.0 mg/mL), and type of image guidance (none, US, CT). All the injections were performed by, or with the supervision of, an interventional radiologist (S.F.M.J.). After injection, all the samples were fixed with 4% formaldehyde within 4 h to prevent decomposition of the autolytic pancreatic parenchyma. After at least 48 h of fixation, the tissues were sliced, histologically stained (hematoxylin–eosin), and analyzed by the Department of Pathology.

Outcome measures

Injection feasibility was achieved if intratumoral ^{165}HoMS deposition and distribution were observed and visualized via CT and/or MRI. Extratumoral ^{165}HoMS leakage is defined as extratumoral ^{165}HoMS deposition on CT and/or MRI or by visual confirmation of fluid leakage. Feasibility was

pursued by adjusting IIPs with an iteration-based approach to find the optimal intratumoral injection control and distribution. Note that IIPs were also adjusted to find feasibility limitations and cutoff values. The feasibility outcomes included intratumoral microsphere deposition (yes/no), extratumoral microsphere leakage (yes/no), injection observations (descriptive), and type of image guidance. Furthermore, outcomes included advised ISPs (syringe volume, rotation time, rotation speed, flush volume, minimal injection volume, and injection speed) and advised IIPs (tumor volume, injection volume, syringe angle, needle insertions, deposits, volume per deposit, ^{165}HoMS concentration, needle diameter, and needle length).

Data processing

Because of the iterative-based study approach, most findings are descriptive or measurements. All reported measurements are described as medians (ranges) unless stated otherwise. Graphs were created in Microsoft Office 365, Excel. MRI and CT processing was performed with RadiAnt DICOM viewer (version 2021.2.2), Q-Suite (version 2.1), and the open-source software 3D Slicer (version 4.11–5.6).[24]

Results

Injection system development

Initial laboratory testing resulted in the development of the injection system described previously in Section 2.1, and in the ISPs described in Table 2. A more extensive description of the findings during injection system development is shown in Appendix S1.

Table 2. *Established and laboratory-validated injection system parameters (ISPs). These parameters were used to establish a homogeneous and constant holmium-165 microsphere concentration during injection.*

Parameter	Limit	Unit
Syringe volume	3.0	mL
Minimal rotation time	120	seconds
Intermittent rotation time[1]	10	seconds
Rotation speed	60	RPM
Minimal flush volume	1.0	mL
Minimal injection volume without tube	0.3	mL
Minimal injection volume with tube	0.5	mL
Injection speed	0.2	mL/s

[1] *The minimal rotation time required between succeeding injections or after every 0.5 mL injected*

Injection system validation

The established injection system and determined ISPs (Table 2) were validated. Four syringes were prepared with a median ^{165}HoMS concentration of 17.4 mg/mL (range 16.7–18.6). An overall R_{HoMS} of 65% (range 63–68%) was injected for the complete 3.0 mL syringe. The flush was the first 1.0 mL ejected from the injection system and resulted in a lower $R_{HoMS-flush}$ of 44.1% (range 34.7–48.8%).

The remaining 2.0 mL resulted in a $R_{HoMS-injection}$ of 81% (range 69–93%); see Figure 2. The $R_{HoMS-injection}$ was applied for ex vivo intratumoral injections.

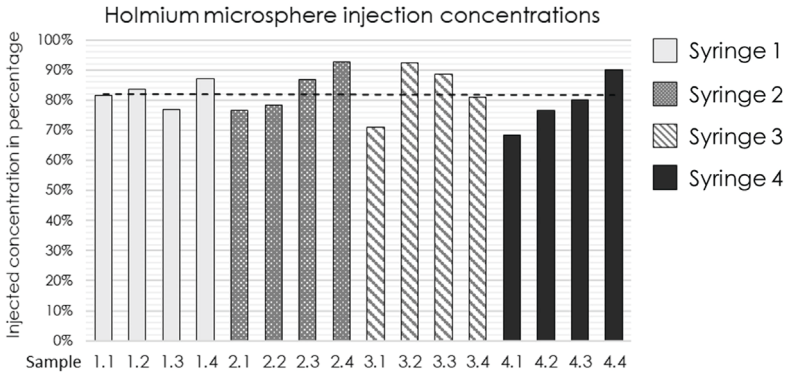


Figure 2. *Bar graph of the ^{165}HoMS concentration recovered ($R_{HoMS-injection}$) after injection in 16 samples of 0.5 mL compared with the prepared syringe concentration. The black dotted line indicates a median recovered concentration of 81% (range: 69–93%).*

Ex vivo intratumoral injection

Overall, ten ex vivo PDAC tumor samples were injected and analyzed. The tumor volume on diagnostic CECT ranged from 2.5 to 15.6 mL, with a median of 6.9 mL. A sample containing the tumor often includes healthy pancreatic parenchyma, (part of) the duodenum, resected vasculature, and, sometimes, the spleen, clips, staples, stents, or stitches. The established IIPs based on ex vivo intratumoral injection are presented in Table 3. The most notable results are described below. An overview of all the tissue samples and injection results is presented in Table 4.

Table 3. *Intratumoral injection parameters (IIPs) that were established after intratumoral holmium-165 microsphere injection in ex vivo pancreatic ductal adenocarcinoma samples.*

Parameter	Limit	Unit
Tumor volume[1]	*2.5–15.6*	*mL*
Maximum injection volume	*20.0*	*% of tumor volume*
Maximum syringe angle to horizontal	*10.0*	*Degrees*
Number of needle insertions	*1–3*	*-*
Number of deposits	*1–3*	*-*
Volume per deposit[1]	*0.3–1.0*	*mL*
Holmium microsphere concentration in syringe[1]	*5.0–50.0*	*mg/mL*
Needle diameter[1]	*18–21*	*Gauge*
Needle length[1]	*50–150*	*mm*

[1] *Parameters outside this range (one- or two-sided) were not applied in this study.*

Table 4. *Overview of tissue samples 1–10, intratumoral injection parameters, and observations.*

Characteristics	Unit	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
Tumor volume	mL	14.3	6.7	9.6	2.5	15.6	3.6	5.6	11.6	7.1	2.5
Extratumoral leakage observed	Yes/no	yes	yes	no	no	no	yes	no	no	yes	yes
Observation remark	-	Over fractionated deposits	Needle overshoot	-	HVC [2] instead of injection fluid	-	Intentionally increased injection volume	Needle blockage due to syringe angle	-	Leakage through pancreatic duct	Intentionally increased injection volume
Image guidance	-	-	-	US	US	US	US + CT	US + CT	US + CT	US + CT	US + CT
Injection volume (as % of tumor volume)	mL (%)	4.5 (31)	2.3 (35)	1.8 (19)	0.2 (8)	3.0 (19)	3.0 (83)	0.7 (13)	2.0 (17)	0.7 (10)	1.0 (40)
Number of needle insertions	-	13	5	3	1	3	1	1	2	1	2
Number of deposits x volume per deposit	mL	45 × 0.1	3 × 0.5 2 × 0.3	1 × 0.8 2 × 0.5	1 × 0.2	3 × 1.0	6 × 0.5	1 × 0.7	2 × 1.0	1 × 0.7	2 × 0.5
Concentration of ^{165}HoMS in syringe	mg/mL	5.0	5.0	5.0	10.0	10.0	10.0	20.0	20.0	25.0	25.0 50.0
HoMS injected[1]	mg (mg/mL)	18.5 (4.1)	9.4 (4.1)	7.4 (4.1)	2.0 (10.0)[2]	24.6 (8.2)	24.6 (8.2)	11.5 (16.4)	32.8 (16.4)	13.4 (20.5)	10.3 (20.5) 20.5 (41.0)

[1] $R_{HoMS\text{-}injection}$ was applied, which assumes 81% of the prepared ^{165}HoMS concentration was injected, as determined during validation testing. [2] High-viscosity compound (HVC) was used instead of the injection fluid. For HVC, an R_{HoMS} of 100% was assumed since it does not suffer from accumulation within the injection system. HVC is currently not available in standard care.

Image guidance

In the first two samples, periprocedural needle guidance was visual and performed using tumor palpation without image guidance. On the tissue surface, no visual distinction could be made between healthy pancreatic parenchyma and tumor (see Figure 3). Distinguishing between harder and dense tumor tissue and softer, healthy pancreatic parenchyma was possible via palpation. Estimating needle depth by palpation was more challenging, and multiple needle overshoots occurred in samples 1 and 2. Thereafter, US was introduced for periprocedural image guidance of the needle. In samples 3–10, no needle overshoots were observed. Owing to the magnetic and spatial limitations of MRI, it could not be used for periprocedural needle guidance. Additionally, heterogeneous tissues in and around the tumor, air in the samples, and a low spatial resolution made MRI ineligible for ^{165}HoMS distribution assessment (see Figure 4). In samples 4–10, ^{165}HoMS deposits became clearly visible on post-injection CT because of an increased prepared ^{165}HoMS concentration. An overview is presented in Figure 5. For samples 6 to 10, periprocedural CT was added for needle guidance and periprocedural ^{165}HoMS visualization (see Figure 6). However, needle guidance by CT was challenging since the ex vivo tissues were not perfused, and therefore CT contrast was unavailable.

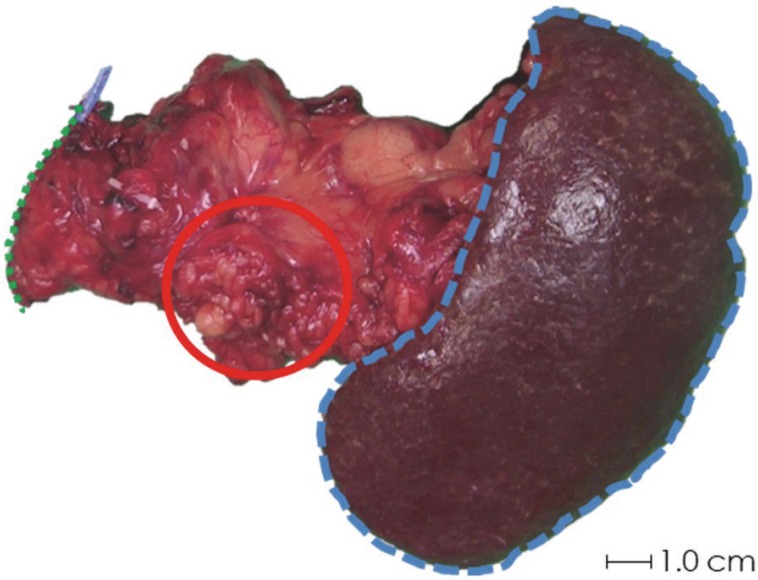


Figure 3. *Photograph of the anterior side of ex vivo tissue sample 1 after pancreatic tail resection with splenectomy. Schematic estimations of the pancreatic resection surface (green dotted line), spleen (blue striped line), and rough approximation of the tumor (red circle) are shown. Accurate identification of the tumor by visual inspection was not feasible, and palpation was not sufficient for needle depth estimation.*

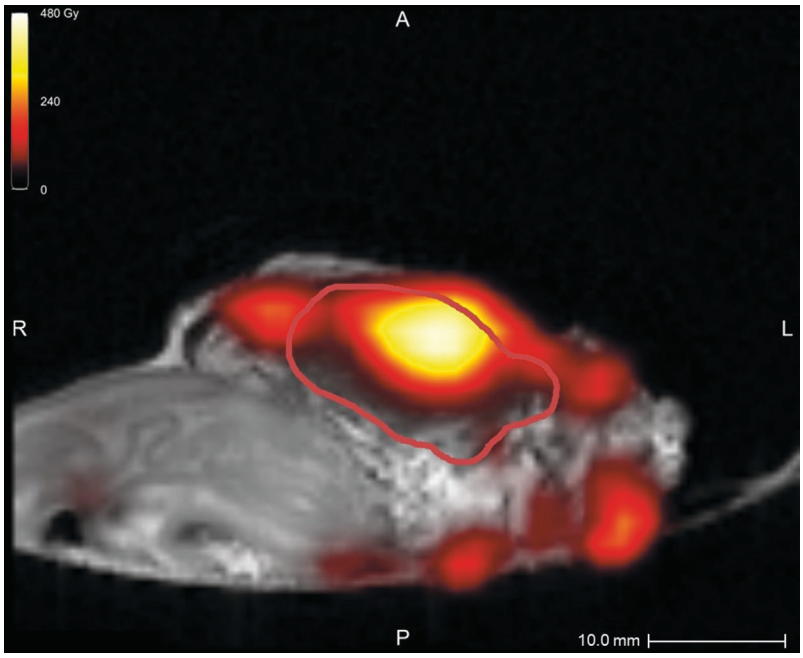


Figure 4. *MRI quantification after nonradioactive intratumoral ^{165}HoMS injection in sample 5, with a theoretical specific activity of 12.00 MBq/mg ^{165}HoMS. The red line delineates the tumor. The highest dose, at the location of the ^{165}HoMS deposition, was observed within the tumor. Multiple MRI artifacts caused inaccurate dose estimations surrounding the tissue, which were created by air and heterogeneous tissues. This figure was created in Q-Suite (version 2.1).*

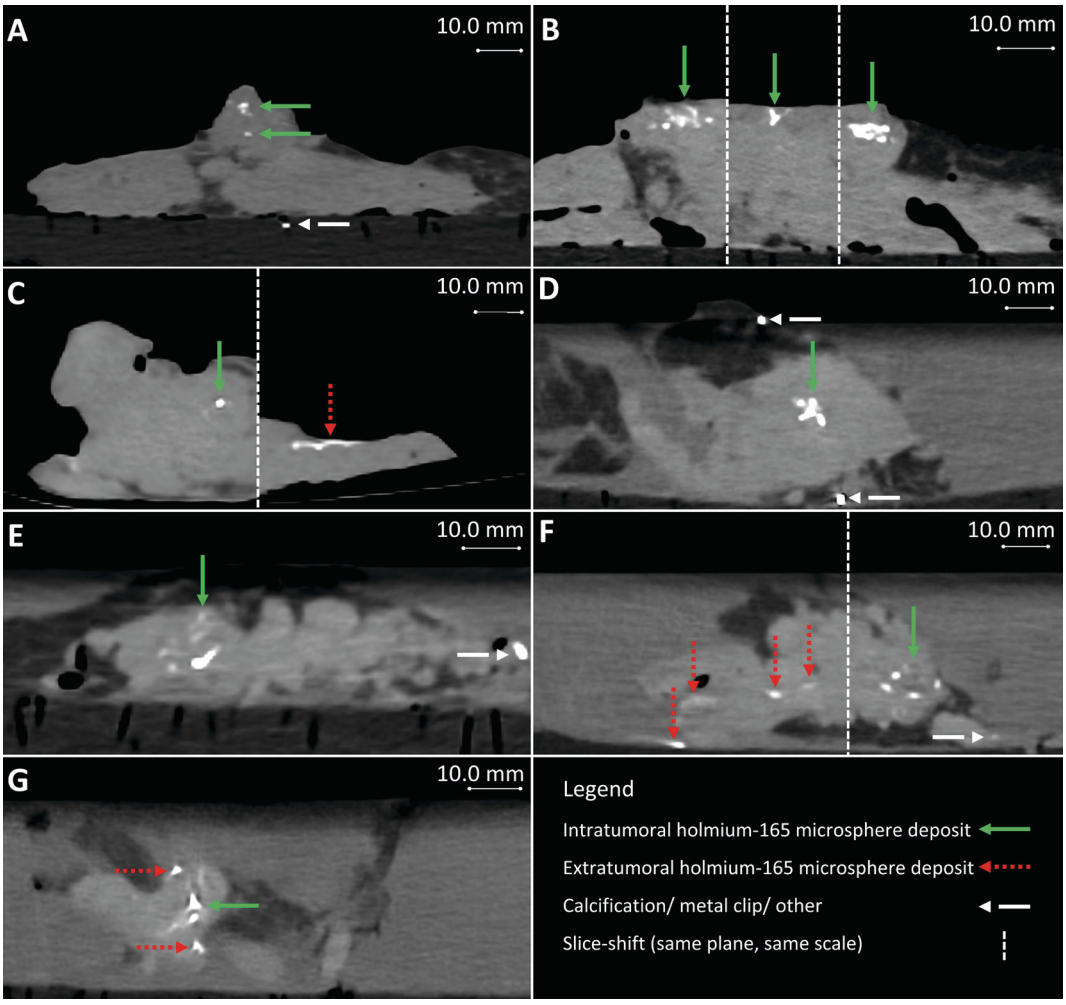


Figure 5. *Post-injection CT from samples 4–10 (**A**–**G**), respectively. (**B**,**C**,**F**) contain multiple slices of the same plane and scale. Extratumoral holmium-165 microsphere deposits occurred and are visible in images (**C**,**F**,**G**), for samples 6, 9, and 10, respectively.*

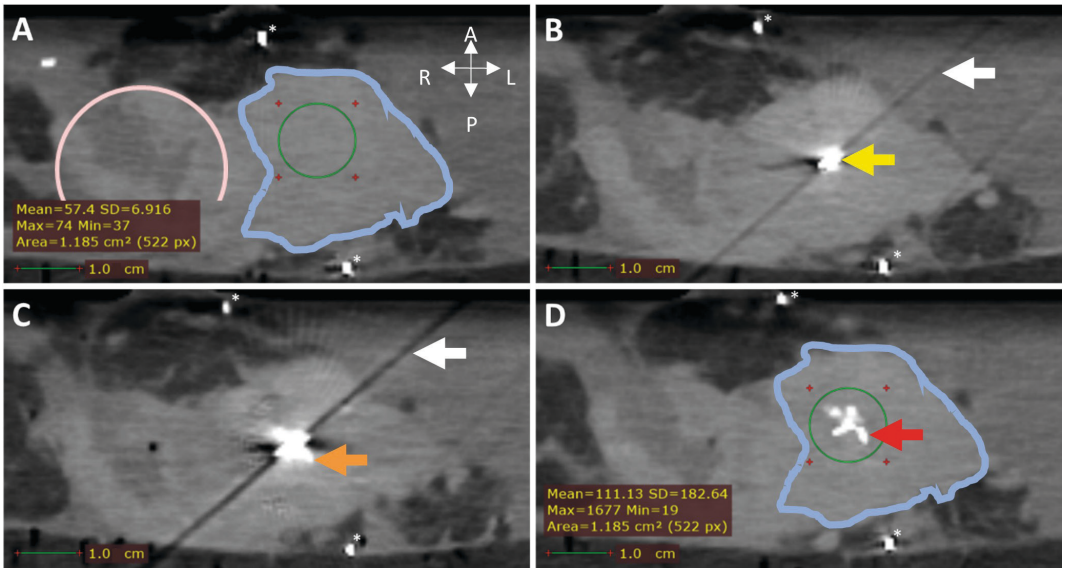


Figure 6. *CT (0.5 mm isotropic) of intratumoral [165]HoMS injection in ex vivo pancreatic cancer sample 7. (**A**) CT before [165]HoMS injection showing the sample anterior (**left**), posterior (**right**) (white arrows), duodenum (pink circle), tumor border approximation (blue contour), and HU values of interest (**bottom left**) in the region of interest (ROI; green circle). (**B**) CT with a 20G needle in situ. The needle tip is in the viewed slice, causing the hyperdense artifact (yellow arrow) and the diagonal shading artifact (white arrow in **B**,**C**). (**C**) CT scan directly following the injection of 11.5 mg of [165]HoMS in 0.7 mL suspension with the needle in situ. Deposition of [165]HoMS around the needle tip causes the hyperdense artifact to increase in size (orange arrow). (**D**) CT scan after needle extraction with [165]HoMS (red arrow) visible as hyperdense area in the center of the tumor (blue contour); HU values of interest (**bottom left**) in the region of interest (ROI; green circle). * Calcifications.*

Injection volume, concentration, and deposits

The total injection volume per sample ranged from 0.2 to 4.5 mL, with a median injection volume of 1.9 mL. When injection volumes > 2.0 mL were needed, multiple [165]HoMS syringes could be prepared and replaced between injections. The injection volumes with respect to the tumor volume ranged from 8 to 83%, with a median of 19%. The injection concentration did not affect feasibility during injection, but higher concentrations did result in increased Hounsfield units (HUs) of the [165]HoMS deposits, and thus a better contrast-to-background ratio on CT.[15] When the prepared [165]HoMS concentration was ≥10.0 mg/mL (samples 4–10), [165]HoMS deposits became clearly disguisable on post-injection CT, as presented in Figure 5. On MRI, highly concentrated [165]HoMS deposits cause large susceptibility artifacts, also called signal voids, which cause an underestimation of [165]HoMS when quantified. The number of deposits per sample ranged between 1 and 45, with a median of 2 deposits per sample. The first sample had 45 deposits, which showed severe leakage due to needle tract contact and needle overshoot through the tumor. All remaining samples had between one and five deposits. The injection volume per deposit ranged from 0.1 to 1.0 mL.

Leakage

Leakage of needle tracts was observed when two or more needle tracts touched or crossed paths, which occurred only in sample 1. Leakage due to backflow was observed once during injection in a tumor region with high interstitial pressure. Manual injection allowed haptic feedback on injection resistance, which could be used to estimate the risk of injection in a duct or vessel when low resistance was felt or the risk of needle tract leakage when high resistance was felt to due high interstitial pressure. High interstitial pressure was observed multiple times; however, leakage could easily be averted by slightly changing the needle tip position and continuing the injection when normal resistance was felt. Extratumoral leakage, other than leakage by needle tracts, was not observed when the injection volume remained below 20% of the total tumor volume and when needle placement was central in the tumor as confirmed by US. US was used to identify vital structures such as major blood vessels and the pancreatic duct. In the tumor, the pancreatic duct was unidentifiable via imaging due to complete obstruction. Nevertheless, in sample 9, leakage through the pancreatic duct toward the duodenum was observed on post-injection CT without an indication of hitting the pancreatic duct or injecting into it (see Figure 7).

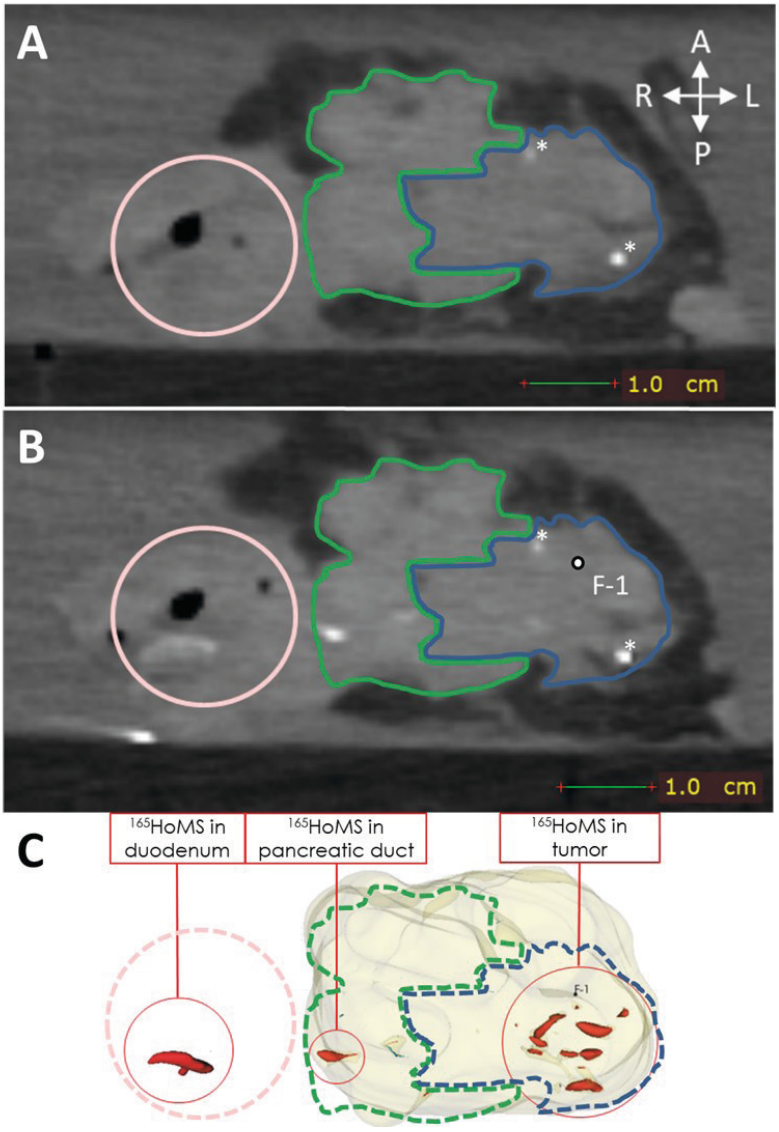


Figure 7. *CT (0.5 mm isotropic) of intratumoral ^{165}HoMS injection in ex vivo pancreatic cancer sample 9 (vertically aligned). (**A**) CT before ^{165}HoMS injection showing the patient's anterior (**left**), posterior (**right**) (white arrows), duodenum (pink circle), tumor border approximation (blue contour), and healthy pancreas border approximation (green contour). (**B**) CT scan directly following the injection of 13.4 mg of ^{165}HoMS in a 0.7 mL suspension with the needle removed. Leakage of the ^{165}HoMS occurred from the injection location (F-1, out of plane) through the pancreatic duct toward the duodenum. (**C**) Three-dimensional segmentation of the pancreatic head (beige), including the healthy pancreas and tumor, and the ^{165}HoMS (red).*

Needle blockage

For sample 7, the ^{165}HoMS syringe was tilted approximately 30 degrees vertically due to a lack of space for the injection system. This caused the ^{165}HoMS to agglomerate in the syringe tip. During injection, a needle blockage was observed after 0.7 mL. This block could be resolved only by extracting the needle from the tissue. To prevent space limitations for the injection system, tubing was added between the injection system and the needle as described in Section 2.1. Furthermore, an unresolvable needle block never occurred with the ^{165}HoMS syringe in a near-horizontal position (0–10 degrees).

Histopathology

Histological images of all ten tissues confirmed that ^{165}HoMS lodged within the tumor. ^{165}HoMS were observed to have lodged within existing lumen, such as minor blood vessels and neoplastic ducts, and also in interstitial lumen newly formed by the injection pressure (see Figure 8). Large, empty, newly formed interstitial lumen were also observed and suggest that some of the ^{165}HoMS were washed out of the samples during the multiple tissue slicing, staining, and washing steps. Therefore, proper distribution patterns could not be established via this method.

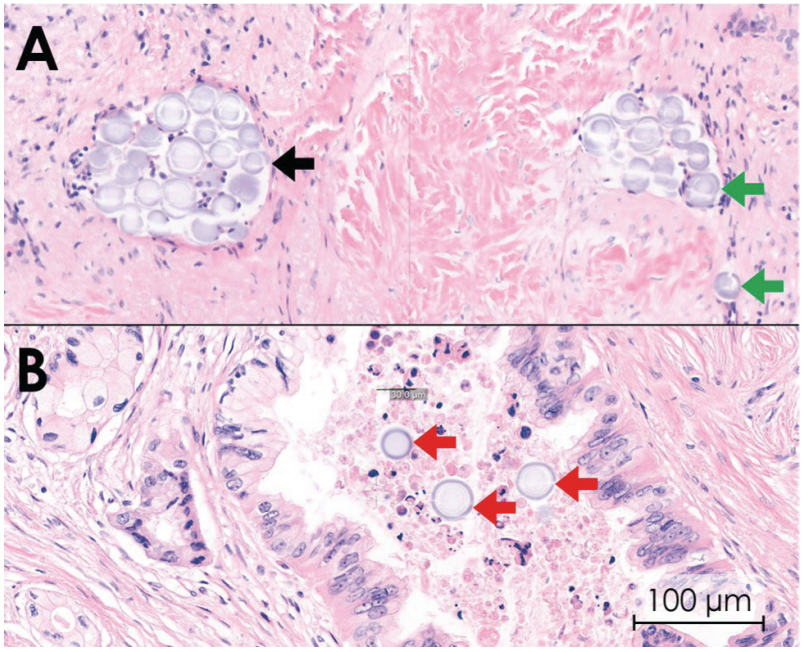


Figure 8. *Hematoxylin–eosin staining of pancreatic cancer tissue after intratumoral holmium-165 microsphere (^{165}HoMS) injection. Herein, purple-stained cell nuclei and gray–purple-stained ^{165}HoMS (±30 µm; circles) are visible. (**A**) Purple-stained tumor cell nuclei in a network of fibrosis (pink) with ^{165}HoMS deposition in lymphatic lumen (black arrow) and interstitial lumen (green arrows). (**B**) ^{165}HoMS deposition in a neoplastic duct (red arrows).*

Discussion

Minimally invasive therapies for downstaging or reducing the tumor burden of PDAC, especially in patients with LAPC, remain limited. Radioactive holmium-166 microspheres administered through intratumoral injection present a promising therapeutic option for this patient population. This study aimed to develop an intratumoral ^{165}HoMS injection method in ex vivo human PDAC samples and to optimize injection parameters by using a novel injection system designed for ^{165}HoMS suspension homogenization. These findings provide a foundation for future studies exploring intratumoral ^{166}HoMS injection as a minimally invasive treatment approach for PDAC.

