MULTIMODALITY TREATMENT IN PANCREATIC AND PERIAMPULLARY CANCER

Marjolein J.M. Morak

Financial support for the printing of this thesis was obtained from:
Covidien, Chipsoft, Krijnen Medical, W. L. Gore & Associates, Ipsen Farmaceutica, Vascutek, Cook, ABN AMRO, Care10, Erasmus Universiteit Rotterdam, Afdeling Heelkunde Erasmus Universiteit Rotterdam

ISBN: 978-94-6108-952-6

Layout: Nicole Nijhuis - Gildeprint
Printed by: Gildeprint, Enschede, The Netherlands

MULTIMODALITY TREATMENT IN PANCREATIC AND PERIAMPULLARY CANCER

MULTIMODALE BEHANDELING VAN PANCREAS EN PERIAMPULLAIR CARCINOOM

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus

Prof.dr. H.A.P. Pols
en volgens besluit van het College voor Promoties

De openbare verdediging zal plaatsvinden op
Woensdag 22 april 2015 om 15.30 uur
door

Marjolein Johanna Maria Morak
geboren te Rotterdam

Erasmus University Rotterdam

PROMOTIECOMMISSIE

Promotor:	Prof. dr. C.H.J. van Eijck
Overige leden:	Prof. dr. L.J. Hofland
	Prof. dr. H.W. Tilanus
	Prof. dr. M.J. Bruno
	Prof. dr. S. Sleijfer
	Prof. dr. O.R.C. Busch
	Prof. dr. G. Kazemier
	Dr. K. Biermann

CONTENTS

CHAPTER 1

General Introduction

Pancreatic cancer is the eight most common form of cancer in Europe with 96.000 new cases yearly. This incidence closely matches the mortality rate, thus revealing the aggressive behaviour of this tumour. Five-year survival after diagnosis is only 5% with a median overall survival of 2-8 months. For the patients undergoing surgery, the 5 year survival rate increases to 5% to 25%[1-4] with a median survival of 12- 15 months.[3, 5] At presentation only 15-20% of the patients have a resectable, and thus possibly curative disease, while the majority of the patients already has locally advanced (i.e. unresectable) or even metastasized pancreatic cancer. The retroperitoneal location of the pancreas plays an important role in the absence of specific complaints. Obstructive jaundice, caused by obstruction of the distal common bile duct by tumours located in the pancreatic head, is a late symptom. This lack of evident clinical manifestations frequently results in a delayed diagnosis and is one of the reasons why pancreatic cancer is usually detected in late stages where curation is no longer an option.

In the pancreatic head a variety of tumours can be found, all with its own biological behaviour. Pancreatic ductal adenocarcinoma originating in the pancreatic ducts is the most common and most aggressive tumour resulting in the shortest survival.

Periampullary tumours are also often encountered in the pancreatic head. Several definitions of periampullary cancer co-exist.[6] Most authors include cancers originating from distal common bile duct, ampulla of Vater or duodenum nearby the ampulla. Some also include cancers located within 2 cm of the ampulla of Vater. This definition may even include "periampullary" cancers of pancreatic ductal origin.

Furthermore cystic lesions such as mucinous cystic neoplasms and intraductal papillary mucinous neoplasms (IPMN) occur in the pancreas. Even if malignant degeneration is present in these lesions, survival is beneficial compared to ductal adenocarcinomas (5 year survival 24-74%).[7] Less frequently pancreatic neuroendocrine tumours (PNETs) or metastasis especially from renal cell carcinomas could be localised in the pancreatic head. In this thesis we have studied several clinical aspects of only patients with pancreatic ductal adenocarcinomas and periampullary tumours and performed experimental in vivo studies using human pancreatic cancer cells.

Resectable pancreatic cancer

Pancreatic and periampullary cancer is considered resectable if there are no metastases, no pathological lymph nodes outside the resection area and no encasement of the tumour ito the superior mesenteric artery, celiac trunk or hepatic artery. Less than 270° encasement of the portal vein and/ or superior mesenteric vein is considered resectable although for these tumours several surgeons also use the term "borderline resectable". In fact, there is no general consensus regarding resectability of pancreatic tumours with venous involvement. This often depends on

the exact location of the tumour. Tumours located in the uncinate process with ingrowth in the distal superior mesenteric vein are in general more difficult to resect than cranial tumours with involvement of the portal vein.
In case of malignancies in the pancreatic tail, ingrowth into the splenic artery does not result in unresectability, because a splenectomy can safely be performed.

Survival
Due to locoregional recurrence and/ or the development of distant metastases median survival, even after radical resection, is 12- 15 months[3, 5] with a 5-year survival rate ranging from 5% to 25%.[1-4] The majority of the tumours described in these studies are pancreatic ductal adenocarcinomas located in the head of the pancreas. Patients with periampullary carcinoma have a more favourable outcome, with a median survival after resection of 25- 42 months[8, 9] and a 5-year survival rate of 40% to 70%.[10-17] In the Netherlands median survival after radical resection is about 15 months for pancreatic cancer and 45 months for periampullary tumours, which is similar to studies from other countries[6, 8, 18, 19].
Cause for the survival difference between pancreatic head and periampullary tumours is unclear. The classical hypothesis is that these tumours are diagnosed in an earlier stage because of their anatomical location, leading to early jaundice and clinical manifestation. An additional cause for early detection is that they may be discovered at routine endoscopy for example, in patients with familiar adenomatous polyposis. For these reasons periampullary tumours may more frequently be amenable to surgical resection than their pancreatic counterparts. Another hypothesis is that periampullary tumours represent a separate family of tumours with distinct biological behaviour. Although histological they appear very similar, periampullary cancers seem to have a different biological basis.[20, 21]
Factors negatively influencing survival are a positive resection margin, lymph node metastases, a larger tumour size, poor differentiation grade, vaso-invasive and perineural growth. Although pathological review reports N0 resection according to standard pathological review, micrometastases could be found in 53-73% of patients when lymph nodes are examined with advanced techniques such as immunohistochemistry or PCR.[22, 23] Furthermore in 37-73% of the R0 resections, nerve plexus invasion outside the pancreas can be found.[24]

Adjuvant chemo(radio)therapy
One of the causes of a poor survival after resection is the development of local progression or distant metastases with recurrence rates as high as 90%. Recurrence occurs locoregional in 50%-80%, peritoneal in 25% and in the liver in 50% of patients.[25] This implies that at the

time of diagnosis, pancreatic cancer is already a systemic disease and treatment should not be confined to surgical resection. To obtain both local and distant control, the first trials on adjuvant therapy included both radiotherapy and chemotherapy. The Gastrointestinal Tumour Study Group (GITSG) randomised 43 patients between surgery alone or an adjuvant schedule including both chemoradiotherapy and chemotherapy. Adjuvant treatment resulted in better overall survival (20 vs. 11 months), but the effect could be caused by either chemotherapy or chemoradiotherapy. The European Organisation for Research and Treatment of Cancer (EORTC) randomised patients between surgery alone or adjuvant chemoradiotherapy but found no benefit of either. The European Study group for Pancreatic Cancer (ESPAC) randomised between surgery alone, surgery combined with chemotherapy and chemoradiotherapy in a 2x2 factorial design. Although chemotherapy resulted in survival benefit, chemoradiotherapy did not. However, in all these studies a split course (2 weeks 20 Gray, 2 weeks rest followed by 2 weeks 20 Gray) was used, which nowadays is obsolete. Thus, although no significant benefit has yet been established, the possible effect of chemoradiotherapy has not been investigated to its full extent.
Adjuvant chemotherapy on the other hand results in a longer disease free survival as well as in a prolonged survival. The ESPAC group treated patients with 5-FU combined with folinic acid during 28 weeks resulting in a survival of 20.1 months (15.5 months in patients not receiving chemotherapy). The CONKO-001 treated patients with gemcitabine during 6 months resulting in 13.4 months median survival (6.9 after surgery alone) and 23.5% disease free survival after 3 years (8.5% after surgery alone). A recent trial performed by the ESPAC randomising between 5-FU combined with leucovorin vs. gemcitabine did not result in a prolonged disease free or overall survival in either group (23 vs. 23.6 months median overall survival).[26-28]

Unresectable pancreatic cancer

In the work-up of patients with suspected pancreatic usually a computed tomography (CT) scan is performed. Although reliable for pancreatic masses, enlarged lymph nodes and vascular ingrowth, especially small (<1cm) peritoneal and liver lesions can be missed. [29] The extend of vascular tumour growth determines the resectability of the pancreatic lesion. Unresectable pancreatic cancer should be discriminated from metastasized pancreatic cancer by ruling out metastases since only in these patient locoregional radiotherapy either combined with chemotherapy might be a therapeutic option.

Locally advanced unresectable cancer is defined in the Netherlands according to Table 1:

	SMA	Celiac axis	CHA	SMV-PV
Resectable (all four required)	no contact	no contact	no contact	≤ 90˚ contact
Borderline resectable (minimally one required)	≤ 90˚ contact	≤ 90˚ contact	≤ 90˚ contact	90˚-270˚ contact, and no occlusion
Irresectable (minimally one required)	contact > 90˚	contact > 90˚	contact > 90˚	contact > 270˚ or occlusion

SMA: superior mesenteric artery
CHA: common hepatic artery
SMV-PV: superior mesenteric vein-portal vein

Chemo(radio)therapy

Since the publication of the Gastro Intestinal Tumour Study Group (GITSG) in 1981[30], patients with locally advanced pancreatic cancer are treated with radiotherapy in combination with 5 fluorouracil (5-FU). The median survival after 5-fluorouracil (5-FU) based chemoradiotherapy treatment in literature varies between 7- 14 months.[31-39] In a number of studies 5-FU has been replaced by gemcitabine in the treatment of unresectable pancreatic cancer, either alone, in combination with radiotherapy or in combination with other drugs. After treatment with gemcitabine either with or without concurrent radiotherapy, median survival varies between 10-17 months.[32, 35, 37, 39-43]

One of the causes for these discouraging results might be the pre-treatment staging because small metastatic liver or peritoneal lesions remain undetected by CT scanning.

To prevent local progression, radiotherapy might play a more important role in unresectable than in resectable pancreatic cancer. Besides 5-FU and gemcitabine radiosensitizing properties of interferon alpha (IFNα) and interferon beta (IFNβ) have been demonstrated in several tumours in vitro[44-49] as well as in vivo.[50, 51] In pancreatic cancer cell lines, IFNα has already shown to act as radiosensitizer[52] and in vivo promising therapy results have been reported combining 5-FU, cisplatinum, and radiation therapy with IFNα alone (5-year survival rate of 55%) or followed by 2 cycles of gemcitabine (median survival 25 months) in patients with resected pancreatic adenocarcinoma.[53, 54]

In vivo studies showed that approximately 20% of pancreatic cancers express interferon receptors and the expression of the interferon receptor was found to correlate with a significant survival benefit in patients with resected pancreatic cancer.[55]

OUTLINE OF THE THESIS

Intra-arterial chemotherapy is a promising way of administering drugs. Celiac artery infusion delivers the drugs not only directly to the liver through the common hepatic artery, but also indirectly through the splenic and left gastric artery and subsequent portal vein. One of the benefits of intra-arterial admission of chemotherapy is obtaining a higher local dose with tolerable toxicity. Two clinical phase II studies with celiac artery infusion (CAI) suggested that regional chemotherapy might extend survival in locally advanced non-resectable as well as in resected pancreatic cancers. Intra-arterial chemotherapy reduced hepatic progression from 62% to 17% and hepatic metastases related death from 33% to 8%. No reduction in local recurrence rates was found in these studies.[26-28] Therefore we hypothesized that CAI combined with locoregional radiotherapy might prolong survival of patients after curative resection of either pancreatic or periampullary cancer. In **Chapter 2** we discussed the results of a randomised controlled trial we performed in order to investigate our hypothesis. As a consequence of the intra-arterial chemotherapy, patients in the treatment arm had an indwelling catheter and were obliged to stay in bed throughout all six cycles which might affect quality of life. Patients in the surgery alone arm on the other hand, were regularly followed up on outpatient basis, but did not suffer from regular admissions or toxicity. We addressed the effect of our regime on quality of life in all these patients in **Chapter 3**. In **Chapter 4** we further investigated the long term survival of patients treated with intra-arterial chemotherapy and radiotherapy or surgery alone, especially considering tumour origin.

In **Part II** we focus on patients with locally advanced/ unresectable pancreatic cancer. Diagnosis in these patients can be difficult and multiple modalities are available for obtaining optimal staging. Besides criteria for unresectability based on computed tomography (CT) scan, the role of a diagnostic laparoscopy in these patients is addressed in **Chapter 5**. Patients with unresectable pancreatic cancer are known to be at risk for small peritoneal and liver metastases, undetectable for CT scan and during a diagnostic laparoscopy these lesions can be detected. The quality of the CT scan and the presence of a dedicated radiologist in different centres might also influence the detection rate. In **Chapter 6** a multicenter trial with the Academic Medical Center, Amsterdam is described. Patients with unresectable, non-metastasised pancreatic cancer were treated with Uracil/Tegafur (UFT) plus leucovorin and celecoxib combined with radiotherapy in a short, intensive schedule. The combination of drugs was in part based on promising research and the possibility of oral intake (UFT plus leucovorin) and in part on radiosensitising properties of selective inhibition of cyclooxygenase-2 by celecoxib and the presence of the COX-2 receptor in pancreatic cancer.

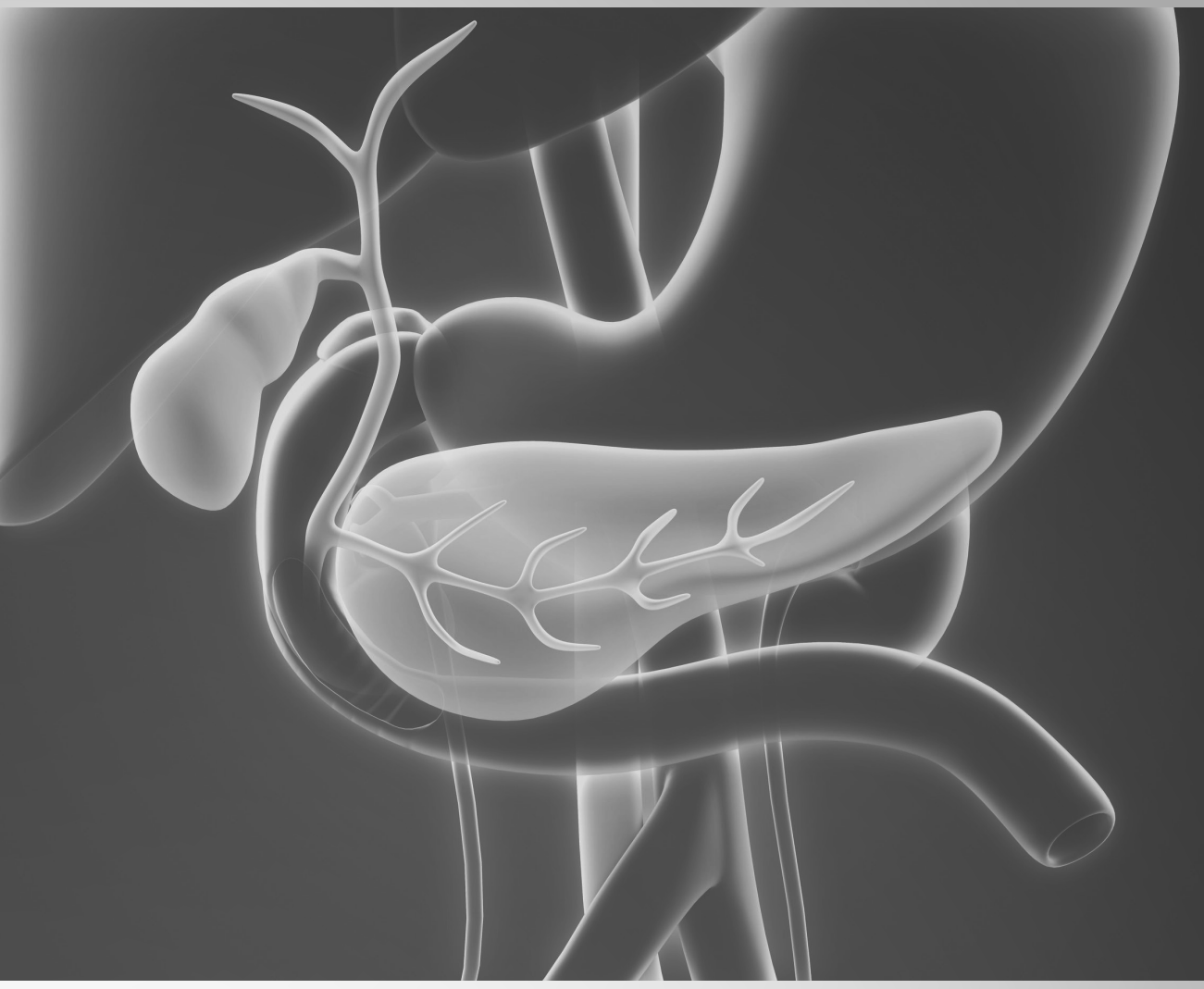
In **Part III** we took research back into the laboratory. Based on the results of the Virginia Mason Clinic, the radiosensitising activity of both interferon (IFN) α and β was further investigated. We already described the presence of the IFN receptor on both membrane as well as intracellular in different cell lines. The aim of the study presented in **Chapter 7** was to assess the radiosensitising properties of both types of interferon in a colony forming assay. This method was chosen because both the apoptotic effects of radiation and IFN as well as the reproductive integrity of tumour cells after treatment is of pivotal importance.
The general discussion (**Chapter 8**) and summary and conclusions (**Chapter 9**) are presented in **Part IV** of this thesis.

REFERENCES

1. Bassi C, Stocken DD, Olah A, et al. Influence of surgical resection and post-operative complications on survival following adjuvant treatment for pancreatic cancer in the ESPAC-1 randomized controlled trial. Dig Surg 2005; 22(5):353-63.
2. Kennedy EP, Yeo CJ. The case for routine use of adjuvant therapy in pancreatic cancer. J Surg Oncol 2007; 95(7):597-603.
3. Neoptolemos JP, Dunn JA, Stocken DD, et al. Adjuvant chemoradiotherapy and chemotherapy in resectable pancreatic cancer: a randomised controlled trial. Lancet 2001; 358(9293):1576-85.
4. Oettle H, Post S, Neuhaus P, et al. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. Jama 2007; 297(3):267-77.
5. Willett CG, Czito BG, Bendell JC. Adjuvant therapy of pancreatic cancer. Cancer J 2007; 13(3):185-91.
6. Sarmiento JM, Nagomey DM, Sarr MG, Farnell MB. Periampullary cancers: are there differences? Surg Clin North Am 2001; 81(3):543-55.
7. Sugiyama M, Suzuki Y, Abe N, et al. Management of intraductal papillary mucinous neoplasm of the pancreas. J Gastroenterol 2008; 43(3):181-5.
8. Smeenk HG, van Eijck CH, Hop WC, et al. Long-term Survival and Metastatic Pattern of Pancreatic and Periampullary Cancer After Adjuvant Chemoradiation or Observation: Long-term Results of EORTC Trial 40891. Ann Surg 2007; 246(5):734-40.
9. Tran KT, Smeenk HG, van Eijck CH, et al. Pylorus preserving pancreaticoduodenectomy versus standard Whipple procedure: a prospective, randomized, multicenter analysis of 170 patients with pancreatic and periampullary tumors. Ann Surg 2004; 240(5):738-45.
10. Baumel H, Huguier M, Manderscheid JC, et al. Results of resection for cancer of the exocrine pancreas: a study from the French Association of Surgery. Br J Surg 1994; 81(1):102-7.
11. Klinkenbijl JH, Jeekel J, Schmitz PI, et al. Carcinoma of the pancreas and periampullary region: palliation versus cure. Br J Surg 1993; 80(12):1575-8.
12. Lillemoe KD. Current management of pancreatic carcinoma. Ann Surg 1995; 221(2):133-48.
13. Nitecki SS, Sarr MG, Colby TV, van Heerden JA. Long-term survival after resection for ductal adenocarcinoma of the pancreas. Is it really improving? Ann Surg 1995; 221(1):59-66.
14. Trede M. The surgical treatment of pancreatic carcinoma. Surgery 1985; 97(1):28-35.
15. Trede M, Schwall G, Saeger HD. Survival after pancreatoduodenectomy. 118 consecutive resections without an operative mortality. Ann Surg 1990; 211(4):447-58.
16. Wade TP, Coplin MA, Virgo KS, Johnson FE. Periampullary cancer treatment in U.S. Department of Veterans Affairs hospitals: 1987-1991. Surgery 1994; 116(4):819-25; discussion 825-6.
17. Yeo CJ, Cameron JL, Lillemoe KD, et al. Pancreaticoduodenectomy for cancer of the head of the pancreas. 201 patients. Ann Surg 1995; 221(6):721-31; discussion 731-3.
18. Winter JM, Cameron JL, Olino K, et al. Clinicopathologic analysis of ampullary neoplasms in 450 patients: implications for surgical strategy and long-term prognosis. J Gastrointest Surg; 14(2):379-87.
19. Yeo CJ, Cameron JL, Sohn TA, et al. Six hundred fifty consecutive pancreaticoduodenectomies in the 1990s: pathology, complications, and outcomes. Ann Surg 1997; 226(3):248-57; discussion 257-60.
20. Hatzaras I, George N, Muscarella P, et al. Predictors of survival in periampullary cancers following pancreaticoduodenectomy. Ann Surg Oncol; 17(4):991-7.
21. Westgaard A, Tafjord S, Farstad IN, et al. Pancreatobiliary versus intestinal histologic type of differentiation is an independent prognostic factor in resected periampullary adenocarcinoma. BMC Cancer 2008; 8:170.

22. Kurahara H, Takao S, Maemura K, et al. Impact of lymph node micrometastasis in patients with pancreatic head cancer. World J Surg 2007; 31(3):483-90; discussion 491-2.
23. Niedergethmann M, Rexin M, Hildenbrand R, et al. Prognostic implications of routine, immunohistochemical, and molecular staging in resectable pancreatic adenocarcinoma. Am J Surg Pathol 2002; 26(12):1578-87.
24. Chatterjee D, Katz MH, Rashid A, et al. Perineural and intraneural invasion in posttherapy pancreaticoduodenectomy specimens predicts poor prognosis in patients with pancreatic ductal adenocarcinoma. Am J Surg Pathol; 36(3):409-17.
25. Gudjonsson B. Survival statistics gone awry: pancreatic cancer, a case in point. J Clin Gastroenterol 2002; 35(2):180-4.
26. Beger HG, Gansauge F, Buchler MW, Link KH. Intraarterial adjuvant chemotherapy after pancreaticoduodenectomy for pancreatic cancer: significant reduction in occurrence of liver metastasis. World J Surg 1999; 23(9):946-9.
27. Hayashibe A, Kameyama M, Shinbo M, Makimoto S. Clinical results on intra-arterial adjuvant chemotherapy for prevention of liver metastasis following curative resection of pancreatic cancer. Ann Surg Oncol 2007; 14(1):190-4.
28. Ishikawa O, Ohhigashi H, Sasaki Y, et al. Extended pancreatectomy and liver perfusion chemotherapy for resectable adenocarcinoma of the pancreas. Digestion 1999; 60 Suppl 1:135-8.
29. Liu RC, Traverso LW. Laparoscopic staging should be used routinely for locally extensive cancer of the pancreatic head. J Gastrointest Surg 2004; 8(8):923-4.
30. Moertel CG, Frytak S, Hahn RG, et al. Therapy of locally unresectable pancreatic carcinoma: a randomized comparison of high dose (6000 rads) radiation alone, moderate dose radiation (4000 rads + 5-fluorouracil), and high dose radiation + 5-fluorouracil: The Gastrointestinal Tumor Study Group. Cancer 1981; 48(8):1705-10.
31. Ceha HM, van Tienhoven G, Gouma DJ, et al. Feasibility and efficacy of high dose conformal radiotherapy for patients with locally advanced pancreatic carcinoma. Cancer 2000; 89(11):2222-9.
32. Chauffert B, Mornex F, Bonnetain F, et al. Phase III trial comparing intensive induction chemoradiotherapy (60 Gy, infusional 5-FU and intermittent cisplatin) followed by maintenance gemcitabine with gemcitabine alone for locally advanced unresectable pancreatic cancer. Definitive results of the 2000-01 FFCD/SFRO study. Ann Oncol 2008; 19(9):1592-9.
33. Cohen SJ, Dobelbower R, Jr., Lipsitz S, et al. A randomized phase III study of radiotherapy alone or with 5-fluorouracil and mitomycin-C in patients with locally advanced adenocarcinoma of the pancreas: Eastern Cooperative Oncology Group study E8282. Int J Radiat Oncol Biol Phys 2005; 62(5):1345-50.
34. Isacoff WH, Bendetti JK, Barstis JJ, et al. Phase II trial of infusional fluorouracil, leucovorin, mitomycin, and dipyridamole in locally advanced unresectable pancreatic adenocarcinoma: SWOG S9700. J Clin Oncol 2007; 25(13):1665-9.
35. Li CP, Chao Y, Chi KH, et al. Concurrent chemoradiotherapy treatment of locally advanced pancreatic cancer: gemcitabine versus 5-fluorouracil, a randomized controlled study. Int J Radiat Oncol Biol Phys 2003; 57(1):98-104.
36. Luderhoff EC, Gonzalez Gonzalez D, Bakker P. Pilot study in locally advanced unresectable pancreas carcinoma using a combination of accelerated radiotherapy and continuous infusion of 5-fluorouracil. Radiother Oncol 1996; 40(3):241-3.
37. Park JK, Ryu JK, Lee JK, et al. Gemcitabine chemotherapy versus 5-fluorouracil-based concurrent chemoradiotherapy in locally advanced unresectable pancreatic cancer. Pancreas 2006; 33(4):397-402.
38. Smeenk HG, de Castro SM, Jeekel JJ, et al. Locally advanced pancreatic cancer treated with radiation and 5-fluorouracil: a first step to neoadjuvant treatment? Dig Surg 2005; 22(3):191-7.
39. Tada M, Arizumi M, Nakai Y, et al. Efficacy of gemcitabine for locally advanced pancreatic cancer: comparison with 5-fluorouracil-based chemoradiotherapy. Chemotherapy 2008; 54(4):302-8.

40. Goldstein D, Van Hazel G, Walpole E, et al. Gemcitabine with a specific conformal 3D 5FU radiochemotherapy technique is safe and effective in the definitive management of locally advanced pancreatic cancer. Br J Cancer 2007; 97(4):464-71.
41. Haddock MG, Swaminathan R, Foster NR, et al. Gemcitabine, cisplatin, and radiotherapy for patients with locally advanced pancreatic adenocarcinoma: results of the North Central Cancer Treatment Group Phase II Study N9942. J Clin Oncol 2007; 25(18):2567-72.
42. Hong SP, Park JY, Jeon TJ, et al. Weekly full-dose gemcitabine and single-dose cisplatin with concurrent radiotherapy in patients with locally advanced pancreatic cancer. Br J Cancer 2008; 98(5):881-7.
43. Murphy JD, Adusumilli S, Griffith KA, et al. Full-dose gemcitabine and concurrent radiotherapy for unresectable pancreatic cancer. Int J Radiat Oncol Biol Phys 2007; 68(3):801-8.
44. Angioli R, Sevin BU, Perras JP, et al. In vitro potentiation of radiation cytotoxicity by recombinant interferons in cervical cancer cell lines. Cancer 1993; 71(11):3717-25.
45. Chang AY, Keng PC. Potentiation of radiation cytotoxicity by recombinant interferons, a phenomenon associated with increased blockage at the G2-M phase of the cell cycle. Cancer Res 1987; 47(16):4338-41.
46. Dritschilo A, Mossman K, Gray M, Sreevalsan T. Potentiation of radiation injury by interferon. Am J Clin Oncol 1982; 5(1):79-82.
47. Gould MN, Kakria RC, Olson S, Borden EC. Radiosensitization of human bronchogenic carcinoma cells by interferon beta. J Interferon Res 1984; 4(1):123-8.
48. Hoffmann W, Blase MA, Santo-Hoeltje L, et al. Radiation sensitivity of human squamous cell carcinoma cells in vitro is modulated by all-trans and 13-cis-retinoic acid in combination with interferon-alpha. Int J Radiat Oncol Biol Phys 1999; 45(4):991-8.
49. Schmidberger H, Rave-Frank M, Lehmann J, et al. Radiosensitizing effect of natural and recombinant beta-interferons in a human lung carcinoma in vitro. J Cancer Res Clin Oncol 1999; 125(6):350-6.
50. Basu P, Biswas J, Mandal R, Choudhury P. Is interferon-alpha and retinoic acid combination along with radiation superior to chemo-radiation in the treatment of advanced carcinoma of cervix? Indian J Cancer 2006; 43(2):54-9.
51. Holsti LR, Mattson K, Niiranen A, et al. Enhancement of radiation effects by alpha interferon in the treatment of small cell carcinoma of the lung. Int J Radiat Oncol Biol Phys 1987; 13(8):1161-6.
52. Ma JH, Patrut E, Schmidt J, et al. Synergistic effects of interferon-alpha in combination with chemoradiation on human pancreatic adenocarcinoma. World J Gastroenterol 2005; 11(10):1521-8.
53. Linehan DC, Tan MC, Strasberg SM, et al. Adjuvant interferon-based chemoradiation followed by gemcitabine for resected pancreatic adenocarcinoma: a single-institution phase II study. Ann Surg 2008; 248(2):145-51.
54. Picozzi VJ, Kozarek RA, Traverso LW. Interferon-based adjuvant chemoradiation therapy after pancreaticoduodenectomy for pancreatic adenocarcinoma. Am J Surg 2003; 185(5):476-80.
55. Saidi RF, Williams F, Silberberg B, et al. Expression of interferon receptors in pancreatic cancer: identification of a novel prognostic factor. Surgery 2006; 139(6):743-8.