The injection system demonstrated promising results during both validation testing and intratumoral injection in ex vivo PDAC samples. For the ISPs, a ^{165}HoMS syringe of 3.0 mL was optimal, balancing control over the injection volume with sufficient capacity. Pre-injection homogenization was achieved by rotating the syringe for at least 2 min, which was confirmed visually and through validation testing. Homogeneity was effectively maintained by rotating the syringe for at least 10 s between injections or after every 0.5 mL during consecutive injections. A rotation speed of 60 RPM minimized suspension idleness and could be comfortably sustained by hand for several minutes. An injection speed of 0.2 mL/s was identified as optimal for volume control while ensuring suspension homogeneity. To achieve a constant ^{165}HoMS concentration, a minimum injection volume of 0.3 mL was required without an extension tube, and 0.5 mL was needed with an extension tube attached.

These parameters, along with the finalized injection system, resulted in a more constant injection of the ^{165}HoMS concentration ($R_{HoMS-injection}$) compared with previous injection techniques. In a study of head and neck squamous cell carcinoma, the injected ^{166}HoMS concentrations ranged between 17.8 and 84.3%, whereas this study achieved a range of 69% to 93%.[23] In feline oral squamous cell carcinoma, the mean injected ^{166}HoMS concentration reported was 59.8%, with a standard deviation (SD) of ±17.6%, whereas it was 81% with an SD of ±7.3% in this study.[22] Importantly, these comparisons involve both in vivo and laboratory settings (further discussed below). Nevertheless, the injection system and ISPs appear to enhance the ^{165}HoMS injection efficiency while reducing the overall variation. The validated injection system and ISPs have been tested independently of PDAC, allowing their potential application in research involving intratumoral injections in animal models or other solid tumor types as a versatile platform technique.[12]

A key consideration for the feasibility of intratumoral injection in PDAC was whether the exceptionally high interstitial pressure associated with this cancer type would permit fluid injections at all.[25] This elevated interstitial pressure is attributed to a dense extracellular matrix rich in activated fibroblasts and hyaluronan.[25] High interstitial pressure results in injection resistance, which can be sensed through

haptic feedback from the syringe plunger. Accurately estimating interstitial pressures is crucial for ensuring safe intratumoral ^{165}HoMS injection.

When considering the number of deposits injected per tumor, dividing the injection volume does not completely prevent leakage and introduces risks such as needle overshoot and increased tissue trauma. However, in tumors exceeding 10.0 mL, administering double or triple fractionated deposits may increase tumor dose coverage and minimize local saturation, thereby reducing the risk of unexpected fluid leakage. While not experimentally validated, it is recommended to limit the injection volume per deposit to a maximum of 1.0 mL to mitigate the risk of unpredictable distribution patterns. Experimental results indicate that deposit volumes of ≤1.0 mL result in localized deposition of ^{165}HoMS as presented in Figure 5, demonstrating a feasible degree of spatial control. Overall, overcoming interstitial pressure and effectively controlling intratumoral ^{165}HoMS injections in ex vivo PDAC via the developed injection method, including the recommended ISPs and IIPs shown in Table 2 and Table 3, respectively, is feasible. Disregarding these parameters could result in the loss of ^{165}HoMS concentration control or overall loss of injection feasibility.

Although multiple intratumoral injection therapies have been studied, detailed descriptions of injection parameters and techniques are scarce.[11] The in vivo intratumoral injection of ^{32}P microparticles, which can be delivered either percutaneously[26-28] or via EUS[14,29], serves as the most comparable approach to the injection of ^{165}HoMS. The OncoPaC-1 trial, which employs intratumoral injection parameters similar to those established in this study, notably differs in the maximum injection volume (8% in OncoPaC-1 vs. 20% advised in this study) and only applies a single deposition per tumor.[14] A smaller injection volume might result in lower leakage risks and a lower tumor coverage; however, the results of the OncoPac-1 trial might also suggest that complete tumor coverage is unnecessary to establish a sufficient immunologic response and increase patient outcomes.[14]

This study has several limitations regarding the generalizability of the results. The investigation involved a small sample size with significant variation in PDAC characteristics and with limited selection of patient demographics, including tumor morphology, location, intratumoral heterogeneity, vascularization, comorbidities, and prior anticancer treatment. Injection feasibility may vary among these factors, for example, due to changes in the tumor microenvironment following chemotherapy, which can differ between responders and nonresponders.[30] In addition to sample variation, the injection methodology and distribution evaluation also varied due to the exploratory and iterative design of this study, preventing further statistical analysis. Nuclear imaging with SPECT/CT is the standardized modality for observing ^{166}HoMS distributions in humans[31] but is unavailable for nonradioactive ^{165}HoMS; thus, other methods (CT, MRI, US, pathology) were explored for further distribution assessment.

When IIPs are applied in vivo, additional factors such as vascularity, blood flow, and surrounding tissues may alter interstitial pressure, impacting feasibility. Thus, it should be recognized that the ex vivo results observed in this study may change when extrapolated to in vivo intratumoral injections. Further in vivo studies in human patients or animal models are necessary to validate these findings. Given the inherent variability of PDAC tumors within the patient population, specific injection limitations for guaranteed feasibility may not exist at all, emphasizing the need for a more patient-specific and image-guided approach. Thus, while the identified IIPs and cutoff values can be used as guidelines and may provide a foundation for future studies exploring intratumoral ^{166}HoMS injections in PDAC, they may not be generalizable for all PDAC tumors, nor can they be directly extrapolated to an in vivo setting without further investigation.

In future investigations, several factors should be considered. High interstitial pressure plays a critical role in injection feasibility, necessitating assessment in an in vivo setting with a patient-specific methodology. The approach is also essential; options such as endoscopic ultrasound (EUS), open surgery, or percutaneous image-guided techniques are currently viable.[11] EUS guidance offers superior real-time visualization of the tumor and needle, although the visibility of the microspheres and the degrees of freedom for needle insertion are limited. While the ultrasound-guided approach utilized in this study could translate well to open surgical methods, surgery remains highly invasive, contradicting the goal of minimally invasive microbrachytherapy. Thus, a percutaneous, periprocedural image-guided approach might be the superior choice. Owing to the multimodality imaging characteristics of ^{166}HoMS, therapy guidance enables quantification of the ^{166}HoMS distribution and further optimizes a patient-specific approach. Periprocedural imaging can be performed with MRI[18]; however, factors such as cost, acquisition speed, availability, standardized needle guidance, resolution, and magnetic restrictions favor CT guidance. Moreover, the clear visibility of the main ^{165}HoMS deposits on CT in this study substantiate the use of CT guidance for both needle and therapy guidance. Still, post-injection validation by standardized SPECT/CT is necessary for confirmation of extratumoral and intratumoral distributions and for validation of novel dosimetry modalities. With respect to the target population, PDAC is known for its rapid systemic progression, meaning that a local therapy should always be combined with systemic anticancer treatment, such as conventional FOLFIRINOX chemotherapy.[32] Patients who may benefit most from enhanced local tumor control and potentially even from downstaging are the patients diagnosed with unresectable LAPC and who are receiving, or have just finished, systemic chemotherapy.

Conclusion

A feasible method was developed for intratumoral injection of holmium-165 microspheres in ex vivo pancreatic ductal adenocarcinoma. Adherence to the developed injection methods and guidelines is recommended for effective intratumoral deposition and minimal extratumoral leakage. The injection system and parameters developed here provide a foundation for future studies on holmium-166 microsphere injections in pancreatic cancer patients, with the aim to improve local tumor control and potentially broaden treatment options.

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Appendices

Appendix S1 Extensive injection system development results

An elaborated description of the established injection system parameters and findings during initial laboratory testing are described here.

1. Syringe volume: 3.0 mL (range: 1.0–10.0 mL)

Syringe volumes of 1.0 mL resulted in less homogeneous suspensions and faster agglomeration, whereas syringes up to 10.0 mL resulted in excessive variation in the volume injected by manual injection due to the larger diameter.

2. Rotation time: 120 s (range: ~1–120 s)

Visual homogenization of the ^{165}HoMS suspension was achieved after 120 s in all the cases, even if the ^{165}HoMS accumulated to one side of the syringe before rotation. There were no clear differences in injection concentrations when rotating over 120 s.

3. Rotation speed: 60 RPM (range: 30–120 RPM)

Since rotation of the syringe was performed manually, a rotation speed was evaluated which could be achieved for multiple minutes without exhausting the operator and did not cause visual agglomeration. Rotation speeds below 60 RPM caused moments of idleness, which resulted in agglomeration. When the suspension was injected, rotation of the syringe was not possible, and agglomeration of the suspension occurred within seconds. Therefore, between every separate injection, or every 0.5 mL of injection volume, the syringe was rotated again for at least 10 s.

4. Injection speed: 0.2 mL/s (range: 0.05–1.0 mL/s)

Faster injection up to 1.0 mL/s could cause volume overshoots and thus increase the chance of leakage. Since the syringe could not be rotated during injection, lower injection speeds caused more ^{165}HoMS agglomeration and more injection pauses to homogenize the suspension again.

5. Injection volumes: 0.3–1.0 mL (range: 0.1–1.0 mL)

Because the injection system contains a dead volume, which is the lumen volume between the syringe exit and needle tip in which the ^{165}HoMS agglomerate, a minimal injection volume of 0.3 mL was used to ensure complete flow through the dead volume, and 0.5 mL when the plastic extension tube was attached.

6. System component diameters:

System components with adjacent internal diameter changes, especially downstream narrowing, showed increased visual agglomeration of the ^{165}HoMS. Additionally, relatively large dead volumes, commonly found near Luer-lock connections, showed increased agglomeration when in a horizontal position. ^{165}HoMS agglomeration within dead volumes caused a lower ^{165}HoMS concentration; however, it could unexpectedly dislodge during injection and therefore suddenly increase the injected ^{165}HoMS concentration.

Chapter 4

INTRATUMORAL HOLMIUM-166 MICROSPHERE INJECTION IN PATIENTS WITH UNRESECTABLE PANCREATIC DUCTAL ADENOCARCINOMA: A SINGLE-CENTER, SINGLE-ARM, OPEN-LABEL, FEASIBILITY, AND SAFETY STUDY

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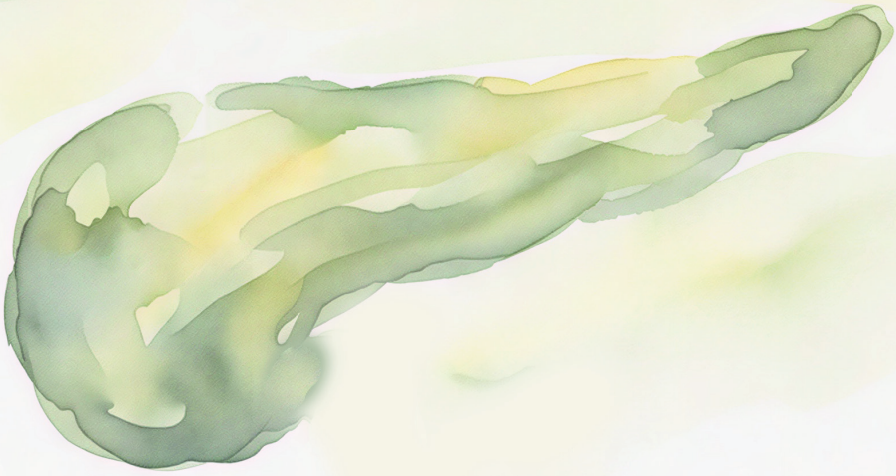

Abstract

Introduction: Pancreatic ductal adenocarcinoma (PDAC) has a poor prognosis and lacks local treatment options. This study aimed to assess the feasibility and safety of the first-in-human intraoperative ultrasound-guided intratumoral injection of radioactive holmium-166 microsphere in patients with PDAC.

Materials and methods: Patients with proven PDAC eligible for open surgical resection were included. If resection was abandoned during exploration, study intervention was performed. Feasibility was defined by injection success and on/off-target radiation. Safety was based on adverse event (AE) monitoring for 12 weeks categorized by severity grade and study attribution.

Results: Three of the thirteen included patients received study intervention. Injection was successful in all three patients. Mean tumor doses of 5.0, 17.0 and 39.0 Gy and maximum tumor doses of 25.0, 41.0 and 256.0 Gy were achieved. Off-target radiation was found once in the lungs and once in the colon with a mean dose <1.0 Gy. There were no AEs with high study attribution, 16, 14 and 19 AEs with low study attribution, including 3, 2 and 4 AEs with grade ≥3. Holmium-166 microspheres appeared hyperdense on CT.

Conclusion: Intratumoral injection of holmium-166 microspheres in patients with unresectable PDAC seems feasible and safe. Research into minimally invasive image-guided application is advised.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) accounts for more than 85% of all primary pancreatic neoplasm diagnoses.[1] The low 5-year relative survival rate of 13% is mostly caused by the small proportion of patients eligible for potentially curative surgical resection.[2] Although patients with locally advanced pancreatic cancer (LAPC) suffer no metastatic disease, 77.8% is ineligible for surgical resection after neoadjuvant therapy because of local unresectability.[3] This underscores the potential gain that can still be achieved at the local treatment level for patients with PDAC to reach a resectable tumor stage, with less abandonment of resection and increased R0 resection rates.

Multiple local treatment modalities have been studied and gained popularity over the past few years. However, recent randomized controlled trials (RCT) to the effectiveness of radiofrequency ablation (RFA), irreversible electroporation (IRE), and MR-guided stereotactic ablative body radiotherapy (MR-SABR) in LAPC did not find significant benefits for IRE over MR-SABR (or vice versa) or for RFA with chemotherapy over chemotherapy alone, while introducing considerable side-effects.[4,5] A notable local treatment for LAPC with less side-effects is intratumoral injection (ITI) with radioactive phosphorus-32 (^{32}P) microparticles. In a single-arm trial in 42 patients, the use of ^{32}P ITI by endoscopic ultrasound (EUS) demonstrated good local disease control of 82% after 16 weeks and a median overall survival of 15.5 months, however no advanced dosimetry or a RCT is currently available.[6]

Radioactive holmium-166 (^{166}Ho) poly-L-lactic acid microspheres (^{166}HoMS) represent another promising local intervention for treating PDAC by ITI.[7,8] The short half-life of 26.83 hours (versus 14.3 days for ^{32}P) induces a high dose-rate for the same total dose, while the similar limited tissue penetration of the Beta$^-$ radiation results in a sharp dose drop-off, sparing adjacent healthy organs and critical vasculatures, as demonstrated in previous preclinical studies.[9-11] A key attribute of ^{166}HoMS, and a distinction from most other therapeutics, is its multimodality imaging capability, enabling conventional SPECT, computed tomography (CT) and magnetic resonance imaging (MRI) visualization and quantification, thus facilitating enhanced analysis for periprocedural and postprocedural therapy delivery and optimization.[12-15] Previous research on ITI in *ex vivo* PDAC tumors has established a proof-of-concept and guidelines for this novel intervention.[16]

This first in-human study aimed to assess the feasibility and safety of ultrasound-guided intratumoral injection of holmium-166 microspheres in patients with primary PDAC, which were found to be unresectable during exploratory surgery due to blood vessel involvement or metastatic disease.

Materials and methods

Here the condensed version of the Materials and Methods is presented. The complete version can be found in the Appendix S1. This prospective, single-center, single-arm, open-label, feasibility, and safety, phase-1 study was registered on clinicaltrials.gov (ID: NCT05191498). The study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Research Ethics Committee (NL76311.091.20). The expected enrollment was 3 patients.

Patient selection and planning

Eligible participants had pathologically proven PDAC of either resectable pancreatic cancer (RPC), borderline resectable pancreatic cancer (BRPC) or LAPC according to the Dutch Pancreatic Cancer Group (DPCG) criteria[17] after neoadjuvant chemotherapy. Eligible participants had no evidence of metastatic disease and were eligible and willing to undergo surgical exploration with the purpose of surgical resection of the primary tumor. All patients were discussed in a multidisciplinary panel preceding inclusion, regarding resectability status and surgical expectations.[17] After all above mentioned criteria were met, but before surgery commenced, the patients were provided with study information and could provide written informed consent. Thereafter, demographic data was collected at a baseline visit, including age, sex, WHO performance score, body mass index (BMI), tumor characteristics (location and resectability status according to the DPCG criteria[17], largest diameter, and volume), and neoadjuvant chemotherapy.

Patients underwent preoperative CECT for the purpose of patient-specific ^{166}HoMS ITI 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.), as shown in Figure 1A. The CT image acquisition parameters and reconstruction parameters are shown in Appendix S2.

Holmium microsphere preparation

Radioactive holmium-166 microspheres in a 0.1% Pluronic phosphate-buffer suspension (Quirem Medical, Deventer, The Netherlands) were delivered to the hospital. A detailed description of the ^{166}HoMS characteristics is given in Appendix S3. ^{166}HoMS in suspension were loaded in 3 mL syringes with a Luer-lock connection (Plastipak, BD Medical, New Jersey, USA). The activity required per patient was based on a dose coefficient of 15.87 mJ/MBq/kg tissue[18,] which resulted in equation (1).

$$A = \frac{D * M}{15.87} \quad (1)$$

The required injection activity (A) in MBq was calculated for a maximum achievable mean tumor dose (D) of 125 Gray (Gy) in tumors with a mass M in grams (g) and an assumed tumor density of 1,0 g/cm^3.

Treatment

All patients received piperacillin/tazobactam as antibiotic prophylaxis before surgery. Immediately following open exploratory surgery, patients either underwent surgical resection of the tumor or received ^{166}HoMS ITI if surgical resection was determined to be not feasible by the surgeon. In the case of surgical resection, patients were excluded. In the case of no surgical resection, either caused by local blood vessel involvement or by the perioperative observation of distant metastatic disease, ITI was performed using intraoperative ultrasound (US, Canon Medical Systems Corporation, Amstelveen, the Netherlands), as shown in Figure 1B. ITI was performed with 110–150 mm 20G needles (MediPlast Special Cannula, MediPlast, Malmö, Sweden).

Follow-up

Patients were hospitalized for at least 48 hours for contamination monitoring. Any excretion of holmium-166 was measured in a dose calibrator (VIK-202, Comecer, Joure, the Netherlands; lower detection limit 0.01 MBq). The monitoring of complications by CTCAE v4.0 was performed continuously during hospitalization including conventional blood tests. CECT, planar thorax-abdomen gamma camera imaging, and single-photon emission computed tomography (SPECT)/CT were acquired within 72 hours after intervention. Planar and SPECT acquisition parameters are presented in Appendix S4. Outpatient clinics were used for complication registration at one, eight and twelve weeks after intervention. CECT was repeated twelve weeks after the intervention.

Data analysis

The total injected activity (MBq) was determined by measuring all residual activity post-procedure and subtracting from the starting activity.

The planar images were analyzed with Hermia Gold Smart (version: 2.17.0.87), background corrected and anterior-posterior fused by geometric mean.[19] The activity per Region of Interest (ROI) was determined as fraction from the total injected activity and then translated to the respective SPECT/CT Volume of Interest (VOI) with Qsuite (version 2.2, build 2.2.0.6237, Quirem Medical, Deventer, The Netherlands). With the SPECT/CT, the mean dose (Gy) and maximum dose (Gy) in on- and off-target VOIs were calculated. If structures/organs were not completely visible on SPECT/CT, the mean dose was calculated using equation (1), with the calculated activity from planar imaging in MBq (A) and the reference organ mass from IRCP-089 in grams (D).[20]

Study outcomes

The primary outcome feasibility, the ability to inject ^{166}HoMS 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. The primary outcome safety, 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). Only the highest-grade AE was reported. AEs were also categorized by likelihood of study attribution(*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. Only 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 (H_p 0.07) hands and body and the deep dose equivalent (H_p 10) of the body, with a lower detection limit of 0.15 mSv for the hands and 0.04 mSv for the body. 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.

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.

Results

Patient demographics

Between December 2021 and November 2023, thirteen patients were included. Nine underwent surgical resection, one was excluded due to pancreatitis, and three were ineligible for resection (caused by unresectable vascular involvement of the hepatic artery and superior mesenteric artery in patients 1, celiac axis and splenic artery in patient 3, and peritoneal metastases patient 2), and received ITI. An overview of the baseline characteristics is presented in Table 1. Summation of results are for patients 1, 2 and 3, respectively, unless stated otherwise.

Table 1. *Baseline characteristics of the nonholmium-166 microsphere (166HoMS) population (NP) and the ^{166}HoMS intervention population (IP).*

Baseline characteristics*	Non-^{166}HoMS population (NP)		^{166}HoMS intervention Population (IP)	
Total, n	10		3	
Median age (range), years	68	(57 – 78)	58	(52 – 73)
Sex				
Female (%)	5	(50)	2	(67)
Male (%)	5	(50)	1	(33)
WHO performance score				
0 (%)	8	(80)	1	(33)
1 (%)	2	(20)	2	(67)
Median BMI (range), kg/m^2	25.8	(22.2 – 39.9)	32.7	(29.5 – 43.2)
Pancreatic tumor location				
Head (%)	8	(80)	1	(33)
Body (%)	2	(20)	1	(33)
Tail (%)	0	(0)	1	(33)
Resection status (DPCG criteria)				
Resectable (%)	3	(30)	0	(0)
Borderline resectable (%)	5	(50)	2	(67)
Locally advanced (%)	2	(20)	1	(33)
Vascular involvement				
Superior mesenteric artery <90 degrees (%)	1	(10)	0	(0)
Superior mesenteric artery ≥90 degrees (%)	1	(10)	1	(33)
Celiac axis <90 degrees (%)	1	(10)	1	(33)
Celiac axis ≥90 degrees (%)	1	(10)	0	(0)
Common hepatic artery <90 degrees (%)	3	(30)	0	(0)
Common hepatic artery ≥90 degrees (%)	0	(0)	0	(0)
Superior mesenteric / portal vein 90-270 degrees (%)	3	(30)	1	(33)
Superior mesenteric / portal vein ≥270 degrees (%)	0	(0)	0	(0)
Median largest tumor diameter (range), mm	24	(15 – 40)	28	(27 – 50)
Median tumor volume (range), mL	6.1	(2.5 – 11.6)	18.3	(6.1 – 30.0)
Neoadjuvant chemotherapy**				
Yes (%)	9	(90)	3	(100)
No (%)	1	(10)	0	(0)

**All baseline characteristics were reported <14 days before surgery and (if applicable) after neoadjuvant chemotherapy. **Neoadjuvant chemotherapy in the form of FOLFIRINOX. WHO, World Health Organization; BMI, Body Mass Index; DPCG, Dutch Pancreatic Cancer Group.*

Treatment and hospitalization

An overview of the results from the ITI is shown in Table 2. No procedural failures or complications occurred. The ITI procedure time was between 30–45 minutes. The injection volume was reduced because of a smaller tumor volume on US in patient 1 and because of limited sight of the tumor by US caused by tumor growth into the stomach in patient 3. Minimal radioactive contaminations of 7.3, 0.0, and 3.8 MBq (16%, 0% and 5% of the injected activity) occurred.

No extracorporeal contaminations were found during hospitalization except in a fecal sample from patient 1 of 2.1 MBq (4.7% of the injected activity). Contamination monitoring was released for all patients within 72 hours after intervention did not require extended hospitalization. All patients were discharged from the hospital after recovery from surgery after 6, 8 and 5 days.

Table 2. *Overview of the intratumoral holmium-166 microsphere (^{166}HoMS) injection procedures.*

#Result	Description	Patient 1		Patient 2		Patient 3	
#1	Tumor volume, mL	18.3		6.1		30.0	
#2.1	Planned injection volume, mL (% of #1)	3.6	(20)	0.6	(10)	3.0	(10)
#2.2	Injected volume, mL (% of #1; % of #2.1)	2.6	(14; 72)	0.6	(10; 100)	2.0	(7; 67)
#3.1	Planned injection activity, MBq	144.1		48.1		236.3	
#3.2	Reassessed injection activity, MBq (% of #3.1)	103.8	(72)	48.1	(100)	157.5	(67)
#3.3	Injected activity, MBq (% of #3.2)	44.6	(43)	42.6	(89)	74.1	(47)
#4	Injected ^{166}HoMS, mg	7.3		10.4		19.0	
#4.1	Injected ^{166}HoMS concentration, mg/mL	2.8		17.3		9.5	
#5.1	Planned number of ^{166}HoMS deposits	4		1		3	
#5.2	Injected number of ^{166}HoMS deposits	3		1		2	
#6	Procedure duration, minutes	45		30		45	
#7	Specific activity (MBq/mg)	6.1		4.1		3.9	
#8	Prepared activity concentration, MBq/mL	39.7		99.7		99.7	
#9.1	Residual activity in needle(s), MBq (% of #3.3)	9.2	(21)	3.7	(9)	12.7	(17)
#9.2	Residual activity in injection device, MBq (% of #3.3)	45.9	(103)	65.8	(154)	142.5	(192)
#9.3	Residual activity in disposables, MBq (% of #3.3)	7.3	(16)	0.43	(1)	3.8	(5)
#10.1	Activity in tumor, MBq (% of #3.3)	6.5	(15)	17.3	(41)	74.1	(100)
#10.2	Mean tumor dose, Gy (max tumor dose)	5.0	(25.0)	17.0	(41.0)	39.0	(256.0)
#11.1	Off-target radiation organ	Lungs		Colon		None	
#11.2	Off-target activity, MBq (% of #3.3)	38.1	(85)	25.2	(59)	0.0	(0)
#11.3	Off-target mean dose, Gy (max dose)	<1.0	(1)	<1.0	(4)	0.0	(0)

All activities in MBq refer to the activity at the time of treatment (t = 0). ^{166}HoMS: holmium-166 microspheres.

Feasibility

The injected activity was 44.6 MBq, 42.6 MBq and 74.1 MBq (43%, 89% and 47% of the planned activity). The residual activity found in the injection device was 45.9 MBq, 65.8 MBq and 142.5 MBq (103%, 154% and 192% compared with the injected activity). Mean tumor doses of 5.0, 17.0 and 39.0 Gy and maximum tumor doses of 25.0, 41.0 and 256.0 Gy (shown in Figures 1C and 1F) were achieved. For patient 3, 1/3rd of the tumor was intentionally not treated, resulting in a mean dose of 69.0 Gy to the remaining 2/3rd of the tumor. Off-target radiation of 38.1 MBq (85% of injected activity) was detected in the lungs of patient 1, and 25.2 MBq (39% of injected activity) in the colon of patient 2 (shown in Figure 1D). This resulted in a mean organ dose of <1.0 Gy in both organs, a maximum dose of 1.0 Gy in the lungs and 4.0 Gy in the colon. Patient 3 suffered no off-target radiation.

Safety

Follow-up was 11.0, 12.0 and 11.7 weeks after the intervention. Patient 1 retracted informed consent because of an increased burden of metastatic disease, and patient 3 died (grade 5) 11 weeks and 5 days after intervention because of metastatic disease. This was considered unrelated to ^{166}HoMS ITI. Patient 2 completed follow-up. During follow-up, no adverse events with high study attribution were found in any of the treated patients. In total, 16, 14 and 19 AEs with low study attribution, including 3, 2 and 4 AEs with grades ≥3, were recorded. AEs with a grade ≥3 included increased ASAT, bile duct stenosis caused by progressive disease and requiring intervention and increased blood bilirubin due to bile duct stenosis after 11 weeks (patient 1); abdominal pain, strongly referring to the abdominal wound and fatigue, within the first 48 hours after surgery (patient 2); and dehydration, metastatic disease, nausea, and vomiting, shortly before death at 11 weeks and 5 days after intervention (patient 3). A complete overview of all recorded adverse events, grades and study attribution is presented in Table 3.