PART I

Resectable pancreatic and periampullary cancer

CHAPTER 2

Adjuvant intra-arterial chemotherapy and radiotherapy versus surgery alone in resectable pancreatic and periampullary cancer

A prospective randomized controlled trial

M.J.M. Morak[1], A. van der Gaast[2], L. Incrocci[3], H. van Dekken[4], J.J. Hermans[5], J. Jeekel[1], W.C.J. Hop[6], G. Kazemier[1] and C.H.J. van Eijck[1]

[1]Department of Surgery, Erasmus MC, Rotterdam, The Netherlands
[2]Department of Oncology, Erasmus MC, Rotterdam, The Netherlands
[3]Department of Radiotherapy, Erasmus MC, Rotterdam, The Netherlands
[4]Department of Pathology, Erasmus MC, Rotterdam, The Netherlands
[5]Department of Statistics and Epidemiology, Erasmus MC, Rotterdam, The Netherlands
[6]Department of Radiology, Erasmus MC, Rotterdam, The Netherlands

Ann Surg. 2008 Dec;248(6):1031-41.

ABSTRACT

Background: Success of surgical treatment for pancreatic and periampullary cancer is often limited due to locoregional recurrence and/or the development of distant metastases.

Objective: The survival benefit of celiac axis infusion (CAI) and radiotherapy (RT) *versus* observation following resection of pancreatic or periampullary cancer was investigated.

Methods: In a randomized controlled trial, 120 consecutive patients with histopathologically proven pancreatic or periampullary cancer received either adjuvant treatment consisting of intra-arterial mitoxantrone, 5-FU, leucovorin and cisplatinum in combination with 30x 1.8 Gray radiotherapy (group A) or no adjuvant treatment (group B). Groups were stratified for tumor type (pancreatic vs. periampullary tumors).

Results: After surgery, 120 patients were randomized (59 patients in the treatment group, 61 in the observation group). The median follow up was 17 months. No significant overall survival benefit was seen (median 19 vs. 18 months resp.). Progressive disease was seen in 86 patients: in 37 patients in the CAI/RT group, and in 49 patients in the observation group (log-rank $p<0.02$). Subgroup analysis showed significantly less liver metastases after adjuvant treatment in periampullary tumors (log-rank $p<0.03$) without effect on local recurrence. Nonetheless, there was no significant effect on overall survival in these patients (log-rank $p=0.15$). In patients with pancreatic cancer, CAI/RT had no significant effect on local recurrence (log-rank $p=0.12$) neither on the development of liver metastases (log-rank $p=0.76$) and consequently, no effect on overall survival.

Conclusion: This adjuvant treatment schedule results in a prolonged time to progression. For periampullary tumors, CAI/RT induced a significant reduction in the in the development of liver metastases, with a possible effect on overall survival. Especially in these tumors, CAI/RT might prove beneficial in larger groups and further research is warranted.

INTRODUCTION

Pancreatic cancer is one of today's most fatal malignant diseases and ranks fourth in cancer related mortality world-wide.[1] Curative surgery is only possible in case of early detection of pancreatic cancer, but most patients present with advanced, and thus incurable disease. Resection by partial or total pancreaticoduodenectomy is possible in only 5% - 25% of patients. The success of surgical treatment is often limited due to locoregional recurrence and/ or the development of distant metastases. Even after radical resection, median survival time is 12 - 15 months[2, 3] with a 5-year survival rate ranging from 5% to 25%.[2, 4-6] Patients with periampullary carcinoma have a more favorable outcome, with a 5-year survival rate of 40% to 70%.[7-14]

To improve this dismal course after resection of pancreatic cancer, adjuvant treatment has been studied in several clinical trials. First, the Gastrointestinal Tumor Study Group (GITSG) randomly assigned 43 patients to surgery alone or chemoradiation followed by maintenance chemotherapy.[15, 16] The median survival was significantly longer in the adjuvant treatment group compared with the surgery group (20 months vs. 11 months), with respectively 18% and 8% survival at 5 years.[15-18] However, this was a small study, which was terminated early because of poor accrual. Furthermore, it is unclear whether the survival advantage in the GITSG trial was due to the combination of chemoradiation and maintenance chemotherapy or caused by only one of these modalities.[19] The first large multicenter trial in pancreatic cancer was a randomized phase III trial conducted by the EORTC Gastro-intestinal group, initiated in 1987 (EORTC 40891). Based on 218 patients, this trial did not show a benefit for adjuvant chemoradiation although they suggested a trend in favor of chemoradiation (p = 0.09) in patients with ductal pancreatic adenocarcinoma. However, this trial included patients with pancreatic as well as more favorable periampullary tumors, 20% of the patients randomized for chemoradiotherapy never started therapy and no maintenance chemotherapy was given which could possibly explain the poor results.[20] Recently Smeenk et al. reported the long-term follow-up results of EORTC trial 40891, which assessed the role of chemoradiation in resectable pancreatic cancer. The results confirmed the previous short-term analysis indicating no benefit, neither on progression-free, nor on overall survival, of adjuvant chemoradiation over observation in patients with resected pancreatic or periampullary cancer.[21] In 1994, the European Study Group for Pancreatic Cancer (ESPAC) undertook a multicenter factorial phase III trial to investigate the possible benefits of adjuvant chemoradiation and maintenance chemotherapy in patients with pancreatic cancer. In this trial, a deleterious effect of adjuvant chemoradiation on survival was shown, whereas chemotherapy significantly improved survival in patients with resected pancreatic cancer.[2, 22, 23] However, concerns about trial design and the background therapy effect remain overt.[24, 25]

Two clinical phase II studies with celiac artery infusion (CAI) suggested that regional chemotherapy might extend survival in locally advanced nonresectable as well as in resected pancreatic cancers. Celiac artery infusion delivers the drugs not only directly to the liver through the common hepatic artery, but also indirectly through the splenic and left gastric artery and subsequent portal vein. One of the benefits of intra-arterial admission of chemotherapy is obtaining a higher local dose with tolerable toxicity. This treatment reduced hepatic progression from 62% to 17% and hepatic metastases related death from 33% to 8%. No reduction in local recurrence rates was found in these studies.[26-28]

To reduce local relapse, full dose radiotherapy together with sensitizing chemotherapy was given by Blackstock with promising results. Patients with resected adenocarcinoma of the pancreas were treated with intravenous gemcitabine administered concurrent with upper abdominal radiation followed by gemcitabine in case of no progression. Only 18% of the patients with progression experienced local regional relapse as a component of the first site of failure. [29]

In the present study we investigated whether we could improve survival of resected patients with periampullary and pancreatic cancers by postoperative celiac artery infusion + radiotherapy as adjuvant treatment compared to surgery alone.

METHODS

Study Design and Patients

After resection for cancer of the pancreas or the periampullary (distal common bile duct or papilla of Vater) region, patients were randomized and stratified for tumor localization (pancreas *versus* periampullary) to receive either adjuvant intra-arterial chemotherapy in combination with radiotherapy (CAI/RT) (group A) or no adjuvant therapy (group B).

Resection was by Whipple's procedure or pylorus- preserving pancreaticoduodenectomy including removal of peripancreatic lymph nodes, lymph nodes in the hepatoduodenal ligament, and around the superior mesenteric vein including the tissue on the right side of the superior mesenteric artery for periampullary or pancreatic head tumors. A distal pancreatectomy plus splenectomy was performed in case of a tumor of the body of the pancreas. No extended lymph node resection was performed in this study.

Pathology was performed by one specialised pathologist and tumors were staged according to the UICC's 2002 guidelines.[30] Patients were eligible if they had histologically proven adenocarcinoma of the pancreas (UICC stages IA-III) or periampullary region (UICC stages IB- III, thus excluding T1N0 tumors). Other eligibility criteria included an adequate functioning hematopoiesis (leucocytes

>3.5x10^9/l, thrombocytes >100x10^9/l), kidney (serum creatinine <2 mg/dl) and liver function (total serum bilirubin <2 mg/dl, partial thromboplastin time> 60%).

Duodenal carcinomas, neuroendocrine tumors and cystadenocarcinomas were excluded. Other exclusion criteria included age > 75 yrs, WHO performance status 3-4 or Karnofsky- Index ≤50%, serious active or uncontrolled infection or previous chemo- or radiotherapy. Patients with a vascular supply to the liver by an aberrant right hepatic artery deriving from the superior mesenteric artery were excluded as well as patients with previous or current malignancies at other sites other than carcinoma in situ of the cervix and adequately treated basal cell carcinoma of the skin, no effective contraception and child- bearing or breast feeding women.

Patients were randomized when definitive pathology reports were available and patients had recovered from surgery. Patients were informed at first visit to the outpatient clinic and an informed consent was signed before the start of treatment. The treatment was planned to start after 6 weeks but within 12 weeks after surgery; thus patients with complications resulting in a prolonged hospital stay were not randomized.

Patients were restaged before the start of adjuvant treatment or during the first CAI course, before radiotherapy. All patients were closely followed up for disease progression with clinical and laboratory examinations every three months during the entire follow up period. Imaging techniques were performed every three months during the first two years after resection and every six months afterwards. An increased Ca 19.9 or CEA level or clinical signs of recurrence were indications for additional imaging. The follow up was continued for 5 years or up to decease of the patient.

Adjuvant Treatment

Postoperative regional chemotherapy was performed by celiac axis infusion (CAI) with a planned total of 6 cycles or up to progressive disease. Treatment was delivered via a catheter placed angiographically through Seldinger's technique via the femoral artery with the tip into the celiac trunk and left in place during the five treatment days of each cycle. To prevent thrombosis/ embolism, 20.000 IU/day of heparin were infused continuously via the catheter, exempt during drug infusion. One cycle consisted of mitoxantrone 10mg/m^2 on day 1, folinic acid 170mg/m^2 directly followed by 5-fluorouracil 600mg/m^2 on days 2-4, and cisplatinum 60mg/m^2 on day 5. During and after infusion, toxicity was closely monitored according to WHO criteria, and doses were reduced by 20% in case of toxic events >WHO II. If the catheter could not be placed, the drug regimen was delivered intravenously (iv.) with a dose reduction of 20%.

Two weeks after the first CAI cycle, radiotherapy was started. Radiotherapy was delivered according to the guidelines of the International Commission on Radiation Units and Measurements ICRU 50.

A total dose (TD) of 54 Gray (Gy) was delivered in single doses (SD) of 1.8 Gy 5 days a week using a 3 or 4-field technique. The minimum and maximum doses in the target volume was specified. Three weeks after finishing radiotherapy CAI was restarted up to a total of 6 cycles (including the first cycle given before radiotherapy) with an interval of 4 weeks between each cycle.
Treatment was discontinued if local or systemic tumor progression occurred, in case of irreversible toxicities WHO III or WHO IV, in case of intolerable side effects that could not be controlled by dose reductions or on the patient's demand.

Outcome measures and statistical analysis

Power calculations had led to a total of 120 patients in each group. However, several important clinical trials demonstrated a survival benefit of adjuvant chemotherapy after curative surgery which was not yet reported when this study was initiated.[6, 23] Since our control group (observation group) did not receive any form of adjuvant treatment it was considered unethical to continue to include patients. The decision to stop inclusion was not based on outcome data.
The primary endpoint was overall survival time. Analysis was done with the Statistical Package for the Social Sciences version 10.1 (SPSS). Survival curves were computed according to the Kaplan Meier method from the date of randomization until the date of death from any cause or censored at the last follow up and median survival was calculated based on the curve. Comparison of survival curves was done with the log-rank test.
Secondary endpoints were toxicity of treatment and disease free survival defined as the time between randomization and local or distant recurrence on diagnostic imaging and local recurrence or hepatic progression rates. Patients were stratified for tumor type. Analyses were performed on an intention-to-treat basis.
Our local ethics committee approved this open prospective randomized controlled trial.

RESULTS

Patients

Between June 2000 and March 2007, 256 pancreaticoduodenectomies were performed. One specialized pathologist reviewed all resection specimens. One hundred thirty six patients were excluded for adjuvant treatment because of benign, neuroendocrine or cystic lesions (n=54), patient related factors (n=29) such as age (n=17), patient's refusal (n=6), poor mental status (n=2), language related problems (n=2) or an aberrant right hepatic artery (n=2) or postoperative complications (n=23), other primary tumors (n=19), other neo or adjuvant therapies (n=5) and T1 or metastatic disease (n=6).

After excluding these patients, 120 patients with resected pancreatic or periampullary (distal common bile duct or papilla of Vater) adenocarcinoma were eligible. Fifty-nine patients were randomized to the chemoradiation group (group A) and 61 patients to the surgery alone group (group B). Baseline characteristics were similar among the groups except for a possible relevant difference in mean age (60 years in group A vs. 66 years in group B; p< 0.01) and histopathological grading (more poorly differentiated tumors in the CAI/RT group; p=0.021) (Table 1).

Table 1. Baseline Characteristics of Eligible Patients:

Characteristics	**CAI/RT N= 59**	**Observation N= 61**	p-value
Median age (yr) (range)	62 (33-75)	69 (36-79)	<0.001
Sex; number (%)			
Male	26 (44)	32 (53)	n.s.
Female	33 (56)	29 (47)	n.s.
Type of surgery; number (%)			
Whipple's procedure	16 (27)	14 (23)	n.s.
PPPD	43 (73)	46 (75)	n.s.
Distal pancreatectomy	0 (0)	1 (2)	n.s.
Days from surgery to randomization (range)	27 (15-70)	29 (15-57)	n.s.
Karnofsky performance score; median (range)	90 (80-100)	90 (80-100)	n.s
Pathologic diagnosis; number (%)			
Pancreatic head	31 (53)	31 (51)	n.s
Periampullary cancer	28 (47)	30 (49)	n.s.
T category; number (%) (pancreatic head)			
T2	2 (7)	3 (10)	n.s.
T3	28 (90)	24 (77)	n.s.
T4	1 (3)	4 (13)	n.s.
T category; number (%) (periampullary)			
T2	9 (32)	8 (27)	n.s.
T3	13 (47)	16 (53)	n.s.
T4	6 (21)	6 (20)	n.s.
N category; number (%) (pancreatic head)			
N0	13 (42)	16 (52)	n.s.
N1	18 (58)	15 (48)	n.s.
N category; number (%) (periampullary)			
N0	12 (43)	12 (40)	n.s.
N1	16 (57)	18 (60)	n.s.
Resection status; number (%) (pancreatic head)			
R0	23 (74)	22 (71)	n.s.
R1	8 (26)	9 (29)	n.s.
Resection status; number (%) (periampullary)			
R0	23 (82)	29 (97)	n.s.
R1	5 (28)	1 (3)	n.s.
Histopathological grading; number (%)	6 (10)	7 (12)	
Well differentiated	34 (58)	45 (74)	n.s.
Moderately differentiated	18 (30)	8 (13)	n.s.
Poorly differentiated	1 (2)	1 (2)	0.021
Missing			n.s.

Median time from resection to start of adjuvant treatment was 61 days (range 42-96 days). None of the patients were lost to follow up. Median follow up was 17 months for all patients range 2-78 months). For the patients still alive the median follow up was 30 months (range 4-78 months).

Compliance and adverse effects

Seven patients randomized to group A either refused treatment (n=3), were ineligible because of liver metastases detected on the re-staging CT scan (n=3) or died (n=1) because of congestive heart failure while waiting for start of treatment. Hence, of the 59 patients assigned to CAI/RT a total of 52 patients actually received adjuvant treatment. In the statistical analysis, all 59 patients remained in the assigned group according to the intention to treat principle.

Overall grade 3 and 4 toxicity is shown in Table 2. Adverse toxic events caused discontinuation in 13/ 52 patients (25%), in 7 with pancreatic cancer and in 6 with periampullary cancer. Ten patients suffered from hematological toxicity and the three others because from a skin rash, a gastrointestinal bleeding and a gastrointestinal ulcer. Of these 13 patients, three had to stop treatment after CAI-I, four after CAI-II, one after CAI-III, three after CAI-IV and two after CAI-V. Progressive disease caused termination in 11 patients (21%), in 9 with pancreatic cancer and in 2 with periampullary cancer. Three patients after CAI-I, II and III and two patients after CAI-IV. Three patients (6%) refused to continue treatment after CAI-I, II or III. In three other patients treatment was postponed and finally stopped because of sever hyperglycaemia after CAI-I, cerebral infarction after CAI-IV or a lethal myocardial infarction after CAI-I. Six hundred twenty celiac axis catheterisations were performed and catheter luxations were seen in 14% of the placements. Catheter related complications were seen in five patients resulting in discontinuation of treatment after CAI-II in one patient. A subtotal stenosis of the celiac trunk was found in three patients leading to intravenous administration of chemotherapy. A dissection of the common femoral artery was caused in one patients necessitating stenting and an external iliac artery dissection in another necessitating thrombectomy and a patch angioplasty.

Thirty six per cent (21/ 59) of all patients in the CAI/RT group received all 6 cycles including radiotherapy as specified in the protocol: six patients with pancreatic cancer and 15 patients with periampullary cancer. The median number of cycles administered in these 59 patients was 3. For the patients with pancreatic tumors the median number of cycles was three and the patients with periampullary tumors received median six cycles.

Table 2: Recurrence pattern

	Chemoradiotherapy Number (%)	Observation Number (%)	P value
No progression			
Pancreatic cancer	9 (29)	4 (13)	n.s.
Periampullary cancer	13 (46)	7 (23)	n.s.
Alive with disease			
Pancreatic cancer	2 (6)	3 (10)	n.s.
Periampullary cancer	3 (11)	4 (13)	n.s.
Dead of disease			
Pancreatic cancer	20 (65)	24 (77)	n.s.
Periampullary cancer	12 (43)	19 (63)	n.s.
First site of progression			
Local recurrence			
Pancreatic cancer	6 (19)	11 (35)	n.s.
Periampullary cancer	5 (18)	3 (10)	n.s.
Liver			
Pancreatic cancer	8 (26)	7 (23)	n.s.
Periampullary cancer	4 (14)	11 (37)	n.s.
Both			
Pancreatic cancer	3 (10)	2 (6)	n.s.
Periampullary cancer	1 (4)	2 (7)	n.s.
Other			
Pancreatic cancer	5 (16)	7 (23)	n.s.
Periampullary cancer	5 (18)	7 (23)	n.s.
Overall development of distant progression			
Liver			
Pancreatic cancer	14 (45)	15 (48)	n.s.
Periampullary cancer	8 (29)	17 (57)	0.021
Lung			
Pancreatic cancer	7 (23)	5 (16)	n.s.
Periampullary cancer	3 (11)	5 (17)	n.s.
Other			
Pancreatic cancer	2 (6)	7 (23)	
Periampullary cancer	2 (7)	5 (17)	

Time to Progression

Progressive disease was seen in 87 of the 120 patients. Progressive disease developed in 37 patients in the CAI/RT group, and in 50 patients in the surgery alone group. Median time until progression was 12 months in the CAI/RT group vs. 7 months in the surgery alone group (p=0.015; Figure 1). The distribution of progressive disease is shown in Table 3.

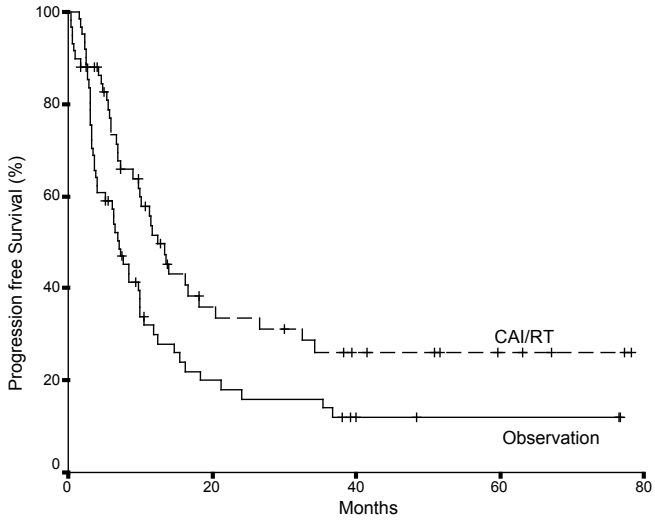


Figure 1: Time to progression after CAI/RT vs. observation (p=0.015). The numbers below the figure denote the numbers at risk.

Table 3: Grade 3 and 4 toxicity

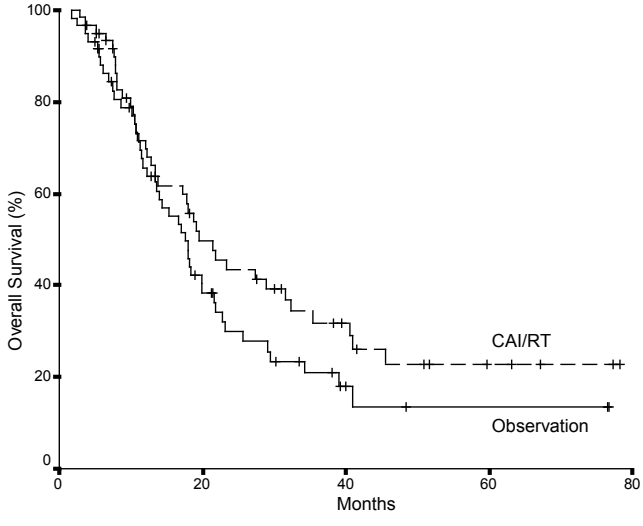
		Grade 3		Grade 4	
Toxicity		**Number**	**(%)**	**Number**	**(%)**
Hematologic					
	Hemoglobin	1	(2)	0	(0)
	Leucocytes	10	(20)	1	(2)
	Platelets	0	(0)	1	(2)
Non-hematologic					
	Nausea	0	(0)	0	(0)
	Vomiting	3	(6)	0	(0)
	Diarrhea	0	(0)	0	(0)
	Alopecia	0	(0)	0	(0)
	Fatigue	0	(0)	0	(0)

Stratification for tumor type, i.e. periampullary vs. pancreas carcinoma, shows a considerable difference in recurrence pattern. Patients with periampullary tumors develop significantly less liver metastases after CAI/RT (log-rank p=0.021), without significant effect on prevention of locoregional recurrence (log-rank p=0.294). In pancreatic tumors on the other hand CAI/RT had no significant effect on the development of liver metastases (log-rank p=0.755) or on local recurrence rate (log-rank p=0.12).

Overall Survival

Survival analysis was based on 82 deaths, of which 72 deaths were cancer related.

Overall thirty-eight patients died in the CAI/RT group as compared to 44 in the surgery alone group. There was no significant survival benefit in the treatment group. After CAI/RT, median survival was 19 months compared to 18 months after surgery alone (p=0.25; Figure 2). Survival in the three patients with IV continuation of treatment was 5, 7 and 39 months respectively.



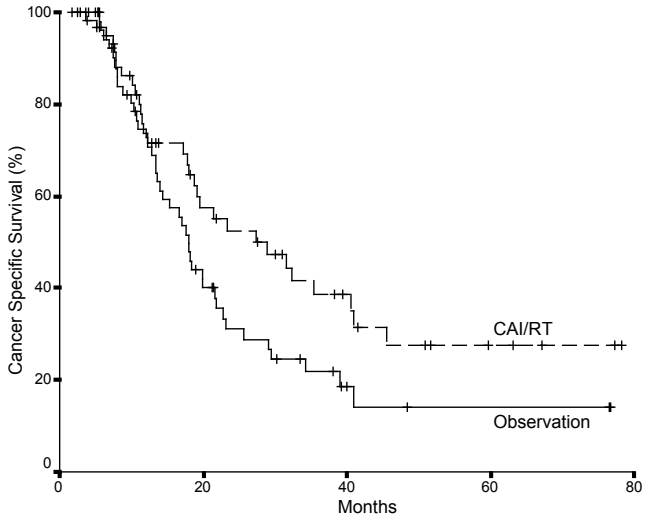
CAI/RT	59	24	11	4
Observation	61	20	5	2

Figure 2: Overall survival after CAI/RT vs. observation (p=0.25). The numbers below the figure denote the numbers at risk.

Ten patients died of non-cancer related causes: Eight patients in group A because of a cerebral infarction (n=2), hypo- and hyperglycaemic coma (n=2), myocardial infarction (n=2), Aspergillus pneumonia (n=1) and a gastric bleeding (n=1) and two patients in group B caused by a gastric bleeding (n=1) and a traffic accident (n=1). There was a clear trend in cancer specific survival between the two treatment groups (log-rank p= 0.05; Figure 3).

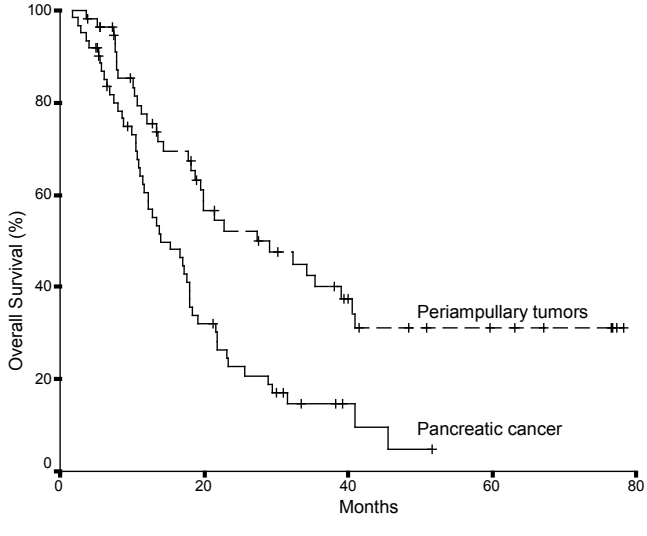
Regardless of treatment group, a significant better overall survival was found in periampullary tumors *vs*. pancreatic cancer (median survival was 27 vs. 14 months; p< 0.001) (Figure 4). However, no significant benefit of treatment on overall survival is seen in either subgroup (p= 0.66 for pancreatic cancer and p= 0.15 for periampullary tumors) (Figure 5a-b).

For all endpoints analyzed, we found no effect of age. The by chance imbalance regarding age between the two treatment groups did not affect our results.



CAI/RT	59	24	11	4
Observation	61	20	5	2

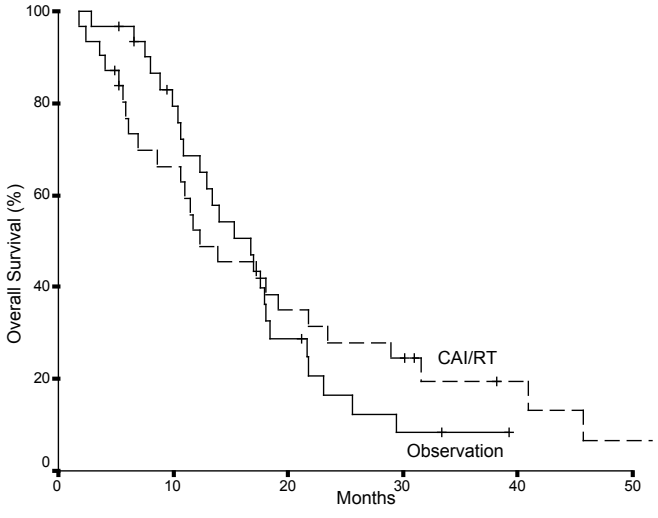
Figure 3: Cancer related survival after CAI/RT vs. observation (p=0.052). The numbers below the figure denote the numbers at risk.



Periamp	58	26	13	6
Pancreas	62	18	3	

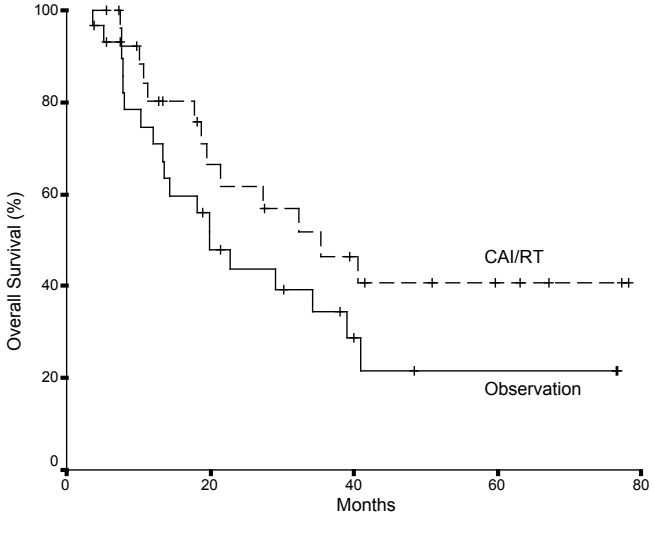
Figure 4: Overall survival in pancreatic cancer vs. periampullary tumors regardless of treatment (p<0.001). The numbers below the figure denote the numbers at risk.

A



CAI/RT	31	19	10	7	3	1
Observation	31	22	8	2		

B



CAI/RT	28	14	8	4
Observation	30	12	5	2

Figure 5a-b: Overall survival after CAI/RT vs. observation in pancreatic carcinoma (a; p=0.66) and periampullary tumors (b; p=0.15). The numbers below the figure denote the numbers at risk.

DISCUSSION

This randomized controlled trial shows a significant improvement of time to progression after intra-arterial chemoradiotherapy compared to observation for both pancreatic as well as periampullary tumors. Most effect is seen in periampullary tumors were liver metastases are significantly reduced. These promising effects on recurrence do not result in a prolonged overall survival, not even after stratification for tumor type. However, cancer related survival seems to improve after intra-arterial chemoradiation (p=0.05). Toxicity of CAI/RT was acceptable (25%) and catheter related problems necessitating intervention occurred in two patients.

Surgery is still considered to be the mainstay of treatment for patients with resectable pancreatic tumors. Mortality and morbidity rates have declined over the years without subsequent effect on 5-year survival.[31]Adjuvant therapies were administered to improve this 5-year survival, so far with disappointing results. Where only the ESPAC-1 showed an disputable overall survival benefit, all other published randomized controlled trials showed only improved disease free survival.[2, 6, 20, 32]

The median survival we presented for all patients after CAI/RT or observation (19 or 18 months) was comparable with the results of the ESPAC-1 trial[2, 23] (median survival depending on the treatment arm varying between 13.9 and 21.6 months) and the CONKO-1 trial[6] (median survival of 22.1 months after gemcitabine and 20.2 months after observation). However these trials pretended to include only patients with pancreatic cancer. We describe the results of both patients with pancreatic (median survival 14 months) and periampullary (median survival 27 months) tumors. In this trial a standardized system of pathologic assessment for quality control was used. One single, specialized pathologist reviewed all specimens to differentiate between pancreatic and periampullary tumors. In both the ESPAC-1 and CONKO-1 trial no central pathology control was performed probable leading to the inclusion of patients with periampullary tumors as well. Furthermore in this trial we performed a restaging before the start of the radiotherapy, which in our opinion is essential to evaluate the result of adjuvant treatment.