Table 3. *Complete overview of recorded adverse events, grading, and study attribution.*

	Patient 1		Patient 2		Patient 3	
Term Adverse Event (alphabetical a-z)	**Grade**	**Study attribution**	**Grade**	**Study attribution**	**Grade**	**Study attribution**
Abdominal pain	2	Unlikely	3	Unlikely	-	-
Alanine aminotransferase increased	1	Unlikely	1	Unlikely	-	-
Alkaline phosphatase increased	2	Unlikely	1	Unlikely	1	Unlikely
Alkalosis	1	Unrelated	-	-	1	Unrelated
Anemia	2	Unlikely	1	Unlikely	2	Unrelated
Ascites	1	Unlikely	-	-	-	-
Aspartate aminotransferase increased	3	Unlikely	1	Unlikely	-	-
Back pain	-	-	1	Unrelated	2	Unrelated
Bile duct stenosis	3	Unlikely	-	-	-	-
Blood bilirubin increased	3	Unlikely	-	-	-	-
Confusion	-	-	-	-	1	Unlikely
Dehydration	-	-	-	-	3	Unlikely
Dysgeusia	-	-	-	-	1	Unrelated
Fatigue	-	-	3	Unlikely	-	-
Fever	-	-	-	-	1	Unlikely
Gamma GT increased	2	Unlikely	2	Unlikely	1	Unlikely
Gastroesophageal reflux disease	2	Unlikely	-	-	-	-
Hemoglobinuria	1	Unlikely	-	-	-	-
Hyperglycemia	2	Unrelated	2	Unrelated	2	Unrelated
Hyperkalemia	1	Unlikely	-	-	1	Unrelated
Hypoalbuminemia	-	-	2	Unlikely	-	-
Hypocalcemia	-	-	-	-	1	Unrelated
Hyponatremia	-	-	1	Unlikely	-	-
Lymphocyte count decreased	1	Unlikely	-	-	-	-
Malaise	-	-	-	-	2	Unrelated
Malignant neoplasm *	-	-	-	-	5	Unlikely
Nausea	-	-	1	Unlikely	3	Unrelated
Pancreatitis	-	-	2	Unrelated	-	-
Peripheral neuropathy	-	-	1	Unrelated	2	Unrelated
Platelet count decreased	1	Unlikely	-	-	-	-
Stomach pain	-	-	-	-	2	Unlikely
Urinary tract infection	-	-	-	-	2	Unrelated
Vomiting	-	-	-	-	3	Unlikely
Total low study attribution adverse events	16		14		19	
Total high study attribution adverse events	0		0		0	
Total adverse events grade 1–2	13		12		15	
Total adverse events grade 3–5	3		2		4	

*Grade: Only the highest study attribution and grade at any time was reported here. 'Unrelated' was assigned only to AEs that were present before the intervention and did not worsen in grade after the intervention. 'Low study attribution' indicates either 'unrelated' or 'unlikely'. 'High study attribution' indicates either 'possible', 'probable', or 'definite'. '-' indicates that the adverse event in that row was not found in the patient of that column. 'Peripheral neuropathy' refers to either peripheral motor or sensory neuropathy. * The patient passed away 11 weeks and 5 days after intervention without further clarification from the general practitioner, likely caused by the diagnosed progressive disease.*

Secondary and exploratory results

All radiation exposure measurements of the operators during the procedures were below the detection limits, with the exception of the operator skin dose on the hands of 0.3 and 1.2 mSv. With respect to tumor response, all patients developed distant metastatic disease, either diagnosed during surgery

(patient 2) or by clinical CT within the follow-up period (patients 1 and 3). Patients 1 and 2 had stable disease of the primary tumor during follow-up, whereas patient 3 had progressive disease of the primary tumor. In patient 2, on the first CECT after ^{166}HoMS injection with the highest ^{166}HoMS concentration (Table 2, #4.1), a hyperdense ^{166}HoMS deposition was visible (shown in Figure 1E), with a maximum intensity of 522 Hounsfield units (HU). Compared with associated SPECT/CT (shown in Figure 1F), CT revealed a more localized deposition of the ^{166}HoMS.

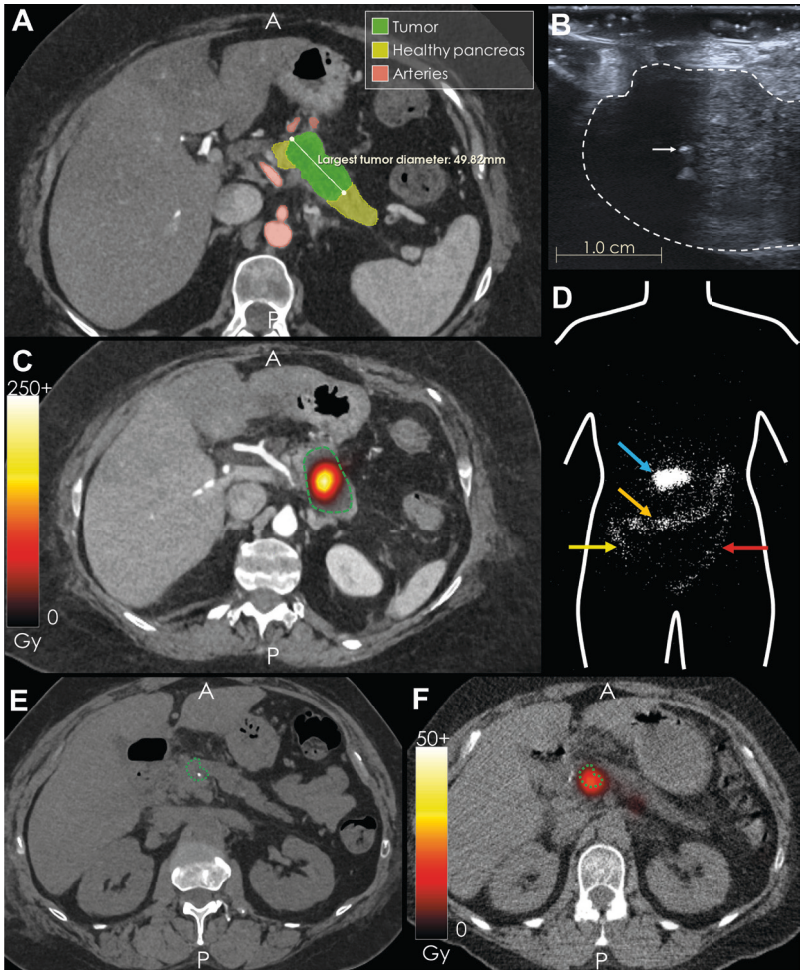

Figure 1. *Results from intratumoral injection (ITI) of holmium-166 microspheres (^{166}HoMS). (**A**) Preoperative ITI planning. Patient 3; (**B**) Intraoperative ultrasound (US) to assess the tumor size and guide the needle during injection. Tumor: white-dotted line, needle tip: white arrow. Patient 2; (**C**) SPECT-CT in plane with Figure 1A. Tumor (green dotted line). Patient 3; (**D**) Planar gamma camera image. Tumor region: blue arrow, ascending, transverse and descending colon: yellow, orange, and red arrows, respectively. Patient 2; (**E**) Noncontrast CT 24 hours after ^{166}HoMS ITI. ^{166}HoMS: white dot, tumor: green dotted line. Patient 2; (**F**) SPECT-CT in plane with Figure 1E. Tumor: green dotted line. Patient 2.*

Discussion

Intratumoral injection of radioactive holmium-166 microspheres could be a viable treatment option for patients with LAPC to further increase local tumor control, decrease the local tumor burden and increase quality of life or even achieve resectability. In this study, the safety and feasibility of intratumoral ^{166}HoMS injection in patients with PDAC were assessed in an open surgical setting.

Regarding the performance of the injections as planned, less volume was injected because of two identified causes. The first was the clear discrepancy between the tumor volume on CT and on US. The second cause was the limited visualization of the tumor via US during open abdominal surgery. Less activity was injected than planned, caused by the residue of the highly concentrated ^{166}HoMS suspension in the injection system and syringe. The percentage of ^{166}HoMS agglomeration within the injection device was previously assessed in a laboratory and *ex vivo* setting[16], but agglomeration seemed greater when it was performed *in vivo.* This could be caused by a less ideal clinical situation, which notably affected the syringe angle (aimed to be horizontal), syringe rotation (aimed to be continuous between injections) and rotation speed (aimed at 60 RPM). These parameters were more difficult to maintain *in vivo* because of the limited intraabdominal space and surgical restrictions. Additionally, injection resistance is expected to be greater *in vivo* than in previous ex vivo experiments because of the presence of blood flow and blood pressure *in vivo*.[21] Therefore, the injection efficacy results from this study could be used to optimize and standardize dosing protocols in future studies.

With respect to injection accuracy, on-target radiation was present in all patients; however, the on-target dose was lower than planned preoperatively in every patient, caused by shunting and leakage. After shunting of the ^{166}HoMS in patient 1, the injection parameters were adapted by decreasing the total injection volume from 20% to 10% in the following patients, with the aim of increasing ITI suspension control. Leakage might occur via the pancreatic duct or side branches of the pancreatic duct. Although these ducts were not visible on US and could therefore not be avoided, passage from the tumor toward the duodenum was clearly visible in patient 2 (Figure 1D). This type of leakage is also known to occur after the ITI of different therapeutics.[22,23] With respect to off-target radiation, because of the wide ^{166}HoMS distribution over large volumes in other organs, the dosage in these organs drops and no severe complications were expected or observed. To reduce shunting or leakage, intraoperative visualization and possibly quantification of the ^{166}HoMS might indicate when leakage occurs.[13,14,24] This is important because when the ^{166}HoMS are retained to the injection side without shunting or leakage, as seen in patient 3, a therapeutic tumor dose can be realized. Currently, image-guided needle placement, needle fixation, injection of no more than 10% of the tumor volume and the use of a modality for intraoperative ^{166}HoMS visualization are strongly advised for future studies.

The practicality of the implementation methodology should also be discussed. With surgery being the primary treatment, ITI was a secondary option for the patients in this study. Since ten out of the thirteen patients in this study received the first-choice treatment, ITI preparation costs were, in a sense, unnecessary. Since surgery is a well-considered treatment, even with the implementation of more strict patient selection and conservative surgery guidelines, resection rates would hardly reduce from 77% (10 out of 13 patients) in this study to 72% (171 out of 238) in LAPC with the National Comprehensive Cancer Network (NCCN) guidelines.[25] Given the current pressure on healthcare costs, a spare treatment option of this proportion is unfeasible. Given the current safety profile of ITI and sensitizer potential for combination therapies such as chemotherapy or immunotherapy, the inclusion of ITI in the neoadjuvant work-up would eventually be more efficient.[6,26]

No direct safety concerns with respect to the injected ^{166}HoMS were observed. More short-term adverse events (weeks 0–6) were either already present before intervention (unrelated to study attribution) or related to open-abdominal surgery, such as abdominal pain specifically aimed at the recovering incision. Late-term adverse events (weeks 6–12) were more often related to disease progression. For the safety of the operators, radiation exposure was far below the advised limits, making multiple procedures per week possible without any necessary radiation restrictions.

Within this first feasibility and safety trial, some limitations are worth noting. The small intervention group inhibits any statistical analysis, generalizability, and comparison to other intratumoral interventions. Furthermore, the specified inclusion and exclusion criteria could introduce selection bias. Still, even in this small sample size, the determined inclusion criteria enabled variation in essential factors for feasibility and safety, such as condition, tumor size or neoadjuvant therapy. Within this study, the effects of these factors remain unclear. Because patients underwent invasive surgery simultaneously with ^{166}HoMS injections and because the reviewers of the AEs study attribution needed to be trained and experienced with both interventions (e.g., study personnel), characterization of complications could also be vulnerable to bias. Because holmium-166 has a half-life of 26.83 hours and the most common complication (pancreatitis) after comparable interventions, such as fine needle aspiration/biopsy, manifests shortly after intervention[27], no radiation- or intervention-induced toxicity was expected to extend beyond the follow-up period. Still, The short follow-up period of 12 weeks can be used to assess short-term toxicity. This first in-human trial of ^{166}HoMS in PDAC had a considerable risk potential. Therefore, safety considerations were strict, limiting the maximum administered activity. Nevertheless, considering the current safety profile and the found dose distribution, the full potential of ^{166}HoMS injection seems yet undiscovered.

In future studies on ^{166}HoMS ITI in patients with PDAC, an early phase (phases I–II) trial should be performed in a larger cohort with a focus on minimally invasive application. The patient population should focus on local disease without signs of metastatic disease on imaging and biomarkers, circumventing the influence of surgery on adverse events and of systemic advanced disease on efficacy outcomes. Owing to the agglomeration of ^{166}HoMS in their current suspension, injection by EUS seems infeasible over the length of the EUS-scopes' injection lumen. Because of the visibility of ^{166}HoMS on CT in this study and the known correlation between holmium concentrations and HU values on CT[28], it seems to be the optimal imaging modality to be applied in future studies for both needle and therapy guidance and possibly even dosimetry. To reduce the variety of the mean tumor doses, refined dose planning and delivery techniques, such as high-viscosity microsphere carriers, are needed to maximize tumor coverage while minimizing off-target radiation. Considering the fast systemic progression of PDAC, ^{166}HoMS ITI should be an addition to systemic anticancer therapy.

Conclusion

In conclusion, intratumoral injection of holmium-166 microspheres in patients with unresectable pancreatic ductal adenocarcinoma seems feasible with a well-tolerated safety profile. Research into minimally invasive image-guided intratumoral injection is advised to visualize the microspheres perioperatively and increase microsphere retention in the tumor to increase the mean tumor dose and the potential efficacy.

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Appendices

Appendix S1 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 ^{166}HoMS intervention group, without a control arm, because of the early research phase of this study. 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*10^9 and platelet count <100*10^9), 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 ^{166}HoMS 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, 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 ^{166}HoMS 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 Appendix S2.

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 ^{166}HoMS characteristics is given in Appendix S3. ^{166}HoMS 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 ^{166}HoMS 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 which resulted in equation (1).

$$A = \frac{D * M}{15.87} \quad (1)$$

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/cm^3. The activity loaded into the syringe (A_{syr}) 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 [16]. 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 ^{166}HoMS 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 ^{166}HoMS 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 ^{166}HoMS injection, a manual rotation device (Figure S1.a), described in detail in previous research[16], 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[16] 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 ^{166}HoMS injection was performed in line with the advised intratumoral injection parameters as described in earlier research.

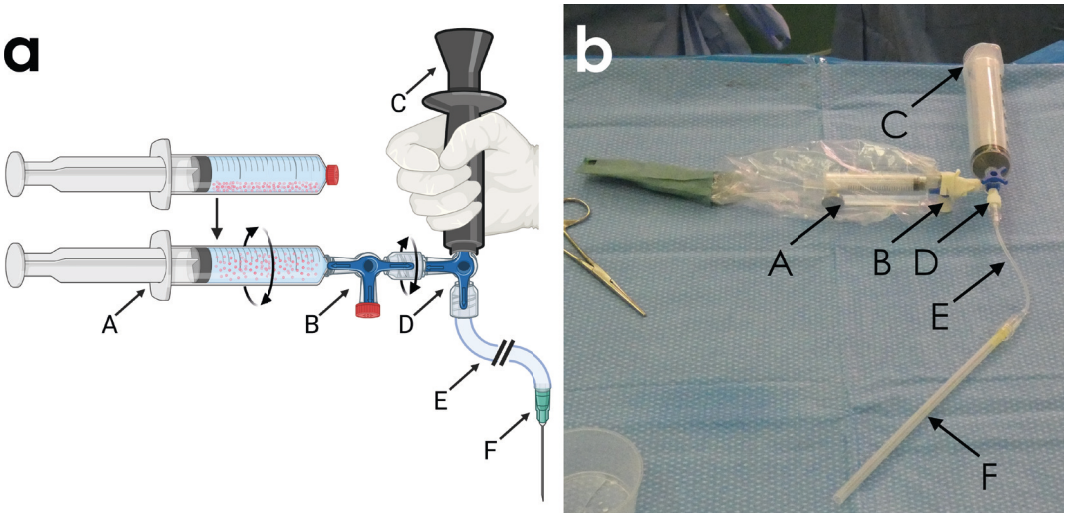

FIGURE S1. *Overview of the holmium-166 microsphere (^{166}HoMS) manual rotation and injection device. (a) Schematic overview. Created with BioRender.com (b) Device during the injection procedure with the therapy syringe with acrylic glass shielding in a sterile plastic sleeve. Components: A) therapy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

Intratumoral ^{166}HoMS 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 ^{166}HoMS suspension by rotating the ^{166}HoMS-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 radiation safety 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. 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 (A_{inj}), 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 (A_{disp}) were converted to the activity at the time of treatment (t = 0) and cumulatively subtracted from the prepared syringe activity at t = 0 (A_{syr}), as shown in equation 2.

(2) $$A_{inj} = A_{syr(t=0)} - A_{disp} * 0.5^{\frac{\Delta t}{t_{\frac{1}{2}}}}$$

Where A_{inj} is the total activity injected (MBq), A_{syr} is the initially prepared syringe activity at t = 0 (MBq), A_{disp} is the cumulative activity from all measured disposables (MBq), Δt is the amount of time between the time of measurement and the time of treatment (hours), and $t_{1/2}$ is the half-life of holmium-166 at 26.83 hours.

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 (cm^2), and the detected counts (C) which was related to the amount of radioactive ^{166}HoMS 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/cm^2) in comparison with an adjacent BR were selected. These RoIs were then background corrected (RoI_{BC}) (equation 3).

(3) $$BC_{RoI(x)} = C_{RoI(x)} - ([C_{BR}] * A_{RoI(x)})$$

Equation (3) is used for each RoI individually, as shown in equations (3), (4) and (5) by *(x)*. In equation 3, BC_{RoI} is the background-corrected number of counts in a RoI (counts), C_{RoI} is the total number of counts in a RoI not compensated for background (counts), $[C_{BR}]$ is the count concentration in the BR adjacent to RoI(x) (counts/cm^2), and A_{RoI} is the surface area of a RoI (cm^2). 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) $$T_{RoI(x)} = \sqrt{BC_{ant(x)} * BC_{post(x)}}$$

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.

(5) $$\frac{T_{ROI(x)}}{\sum_x T_{ROI(x)}} = \frac{A_{ROI(x)}}{A_{inj}}$$

SPECT/CT gamma camera imaging analysis

The calculated activities found via planar gamma camera imaging ($A_{RoI(x)}$) 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 Ho^{166} 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*). 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 ^{166}HoMS 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 (H_p 0.07) hands and body and the deep dose equivalent (H_p 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. 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, the feasibility of CT application for intratumoral ^{166}HoMS 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.

Appendix S2 Computed Tomography acquisition and reconstruction parameters

Parameter	Computed tomography	
Phase	Non-contrast	Arterial/venous
Brand	Canon Medical Systems – Aquillion	Canon Medical Systems – Aquillion
Patient position	Feet-first supine	Feet-first supine
Acquisition type	Spiral	Spiral
Spiral pitch factor	0.813	0.813
Samples per pixel	1	1
Pixels spacing (mm)	0.740/0.740	0.740/0.740
Slice thickness (mm)	0.5	0.5
Reconstruction algorithm and covolution kernel group	Advanced intelligent Clear-IQ Engine (AiCE)	Advanced intelligent Clear-IQ Engine (AiCE)
Convolution kernel	BODY_SHARP	BODY_SHARP
Image filter	Beam hardening correction	Beam hardening correction
Filter type	LARGE	LARGE
Focal spot(s)	0.9/0.8	0.9/0.8
Filter material	Aluminium/copper	Aluminium/copper
Rescale intercept	0	0
Contrast agent	None	Iomeron 300
Contrast bolus (mL)	None	150
Rescale slope	1	1
Kilo voltage peak (kVp)	120	120
Exposure time (ms)	750	500
Tube current (mA)	240	107
Exposure (mAs)	180	54
Revolution time (s)	0.75	0.50
Single collimation width	0.5	0.5
Total collimation width	40	40

Appendix S3 Detailed description of the used holmium-166 poly-L-lactic acid microspheres

The used biocompatible holmium-166 poly-L-lactic acid microspheres (^{166}HoMS) had a mean diameter of 30 micrometer (± 5 micrometer, 97% of the microspheres have a diameter of between 15 and 60 micrometer). The maximum energy of the β^- radiation is 1.85 MeV (50.0%) and 1.77 MeV (48.7%). The maximum range of the emitted beta particles in tissue is 8.7 mm with a mean of 2.5 mm. In addition, holmium-166 emits primary gamma photons (81 KeV). Holmium-166 has a half-life of 26.83 hours. The mass percentage of the element holmium (holmium-165 and holmium-166) in the microspheres was between 19.5—19.7%. ^{166}HoMS can be visualized *in vivo* with MRI and SPECT and with CT when the concentration ^{166}HoMS in tissue is sufficient. Intratumoral holmium-166 microspheres are similar to the CE-marked product QuiremSpheres™, intended to be used for selective internal radiation therapy of liver cancer.

Appendix S4 Planar gamma camera and SPECT acquisition and reconstruction parameters

Parameter	Planar gamma camera	SPECT
Brand	Siemens – Encore 2	Siemens – Encore 2
Patient position	Feet-first supine – arms down	Feet-first supine – arms up
Scan region	Thorax-abdomen	Abdomen
Collimator	Medium energy all purposes – parallel	Medium energy all purposes – parallel
Convolution kernel	n.a.	3D-ordered-subsets implementations
Iterations	n.a.	10
Subsets	n.a.	8
Gaussian filter	None	None
Pixel spacing (mm)	2.4	4.8
Slice thickness (mm)	n.a.	4.8
Number of frames	n.a.	81
Samples per pixel	1	1
Number of energy windows in reconstruction	2	1
Energy window(s) lower limit(s) (KeV)	73.1, 108.2	73.1
Energy window upper limit(s) (KeV)	85.0, 122.1	85.0
Number of Frames in rotation	n.a.	64
Scan speed	8 cm/minute	20 seconds per frame

Chapter 5

CT-GUIDED INTRATUMORAL HOLMIUM-166 MICROSPHERE INJECTION IN PATIENTS WITH LOCALLY UNRESECTABLE PANCREATIC CANCER AFTER FOLFIRINOX INDUCTION CHEMOTHERAPY: A SINGLE-CENTER, SINGLE-ARM, OPEN-LABEL, FEASIBILITY, AND SAFETY STUDY

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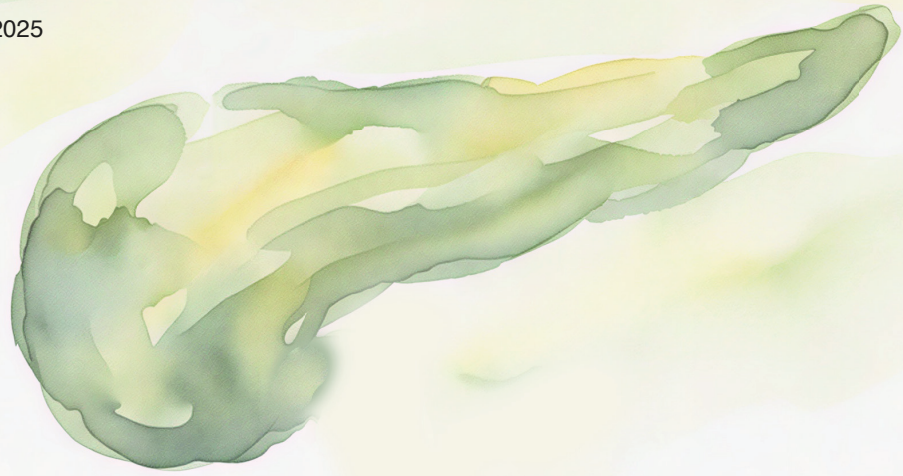
Submitted, 2025



Abstract

Introduction: Pancreatic ductal adenocarcinoma (PDAC) has a high mortality rate, with surgical resection being the only potentially curative option. Patients who are ineligible for surgery after induction chemotherapy, CT-guided percutaneous intratumoral injection of radioactive holmium-166 microspheres (^{166}HoMS) is a promising alternative. This study evaluated the feasibility and safety of fully CT-guided intratumoral ^{166}HoMS injection in patients with unresectable PDAC following induction chemotherapy.

Methods: Six patients with pathologically confirmed PDAC underwent personalized, CT-guided percutaneous ^{166}HoMS injection under general anesthesia. Feasibility was assessed by injection success, and by on-target and off-target dose distribution, evaluated using planar and SPECT/CT imaging within 24 hours post-injection. Safety was assessed through adverse event (AE) monitoring (CTCAE v5.0) for 16 weeks. Local disease control rate (LDCR) at 16 weeks was an exploratory outcome.

Results: Technical feasibility was achieved in all six patients. CT provided clear visualization of hyperdense ^{166}HoMS, aiding needle and therapy guidance. The median of the mean tumor dose (MD) was 25 Gy (range: 14—38 Gy) and the median of the maximum local dose (MLD) was 102 Gy (range: 90—395 Gy). The tumor MD was lowered by off-target radiation in 6/6 (100%) patients, mainly in the needle tract (3/6 patients) and colon (5/6 patients), without causing any related AE ≥grade 3. Off-target MD and MLD ranged from <1–58 Gy and <1–663 Gy, respectively. Of 65 AEs (range: 6—15 per patient), five (7.7%) were intervention-related; four grade 1 and one grade 3. LDCR was 66.7% at 16 weeks.

Conclusion: CT-guided percutaneous ^{166}HoMS injection is feasible and safe in patients with unresectable PDAC. This technique enables safe needle placement and clearly visualizes ^{166}HoMS depositions on CT. Exploratory results indicate local response potential, supporting further study investigations to optimize the tumor dose and reduce off-target radiation.

Introduction

With one of the highest mortality rates of all cancers, pancreatic ductal adenocarcinoma (PDAC) has a clear need for improved treatment options. Currently, complete resection (R0; ≥1mm tumor-free margin) of the tumor is the only treatment with curative intent. At diagnosis, 14% of all patients with PDAC are eligible to undergo surgical resection (resectable PDAC), which grants the highest 5-year relative survival rate after resection of 13%.[1,2] To be eligible for resection, a patient needs to be diagnosed with non-metastatic disease with little to no vascular involvement (resectable PDAC). Primary tumors with more vascular involvement are either characterized as borderline resectable or locally advanced pancreatic cancer (LAPC). Patients diagnosed with LAPC, 30—40% of all patients with PDAC, can receive FOLFIRINOX induction chemotherapy (folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin) to reduce vascular involvement and increase the chance of becoming eligible for surgical resection.[3,4] Approximately a quarter of these patients do receive surgical resection.[5-7] A standardized, safe, and low-burden treatment to further downstage LAPC and increase resection rates is not yet available.

Patients with LAPC could benefit from a supporting minimally invasive local therapy which could also potentially give a synergetic response.[8,9] Furthermore, in palliative care or in vulnerable patients, a minimally invasive intervention could be more accessible to provide local tumor control, reduce tumor burden, and possibly extend survival. A potential minimally invasive local therapy is intratumoral implantation of beta$^-$ (β^-) emitting holmium-166 microspheres (^{166}HoMS) to irradiate the tumor from the inside out. The use of these 30 μm radioactive microspheres is well established for transarterial radioembolization (TARE) in hepatic cancer with its tumor-specific double blood supply.[10,11] However, for PDAC, with a single blood supply, this transarterial approach is expected to be ineffective and unsafe. Intratumoral injection, also called microbrachytherapy, could be an effective treatment method with ^{166}HoMS in PDAC. Because the microspheres can be injected directly into the extracellular matrix, there is no inhibition by the PDAC drug resistant microenvironment and no dependency on tumor vascularization, surpassing limitations of intravenously injected therapies.[12] Because of the β^- radiation penetration depth, >90% within 2.5 mm[13], healthy tissue and nearby vital structures receive minimal irradiation, surpassing limitations of external beam radiation therapy.[14]

Previously, this research group performed the fundamental study on intratumoral ^{166}HoMS injection in PDAC in the form of *ex vivo* non-radioactive ^{165}HoMS injection in surgically resected human PDAC[15] and the first-in-human safety tests by open-abdominal ultrasound-guided intratumoral ^{166}HoMS injection in patients found to be inoperable during open exploratory surgery. The latter showed good safety results, although it lost the minimally invasive and low-burden aspect for which this novel intervention was intended.[16] Injection via percutaneous needle placement is a minimally invasive application that can implant the ^{166}HoMS accurately and can reach the tumor in any and multiple locations of the pancreas

because of the versatile approach angles. Imaging is required to achieve safe needle placement without puncturing any vital tissues, specifically major arteries surrounding the pancreatic head. Computed Tomography (CT) is a common imaging modality used for intraabdominal needle placements or CT-guided interventions.[17] Moreover, because of the inherent high density of ^{166}HoMS, the microspheres can be visualized and possibly quantified by CT.[18] Live periprocedural CT-guidance may contribute to patient-specific therapy planning and execution, and therefore improve therapy safety and efficacy.

The aim of this study was to assess the feasibility and safety of CT-guided percutaneous intratumoral ^{166}HoMS injection in six patients with locally unresectable PDAC after completion, cessation, or refusal of induction chemotherapy.

Materials and methods

This investigator-initiated, single-center, single-arm, and open-label study with a radioactive implant was registered at clinicaltrials.gov (ID NCT05880472). The expected enrollment was six patients in the single ^{166}HoMS intervention arm, without a control arm due to the feasibility and safety phase of this study. All participants were required to give written informed consent before enrollment. A detailed version of the materials and methods, including all equations and image processing steps, is available in Appendix S1.

Patient selection

Eligible participants were males or females aged 18 years or older with a pathologically proven PDAC after completion, cessation, or refusal of induction FOLFIRINOX chemotherapy. Patients were ineligible for, or declined, surgical resection with evidence of no metastatic disease, a World Health Organization (WHO) performance status of 0 or 1, a life expectancy of 16 weeks or longer, and a single solid PDAC tumor ≥20 mm in largest diameter. Exclusion criteria included leukopenia (leukocytes $<3.0\times10^9$/L), renal insufficiency (creatinine >165 μmol/L or eGFR <60 mL/min/1.73 m^2), blood coagulation deficiency (platelet count $<75\times10^9$/L), any unresolved toxicity of ≥grade 3 Common Terminology Criteria for Adverse Events (CTCAE version 5.0), pregnancy, or breastfeeding.