However, the figures presented are not actual median survival figures. We included patients after recovery from surgery and although peri-operative mortality is low in our center, this could have led to a bias.

The similar survival figures illustrate the minor progression achieved on overall survival, despite large volume multicenter trials. Currently applied treatment strategies are based on generally accepted agents such as 5- FU, gemcitabine possibly in combination with cisplatinum. These regimens might need reconsideration since the Virginia Mason, Seattle, USA group reported such encouraging results combining interferon (IFN) alpha with 5-FU, cisplatinum, and radiation therapy after pancreatoduodenectomy. They reported a 2-year survival rate of 64% and a 5-year

survival rate of 55% in patients with high-risk resected pancreatic adenocarcinoma.[33] These promising results are currently reinvestigated in the phase III CapRI study.[34] Results of this study are expected shortly and might give an indication whether immunomodulatory drugs should be added to the standard adjuvant regimens and enhance the effect of both chemo- and/or radiation therapy. In vitro there is clear evidence that differential expression levels and distribution of the interferon receptor subunits play a role in the regulation of the response to type I IFN therapy in pancreatic cancer. Future studies should investigate whether the intensity, subcellular localization, and distribution of these receptor subtypes may predict the response to therapy with different interferons in pancreatic cancer.[35]

An important outcome of our study is the significance of differentiating between periampullary tumors and pancreatic carcinoma. Patients operated for periampullary tumors survived significantly longer compared to patients with pancreatic cancer (27 vs. 14 months; $p<0.001$) and developed less liver metastases after intra-arterial chemotherapy and radiotherapy compared to observation (log-rank $p<0.03$). Patients with pancreatic cancer developed more local recurrences compared to periampullary tumors (log-rank $p<0.01$) but CAI/RT had no significant effect on local recurrence rate (log-rank $p=0.12$) nor on the development of liver metastases compared to the observation group (log-rank $p=0.755$). Consequently, no effect of CAI/RT was found on overall survival. This supports the conclusion of our EORTC trial that there is no role for chemoradiation in the prevention of local recurrence.[20, 21]

The choice for the less frequently used mitoxantrone was based on the study of Beger et al [27], revealing tolerable toxicity after intra-arterial admission if combined with 5-FU and cisplatinum. In addition, mitoxantrone is active in regional chemotherapeutics protocols like hepatic arterial infusion of colorectal liver metastases.[36] If we compare our results with the results published by Beger, the effect of CAI/RT on the development of liver metastases was confirmed, however where they found a consequent survival benefit, where we did not. One of the factors might be that eventually only 21 (36%) patients received treatment as stated in the protocol. However, there is no consensus on the number of cycles gaining the optimal effect of intra-arterial chemotherapy. Seventy three percent of the patients (n=42) for instance received 2 cycles or more of intra-arterial chemotherapy combined with radiotherapy. Based on the intention to treat principle, even with only 21 patients receiving full adjuvant treatment, there is a significant effect on the development of liver metastases in periampullary tumors and time to progression. Furthermore, in considerable number of patients, especially pancreatic cancer patients, adjuvant treatment was stopped due to progressive disease, which explains the low number of patients receiving 6 cycles of CAI.

There were some protocol violations. One patient was included aged 79 years because of younger biological age. And in one patient, due to the holiday, treatment was started after 96 days. We found it unethical to withdraw this patient from the explained treatment arm.

Considering survival after treatment of patients with periampullary tumors, the number of patients in both groups (28 in group A vs. 30 in group B) was not sufficient to prove a significant survival benefit after treatment, but based on the Kaplan Meier curve (Figure 5b) there appears to be an effect of intra-arterial chemoradiation. It seems likely that after a longer follow up a significant survival benefit after CAI/RT in periampullary tumors will be found. However we decided to terminate the trial because of trials published during the inclusion period suggesting it is no longer ethical to have an observation arm and gemcitabine should be standard of care. A complicating factor was the poor patient recruitment, since this trial was designed to include patients in the University Hospital of Ulm as well, but recruitment never started there due to logistic problems. Not surprisingly, for pancreatic carcinoma no overall or disease free survival benefit was found since CAI/RT did not reduce local recurrence nor the development of liver metastases. The imbalance in histopathological grading in favor of the observation group (more poorly differentiated tumors in the CAI/RT group; p=0.021) might have lead to an underestimation of the effect of CAI/RT on overall as well as on disease free survival.

Since differentiating between pancreatic and periampullary cancer pre-operatively by CT scanning or endoscopic ultrasound appears to be difficult if not impossible, new treatment schedules including neoadjuvant therapy for only true pancreatic cancers will be difficult to develop. Nowadays the final diagnosis is based on histopathological findings of the operative specimen, thus requiring resection and consequently eliminating the possibility of tumor specific neoadjuvant treatment. However future improvements in CT scanning techniques, endoscopic ultrasound experience and DNA analysis might facilitate pre-operative differentiation.

Remarkably, more non-cancer related deaths occurred in the CAI/RT group. Cause of this can be twofold: either therapy caused more deaths, or patients in the observation arm were followed less closely. However, death occurred over two months after finishing/ending therapy and patients in both arms were monitored equally. Speculating, patients in the treatment arm were capable of dying from other causes because they survived their cancer, but than you would have expected a survival difference.

One concern that has not been mentioned so far is the impact this treatment could have on the quality of life. This regime is quite intense, necessitating hospital admissions for every cycle and visits to the outpatient clinic daily during radiotherapy. Because we realize it is not ethical to give such an intense treatment to patients with a short prognosis thereby reducing the quality of life, we concomitantly performed a quality of life investigation. Preliminary results of the analysis of quality of life forms, reveals that at least there is no detrimental effect of this treatment on quality of life in general. One of the causes can be that although toxicity occurs in 25% of the patients, this is mainly of hematological origin. However, definite results will be presented in the future.

In conclusion, CAI/RT does result in a significant increase in disease free survival mostly because significantly less liver metastases developed in periampullary tumors after intra-arterial therapy. A clear trend of adjuvant CAI/RT after curative resection was found for overall cancer related survival (p=0.05) and for overall survival in periampullary tumors (p=0.15) but not for overall survival in all patients (p=0.25). Again, no benefit of chemoradiation on local recurrence in neither pancreatic nor periampullary cancers was found. In our opinion, differentiating between periampullary and pancreatic tumors is of paramount importance for further treatment strategies with periampullary tumors being most prone to intra-arterial chemoradiotherapy. For pancreatic cancer another regime has to be considered.

REFERENCES

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2007. CA Cancer J Clin 2007; 57(1):43-66.
2. Neoptolemos JP, Dunn JA, Stocken DD, et al. Adjuvant chemoradiotherapy and chemotherapy in resectable pancreatic cancer: a randomised controlled trial. Lancet 2001; 358(9293):1576-85.
3. Willett CG, Czito BG, Bendell JC. Adjuvant therapy of pancreatic cancer. Cancer J 2007; 13(3):185-91.
4. Bassi C, Stocken DD, Olah A, et al. Influence of surgical resection and post-operative complications on survival following adjuvant treatment for pancreatic cancer in the ESPAC-1 randomized controlled trial. Dig Surg 2005; 22(5):353-63.
5. Kennedy EP, Yeo CJ. The case for routine use of adjuv ant therapy in pancreatic cancer. J Surg Oncol 2007; 95(7):597-603.
6. Oettle H, Post S, Neuhaus P, et al. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. Jama 2007; 297(3):267-77.
7. Baumel H, Huguier M, Manderscheid JC, et al. Results of resection for cancer of the exocrine pancreas: a study from the French Association of Surgery. Br J Surg 1994; 81(1):102-7.
8. Klinkenbijl JH, Jeekel J, Schmitz PI, et al. Carcinoma of the pancreas and periampullary region: palliation versus cure. Br J Surg 1993; 80(12):1575-8.
9. Lillemoe KD. Current management of pancreatic carcinoma. Ann Surg 1995; 221(2):133-48.
10. Nitecki SS, Sarr MG, Colby TV, van Heerden JA. Long-term survival after resection for ductal adenocarcinoma of the pancreas. Is it really improving? Ann Surg 1995; 221(1):59-66.
11. Trede M. The surgical treatment of pancreatic carcinoma. Surgery 1985; 97(1):28-35.
12. Trede M, Schwall G, Saeger HD. Survival after pancreatoduodenectomy. 118 consecutive resections without an operative mortality. Ann Surg 1990; 211(4):447-58.
13. Wade TP, Coplin MA, Virgo KS, Johnson FE. Periampullary cancer treatment in U.S. Department of Veterans Affairs hospitals: 1987-1991. Surgery 1994; 116(4):819-25; discussion 825-6.
14. Yeo CJ, Cameron JL, Lillemoe KD, et al. Pancreaticoduodenectomy for cancer of the head of the pancreas. 201 patients. Ann Surg 1995; 221(6):721-31; discussion 731-3.
15. Further evidence of effective adjuvant combined radiation and chemotherapy following curative resection of pancreatic cancer. Gastrointestinal Tumor Study Group. Cancer 1987; 59(12):2006-10.
16. Kalser MH, Ellenberg SS. Pancreatic cancer. Adjuvant combined radiation and chemotherapy following curative resection. Arch Surg 1985; 120(8):899-903.
17. A multi-institutional comparative trial of radiation therapy alone and in combination with 5-fluorouracil for locally unresectable pancreatic carcinoma. The Gastrointestinal Tumor Study Group. Ann Surg 1979; 189(2):205-8.
18. Radiation therapy combined with Adriamycin or 5-fluorouracil for the treatment of locally unresectable pancreatic carcinoma. Gastrointestinal Tumor Study Group. Cancer 1985; 56(11):2563-8.
19. Neoptolemos JP, Cunningham D, Friess H, et al. Adjuvant therapy in pancreatic cancer: historical and current perspectives. Ann Oncol 2003; 14(5):675-92.
20. Klinkenbijl JH, Jeekel J, Sahmoud T, et al. Adjuvant radiotherapy and 5-fluorouracil after curative resection of cancer of the pancreas and periampullary region: phase III trial of the EORTC gastrointestinal tract cancer cooperative group. Ann Surg 1999; 230(6):776-82; discussion 782-4.
21. Smeenk HG, van Eijck CH, Hop WC, et al. Long-term Survival and Metastatic Pattern of Pancreatic and Periampullary Cancer After Adjuvant Chemoradiation or Observation: Long-term Results of EORTC Trial 40891. Ann Surg 2007; 246(5):734-40.

22. Neoptolemos JP, Stocken DD, Dunn JA, et al. Influence of resection margins on survival for patients with pancreatic cancer treated by adjuvant chemoradiation and/or chemotherapy in the ESPAC-1 randomized controlled trial. Ann Surg 2001; 234(6):758-68.
23. Neoptolemos JP, Stocken DD, Friess H, et al. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. N Engl J Med 2004; 350(12):1200-10.
24. Oettle H, Neuhaus P. Adjuvant therapy in pancreatic cancer: a critical appraisal. Drugs 2007; 67(16):2293-310.
25. Picozzi VJ, Pisters PW, Vickers SM, Strasberg SM. Strength of the evidence: adjuvant therapy for resected pancreatic cancer. J Gastrointest Surg 2008; 12(4):657-61.
26. Hayashibe A, Kameyama M, Shinbo M, Makimoto S. Clinical results on intra-arterial adjuvant chemotherapy for prevention of liver metastasis following curative resection of pancreatic cancer. Ann Surg Oncol 2007; 14(1):190-4.
27. Beger HG, Gansauge F, Buchler MW, Link KH. Intraarterial adjuvant chemotherapy after pancreaticoduodenectomy for pancreatic cancer: significant reduction in occurrence of liver metastasis. World J Surg 1999; 23(9):946-9.
28. Ishikawa O, Ohhigashi H, Sasaki Y, et al. Extended pancreatectomy and liver perfusion chemotherapy for resectable adenocarcinoma of the pancreas. Digestion 1999; 60 Suppl 1:135-8.
29. Blackstock AW, Mornex F, Partensky C, et al. Adjuvant gemcitabine and concurrent radiation for patients with resected pancreatic cancer: a phase II study. Br J Cancer 2006; 95(3):260-5.
30. Sobin LH, Wittekind C. TNM Classification of Malignant Tumours, 6th Edition, 2002.
31. Cameron JL, Riall TS, Coleman J, Belcher KA. One thousand consecutive pancreaticoduodenectomies. Ann Surg 2006; 244(1):10-5.
32. Takada T, Amano H, Yasuda H, et al. Is postoperative adjuvant chemotherapy useful for gallbladder carcinoma? A phase III multicenter prospective randomized controlled trial in patients with resected pancreaticobiliary carcinoma. Cancer 2002; 95(8):1685-95.
33. Picozzi VJ, Kozarek RA, Traverso LW. Interferon-based adjuvant chemoradiation therapy after pancreaticoduodenectomy for pancreatic adenocarcinoma. Am J Surg 2003; 185(5):476-80.
34. Knaebel HP, Marten A, Schmidt J, et al. Phase III trial of postoperative cisplatin, interferon alpha-2b, and 5-FU combined with external radiation treatment versus 5-FU alone for patients with resected pancreatic adenocarcinoma -- CapRI: study protocol [ISRCTN62866759]. BMC Cancer 2005; 5(1):37.
35. Vitale G, van Eijck CH, van Koetsveld Ing PM, et al. Type I interferons in the treatment of pancreatic cancer: mechanisms of action and role of related receptors. Ann Surg 2007; 246(2):259-68.
36. Link KH, Kornmann M, Leder G, et al. Basic research supported developments of chemotherapy in nonresectable isolated colorectal liver metastases to a protocol of hepatic artery infusion using mitoxantrone, 5-FU + folinic acid and mitomycin C. Gan To Kagaku Ryoho 1999; 26(3):269-81.

CHAPTER 3

Quality of life after adjuvant intra-arterial chemotherapy and radiotherapy versus surgery alone in resectable pancreatic and periampullary cancer

A prospective randomized controlled trial

M.J.M. Morak[1], C.J. Pek[1], E.J.O. Kompanje[2], W.C.J. Hop[3], G. Kazemier[1] and C.H.J. van Eijck[1]

[1] Department of Surgery, Erasmus Medical Centre, Rotterdam, The Netherlands
[2] Department of Intensive Care, Erasmus Medical Centre, Rotterdam, The Netherlands
[3] Department of Statistics and Epidemiology, Erasmus Medical Centre, Rotterdam, The Netherlands

Cancer 2010 Feb 15;116(4):830-6.

ABSTRACT

Background: Adjuvant therapies in pancreatic and periampullary cancer have been shown to achieve only marginal survival benefit. In this randomized controlled trial, 120 patients with resected pancreatic or periampullary cancer received either adjuvant celiac axis infusion combined with radiotherapy (CAI/RT) or no adjuvant treatment.

Aim of the study: To compare Quality of Life (QoL) in patients receiving CAI/RT after pancreatoduodenectomy with QoL in patients without adjuvant treatment.

Methods: During and after CAI/RT, QoL was assessed using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire C30 (EORTC QLQ-C30) every 3 months during the first 24 months after randomization.

Results: Eighty-six per cent (n=103) of the patients completed one or more questionnaires. In total, 355 questionnaires were completed. CAI/RT did not impair physical, emotional or social functioning. During and after CAI/ RT, patients had significantly less pain (p=0.02) and less nausea and vomiting (p=0.01). Overall QoL (global functioning) tended to be better (p=0.08) after CAI/RT.

Conclusion: Over a period of 24 months, CAI/RT improved quality of life compared to observation alone in patients with resected pancreatic and periampullary cancer. This beneficial effect of CAI/RT is most prominent in the latter half of the follow-up.

INTRODUCTION

Pancreatic cancer is currently one of the world's most fatal malignant diseases and ranks fourth in cancer related mortality.[1] Even after radical resection, median survival time is 12 - 15 months[2, 3] with 5-year survival rate ranging from 5% to 25%.[2, 4-6]

Considering this short life expectancy after radical resection and morbidity related to a pancreatoduodenectomy[7], the value of resection has been discussed. However, several trials showed that after pancreatoduodenectomy quality of life (QoL) returned to pre-operative values three months after surgery[8] or was even better than before surgery measured up to 6 months after surgery in patients with localized pancreatic cancer.[9, 10] However, compared to laparoscopic surgery for a benign cause, i.e. laparoscopic cholecystectomy, patients after pylorus preserving pancreatoduodenectomy for pancreatic adenocarcinoma have a significantly worse quality of life.[11] This deleterious effect on the quality of life might be explained by both the extend of the surgery as well as the primary indication for surgery, i.e. a benign vs. a malign lesion.

To improve survival after pancreatoduodenectomy in patients with pancreatic tumors as well as periampullary tumors, several clinical trials have been performed studying the efficacy of chemotherapy and/or radiotherapy. Most of the studies investigated improvement of disease free or overall survival by adjuvant therapy.[2, 6, 12-17] In only two randomized controlled trials, the effect of adjuvant chemotherapy or chemoradiotherapy on quality of life was also measured. The ESPAC-I trial found similar quality of life after 5-FU based chemotherapy (425 mg/m^2 on five consecutive days for six cycles) vs. no chemotherapy and 5-FU based chemoradiotherapy (500 mg/m^2 on the first three days of each week of radiotherapy (10x 2 Gray)) vs. no chemoradiotherapy. The CONKO-001 trail compared chemotherapy (6 cycles of three weekly infusions of gemcitabine 1000 mg/m^2) with observation and found no adverse effect on quality of life.[6, 18]

In a randomized controlled trial we compared intra-arterial chemotherapy plus radiotherapy (CAI/RT) with observation after pancreatoduodenectomy in patients with periampullary or pancreatic cancer.[19] This therapy involves six separate weeks of hospitalization, indwelling catheters, several angiographies, six weeks of daily radiotherapy on outpatient basis and considerable toxicity, mainly of hematological origin; the therapeutic results were reported previously.

To investigate whether the efficacy of this treatment was counterbalanced with detrimental effects on quality of life, analysis of the quality of life was a predefined secondary endpoint. The results of the quality of life analysis are described in this report.

METHODS

Because the trial design has been mentioned extensively before, we summarize only the main aspects.[19]

Study design

After pancreatoduodenectomy, patients with histological proven adenocarcinoma of the pancreas or the periampullary region were randomly assigned to receive either adjuvant intra-arterial chemotherapy combined with radiotherapy (CAI/RT) or observation. Intra-arterial chemotherapy consisted of 6 cycles of mitoxantrone (10mg/m^2 on day 1), 5-fluorouracil+ folinic acid (600mg/m^2 +170mg/m^2 on days 2-4) and cisplatinum (60mg/m^2 on day 5); 54 Gray (total dose) radiotherapy was also given. During each cycle of chemotherapy, patients were admitted to hospital, had an indwelling intra-arterial catheter, and were obliged to stay in bed for the entire week. During radiotherapy, patients were treated on an outpatient basis, receiving 1.8 Gray each weekday for 6 weeks. Including intervals, the entire schedule lasted over 8 months. None of the patients received further chemotherapy or radiotherapy in case of recurrence.

Quality of Life Assessments

Quality of life was measured using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire C30 (EORTC QLQ-C30) version 3.0 [20] which is a 30-item self-reporting questionnaire developed to assess the quality of life of cancer patients. It is grouped into five functional subscales (role, physical, cognitive, emotional and social functioning). It also contains three multi-item symptom scales (fatigue, pain, and nausea and vomiting); individual questions concerning common symptoms in cancer patients (dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties); and two questions assessing overall quality of life (global functioning).

Quality of life was assessed every 3 months in the first 24 months after randomization. The first quality of life questionnaire was filled out at three months and was used as baseline. The quality of life forms were sent to the patients prior to an outpatient clinic visit, where filled out forms were collected.

Outcome measures and statistical analysis

Survival curves were computed according to the Kaplan Meier method from the date of randomization until the date of death from any cause or censored at the last follow-up. Median survival was calculated on the basis of the curve. Survival curves were compared using the log-rank test.

All scores from the EORTC QLQ-C30 (version 3) questionnaire were transformed to a 0-100 scale using a linear transformation.[20] Higher scores for a functional scale represent a higher level of functioning, i.e. a better state of the patient. Conversely, higher scores for symptoms indicate a higher level of complaints.

Nine scales of the EORTC QLQ-C30 were selected a priori for this analysis: physical, emotional and social functioning; fatigue; pain; nausea and vomiting; loss of appetite; diarrhea; and global functioning.

Compliance was calculated as the number of completed questionnaires expressed as a percentage of the number of questionnaires expected (per time interval). Fifty per cent of the standard deviation of any QoL tool equivalent to 8-10 points is usually considered as clinically significant [21]

All quality of life scales were compared between randomized arms with repeated measurement ANOVA using SAS PROC MIXED (version 8.2). For all scales it was evaluated whether the treatment effect differed between time points of assessment with interaction terms. Other analyses were done using SPSS version 11.5. All evaluations were done using an intention to treat basis, and p=0.05 (two-sided) was considered the limit of significance.

RESULTS

Summary of Clinical Results[19]

Between June 2000 and March 2007, 120 patients with resected pancreatic or periampullary adenocarcinoma were randomized to intra-arterial chemoradiotherapy (n=59) or observation (n=61). Table 1 shows the characteristics of these patients. The median follow up was 17 months. Thirty six per cent (21/ 59) of all patients in the CAI/RT group received all 6 cycles including radiotherapy as specified in the protocol. Causes for discontinuation of treatment as planned were adverse toxic events in 13/ 52 patients (25%), progressive disease in 11 patients (21%), patient's refusal in 3 patients (6%) and co-morbidity in three others. Six hundred twenty celiac axis catheterizations were performed and catheter luxations were seen in 14% of the placements. After CAI/RT patients had a significantly prolonged time to progression (12 vs. 7 months; log-rank p<0.02). Overall, no significant survival benefit was seen after CAI/RT compared to observation (median 19 vs. 18 months; p=0.25) although cancer specific survival tended towards significance (29 vs. 18 months; log-rank p= 0.052).

Table 1. Baseline Characteristics of Eligible Patients:

Characteristics	CAI/RT ***N= 59***	Observation N= 61	p-value
Median age (yr) (range)	62 (33-75)	69 (36-79)	<0.001
Sex; number (%)			
Male	26 (44)	32 (53)	n.s.
Female	33 (56)	29 (47)	n.s.
Type of surgery; number (%)			
Whipple's procedure	16 (27)	14 (23)	n.s.
PPPD	43 (73)	46 (75)	n.s.
Distal pancreatectomy	0 (0)	1 (2)	n.s.
Days from surgery to randomization (range)	27 (15-70)	29 (15-57)	n.s.
Karnofsky performance score; median (range)	90 (80-100)	90 (80-100)	n.s
Pathologic diagnosis; number (%)			
Pancreatic head	31 (53)	31 (51)	n.s
Periampullary cancer	28 (47)	30 (49)	n.s.
T category; number (%) (pancreatic head)			
T2	2 (7)	3 (10)	n.s.
T3	28 (90)	24 (77)	n.s.
T4	1 (3)	4 (13)	n.s.
T category; number (%) (periampullary)			
T2	9 (32)	8 (27)	n.s.
T3	13 (47)	16 (53)	n.s.
T4	6 (21)	6 (20)	n.s.
N category; number (%) (pancreatic head)			
N0	13 (42)	16 (52)	n.s.
N1	18 (58)	15 (48)	n.s.
N category; number (%) (periampullary)			
N0	12 (43)	12 (40)	n.s.
N1	16 (57)	18 (60)	n.s.
Resection status; number (%) (pancreatic head)			
R0	23 (74)	22 (71)	n.s.
R1	8 (26)	9 (29)	n.s.
Resection status; number (%) (periampullary)			
R0	23 (82)	29 (97)	n.s.
R1	5 (28)	1 (3)	n.s.
Histopathological grading; number (%)			
Well differentiated	6 (10)	7 (12)	n.s.
Moderately differentiated	34 (58)	45 (74)	n.s.
Poorly differentiated	18 (30)	8 (13)	0.021
Missing	1 (2)	(2)	n.s.

Abbreviations: CAI/RT= intra-arterial chemoradiotherapy, PPPD= pylorus preserving pancraetoduodenectomy

Quality of Life

Table 2 shows the proportion of patients with assessable quality of life forms in each time interval. Eighty-six per cent (n=103) of the patients completed one or more questionnaires, 51 in the treatment arm and 52 in the observation arm with 71% of the patients completing at least two questionnaires. A total of 355 questionnaires were completed, with a median number of 4 (range 1-7) in patients undergoing CAI/RT and a median number of 3 (range 1-7) in patients in the observation arm. Overall, 60% of the expected questionnaires were completed and assessable for analysis. The rate of assessable questionnaires decreased over time, with more available forms in the CAI/RT arm in the first 6 months, but no difference after the end of the treatment. The decrease in the number of questionnaires was not related to toxicity. The median number of questionnaires obtained from the 13 patients suffering from toxicity was 5.

Table 2. Response rates/ compliance

	CAI/RT		Observation		p-value
	Patients in time window (number)	Patients with assessable questionnaires (%)	Patients in time window (number)	Patients with assessable questionnaires (%)	
3 months	57	81	59	63	<0.05
6 months	52	87	56	43	<0.01
9 months	45	73	49	59	n.s.
12 months	36	44	40	53	n.s.
15 months	34	50	32	59	n.s.
18 months	29	48	23	52	n.s.
21 months	24	63	20	55	n.s.
24 months	21	43	16	44	n.s.

Abbreviations: CAI/RT= intra-arterial chemoradiotherapy.

After CAI/RT no significantly better physical, emotional and social functioning scores were found during the total observation period, although physical functioning tended towards significance (p=0.10; mean difference 5.4 points) in favor of the treatment arm (Table 3 and Figure 1). Although the treatment effect did not significantly differ between time points, better mean values were found for CAI/RT at 15 and 18 months (both p<0.05).

Table 3. Quality of Life scores

	Adjusted mean values*		Difference	95% CI Difference	Overall p-value
	CAI/RT	Observation			
FUNCTIONING SCALES					
Social Functioning	85	81	4.0	-3.7 to 11.5	0.30
Physical Functioning	85	79	5.4	-1.0 to 11.8	0.10
Emotional Functioning	81	75	6.3	-1.2 to 13.9	0.11
SYMPTOM SCALES					
Pain	17	26	-9.7	-17.9 to -1.5	**0.02**
Fatigue	25	30	-6.3	-14.2 to 1.5	0.12
Nausea & Vomiting	6	12	-5.9	-10.4 to -1.4	**0.01**
Loss of appetite	11	16	-4.4	-11.1 to 2.3	0.20
Diarrhea	11	16	-5.0	-12.5 to 2.6	0.20
OVERALL Quality of Life					
Global functioning	73	66	6.5	-0.6 to 13.6	0.08

*Adjusted for age and gender
Abbreviations: CAI/RT= intra-arterial chemoradiotherapy, 95% CI= 95% confidence interval.

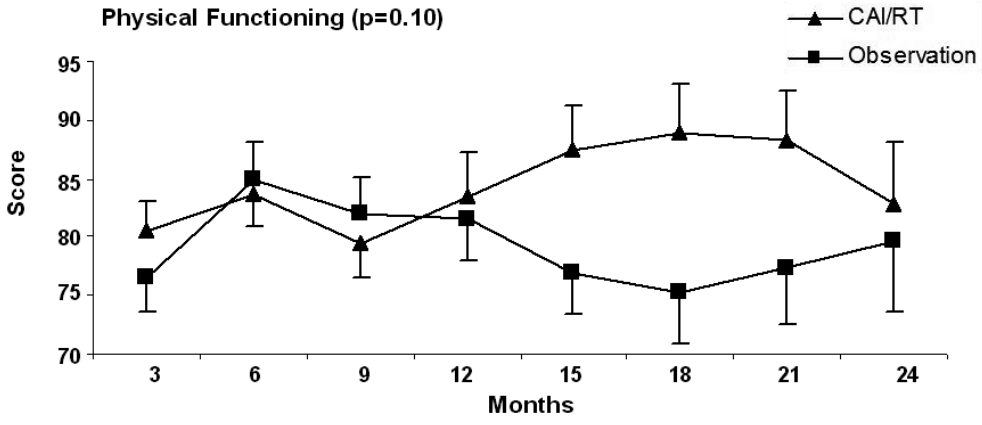


Figure 1: Mean EORTC QLQ-C30 score on physical functioning after adjuvant intra-arterial chemotherapy combined with radiotherapy or observation in resected pancreatic and periampullary cancer according to the number of months after randomization. Note that a higher score corresponds with a better physical functioning. Error bars represent standard errors. Denoted p-value denotes overall p-value.

For the symptom scales, patients had significantly less pain (overall p=0.02; mean difference 9.7 points) (Figure 2). This difference was most outspoken at time points 15 and 18 months (both p<0.05). There was also significantly less nausea and vomiting (p=0.01; mean difference 5.9 points) after CAI/ RT with the most eminent difference at 15 and 24 months (both p<0.05) (Figure 3).

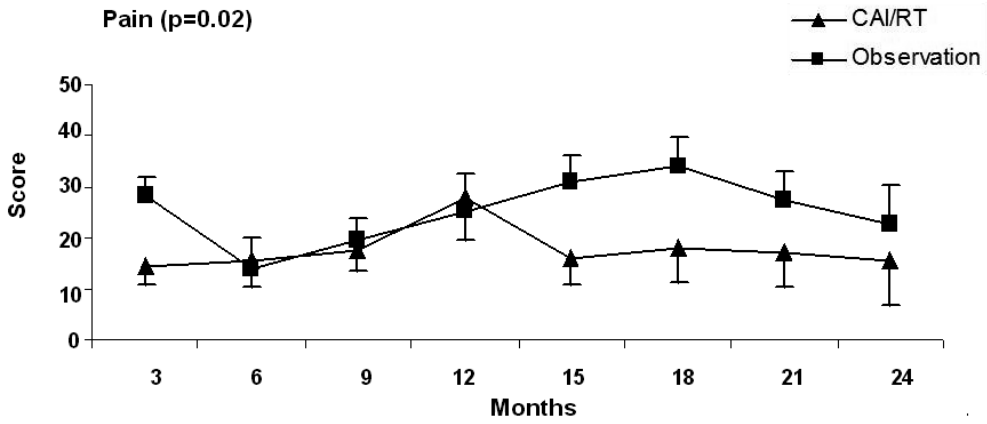


Figure 2: Mean EORTC QLQ-C30 pain scores after adjuvant intra-arterial chemotherapy combined with radiotherapy and observation in resected pancreatic and periampullary cancer. Note that a higher score corresponds with more pain.

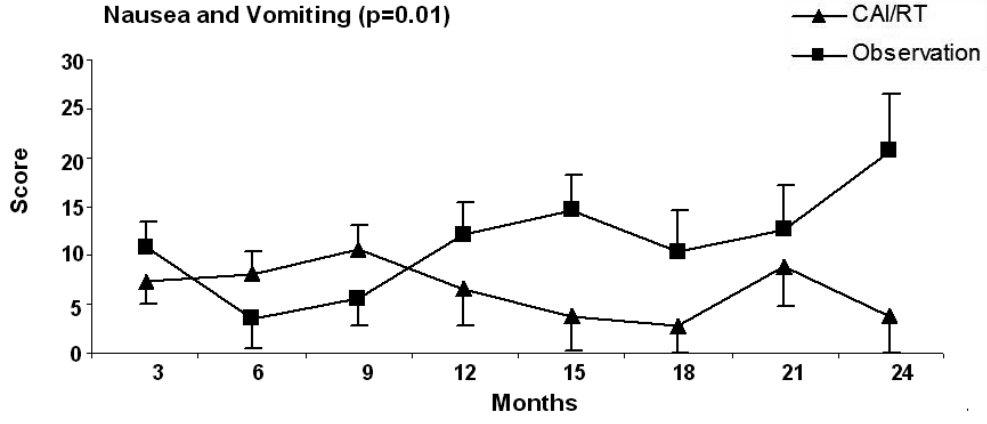


Figure 3: Mean EORTC QLQ-C30 nausea and vomiting scores after adjuvant intra-arterial chemotherapy combined with radiotherapy and observation in resected pancreatic and periampullary cancer. Note that a higher score corresponds with more nausea and vomiting.

For the other symptom scales there was no significant difference between randomized arms. Especially fatigue did not occur more often during or after CAI/RT (p=0.12; mean difference 6.3 points) (Figure 4).

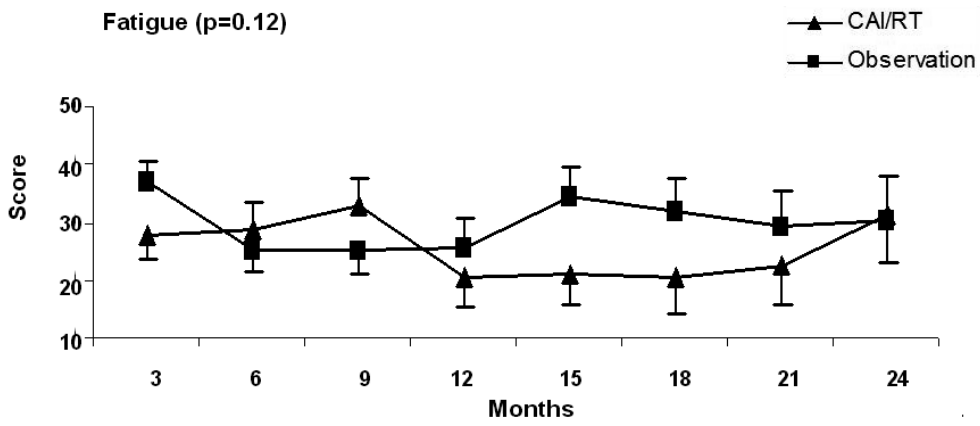


Figure 4: Mean EORTC QLQ-C30 fatigue scores after adjuvant intra-arterial chemotherapy combined with radiotherapy and observation in resected pancreatic and periampullary cancer. Note that a higher score corresponds with more fatigue.

Toxicity occurred in 13 patients and did not negatively influence quality of life compared to patients receiving the entire schedule or patients in the observation arm.

Overall quality of life (global functioning) tended towards significance (p=0.08; mean difference 6.5 points) in favor of the CAI/RT arm (Figure 5).

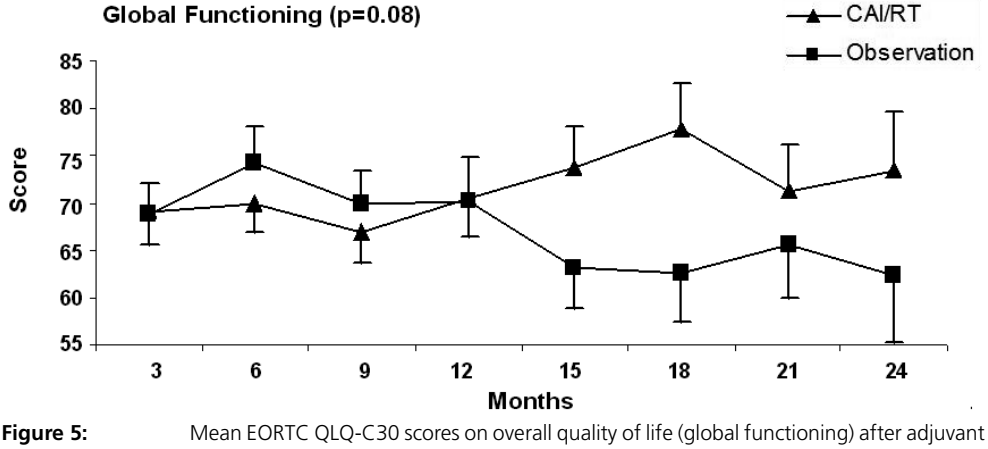


Figure 5: Mean EORTC QLQ-C30 scores on overall quality of life (global functioning) after adjuvant intra-arterial chemotherapy combined with radiotherapy and observation in resected pancreatic and periampullary cancer. Note that a higher score corresponds with a better overall quality of life.

DISCUSSION

In this randomized controlled trial[19], we compared intra-arterial chemoradiotherapy (CAI/RT) with observation after R0/R1 pancreatoduodenectomy. Patients randomized to the treatment arm received 6 cycles of chemotherapy and 30x 1.8 Gray radiation. Including intervals, the entire schedule lasted a minimum of 8 months with intermittent hospital admissions. Although there was no overall survival benefit after intra-arterial chemoradiotherapy, cancer-related survival tended to be better in the treatment arm. Intra-arterial chemoradiotherapy also significantly extended time to progression in all patients and reduced the number of liver metastases in patients with periampullary tumors.

In this study, quality of life measured according to the EORTC QLQ-C30 principles was improved from baseline after CAI/RT for all endpoints analyzed. On all functioning scales higher mean scores indicating better functioning were found, although none of these scores were significant. For all symptom scores, patients reported fewer complaints, leading to lower mean scores. For pain and nausea and vomiting, these scores were significantly lower after CAI/RT than the scores in the observation arm for the first 24 months after randomization. For pain score, for which there was a mean difference of 9.7 points between CAI/RT and surgery alone, this difference was also clinically relevant. For all QoL scores, the difference between the CAI/RT arm and the observation arm was constant over the 24 months, but the effect of CAI/RT on QoL was most apparent 12- 24 months after the start of therapy. In the first 12 months of quality of life assessment, i.e. during CAI/RT, symptoms such as fatigue, nausea and vomiting or pain did not occur more often. Overall quality of life tended to be better after CAI/RT ($p=0.08$).

A limitation of our study is the absence of questionaires at baseline. Therefore we could not evaluate changes from baseline, which are expected to give more precise results. Our results are valid though because from the randomisation one may expect that the baseline quality of life is comparable between both groups. Also the number of questionaires returned per patient is comparable.

One important feature of quality of life assessment is that it is subjective. This is particularly relevant as CAI/RT might be expected to negatively influence patient's quality of life due to the impact of the number and length of hospital admissions, outpatient visits and the toxicity of the therapy itself.

However, chemotherapy and/or radiotherapy is not necessarily associated with an impaired quality of life. More effective therapies produce a better quality of life because minor tumor shrinkage relieves some of the symptoms, or because they produce a longer period without disease and thus less time involving the problems of recurrent disease and its treatment. Thus a longer or more toxic treatment is not always associated with lower quality of life. Quality of life

can even improve when therapy has no objective effect; this may be due to the placebo effect, to the provision of hope, or to the increased medical attention associated with being in a study. Neither are side-effects the major determinants of quality of life.[22]

Comparison of our results with previous randomized controlled trials on adjuvant therapies in pancreatic and periampullary cancer[2, 6] shows that this is the first trial to demonstrate that adjuvant therapy benefited quality of life up to 24 months after resection. One of the reasons for this difference is that the CONKO-001 trial used the Spitzer Quality of Life Index, a questionnaire focusing on more non-specific quality of life parameters, such as daily activity, social support and mental well being. Our use of the EORTC QLQ-C30, which focuses both on functioning scales and on cancer-specific complaints such as pain, fatigue and nausea and vomiting, is important, as it is especially on these symptom scores that we found a beneficial difference for the CAI/RT arm. Although the ESPAC-1 trial also evaluated quality of life with the EORTC QLQ-C30 questionnaire, it produced a quality of life response percentage of 53%, and measured quality of life for only the first 12 months after resection. In our trial, the most noticeable differences in all quality of life dimensions occurred after these first 12 months. If the ESPAC-1 trial had continued to assess quality of life over a longer period, it might have found a similar difference, although this effect might also have been reduced by the two-by-two factorial design.

Since survival after adjuvant therapy for resected pancreatic or periampullary cancer has not lengthened substantially over the years, the aim of adjuvant therapy in these patients should at least be to improve the quality of life during the short remaining lifetime. We have shown that CAI/RT, although a demanding therapy, improves quality of life from baseline. We therefore state that it is incorrect to deny cancer patients intensive therapies only because they do not result in a significant survival benefit. One of the possible objective factors underlying this beneficial effect on the quality of life is the fact that time to progression is extended. Where patients with recurrent disease develop duodenal obstruction and especially pain, the postponement of recurrence leads to a longer complaint-free period and thus a higher quality of life. Because none of our patients were treated with chemotherapy or radiotherapy after recurrence, there is no treatment related effect causing a decline in quality of life in the second half of the follow up.

In conclusion, 5-FU based intra-arterial chemotherapy combined with radiotherapy improves quality of life compared to observation alone in patients with resected pancreatic and periampullary cancers. Over a period of 24 months, patients suffer from significantly less pain and significantly less nausea and vomiting. This beneficial effect of CAI/RT is most prominent in the latter half of the follow-up and is probably caused by a delay in tumor recurrence and related symptoms. Given the disappointing effect on overall survival of adjuvant therapy after pancreatoduodenectomy, quality of life during this period is of eminent importance. Impairment in quality of life during or after treatment is no longer a reason to deny patients adjuvant therapies.

REFERENCES

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007;57(1): 43-66.
2. Neoptolemos JP, Dunn JA, Stocken DD, Almond J, Link K, Beger H, Bassi C, Falconi M, Pederzoli P, Dervenis C, Fernandez-Cruz L, Lacaine F, Pap A, Spooner D, Kerr DJ, Friess H, Buchler MW. Adjuvant chemoradiotherapy and chemotherapy in resectable pancreatic cancer: a randomised controlled trial. Lancet 2001;358(9293): 1576-1585.
3. Willett CG, Czito BG, Bendell JC. Adjuvant therapy of pancreatic cancer. Cancer J 2007;13(3): 185-191.
4. Bassi C, Stocken DD, Olah A, Friess H, Buckels J, Hickey H, Dervenis C, Dunn JA, Deakin M, Carter R, Ghaneh P, Neoptolemos JP, Buchler MW. Influence of surgical resection and post-operative complications on survival following adjuvant treatment for pancreatic cancer in the ESPAC-1 randomized controlled trial. Dig Surg 2005;22(5): 353-363.
5. Kennedy EP, Yeo CJ. Pancreaticoduodenectomy with extended retroperitoneal lymphadenectomy for periampullary adenocarcinoma. Surg Oncol Clin N Am 2007;16(1): 157-176.
6. Oettle H, Post S, Neuhaus P, Gellert K, Langrehr J, Ridwelski K, Schramm H, Fahlke J, Zuelke C, Burkart C, Gutberlet K, Kettner E, Schmalenberg H, Weigang-Koehler K, Bechstein WO, Niedergethmann M, Schmidt-Wolf I, Roll L, Doerken B, Riess H. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. Jama 2007;297(3): 267-277.
7. Fingerhut A, Vassiliu P, Dervenis C, Alexakis N, Leandros E. What is in a word: Pancreatoduodenectomy or pancreaticoduodenectomy? Surgery 2007;142(3): 428-429.
8. Nieveen van Dijkum EJ, Kuhlmann KF, Terwee CB, Obertop H, de Haes JC, Gouma DJ. Quality of life after curative or palliative surgical treatment of pancreatic and periampullary carcinoma. Br J Surg 2005;92(4): 471-477.
9. Crippa S, Dominguez I, Rodriguez JR, Razo O, Thayer SP, Ryan DP, Warshaw AL, Fernandez-del Castillo C. Quality of life in pancreatic cancer: analysis by stage and treatment. J Gastrointest Surg 2008;12(5): 783-793; discussion 793-784.
10. Kokoska ER, Stapleton DR, Virgo KS, Johnson FE, Wade TP. Quality of life measurements do not support palliative pancreatic cancer treatments. Int J Oncol 1998;13(6): 1323-1329.
11. Huang JJ, Yeo CJ, Sohn TA, Lillemoe KD, Sauter PK, Coleman J, Hruban RH, Cameron JL. Quality of life and outcomes after pancreaticoduodenectomy. Ann Surg 2000;231(6): 890-898.
12. A multi-institutional comparative trial of radiation therapy alone and in combination with 5-fluorouracil for locally unresectable pancreatic carcinoma. The Gastrointestinal Tumor Study Group. Ann Surg 1979;189(2): 205-208.
13. Further evidence of effective adjuvant combined radiation and chemotherapy following curative resection of pancreatic cancer. Gastrointestinal Tumor Study Group. Cancer 1987;59(12): 2006-2010.
14. Kalser MH, Ellenberg SS. Pancreatic cancer. Adjuvant combined radiation and chemotherapy following curative resection. Arch Surg 1985;120(8): 899-903.
15. Klinkenbijl JH, Jeekel J, Sahmoud T, van Pel R, Couvreur ML, Veenhof CH, Arnaud JP, Gonzalez DG, de Wit LT, Hennipman A, Wils J. Adjuvant radiotherapy and 5-fluorouracil after curative resection of cancer of the pancreas and periampullary region: phase III trial of the EORTC gastrointestinal tract cancer cooperative group. Ann Surg 1999;230(6): 776-782; discussion 782-774.
16. Neoptolemos JP, Cunningham D, Friess H, Bassi C, Stocken DD, Tait DM, Dunn JA, Dervenis C, Lacaine F, Hickey H, Raraty MG, Ghaneh P, Buchler MW. Adjuvant therapy in pancreatic cancer: historical and current perspectives. Ann Oncol 2003;14(5): 675-692.

17. Smeenk HG, van Eijck CH, Hop WC, Erdmann J, Tran KC, Debois M, van Cutsem E, van Dekken H, Klinkenbijl JH, Jeekel J. Long-term survival and metastatic pattern of pancreatic and periampullary cancer after adjuvant chemoradiation or observation: long-term results of EORTC trial 40891. Ann Surg 2007;246(5): 734-740.
18. Neoptolemos JP, Stocken DD, Friess H, Bassi C, Dunn JA, Hickey H, Beger H, Fernandez-Cruz L, Dervenis C, Lacaine F, Falconi M, Pederzoli P, Pap A, Spooner D, Kerr DJ, Buchler MW. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. N Engl J Med 2004;350(12): 1200-1210.
19. Morak MJ, van der Gaast A, Incrocci L, van Dekken H, Hermans JJ, Jeekel J, Hop WC, Kazemier G, van Eijck CH. Adjuvant intra-arterial chemotherapy and radiotherapy versus surgery alone in resectable pancreatic and periampullary cancer: a prospective randomized controlled trial. Ann Surg 2008;248(6): 1031-1041.
20. Aaronson NK, Ahmedzai S, Bergman B, Bullinger M, Cull A, Duez NJ, Filiberti A, Flechtner H, Fleishman SB, de Haes JC, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. J Natl Cancer Inst 1993;85(5): 365-376.
21. Sloan J. Asking the obvious questions regarding patient burden. J Clin Oncol 2002;20(1): 4-6.
22. Slevin ML. Quality of life: philosophical question or clinical reality? Bmj 1992;305(6851): 466-469.

CHAPTER 4

Long-term Survival after Adjuvant Intra-Arterial Chemotherapy and Radiotherapy versus Surgery Alone in Resectable Pancreatic and Non-Pancreatic Periampullary Cancer

Improved survival by prevention of liver metastases in non-pancreatic cancers

J. Erdmann[1], M.J.M. Morak[1], H.J. Duivenvoorden[2], H.van Dekken[3], G. Kazemier[1], N.F.M. Kok[1], C.H.J. van Eijck[1]

[1] Department of Surgery Erasmus MC, Rotterdam The Netherlands.
[2] Methodologist-statistician, Erasmus MC, Rotterdam, The Netherlands.
[3] Department of Pathology Erasmus MC, Rotterdam, The Netherlands.

Accepted HPB

ABSTRACT

Background: In pursuit of improvement of survival after surgery for pancreatic cancer, several randomized trials have been conducted offering adjuvant chemotherapy with or without concomitant radiotherapy. For non-pancreatic periampullary cancers however, no proven adjuvant therapy is available.

Objective: To compare the effect on suvival of adjuvant intra-arterial chemotherapy with concomitant radiotherapy (CAI/RT) to surgery alone.

Methods: In 2008 we published the results of a randomized controlled trial comparing intra-arterial chemotherapy with concomitant radiotherapy (CAI/RT) to observation. We now present the data after complete 5-year follow-up of all patients.

Results: Long-term survival (>60 months) was observed in 19 patients. CAI/RT did not influence survival for pancreatic cancer (20 vs 21 months p=0.9), For CAI/RT in non-pancreatic periampullary cancers, median survival was 37 vs 28 months and a doubling of the number of long-term survivors from 5 to 10 was seen. Interestingly, occurrence of livermetastases was also reduced by CAI/RT, from 57% to 29% (p=0.04). Cox regression revealed a substantial effect of CAI/RT on survival (hazard ratio 0.436, 95% CI: 0.231 to 0.825, p=0.01). The other independent factor was differentiation grade: p<0.01 (hazard ratio 2.548, 95% CI: 1.529 to 4.242).

Conclusions: This long term-analysis shows that for non-pancreatic periampullary cancers median and long-term survival is greatly improved after CAI/RT, probably because of the effective reduction of livermetastases. We advocate further investigation of this concept of CAI/RT.

INTRODUCTION

The treatment of adenocarcinoma of the pancreas and periampullary region remains a challenge. Even for those few patients who are amenable to resection with curative intent overall survival remains poor. Most tumors arise in the pancreatic head near the ampulla of Vater. The majority of these tumors are of pancreatic ductal origin. Less frequently tumors in the pancreatic head region arise from the distal common bile duct, ampulla of Vater and duodenum, collectively and sometimes confusingly, known as periampullary cancers.[1] Although histologically very similar, these tumors carry a more favorable prognosis. The classical hypothesis is that these tumors are diagnosed at an earlier stage because they lead to jaundice early due to their anatomical location. Another reason for early detection may be that some could be discovered at routine endoscopy. For these reasons non-pancreatic periampullary tumors may more frequently be amenable to surgical resection than their pancreatic counterparts.

On the other hand non-pancreatic periampullary cancers may represent a separate family of tumors with a different biological behavior. This hypothesis is supported by the superior survival of periampullary cancers, even after having taken into account tumor size, positive lymph nodes and stage.[2, 3]

In pursuit of improvement of survival after surgery for pancreatic cancer, several randomized trials have been conducted offering adjuvant chemotherapy both with and without concomitant radiotherapy. This has recently led to the consensus that gemcitabine based adjuvant chemotherapy improves outcomes after surgery for pancreatic cancer. The role of adjuvant radiotherapy remains unclear.[4]

On the other hand, the evidence that adjuvant chemotherapy is effective in patients with non-pancreatic periampullary cancers is limited. The first large trial was conducted offering a 5-fluorouracil based regimen with concomitant radiotherapy, but there was no effect on survival.[5] Another, recent, report on gemcitabine based adjuvant therapy showed a small albeit significant improvement in survival after adjuvant chemotherapy.[6]

In 2008 we published the results of a randomized controlled trial comparing intra-arterial chemotherapy with concomitant radiotherapy (CAI/RT) to surgery alone.[7] The groups were stratified for the location of the tumor (i.e. pancreatic or non-pancreatic periampullary),This study showed a prolonged time to progression after CAI/RT. For non-pancreatic periampullary tumors, CAI/RT induced a significant reduction in liver metastases. Median follow-up at the time of publication was 17 months. Since recurrence can be observed up to years after resection and only estimated survival could be calculated from this follow-up, we currently present the data after 5-year follow-up of all patients.

METHODS

The study design was described in the primary analysis in detail[7]. Briefly, patients were randomized after resection into two groups: CAI/RT or no adjuvant therapy. All specimens were reviewed by one specialized pathologist (HvD), graded and staged according to UICC's 2002 guidelines. Stage 1A (T1N0) tumors of the ampullary region were excluded. Determination of tumor origin was based on micro- and macroscopic evaluation of the resected specimen. Duodenal, cystic and neuro-endocrine tumors were excluded. Other exclusion criteria were: age>75, karnofsky-Index =/< 50%, uncontrolled infection, previous chemo- or radiotherapy and aberrant vascular supply to the liver. Enrolled patients were prestratified for tumor origin (pancreatic or non-pancreatic periampullary adenocarcinoma). After recovery from surgery patients were randomized during their first visit to the outpatient clinic, according to a computer generated randomization list provided by the trial statistician. Treatment started within 6-12 weeks after surgery. Patients with complications resulting in a prolonged hospital stay were not randomized. During or before the first CAI and before RT, all patients were restaged by Computed Tomography (CT). Follow-up consisted of clinical and laboratory examinations every 3 months. During the first two years CT was performed every 3 months and every 6 months afterwards. Clinical signs of recurrence were indications for additional imaging. All patients were monitored to 5 years or up to death. All survival data was cross-checked with the national population registry.

Adjuvant treatment

The treatment schedule was described in full detail previously.[7] Chemotherapy was administered through a catheter placed in the celiac trunk and left in place during the five treatment days of each cycle. Heparin was infused to prevent thrombosis. Cycles consisted of mitoxantrone on day one, followed by 5-fluorouracil/ folinic acid on days two to four and cisplatinum on day five. Toxicity was monitored and the dose was reduced 20% in case of toxicity >World Health Organization (WHO) degree II. After two weeks radiotherapy was started. A total of 54Gy was delivered in a single dose of 1.8 Gy five days a week. CAI was continued afterwards up to a total of six cycles with an interval of four weeks between each cycle. Therapy was discontinued in case of serious toxicity, WHO III/IV.

Statistics

Primary outcome was overall survival. Secondary endpoints were toxicity and disease-free survival. Initial power calculations led to a total of 120 patients in each group. The trial was approved by the local ethics committee. Inclusion was stopped because of gemcitabine based adjuvant therapy had become standard-care and an observation-only group was therefore considered unethical.

Analyses were performed on an intention-to-treat basis. IBM SPSS Statistics version 20 for Windows was used for the current analyses. Significance was calculated using Chi-square for categorical and student-t for continuous variables. Statistical significance was defined at p=0.05 (two-sided). All p-values were rounded to two decimals. Survival was estimated using the Kaplan Meier method, significance calculated by log-rank test.
In addition, significant factors from the univariate analysis were entered in a multivariate cox-proportional hazards model. Hazard ratios are shown with 95% confidence interval (95% CI). Grade variables were considered of ordinal level and therefore coded as dummy variables.

RESULTS

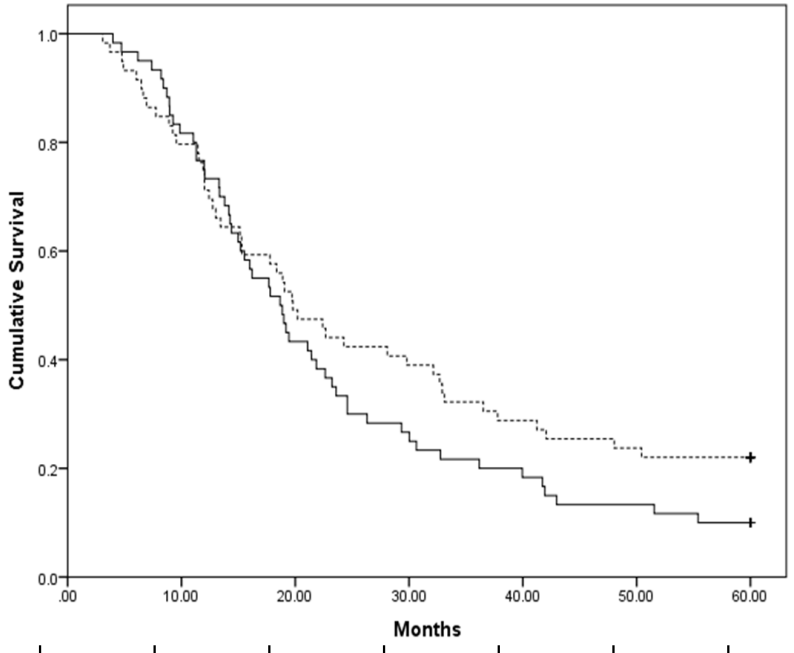
Patients

From a cohort of 256 pancreatoduodenectomies, 120 patients were included in this trial. Fifty-nine patients were randomized to the chemoradiation group and 61 to surgery alone. Baseline characteristics were not statistically significant different between groups except for differentiation grade (more poorly differentiated tumors in the CAI/RT group) and age (60 vs. 66 years).
TNM staging was not different between the groups; neither were presence of lymph nodes (60%) and radicality of resection (73-97%).
Median time from resection to start of CAI/RT was 61 days (range 42 to 96). Full 5-year follow-up was completed for all patients; one patient was lost to follow-up due to emigration.
Of the 59 patients assigned to CAI/RT, three patients refused treatment, three developed liver metastases as seen on the restaging CT-scan and one died waiting for the start of treatment. Therefore 52 patients received adjuvant treatment. Overall toxicity was mild, and led to discontinuation of CAI/RT in 13 patients, seven patients in the pancreatic cancer group and six in the non-pancreatic periampullary group). Thirty-six percent of all patients received the full six cycles of CAI/RT: Six in the pancreatic cancer group and 15 in the non-pancreatic periampullary group. The median number of cycles administered was three in the pancreatic cancer group and six in the non-pancreatic periampullary group.

Overall Survival

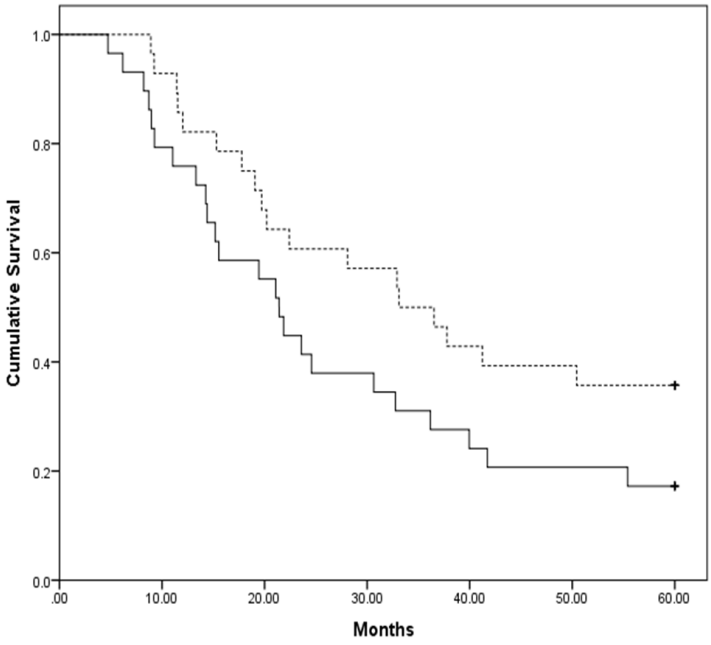
The primary analysis was based on 82 deaths. Longer follow-up led to registration of 18 more events. Therefore survival analysis was based on 100 deaths. Long-term survival (defined as >60 months) was observed in 19 patients (Figure 1). Overall survival for patients suffering from pancreatic cancer is worse than survival for patients suffering from non-pancreatic periampullary cancer, regardless of therapy (median survival 20 vs. 32 months, p<0.01). For the whole study

group CAI/RT did not improve survival (primary endpoint, Figure 1). CAI/RT did not influence survival for pancreatic cancer (20 vs. 21 months p= 0.87 Cox-regression). However, for the stratum non-pancreatic periampullary cancer the number of long-term survivors in the CAI/RT group was twofold the number of long-term survivors after surgery alone (5 vs. 10). Overall survival was also superior after CAI/RT, with a median actual survival of 37 vs. 28 months (Figure 2).The log rank test approached statistical significance (P=0.08). Furthermore, Cox regression revealed a substantial effect of CAI/RT on survival (hazard ratio 0.44, 95% CI: 0.23 to 0.83, p=0.01).The other independent factor in the same regression model was differentiation grade (p<0.01 hazard ratio 2.55, 95% CI: 1.53 to 4.24). Factors were tested for their independent contribution in the model. Kaplan-Meier analysis of well, moderate and poorly differentiated carcinomas showed that well differentiated non-pancreatic periampullary cancers did not benefit from CAI/RT (median survival 54 months after surgery only vs. 44 months with CAI/RT, p=0.38). When only moderate/ poor differentiated tumors are included in the Kaplan Meier analyses, significant improvement of survival after CAI/RT is found (median 22 vs. 36 months, p<0.01 log-rank).