Baseline visit

During a baseline visit preceding intervention, demographic data (age, sex, body mass index (BMI)), WHO performance status, tumor location, resection status by the Dutch Pancreatic Cancer Group (DPCG) guidelines[19], previous anti-cancer therapy, current adverse events (AEs), and numeric pain rating scale (NRS) were noted. A three-phase contrast-enhanced CT (CECT) containing a holmium-specific non-contrast CT, arterial phase, and venous phase (image acquisition parameters are described in Appendix S2) was made, and a laboratory blood examination was performed for AE monitoring.

Patient-specific planning

A patient-specific intervention plan was made, determining the maximum injection volume, injection activity required for a targeted maximum achievable mean absorbed tumor dose, needle path, number of deposits, and injection volume per deposit. The maximum injection volume was calculated at 10% of the tumor volume and for safety standards, the targeted maximum achievable mean absorbed tumor dose was set between 125 and 150 Gray (Gy), only achievable with a 100% intratumoral and homogeneous distribution. The maximum injection volume was fractionated into deposits of 0.3 to 1.0 mL and needle paths were determined in an anterior-posterior direction, evading major blood vessels, ducts, healthy pancreatic tissue, or other organs. If possible, needle paths through intestines were avoided. Patients did not receive radiation therapy within 4 weeks before intervention or chemotherapy within 2 weeks before intervention.

Holmium microspheres preparation

A suspension of radioactive holmium-166 poly-L-lactic acid microspheres in a phosphate buffer with 0.1% Pluronic F-68 was delivered (Quirem Medical, Deventer, The Netherlands). The specific activity of the ^{166}HoMS was 1.5 to 2.5 MBq/mg (^{166}Ho half-life: 26.83 hours), at the time of treatment (t = 0). The patient-specific suspension was loaded in 3 mL syringes (Plastipak, BD Medical, New Jersey, USA) shielded by 9 mm thick acrylic glass.

Treatment procedure

The treatment procedure was performed in a CT suite (Canon Medical Systems – Aquilion, Amstelveen, the Netherlands). Patients received general anesthesia by intravenous induction and ventilation was performed using oral endotracheal intubation. After anesthesia was induced, a three-phase CECT was performed. All scans and needle (re-)placements were performed during end-expiration apnea.

For intratumoral injection, 20 gauge (G), 150 to 200 mm injection needles were used (Special Cannula, Mediplast, Malmö, Sweden) with an 18G 100 to 150 mm guidance needles (Chiba, Cook Medical, Bloomington, Indiana, US). Needle placement was performed with intermittent low-dose volume CT scans (LDCT) after every needle (re-)placement. The LDCT was registered (deformable registration) on the arterial or venous CT upon request of the interventional radiologist by Vitrea (Canon Medical Systems – Aquilion, Amstelveen, the Netherlands) to assess the relation between the needle, nearby blood vessels, and the tumor. After confirmation of the correct needle position, the intratumoral ^{166}HoMS injection was performed. An LDCT was made after every 0.2 to 0.5 mL injection to assess the ^{166}HoMS distribution. If the distribution was visible outside of the intended treatment area, further deposition at that needle location was abandoned. A holmium-specific non-contrast CT (see Appendix S2) was made after every completed deposit in a needle position, up to 1.0 mL per deposit.

When all injections were completed, a three-phase abdominal CT was repeated. The duration of the procedure was logged as the time from the first to the last CT scan.

All operators (interventional radiologists and researchers) working in close contact with the ^{166}HoMS wore a personal body dosimeter (Mirion Technologies, Atlanta, Georgia, US, lower detection limit 0.04 mSv) on the outside of a lead apron, and personal ring dosimeters (SCK CEN, Mol, Belgium, lower detection limit 0.15 mSv) on both hands. If radioactive contamination occurred (at least twice the background counts per second (cps) on Mini 900 type 44A, Mini-Instruments, Essex, U.K.; CoMo 170, PI Medical, Raamsdonksveer, The Netherlands), the contaminated item or waste was transported to the department of Nuclear Medicine for laboratory measurements and storage.

Follow-up

All patients were hospitalized for at least 24 hours with continuous monitoring for AEs and contamination. No radiation safety restrictions were required for medical staff or family. Patients received planar gamma camera imaging and single-photon emission computed tomography (SPECT) within 24 hours. Imaging parameters are described in Appendix S2.

The AE monitoring, laboratory blood examination, WHO performance status, and NRS were repeated at 1 week (w1), 8 weeks (w8), and 16 weeks (w16) after the treatment procedure at an outpatient clinic. A three-phase CECT was made for tumor staging at w8 and w16, after which follow-up was concluded. No adjuvant therapy was given during follow-up.

Data analysis

A more detailed description of the data analysis, including equations, is presented in Appendix S1.

Laboratory measurements

The total injected activity (A_{inj}, MBq) was calculated by subtracting all residual activity (A_{rest}, MBq) from the prepared activity in the syringe ($A_{syringe}$, MBq).

Planar and SPECT dosimetry

The planar and SPECT analyses is described in previous research.[16] The activity present in regions was determined by planar imaging (A_{region}, MBq) and the mean absorbed dose (MD, Gy) and max local absorbed dose (MLD, Gy) were calculated by SPECT for the tumor and off-target organs.

CT quantification

^{166}HoMS quantification by CT is based on the density of a voxel given in Hounsfield Units (HU),

which increases when a larger concentration of holmium ([Ho]) is present.[18] ^{166}HoMS deposits were identified, pre-existing calcifications or metallic endoprosthesis excluded, and all voxels >100 HU were selected for CT quantification by thresholding. Per region the CT detection rate (DR_{CT}, %) was calculated by dividing the amount of ^{166}HoMS (mg) recovered on CT by the amount of ^{166}HoMS (mg) recovered on SPECT. The amount recovered on SPECT was calculated by dividing the A_{region} (MBq, see above) by the ^{166}HoMS specific activity (MBq/mg) at t=0.

Study outcomes

Primary outcomes: feasibility and safety

The primary outcomes were patient safety and treatment feasibility. Feasibility was assessed by whether the intervention could be performed or had to be abandoned for any reason, by the percentages of the planned injection volume and activity actually injected, and by on-target and off-target radiation in MD (Gy) and MLD (Gy) defined by SPECT dosimetry.

Safety was determined by AE monitoring (CTCAE 5.0)[20] from the time of enrollment until w16 after the intervention. Each AE was labeled with its study attribution, which includes AEs caused by the effects of the intervention, the postprocedural effects of the ^{166}HoMS implants, or the radiation, with low study attribution (*unrelated, unlikely)* or high study attribution (*possibly, probably,* or *definitely*). If an AE developed over multiple affinities to study attribution, only the highest affinity was reported. An AE was only labeled as *unrelated* if the AE was present before intervention and did not worsen in severity grade at any time during follow-up. If an AE developed over multiple severity grades, only the highest grade was reported.

Secondary outcomes: radiation safety, CT guidance feasibility and CT quantification

As a secondary outcome, operator radiation safety from the combined ^{166}HoMS and CT radiation exposure was determined by dose (mSv) to the skin of the hands and body ($H_p0.07$) and deep dose equivalent of the body (H_p10). The maximum radiation exposure was determined in line with ICRP recommended guidelines for exposed operators (skin dose: 500 mSv/year, deep dose equivalent: 20 mSv/year).[21]

Feasibility of CT for ^{166}HoMS guidance was assessed by periprocedural ^{166}HoMS visibility and by periprocedural treatment adaptations caused by CT findings. Feasibility of CT quantification was determined by the DR_{CT} in the tumor. A DR_{CT} between 70% and 130% of the expected quantity was deemed feasible, in line with previous research.[18]

Exploratory outcomes: response and patient welfare

Exploratory response outcomes included overall survival (OS), progression-free survival (PFS) and

local progression-free survival (LPFS), defined as the time from enrollment to the date of an event or death from any cause. The event for PFS was the confirmation of a new lesion on CECT and for LPFS the confirmation of local progressive disease (≥20% increase in largest diameter), according to RECIST 1.1.[22] If an event or death occurred after w16, it was recorded retrospectively from standard clinical care. A local disease control rate (LDCR) was determined at w16 and tumor markers (CA19.9) were measured from baseline to final follow-up.

Patient welfare was assessed by WHO performance status and by NRS pain scale alterations from baseline to final follow-up. A WHO performance status difference of less than 2 and either a mean or worst NRS difference of 2 points or less was considered irrelevant.

Results

Patient demographics

Between May 2023 and December 2024, six patients were included and received intratumoral ^{166}HoMS injection. A demographic overview of the included patients at baseline is presented in Table 1. All patients were deemed surgically unresectable by a multidisciplinary board or declined surgery following induction chemotherapy, except for patient 2, who refused both chemotherapy and surgery.

Table 1. *Baseline demographic overview of the intervention group.*

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Median
Age, years	80	83	68	65	61	73	71
Sex	Female	Male	Male	Female	Male	Male	N.a.
BMI, kg/m^2	22.5	24.7	26.6	19.9	25.2	22.2	23.6
WHO performance status, 0–1	0	1	0	0	1	0	0
Tumor location, pancreas head/ body/ tail	Head	Head	Head	Head	Body	Head	N.a.
Resection status, DPCG guidelines[19]	LAPC	Borderline	LAPC	LAPC	LAPC	LAPC	N.a.
Tumor volume, mL	20.2	52.6	7.4	19.6	10.7	6.6	15.2
Induction FOLFIRINOX, cycles	11	0	12	8	8	7	8
NRS average (worst), 0 – 10	1 (3)	2 (2)	0 (0)	0 (0)	4 (7)	0 (0)	1 (1)
CA 19-9, E/mL	151	8345	<2	36	17	99	68
CEA, μg/L	4.3	2	9	11	2.2	2.6	3.5

BMI, body mass index; WHO, World Health Organization; N.a., not applicable; DPCG, Dutch Pancreatic Cancer Group; LAPC, locally advanced pancreatic cancer; NRS, numeric pain rating scale; CA 19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen.

Feasibility

CT effectively guided needle placement and visualized ^{166}HoMS in every patient and no procedures were abandoned. No acute complications or major radioactive contamination occurred. A median of 93% (range 75—100%) of the planned volume and a median of 112% (range 82.7—138.3%) of the planned activity was injected. A median MD of 24.6 Gy (range 14—38 Gy) and a MLD of 101.5 Gy (range 90—395 Gy) in the tumors was achieved. Off-target radiation was detected by SPECT in 6

out of 6 patients (100%), without causing any related AE ≥grade 3, in the needle tract (patients 1, 2, and 6), pancreatic tail (patient 6), duodenum (patient 3), liver (patient 4), and lungs (patient 4). Microsphere leakage into the intestines via the pancreatic duct was visible on periprocedural CT and resulted in radiation to the colon, visible on SPECT, in five patients (patients 2—6). Overall, the off-target MD ranged from <1–58 Gy and the off-target MLD ranged from <1–663 Gy. The results of all patients' injected activities, on-target and off-target radiation distribution and dosimetry are presented in Table 2.

Table 2. *Results of injected activity, on-target and off-target radiation distribution and dosimetry.*

	Unit	Patient 1	Patient 2		Patient 3		Patient 4			Patient 5	Patient 6			Median
Injected activity	*MBq*	193.5	426.9		79.6		184.1			83.6	86.3			135.2
Percent from planned activity	%	121.6	103.0		136.6		99.3			82.7	138.3			112.3
Activity in tumor	*MBq*	27.8	142.4		26.6		17.3			13.0	15.8			22.0
Percent from injected	%	14.3	33.4		33.4		9.4			15.5	18.3			16.9
MD tumor	*Gy*	17	38		35		14			19	30			24.5
MLD tumor	*Gy*	337	395		94		105			90	98			101.5
Off-target location		N.T.	N.T.	Co.	Du.	Co.	Lu.	Li.	Co.	Co.	N.T.	Pa.	Co.	N.a.
Activity in off-target	*MBq*	165.7	29.0	255.5	31.3	21.7	31.2	29.4	106.2	70.6	4.1	1.6	64.8	118.2
Percent from injected	%	85.7	6.8	59.9	39.3	27.3	17.0	16.0	57.7	84.5	4.8	1.8	75.1	83.1
MD off-target	*Gy*	58	41	11	8	<1	<1	<1	5	3	11	<1	3	4
MLD off-target	*Gy*	663	192	10	30	1	<1	1	10	3	60	14	3	10

MD, mean absorbed dose; MLD, maximum local absorbed dose. N.T., needle tract; Co., colon; Du., duodenum; Lu., lungs; Li., liver; Pa., pancreas; N.a., not applicable.

Safety

All patients were discharged from the hospital after 24 hours. Patient 1 withdrew consent after 15.6 weeks of follow-up (last completed visit: w8) because of local tumor progression and hepatic and peritoneal metastasis. Patient 2 died of metastatic pancreatic cancer after 6.1 weeks of follow-up (last completed visit: 1 week). Patients 3, 4, 5, and 6 (66.7%) completed the w16 follow-up.

An AE summary is presented in Table 3. A detailed description of all reported AEs is presented in Appendix S3. The total number of AEs reported was 65, of which 52 (80.0%) were grade 1—2, and 60 (92.3%) had low study attribution. The 5 AEs (7.7%) that had high study attribution include an increased lactate dehydrogenase (patient 2, grade 1, resolved), decreased platelet count (patient 3, grade 1, resolved), allergic reaction (patient 4, grade 3, resolved), mesenteric hematoma (patient 6, grade 1, resolved), diarrhea (patient 6, grade 1, resolved). Besides the mesenteric hematoma and the allergic reaction to the CT contrast agent, no acute complications occurred during treatment or post-procedure hospitalization. Twelve AEs ≥grade 3 were unrelated to study intervention and include increased alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in patient 1 (grade 3), increased alanine aminotransferase and aspartate aminotransferase in patient

6 (grade 3), and a duodenum obstruction, presenting with nausea, vomiting, dehydration, fatigue, and increased liver enzymes in patient 2 (grade 3), which was caused by progressive local disease from which the patient died (grade 5).

Table 3. *Adverse Event (AE) summary table.*

	Total (n = 6) Number of patients (%)		Total (n = 6) Number of AEs (%)		Per patient Median number of AEs (range)	
Any AE	6	(100)	65	(100)	10.5	(6—15)
Any AE with high study attribution	4	(67)	5	(8)	1	(0—2)
Any AE ≥grade 3	4	(67)	13	(20)	1.5	(0—7)
Any AE ≥grade 3 with high study attribution	1	(17)	1	(2)	0	(0—1)

Secondary objectives: radiation safety, CT guidance feasibility, and CT quantification

For the operator's radiation safety, the skin dose to the hands and to the body ($H_p0.07$) as well as the deep dose equivalent to the body (H_p10) were below the detection limit of the dosimeters ($H_p0.07$ <0.15 mSv, H_p10 <0.04 mSv) after every procedure except one. After the procedure of patient 2, with more than twice the prepared activity and procedure time of the second highest case (see Appendix S4.1), the skin dose was 1.75 mSv to the hands, and 0.28 mSv to the body, while the deep dose equivalent remained below the detection limit.

Because of CT guidance by intermittent periprocedural imaging, ^{166}HoMS leakage through the needle tract or through the pancreatic duct could be seen before the injection in that location was completed. If leakage occurred, the injection in that location was then abandoned, and, if possible, it was continued in another location. Additional injection results are presented in Appendix S4.1. An overview and description of every injection, including associated CT scans, is presented in Appendix S4.2.

The results of the CT quantifications are presented in Table 4. The median DR_{CT} was 37.1% (range: 13.2—63.1%) in all areas, 67.9% (range: 10.8—172.0%) in the tumor, and 24.8% (range: 15.5—417.5%) in off-target regions. From the ^{166}HoMS CT quantification in the tumor, 2 out of 6 quantifications were between 70—130% and were thus considered feasible. Periprocedural CT showed a more detailed and more heterogeneous distribution of the ^{166}HoMS deposits compared with conventional imaging such as SPECT, as presented in Figure 1. The intratumorally injected ^{166}HoMS remained implanted and visible on all follow-up CT scans, as presented in Figure 2.

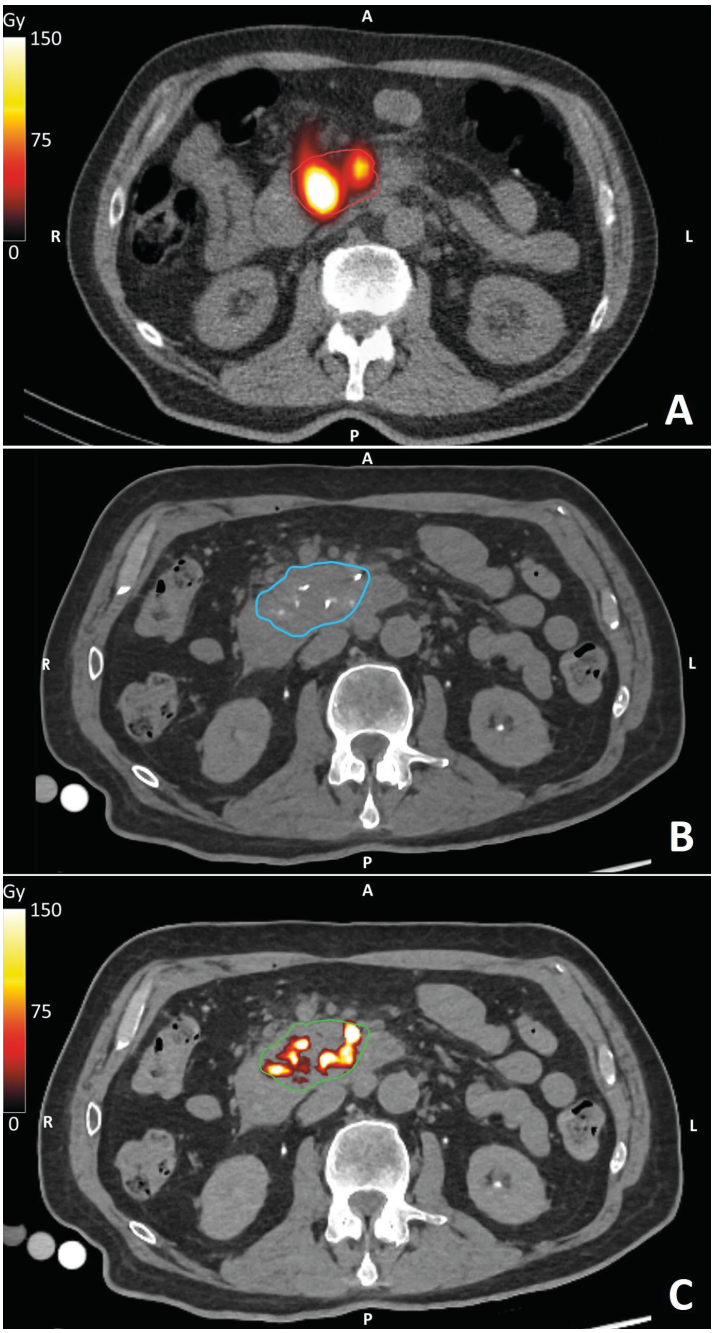


Figure 1. *Image overview showing the distinction between SPECT and CT imaging, and quantification in the same patient and position (patient 2). (**A**) Postprocedural SPECT dose map (Gy) showing the irradiated area in red, yellow, white within the tumor, outlined in red. (**B**) Periprocedural CT with hyperdense holmium-166 microsphere deposits within the tumor, outlined in blue. (**C**) Postprocedural CT dose map (Gy) showing the irradiated area in red, yellow, and white within the tumor, outlined in green.*

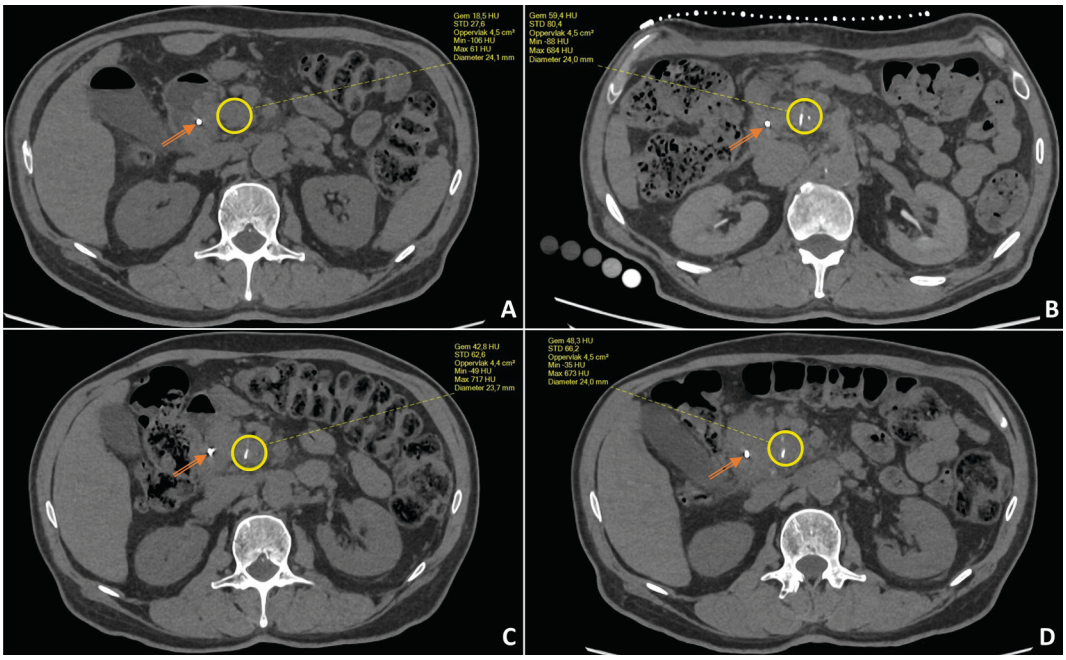


Figure 2. *Patient 3: Holmium-specific non-contrast CTs with Hounsfield Unit specifications from within the tumor (yellow circle) (**A**) at baseline, (**B**) directly after ^{166}HoMS injection, (**C**) 8 weeks after ^{166}HoMS injection, (**D**) and 16 weeks after ^{166}HoMS injection. The orange arrow indicates a biliary endoprosthesis.*

Table 4. *Results of the ^{166}HoMS quantification based on the post-injection CT, compared to SPECT.*

	Unit	Patient 1	Patient 2		Patient 3	Patient 4		Patient 5	Patient 6		
Total ^{166}HoMS injected	mg	102.9	189.7		37.0	92.5		38.9	40.1		
Total ^{166}HoMS recovered by CT (percent from injected)	mg (%)	22.0 (21.4)	119.7 (63.1)		4.9 (13.2)	32.8 (52.8)		9.9 (25.4)	19.5 (48.7)		
Intratumoral ^{166}HoMS recovered by SPECT	mg	14.8	63.3		12.4	8.7		6.0	7.3		
Intratumoral ^{166}HoMS recovered by CT	mg	1.6	76.2		4.9	14.93		3.55	5.67		
DRCT in tumor	%	10.9	120.2		39.5	172.0		59.0	77.0		
Off-target region on CT		N.T.	N.T.	Du.	None	Pa. tail	Du.	Pa. + Du.	N.T.	Pa. tail	Pa.
Off-target region on SPECT*		N.T.	N.T.	Co.	N.a.	Pa. tail	Co.	Co.	N.T.	Pa. tail	Co.
Off-target ^{166}HoMS recovered by SPECT	mg	88.1	12.9	113.6	N.a.	0.0	53.4	32.8	1.9	0.7	30.1
Off-target ^{166}HoMS recovered by CT	mg	20.4	2.0	41.4	N.a.	4.83	13.0	5.02 + 1.32	3.22	3.07	7.59
DRCT in off-target	%	23.2	15.5	36.4	N.a.	N.a.	24.4	19.3	167.6	417.5	25.2

**Since SPECT was acquired 24 hours after CT, some of the off-target locations may differ between modality depending on expected movement of the 166HoMS. It is expected that all ^{166}HoMS visible in the draining pancreatic duct and duodenum drained towards the colon within 24 hours, as was visible on SPECT. DRCT, CT detection rate; calculated by the 166HoMS recovered on CT divided by the 166HoMS recovered on SPECT; NT, needle tract; Du, duodenum; Co, colon; Pa, pancreas; N.a., not applicable.*

Exploratory objectives: Response and patient welfare

The median OS was 6.7 months, with two patients still alive at 8.1 and 5.1 months after enrollment. Median LPFS was 6.7 months (range: 2.8—8.1 months) and median PFS was 4.4 months (range: 2.8— 8.1months). The response results are presented in Figure 3 and Table 5.

After the w16 follow-up, a local disease control was seen in 4 out of 6 (67%) patients (patients 3—6). One of these patients (patient 5) presented with expected lung metastases on CECT at w16. Locally and distant progressive disease was diagnosed in one patient (patient 1) and was suspected by the general practitioner due to rapid clinical deterioration, but was not confirmed on imaging, in a second patient (patient 2).

The CA19.9 values were elevated at baseline (CA19.9 >34 E/ml) in 4 out of 6 patients and kept rising until the final follow-up in all patients with the exception of patient 4, whose CA19.9 decreased from 36 E/mL at baseline to <34 E/mL after treatment which persisted throughout follow-up. No relevant changes in WHO performance status (≥2 points) or in average or worst NRS pain score (>2 points) occurred in any patient at any time during follow-up.

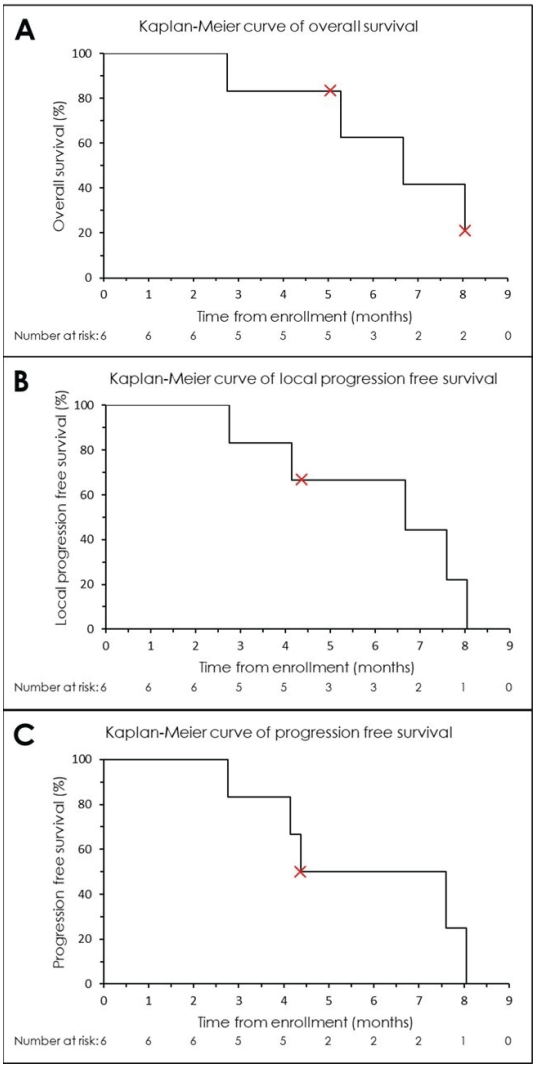


Figure 3. *Kaplan-Meier curves of the overall survival (**A**), local progression free survival (**B**), and progression free survival (**C**). Censored data are presented by a red 'X'.*

Table 5. *Results of the overall survival, local progression-free survival, and progression free survival.*

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Median*
Overall survival from enrollment, months	5.3	2.8	8.1	8.1*	6.7	5.1*	6.7
Local progression-free survival from enrollment, months	4.1	2.8	8.1	7.6	6.7	4.4*	6.7
Progression-free survival from enrollment, months	4.1	2.8	8.1	7.6	4.4	4.4*	4.4

**Censored data*

Discussion

This phase-1 study showed the initial feasibility and safety of CT-guided intratumoral holmium-166 microsphere injection in patients with locally unresectable pancreatic cancer after FOLFIRINOX induction chemotherapy. To the investigators' knowledge the treatment in this context has not been performed before and is a unique endeavor toward fully image-guided minimally invasive microbrachytherapy.

The primary concern regarding both the feasibility and safety was the extratumoral ^{166}HoMS deposition, which although it did not lead to any major adverse events in this study, caused considerable loss of the total dose delivered to the tumor. The MLD in the tumor that was achieved (90–395 Gy) was sufficient to induce local tumor cell death. However, due to the heterogeneous distribution of ^{166}HoMS, the total tumor volume receiving a high absorbed dose was expected to be insufficient for an optimal therapeutic response, resulting in the absence of partial response and limited OS after enrollment. Although the LDCR and LPFS may suggest a slightly beneficial local response, this effect is expected to improve if a higher MD could be achieved in the tumor, in which image-guidance may have a critical role.

Since CT guidance of an intratumoral injection with a radioactive suspension has not previously been performed in this context, valuable insights were discovered. CT showed new information regarding the heterogeneous intratumoral distribution of ^{166}HoMS, as compared to the homogeneous distribution shown by conventional SPECT imaging (Figure 2). Periprocedural intermittent CT provided direct feedback of the intratumoral distribution and potential extratumoral leakage of the ^{166}HoMS, enabling treatment adaptation. In this pilot study, extratumoral ^{166}HoMS deposition was identified often on CT, especially through the draining pancreatic duct or through the needle tract with the needle still in situ. The latter could be explained by the exceptionally high intratumoral pressure present in PDAC.[23] If extratumoral deposition was identified, injection could be halted in that position and injection fractionation could be applied to prevent complete off-target radiation and enable safe injection of the prepared activity. It remains uncertain whether the distribution pattern observed is specific to ^{166}HoMS injection only, since limited dosimetry data are available from comparable microbrachytherapy treatments. In the literature, when dosimetry is performed, it is with low-resolution SPECT, limiting the ability to conclusively identify or exclude extratumoral needle tract leakage[24], as seen in the contrast between SPECT and high-resolution CT in this study (see Figure 4). Leakage into the duodenum has also been reported in previous studies, but no follow-up was conducted on the activity within the colon.[25] There are indications that leakage remains a recurring challenge, as evidenced by reports of co-injecting microaggregate albumin to help retain the activity at the injection site.[24,25] Future studies could explore injecting ^{166}HoMS in a more viscous carrier to determine whether this approach improves retention of the ^{166}HoMS in the tumor.

Regarding the safety profile following CT-guided intratumoral injection of ^{166}HoMS, the mild AEs and few AEs caused by the study intervention make it appear safe and show potential for minimally invasive treatment. Most adverse events identified were of a low severity grade, either present before the study intervention, related to PDAC, aftereffects of chemotherapy, or tumor progression in a later phase. More importantly, none of the anticipated and potentially severe complications with high study attribution, such as abdominal pain, pancreatitis, gastrointestinal bleeding, fistula, or emesis, were observed.

The safety of this intervention is attributed to three unique factors: 1) the fractionated injection approach, 2) the distribution pattern of the ^{166}HoMS, and 3) the limited penetration depth of the β^--radiation. 1) With fractionated injections, a more homogeneous microsphere distribution in the tumor is pursued while suboptimal ^{166}HoMS injection or leakage from the tumor affects only a fraction of the activity rather than the entire therapeutic dose. 2) Since the therapeutic effects are related to the local concentration of the ^{166}HoMS and fixation over a longer period of time, as soon as the microspheres distribute out of the tumor the concentration drops and the ^{166}HoMS are expelled more quickly, for example in the colon. 3) The limited penetration depth of the β^- radiation (>90% within 2.5 mm) ensures that even in the event of needle tract leakage, a carefully placed CT-guided needle path through intraabdominal fat minimizes the risk of damaging nearby arteries or veins. These factors all contributed to an acceptable safety profile.

In terms of operator safety, the use of acrylic glass shielding around the injection syringe shields the operator and sufficiently reduces the absorbed radiation dose. The highest measured skin dose (1.75 mSv), associated with the highest activity, longest procedure time, and most CT scans, still indicates that an operator could safely perform this procedure over 250 times per year before exceeding the ICRP-recommended skin dose limits (500 mSv).[21] For patients, no contact restrictions were necessary. Previous research on ^{166}HoMS radioembolization has shown that contact restrictions are unnecessary beyond six hours post-treatment when the total activity is less than 7 GBq, which is nearly ten times higher than the maximum activity used in this study.[26]

CT quantification was feasible in 2 out of 6 patients, with a DR_{CT} in the tumor of between 70% and 130% when compared with SPECT. The differences in ^{166}HoMS detection rates between SPECT and CT could be attributed to different moments of acquisition. CT was acquired during treatment while SPECT was acquired almost 24 hours later, allowing for microsphere outflow from the tumor into the gastrointestinal tract, resulting in an overestimation of the DR_{CT} in the tumors of patients 2 and 4. In patient 1, injected air adjacent to the ^{166}HoMS likely reduced the measured HU, causing the largest underestimation of the ^{166}HoMS concentration.[18] The ^{166}HoMS leakage into the duodenum

could partly go undetected on CT when a biliary metallic endoprosthesis was present, as the hyperdense microspheres were indistinguishable from the metallic endoprosthesis as seen in patients 3 and 6 (see Appendix S4.2). Interestingly, CT quantification appears to improve when a higher total amount of ^{166}HoMS is injected, which aligns with findings from previous research indicating significant improvements in detection for larger *in vivo* amounts or concentrations.[18] These findings should be considered for future applications to improve detection and quantification of ^{166}HoMS on CT. Finally, further investigation to improve CT quantification and dosimetry is required since it could potentially establish a detailed dose-response on a new level, not only describing the mean tumor dose, but also dose coverage, dose homogeneity, and minimal local dose both post and periprocedural.

When interpreting the results of this study, several limitations must be considered. First, in this small cohort even minor patient variability could have a large impact on the results. For example, patient 2 was the only patient without prior chemotherapy, and although within inclusion criteria, was an outlier in terms of tumor size and CA19.9 levels, which in hindsight may all have contributed to the rapid onset of tumor progression. This likely affected both the primary safety outcomes and exploratory efficacy results substantially. Work-up from diagnosis until study intervention also varied between patients affecting follow-up outcomes like OS, LPFS, and PFS. Since the risks associated with this novel intervention were yet unclear, it could not be combined in parallel with induction chemotherapy. However, in most research on local therapies, they are applied in combination with systemic chemotherapy, making any comparisons with existing literature currently unclear and unreliable.[27] Additionally, minor procedural adjustments and an operator learning curve may have influenced outcomes, creating variability that should be considered in future analyses. Another limitation is the study's reliance on CT and SPECT for post-treatment evaluation. A known challenge of SPECT with relatively low activities is the detection deficit caused by the relatively large amount of scatter and background noise.[28] This may introduce discrepancies in quantification and cause underestimations in the recovered absorbed tumor dose. Potentially, as indicated by the results of this study, CT may also be limited in detecting and accurately quantifying low ^{166}HoMS concentrations. Overall, this study presents the preliminary feasibility and safety for CT-guided intratumoral ^{166}HoMS injection in unresectable PDAC but requires additional research to deliver firm conclusions.

Given the acceptable safety profile, low patient burden, and local disease control in 4 out of 6 patients after 16 weeks, a larger cohort study is warranted to fully assess the therapeutic potential, optimal dosing, and risks of this intervention. Future research should focus on optimizing the intervention to enhance its therapeutic effect. Therefore, it is crucial to increase the mean absorbed tumor dose by either improving intratumoral deposition and minimizing extratumoral leakage or by safely increasing the total injected activity. Additionally, continuous research toward a fully CT-guided approach is

highly recommended. The population expected to benefit most from additional local therapy includes patients without metastasis but with surgically unresectable disease. Given the unpredictable progression of local and locoregional PDAC, local interventions such as intratumoral ^{166}HoMS injection should always be preceded by, though preferably combined with, chemotherapy. The ^{166}HoMS microbrachytherapy could be implemented between the fourth and fifth out of 8-12 cycles FOLFIRINOX, when response for surgical resection is inadequate but progressive disease remains absent, in line with European Society for Medical Oncology (ESMO) guidelines.[29] In terms of a control group, it is too soon to perform a randomized controlled trial. However, recent trials with comparable methods to the one described here are available to perform an extensive retrospective comparison, including radiofrequency ablation[30], IRE and MRI-guided radiotherapy[31]. Primary outcomes should focus on a quality-of-life-related or event-free survival rather than overall survival alone to include the impact and burden of the interventions as well. Secondary outcomes should include (local) tumor response in terms of RECIST 1.1, CA19.9 levels, FDG-PET imaging, and/or tumor resection rates.

Conclusion

CT-guided percutaneous ^{166}HoMS injection is a feasible and safe approach for locally unresectable PDAC. This technique enables effective and safe needle placement and visualizes ^{166}HoMS depositions clearly. Exploratory results suggest a beneficial local response, warranting further investigation. Although this intervention is safe and minimally invasive, optimization is required to enhance CT quantification, further minimize off-target radiation, and increase the mean absorbed tumor dose to fully assess the therapeutic potential of this novel intervention.

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Appendices

Appendix S1 Detailed materials and methods

This investigator-initiated, single-center, single-arm, and open-label study with a radioactive implant was registered at clinicaltrials.gov (ID NCT05880472). The enrollment was six patients in the single ^{166}HoMS intervention arm without a control arm due to the feasibility and safety phase. All participants were required to give written informed consent before enrollment.

Patient selection

Eligible participants were males or females of 18 years and older with a pathologically proven PDAC. If first-line FOLFIRINOX chemotherapy was completed after 8-12 cycles, or chemotherapy was abandoned because of toxicity or personal choice, patients were screened for inclusion. Patients were required to be ineligible for surgical resection or chose not to undergo surgical resection with evidence of no (active) metastatic disease, a World Health Organization (WHO) Performance status of 0 or 1, a life expectancy of 16 weeks or longer, and a single solid PDAC tumor ≥20 mm in largest diameter. Exclusion criteria included immune, kidney, or blood coagulation deficiency (leukocytes $<3.0\times10^9$/L, creatinine >165 μmol/L, eGFR <60 ml/min/1.73m^2, and/or platelet count $<75\times10^9$/L), any unresolved toxicity of ≥grade 3 from previous anticancer therapy was present (National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE version 5.0)), pregnancy, or breastfeeding.

Baseline visit

Before the study intervention a baseline visit was performed. During the baseline visit demographic data (age, sex, body-mass index (BMI)), WHO performance status, tumor location, resection status by the Dutch Pancreatic Cancer Group (DPCG) guidelines[19], previous anti-cancer therapy, current AEs, and numeric pain rating scale (NRS) of the average and worst pain of the past 7 days (scale of 0, no pain, to 10, worst pain) was noted. Contrast-enhanced CT (CECT; image acquisition parameters are described in Appendix S2) was made and a laboratory blood examination was performed and included in the AE monitoring. The laboratory blood examination included aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), total bilirubin, C-reactive protein (CRP), hemocytometry, creatinine, amylase, lipase, carbohydrate antigen 19.9 (CA19.9), and carcinoembryonic antigen (CEA).

Patient-specific planning

A patient-specific ^{166}HoMS planning was made, determining the maximum injection volume (V_{inj}), injection activity (A_{req}) required for a targeted maximum achievable mean absorbed tumor dose (D), needle path, number of deposits, and injection volume per deposit, for each patient. First a three-dimensional (3D) segmentation was made using open-source image progressing software 3D Slicer (version 4.11 – 5.4).

The primary tumor, nearby structures, and organs on arterial or venous CECT were segmented in consultation with an expert radiologist (J.H). The tumor volume (V_{tumor}) in mL was automatically calculated from the 3D segmentation and the maximum injection volume was calculated at 10% of the tumor volume. The required injection activity (A_{req}) in MBq was calculated using Equation 1.[32]

(1) $$A_{req} = \frac{D * M}{e}$$

For safety standards targeted maximum achievable mean absorbed tumor dose (D) was set between 125 and 150 Gray (Gy), only achievable with a 100% intratumoral and homogeneous distribution. The tumor mass (*M*) in gram (g) was determined by multiplying the tumor volume with the tumor density (ρ) which was set at 1.0 g/mL. The used holmium-166 dose coefficient (*e*) was 15.87 mJ/MBq/kg.[32] The maximum injection volume was fractionated into deposits of 0.3 – 1.0 mL and needle paths were determined in an anterior-posterior direction, evading major blood vessels, ducts, healthy pancreatic tissue, or other organs. If possible, planning of needle paths through intestines was evaded. Patients did not receive radiation therapy <4 weeks before intervention, or chemotherapy <2 weeks before intervention.

Holmium microspheres preparation and injection system

A suspension of radioactive holmium-166 poly-L-lactic acid microspheres in a phosphate buffer with 0.1% Pluronic F-68 was delivered (Quirem Medical, Deventer, The Netherlands). The specific activity of the ^{166}HoMS was 1.5 – 2.5 MBq/mg (^{166}Ho half-life: 26.83 hours), at the planned moment of treatment (t = 0). The patient-specific suspension was loaded in 3 mL syringes (Plastipak, BD Medical, New Jersey, USA) shielded by 9 mm thick acrylic glass against the β^- radiation. The syringe was connected to a manual rotation injection system, elaborately described in previous research.[15] The activity loaded into the syringe was compensated for an expected agglomeration loss of 30% and 1.0 mL suspension with the same concentration (MBq per mL) was added to flush the injection system of any air before intervention.

Treatment procedure

Intratumoral ^{166}HoMS injection was performed by an interventional radiologist with the assistance of a researcher and under the supervision of a nuclear medicine physician or radiation safety expert. The treatment procedure was performed in a CT suite (Canon Medical Systems – Aquilion, Amstelveen, the Netherlands), with an adjacent CT monitoring room. All operators working with in close contact with the ^{166}HoMS (interventional radiologists and researchers) wore a personal body dosimeter (Mirion Technologies, Atlanta, Georgia, US, lower detection limit 0.04 mSv) on the outside of a lead protection apron, and personal ring dosimeters (SCK CEN, Mol, Belgium, lower detection limit 0.15 mSv).

Operators, equipment, and waste was intermittently monitored for contaminations. A contamination was defined as ≥2 times the background in counts-per-second (cps), measured on a contamination monitor (Mini 900 type 44A, Mini-Instruments, Essex, U.K.; CoMo 170, PI Medical, Raamsdonksveer, The Netherlands). If contamination occurred, the contaminated item or waste was transported to the department of Nuclear Medicine for laboratory measurements (see below) and storage.

Patients received general anesthesia by intravenous induction and ventilation was performed using oral endotracheal intubation. After anesthesia commenced, a three-phase (holmium-specific non-contrast, arterial phase, and venous phase) abdominal CECT was made. The patient-specific planning was reassessed. All scans and needle (re-)placements were performed during end-expiration apnea.

During the procedure, the injection system was suspended above the patient's abdomen, just outside the scanning area, by a three section double articulated arm. For intratumoral injection, 20 gauge (G), 150 – 200 mm injection needles were used (Special Cannula, Mediplast, Malmö, Sweden) with an 18G 100 – 150 mm guidance needles (Chiba, Cook Medical, Bloomington, Indiana, US). Needle placement was performed with intermittent low-dose volume CT-scans (LDCT) after every needle (re-)placement. The LDCT was registered (deformable registration) on the arterial or venous CT upon request of the interventional radiologist by Vitrea (Canon Medical Systems – Aquilion, Amstelveen, the Netherlands) to assess the relation between the needle, nearby blood vessels, and the tumor. After confirmation of the correct needle position, the intratumoral ^{166}HoMS injection was performed. A LDCT was made after every 0.2 – 0.5 mL injection to assess the ^{166}HoMS distribution. If the distribution was visible outside of the intended treatment area, further deposition at that needle location was abandoned. A holmium-specific non-contrast CT was made after every completed deposit in a needle position, up to 1.0 mL per deposit. When all injections were completed, a three-phase abdominal CT was repeated. The duration of the procedure was logged as the time from the first to the last CT-scan.

Follow-up

All patients were hospitalized at the Department of Medical Oncology for at least 24 hours. No radiation safety restrictions were required for medical staff or family. During hospitalization continuous AE and contamination monitoring was performed. If a contamination was found, the contaminated item or waste was transported to the department of Nuclear Medicine for laboratory measurements (see below) and storage. Patients received planar gamma camera imaging (planar) and single-photon emission computed tomography (SPECT)/CT the following day, parameters are described in Appendix S2. Patients were discharged after medical confirmation from a medical oncologist or physician-assistant and radiation safety confirmation from a nuclear medicine physician.

The AE monitoring, laboratory blood examination, WHO performance status and NRS was repeated at one week (w1), 8 weeks (w8), and 16 weeks (w16) after the treatment procedure at an outpatient clinic. A three-phase CECT was made for tumor staging at w8 and w16, after which follow-up was secluded. No adjuvant therapy was given during follow-up.

Data analysis

Laboratory measurements

The total activity that was injected into the patient (A_{inj}, MBq) was determined by measuring all residual activity (A_{rest}, MBq) and subtracting it from the initial prepared activity in the syringe ($A_{syringe}$, MBq), compensated for the decay in the time between treatment (t = 0) and the laboratory measurements, as shown in Equation 2.

(2) $$A_{inj} = A_{syringe(t=0)} - A_{rest} * 0.5^{\left(\frac{\Delta t}{t_{\frac{1}{2}}}\right)}$$

The amount of time between the time of measurement and treatment (t=0) is given presented by Δt and the half-life of holmium-166 ($t_{1/2}$) is 26.83 hours.[33] All residual activity was measured in a dose calibrator (VIK-202, Comecer, Joure, The Netherlands) with a lower detection limit of 0.01 MBq.

Planar and SPECT dosimetry

Here a summarized description of planar and SPECT imaging analysis is given. A more detailed description is given in previous research.[16] Planar images were exported to Hermia Gold Smart (version 2.17.0.87) to analyze the distribution of the ^{166}HoMS within the thorax-abdomen (2.4 x 2.4 mm; gamma-counts (C)). Regions of interest (RoIs) and background regions (BR) were manually drawn. ROIs with more than twice the counts/cm^2 as the BR were selected for further analysis and were corrected for background scatter and noise and anterior-posterior captures were fused using the geometric mean method to attain the total counts in a region (TC_{region}).[34] The sum of all counts within the selected, background corrected and fused ROIs ($\sum TC_{region}$) represents the total injected activity (A_{inj}). Therefore, the activity per ROI (A_{region}; in MBq) could be determined as shown in Equation 3.

(3) $$\frac{TC_{region}}{\sum TC_{region}} = \frac{A_{region}}{A_{inj}}$$

The A_{region} was translated to their corresponding 3D areas of interest (AoIs) on SPECT with the use of Qsuite (Research version 2.2, build 2.20.6237,Quirem Medical, Deventer the Netherlands). Further segmentation of the tumor and off-target AoIs on SPECT/CT was used to calculate the mean

absorbed dose (MD) and max local absorbed dose (MLD) in Gy. Segmentation of the tumor was performed in line with the preprocedural CECT with the same position and volume. If off-target AoIs were not fully visible on SPECT/CT (for example lungs or colon), the mean absorbed dose (D) was calculated using Equation 1, with as activity (A) input the activity determined by planar imaging (A_{region}) and as mass input the reference organ mass from IRCP-089.[35]

CT quantification

^{166}HoMS quantification by CT is based on the density of a voxel given in Hounsfield Units (HU), which increases when a larger concentration of holmium ([Ho]) is present.[18] The linear relationship between the HU and [Ho] for the used scanner and parameters, a calibration slope (a) and intercept (b) were determined as shown in Equation 4.

(4) $$[Ho] = \frac{HU - b}{a}$$

Five 15 mL Eppendorf tubes containing a holmium-165 (non-radioactive) chloride solution (HoCl; Quirem Medical, Deventer, The Netherlands) with different [Ho] (0.0, 0.2, 1.0, 4.0, and 10.0 mg/mL) were scanned. The HoCl in all visible tubes was segmented (10 mm diameter, 30 mm length) in 3D Slicer (version 4.11–5.4), excluding air and edges. A linear calibration curve was fitted from the [Ho] and associated HU to determine the slope (a), and intercept (b; at 0.0 mg/ml [Ho]).

Equation 4 was used to determine the [Ho] for selected voxels within the patient. Volumes of interest (VoIs) were segmented in 3D Slicer (version 4.11 – 5.4). VoIs include the tumor segmentation from the patient-specific planning, manually registered to the post-injection CT, and all areas with visual indication of the presence of ^{166}HoMS by increased HU such as adjacent extratumoral tissue, duodenum, or other nearby structures. VOIs segmentations were exported to NIFTI files and together with the post-injection CT analyzed in Python (version 3.12).

Within the VoIs, voxels containing Ho were selected using a thresholding method.[18] All voxels containing ≥100 HU were selected, all other voxels (>100 HU) were ignored. Previously present voxels ≥100 HU, such as calcifications, drains or other radiodensities, were manually excluded from the selection. The [Ho] was calculated per selected voxel with Equation 4. The total ^{166}HoMS (mg) was calculated per VoI. The CT detection rate of ^{166}HoMS (DR_{CT}; %) was calculated by dividing the ^{166}HoMS (mg) recovered on CT per VOI with the ^{166}HoMS recovered by SPECT in the same VOI. The quantity of ^{166}HoMS (mg) in a VOI on SPECT was calculated by dividing the activity (MBq) with the specific activity (MBq/mg) at t=0.

Study outcomes

Primary outcomes

The primary outcomes were the safety and feasibility of CT-guided percutaneous intratumoral ^{166}HoMS injection in patients with locally unresectable pancreatic cancer after FOLFIRINOX induction chemotherapy. Feasibility was assessed by being able to perform the intratumoral ^{166}HoMS injection, versus intervention abandonment. Furthermore, feasibility was assessed by the percentages of the planned injection volume and activity actually injected and by on-target and off-target radiation in MD and MLD (Gy) defined by SPECT/CT dosimetry.

Safety was determined by AE monitoring (CTCAE 5.0)[20] from the time of enrollment until w16. Each AE was labeled with its study attribution, which entails AEs caused by the effects of either needle injection or the postprocedural effects from the ^{166}HoMS implants or the radiation, from *unrelated, unlikely, possibly, probably,* to *definitely* related. Study attribution was further categorized as low (*unrelated, unlikely*) or *high* (*possibly, probably, and definitely*). If an AE developed over multiple affinities to study attribution, only the highest affinity was reported. An AE was only labeled as *unrelated* if the AE was present before intervention and did not worsen in severity grade after intervention. A severity grade was appointed to every AE from grade 1 (mild) to grade 5 (fatal). If an AE developed over multiple severity grades, only the highest grade was reported.

Secondary outcomes

As a secondary outcome safety for operators (interventional radiologist and researcher) was determined by dose (mSv) for the skin ($H_p0.07$) of the hands and body and deep dose equivalent (H_p10) of the body. The maximum radiation exposure was determined in line with ICRP recommended guidelines for exposed operators (skin dose: 500 mSv/year, deep dose equivalent: 20 mSv/year).[21] Feasibility of CT for ^{166}HoMS guidance was assessed by noting all treatment adaptations caused by CT findings during treatment. Feasibility of CT quantification was determined by the DR_{CT} in the tumor. A DR_{CT} between 70—130% of the expected quantity was deemed feasible.[18]

Exploratory outcomes

As exploratory outcome the primary tumor response was estimated on CECT at w8 and w16 in line with RECIST 1.1.[22] Outcomes were complete response, partial response, stable disease, or progressive disease. Additionally, the absence or presence of distant disease progression was noted. Response was also explored by tumor marker response (CA19.9) over time. Patient welfare was assessed by WHO performance status and by NRS pain scale. A mean or worst NRS difference over time of ≤2 points was considered irrelevant.

Appendix S2 Imaging acquisition parameter

Computed tomography acquisition and reconstruction parameters

Parameter	Computed tomography		
Phase	Non-contrast	Arterial/venous	Low dose
Brand	Canon Medical Systems – Aquillion	Canon Medical Systems – Aquillion	Canon Medical Systems - Aquilion
Patient position	Feet-first supine	Feet-first supine	Feet-first supine
Acquisition type	Spiral	Spiral	Spiral
Spiral pitch factor	0.813	0.813	0.813
Samples per pixel	1	1	1
Pixels spacing (mm)	0.740/0.740	0.740/0.740	0.846/0.846
Slice thickness (mm)	0.5	0.5	0.5
Reconstruction algorithm and covolution kernel group	Advanced intelligent Clear-IQ Engine (AiCE)	Advanced intelligent Clear-IQ Engine (AiCE)	Advanced intelligent Clear-IQ Engine (AiCE)
Convolution kernel	BODY_SHARP	BODY_SHARP	BODY_SHARP
Image filter	Beam hardening correction	Beam hardening correction	Beam hardening correction
Filter type	LARGE	LARGE	LARGE
Focal spot(s)	0.9/0.8	0.9/0.8	0.9/0.8
Filter material	Aluminium/copper	Aluminium/copper	Aluminium/copper
Rescale intercept	0	0	0
Contrast agent	None	Iomeron 300	None
Contrast bolus (mL)	None	150	None
Rescale slope	1	1	1
Kilo voltage peak (kVp)	120	120	120
Exposure time (ms)	750	500	500
Tube current (mA)	240	107	50
Exposure (mAs)	180	54	25
Revolution time (s)	0.75	0.50	0.5
Single collimation width	0.5	0.5	0.5
Total collimation width	40	40	80

Planar gamma camera and SPECT acquisition and reconstruction parameters

Parameter	Planar gamma camera	SPECT
Brand	Siemens – Encore 2	Siemens – Encore 2
Patient position	Feet-first supine – arms down	Feet-first supine – arms up
Scan region	Thorax-abdomen	Abdomen
Collimator	Medium energy all purposes – parallel	Medium energy all purposes – parallel
Convolution kernel	N.a.	3D-ordered-subsets implementations
Iterations	N.a.	10
Subsets	N.a.	8
Gaussian filter	None	None
Pixel spacing (mm)	2.4	4.8
Slice thickness (mm)	N.a.	4.8
Number of frames	N.a.	81
Samples per pixel	1	1
Number of energy windows in reconstruction	2	1
Energy window(s) lower limit(s) (KeV)	73.1, 108.2	73.1
Energy window upper limit(s) (KeV)	85.0, 122.1	85.0
Number of Frames in rotation	N.a.	64
Scan speed	8 cm/minute	20 seconds per frame

**N.a., not applicable.*

Appendix S3 Detailed overview of all reported adverse events

Adverse event definition	Patient 1			Patient 2			Patient 3		
	Grade	S.A.	T.o.D.	Grade	S.A.	T.o.D.	Grade	S.A.	T.o.D.
Abdominal pain	1	Unlikely	>w8	-	-	-	-	-	-
Description	Likely related to disease progression.			-			-		
ALT increased	3	Unlikely	>w8	2	Unlikely	>w1	1	Unrelated	Baseline
Description	Present before intervention, increased grade >w8. Likely related to disease progression.			No clear study attribution.			Present before intervention.		
Allergic reaction	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Alkaline phosphatase increased	3	Unlikely	>w8	2	Unlikely	>w1	2	Unrelated	Baseline
Description	Likely related to disease progression.			No clear study attribution.			Present before intervention.		
Anemia	1	Unrelated	Baseline	1	Unrelated	Baseline	1	Unrelated	Baseline
Description	Present before intervention.			Present before intervention. Resolved.			Present before intervention.		
Ascites	2	Unlikely	>w8	-	-	-	-	-	-
Description	Likely related to disease progression.			-			-		
AST increased	3	Unlikely	>w8	1	Unlikely	>w1	1	Unrelated	Baseline
Description	Present before intervention, increased grade at >w8. Likely related to disease progression.			No clear study attribution.			Present before intervention.		
Back pain	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Bloating	-	-	-	1	Unrelated	Baseline	1	Unrelated	Baseline
Description	-			Present before intervention.			Present before intervention.		
Blood bilirubin increased	-	-	-	-	-	-	1	Unrelated	Baseline
Description	-			-			Present before intervention.		
Dehydration	-	-	-	3	Unlikely	>w1	-	-	-
Description	-			See 'Duodenal obstruction'.			-		
Dizziness	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Diarrhea	-	-	-	1	Unrelated	Baseline	-	-	-
Description	-			Present before intervention.			-		
Dry skin	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Duodenal obstruction	-	-	-	3	Unlikely	>w1	-	-	-
Description	-			Caused by PDAC. Hospitalized 3 weeks after intervention for expected blockage causing increased GGT, vomiting, dehydration fatigue, and malaise.			-		
Dyspnea	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Fatigue	1	Unlikely	>w8	3	Unlikely	>w1	-	-	-
Description	Likely related to disease progression.			See 'Duodenal obstruction'.			-		
Gamma GT increased	-	-	-	3	Unlikely	>w1	-	-	-
Description	-			No clear study attribution.			-		
Gastroesophageal reflux	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Headache	-	-	-	1	Unrelated	Baseline	-	-	-
Description	-			Present before intervention. Resolved.			-		
Hematoma	-	-	-	-	-	-	-	-	-
Description	-			-			-		

Adverse event definition	Patient 4			Patient 5			Patient 6		
	Grade	S.A.	T.o.D.	Grade	S.A.	T.o.D.	Grade	S.A.	T.o.D.
Abdominal pain	-	-	-	-	-	-	-	-	-
Description	-			-			-		
ALT increased	2	Unrelated	Baseline	1	Unlikely	w16	3	Unlikely	w16
Description	Present before intervention. Resolved.			Likely related to disease progression.			Present before intervention, increased grade at w16.		
Allergic reaction	3	Probably	Intervention	-	-	-	-	-	-
Description	Probable reaction to CT contrast-agent. Resolved.			-			-		
Alkaline phosphatase increased	-	-	-	1	Unrelated	Baseline	2	Unrelated	Baseline
Description	-			Present before intervention.			Present before intervention		
Anemia	-	-	-	2	Unlikely	w16	1	Unrelated	Baseline
Description	-			Likely related to disease progression.			Present before intervention		
Ascites	-	-	-	-	-	-	-	-	-
Description	-			-			-		
AST increased	2	Unrelated	Baseline	1	Unlikely	w16	3	Unlikely	w16
Description	Present before intervention. Resolved.			Likely related to disease progression.			Present before intervention, increased grade at w16.		
Back pain	-	-	-	2	Unrelated	Baseline	-	-	-
Description	-			Present before intervention.			-		
Bloating	-	-	-	1	Unrelated	Baseline	-	-	-
Description	-			Present before intervention. Resolved.			-		
Blood bilirubin increased	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Dehydration	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Dizziness	-	-	-	1	Unlikely	w1	-	-	-
Description	-			No clear study attribution.			-		
Diarrhea	-	-	-	-	-	-	1	Probably	>Intervention
Description	-			-			On day 2 after intervention. Resolved next morning.		
Dry skin	1	Unrelated	Baseline	1	Unrelated	Baseline	-	-	-
Description	Present before intervention. Resolved.			Present before intervention.					
Duodenal obstruction	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Dyspnea	-	-	-	1	Unrelated	Baseline	-	-	-
Description	-			Present before intervention.			-		
Fatigue	-	-	-	1	Unrelated	Baseline	-	-	-
Description	-			Present before intervention. Resolved.			-		
Gamma GT increased	-	-	-	1	Unrelated	Baseline	2	Unrelated	Baseline
Description	-			Present before intervention.			Present before intervention. Resolved >w8.		
Gastroesophageal reflux	-	-	-	1	Unrelated	Baseline	-	-	-
Description	-			Present before intervention. Resolved.			-		
Headache	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Hematoma	-	-	-	-	-	-	1	Definitely	Intervention
Description	-			-			Mesenteric hematoma caused by needle. Resolved.		