Months	0	10	20	30	40	50	60
CAI/RT	59	48	30	24	18	15	13
Surgery only	61	50	27	17	12	9	6

Figure 1: Kaplan Meier, overall survival CAI/RT (dotted line) vs Surgery alone, P= 0.163 log-rank.

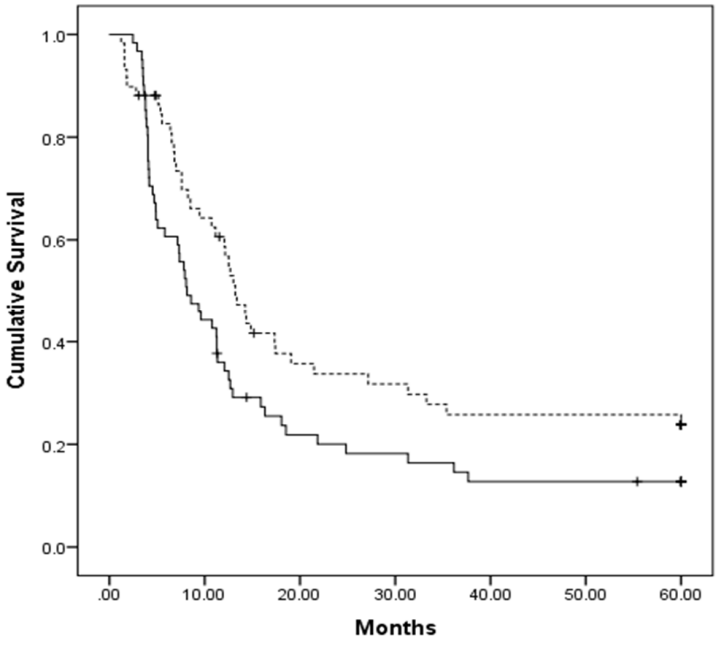


Months	0	10	20	30	40	50	60
CAI/RT	28	27	20	17	13	12	10
Surgery only	30	23	17	12	8	7	5

Figure 2: Kaplan Meier, overall survival, non-pancreatic periampullary cancer: CAI/RT (dotted line) vs Surgery only, p = 0.08 log-rank, hazard ratio 0.436, 95% CI: 0.231 to 0.825, p=0.01.

Disease free survival

Time to progression was longer for patients treated by CAI/RT for the whole study group, (13 vs. 8 months p=0.03, Figure 3). There was no significant effect of CAI/RT on time to progression in pancreatic cancer (12 vs. 8 months, p=0.21 log-rank, cox-regression p=0.24). For non-pancreatic periampullary cancer prolonged time to progression was assessed for the CAI/RT group (Figure 4, 19 vs. 8months, P=0.10 log-rank, Hazard ratio for recurrent disease 0.48 95% CI: 0.25 to 0.90 p= 0.02). Again, when well-differentiated cancers were excluded, log-rank test of Kaplan-Meier became significant (p=0.01). Recurrences were observed in 93 patients. The recurrence pattern is shown in Table 1. Interestingly, occurrence of liver metastases in patients with non-pancreatic periampullary cancer was identical to the primary analysis; CAI/RT effectively reduced the occurrence from 17 to 8 cases (p = 0.04 Chi-square, hazard ratio 3.27 95% CI: 1.10 to 9.80), no effect could be shown in time to occurrence of these liver metastasis.

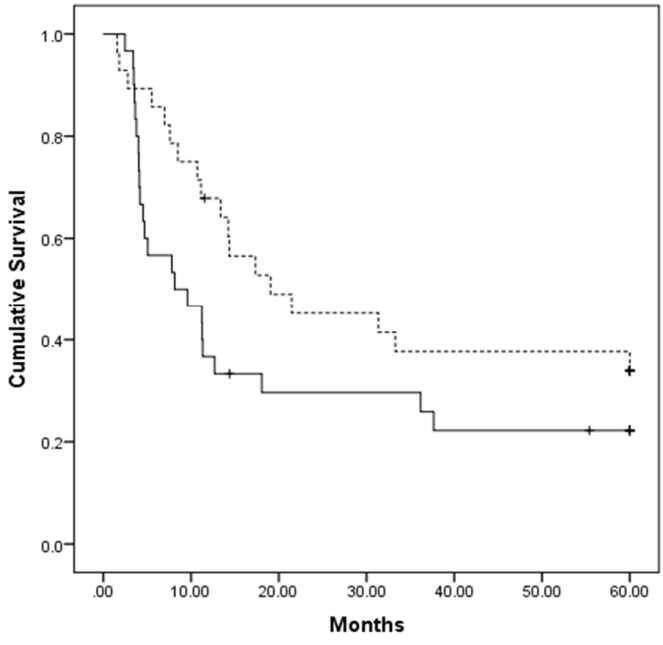


Months	0	10	20	30	40	50	60
CAI/RT	59	36	19	17	13	13	13
Surgery only	61	27	12	10	7	6	6

Figure 3: Kaplan Meier, disease-free survival, CAI/RT (dotted line) vs Surgery only, p = 0.04.

Table 1. Recurrence pattern, PC: pancreatic cancer, NPAC: non-pancreatic periampullary cancer.

Recurrence pattern	PC					NPAC				
	CAI/RT	%	Control	%	P	CAI/RT	%	Control	%	P
N	31		31			28		30		
First Recurrence										
Local	14	45%	19	61%	0.30	11	39%	9	30%	0.60
Liver	14	45%	11	35%	0.60	7	25%	13	43%	0.17
Local and Liver	6	19%	4	13%	0.73	2	7%	3	10%	1.00
Lung	5	16%	3	10%	0.71	3	11%	3	10%	1.00
Other	0	0%	6	19%	0.03	3	11%	3	10%	1.00
Cumulative Recurrence										
Local	15	48%	22	71%	0.12	13	46%	12	40%	0.79
Liver	15	48%	16	52%	1.00	8	29%	17	57%	0.04
Local and Liver	7	23%	11	35%	0.26	4	14%	7	23%	0.51
Lung	5	16%	3	10%	0.71	3	11%	5	17%	0.71
Other	1	3%	9	29%	0.01	5	18%	5	17%	1.00



Months	0	10	20	30	40	50	60
CAI/RT	28	21	13	12	10	10	10
Surgery only	30	14	8	8	6	5	5

Figure 4: Disease-free survival, non-pancreatic periampullary cancer, Kaplan Meier, CAI/RT (dotted line) vs. Surgery only, (19 vs. 8months, P = 0.10 log-rank, Hazard ratio for recurrent disease 0.48 95% CI: 0.25 to 0.90 p = 0.02).

DISCUSSION

For pancreatic cancer there appears to be no survival benefit for adjuvant CAI/RT and gemcitabine-based regimens have proven their effectivity.[6] The effect of CAI/RT in this group is disappointing and confirms that true pancreatic cancer has a dismal prognosis despite the addition of chemotherapy and radiotherapy. However, for non-pancreatic periampullary cancer, adjuvant CAI/RT improves overall survival and disease-free survival. This is remarkable and therefore we would like to draw attention to the benefit of the aforementioned adjuvant regimen in the latter group.

The evidence in the literature for multimodality treatment of non-pancreatic periampullary tumors is sparse. The first large randomized controlled trial to investigate non-pancreatic periampullary cancers was published by Klinkenbijl et al. in 1999.[5] Long-term follow-up was published in 2007.[8]

In this EORTC trial adjuvant 5-fluorouracil based chemotherapy with concomitant radiotherapy (40Gy) was compared to observation. No significant differences were found regarding survival. Liver metastases occurred in 50 % of the patients. There were no significant differences in occurrence of these liver metastases between treatment groups. Recently, the ESPAC-3 trial for periampullary cancer was published.[6] This trial compared three study groups; 5-fluorouracil based chemotherapy, gemcitabine based chemotherapy and observation only. In analysis of the primary endpoint, significance with regard to overall survival was not observed. After taking into account other variables (i.e. differentiation grade) in secondary analyses they demonstrated a beneficial effect for adjuvant chemotherapy (hazard ratio 0.75, 95% CI: 0.57 to 0.98 p= 0.03). This effect was smaller than the effect of CAI/RT as observed in the current study.

The rationale for CAI/RT in the present study was twofold: reduction of liver metastases by CAI and improved local control by RT. And indeed, for non-pancreatic periampullary cancers CAI/RT led to reduction of occurrence of liver metastases and a significant effect was observed on median survival, disease-free survival and also more long-term survivors (5 patients in observation group vs. 10 in CAI/RT). We could not show a significant effect on local recurrence. The EORTC study also included RT, 40Gy compared to the 54Gy in the present study. Bearing in mind the effects of RT in the ESPAC-1 trial[9] where it was even detrimental for survival, it is more likely that CAI is responsible for fewer liver metastases and consequent effect on survival and not RT. This strengthens the conclusions of both the EORTC[5] and ESPAC-1[9] trials that the role for adjuvant RT in the prevention of local recurrence of (non-)pancreatic periampullary cancers is doubtful.

Two phase II clinical trials and a case study preceded this trial.[10-12] Both trials showed a decrease in liver metastases and improved survival after CAI. The rationale is that by infusing selectively, a much higher dose can be achieved in the target organ, in this case the liver. We could not confirm these results for pancreatic cancers, but a clear effect was observed in the non-pancreatic periampullary cancer group. It is not precluded that for pancreatic cancers, the mitoxantrone, 5-flourouracil and cisplatinum combination used in this study may be inferior to a gemcitabine based regimen. Furthermore, recent developments of new therapeutic agents and combination therapy have led to more effective systemic therapy in metastatic pancreatic cancer using a 5-fluorouracil, leucovorin, irinotecan en oxaliplatin (Folfirinox) regimen.[13] We speculate that a different agent or combination could be effective as CAI in pancreatic cancers.

In the Cox regression analysis we showed that both differentiation grade and adjuvant treatment were of independent influence on overall and disease-free survival. Differentiation grade was inversely correlated with survival, which is concordant with other studies.[6, 8] Since survival after resection of well differentiated cancers is already much better than for moderately/poorly differentiated tumors, the effect of adjuvant therapy may be concealed. In our study we found

no significant difference between treatment and observation for these patients (54 vs 44 months median survival, p=0.38). When we corrected for this effect in the multivariate analysis, the treatment effect proved to be significant. Also the survival benefit for moderate and poorly differentiated tumors proved to be larger after CAI/RT compared to observation (median 36 vs. 22 months, p<0.01 log-rank).

This long term analysis shows interesting effects of adjuvant CAI/RT on survival in patients with non-pancreatic periampullary cancer that were not revealed in the primary analysis. Strengths of this study include its randomized design with prestratification by tumor origin. We presented follow-up of all patients to at least 5 years or to death. However, we admit the weaknesses of the study. First, the observational group did not receive adjuvant treatment, which is standard care nowadays. When we initiated this trial, studies like the ESPAC-1 or CONKO-1 were not published yet. We included radiotherapy as part of the protocol because we expected to be able to reduce local relapse. Analysis of our results reveals that, although some effect can be seen in patients with pancreatic cancer, there is no significant effect of radiotherapy on local recurrence rates. Second, the early break-up of the study led to groups which were twofold smaller than originally planned and thus a type two statistical error could occur in the pancreatic cancer group. Nevertheless, we addressed a significant and substantial effect of CAI/RT in non-pancreatic periampullary tumors in this relatively small group. Furthermore, one could argue that the majority of patients did not receive all planned cycles of CAI. We acknowledge this, but would like to stress that for most patients it was because of early progression of the disease. The timing of randomization after recovery from surgery may be debated. Ideally, further randomized studies on adjuvant therapies in patients with either pancreatic cancer or non-pancreatic periampullary cancer would rather assign patients to either treatment before surgery. Probably a significant number of patients would drop out of the study because of irresectability, postoperative morbidity and mortality and withdrawal. Finally, one could argue that the proposed schedule of CAI/RT is intense and would interfere with quality of life during the short time most of the patients live after "curative" resection. We previously published that quality of life[14] was not adversely affected by this adjuvant regimen and toxicity was relatively mild.[7]

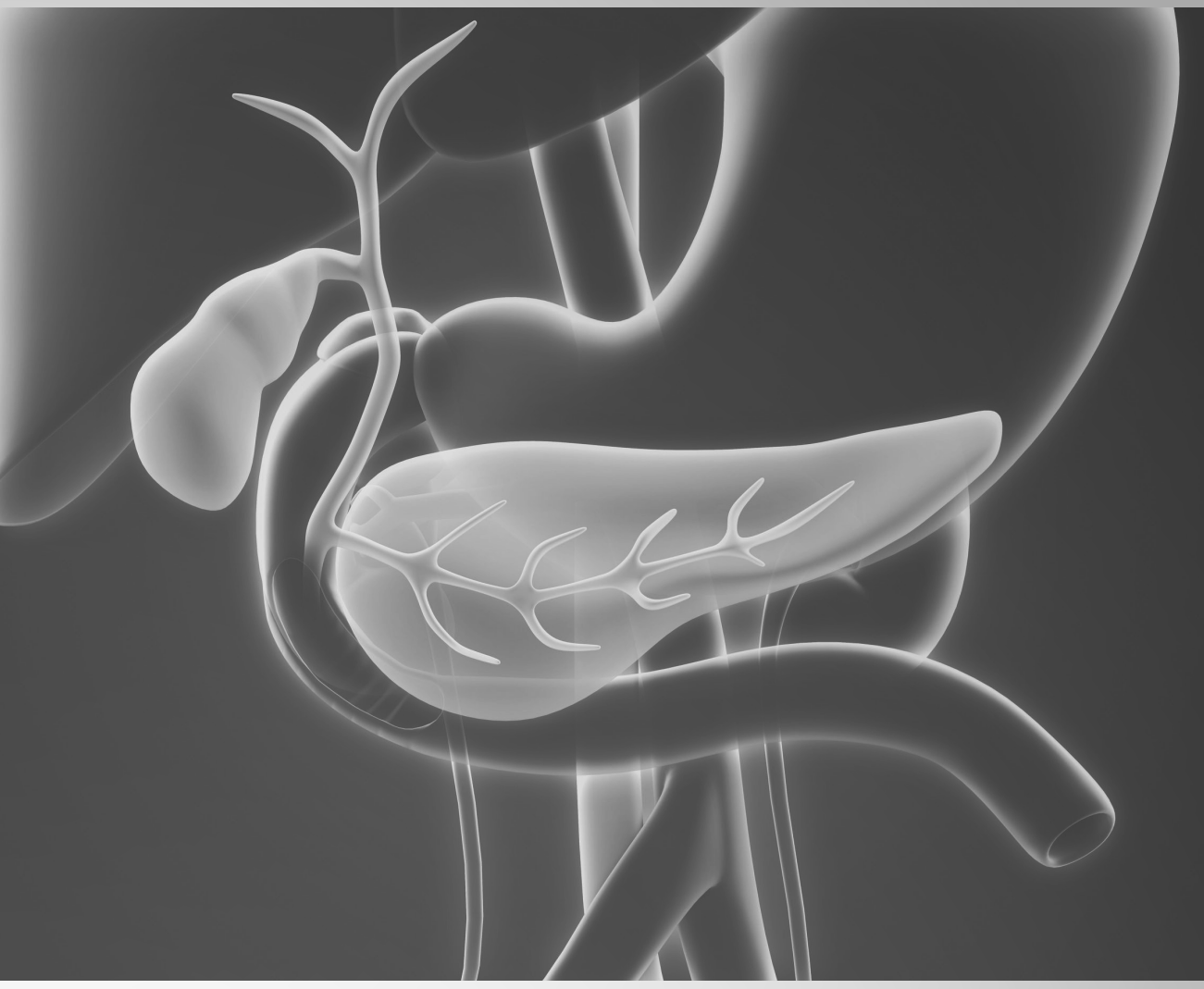
In the current study we deliberately differentiate between pancreatic and non-pancreatic periampullary cancers. Interestingly, "periampullary" cancers were only intentionally included in three other randomised trials.[5, 6, 15] As was also clearly described in the recent paper by Verbeke et al[1, 16], correct classification of periampullary cancers can be difficult for inexperienced pathologists. Especially large multicenter studies without central pathology control are at risk of falsely including non-pancreatic tumors in a pancreatic cancer study group and vice versa. When considering the difference in effect on survival by CAI/RT between pancreatic and non-pancreatic periampullary

cancers in our study, it is clear that correct differentiation between these two groups is essential for the interpretation of effects of adjuvant therapy in randomized trials.

In conclusion, patients with resectable non-pancreatic periampullary cancer may benefit from adjuvant CAI/RT as it significantly prolongs overall and disease-free survival, probably because of the effective reduction of liver metastasis. Further trials should clearly distinguish non-pancreatic periampullary cancer from pancreatic cancer. In the past, the differences between these two cancers had impact only on prognosis. Now, it may guide clinical management.

REFERENCES

1. Verbeke CS, Gladhaug IP. Resection margin involvement and tumour origin in pancreatic head cancer. Br J Surg; 99(8):1036-49.
2. Klempnauer J, Ridder GJ, Pichlmayr R. Prognostic factors after resection of ampullary carcinoma: multivariate survival analysis in comparison with ductal cancer of the pancreatic head. Br J Surg 1995; 82(12):1686-91.
3. Smeenk HG, Erdmann J, van Dekken H, et al. Long-term survival after radical resection for pancreatic head and ampullary cancer: a potential role for the EGF-R. Dig Surg 2007; 24(1):38-45.
4. Sultana A, Cox T, Ghaneh P, Neoptolemos JP. Adjuvant therapy for pancreatic cancer. Recent Results Cancer Res; 196:65-88.
5. Klinkenbijl JH, Jeekel J, Sahmoud T, et al. Adjuvant radiotherapy and 5-fluorouracil after curative resection of cancer of the pancreas and periampullary region: phase III trial of the EORTC gastrointestinal tract cancer cooperative group. Ann Surg 1999; 230(6):776-82; discussion 782-4.
6. Neoptolemos JP, Moore MJ, Cox TF, et al. Effect of adjuvant chemotherapy with fluorouracil plus folinic acid or gemcitabine vs observation on survival in patients with resected periampullary adenocarcinoma: the ESPAC-3 periampullary cancer randomized trial. JAMA; 308(2):147-56.
7. Morak MJ, van der Gaast A, Incrocci L, et al. Adjuvant intra-arterial chemotherapy and radiotherapy versus surgery alone in resectable pancreatic and periampullary cancer: a prospective randomized controlled trial. Ann Surg 2008; 248(6):1031-41.
8. Smeenk HG, van Eijck CH, Hop WC, et al. Long-term survival and metastatic pattern of pancreatic and periampullary cancer after adjuvant chemoradiation or observation: long-term results of EORTC trial 40891. Ann Surg 2007; 246(5):734-40.
9. Neoptolemos JP, Dunn JA, Stocken DD, et al. Adjuvant chemoradiotherapy and chemotherapy in resectable pancreatic cancer: a randomised controlled trial. Lancet 2001; 358(9293):1576-85.
10. Hayashibe A, Kameyama M, Shinbo M, Makimoto S. Clinical results on intra-arterial adjuvant chemotherapy for prevention of liver metastasis following curative resection of pancreatic cancer. Ann Surg Oncol 2007; 14(1):190-4.
11. Beger HG, Gansauge F, Buchler MW, Link KH. Intraarterial adjuvant chemotherapy after pancreaticoduodenectomy for pancreatic cancer: significant reduction in occurrence of liver metastasis. World J Surg 1999; 23(9):946-9.
12. Ishikawa O, Ohhigashi H, Sasaki Y, et al. Extended pancreatectomy and liver perfusion chemotherapy for resectable adenocarcinoma of the pancreas. Digestion 1999; 60 Suppl 1:135-8.
13. Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med; 364(19):1817-25.
14. Morak MJ, Pek CJ, Kompanje EJ, et al. Quality of life after adjuvant intra-arterial chemotherapy and radiotherapy versus surgery alone in resectable pancreatic and periampullary cancer: a prospective randomized controlled study. Cancer; 116(4):830-6.
15. Bakkevold KE, Arnesjo B, Dahl O, Kambestad B. Adjuvant combination chemotherapy (AMF) following radical resection of carcinoma of the pancreas and papilla of Vater--results of a controlled, prospective, randomised multicentre study. Eur J Cancer 1993; 29A(5):698-703.
16. Erdmann J, van Eijck CH. Resection margin involvement and tumour origin in pancreatic head cancer (Br J Surg 2012; 99: 1036-1049). Br J Surg; 100(2):299.



PART II

Unresectable pancreatic and periampullary cancer

CHAPTER 5

Staging for locally advanced pancreatic cancer

M.J.M. Morak[1], J.J. Hermans[2], H.G. Smeenk[1], W.M. Renders[1],
J.J.M.E. Nuyttens[3], G. Kazemier[1] and C.H.J. van Eijck[1]

[1]Department of Surgery, Erasmus Medical Centre, Rotterdam, The Netherlands
[2]Department of Radiology, Erasmus Medical Centre, Rotterdam, The Netherlands
[3]Department of Radiotherapy, Erasmus Medical Centre, Rotterdam, The Netherlands

Eur J Surg Oncol. 2009 Sep;35(9):963-8.

ABSTRACT

Aim: To address the role of a dedicated radiologist and high quality CT scanning in staging of patients referred with suspected locally advanced pancreatic cancer. Furthermore, the value of laparoscopy in detecting CT occult metastases in these patients was assessed.

Methods: In a prospective cohort study, 116 patients with suspected unresectable pancreatic cancer referred from peripheral hospitals (107) or our own gastroenterology department (9) were analysed. CT scans from referral centres were reviewed and in case of locally advanced disease or uncertain metastatic disease, patients underwent a laparoscopy to detect CT occult metastases. Patients without metastases were offered 5-FU based chemoradiotherapy.

Results: After reviewing 107 abdominal CT scans from referral centres, 73 (68%) scans had to be repeated due to unacceptable quality. Locally advanced disease was confirmed in 59 (55%) patients and metastatic disease was found in 24 patients (22%). During laparoscopy, metastases were found in 24/ 68 (35%) patients with locally advanced disease on CT scan and metastases were confirmed in 3/5 (60%) with suspected metastases.
Overall, only 46/ 116 (40%) patients with suspected unresectable disease appeared to have locally advanced pancreatic cancer after adequate staging including laparoscopy in our centre.

Conclusion: Correct staging is difficult in patients with suspected locally advanced pancreatic cancer and should preferably be performed in centres with technically advanced equipment and experienced radiologists. Laparoscopy should be offered to patients before locoregional therapy.

INTRODUCTION

Pancreatic carcinoma is the 4th most common cause of cancer related death worldwide.[1] Despite improved quality of surgery, the prognosis of patients with pancreatic cancer is still dismal. Additional treatment seems essential to improve outcome in these patients. Several studies have shown benefit for neoadjuvant or adjuvant treatment in patients with resectable pancreatic cancer.[2-4] In contrast, 80-90% of patients suffer from either unresectable or metastatic disease at the time of diagnosis. In these patients, no curation can be established and survival relies on the effectiveness of palliative chemotherapy and/ or radiation.

The introduction of spiral or helical computed tomography (CT) scanning has greatly affected the selection of patients eligible for surgical exploration, thus increasing overall resectability rates in these patients. Vascular tumour ingrowth for example, can be visualised with pre-operative helical scanning with high accuracy.[5] However, small metastatic lesions can be missed.[6-8]

Laparoscopy has proven to be able to detect these metastases that mainly consist of peritoneal and superficial liver lesions. As a result several groups recommend diagnostic laparoscopy in combination with or without laparoscopic ultrasonography as a standard procedure in the staging of all patients with resectable as well as unresectable pancreatic cancer.[9,10] However recent studies using high quality CT-scanning in resectable pancreatic cancer patients revealed that only a small percentage of these patients would benefit from a routine diagnostic laparoscopy.[11,12] Nieveen et al. found a 13% detection rate of metastatic disease during laparoscopy in a group of 297 consecutive patients with resectable disease. The low detection rate and subsequent need for, and excellent long term results of, bypass surgery validate the conclusion that general use of diagnostic laparoscopy in all pancreatic cancer patients is not cost-effective and not justified.[12] Tilleman et al. concluded that there is no indication to perform a routine laparoscopy in patients with resectable pancreatic cancer, but that there might be an indication in a, non-specified, well-defined high-risk subgroup.[11]

Patients with locally advanced disease in the work-up, are known to have this higher incidence of CT-occult liver and peritoneal metastases[13] and laparoscopy has been reported to be of additional value to improve assessment of tumour stage in these patients with metastatic detection rates varying between 34-37%.[6,14,15]

Because a laparoscopy appears most valuable in patients with locally unresectable but not in resectable disease, cautious staging based on CT scanning is of utmost importance. However, patients with pancreatic cancer often present in non-specialised centres without high quality CT scans or specific pancreatic imaging protocols. After referral to specialised centres, the quality of the CT scans has to be assessed and designated radiologists review appropriate scans. New scans are made in case of poor quality.

Several studies imply that chemotherapy should be offered to patients with locally advanced pancreatic cancer as in metastatic disease because of disappointing results of chemoradiotherapy. However, laparoscopy was no part of the staging regime in these studies resulting in the inclusion of metastasised disease and thus, considering the fact that chemoradiotherapy only has an effect on localised disease, an underestimation of the effect of chemoradiotherapy.[16-18] Based on previous results in our centre, chemoradiotherapy is a promising option in patients with unresectable, non-metastasised pancreatic cancer based on laparoscopy.[19]
The aim of this study was to address the role of a dedicated radiologist and high quality CT scanning in staging of patients referred with suspected locally advanced pancreatic cancer. Furthermore, the value of laparoscopy in detecting CT occult metastases in these patients was assessed.

METHODS

In this study we evaluated all patients referred to the surgical department of the Erasmus Medical Centre (Erasmus MC) between January 1995 and March 2007 with the diagnosis of locally advanced pancreatic cancer based on abdominal CT scan. In most patients with obstructive jaundice, preoperative biliary drainage was performed by endoscopic retrograde cholangiopancreatography (ERCP) and placement of an endoprosthesis; others received either a papillotomy or percutaneous transhepatic drainage.

Abdominal CT Scan

From 1995- 1999 CT scanning in the Erasmus MC was performed using a Siemens Somatom Plus single slice CT (Siemens Medical Solutions; Erlangen, Germany). After 1999 a Siemens multislice spiral CT scanner was used (Siemens Medical Solutions; Erlangen, Germany).
Our standard triphasic pancreatic protocol consists of a non contrast enhanced scan with a slice thickness of 5.0 mm, an arterial/ pancreatic phase at 40 seconds after the initiation of contrast injection (Visipaque 320 mg/ml) with a slice thickness of 3.0 mm, followed by 5.0-mm-thick slices at 80 seconds (portal venous phase). One hundred fifty milliliters of contrast material is injected intravenously at a rate of 3.5 ml/ sec.
One of our designated radiologists (Hermans) reviewed all abdominal CT scans. CT scans from peripheral hospitals were judged as of sufficient quality in case of triphasic scanning, a comparable scanning protocol, a maximum slice thickness of 3.0 mm in arterial/ pancreatic phase and 5.0 mm in portal venous phase and no movement artefacts.

A pancreatic tumour was classified as resectable, uncertain resectable, unresectable, suspected metastatic or metastatic, based on CT scan. In case of metastatic disease, the resectability of the primary tumour was not addressed and could be either resectable or unresectable. All metastatic lesions were histopathologically proven.

In case of (uncertain) resectable disease, a laparotomy was performed outside the perspectives of this study. All patients with unresectable disease and uncertain metastatic disease underwent a laparoscopy.

Unresectable disease was described by Loyer and Phoa defined as: i) infiltration of peripancreatic fatplanes, the hepatoduodenal ligament and the mesentery (Figure 1), ii) >180° encasement of the portal or superior mesenteric vein or of the hepatic or superior mesenteric artery (Figure 1), iii) infiltration of the vessel, i.e. ingrowth or thrombosis (Figure 2).[20,21]

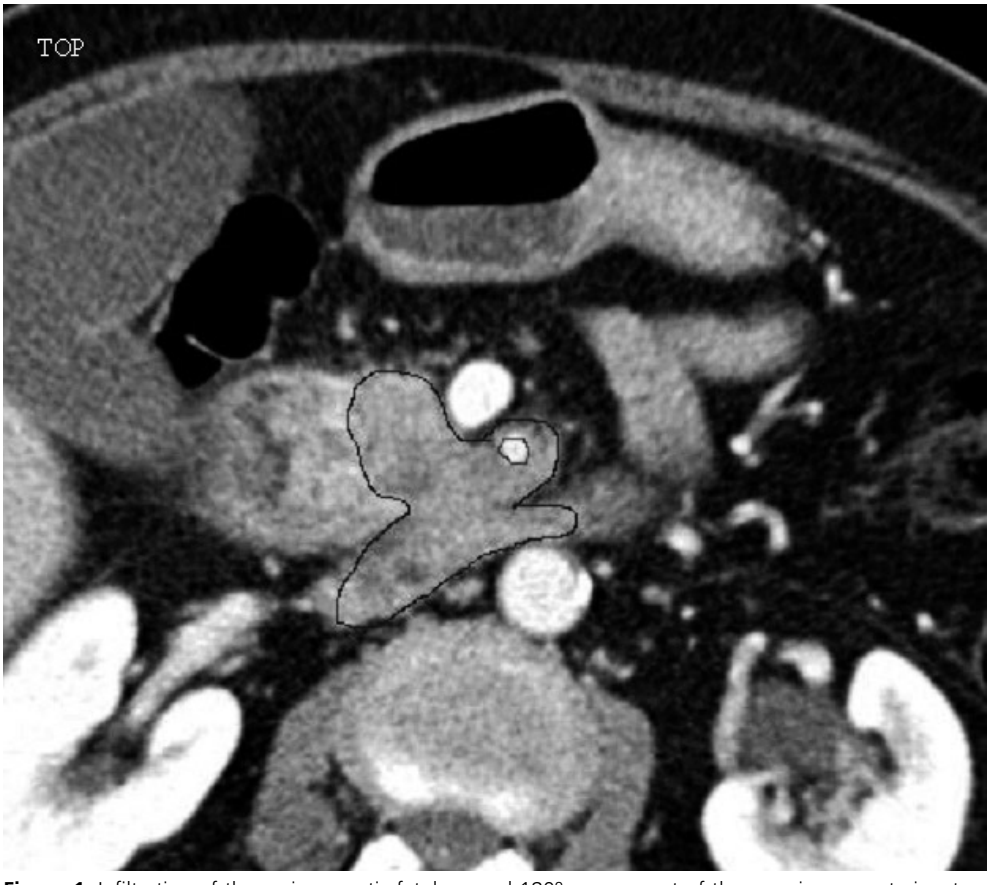


Figure 1: Infiltration of the peripancreatic fatplane and 180° encasement of the superior mesenteric artery (white) by tumour (grey).