Appendix S3 Detailed overview of all reported adverse events (continuation)

Adverse event definition	Patient 1			Patient 2			Patient 3		
	Grade	S.A.	T.o.D.	Grade	S.A.	T.o.D.	Grade	S.A.	T.o.D.
Hyperkalemia	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Hypoalbuminemia	1	Unlikely	>w8	-	-	-	1	Unrelated	Baseline
Description	Likely related to disease progression.			-			Present before intervention.		
Lactate dehydrogenase increased	1	Unlikely	>w8	1	Probably	w1	-	-	-
Description	Likely related to disease progression. Resolved.			Resolved.			-		
Lymphocyte count decreased	2	Unlikely	>w8	-	-	-	-	-	-
Description	Likely related to disease progression.			-			-		
Malaise	-	-	-	3	Unlikely	>w1	-	-	-
Description	-			See 'Duodenal obstruction'.			-		
Malignant neoplasms	-	-	-	5	Unlikely	>w1	-	-	-
Description	-			Likely related to disease progression.			-		
Nausea	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Neuropathy	1	Unrelated	Baseline	-	-	-	1	Unrelated	Baseline
Description	Present before intervention.			-			Present before intervention.		
Platelet count decreased	-	-	-	-	-	-	1	Possibly	>Intervention
Description	-			-			Resolved.		
Vomiting	-	-	-	3	Unlikely	>w1	-	-	-
Description	-			See 'Duodenal obstruction'.			-		
Total low study attribution	11			14			8		
Total high study attribution	0			1			1		
Total grade 1-2	8			8			9		
Total grade 3-5	3			7			0		

*The '-' indicates the adverse event (AE) was not found in this patient. *Neuropathy indicates either peripheral motor neuropathy, sensory neuropathy, or both. S.A.: Study attribution. T.o.D.: Time of Diagnosis indicates diagnosis of an AE at the baseline visit (baseline), during intervention (Intervention), or at a follow-up visit at week 1 (w1), week 8 (w8) or week 16 (w16), or after an intervention or visit by conventional care (>).*

Adverse event definition	Patient 4			Patient 5			Patient 6		
	Grade	S.A.	T.o.D.	Grade	S.A.	T.o.D.	Grade	S.A.	T.o.D.
Hyperkalemia	-	-	-	-	-	-	1	Unlikely	>w8
Description	-			-			No clear study attribution.		
Hypoalbuminemia	1	Unrelated	Baseline	-	-	-	-	-	-
Description	Present before intervention. Resolved.			-			-		
Lactate dehydrogenase increased	-	-	-	-	-	-	1	Unrelated	Baseline
Description	-			-			Present before intervention. Resolved >w8.		
Lymphocyte count decreased	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Malaise	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Malignant neoplasms	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Nausea	-	-	-	1	Unrelated	Baseline	-	-	-
Description	-			Present before intervention. Resolved.			-		
Neuropathy	1	Unrelated	Baseline	-	-	-	-	-	-
Description	Present before intervention. Resolved.			-			-		
Platelet count decreased	-	-	-	1	Unrelated	Baseline	1	Unrelated	Baseline
Description	-			Present before intervention. Resolved.			Present before intervention.		
Vomiting	-	-	-	-	-	-	-	-	-
Description	-			-			-		
Total low study attribution	5			14			8		
Total high study attribution	1			0			2		
Total grade 1-2	5			14			8		
Total grade 3-5	1			0			2		

Appendix S4.1 Overview of the planning and procedure parameters

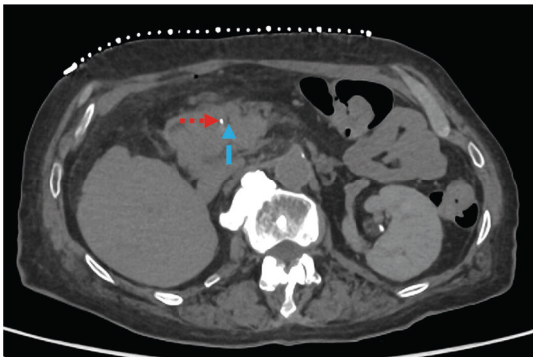
Nr.	Planning and treatment results	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
1	Tumor volume, mL	20.2	52.6	7.4	19.6	10.7	6.6
2.1	Planned injection volume, mL (% of Nr. 1)	2.0 (9.9)	5.3 (10.1)	0.7 (9.5)	2.0 (10.2)	1.1 (10.3)	0.7 (10.6)
2.2	Injected volume, mL (% of Nr. 1)	1.7 (8.4)	4.0 (7.6)	0.7 (9.5)	2.0 (10.2)	0.9 (8.4)	0.7 (10.6)
3.1	Activity prepared in syringe(s), MBq	365.29	727.11	207.59	372.46	263.01	217.57
	- Residual activity in needle(s), MBq	-19.17	-28.00	-13.47	-24.07	-12.42	-16.25
	- Residual activity in injection system, MBq	-67.34	-109.51	-86.12	-118.75	-89.41	-74.82
	- Residual activity in disposables, MBq	-1.44	-41.00	-0.25	-0.34	-15.06	-0.58
	- Residual activity in syringe(s), MBq	-51.75	-98.28	-5.31	-18.89	-30.72	-6.46
	- Residual activity in system flush, MBq	-32.14	-23.40	-22.81	-26.34	-31.82	-33.18
3.2	Injected activity, MBq (Δ% of 3.3)	193.45 (21.6)	426.92 (3.0)	79.63 (36.6)	184.07 (99.3)	83.58 (82.7)	86.28 (138.3)
3.3	Planned injection activity, MBq	159.10	414.30	58.30	185.3	101.1	62.4
4.1	Specific activity at treatment t = 0, MBq/mg ^{166}HoMS	1.88	2.25	2.15	1.99	2.15	2.15
4.2	Ho content, w/w%	19.69	19.66	20.05	19.58	19.58	19.82
5.1	Injected ^{166}HoMS, mg	102.90	189.74	37.04	92.50	38.87	40.13
5.2	^{166}HoMS per mL suspension prepared, MBq/mL (mg/mL)	125.96 (67.00)	127.56 (56.69)	129.74 (60.34)	92.04 (46.25)	92.86 (43.19)	130.72 (60.80)
	^{166}HoMS prepared, mg	194.30	323.16	96.55	187.17	122.33	101.20
	Suspension volume prepared, mL	2.9	5.7	1.6	3.0	2.1	1.7
5.3	^{166}HoMS per mL suspension injected, MBq/mL (mg/mL)	113.79 (60.53)	106.7 (47.44)	113.76 (52.91)	92.0 (46.25)	92.9 (43.19)	130.72 (60.80)
5.4	^{166}HoMS per ml tumor injected, MBq/mL (mg/mL)	9.58 (5.09)	8.12 (3.61)	10.76 (5.01)	9.39 (4.72)	7.80 (3.63)	13.07 (6.08)
6.1	Number of depositions planned	2 – 3	3 – 5	1	2	1	1
6.2	Number of depositions injected	2	5	1	2	2	2
7	Procedure duration, minutes	99	162	80	68	57	72

Appendix S4.2 CT ^{166}HoMS periprocedural findings and guidance

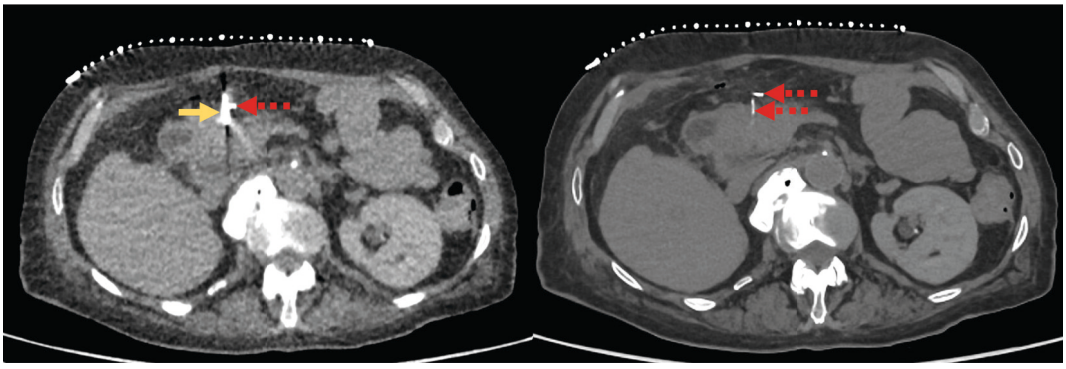
Legend: Needle ^{166}HoMS Air Stent

Arrows indicate the needle and holmium-166 microsphere (^{166}HoMS) deposit from a single injection location.
Location: (left-right, anterior-posterior, superior-inferior)

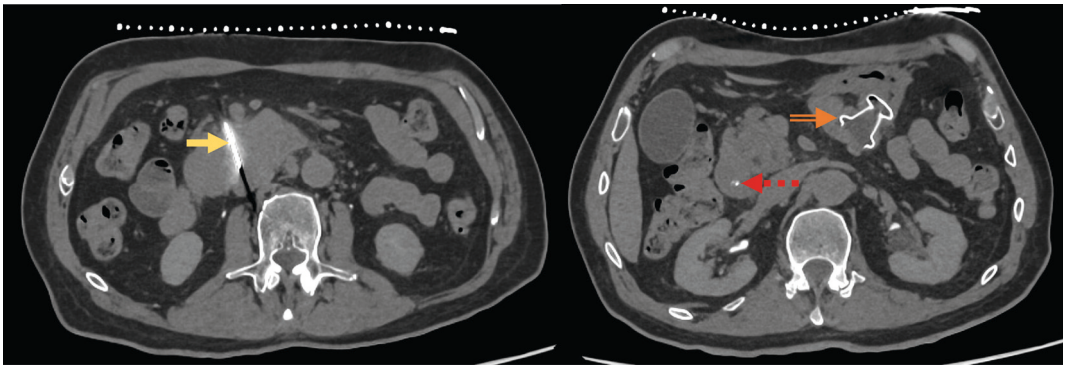
1.1 In patient 1, after injection 1 (location: center, center, inferior; 0.7 mL) intratumoral ^{166}HoMS and air deposition was visible.



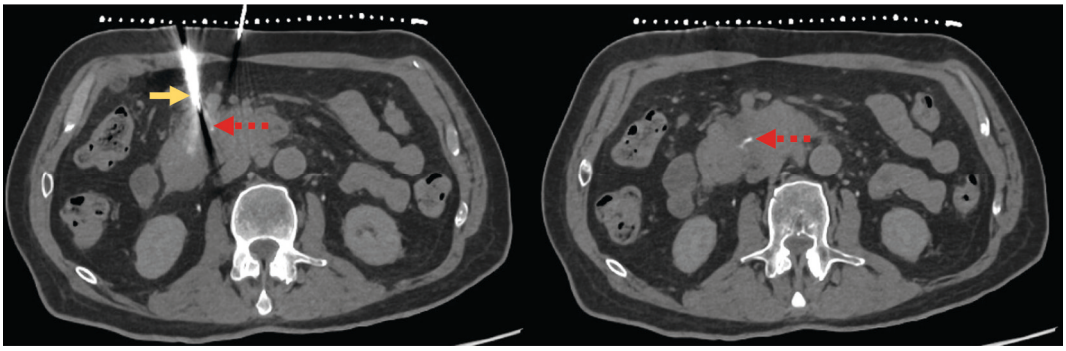
1.2 In patient 1, after injection 2 (location: center, center, superior; 1.0 mL) intratumoral and extratumoral ^{166}HoMS and air deposition was visible with the disconnected injection needle still in situ. The needle tip location was moved towards anterior during the injection between 0 – 5 mm. Although the needle angles between injection 1 and 2 were different, the needle tip locations were similar.



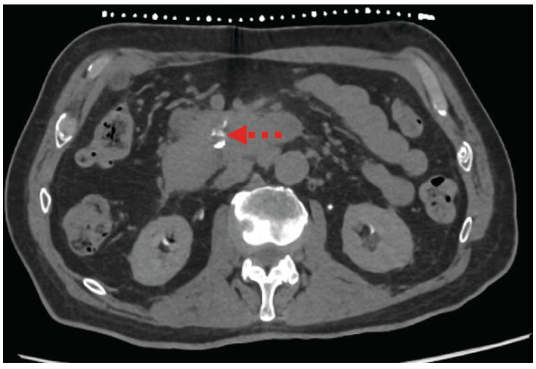
2.1 In patient 2, during injection 1 (location in tumor: right, posterior, inferior; 0.6 mL) ^{166}HoMS deposition was visible in the duodenum. Injection in this location was thereafter abandoned.



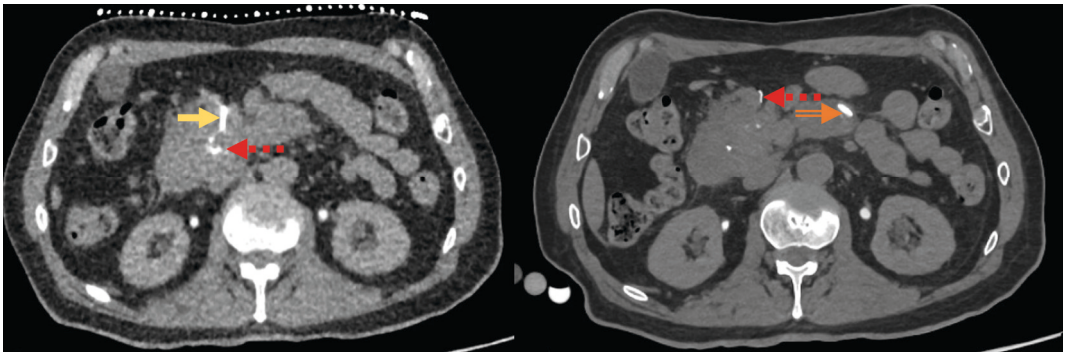
2.2 In patient 2, during injection 2 (location in tumor: right, superior, center; 0.85 mL) intratumoral ^{166}HoMS deposition became visible right in the tumor.



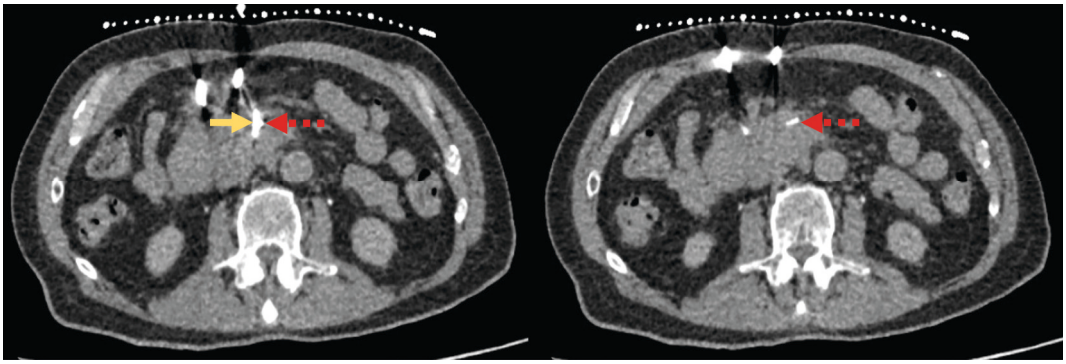
2.3 In patient 2, during injection 3 (location in tumor: center, inferior, center; 0.85 mL) intratumoral ^{166}HoMS deposition became visible centrally in the tumor.



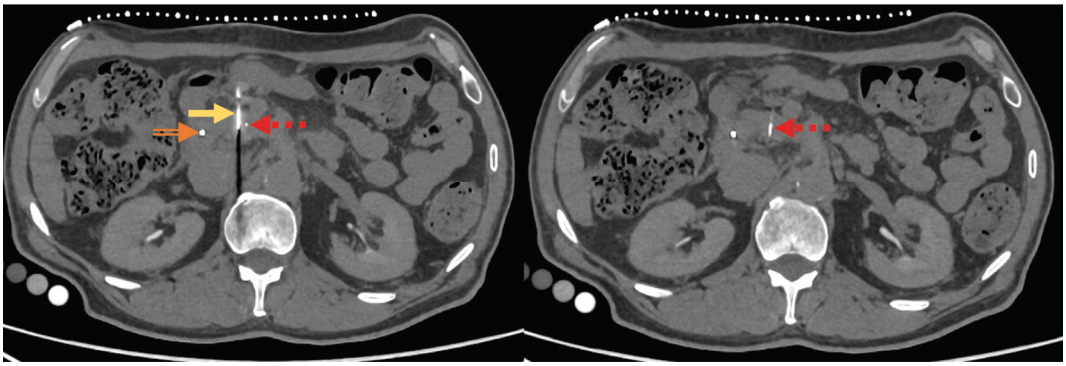
2.4 In patient 2, during injection 4 (location in tumor: centered, superior, center; 0.85 mL) intratumoral ^{166}HoMS deposition increased centrally in the tumor. After needle removal, ^{166}HoMS deposition in this needle tract became visible.



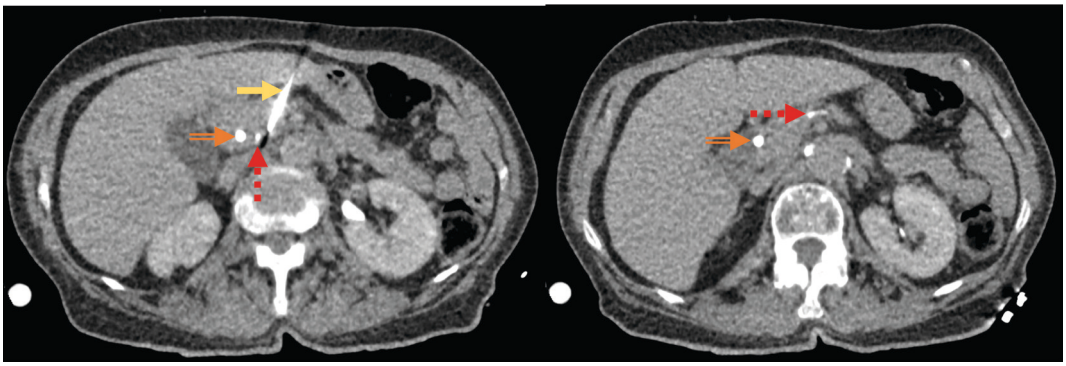
2.5 In patient 2, during injection 5 (location in tumor: left, center, center; 0.85 mL) intratumoral ^{166}HoMS deposition became visible left in the tumor.



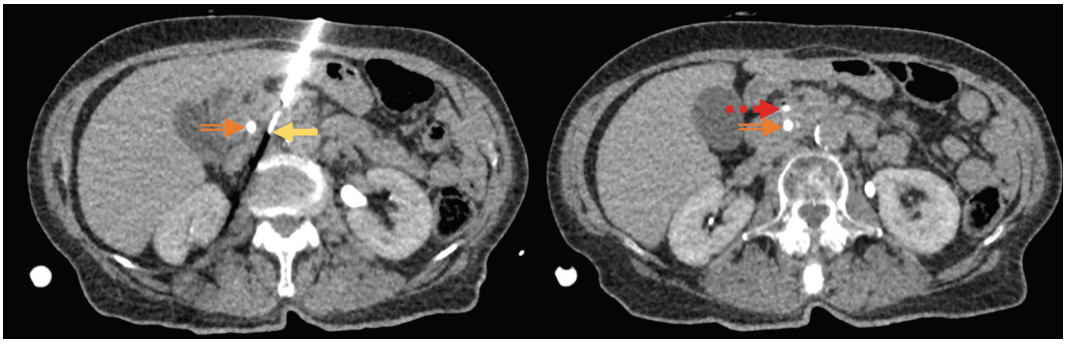
3.1 In patient 3, during injection 1 (location in tumor: center, center, center; 0.7 mL) intratumoral ^{166}HoMS deposition became visible in the tumor.



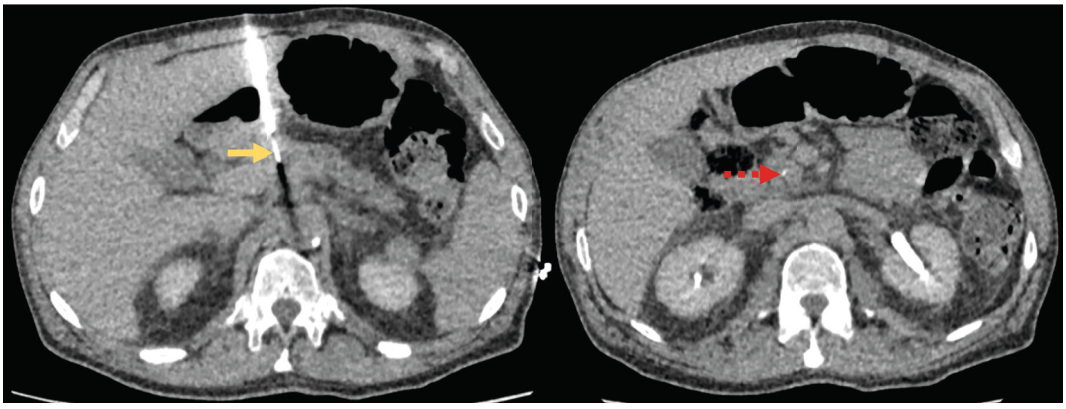
4.1 In patient 4, during injection 1 (location in tumor: center, superior, center; 1.0 mL), intratumoral ^{166}HoMS deposition became visible in the tumor near the stent and in the pancreatic duct in the pancreatic tail.



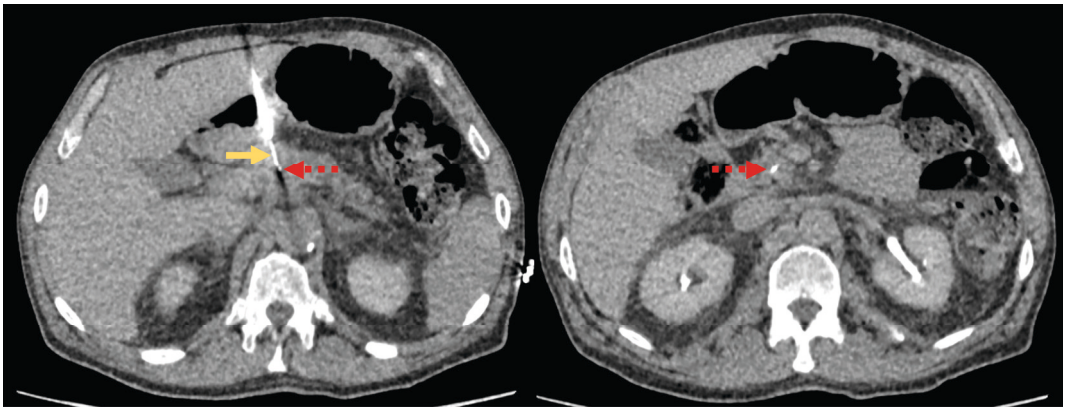
4.2 In patient 4, during injection 2 (location in tumor: center, inferior, center; 1.0 mL), needle overshoot in the renal vein was visible before injection. Retraction of needle and injection showed deposition in tumor and duodenum.



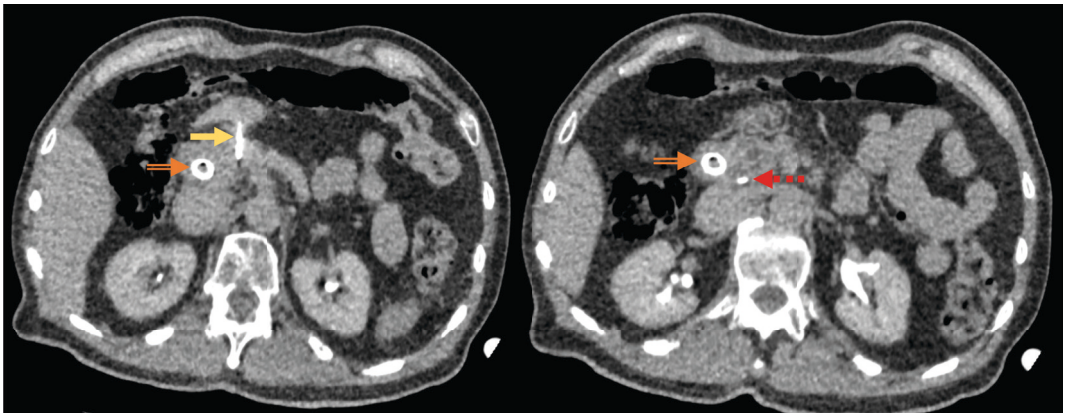
5.1 In patient 5, during injection 1 (location in tumor: center, center, anterior; 0.3 mL), ^{166}HoMS deposition became visible in the draining pancreatic duct and duodenum.



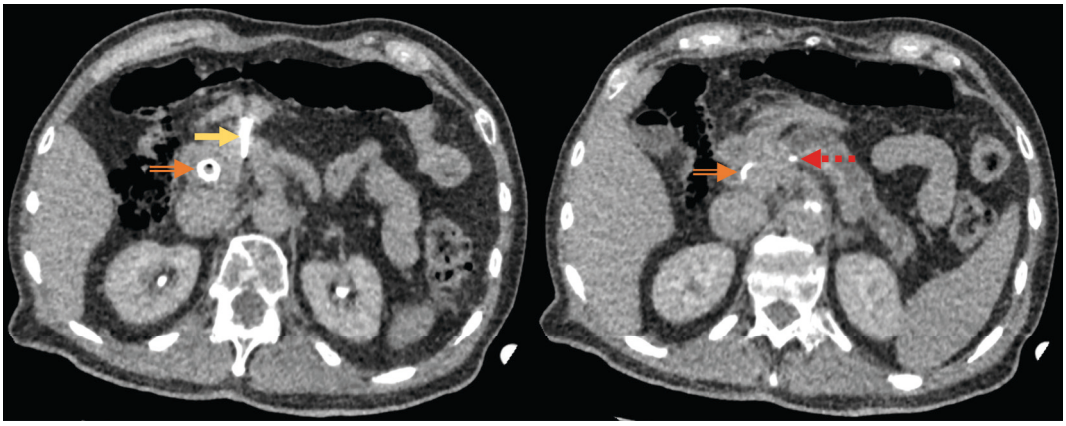
5.2 In patient 5, during injection 2 (location in tumor: center, center, posterior; 0.7 mL), the needle position was advanced towards posterior (~4 mm). ^{166}HoMS deposits became visible in the tumor but increased intensity was visible in the pancreatic duct.



6.1 In patient 6, during injection 1 (location in tumor: center, center, posterior; 0.2 mL), ^{166}HoMS became visible in tumor and draining pancreatic duct.

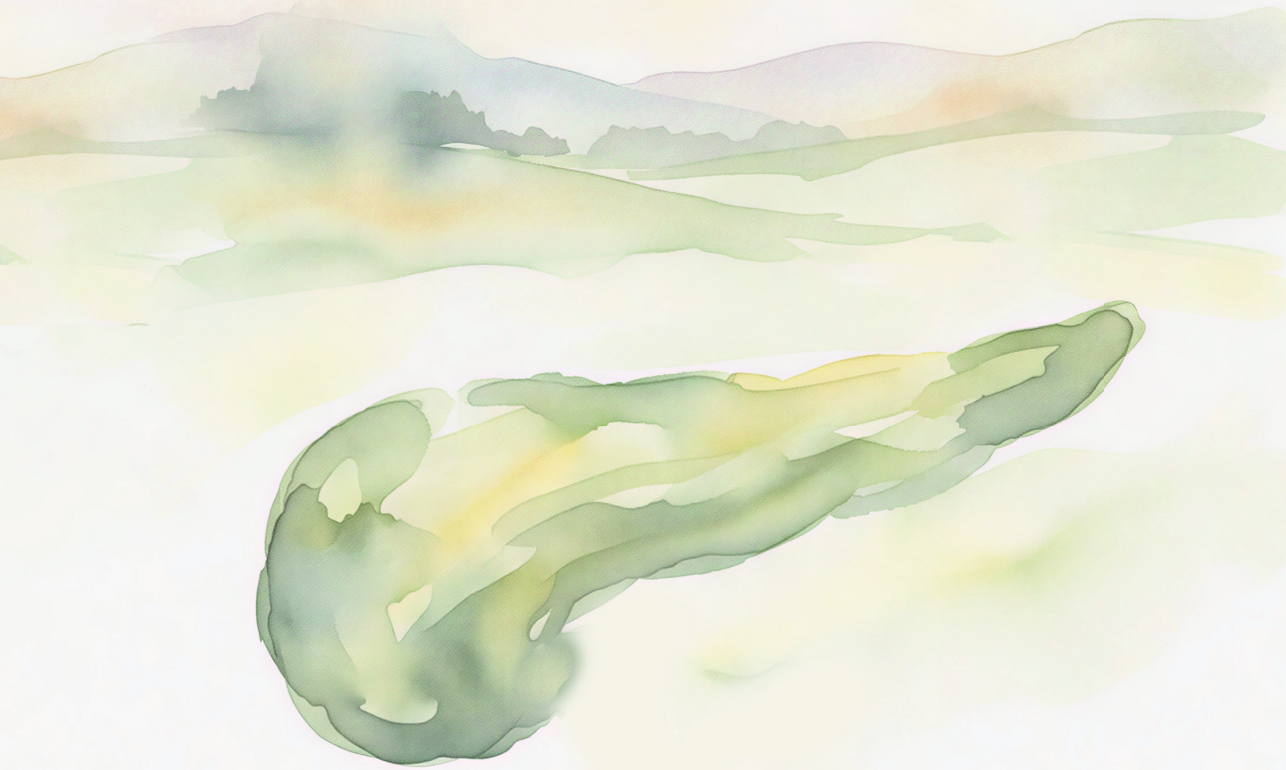


6.2 In patient 6, during injection 2 (location in tumor: center, center, superior; 0.46 mL), the needle was retracted towards anterior (~4mm). ^{166}HoMS deposits became visible in tumor and in the pancreatic duct in the pancreatic tail.



Chapter 6

GENERAL DISCUSSION AND FUTURE PERSPECTIVES



General discussion and future perspectives

Intratumoral injection of holmium-166 microspheres (^{166}HoMS) is a novel treatment option with high potential for patients with pancreatic ductal adenocarcinoma (PDAC), which to the best of our knowledge has not been studied outside the scope of this dissertation. Previous studies have explored treatment of PDAC by intratumoral injection of therapeutic agents such as chemotherapy and immunotherapy, as well as (micro)brachytherapy using phosphorus-32 microparticles, iodine-125 seeds, or palladium-103 seeds, as summarized in Chapter 2. These studies reported varying outcomes in terms of treatment response and provided only limited information on injection methods and treatment confirmation. The use of intratumoral injection with ^{166}HoMS has been studied in various other tumor types, including subcutaneous liver tumor models (VX2 in rabbits)[1], renal tumor models (Renca in Balb/C mice)[2], and various spontaneous tumors in felines such as hepatocellular carcinoma, cholangiocarcinoma, malignant epithelial liver tumors, and oral squamous cell carcinoma.[3, 4] Additionally, it has been investigated in pituitary adenoma and spindle cell sarcomas in canines[5, 6], as well as in recurrent head and neck squamous cell carcinoma in human patients[7]. Although reported outcomes varied in feasibility, these studies showed safety of the treatment and a correlation between the mean tumor dose and tumor response, which could only be accurately assessed with dedicated therapy confirmation by imaging modalities (SPECT, MRI, or CT).

The primary objective of this thesis was to develop and evaluate the clinical application of ^{166}HoMS for intratumoral injection in PDAC, focusing on feasibility and safety. In this overarching discussion the most significant findings are summarized and considered in the context of the rapidly evolving field of local tumor therapies. Finally, the future perspectives are provided.

Main results of this thesis

The systematic review in Chapter 2 provided a comprehensive overview of intratumoral injection strategies for PDAC utilizing five different therapeutic agents, as described above. Although the available data was insufficient to draw definitive conclusions about the impact of intratumoral injection therapies on overall survival, the review identified potential clinical benefits, including improved local tumor control, pain relief, and enhanced quality of life (QoL). Moreover, the review offered valuable insights into injection methodologies, routes of administration, limitations, and potential complications.

Building on these insights, Chapter 3 focused on developing an administration method for holmium microspheres (HoMS). This was first validated in an *in vitro* laboratory setup, followed by extensive testing and development in surgically removed ex vivo pancreatic specimens with PDAC localization. Intratumoral injection of HoMS proved feasible, provided that established injection parameters were followed to ensure an effective intratumoral deposition and minimize extratumoral leakage. These parameters were fundamental for clinical research to follow, and already suggested the movement towards an image-guided intervention.

In Chapter 4, the first-in-human application was conducted in an open abdominal setting. In this phase, intraoperative ultrasound-guided needle placement was employed to ensure needle placement centrally in the tumor without damaging nearby vasculature. However, a disadvantage of ultrasound was that it could not be utilized for real-time visualization of the injected ^{166}HoMS and thus the therapy distribution. In two of the first three patients treated, postoperative imaging revealed extratumoral leakage of microspheres to the lungs and colon (<1 Gy). Despite this, there were no severe therapy-related complications or side effects, explained by the relatively low total activity that was used and the rapid dispersal of the ^{166}HoMS in these organs. The intervention was proven safe for further exploratory research.

The development of intratumoral injection of ^{166}HoMS advanced further in Chapter 5, accumulating in a minimally invasive, fully image-guided procedure applied in six patients. This approach demonstrated minimal side effects, underscoring the minimally invasive nature of the intervention, without any acute complications and fast mobilization of the patients. Safety remained unchallenged, however, drainage of the ^{166}HoMS towards the intestines persisted as the primary limitation, significantly reducing the mean absorbed tumor dose.

To summarize, the main results of this thesis demonstrate that with the novel developed image-guided intratumoral injection method, ^{166}HoMS injection in PDAC is feasible and safe, with minimal

adverse effects observed throughout the clinical studies. The results provide a crucial foundation for the continued development of this therapy, both for PDAC and other tumor types. While minimally invasive image-guided application is feasible and safe, the key challenge remains delivering a maximally effective tumor dose to evaluate the optimal therapeutic potential of this intervention.

Alternative local therapies

With similar long-term visions as the one of this thesis, to reduce the tumor burden or downstage locally advanced pancreatic cancer (LAPC) in order to improve survival in better quality of life for patients suffering from PDAC, several other locoregional interventions have been studied. In addition to the intratumoral injection therapies detailed in Chapter 2, radiofrequency ablation (RFA), irreversible electroporation (IRE), and (MR-guided) stereotactic body radiation therapy (SBRT) are the most studied locoregional treatment methods, warranting discussion here.

RFA employs alternating electric currents delivered through intratumorally placed needles to induce central acute lethal hyperthermia and peripheral (sub)lethal hyperthermia. While RFA is predominantly used for liver tumors[8], it has also been investigated for bone metastases[9], renal tumors[10], cholangiocarcinoma[11], and in multiple trials for PDAC, particularly LAPC.[12-14] Unlike RFA, irreversible electroporation (IRE) is a non-thermal ablative technique that utilizes high-voltage pulses to create microscopic damage and permeabilize cell membranes between two or more needles inserted in or around the tumor.[15] Meanwhile, SBRT delivers a high tumor dose by external radiation with precise targeting, often achieving mean tumor doses exceeding 50 Gy while sparing adjacent critical organs.[16] Recent advancements, such as MR-guided SBRT (MR-Linac), further enhance targeting accuracy and therapeutic precision.

While RFA, IRE, and SBRT have occasionally demonstrated promising results and suggest potential survival benefits when combined with chemotherapy, the evidence remains inconsistent.[12] Discrepancies between prospective and retrospective studies suggest selection bias, and the most promising survival benefits are rarely replicated in randomized controlled trials (RCTs).[12, 13] A clear example is the international RCT PELICAN study (ClinicalTrials.gov ID: NCT03690323) investigating RFA combined with chemotherapy versus chemotherapy alone in patients with stable LAPC after four cycles of FOLFIRINOX (or two cycles of gemcitabine/nab-paclitaxel).[17] Preliminary and unpublished results indicate no improvement in overall survival and an inferior quality of life (QoL) in the RFA group (Pancreasdag 2024, Kamerik, The Netherlands). Similarly, the Dutch CROSSFIRE RCT compared SBRT and IRE in LAPC patients who had received 3–8 cycles of FOLFIRINOX.[18] The study found no significant survival advantage, with median overall survival from randomization reported at 16.1 months in the SBRT group versus 12.5 months in the IRE group (p=0.21) and treatment-related

severe adverse events (grade 3–4) occurred more frequently in the IRE group (22%) compared to the SBRT group (6%).[18] Most recently, the Dutch LAPSTAR multicenter RCT (ClinicalTrials.gov ID: NCT06272162) was initiated to evaluate the impact of MR-guided SBRT combined with standard therapy on health-related QoL in patients with LAPC. As of yet, clear substantiating evidence for the benefit of locoregional intervention over the standard of care is lacking.

A possible explanation for the lack of benefit from RFA is that thermoablation is often employed for tumor debulking rather than radical ablation due to the proximity of major blood vessels or other thermo-sensitive tissues such as healthy pancreatic parenchyma, bile ducts, and the intestines.[12] The blood vessels also create a heat-sink effect impairing the effectiveness of the ablation. The non-thermal modalities IRE and SBRT are associated with substantial risk of complications such as cholangitis, pancreatitis, pancreatic and duodenal fistulas, duodenal perforations, and blood vessel thrombosis or stenosis.[18, 19] Given the high complication rate associated with IRE, careful patient selection is crucial to utilize the potential therapeutic benefit of the treatment.[18, 19] Overall, the anatomical position of the pancreas, surrounded by vital and fragile structures, further complicates the use of radical ablative methods.[12, 13]

However, ablative treatment methods may serve a purpose, even when not performed with radical intent and thus with a lower complication rate. Emerging evidence highlights immunomodulation therapy as a key area of potential. Immunomodulation, the upregulation of immune activation pathways or downregulation of immunosuppressive pathways, is an increasingly prominent focus for interventional oncology. Although not yet fully understood in PDAC, locoregional therapies like RFA, IRE, and SBRT appear to have significant immunomodulatory effects that may extend beyond the local treatment site. For example, 30 days after RFA, studies observed activation of the adaptive immune response, characterized by increased CD4+, CD8+, and terminal effector memory T-cells, with stable levels of immunosuppressive T-regulatory (Treg) cells.[20] Similarly, two weeks after IRE or SBRT, the ratio of $CD8^+$ (and $CD4^+$ for IRE) to Treg cells increased, though these effects returned to baseline after three months.[18] While these immunomodulatory effects appear transient, they create a treatment window during which combination therapies, such as immunotherapy, could amplify a systemic anti-tumor immune response and induce an abscopal effect. When intratumoral injection with ^{166}HoMS is performed, the central tumor dosages may reach >200 Gy, creating cell necrosis over time, while at the periphery of the ^{166}HoMS deposits, cell apoptosis may be initiated by a lower dose. It is expected that this combination creates a large and diverse release of tumor-specific antigens which may serve as an immunomodulation jump-start for immunotherapy.[21]

A promising preclinical immunomodulation example is the combination of SBRT followed by intratumoral injection of interleukin-12 (IL-12), encapsulated in polylactic acid microspheres, in a mouse model. This approach uniquely eradicated pancreatic tumors and demonstrated an abscopal effect, effectively eliminating hepatic metastases in multiple mice.[22] Supplementing research showed that intratumorally injected agents and tumor-specific antigens drained, and therefore targeted, towards the nearby lymph nodes of the mice within two hours, initiating a further antitumor immune response.[23] These findings underscore the potential of combining locoregional therapies that initiate immunomodulation with immunotherapy to enhance systemic anti-tumor responses and improve outcomes in PDAC.

Future perspectives

The target population

Intratumoral ^{166}HoMS injection presents a promising treatment for various PDAC patient groups. In LAPC, it could serve as a neoadjuvant therapy alongside chemotherapy to increase downstaging. In palliative care, it may increase local tumor control, reduce tumor burden, and extend survival. Additionally, in metastatic PDAC, combining ^{166}HoMS with immunotherapy could enhance systemic immune responses.[24]

Given the diversity of PDAC presentations, patient selection is crucial for maximizing treatment efficacy. Future studies could refine eligibility by assessing tumor microenvironment (TME) factors such as perfusion, stroma density, FDG uptake, and radiation-resistant mutations.[25] These factors should be incorporated into major clinical trials to identify predictive markers of treatment response. Preclinical PDAC models may provide initial insights into these correlations.[26]

Currently, the most suitable candidates for ^{166}HoMS are patients with locally advanced, unresectable PDAC who have no metastases and have undergone induction chemotherapy. However, its true clinical utility will ultimately depend on demonstrating its efficacy, which remains to be fully evaluated. If proven effective, it may one day become part of a curative combination treatment.[22]

Application, availability, and cost

Intratumoral ^{166}HoMS injection could have a wide application due to its low patient burden, minimally invasive nature, and favorable safety profile. The results of Chapter 5 suggest it can be performed as a single-day outpatient procedure, eliminating hospitalization. Compared to SBRT, which requires 3–7 visits often on non-consecutive days, a one-time ^{166}HoMS treatment significantly reduces both treatment burden and healthcare costs.[16] Depending on treatment effectiveness, the treatment could also be repeated after 2–4 months, considering conventional tumor reevaluation after 2-months chemotherapy and the fact that tumor response rate might still improve after 1 months, as seen in ^{166}HoMS TARE.[27]

The low complication rates seen in Chapters 4 and 5 further support its safety, with most adverse events being mild or unrelated to the intervention. This advantage enhances post-procedural QoL compared to other local therapies, described above. Additionally, performing ^{166}HoMS injections on a conventional CT scanner, rather than requiring advanced MRI/radiotherapy units or ablation stations, improves accessibility and cost-effectiveness. Simple and affordable radiation safety measures, such as fixation arms and acrylic syringe covers, make implementation straightforward. Further cost reductions could be achieved through automation. Automatic segmentation and injection planning software could streamline preprocedural work, while modifications to the injection system, such as automated syringe rotation or more viscous microsphere carriers preventing agglomeration, could allow a single interventional radiologist or nuclear medicine physician to perform the procedure independently.

The intervention

As highlighted earlier, a key limitation remains in delivering a maximally effective tumor dose to evaluate the optimal therapeutic potential of intratumoral ^{166}HoMS injection in PDAC**.** Here, several methods are proposed to address this limitation. The most direct approach involves increasing the specific activity of the microspheres. For example, the specific activity could be increased from the current mean of 2.0 MBq/mg (as described in Chapter 5) to the conventionally available 14.0 MBq/mg used in TARE (QuiremSpheres, Quirem Medical, Deventer, Netherlands), increasing both the tumor dose and off-target radiation dose by 700%. When the results from Chapter 5 are extrapolated, the tumor and colon would receive an estimated mean dose of 98–266 Gy and 0–32.9 Gy, respectively, assuming the ^{166}HoMS remains within the colon indefinitely without clearance. This adjustment would not affect ^{166}HoMS concentrations, CT imaging capabilities, injection feasibility, or distribution. Still, the application of this higher specific activity raises safety concerns, particularly regarding gastrointestinal radiation injury. These risks must be carefully evaluated in a small cohort before proceeding to larger-scale efficacy studies.

A safer and more robust alternative involves improving ^{166}HoMS retention within the tumor while minimizing extratumoral leakage. Further reduction of the overall injection volume is not feasible since leakage through the pancreatic duct was seen on CT, even after the initial injection fraction of 0.2 mL. Furthermore, this approach may negatively impact the microsphere distribution within the tumor. A promising strategy is to modify the ^{166}HoMS carrier fluid. In the current studies, the carrier fluid was a low-viscosity, water-based isotonic phosphate buffer. To reduce leakage through the pancreatic duct or needle tract, a high-viscosity or glue-like carrier fluid could be employed. For example, in Chapter 3, sample 4 a high-viscosity carrier (HVC) was utilized with encouraging results. A similar HVC containing microcrystalline cellulose, has undergone biocompatibility testing in mice and is currently being investigated for intratumoral injection in glioblastomas in pigs (N.J.M. Klaassen, unpublished data). However, provided data was yet insufficient for clinical application in Chapters 4 and 5.

It is important to recognize that achieving complete (100%) retention of the ^{166}HoMS in all PDAC tumors may be unfeasible when using a liquid carrier fluid, as was described in this dissertation. By using a formulation with a high-viscosity carrier diluent it can be expected that retention will be increased as described in several studies. In a study by Ross et al. (2021), 42 patients with PDAC underwent intratumoral injection of phosphorus-32 (^{32}**P**) using a high-viscosity carrier diluent (OncoSil Diluent, OncoSil, Sydney, Australia), aiming for a mean absorbed tumor dose 100 Gy.[28] While post-injection dosimetry was not performed, Bremsstrahlung SPECT imaging demonstrated the localization of microparticles 4 hours after injection. Despite using a high-viscosity carrier diluent and limiting the injection volume to a maximum of 8% of the tumor volume, 2–6 patients (4.8–14.3%) showed no detectable radiation uptake in the tumor[28, 29], 10 patients (23.8%) exhibited radiation uptake in other regions of the pancreas, and 19 patients (45.2%) displayed uptake in the gastrointestinal tract.[29] The study did not quantify extratumoral activity in MBq or as a fraction of the injected dose, nor did it report the achieved mean tumor dose. Nevertheless, the oncological outcomes were notable, with a local disease control rate of 82% (95% CI: 68.6–90.9%) at 16 weeks and a median overall survival of 15.5 months. However, the potential influence of selection bias on these results should not be overlooked.[28]

Image-guidance

Within the studies included in this dissertation, four different imaging modalities were tested and evaluated for their utility in periprocedural image-guidance of the needle and/or ^{166}HoMS**,** as well as for post-injection evaluation and quantification in PDAC tumors. To maintain a focused scope, not all results were reported. The evaluated modalities include ultrasound (US; Chapters 3 and 4), magnetic resonance imaging (MRI; Chapters 3, 4, and 5), single photon emission computed tomography (SPECT; Chapters 4 and 5), and computed tomography (CT; Chapters 3, 4, and 5). Table 1 provides an overview of the potential applications of each modality for intratumoral ^{166}HoMS injection in PDAC.

Table 1. *A feasibility overview of the application of imaging modalities during or after intratumoral injection of holmium-166 microspheres in pancreatic ductal adenocarcinoma.*

Modality	Periprocedural needle guidance	Periprocedural ^{166}HoMS guidance	Post-injection ^{166}HoMS quantification
US	+++	–	–
MRI	+ –	+ –	+ –
SPECT	–	–	+ –
CT	++	++	+ –

"+" indicates the application has been performed and is feasible. "+-" indicates the application could be feasible, although several limitations are warranted. "-" indicates the application is currently unfeasible.

- US proved feasible for needle guidance, as both the tumor and needle were clearly visible during the procedure. While the movement of the ^{166}HoMS suspension could be visualized on US during the injection, it was indistinguishable from surrounding tissue when stagnated, making post-injection evaluation or quantification unachievable.
- MRI was suitable for both visualization and quantification of ^{166}HoMS and has even been utilized for intraprocedural dosimetry during transarterial radioembolization (TARE).[30] However, the inherent limitations of MRI, such as the magnetic field, narrow bore, and the need for coils, restrict its use during percutaneous needle placement or for periprocedural guidance of ^{166}HoMS. However, novel low-field MRI developments and MR-safe needles could overcome these limitations.[31] Post-injection evaluation and quantification of ^{166}HoMS deposits in PDAC seems feasible, but challenges such as intestinal air, organ size, resolution, and adjacent tissue interfaces render MRI more prone to artifacts, which sometimes can be mistaken for ^{166}HoMS, in PDAC compared to liver tumors.
- SPECT is valuable for detecting systemic and low-concentration distributions of ^{166}HoMS, such as in the lungs or colon. However, its low spatial resolution and long acquisition times limit its utility in periprocedural applications.
- CT demonstrated feasibility for both periprocedural needle guidance and ^{166}HoMS visualization. In exploratory settings, injections in phantom models were performed during 4D CT acquisition to enable real-time visualization of the injected ^{166}HoMS. However, in Chapter 5, 4D CT was infeasible due to radiation safety restrictions, which could be easily overcome in future setups if remote injections become available. CT quantification of ^{166}HoMS deposits was performed in Chapter 5, but the recovered amounts varied significantly compared to SPECT imaging, potentially influenced by the distribution differences between acquisition times (24 hours). Additionally, the impact of low concentrations of ^{166}HoMS (<100 HU) on CT quantification and distribution visualization remains unclear. Future advancements could include the development high holmium-content microspheres, dedicated software with dose-point-kernels for the high resolution of clinical CT scanners, alongside standardized quantification methods, to enhance the accuracy and utility of CT for ^{166}HoMS quantification.[32]

The application of advanced imaging techniques, such as dual-energy CT, photon-counting CT, or low-field MRI, as well as the integration of modalities (e.g., combining SPECT with high-resolution contrast-enhanced CT), holds promise for enhancing periprocedural therapy guidance. These, and previously mentioned, advancements have the potential to provide more accurate and reliable data on tumor dosimetry, including essential insights into dose-response relationships, tumor dose coverage, and dosimetry related therapeutic outcomes.

Combination therapy

PDAC is characterized by its aggressive biology, rapid progression, and early systemic dissemination with often present but undetectable micrometastatic disease at diagnosis, making it a fundamentally systemic disease even in its localized stages. Consequently, local therapies alone are unlikely to provide durable tumor control or significantly improve long-term survival outcomes. Combining local therapies, such as intratumoral ^{166}HoMS injections, with systemic treatments like FOLFIRINOX chemotherapy is essential to address both the primary tumor and systemic dissemination. Therefore, local therapies remain dependent on systemic therapies.

The integration of ^{166}HoMS injection with systemic treatments, such as FOLFIRINOX or intravenously administered immunotherapies, could provide a synergistic effect, enhancing the efficacy of systemic therapies. A major obstacle limiting the success of systemic treatments in PDAC is the TME, which acts as a physical, immunological, and therapeutic barrier, preventing circulating drugs and immune cells from effectively reaching the cancer cells. High-dose intratumoral injection of ^{166}HoMS, beyond its direct tumoricidal effects, may help disrupt these TME barriers. Similar immunomodulatory effects have been reported with other local therapies such as RFA, SBRT, and IRE.[18, 20] By improving the accessibility of circulating therapeutic molecules to the tumor, this approach could not only increase treatment efficiency but also allow for dose reductions, thereby mitigating the toxicity that currently limits the broader application of both immunotherapies and chemotherapies.[33] Thus, combining intratumoral ^{166}HoMS injection with systemic therapies has the potential to make these treatments more effective and less burdensome for patients.

The timing of integrating intratumoral ^{166}HoMS injection with conventional chemotherapy is crucial. A promising approach would be to introduce the minimally invasive intratumoral injection of ^{166}HoMS during the treatment breaks of FOLFIRINOX chemotherapy, typically after 4, 8, or 12 cycles (or after 2, 4, or 6 cycles of gemcitabine plus nab-paclitaxel). This corresponds to the treatment breaks at 2, 4, or 6 months for both types of chemotherapy. However, since many patients do not complete all intended cycles of chemotherapy due to poor tolerability, early integration of ^{166}HoMS after 4–8 cycles (or 2–4 months) of FOLFIRINOX is recommended.[34] This approach mirrors that of several multicenter randomized controlled trials, which implemented local interventions after a few months of chemotherapy. Examples include the PELICAN trial with RFA following 2 months of chemotherapy[35], the CROSSFIRE trial with IRE or SBRT after 2–4 months of chemotherapy[18], and the LAPSTAR trial (NCT06272162) with MR-guided SBRT after 2–4 months of chemotherapy.

Outcome measures

Given the often-incurable nature of PDAC, coupled with frequent comorbidities and treatment-related toxicities, there has been a movement away from traditional oncological outcomes such as overall survival (OS), progression-free survival (PFS), disease-free survival (DFS), and surgical resection rates, as primary outcome. Instead, the focus has increasingly moved toward survival with an acceptable quality of life (QoL) as a primary outcome. QoL can be assessed using validated questionnaires, such as the EORTC QLQ-C30 or EQ-5D-5L. Since most PDAC patients are in the terminal stages of the disease, a severe loss of QoL or death, referred to as time until definitive deterioration (TUDD), is often inevitable. TUDD is measured from the time of randomization until a clinically significant deterioration in health occurs, defined by a specific reduction in points (e.g., using the results of the EORTC QLQ-C30 questionnaires). Thus, QoL is continuously monitored until a defined health decline, at which point the TUDD is determined.

To ensure comparability with the latest interventions and chemotherapy regimens, it is essential to define outcomes clearly and ensure robust homogeneity in their measurement. While guidelines for time-to-event outcomes in PDAC have been established, these guidelines were set over a decade ago.[36] As such, an updated version of these guidelines is expected soon, as part of an initiative by the Dutch Pancreatic Cancer Group (DPCG).

Author's opinion: In this section, the author presents their personal opinion on the essential steps for advancing future studies on intratumoral injection of ^{166}HoMS in PDAC. First, a small cohort feasibility study using a high-viscosity carrier (HVC) should be conducted to potentially enhance tumor dose and reduce gastrointestinal leakage. Here, immunomodulation effects should already be monitored as secondary or exploratory outcomes. Following this, the results regarding ^{166}HoMS retention in the tumor will inform decisions on increasing the microsphere activity to achieve a mean absorbed tumor dose of >200 Gy. This approach should be tested in a multicenter, randomized controlled superiority trial comparing ^{166}HoMS with FOLFIRINOX versus FOLFIRINOX alone. The target population should consist of patients with locally unresectable PDAC who show no metastatic disease after 4 cycles of (modified, m)FOLFIRINOX chemotherapy. Patients would be randomized into two groups: the intervention arm receiving CT-guided ^{166}HoMS injection, closely followed by 4–8 more cycles of (m)FOLFIRINOX, and the control arm receiving only 4–8 additional cycles of (m)FOLFIRINOX. The primary outcome should likely be time until definitive deterioration (TUDD), defined by a significant decrease in QoL or death, or another appropriate outcome in line with the current international guidelines. Secondary outcomes should include intervention-related, chemotherapy-related, and other toxicity, overall survival, chemotherapy completion rate, CA19.9 response, and immune response. If no superiority is demonstrated for the intervention arm, further investigation into the immune response should

be conducted, particularly to assess potential immunomodulation windows to combine with immunotherapy. If no significant immunomodulation is observed, the application of intratumoral ^{166}HoMS in PDAC should be re-evaluated, and its potential use in other tumor types should be explored.

Platform technique

Given that survival rates for PDAC have scarcely improved over the past few decades and the tumor presents numerous challenges for intratumoral injection, it is clear that PDAC remains a particularly difficult tumor to treat.[37, 38] This dissertation presents the development of an intratumoral injection method for ^{166}HoMS, providing valuable new insights. The use of periprocedural image guidance for intratumoral ^{166}HoMS injection could serve as a platform technique, facilitating easy translation to other tumor types with minimal modifications to the injection methods. This platform technique could potentially be applied to various solid tumors, including brain, head and neck, lung, skin, breast, ovarian, renal, prostate, bladder, and cervical cancers.[37, 39] However, established outcomes, potential benefits, and treatment costs must be carefully considered. By expanding the application of intratumoral ^{166}HoMS injection to a broader range of tumor types, its clinical use could be further developed and optimized.