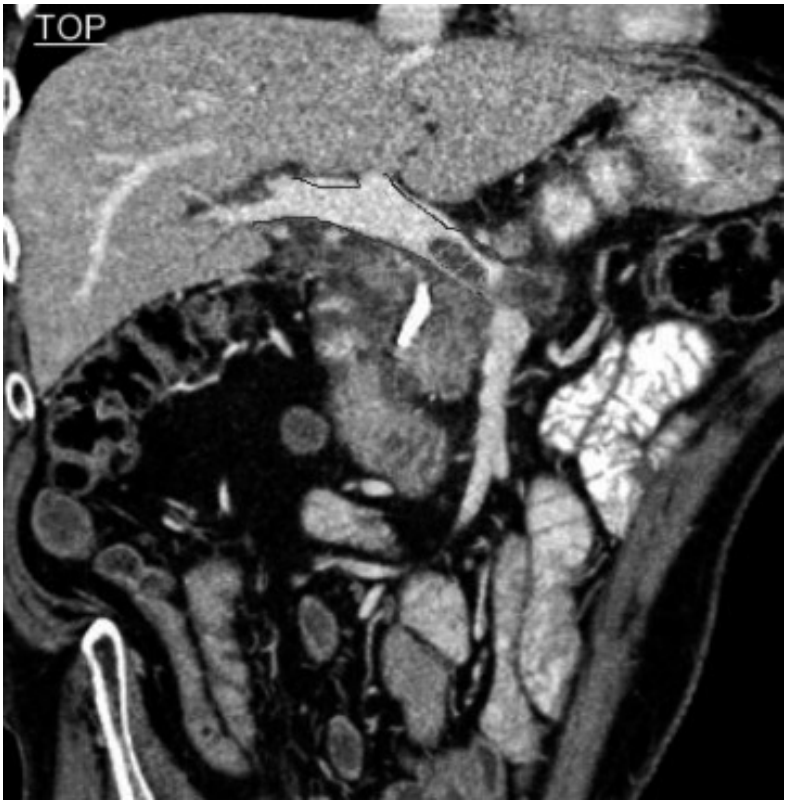


Figure 2: Thrombus (grey) located in the portal vein (white).

Diagnostic Laparoscopy

Patients undergo examination under general anaesthesia as outpatients or with a 24-hour observation stay. Following establishment of pneumoperitoneum, a 10 mm trocar is inserted through an infraumbilical incision. The laparoscope is introduced through this trocar, and examination begins by inspection of the lower abdomen and pelvis. The laparoscope is then rotated for examination of the upper quadrants. Inspection begins by assessment of the omentum and subdiaphragmatic spaces. Meticulous examination of the liver surface is essential. Insertion of a second and third 5-mm trocar in the right upper quadrant is necessary for adequate evaluation of the posterior aspect of the liver and biopsies of suspected lesions. Elevation of the transverse colon and inspection of the mesentery and Treitz' ligament is performed with the patient placed in anti- Trendelenburg position. Finally biopsies of suspected lesions are taken with biopsy forceps (superficial lesions) or with Rotex or Tru-cut needles (deeper lesions) under guidance of the laparoscope. We do not perform extensive dissection of the retrogastric space and lesser sac routinely, nor peritoneal lavage. Laparoscopic ultrasound was not used routinely.

Treatment

If no metastases were found during laparoscopy, a percutaneous or endoscopic ultrasound fine needle aspiration of the primary tumour was performed to confirm malignancy.

All patients with true (laparoscopy based, histopathologically proven) locally advanced, i.e. non metastasised, pancreatic cancer were offered chemoradiotherapy consisting of 5-FU in combination with radiotherapy.

Patients with metastatic disease based on laparoscopy were offered chemotherapy.

Statistics

Analysis was done with the Statistical Package for the Social Sciences version 11.5 (SPSS). Survival curves were computed according to the Kaplan Meier method and comparison of survival curves was done with the log-rank test.

RESULTS

From January 1995 until March 2007 116 consecutive patients referred with the presumed diagnosis of locally advanced pancreatic tumours according to CT scan were incorporated in this prospective cohort study. The median age in these patients was 63 years (range 43-92 years), and 68 of the patients were men. Presenting symptoms were weight loss (n=94), pain (n=87), obstructive jaundice (n=68) and diabetes mellitus de novo (n=13). Ca 19.9 was elevated (>34kU/l) in 102 patients, whereas CEA was elevated (>4.99µg/l) in 56 patients. Tumour size was larger than 3 cm radiologically in 95 patients.

CT Scan

Of the 116 patients, 107 patients were referred from peripheral hospitals, while 9 patients were referred from our own gastroenterology department. CT scans of seventy-three (68%) of the 107 referred patients had to be repeated while the other scans were of predefined quality. Unresectable disease was found in 59 patients and uncertain metastatic disease in 5 patients. Furthermore, resectable disease was found in 6 patients, uncertain unresectable disease in 13 patients and metastatic disease in 24 patients.

The cause of unresectability in all 68 (59 plus the nine patients from our own gastroenterology department) patients is shown in Table I.

Table I: CT criteria for unresectability (n=68)

Infiltration of peripancreatic fatplanes, HDL +/- mesentery	N=1
>180º encasement of the PV, SMV, HA or SMA	N=38
Infiltration of the vessel (i.e. ingrowth or thrombosis)	N=16
Combination of encasement and infiltration of the vessel	N=13

HDL= hepatoduodenal ligament, PV= portal vein, SMV= superior mesenteric vein, HA= hepatic artery, SMA= superior mesenteric artery.

Diagnostic Laparoscopy

The mean amount of days between CT scan and laparoscopy was 13 (range 0- 49 days, SD ±10). The median operative time, including anaesthesia, was 79 minutes (range 41- 130 min). In two patients there was an indication for a consecutive laparoscopic gastroenterostomy.

Twenty-four (35%) of the 68 patients with unresectable disease appeared to have metastatic disease at laparoscopy. Metastases were mainly located on the liver and peritoneum (Table II). No correlation could be found between the reason for unresectability (i.e. ingrowth into peripancreatic fatplane, into adjacent vessels or encasement of these vessels) on CT scan and the presence of metastases at laparoscopy. There was no difference in detection rate between CT scans performed before 1999 (6/16= 27%) and CT scans performed in/or after 1999 (18/28=39%). All metastases were proven histopathologically.

Table II: Laparoscopic findings

No signs of metastases on laparoscopy	N=44 (65%)
Metastases:	N=24 (35%)
liver	*N= 5 (21%)*
peritoneum/ ascites	*N=14 (58%)*
lymph nodes near celiac trunk	*N= 3 (13%)*
liver and peritoneum	*N= 2 (8%)*

There were 2 major complications in the entire group: In one patient the laparoscopic gastroenterostomy was insufficient for which a relaparotomy was necessary and in another patient a prolonged paralytic ileus was seen after laparoscopy. The mean overall hospital stay was 5 days (range 1- 50 days).

Of the 5 patients with suspected metastatic lesions based on CT scan, all lesions were located in the liver. Laparoscopy confirmed these metastases in only three of the patients.

After laparoscopy 44 (65%) of the 68 patients with locally advanced pancreatic cancer on abdominal CT scan were diagnosed with true, non-metastatic, locally advanced disease and two (40%) out of five patients with uncertain metastatic disease. A percutaneous biopsy or fine needle aspiration during subsequent endoscopic ultrasound confirmed malignancy in all these patients.

In conclusion, after reviewing abdominal CT scan and laparoscopy in all 116 patients with suspected unresectable disease at referral, 46/ 116 patients (40%) appeared to have non-metastatic unresectable pancreatic cancer and chemoradiotherapy was offered to all these patients.

DISCUSSION

Because the complexity of pancreatic cancer, the different stages it presents in, the need for exact imaging, the difficulty of surgery and the ever changing non-surgical or adjuvant treatments, it remains a disease best treated in specialised centres. Although for diagnosing pancreatic cancer abdominal CT scan remains an important imaging module, the quality of scans performed in non-specialised centres remains poor and a high quality pancreatic cancer specified protocol CT scan needs to be performed to be able to draw conclusions concerning resectability and the presence of metastases. On the other hand radiologists need to be trained to be able to address pancreatic cancer specific issues, as there are vascular invasion and or encasement. This is based on our results where CT scans had to be repeated in our institution in 68% of the patients and only 55% of the patients referred for unresectable disease based on CT scan, did have an unresectable pancreatic tumour after reviewing. And even our staging methods and radiologist's experience are not flawless. Although we diagnosed locally advanced disease on abdominal CT scan and laparoscopy in 46 patients (i.e. only 40% of the originally referred patients), a laparotomy and thoracic CT scan revealed metastases in six more patients. Because a thoracic CT scan is less invasive than a laparoscopy, a thoracic CT scan before laparoscopy should be considered in future studies on unresectable pancreatic cancer.

Literature

In this second largest group of patients reported in literature with locally advanced pancreatic cancer that underwent a laparoscopy we can confirm the 37% detection rate of CT occult metastases found by Shoup et al.[14] However, where they used a single site for the laparoscope and only inserted a 5-mm trocar in case of suspected metastatic disease, we routinely inserted a second and third 5-mm trocar in the right upper quadrant for adequate evaluation of the posterior aspect of the liver. Consequently, the 37% detection rate after laparoscopy mentioned by Shoup et al. might even be an underestimation. One smaller study performed by Liu found similar results (34% detection rate), but metastases were mainly found through peritoneal lavage cytology during laparoscopy.[6] We did not perform peritoneal lavage cytology. A combination of standard laparoscopy and peritoneal lavage might have led to an even higher detection rate.

Locally advanced vs. metastatic disease

In our opinion it is important to discriminate between locally advanced and metastatic disease because of the implications for further treatment. In the past, locally advanced and metastatic pancreatic cancer was described as one disease entity, i.e. advanced pancreatic cancer, and was consequently treated identical. In this combined group, chemotherapy containing gemcitabine is the better treatment option although, as far as we know, randomised controlled trials containing radiotherapy have not been performed.[22-24]

Locally advanced and metastatic disease should be considered as two different entities. Where the optimal treatment, i.e. chemotherapy or chemoradiotherapy, is still disputable in locally advanced cancer, chemotherapy is the only option for patients with metastasised disease. Overall survival figures in locally advanced disease vary between 11- 14 months after chemotherapy[25,26] and between 7- 15 months after chemoradiotherapy depending on the used chemotherapeutics.[16,17,19,26] In metastatic disease, median survival figures vary between 8- 11 months.

The staging however, remains inadequate because these studies are based on CT imaging only, consequently incorporating metastatic disease in over a third of the included patients. This resulted in underestimating the effect of locoregional therapy, i.e. chemoradiotherapy, in favour of systemic therapy, which proved to be effective in metastatic disease.

In two studies, patients with locally advanced pancreatic cancer were adequately staged.[27,28] Both studies addressed the effect of chemoradiation either in combination with 5-FU and mitomycin-C or with gemcitabine and irinotecan. Cohen et al. reported a median survival of 8.4 *vs.* 7.1 months after radiotherapy with 5-FU and mitomycin-C compared to radiotherapy alone. Mishra et al. preliminary stopped their study after accruing 20 patients because of disappointing results (median survival of 8.8 months). However both studies used a laparotomy as staging module: Cohen staged all patients with laparotomy and Mishra staged at least part of his patients with laparotomy. Their poor results might be explained by the fact that major operative trauma may favour the development of tumour recurrence. An invasiveness dependent association between surgical trauma and tumour recurrence has been supported by previous in vivo and in vitro studies, showing less metastases after laparoscopy compared to laparotomy.[29,30] In our study all patients were staged by laparoscopy, resulting in less surgical stress and indeed a better median overall survival (11.7 months).

Conclusion

In conclusion we think that adequate staging should be performed in a specialised centre because of the considerable percentage of incorrectly staged patients referred from peripheral centres. Our study shows that after reviewing CT scans and laparoscopy in patients with suspected locally advanced pancreatic cancer, only 40% of these patients appeared to have true unresectable, non-metastasised pancreatic cancer. Only after careful staging including laparoscopy, results of future studies on either locoregional therapy or systemic therapy for locally advanced pancreatic cancer can be interpreted correctly.

REFERENCES

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007;57(1):43-66.
2. Oettle H, Post S, Neuhaus P, et al. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. Jama 2007;297(3):267-77.
3. Neoptolemos JP, Dunn JA, Stocken DD, et al. Adjuvant chemoradiotherapy and chemotherapy in resectable pancreatic cancer: a randomised controlled trial. Lancet 2001;358(9293):1576-85.
4. Beger HG, Gansauge F, Buchler MW, Link KH. Intraarterial adjuvant chemotherapy after pancreaticoduodenectomy for pancreatic cancer: significant reduction in occurrence of liver metastasis. World J Surg 1999;23(9):946-9.
5. Buchs NC, Frossard JL, Rosset A, et al. Vascular invasion in pancreatic cancer: evaluation of endoscopic ultrasonography, computed tomography, ultrasonography, and angiography. Swiss Med Wkly 2007;137(19-20):286-91.
6. Liu RC, Traverso LW. Diagnostic laparoscopy improves staging of pancreatic cancer deemed locally unresectable by computed tomography. Surg Endosc 2005;19(5):638-42.
7. Traverso LW. Pancreatic cancer: surgery alone is not sufficient. Surg Endosc 2006;20 Suppl 2:S446-9.
8. Butturini G, Crippa S, Bassi C, Salvia R, Piccoli M, Pederzoli P. The role of laparoscopy in advanced pancreatic cancer diagnosis. Dig Surg 2007;24(1):33-7.
9. Doucas H, Sutton CD, Zimmerman A, Dennison AR, Berry DP. Assessment of pancreatic malignancy with laparoscopy and intraoperative ultrasound. Surg Endosc 2007;21(7):1147-52.
10. Thomson BN, Parks RW, Redhead DN, et al. Refining the role of laparoscopy and laparoscopic ultrasound in the staging of presumed pancreatic head and ampullary tumours. Br J Cancer 2006;94(2):213-7.
11. Tilleman EH, Kuiken BW, Phoa SS, et al. Limitation of diagnostic laparoscopy for patients with a periampullary carcinoma. Eur J Surg Oncol 2004;30(6):658-62.
12. Nieveen van Dijkum EJ, Romijn MG, Terwee CB, et al. Laparoscopic staging and subsequent palliation in patients with peripancreatic carcinoma. Ann Surg 2003;237(1):66-73.
13. Liu RC, Traverso LW. Laparoscopic staging should be used routinely for locally extensive cancer of the pancreatic head. J Gastrointest Surg 2004;8(8):923-4.
14. Shoup M, Winston C, Brennan MF, Bassman D, Conlon KC. Is there a role for staging laparoscopy in patients with locally advanced, unresectable pancreatic adenocarcinoma? J Gastrointest Surg 2004;8(8):1068-71.
15. Stefanidis D, Grove KD, Schwesinger WH, Thomas CR, Jr. The current role of staging laparoscopy for adenocarcinoma of the pancreas: a review. Ann Oncol 2006;17(2):189-99.
16. Goldstein D, Van Hazel G, Walpole E, et al. Gemcitabine with a specific conformal 3D 5FU radiochemotherapy technique is safe and effective in the definitive management of locally advanced pancreatic cancer. Br J Cancer 2007;97(4):464-71.
17. Haddock MG, Swaminathan R, Foster NR, et al. Gemcitabine, cisplatin, and radiotherapy for patients with locally advanced pancreatic adenocarcinoma: results of the North Central Cancer Treatment Group Phase II Study N9942. J Clin Oncol 2007;25(18):2567-72.
18. Sultana A, Tudur Smith C, Cunningham D, et al. Systematic review, including meta-analyses, on the management of locally advanced pancreatic cancer using radiation/combined modality therapy. Br J Cancer 2007;96(8):1183-90.
19. Jeekel J, Treurniet-Donker AD. Treatment perspectives in locally advanced unresectable pancreatic cancer. Br J Surg 1991;78(11):1332-4.

20. Loyer EM, David CL, Dubrow RA, Evans DB, Charnsangavej C. Vascular involvement in pancreatic adenocarcinoma: reassessment by thin-section CT. Abdom Imaging 1996;21(3):202-6.
21. Phoa SS, Tilleman EH, van Delden OM, Bossuyt PM, Gouma DJ, Lameris JS. Value of CT criteria in predicting survival in patients with potentially resectable pancreatic head carcinoma. J Surg Oncol 2005;91(1):33-40.
22. Boeck S, Hoehler T, Seipelt G, et al. Capecitabine plus oxaliplatin (CapOx) versus capecitabine plus gemcitabine (CapGem) versus gemcitabine plus oxaliplatin (mGemOx): final results of a multicenter randomized phase II trial in advanced pancreatic cancer. Ann Oncol 2007.
23. Moore MJ, Goldstein D, Hamm J, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 2007;25(15):1960-6.
24. Sultana A, Smith CT, Cunningham D, Starling N, Neoptolemos JP, Ghaneh P. Meta-analyses of chemotherapy for locally advanced and metastatic pancreatic cancer. J Clin Oncol 2007;25(18):2607-15.
25. Isacoff WH, Bendetti JK, Barstis JJ, Jazieh AR, Macdonald JS, Philip PA. Phase II trial of infusional fluorouracil, leucovorin, mitomycin, and dipyridamole in locally advanced unresectable pancreatic adenocarcinoma: SWOG S9700. J Clin Oncol 2007;25(13):1665-9.
26. Park JK, Ryu JK, Lee JK, et al. Gemcitabine chemotherapy versus 5-fluorouracil-based concurrent chemoradiotherapy in locally advanced unresectable pancreatic cancer. Pancreas 2006;33(4):397-402.
27. Cohen SJ, Dobelbower R, Jr., Lipsitz S, et al. A randomized phase III study of radiotherapy alone or with 5-fluorouracil and mitomycin-C in patients with locally advanced adenocarcinoma of the pancreas: Eastern Cooperative Oncology Group study E8282. Int J Radiat Oncol Biol Phys 2005;62(5):1345-50.
28. Mishra G, Butler J, Ho C, et al. Phase II trial of induction gemcitabine/CPT-11 followed by a twice-weekly infusion of gemcitabine and concurrent external beam radiation for the treatment of locally advanced pancreatic cancer. Am J Clin Oncol 2005;28(4):345-50.
29. Bouvy ND, Marquet RL, Jeekel J, Bonjer HJ. Laparoscopic surgery is associated with less tumour growth stimulation than conventional surgery: an experimental study. Br J Surg 1997;84(3):358-61.0
30. Shiromizu A, Suematsu T, Yamaguchi K, Shiraishi N, Adachi Y, Kitano S. Effect of laparotomy and laparoscopy on the establishment of lung metastasis in a murine model. Surgery 2000;128(5):799-805.

CHAPTER 6

Phase II trial of Uracil/Tegafur plus leucovorin and celecoxib combined with radiotherapy in non-metastatic locally advanced pancreatic cancer

M.J.M. Morak[1], G. van Tienhoven[2], J.J.M.E. Nuyttens[3], A. van der Gaast[4], O.R.C. Busch[5], C.H.J. van Eijck[1] and D.J. Richel[6]

Erasmus Medical Centre, Rotterdam, The Netherlands
[1]Department of Surgery
[3]Department of Radiation Oncology
[4]Department of Medical Oncology

Academic Medical Centre, Amsterdam, The Netherlands
[2]Department of Medical Oncology
[6]Department of Surgery
[7]Department of Radiation Oncology

Radiother Oncol. 2011 Feb;98(2):261-4.

ABSTRACT

Background and Purpose: To investigate the efficacy and toxicity of a short intensive Uracil/Tegafur (UFT) based chemoradiotherapy scheme combined with Celecoxib in locally advanced pancreatic cancer.

Material and Methods: The Academic Medical Centre, Amsterdam and the Erasmus Medical Centre, Rotterdam enrolled eighty-three eligible patients with unresectable pancreatic cancer in a prospective multicentre phase II study. Median age was 62 years, median tumour size 40 mm and the majority of the patients (85%) had pancreatic head cancers. Treatment consisted of 20 x 2.5 Gy radiotherapy combined with UFT 300 mg/m^2 per day, Leucovorin (folinic acid) 30 mg and Celecoxib 800 mg for 28 days concomitant with radiotherapy. Four patients were lost to follow-up.

Results: Full treatment compliance was achieved 55% of patients, 80% received at least three weeks of treatment. No partial or complete response was observed. Median survival was 10.6 months and median time to progression 6.9 months. Toxicity was substantial with 28% grade III-IV gastrointestinal toxicity and two early toxic deaths.

Conclusions: Based on the lack of response, the substantial toxicity of mainly gastro-intestinal origin and the reported mediocre overall and progression free survival, we can not advise our short intensive chemoradiotherapy schedule as standard treatment.

INTRODUCTION

Pancreatic carcinoma is the 4th most common cause of cancer related death worldwide, with an overall 5 year survival rate of < 5%.[1] At presentation, only 10-20% of patients have resectable disease, half of the remaining patients has already distant metastases and the other half has locally advanced disease not amenable for a curative resection.[2]

Since the publication of the Gastro Intestinal Tumour Study Group (GITSG) in 1981[3], patients with locally advanced pancreatic cancer are treated with radiotherapy in combination with 5 fluorouracil (5-FU). In a number of studies 5-FU has been replaced by gemcitabine in the treatment of unresectable pancreatic cancer, either alone, in combination with radiotherapy or in combination with other drugs. However, at the start of this study, no clear benefit of either treatment had been established.[4-6]

In the Academic Medical Centre in Amsterdam and the Erasmus Medical Centre Rotterdam, various studies have been performed to improve the results of radiotherapy or 5-FU based chemoradiotherapy in patients with unresectable pancreatic cancer.[7, 8-11] So far, most studies showed similar results with a median overall survival between 8 and 11 months. Studies in metastatic colorectal cancer have demonstrated that oral Uracil/ Tegafur (UFT) combined with leucovorin has an equivalent efficacy as intravenous 5-FU with leucovorin, but is safer and more convenient.[12, 13] In a phase I trial in patients with locally advanced and metastatic pancreatic cancer, UFT combined with leucovorin and radiotherapy was feasible, with the obvious benefit of an oral treatment.[14]

In animal models, selective inhibition of cyclooxygenase-2 (COX-2) activity is associated with enhanced radiation sensitivity of tumours without enhancement of the damage to normal tissue.[15] Pancreatic cancers overexpress COX-2 of in 31- 90 % of the tumours. Theoratically COX-2 inhibitors could therefore enhance the effect of radiotherapy.[15, 16]

In the present study we investigated the safety and efficacy of a short intensive course of radiotherapy (50 Gy in 4 weeks) with UFT, leucovorin and celecoxib in patients with pathologically proven locally advanced pancreatic cancer.

METHODS

Patients

Eligibility criteria included a cytologically or histologically confirmed unresectable tumour of the pancreas, smaller than 7x7x7 cm and without metastatic disease. Further inclusion criteria were an adequate hematologic parameters (leucocytes > $3.0x10^9$/l, platelets > $100x10^9$/l, haemoglobin

> 5.6 mmol/l), renal function (creatinine < 135 μmol/l) and liver function (total serum bilirubin < 2 mg/dl, partial thromboplastin time > 60%), age > 18 years and ECOG performance status ≤ 2. Patients with prior radiotherapy or chemotherapy, lymph node metastases outside the radiation target volume, hypersensitivity to celecoxib or other COX-2 inhibitors or serious bleeding disorders were excluded as well as patients with previous or current malignancies at other sites, no effective contraception and child- bearing or breast-feeding women. All patients signed an informed consent before inclusion.

Resectability was assessed by CT scan or at explorative laparotomy. Unresectable disease determined by triphasic contrast enhanced helical CT-scan was based on criteria defined by Loyer and Phoa as: i) infiltration of peripancreatic fatplanes, the hepatoduodenal ligament and the mesentery, ii) >180° encasement of the portal or superior mesenteric vein or of the hepatic or superior mesenteric artery, iii) infiltration of the vessel, i.e. ingrowth or thrombosis.[17, 18]

During treatment patients were weekly monitored for toxicity or signs of early disease progression. After treatment, patients were followed up with clinical and laboratory examinations and CT scans every three months during the first two years and every six months thereafter. Clinical signs of recurrence were indications for additional examinations.

Toxicity was scored according to the National Cancer Institute (NCI) Common Toxicity Criteria, Version 2.[19] Tumour response was measured according to RECIST as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD).[20]

Treatment

Radiotherapy was delivered according to the guidelines of the International Commission on Radiation Units and Measurements (ICRU) report 50 using a linear accelerator with photon beams of at least 6MV energy. The Clinical Target Volume (CTV) consisted of the macroscopically visible tumour on CT scan plus assumed microscopic extension. The Planning Target Volume (PTV) was defined as the CTV plus 15 mm margin in cranial and caudal direction and 10 mm in all other directions. Typically the PTV volume varied between 250 and 350 cubic centimetre (cc). A total dose (TD) of 50 Gray (Gy) was delivered in 20 fractions of 2.5 Gy, five days a week in four weeks using a three dimensional conformal technique with a minimum of three, usually 5-7, beams. The minimum and maximum doses in the PTV were not allowed to exceed 5% of the planned dose. Organs at risk were the spinal cord, kidneys, liver and small bowel. The maximum allowed dose to any part of the spinal cord was an equivalent dose of 46 Gy/23 fractions, the maximum allowed dose to one whole kidney was 25 Gy. Part of one kidney was allowed a higher dose provided the other (functioning) kidney was spared. The entire liver was allowed a maximum dose of 30 Gy and the small bowel, except for a small part of the duodenum, was not to exceed 50 Gy.

Tegafur/ Uracil (UFT) was administered orally, three doses of 100 mg/m^2 per day for 28 days concomitant with radiotherapy, combined with 30 mg of leucovorin (folinic acid) per dose UFT and celecoxib 400 mg twice a day.
Treatment was interrupted in case of ≥ grade III toxicity. Anti-emetics or anti-diarrheic medication was provided if necessary. Furthermore, an H_2-receptor antagonist was offered during the first 6 months to diminish prevent peptic ulcers.

Outcome measures and statistical analysis

The primary endpoint was time to progression. Secondary endpoints were overall survival, response rate, toxicity and resectability rate following treatment. Eighty patients were included as stated in the protocol.
Analysis was performed with the Statistical Package for the Social Sciences version 15.0 (SPSS) using the Kaplan Meier method. Survival curves were compared with the log-rank test. All analyses were performed on an intention-to-treat basis.

RESULTS

Patients

From August 2003 until March 2007 109 consecutive patients with locally advanced pancreatic cancer presented in the Academic Medical Centre, Amsterdam or the Erasmus Medical Centre, Rotterdam. Of these 109 patients, 83 patients were included in this prospective phase II study. Twenty six patients were not included because they did not want to participate in the study (n=8), had metastatic disease at laparoscopy/laparotomy (n=7), had a performance of WHO>2 (n=6), pancreatic malignancy was not proven (n=2), had a previous bladder carcinoma (n=1), were hypersensitive for celecoxib (n=1) or had too high levels of bilirubin (n=1)
Patient characteristics are listed in Table 1. The median tumour size (largest diameter) was 40mm(range 24 - 70mm). The majority of the tumours were localised in the pancreatic head (83%). Enlarged peripancreatic lymph nodes were present in 53%of the patients and unresectability was mainly caused by vascular involvement (81%). Forty five patients underwent surgery before inclusion and bypasses were performed in 76% (n = 34) of these patients.
Median time from informed consent to start of treatment was 29 days (7-55)
Four patients (5%) were lost to follow up during or shortly after treatment. These patients all died, and the date, but not the cause of death was retrieved. Four patients were still alive at the time of the last analysis. Median follow up for these four patients was 28 months (range 16 - 43).

Table 1. Baseline Characteristics of Eligible Patients:

	N= 83
Hospital; number (%)	
Academic Medical Centre	61 (74)
Erasmus Medical Centre	22 (27)
Mean age; yr (range)	62 (39-79)
Sex; number (%)	
Male	42 (51)
Female	41 (49)
Median tumour size; mm (range)	40 (24 -70)
Enlarged peripancreatic lymph nodes; number (%)	44 (53)
Cause of unresectability; number (%)	
Vascular involvement	67 (84)
Lympnodes outside the resection field	4 (5)
Combination of above	8 (10)
Ingrowth into surrounding tissue	4 (5)

Compliance and adverse effects

In total, 46 patients (55%) received the entire treatment schedule as planned in the protocol. Eight patients (10%) interrupted therapy for 1 - 3 days due to gastro-intestinal toxicity (n = 4), a skin rash (n = 2), leucopenia (n = 1) or problems with oral UFT intake (n = 1). Two patients had to stop celecoxib after 11 and 14 days due to a skin rash but continued the chemoradiotherapy. Twenty six patients had to stop treatment due to gastro-intestinal toxicity (n = 22; 27%) with (n = 3; 4%) or without haematological toxicity, skin rash (n = 3; 4%) or pneumonia (n = 1; 1%). The first included patient never started UFT, because it was not yet available (n = 1; 1%). Despite toxicity, 65 patients (78%) received the planned dose (50 Gray) of radiotherapy. Eighty per cent of the patients (n=66) received at least 3 weeks of therapy. Of the 27 patients who stopped treatment, three patients did so during the first week of therapy, three during the second week, eleven during the third week and ten during the fourth week.

Overall grade III and IV and (early) grade V toxicity is shown in Table 2. Twenty-five patients (30%) were admitted into the hospital during or after treatment due to gastro-intestinal toxicity (n=20) or cholangitis (n=4). One patient developed an irreversible renal failure due to (biliary) sepsis (without leucopenia) and died the day of admission. Another patient was admitted to the hospital with dehydration and a severe decline in physical condition without disease progression, caused by nausea and vomiting CTC grade III, was rehydrated and treated with anti-emetics but died shortly thereafter.

Table 2. Toxicity

		Grade III-IV Number (%)
Haematological		
	Leucocytes	2 (2)
	Potassium	1 (1)
Non-haematological		
	Anorexia	6 (7)
	Nausea	11 (13)
	Vomiting	8 (10)
	Mucositis	3 (4)
	Diarrhoea	9 (11)
	Pain	4 (5)
	Fever	1 (1)
	Fatigue	4 (5)

Treatment efficacy

Response could be assessed in 79 patients. None of the patients had a partial or complete response and none underwent further exploratory surgery. Progression of disease occurred in 67 patients (81%). Median time to progression in all 83 patients was 6.9 months (Figure 1). Forty two patients (51%) developed local progression, 25 patients (30%) developed distant progression and 21 (25%) patients developed both local and distant metastases simultaneously, Nine patients received further treatment consisting of gemcitabine (n=8) or radiotherapy (n=1).

Overall survival was based on 79 deaths. Cancer-related death accounted for 77% of the deaths (n = 61). In 7 patients (including the four lost to follow-up) the cause of death was unknown. Other causes of death were (biliary) sepsis (n = 4), pneumonia (n = 1), renal failure during treatment (n = 1), duodenal perforation due to stent (n = 1), haematemesis (n = 1), epileptic insults leading to coma (n = 1) and dehydration and diminished general condition caused by nausea and vomiting without tumour progression (n = 2), one during treatment. All but two of these non cancer-related deaths occurred more than two months after study treatment.

Median overall survival in all patients was 10.6 months (Figure 1). Single log-rank tests showed that survival was similar in both hospitals (10.5 vs. 10.8 months; p=0.983), was independent of the performance of explorative surgery before the start of treatment, the localisation of the tumour (head vs. elsewhere), tumour size (≤ 40mm vs. > 40mm) or the presence of enlarged peripancreatic lymph nodes or vascular involvement. Furthermore, survival was not determined by the number of treatment weeks (≤ 3 vs. > 3 nor full treatment vs. partial treatment).

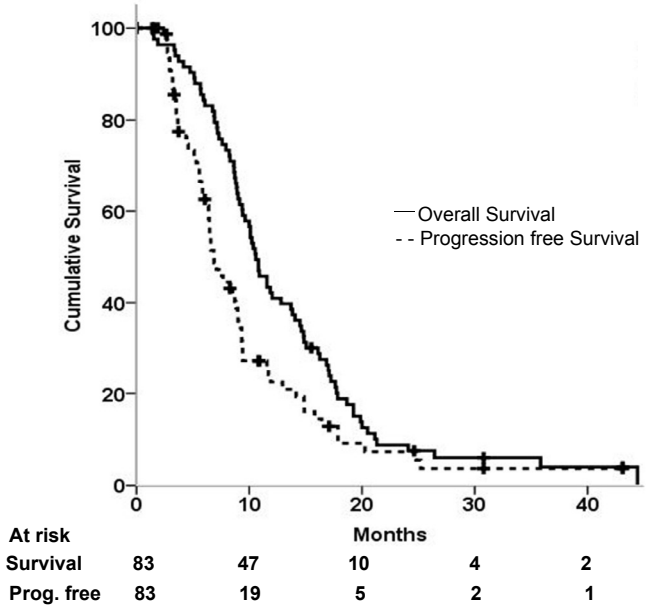


Fig. 1. Actuarial overall survival and progression free survival of all 83 patients.

DISCUSSION

In this multicentre phase-II trial the median overall survival was 10.6 months with a median time to progression of 6.9 months after oral Tegafur/ Uracil (UFT) combined with leucovorin (folinic acid), celecoxib and radiotherapy in patients with unresectable pancreatic cancer. Nineteen per cent of the patients did not progress during follow-up. None of the patients had a complete or partial response and none of the patients underwent an exploratory laparotomy to re-asses resectability.

Toxicity was substantial: Two patients died during therapy, CTC grade III-IV toxicity occurred in 23 patients (28%), and 80% (n = 66) of the patients had CTC grade ≥ II. However, compared to other studies with similar regimens in both pancreatic and rectal cancer[21, 22], grade III-IV toxicity occurred in equivalent frequency. The high fraction doses of radiotherapy combined with celecoxib in such short interval may have accounted partly to the toxicity.

The survival of our patient group is poor and is in line with median survival after 5-fluorouracil (5-FU) based chemoradiotherapy treatment in literature (7- 14 months).[9-11, 23-28] After treatment with gemcitabine either with or without concurrent radiotherapy, median survival varies between 10- 17 months.[23, 26-32]

A recent randomised controlled trial performed by Chauffert[23] revealed a significant survival benefit of gemcitabine alone, over intensive 5-FU and cisplatin based chemoradiotherapy followed by the same maintenance gemcitabine therapy (median survival 13 vs. 8.6 months; p= 0.03). Based on this study, one could question whether radiotherapy should remain part of the treatment at all. However, this was an analysis of an unplanned interim analysis with only 42% of the patients receiving at least 75% of the protocol. Furthermore, no other study could reproduce the high survival in the gemcitabine arm.

More than half of the recurrences/progressions of pancreatic cancer occur locally, and cause severe pain in a high proportion of patients. Chemoradiotherapy has a potent effect on this pain.[10, 33] Hence we believe that the search for better chemoradiotherapy remains valid. Probably chemoradiotherapy should be gemcitabine-based and combined with other new drugs with radio-, and/or chemo-enhancing potency.

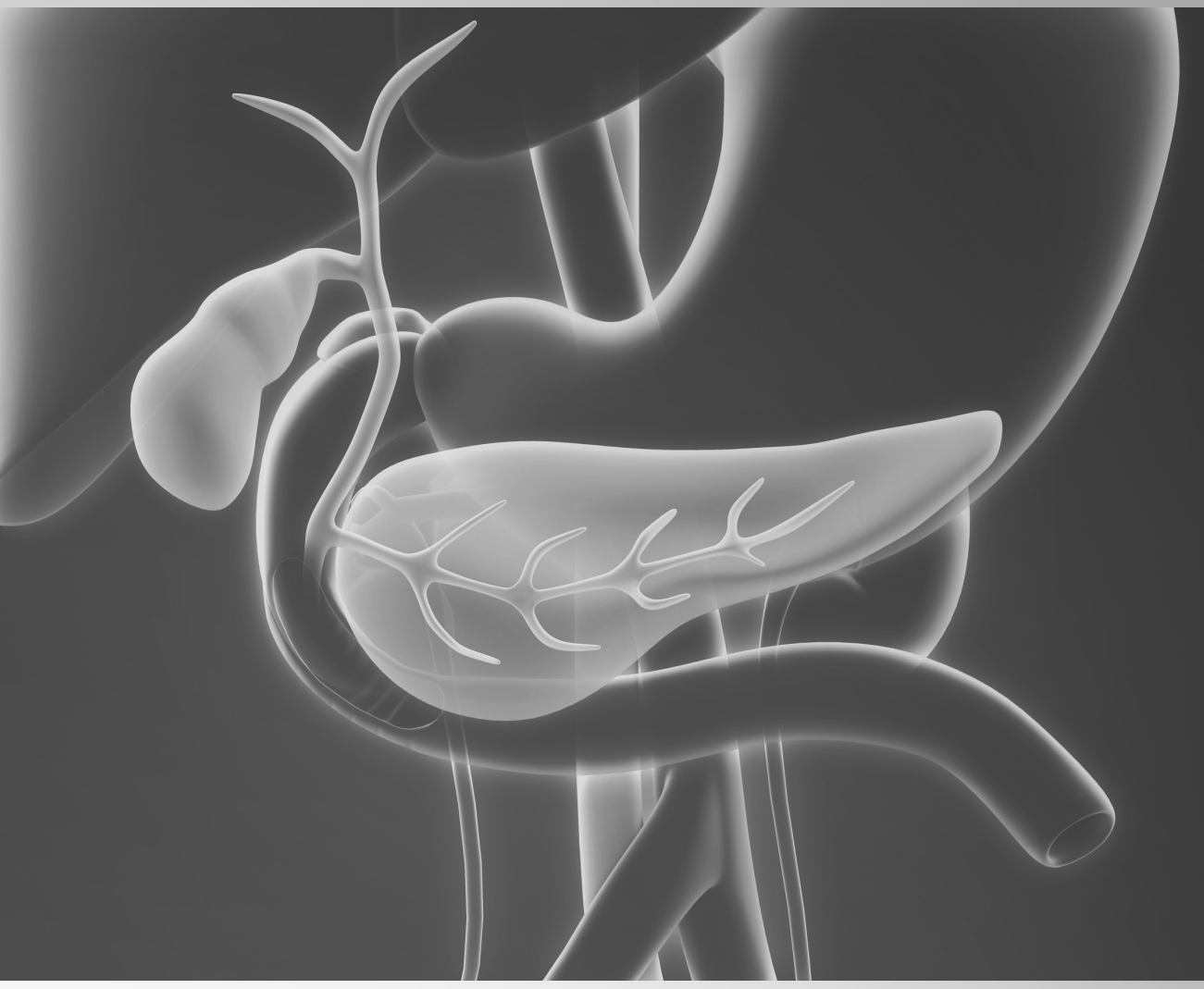
In conclusion, based on the lack of response, the accompanying toxicity of mainly gastro-intestinal origin and the reported mediocre overall and progression free survival, we can not advise our short intensive chemoradiotherapy schedule consisting of radiation, 50 Gy in four weeks, oral UFT, leucovorin, and celecoxib as standard treatment in patients with locally advanced pancreatic cancer.

REFERENCES

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. CA Cancer J Clin 2008;58(2): 71-96.
2. Parsons CM, Sutcliffe JL, Bold RJ. Preoperative evaluation of pancreatic adenocarcinoma. J Hepatobiliary Pancreat Surg 2008;15(4): 429-435.
3. Moertel CG, Frytak S, Hahn RG, O'Connell MJ, Reitemeier RJ, Rubin J, Schutt AJ, Weiland LH, Childs DS, Holbrook MA, Lavin PT, Livstone E, Spiro H, Knowlton A, Kalser M, Barkin J, Lessner H, Mann-Kaplan R, Ramming K, Douglas HO, Jr., Thomas P, Nave H, Bateman J, Lokich J, Brooks J, Chaffey J, Corson JM, Zamcheck N, Novak JW. Therapy of locally unresectable pancreatic carcinoma: a randomized comparison of high dose (6000 rads) radiation alone, moderate dose radiation (4000 rads + 5-fluorouracil), and high dose radiation + 5-fluorouracil: The Gastrointestinal Tumor Study Group. Cancer 1981;48(8): 1705-1710.
4. Morganti AG, Massaccesi M, La Torre G, Caravatta L, Piscopo A, Tambaro R, Sofo L, Sallustio G, Ingrosso M, Macchia G, Deodato F, Picardi V, Ippolito E, Cellini N, Valentini V. A systematic review of resectability and survival after concurrent chemoradiation in primarily unresectable pancreatic cancer. Ann Surg Oncol;17(1): 194-205.
5. Ryan DP, Willett CG. Management of locally advanced adenocarcinoma of the pancreas. Hematol Oncol Clin North Am 2002;16(1): 95-103.
6. Wilkowski R, Boeck S, Ostermaier S, Sauer R, Herbst M, Fietkau R, Flentje M, Miethe S, Boettcher HD, Scholten T, Bruns CJ, Rau HG, Hinke A, Heinemann V. Chemoradiotherapy with concurrent gemcitabine and cisplatin with or without sequential chemotherapy with gemcitabine/cisplatin vs chemoradiotherapy with concurrent 5-fluorouracil in patients with locally advanced pancreatic cancer--a multi-centre randomised phase II study. Br J Cancer 2009;101(11): 1853-1859.
7. Schuster-Uitterhoeve AL, Gonzalez Gonzalez D, Blank LE. Radiotherapy with multiple fractions per day in pancreatic and bile duct cancer. Radiother Oncol 1986;7(3): 205-213.
8. Jeekel J, Treurniet-Donker AD. Treatment perspectives in locally advanced unresectable pancreatic cancer. Br J Surg 1991;78(11): 1332-1334.
9. Luderhoff EC, Gonzalez Gonzalez D, Bakker P. Pilot study in locally advanced unresectable pancreas carcinoma using a combination of accelerated radiotherapy and continuous infusion of 5-fluorouracil. Radiother Oncol 1996;40(3): 241-243.
10. Ceha HM, van Tienhoven G, Gouma DJ, Veenhof CH, Schneider CJ, Rauws EA, Phoa SS, Gonzalez Gonzalez D. Feasibility and efficacy of high dose conformal radiotherapy for patients with locally advanced pancreatic carcinoma. Cancer 2000;89(11): 2222-2229.
11. Smeenk HG, de Castro SM, Jeekel JJ, Kazemier G, Busch OR, Incrocci L, Erdmann J, Hop WC, Gouma DJ, van Eijck CH. Locally advanced pancreatic cancer treated with radiation and 5-fluorouracil: a first step to neoadjuvant treatment? Dig Surg 2005;22(3): 191-197.
12. Carmichael J, Popiela T, Radstone D, Falk S, Borner M, Oza A, Skovsgaard T, Munier S, Martin C. Randomized comparative study of tegafur/uracil and oral leucovorin versus parenteral fluorouracil and leucovorin in patients with previously untreated metastatic colorectal cancer. J Clin Oncol 2002;20(17): 3617-3627.
13. Douillard JY, Hoff PM, Skillings JR, Eisenberg P, Davidson N, Harper P, Vincent MD, Lembersky BC, Thompson S, Maniero A, Benner SE. Multicenter phase III study of uracil/tegafur and oral leucovorin versus fluorouracil and leucovorin in patients with previously untreated metastatic colorectal cancer. J Clin Oncol 2002;20(17): 3605-3616.
14. Childs HA, 3rd, Spencer SA, Raben D, Bonner JA, Newsome J, Robert F. A phase I study of combined UFT plus leucovorin and radiotherapy for pancreatic cancer. Int J Radiat Oncol Biol Phys 2000;47(4): 939-944.

15. Choy H, Milas L. Enhancing radiotherapy with cyclooxygenase-2 enzyme inhibitors: a rational advance? J Natl Cancer Inst 2003;95(19): 1440-1452.
16. Molina MA, Sitja-Arnau M, Lemoine MG, Frazier ML, Sinicrope FA. Increased cyclooxygenase-2 expression in human pancreatic carcinomas and cell lines: growth inhibition by nonsteroidal anti-inflammatory drugs. Cancer Res 1999;59(17): 4356-4362.
17. Loyer EM, David CL, Dubrow RA, Evans DB, Charnsangavej C. Vascular involvement in pancreatic adenocarcinoma: reassessment by thin-section CT. Abdom Imaging 1996;21(3): 202-206.
18. Phoa SS, Tilleman EH, van Delden OM, Bossuyt PM, Gouma DJ, Lameris JS. Value of CT criteria in predicting survival in patients with potentially resectable pancreatic head carcinoma. J Surg Oncol 2005;91(1): 33-40.
19. Trotti A, Byhardt R, Stetz J, Gwede C, Corn B, Fu K, Gunderson L, McCormick B, Morrisintegral M, Rich T, Shipley W, Curran W. Common toxicity criteria: version 2.0. an improved reference for grading the acute effects of cancer treatment: impact on radiotherapy. Int J Radiat Oncol Biol Phys 2000;47(1): 13-47.
20. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92(3): 205-216.
21. Feliu J, Mel R, Borrega P, Lopez Gomez L, Escudero P, Dorta J, Castro J, Vazquez-Estevez SE, Bolanos M, Espinosa E, Gonzalez Baron M. Phase II study of a fixed dose-rate infusion of gemcitabine associated with uracil/tegafur in advanced carcinoma of the pancreas. Ann Oncol 2002;13(11): 1756-1762.
22. Fernandez-Martos C, Aparicio J, Bosch C, Torregrosa M, Campos JM, Garcera S, Vicent JM, Maestu I, Climent MA, Mengual JL, Tormo A, Hernandez A, Estevan R, Richart JM, Viciano V, Uribe N, Campos J, Puchades R, Arlandis F, Almenar D. Preoperative uracil, tegafur, and concomitant radiotherapy in operable rectal cancer: a phase II multicenter study with 3 years' follow-Up. J Clin Oncol 2004;22(15): 3016-3022.
23. Chauffert B, Mornex F, Bonnetain F, Rougier P, Mariette C, Bouche O, Bosset JF, Aparicio T, Mineur L, Azzedine A, Hammel P, Butel J, Stremsdoerfer N, Maingon P, Bedenne L. Phase III trial comparing intensive induction chemoradiotherapy (60 Gy, infusional 5-FU and intermittent cisplatin) followed by maintenance gemcitabine with gemcitabine alone for locally advanced unresectable pancreatic cancer. Definitive results of the 2000-01 FFCD/SFRO study. Ann Oncol 2008;19(9): 1592-1599.
24. Cohen SJ, Dobelbower R, Jr., Lipsitz S, Catalano PJ, Sischy B, Smith TJ, Haller DG. A randomized phase III study of radiotherapy alone or with 5-fluorouracil and mitomycin-C in patients with locally advanced adenocarcinoma of the pancreas: Eastern Cooperative Oncology Group study E8282. Int J Radiat Oncol Biol Phys 2005;62(5): 1345-1350.
25. Isacoff WH, Bendetti JK, Barstis JJ, Jazieh AR, Macdonald JS, Philip PA. Phase II trial of infusional fluorouracil, leucovorin, mitomycin, and dipyridamole in locally advanced unresectable pancreatic adenocarcinoma: SWOG S9700. J Clin Oncol 2007;25(13): 1665-1669.
26. Li CP, Chao Y, Chi KH, Chan WK, Teng HC, Lee RC, Chang FY, Lee SD, Yen SH. Concurrent chemoradiotherapy treatment of locally advanced pancreatic cancer: gemcitabine versus 5-fluorouracil, a randomized controlled study. Int J Radiat Oncol Biol Phys 2003;57(1): 98-104.
27. Park JK, Ryu JK, Lee JK, Yoon WJ, Lee SH, Kim YT, Yoon YB. Gemcitabine chemotherapy versus 5-fluorouracil-based concurrent chemoradiotherapy in locally advanced unresectable pancreatic cancer. Pancreas 2006;33(4): 397-402.
28. Tada M, Arizumi M, Nakai Y, Sasaki T, Kogure H, Togawa O, Matsubara S, Tsujino T, Hirano K, Sasahira N, Isayama H, Kawabe T, Omata M. Efficacy of gemcitabine for locally advanced pancreatic cancer: comparison with 5-fluorouracil-based chemoradiotherapy. Chemotherapy 2008;54(4): 302-308.
29. Goldstein D, Van Hazel G, Walpole E, Underhill C, Kotasek D, Michael M, Shapiro J, Davies T, Reece W, Harvey J, Spry N. Gemcitabine with a specific conformal 3D 5FU radiochemotherapy technique is safe and effective in the definitive management of locally advanced pancreatic cancer. Br J Cancer 2007;97(4): 464-471.

30. Haddock MG, Swaminathan R, Foster NR, Hauge MD, Martenson JA, Camoriano JK, Stella PJ, Tenglin RC, Schaefer PL, Moore DF, Jr., Alberts SR. Gemcitabine, cisplatin, and radiotherapy for patients with locally advanced pancreatic adenocarcinoma: results of the North Central Cancer Treatment Group Phase II Study N9942. J Clin Oncol 2007;25(18): 2567-2572.
31. Murphy JD, Adusumilli S, Griffith KA, Ray ME, Zalupski MM, Lawrence TS, Ben-Josef E. Full-dose gemcitabine and concurrent radiotherapy for unresectable pancreatic cancer. Int J Radiat Oncol Biol Phys 2007;68(3): 801-808.
32. Hong SP, Park JY, Jeon TJ, Bang S, Park SW, Chung JB, Park MS, Seong J, Lee WJ, Song SY. Weekly full-dose gemcitabine and single-dose cisplatin with concurrent radiotherapy in patients with locally advanced pancreatic cancer. Br J Cancer 2008;98(5): 881-887.
33. van Geenen RC, Keyzer-Dekker CM, van Tienhoven G, Obertop H, Gouma DJ. Pain management of patients with unresectable peripancreatic carcinoma. World J Surg 2002;26(6): 715-720.



PART III

In vitro

CHAPTER 7

Type I interferons as radiosensitizers in pancreatic cancer cell lines

M.J.M. Morak[1], P.M. van Koetsveld[2], R. Kanaar[3], L.J. Hofland[2] and C.H.J. van Eijck[1]

[1]Department of Surgery
[2]Department of Internal Medicine, Division Endocrinology
[3]Department of Cell Biology and Genetics, Cancer Genomics Centre, and Department of Radiation Oncology
Erasmus Medical Centre, Rotterdam, The Netherlands

Eur J Cancer. 2011 Sep;47(13):1938-45.

ABSTRACT

Background: Radiotherapy is an established treatment for malignant localized disease. Pancreatic cancer however seems relatively insensitive to this form of therapy.

Methods: Pancreatic cancer cell lines MiaPaca-2 and Panc-1 were pre-treated with 3000 IU/ml IFNα or 100 IU/ml IFNβ followed by 0, 2, 4 or 6 Gray (Gy) irradiation. Colony forming assay was used to assess the effects on cellgrowth. To measure the surviving fraction at the clinically relevant dose of 2 Gy (SF2), cells were pre-treated with 1000- 10.000 IU/ml IFNα or 50-500 IU/ml IFNβ followed by 2 Gy irradiation.

Results: The plating efficiency was 49% for MiaPaca-2 and 22% for Panc-1. MiaPaca-2 was more radiosensitive than Panc-1 (surviving fraction of 0.28 vs. 0.50 at 4 Gray). The SF2 of MiaPaca-2 was 0.77 while the SF2 of Panc-1 was 0.70. The SF2 significantly decreased after pretreatment with IFNα 1000 IU/ml ($p<0.001$) and IFNβ 100 IU/ml ($p<0.001$) in MiaPaca-2 and with IFNα 5000 IU/ml ($p<0.001$) and IFNβ 100 IU/ml ($p<0.01$) in Panc-1. The sensitizing enhancement ratio (SER) for IFNα 3000 IU/ml was 2.15 in MiaPaca-2 and 1.90 in Panc-1. For IFNβ 100 IU/ml the SER was 1.72 for in MiaPaca-2 and 1.51 in Panc-1.

Conclusions: Type I interferons have radiosensitizing effects in pancreatic cancer cell lines. This radiosensitising property might lead to an improved response to treatment in pancreatic cancer. Interferon β is the most promising drug due to its effect in clinically obtainable doses.

INTRODUCTION

Radiotherapy is an important treatment in cancer, especially for patients with advanced localized disease, with proven efficacy in many tumours.[1-5] Based on several randomised controlled trials studying the effect of adjuvant (chemo)radiotherapy, pancreatic and periampullary cancers are fairly radiotherapy resistant.[6-10]

Often chemotherapeutics (5-fluorouracil (5-FU), gemcitabine) are used as radiosensitizers. Besides their direct cytotoxic effects caused by incorporation of the drugs as modified nucleotides into the DNA, even low doses of these drugs can be effective in radiosensitisation. Interference with normal repair of radiation-induced DNA damage with an inappropriate progression through S phase is key in their radiosensitizing properties causing late, unmanageable toxicities. A favourable side effect of their direct cytotoxicity is a relative increase in oxygenation, leading to an increased vulnerability for radiotherapy.[11, 12] However, as mentioned, results of chemoradiotherapy are disappointing in pancreatic cancer.[6-10]

In several tumours radiosensitizing properties of interferon alpha (IFNα) and interferon beta (IFNβ) have been demonstrated in vitro[13-17] as well as in vivo.[18, 19] In pancreatic cancer cell lines, IFNα has already shown to act as radiosensitizer[20] and in vivo promising therapy results have been reported combining 5-FU, cisplatinum, and radiation therapy with IFNα alone (5-year survival rate of 55%) or followed by 2 cycles of gemcitabine (median survival 25 months) in patients with resected pancreatic adenocarcinoma.[21, 22] These results are currently reinvestigated in the phase III CapRI study.[23]

Type I interferons such as IFNα and IFNβ, sort their effect through the same interferon receptor (IFNAR) with IFNβ having a higher affinity. In vivo studies showed that approximately 20% of pancreatic cancers express IFNARs and that expression of the interferon receptor correlates with a significant survival benefit in patients with resected pancreatic cancer.[24]

The exact mechanism by which type I interferons cause radiosensitisation is unclear. Possibly, concomitant treatment with IFNs causes an inappropriate progression of cells into S-phase, thereby interfering with repair of radiation-induced damage or increasing the proportion of lethal to sublethal damage.[17, 25]

In this study we aim to gain insight in the radiosensitizing abilities of type I interferons, especially IFNβ, in pancreatic cell lines. We decided to address these issues in colony forming assays, because besides the apoptotic effects of radiation and IFNs, the reproductive integrity of tumour cells (i.e. the capacity to produce an expanding colony of descendants, and therefore to regrow the tumour if left intact at the end of treatment) is of pivotal importance.

MATERIALS AND METHODS

Cell lines and culture conditions

The human pancreatic cell lines MiaPaCa-2 and Panc-1 were purchased from the American Type Culture Collection. The cells were cultured in a humidified incubator containing 5% CO_2 at 37°C. MiaPaca-2 was cultured in RPMI 1640 and Panc-1 in DMEM both supplemented with 10% FCS, penicillin ($1x10^5$ U/l), fungizone (0.5 mg/l) and L-glutamine (2 mmol/l). Periodically, the cells were tested for *Mycoplasma* contamination, which was not detected. Cells were harvested with trypsin (0.05%), EDTA (0.02%) and resuspended in medium. Before plating, the cells were counted microscopically using a standard haemocytometer. Tryphan Blue staining was used to assess cell viability, which always exceeded 95%. Media and supplements were obtained from GIBCO Biocult Europe (Invitrogen, Breda, The Netherlands).

Drugs and Reagents

Human recombinant IFN-α-2b (Intron-A) was obtained from Schering-Plough Corporation (Utrecht, The Netherlands), while human recombinant IFN-β-1a (Rebif) was acquired from Serono Benelux BV (Den Haag, The Netherlands). All compounds were stored at -20°C, and the stock solution was constituted in distilled water according to the manufacturer instructions. Doses of 1000- 10.000 IU/ml for IFNα and 50- 500 IU/ml for IFNβ were used.

Irradiation

Cells were exposed to gamma radiation from a ^{137}Cs source at 70.9 cGy/min at room temperature under aerobic conditions. For radiation survival studies, cells were irradiated with 0, 2, 4, 6, 8 or 10 Gray. In the combined modality treatment, the IFN treatment was given before irradiation for 72 hours. Cells were irradiated with 0, 2, 4 or 6 Gray in the presence of the drug.

Colony forming assay

Cells were plated onto poly-L-lysine coated, 60 mm Petri-dishes (6-12 cells/cm^2) and cultured in complete medium for two weeks. Poly-L-lysine (10 μg/ml; Sigma-Aldrich, Zwijndrecht, The Netherlands) inhibited cells from dispersing from the growing colonies.

Dose response curves for IFNα, IFNβ and irradiation were established for both cell lines using a colony-forming assay. Therefore, seeded cells were allowed to attach for 24 hr prior to treatment with 1000- 10.000 IU/ml IFNα, 50- 500 IU/ml IFNβ or 0- 10 Gray irradiation. Cell lines were treated with IFNs continuously and medium plus agents was replaced every three or four days. Fourteen days after seeding, colonies were fixed with 100% ethanol and stained with hematoxicilline to

allow calculation of their average colony-forming efficiency. Colonies containing >50 cells were counted automatically with the MultiImage Light Cabinet from HpH Innitech Corporation.
Plating efficiency was defined as the mean number of colonies divided by the number of inoculated cells for control cultures not exposed to interferons or radiation. The surviving fraction (SF) was calculated as (mean number of colonies)/ (number of inoculated cells × plating efficiency). The curve was plotted using X-Y log scatter (Graph Prism 3.0). Curve-fitting parameters α and β were determined.

Radiation enhancement by type I interferons

To asses radiation enhancement by type I interferons, cells were pretreated with IFNα 3000 IU/ml or IFNβ 100 IU/ml (doses resulting in approximately 50% decrease in surviving fraction in both cell lines) for 72 hours. Cell lines were irradiated with 0, 2, 4 or 6 Gray. Control plates without IFNs were irradiated simultaneously. Cell lines were treated with IFNs continuously and medium plus agents was replaced every three or four days. After two weeks, the formed colonies were fixed and stained to allow counting.
SF2 is the surviving fraction of cells that were irradiated at the clinically relevant dose of 2 Gray. The sensitizing enhancement ratio (SER) for interferon was calculated at the 37% survival level. The radiation dose that reduced the surviving colonies to 37% of the non-treated controls was divided by the radiation dose that reduced survival to 37% after interferon pre-treatment.

Cell proliferation assay

Measurement of total DNA contents, representative for the number of cells, was performed using the bisbenzimide fluorescent dye (Hoechst ™ 33258, Boehring Diagnostics, La Jolla, CA) as previously described.[26]

Measurement of DNA fragmentation (apoptosis)

10.000 cells/dish, depending on the length of the incubation period, were plated on 24-well plates and the cells were allowed to adhere overnight. The next day the cell culture medium was replaced with 1ml/well medium containing 3000 IU/ ml IFN-α or 100 IU/ml IFN-β. Each treatment was performed in quadruplicate. After an additional incubation of 3 days, apoptosis was assessed using a commercially available ELISA kit (Cell Death Detection ELISAPlus, Roche Diagnostic GmbH, Penzberg, Germany). The standard protocol supplied by the manufacturer was used. Relative apoptosis was determined by calculating the ratio of the average absorbance of the treatment dishes to the average absorbance of the control dishes. The data were corrected for the effect on cell number after 3 days of treatment. Intra- and interassay coefficients of variation were 4.2% and 6.3% respectively.