The long-term potential of this platform technique is vast. Automated preprocedural segmentation and advanced injection planning enable a patient-specific approach, which could be further refined by incorporating tumor microenvironment (TME) diagnostics for improved patient selection. Real-time 4D CT imaging could enhance microsphere distribution monitoring, while dedicated quantification software may allow for periprocedural dosimetry. This data-driven feedback could be directly relayed to operators or even be integrated into a fully automated injection system utilizing robotic needle placement. Additionally, fan needles inserted through a single guiding needle could optimize tumor coverage while minimizing healthy tissue trauma and reducing complication rates. Many of these technologies are already under development in other fields, suggesting their integration into clinical practice may happen sooner than expected.

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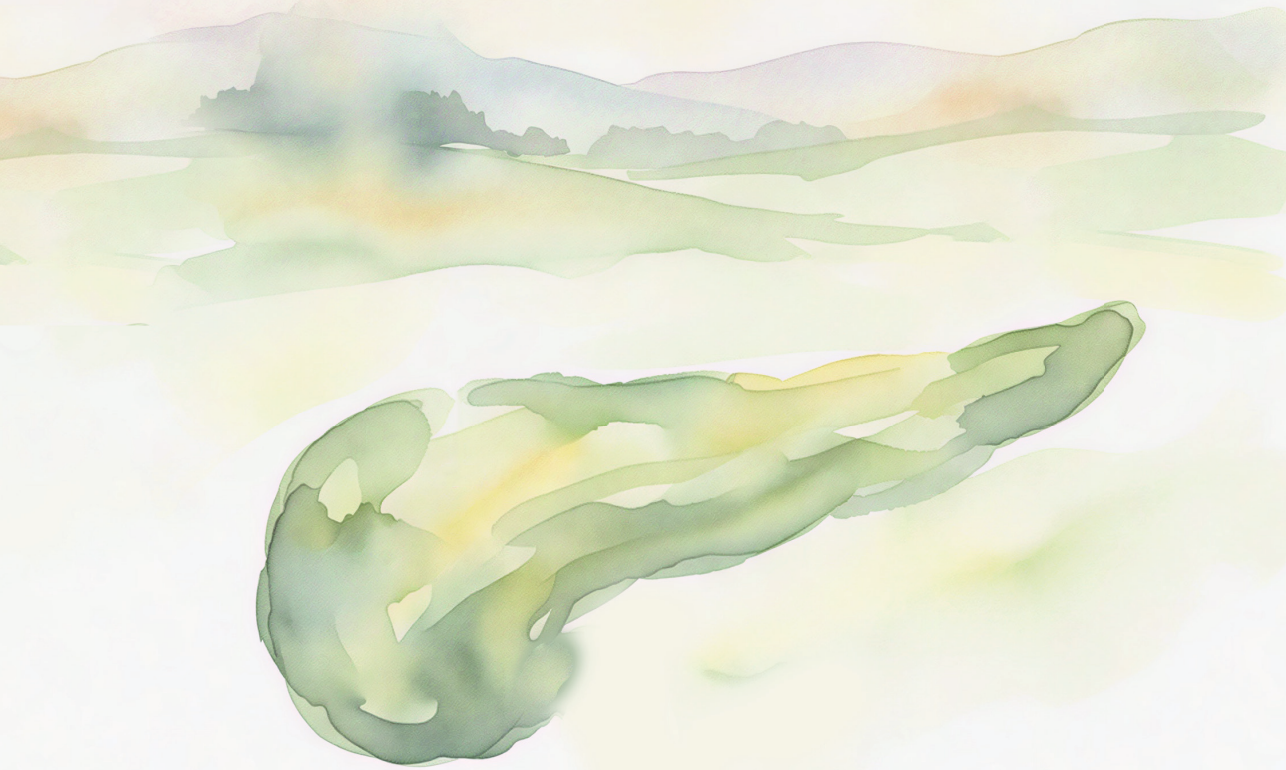
Chapter 7

SUMMARY



Summary

Pancreatic ductal adenocarcinoma (PDAC) is among the three cancers with the highest mortality rates. Surgical resection of the tumor, often preceded by several months of intensive chemotherapy, remains the only potentially curative treatment, eligible for one-third of the patients. Minimally invasive intratumoral injection of holmium-166 microspheres (^{166}HoMS) is a novel treatment approach, offering the potential to downstage local disease to a resectable stage or to improve survival and quality of life in later stages.

The primary aim of this thesis was to develop and evaluate the clinical application of ^{166}HoMS for intratumoral injection in patients with PDAC, focusing on feasibility and safety, and the incorporation of image guidance to confirm therapy delivery both during and after the procedure.

Chapter 2 presents a systematic review of various intratumoral injection therapies for LAPC. Across the 52 included studies, 1,843 patients were treated with intratumoral injection therapies using iodine-125 (^{125}I) seed brachytherapy (32 studies, 1,283 patients), phosphorus-32 (^{32}P) microbrachytherapy (5 studies, 133 patients), palladium-103 (^{103}Pd) seed brachytherapy (2 studies, 26 patients), immunotherapy (9 studies, 330 patients), or chemotherapy (4 studies, 71 patients). While survival outcomes varied widely, even within the same interventions, the safety profiles were generally acceptable. Although the data were insufficient to draw definitive conclusions on overall survival, the review highlighted potential clinical benefits, including improved local tumor control, pain relief, and enhanced quality of life. It also provided valuable insights into injection methodologies, administration routes, limitations, and potential complications.

In **Chapter 3**, these insights were pivotal in the development of an administration method for ^{166}HoMS in ten surgically removed ex vivo PDAC tissues. First, in a controlled laboratory setting, the challenge of microsphere agglomeration, caused by the relatively high density of the microspheres in fluid suspension, was addressed. This issue was resolved by designing a manual rotation and injection system, which prevented agglomeration and ensured homogeneous and consistent injection concentration. Initial feasibility tests were conducted to tackle the high intratumoral pressure characteristic of PDAC, which can cause needle obstruction or leakage along the needle tract. These challenges were managed by implementing specific injection parameters, such as restricting the injection volume, limiting the number of needle passes, and using imaging for needle guidance. Additionally, efforts were made to improve the visibility of ^{166}HoMS on computed tomography (CT). This involved optimizing holmium-specific CT parameters and increasing the concentration of ^{166}HoMS in the injection suspension. The injection system and methodologies developed in this chapter provide a foundation for future studies on the intratumoral injection of ^{166}HoMS in PDAC and potentially other solid tumor types.

In **Chapter 4**, the developed injection system and parameters were applied for the first time in human PDAC patients. Thirteen patients underwent patient-specific planning for ultrasound-guided intratumoral ^{166}HoMS injection in an open-abdominal surgical setting. Intervention was only performed when resection was abandoned during exploratory surgery due to local or distant disease progression. This approach was designed to minimize the impact on conventional treatment, as the intervention's safety was not yet established. Of the 13 patients, three received intervention. Over 12 weeks post-intervention, no adverse events related to the intervention were observed, despite some extratumoral radiation exposure to the lungs and colon (<1 Gy). The achieved mean tumor dose ranged from 5 to 46 Gy, which was lower than expected due to extratumoral radiation and reduced injection volumes caused by limited intraabdominal visibility. Two patients had stable local disease at 12 weeks, while all patients developed distant metastases. While feasibility remained limited, the study established an acceptable safety profile, which led to the initiation of a follow-up trial.

Chapter 5 combines all prior knowledge of intratumoral ^{166}HoMS injection in PDAC. It presents the feasibility and safety results of a minimally invasive approach, with both needle and therapy guidance using periprocedural CT. Six patients with unresectable PDAC after induction FOLFIRINOX received patient-specific treatment. Technical feasibility was achieved in all patients with no severe complications. The ^{166}HoMS deposits were clearly visible on periprocedural CT, directly aiding treatment planning. However, the tumor dose was lower than intended ranging from 14 to 38 Gy, due to off-target radiation in all patients. This was mainly caused by ^{166}HoMS leakage through the pancreatic duct toward the gastrointestinal tract, visible on periprocedural CT, and quantified in the colon on SPECT approximately 24 hours post-injection. Despite this, the procedure demonstrated a favorable safety profile, with only one grade 3 adverse event attributed to the intervention's contrast agent. All other adverse events were either unrelated to the study intervention (92.3%) or were mild (grade 1). At 16 weeks, two-thirds of the patients showed local disease control. This chapter confirms that CT-guided percutaneous ^{166}HoMS injection is a feasible and safe treatment option for unresectable PDAC, with clear periprocedural visibility and encouraging clinical outcomes.

Finally, **Chapter 6** presents the overarching discussion. Key findings are summarized and placed in a broader clinical context. The rapidly evolving field of local tumor therapies and ongoing clinical research are compared to the results of this thesis. Lastly, future perspectives for this novel treatment are explored, along with the further investigations needed to fully assess the therapeutic potential of this novel intervention.

Nederlandse samenvatting

Pancreas ductaal adenocarcinoom (PDAC) behoort tot één van de drie kankersoorten met de hoogste mortaliteit. Chirurgische resectie van de tumor, vaak voorafgegaan door intensieve chemotherapie, is op dit moment de enige curatieve behandeling. Een derde van de patiënten komt hiervoor in aanmerking. Een nieuwe en veelbelovende behandeling is 'minimaal invasieve intratumorale injectie van holmium-166 microsferen (^{166}HoMS)'. Deze vorm van therapie zou lokale ziekte kunnen terugbrengen tot een resectabel stadium of de overleving en kwaliteit van leven in latere stadia kunnen verbeteren.

Het doel van dit proefschrift was het ontwikkelen en evalueren van de klinische toepassing van ^{166}HoMS voor intratumorale injectie in patiënten met PDAC. De nadruk lag op de haalbaarheid en veiligheid van de behandeling en het gebruik van beeldgeleiding om de toediening van de therapie zowel tijdens als na de procedure te kunnen bevestigen.

Hoofdstuk 2 omvat een systematische review van diverse intratumorale injectietherapieën voor lokaal gevorderd pancreascarcinoom (LAPC). In 52 geïncludeerde studies werden in totaal 1843 patiënten behandeld met verschillende vormen van intratumorale therapie: jodium-125 (^{125}I) brachytherapie (32 studies, 1283 patiënten), fosfor-32 (^{32}P) microbrachytherapie (5 studies, 133 patiënten), palladium-103 (^{103}Pd) brachytherapie (2 studies, 26 patiënten), immunotherapie (9 studies, 330 patiënten) en chemotherapie (4 studies, 71 patiënten). Hoewel de overlevingsresultaten sterk varieerden, ook binnen dezelfde interventie, waren de veiligheidsprofielen over het algemeen acceptabel. De beschikbare data waren te beperkt om definitieve conclusies te trekken over de algehele overleving. Toch benadrukte de review mogelijke klinische voordelen, waaronder verbeterde lokale tumorcontrole, pijnverlichting en een verbeterde kwaliteit van leven. Daarnaast bood het overzicht waardevolle inzichten in injectie- en toedieningsmethoden, beperkingen en mogelijke complicaties.

In **Hoofdstuk 3** is beschreven hoe deze inzichten de basis vormden voor de ontwikkeling van een toedieningsmethode voor ^{166}HoMS in tien chirurgisch verwijderde ex vivo PDAC-weefsels. In een gecontroleerde laboratoriumomgeving werd eerst een manueel rotatiesysteem ontwikkeld om bezinking van ^{166}HoMS in een suspensie te verminderen en een consistente injectieconcentratie te realiseren. Tijdens de initiële haalbaarheidstests werd, met behulp van verschillende injectieparameters, de injectiecontrole vastgesteld. De hoge intratumorale druk is kenmerkend voor PDAC en kan resulteren in naaldobstructie of lekkage. Essentiële injectieparameters waren onder andere injectievolume, het aantal naaldpenetraties en het gebruik van beeldvorming voor naaldgeleiding. Daarnaast werd gewerkt aan het verbeteren van de zichtbaarheid van ^{166}HoMS op computertomografie (CT) door holmium specifieke CT-instellingen te optimaliseren en de concentratie van ^{166}HoMS in de injectie-

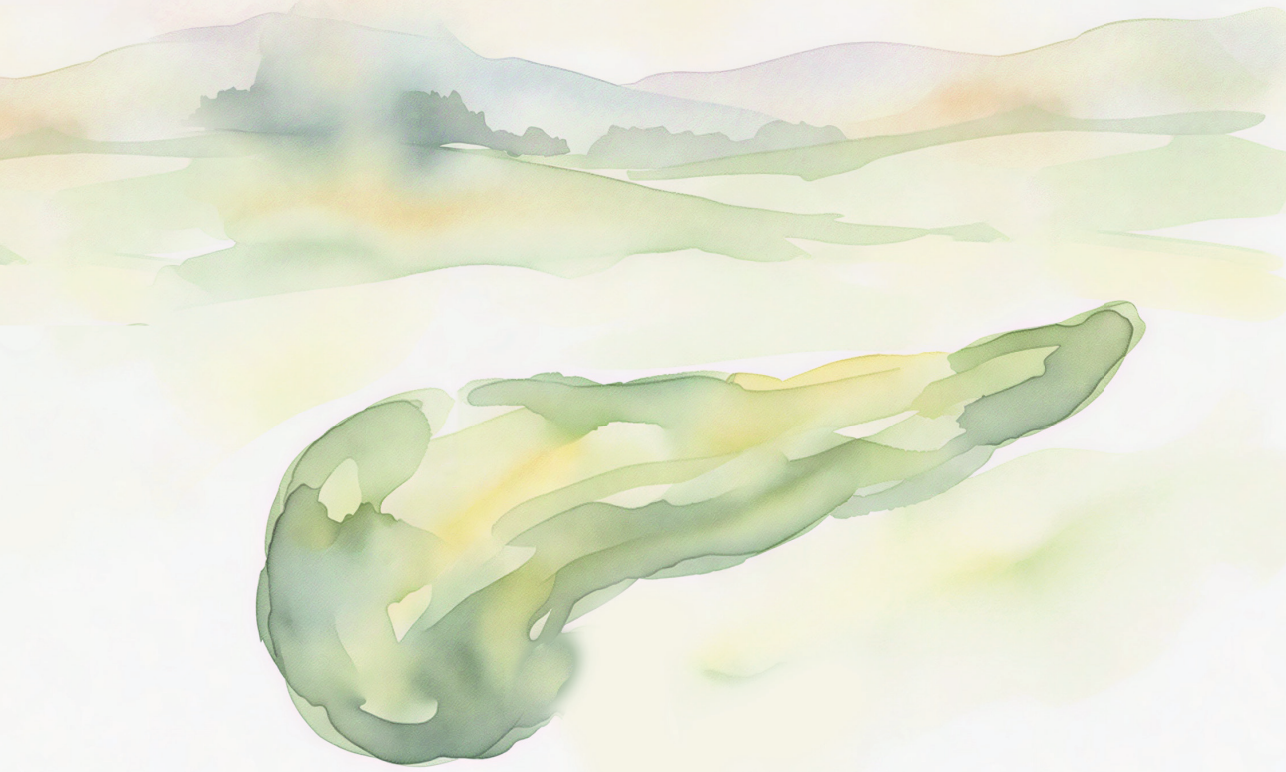
suspensie te verhogen. Het injectiesysteem en de methodologieën die in dit hoofdstuk zijn ontwikkeld vormen in de basis voor toekomstig onderzoek naar intratumorale injectie van ^{166}HoMS bij PDAC en mogelijk andere solide tumoren.

In **Hoofdstuk 4** is beschreven hoe het ontwikkelde injectiesysteem voor het eerst toegepast werd in patiënten met PDAC. Dertien patiënten ondergingen patiënt specifieke planning voor echogeleide intratumorale ^{166}HoMS injectie tijdens een openbuikoperatie. De interventie werd alleen uitgevoerd wanneer werd afgezien van chirurgische resectie vanwege lokale tumorprogressie of afstandsmetastasen. Deze opzet werd gebruikt om minimaal te interfereren met de standaardzorg, aangezien het veiligheidsprofiel van de interventie nog niet was vastgesteld. Drie van de dertien patiënten ondergingen de interventie. Tijdens de 12 weken vervolgperiode werden er geen bijwerkingen geobserveerd die verband hielden met de interventie, ondanks minimale extratumorale irradiatie van de longen en het colon (<1 Gy). De behaalde gemiddelde tumordosis lag tussen de 5 en 46 Gy, wat lager was dan verwacht vanwege de eerdergenoemde extratumorale irradiatie en vanwege een verminderd injectievolume door beperkt zicht van de tumor tijdens de openbuikoperatie. Twee patiënten hadden een stabiele ziekte na 12 weken en alle patiënten ontwikkelden afstandsmetastasen. Hoewel de haalbaarheid beperkt was, werd in dit hoofdstuk een acceptabel veiligheidsprofiel vastgesteld, wat leidde tot een vervolgstudie.

In **Hoofdstuk 5** is de haalbaarheid en veiligheid van een minimaal invasieve toepassing, met zowel therapie- als naaldgeleiding middels periprocedurele CT beschreven en wordt alle opgedane kennis over intratumorale ^{166}HoMS injectie in PDAC gecombineerd. Zes patiënten met niet te verwijderen PDAC na inductie FOLFIRINOX-chemotherapie ondergingen patiënt specifieke interventie. Technische haalbaarheid werd bij alle patiënten bereikt zonder ernstige complicaties. De ^{166}HoMS posities waren duidelijk zichtbaar op CT, wat direct bijdroeg aan de interventieplanning. De gemiddelde tumordosis was echter lager dan gepland, tussen de 14 en 38 Gy, vanwege ^{166}HoMS buiten de tumor in alle patiënten, overigens zonder dat dit ernstige bijwerkingen gaf. Dit werd voornamelijk veroorzaakt door ^{166}HoMS lekkage door het pancreaskanaal naar de darmen, zichtbaar op periprocedurele CT en gekwantificeerd in het colon op SPECT, 24 uur na de interventie. Ondanks deze bevindingen gaf de interventie een gunstig veiligheidsprofiel met slechts één graad 3 bijwerking gerelateerd aan de CT-contrastvloeistof. Alle overige bijwerkingen waren ofwel niet gerelateerd aan de interventie (92,3%), ofwel waren mild (graad 1). Na 16 weken had twee derde van de patiënten een lokaal stabiele ziekte. Dit hoofdstuk bevestigt dat percutane, CT-geleide injectie met ^{166}HoMS een haalbare en veilige behandeloptie is voor patiënten met een niet-resectabel PDAC, met goede periprocedurele zichtbaarheid en veelbelovende initiële klinische uitkomsten.

In **Hoofdstuk 6** wordt de overkoepelende discussie beschreven. Kernresultaten worden samengevat en toegelicht in een breder klinisch perspectief. Het snel ontwikkelende onderzoeksveld rondom lokale therapieën en lopende klinische studies worden vergeleken met de resultaten van dit proefschrift. Tot slot worden toekomstperspectieven voor deze nieuwe behandeling besproken, evenals mogelijke vervolgstudies om de volledige therapeutische potentie van deze interventie in kaart te brengen.

Appendices



About the author

Ysbrand Willink was born in Geldrop on June 28, 1995, and grew up in Winterswijk with his parents, two older sisters, and one older brother. He completed his high school education in 2014 at the Gerrit Komrij College. With a strong foundation in both technical skills and social competencies, he pursued a study path that combined both fields.

In September 2014, he began his bachelor's and subsequent master's in Technical Medicine, a joint degree program involving the Technical University Delft, Leiden University Medical Center, and Erasmus MC in Rotterdam.

Throughout his studies, Ysbrand was actively involved in extracurricular activities, including serving as the financial manager of the study association S.V.K.T. Variscopic during the first year of his Master's in 2017. Shortly after, he took on a student job as a quality controller at a radioactive laboratory in Rotterdam, where he met dr. Frank Nijsen. Under the guidance of dr. Nijsen he completed his master's graduation internship at the Department of Medical Imaging at Radboud University Medical Center, laying the foundation for this thesis. Ysbrand successfully graduated as a Technical Physician in January 2021.

Following his graduation, he immediately continued his research on pancreatic cancer as part of his PhD at the same department. Still supervised by dr. Frank Nijsen, he was now also supported by prof. dr. Jurgen Fütterer, prof. dr. Kees van Laarhoven, and dr. Martijn Stommel. Over the course of four years, he developed an experimental treatment and implemented it in two clinical pilot trials, the results of which are compiled in this thesis.

Beyond his work, Ysbrand enjoys golfing on sunny days, spending time with friends across the Netherlands, and cooking and indulging in delicious food whenever possible.

List of publications

Willink CY, Jenniskens SFM, Stommel MWJ, Westdorp H, Hermans JJ, van Rijk MC, van Laarhoven CJHM, Fütterer JJ, Nijsen JFW. CT-guided Intratumoral Holmium-166 Microsphere Injection in Patients with Locally Unresectable Pancreatic Cancer after FOLFIRINOX Induction Chemotherapy: a Single-Center, Single-Arm, Open-Label, Feasibility, and Safety Study. *Submitted, 2025*.

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PhD Portfolio of Ysbrand Willink

Department: **Medical Imaging**

PhD period: **01/03/2021– 28/02/2025**

PhD Supervisor(s): **Dr. J.F.W. Nijsen, prof. dr. J.J. Fütterer, prof. dr. C.J.H.M. van Laarhoven**

PhD Co-supervisor(s): **Dr. M.W.J. Stommel**

Training activities	Hours
Courses	
Webinar: Intraoperative ultrasound for tumor resection and ablation (2021)	4.00
MRI safety and scanning certificate course (2021)	8.00
RIHS - Introduction course for PhD candidates (2021)	15.00
BLS-AED training (2021)	3.00
Radboudumc - Scientific integrity (2021)	20.00
Boost your writing skills (2022)	1.00
Radboudumc - Scientific integrity (2022)	20.00
Radboudumc - eBROK course (for Radboudumc researchers working with human subjects) (2022)	4.50
CT safety and scanning certificate (2022)	2.00
Nascholing stralingshygiëne kliniek en lab (2024)	1.00
Seminars	
Alvleesklierkanker lotgenotendag (2021)	2.00
Webinar Dutch and non-dutch highlights E-AHBPA 2021 (2022)	2.00
RIHS PhD retreat (2022)	16.00
DPCG meetings (2023)	10.00
NVIR wetenschapsavond^ (2023)	5.00
DPCG meetings (2023)	10.00
Radiation safety course (2023)	1.00
Pancreasdag (2023)	8.00
Active Bystander Course (2023)	1.50
How to prepare your defense (2024)	3.00
Presentation DPCG^ (2024)	3.00
Pancreasdag (2024)	8.00
Conferences	
NVIR sciene-night^ (2022)	5.00
IKNL Symposium pancreas (2022)	4.00
ECIO 2023 – Stockholm^ (2023)	40.00
TiiM (2023)	8.00
Cancer Research Retreat^ (2023)	12.00
EANM^ (2023)	28.00
Cancer Research Retreat^ (2024)	12.00
TiiM (2024)	8.00
SIO^ (Las Vegas) (2025)	24.00
Other	
Radboudumc - Introduction day (2021)	6.00
DPCG meetings (2021)	10.00
PREOPANC-4 webinars (2021)	6.00
Visit Reactor Institute Delft (2023)	4.00

Teaching activities		Hours
Lecturing		
	BMS40 (2021)	16.00
	Biomedical Sciences keuzevak (S. van Lith) (2022)	3.00
	Honour students Holmium-experience (2022)	12.00
	BMS40 (2022)	16.00
	BMS69 (2023)	16.00
	Honour students practice (2023)	4.00
	TG in the NG (2023)	4.00
	Summer school - holmium microsphere brachytherapy (2023)	2.00
	Radiation safety course (2023)	8.00
	BMS69 (2024)	2.00
	Oral presentation Summer School - local applications of dosimetry (2024)	3.00
	Lecture and practical BMS40 (Nanomedicine) (2024)	16.00
Supervision of internships / other		
	Daily mentor of 10 week internship biomedical engineering (2021)	36.00
	Daily mentor 6 months internship (2022)	26.00
	Internship guidance secondary (Na Yu) (2023)	8.00
	Internship guidance secondary (Nikki van Loenen) (2025)	8.00
Total		**495.0**

Research Data Management

Ethics and privacy

Chapters 3, 4 and 5 are based on the results involving human participants. These studies were designed and conducted in accordance with the Declaration of Helsinki, Radboudumc policy and reviewed and approved by the Medical Research Ethics Committee Oost-Nederland. A statement that Chapter 3 was not subject to the Dutch Medical Research Involving Human Subjects Act (WMO), was obtained (CMO 2019-5578). Chapters 4 and 5 were subject to the Dutch Medical Research Involving Human Subjects Acts (WMO) and were approved; NL.76311.091.20 and NL.82292.091.22 for Chapters 4 and 5, respectively.

For Chapter 3, 4 and 5: according to Dutch legislation, data collection from electronic patient files was performed by researcher(s) upon consent by the study participant. The privacy of the participants in these studies was warranted by the use of pseudonymization. The pseudonymization key was stored on a secured network drive that was only accessible to members of the project who needed access to it because of their role within the project. The pseudonymization key is stored and archived separately from the research data.

For Chapter 3, informed consent was obtained from participants to collect and process their (pseudonymized) data. For Chapters 4 and 5, Informed consent was obtained from participants to collect, process, and share their (pseudonymized) data for this research project.

Data collection and storage

Data for Chapter 2 was obtained by systematic literary review of published and peer reviewed publications. Data for Chapters 3, 4 and 5 was obtained from the Electronic Health Records (Epic), laboratory measurements (Excel-files) and from medical imaging (CT, MRI, SPECT; DICOM). Data for Chapters 4 and 5 was collected in electronic Case Report Forms (eCRF) in Caster EDC.

Pseudonymized data from Chapter 3, 4 and 5 were stored and analyzed on the Department of Medical Imaging servers and in Castor EDC (Chapters 4 and 5) and are only accessible by project members working at the Radboudumc. Paper (hardcopy) data is stored and archived in cabinets on the Department of Medical Imaging. The hardcopy pseudonymization key, including informed consents, are sealed, and archived separately on the Department of Medical Imaging.

Data sharing according to the FAIR principles

For Chapter 2, all (meta)data and information for reproducibility is findable and assessable via DOI: https://doi.org/10.1093/bjsopen/zrad052), as by PRISMA-guidelines. In Chapter 3 no consent was

given for sharing pseudonymized data for non-study related purposes and is therefore archived in a data Acquisition collection (DAC) and will not be made accessible (DOI: ru.rumc.slothexv_r0006143a_dac_489). Because of the limited sample size ($n = 3$) in Chapter 4, data cannot be adequately pseudonymized without compromising essential information and is therefore archived in a DAC and will not be made accessible (DOI: ru.rumc.sloth1_r0006143a_dac_001). After publication of the original article, pseudonymized (meta)data from Chapter 5 will be published in a data sharing collections (DSC's) in the Radboud Data Repository (RDR) for long-term archiving and publishing under restricted access and will be made available on reasonable request by the corresponding author (C.Y. Willink), PI (J.F.W. Nijsen) or data steward of the Department of Medical Imaging (V. Souverein) (DOI: ru.rumc.sloth2_r0006143a_dsc_892). All data will be archived for 15 years after completion.

Dankwoord

Tot slot het hoofdstuk dat dit proefschrift en daarmee ook een hoofdstuk van mijn leven, afsluit. Het dankwoord is het meest gelezen deel van elk proefschrift en het deel dat ik het moeilijkst vind om te schrijven. Dit komt omdat ik zoveel mensen wil bedanken die op talloze manieren hebben bijgedragen aan dit proefschrift. Ik vind het belangrijk te benadrukken dat een groot deel van deze bijdragen belangeloos werd geleverd, zonder er iets voor terug te verwachten. Dat is een essentiële menselijke eigenschap in de rumoerige tijd waarin we leven, een eigenschap waarvoor ik niet genoeg waardering kan uitspreken.

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Allereerst wil ik de **patiënten** bedanken die hebben deelgenomen aan een onderzoek in dit proefschrift, evenals hun vrienden en familie. Het is nauwelijks te bevatten welke impact deze ziekte heeft op patiënten en hun naasten, en toch ondergingen zij de onderzoeken met een indrukwekkende inzet en een positieve houding. Voor mij was dit een bron van inspiratie en motivatie. Het vertrouwen dat u in het team en in mij heeft gesteld, gaf mij een gevoel van waardering dat niet in woorden te beschrijven is.

Voor uw inspiratie:
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Voor uw inzicht:
Prof. dr. **Jurgen** Fütterer, beste Jurgen, zoals de TG'er de brug is tussen de kliniek en de techniek, ben jij dat voor mij tussen de interventieradiologie en de nucleaire geneeskunde. Een prachtige samenwerking met waanzinnige uitdagingen die jij graag met beide handen aangrijpt. Jouw inzicht

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Geachte leden van de manuscriptcommissie prof. dr. **Carla** van Herpen, prof. dr. **Marcel** Verheij en prof. dr. **Marc** Besselink: hartelijk dank voor de tijd en aandacht die u hebt gestoken in het lezen en beoordelen van dit manuscript.

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