Statistical analyses

All experiments were carried out in duplicates and gave comparable results. For statistical analysis GraphPad PrismTM 3.0 (GraphPad Software, San Diego, USA) was used. The comparative statistical evaluation among groups was firstly performed by the ANOVA test. When significant differences were found, a comparison between groups was made using the Newman-Keuls test. The unpaired Student t-test was used to analyze the differences in surviving fraction for each dose point.

In all analyses, values of $p<0.05$ were considered statistically significant. Data are reported as mean + SEM. Statistical analysis was made after logarithmic transformation.

RESULTS

In vitro, the control plating efficiency (mean ± SD) was measured and amounted to 49 ± 3% for MiaPaca-2 and 22 ± 3% for Panc-1.

Effect of type I interferons on relative clonogenic survival

Both IFNα and IFNβ inhibited colony formation for both MiaPaca-2 and Panc-1 cells in a dose dependent manner (Figure 1). For all doses analysed, MiaPaca-2 was significantly more vulnerable to both IFNα and IFNβ than Panc-1.

Besides inhibiting colony formation, type I interferon had growth inhibitory effects on both cell lines; after interferon treatment, colonies were significantly smaller than colonies from untreated cells (Figure 1). This finding is consistent with Vitale's manuscript (27) demonstrating both growth inhibitory as well as pro-apoptotic properties of type I interferons with IFNβ being the more potent pro-apoptotic drug than IFNα, where apoptosis only occurs in higher doses.

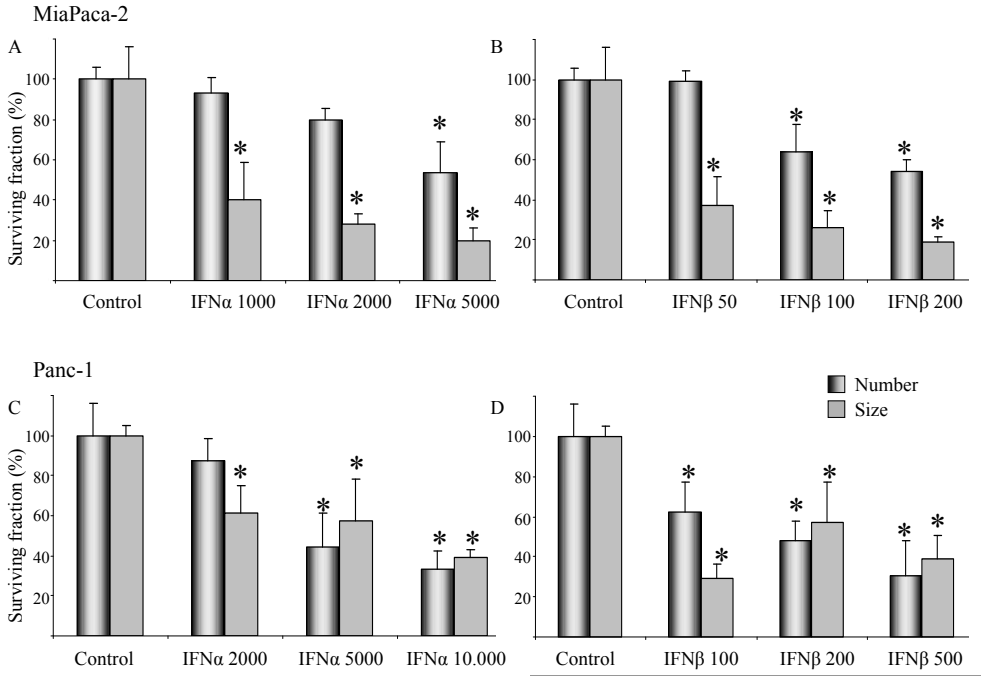


Figure 1 Effect of IFNα and IFNβ treatment on colony forming ability in MiaPaca-2 (A,B) and Panc-1 (C,D). Pancreatic cancer cells were treated with IFNα (A,C) or IFNβ (B,D) during fourteen days and both number and size of colonies were counted automatically. Data are the mean ± SEM. *=p<0.05 vs. control.

Effect of radiation on relative clonogenic survival

MiaPaca-2 was more radiosensitive than Panc-1 for 4, 6 and 8 Gray (Figure 2a). The surviving fraction at 2 Gy (SF2) of MiaPaca-2 was 0.77 while the SF2 of Panc-1 was 0.70. At 4 Gy however, the SF was 0.28 for MiaPaca-2 and 0.50 for Panc-1. The size of the colonies was not considerably influenced by radiotherapy (Figure 2b).

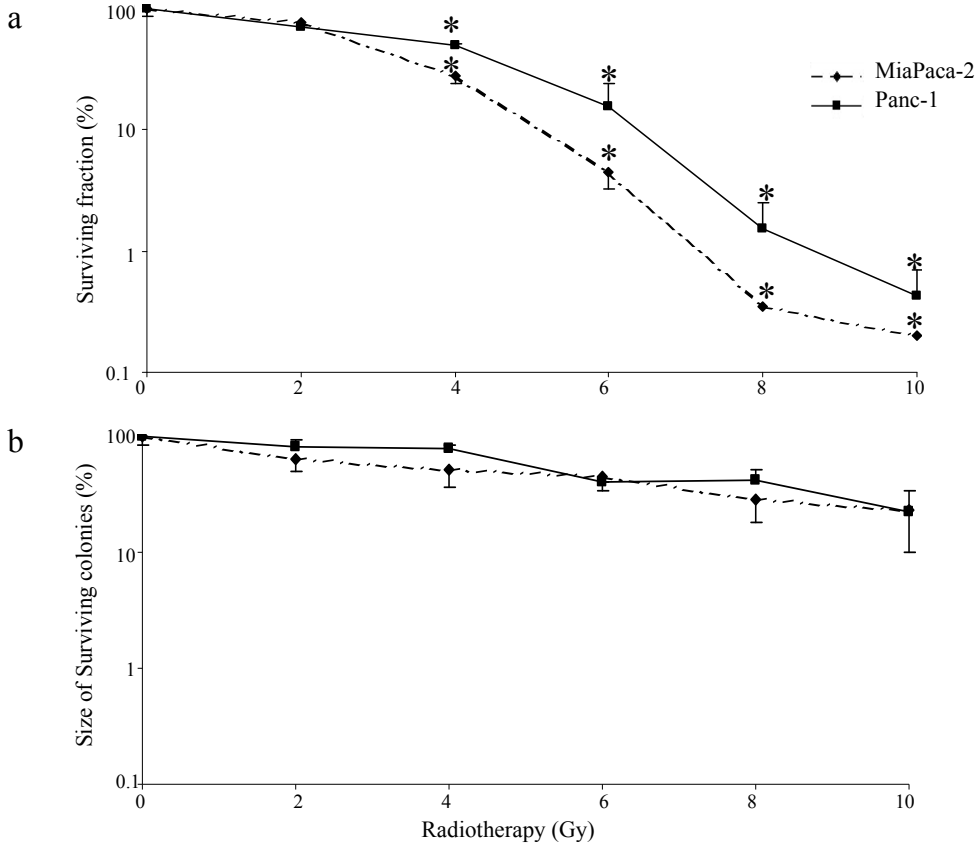


Figure 2 Effect of irradiation on colony forming ability in MiaPaca-2 and Panc-1. Pancreatic cancer cells were treated with irradiation during fourteen days and both number and size of colonies were counted automatically. Data are the mean ± SEM. *=p<0.05 vs. control.

Effect of type I Interferons on apoptosis

Significant apoptosis corrected for DNA content occurred after 72 hours (Figure 3). Apoptosis increased to 802 ± 219% for IFNα 3000 IU/ml and 575 ± 119% for IFNβ 100 IU/ml. In Panc-1 apoptosis after 72 hours was 154 ± 14% for IFNα 3000 IU/ml and 139 ± 7% for IFNβ 100 IU/ml.

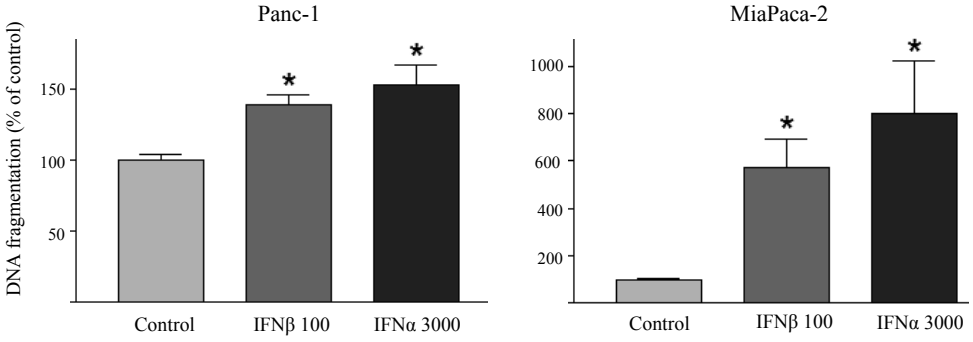


Figure 3 Effect of 3000 IU/ml IFNα and 100 IU/ml IFNβ treatment on apoptosis (DNA fragmentation) in MiaPaCa-2 and Panc-1 cell lines. Cells were incubated for 3 days without (control) or with the drugs indicated. Values are absorbance units and are expressed as percent of the control. Data are the mean ± SEM. *=p<0.05 vs. control.

Surviving fraction at 2 Gray (SF2)

The SF2 of MiaPaca-2 was 0.77 while the SF2 of Panc-1 was 0.70. Pre-incubation with IFNα for 72 hours at doses of 1000, 2000 or 5000 IU/ml changed the SF2 in MiaPaca-2 to 0.56, 0.47 and 0.18 respectively. In Panc-1 pre-incubation with IFNα at doses of 2000, 5000 or 10.000 IU/ml changed the SF2 to 0.66, 0.22 and 0.18 respectively.

Pre-incubation with IFNβ for 72 hours at doses of 50, 100 or 200 IU/ml changed the SF2 in MiaPaca-2 to 0.70, 0.33 and 0.22 respectively. In Panc-1 pre-incubation with IFNβ at doses of 100, 200 or 500 IU/ml changed the SF2 to 0.48, 0.24 and 0.23 respectively.

The decrease of SF2 compared to the SF2 without pre-incubation was significant after IFNα 2000 IU/ml ($p<0.01$) and IFNα 5000 IU/ml ($p<0.001$) and IFNβ 100 IU/ml and IFNβ 200 IU/ml ($p<0.001$) in MiaPaca-2. In Panc-1 concentrations of IFNα 2000 IU/ml ($p<0.01$), IFNα 5000 IU/ml ($p<0.001$) and IFNα 10.000 IU/ml ($p<0.001$) and IFNβ 100 IU/ml ($p<0.001$) IFNβ 200 IU/ml ($p<0.001$) IFNβ 500 IU/ml ($p<0.001$) resulted in significant improved radiosensitivity (Figure 4).

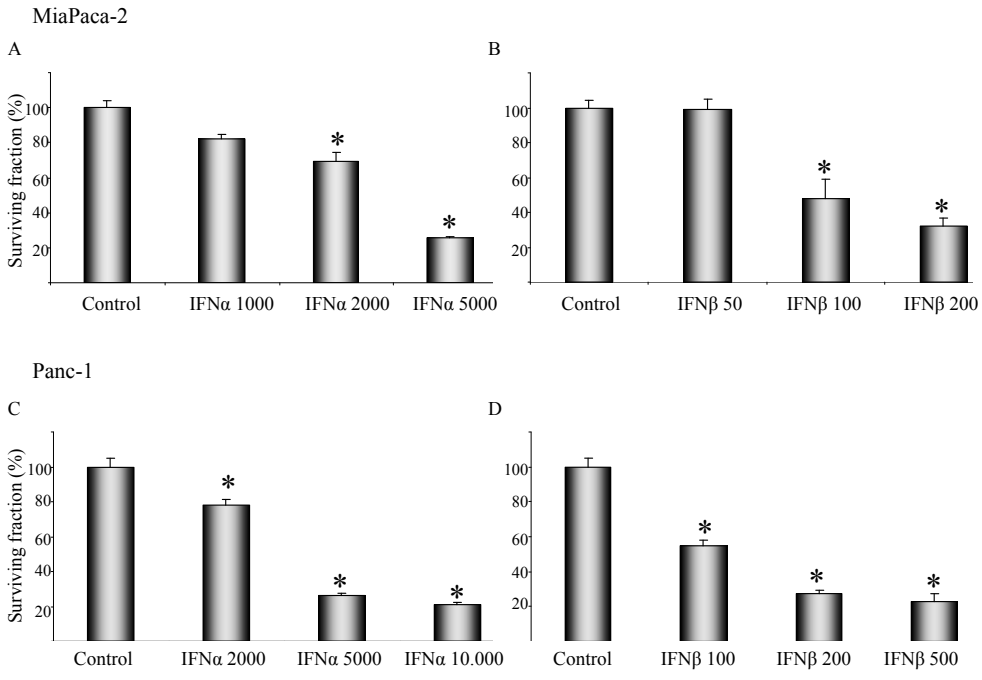


Figure 4 Effect of combined treatment with type I interferons and 2 Gy irradiation on colony forming ability in MiaPaCa-2 (A,B) and Panc-1 (C,D) cell lines. Cells were pretreated with IFNα (A,C) or IFNβ (B,D) for 72 hours followed by irradiation with 2 Gy. The formed colonies were automatically counted after 2 weeks. Data are the mean ± SEM. *=p<0.05 vs. control.

Effect of type I interferon on radiosensitivity

The radiotherapy dose required to reduce the surviving fraction to 37% was 3.98 Gy in MiaPaca-2 for the non-treated controls. After IFNα 3000 IU/ml, the required dose was 1.85 Gy and after IFNβ 100 IU/ml, the required dose was 2.32 Gy. This leads to a sensitizing enhancement ratio (SER) of 2.15 for IFNα 3000 IU/ml and a SER of 1.72 for IFNβ 100 IU/ml in MiaPaca-2.

In Panc-1 the required radiotherapy dose to reduce the SF to 37% was 4.65 Gy for the non-treated controls. After IFNα 3000 IU/ml, the required dose was 2.45 Gy and after IFNβ 100 IU/ml, the required dose was 3.07 Gy. This leads to a sensitizing enhancement ratio (SER) of 1.90 for IFNα 3000 IU/ml and a SER of 1.51 for IFNβ 100 IU/ml in Panc-1.

The shape of the survival curves changed after treatment resulting in an increased steepness of the survival curve with an increase of the α-component after curve fitting (Figure 5). For MiaPaca-2 this increase was significant for both IFNα as IFNβ compared to the non-treated controls. In the Panc-1, we found no significant difference between the α-component after neither IFNα nor IFNβ and the non-treated controls. In this cell line, after comparison of the radiosensitivity between

groups using the Newman-Keuls test, only the SF after 6 Gy of irradiation combined with IFNα or IFNβ was significantly lower than after irradiation alone. All other SFs were similar after treatment or no treatment.

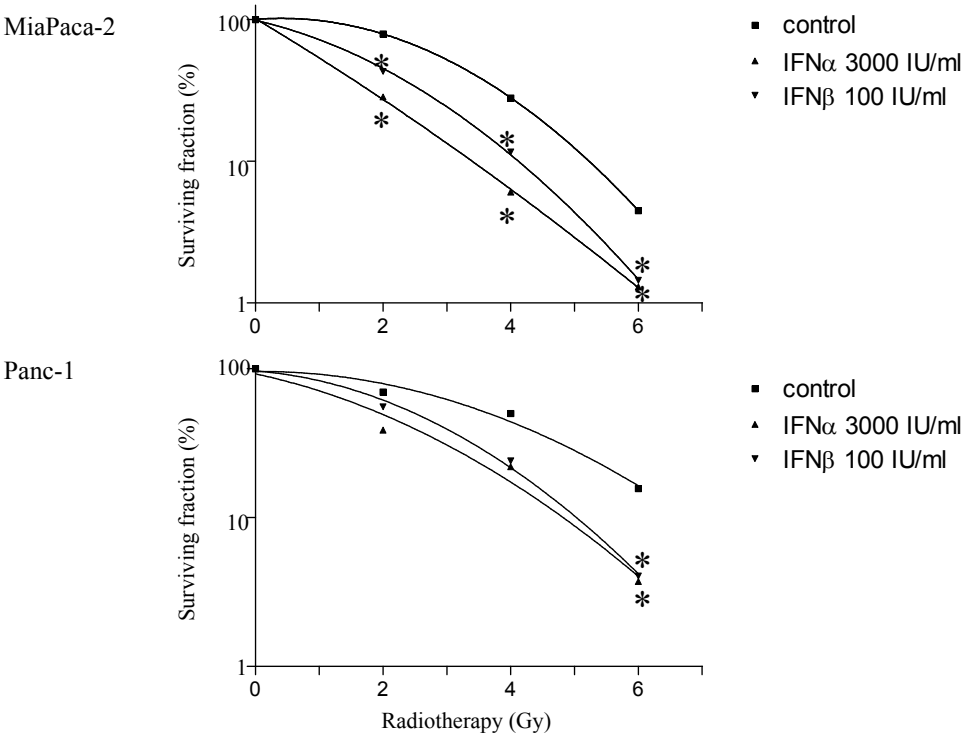


Figure 5 Effect of combined treatment with 3000 IU/ml IFNα and 100 IU/ml IFNβ and irradiation on colony forming ability in MiaPaCa-2 and Panc-1 cell lines. Cells were pretreated with IFNα or IFNβ for 72 hours followed by 0, 2, 4 or 6 Gy irradiation. The formed colonies were automatically counted after 2 weeks. Data are the mean ± SEM. *=p<0.05 vs. control.

DISCUSSION

Our *in vitro* study shows a radiosensitizing effect of type I interferons on pancreatic cancer cell lines MiaPaca-2 and Panc-1. Miapaca-2 was 2.15 times more sensitive to radiotherapy after treatment with IFNα 3000 IU/ml and 1.72 times more sensitive after IFNβ 100 IU/ml. The sensitivity to radiotherapy of Panc-1 increased 1.90 times after treatment with IFNα 3000 IU/ml and 1.51 times after IFNβ 100 IU/ml. Radiosensitization was dose dependently, with higher doses

of interferons resulting in a higher radiosensitivity, i.e. a lower surviving fraction at 2 Gray (SF2). Both overall radiosensitivity and sensitivity to interferon therapy was higher in MiaPaca-2 than in Panc-1. Previously, IFN receptor expression (mRNA and protein) was shown by Vitale et al[27], with MiaPaCa-2 expressing more plasma membrane located IFN receptors than Panc-1. Furthermore, treatment with type I interferons results in more apoptosis in MiaPaca-2 than in Panc-1, although only extremely high doses (>1000 IU/ml) of IFNα cause apoptosis.[27]

Tumours are most vulnerable for radiotherapy in case of adequate vascular supply (proper oxygenation) and appropriate cell cycling.[11, 28] To enhance the effect of radiotherapy, effort has been made to find radiation sensitizers. Because hypoxic cells are known to be up to three-fold more resistant to radiotherapy than well-oxygenated cells[29], several strategies have been investigated to find ways to sensitize hypoxic cells to radiation. These strategies focus at reducing tumour hypoxia by increasing the delivery of oxygen to the tumour, administering oxygen mimetics and mimicking the effect of oxygen in the radiochemical process or by selective destruction of hypoxic cells, for instance by tirapazimine.[30, 31] Trials show that reducing hypoxia by any means, leads to a better locoregional control and an improved survival, especially in head and neck cancer and cervical cancer.[30]

Resistance to apoptosis is the key factor for poor responses to therapies in pancreatic cancer. Ionising radiation alone causes a range of lesions in the DNA of target cells such as base damage, single-strand and double-strand breaks. Double-strand breaks are generally considered the lethal event but can be repaired by DNA repair mechanisms. Inadequately repaired DNA damage causes activation of the mitochondrial pathway of apoptosis by p53 resulting in activation of the caspase cascade. Membrane damage activates the stress-activated protein kinase pathway leading to activation of the mitochondrial pathway as well as a direct activation of caspases. Furthermore radiotherapy activates apoptosis due to stimulation of the death receptor pathway, consisting of the tumour necrosis factor (TNF) receptor superfamily (for instance TRAIL-R1 and TRAIL-R2). Activation causes direct activation of the caspase cascade. These cell death pathways are regulated by numerous signaling molecules, such as nuclear factor-κB (NF-κB), phosphatidylinositol 3-kinase (PI3K), inhibitor of apoptosis proteins (IAPs) and members of the Bcl-2 protein family.[32, 33]

Pancreatic cancer cells have developed multiple resistance mechanisms to therapy-induced apoptosis. The mitochondrial pathway of apoptosis is less activated due to inactivating p53 mutations, present in >70% of the pancreatic cancers. If activated, this pathway has a diminished ability to activate the caspase cascade. Furthermore, pancreatic cancer cells overexpress anti-apoptotic proteins, have inactivated pro-apoptotic genes and express decoy receptors to prevent activation of the death receptor pathway.[34, 35]

In pancreatic cancer cell lines, IFNα has already shown to be able to avoid these resistance mechanisms and act as radiosensitizer.[20] The exact mechanism by which IFN exerts this radiosensitising activity is unclear. Both IFNα and IFNβ have direct anti-tumour effects including apoptosis, cell damage, upregulation of cancer antigens and a growth inhibitory effect with accumulation of cells in S phase. Indirect anti-tumour effects are caused by modulation of the immune system, mainly through activation of T-cells, macrophages and natural killer cells, and anti-angiogenesis activity by downregulation of the vascular endothelial growth factor (VEGF) receptor and an alteration in the expression of various oncogenes.[27, 36-39]

In pancreatic cancer cell lines, type I interferons cause both direct apoptosis (at lower doses in IFNβ) and radiosensitisation. The exact mechanism for enhancing radiosensitivity is unclear. Apparently the accumulation of cells in S phase caused by interferons, which is the most radioresistant part of the cell cycle, does not prevent radiosensitisation. A possible mechanism is the inability of cells to accumulate sublethal DNA damage with interferon interfering in the repair of this kind of DNA damage. Furthermore alteration in oncogene expression levels might sensitize radioresistant cells to radiotherapy. Because not only the pro-apoptotic effect of IFNs combined with radiotherapy but especially the reproductive capacity of the treated cells is related to treatment efficacy, we chose colony forming assays for our study.

Beneficial effects of IFNα combined with chemoradiotherapy were already demonstrated in phase II studies in patients with resectable pancreatic cancer.[21, 22] However, treatment is associated with considerable toxicity[21, 22, 40] and the optimal combination therapy considering efficacy as well as tolerability is yet to be determined.

In conclusion, our in vitro study shows that both IFNα and IFNβ have radiosensitizing effects in pancreatic cancer cell lines that are not based on immunomodulatory properties. Radiosensitizing effects are dose dependent, and lower doses of IFNβ than IFNα cause similar radiosensitisation. The effect of interferon seems related to the receptor status. The radiosensitising property of type I interferons might lead to an improved response to treatment in pancreatic cancer with interferon β being the most promising drug. Therefore, further clinical trials involving combination therapy of type I IFNs and radiotherapy are promising.

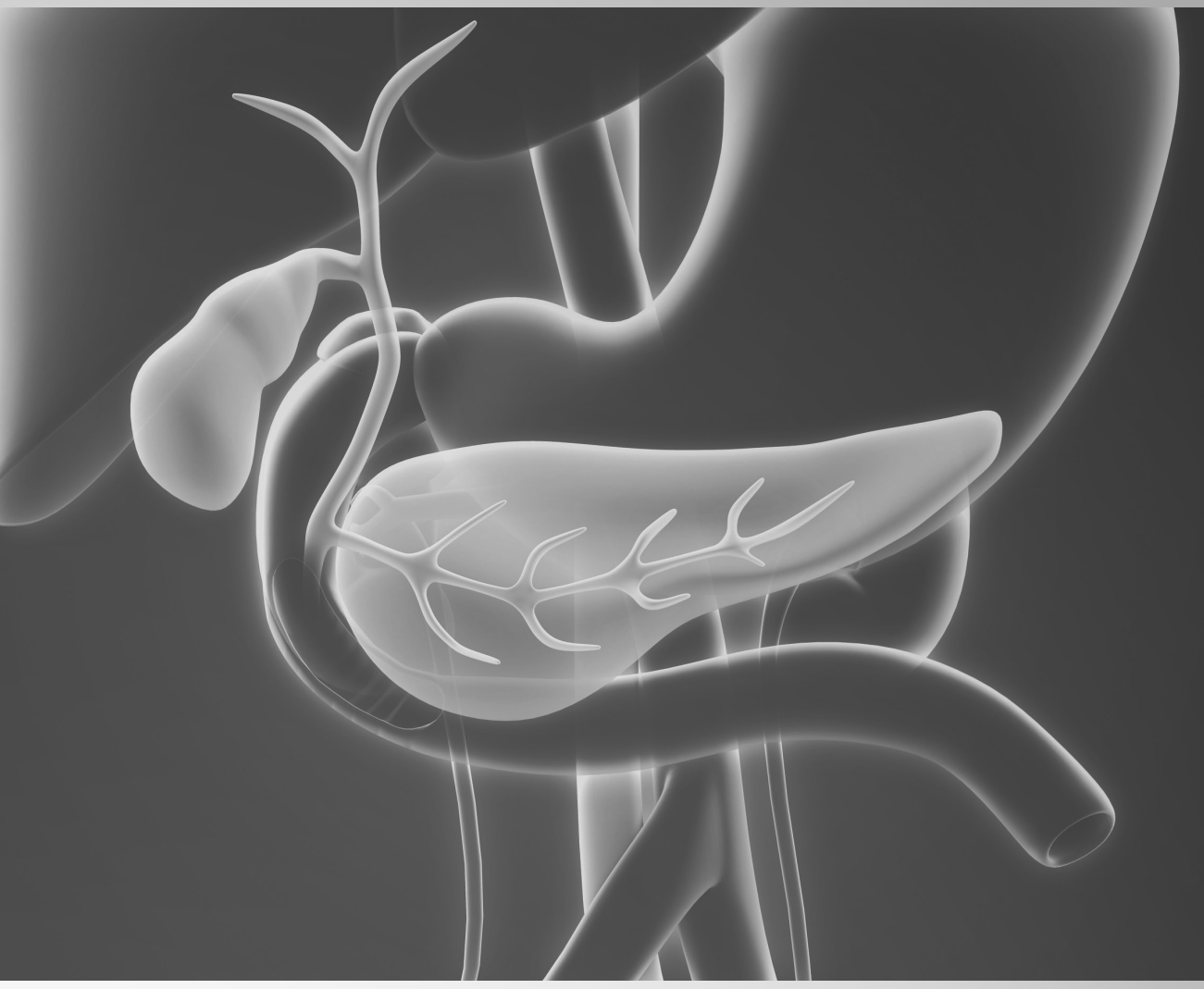
REFERENCES

1. Overgaard J, Hansen HS, Specht L, Overgaard M, Grau C, Andersen E, et al. Five compared with six fractions per week of conventional radiotherapy of squamous-cell carcinoma of head and neck: DAHANCA 6 and 7 randomised controlled trial. Lancet 2003;362(9388):933-40.
2. Picardi M, De Renzo A, Pane F, Nicolai E, Pacelli R, Salvatore M, et al. Randomized comparison of consolidation radiation versus observation in bulky Hodgkin's lymphoma with post-chemotherapy negative positron emission tomography scans. Leuk Lymphoma 2007;48(9):1721-7.
3. Reed NS, Mangioni C, Malmstrom H, Scarfone G, Poveda A, Pecorelli S, et al. Phase III randomised study to evaluate the role of adjuvant pelvic radiotherapy in the treatment of uterine sarcomas stages I and II: an European Organisation for Research and Treatment of Cancer Gynaecological Cancer Group Study (protocol 55874). Eur J Cancer 2008;44(6):808-18.
4. Regine WF, Winter KW, Abrams R, Safran H, Hoffman JP, Konski A, et al. RTOG 9704 a phase III study of adjuvant pre and post chemoradiation (CRT) 5-FU vs. gemcitabine (G) for resected pancreatic adenocarcinoma. Journal of Clinical Oncology 2006;24(2006 ASCO Annual Meeting Proceedings (Post-Meeting Edition).):4007.
5. Trock BJ, Han M, Freedland SJ, Humphreys EB, DeWeese TL, Partin AW, et al. Prostate cancer-specific survival following salvage radiotherapy vs observation in men with biochemical recurrence after radical prostatectomy. Jama 2008;299(23):2760-9.
6. Klinkenbijl JH, Jeekel J, Sahmoud T, van Pel R, Couvreur ML, Veenhof CH, et al. Adjuvant radiotherapy and 5-fluorouracil after curative resection of cancer of the pancreas and periampullary region: phase III trial of the EORTC gastrointestinal tract cancer cooperative group. Ann Surg 1999;230(6):776-82; discussion 782-4.
7. Neoptolemos JP, Dunn JA, Stocken DD, Almond J, Link K, Beger H, et al. Adjuvant chemoradiotherapy and chemotherapy in resectable pancreatic cancer: a randomised controlled trial. Lancet 2001;358(9293):1576-85.
8. Neoptolemos JP, Stocken DD, Friess H, Bassi C, Dunn JA, Hickey H, et al. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. N Engl J Med 2004;350(12):1200-10.
9. Stocken DD, Buchler MW, Dervenis C, Bassi C, Jeekel H, Klinkenbijl JH, et al. Meta-analysis of randomised adjuvant therapy trials for pancreatic cancer. Br J Cancer 2005;92(8):1372-81.
10. Morak MJ, van der Gaast A, Incrocci L, van Dekken H, Hermans JJ, Jeekel J, et al. Adjuvant intra-arterial chemotherapy and radiotherapy versus surgery alone in resectable pancreatic and periampullary cancer: a prospective randomized controlled trial. Ann Surg 2008;248(6):1031-41.
11. Hennequin C, Favaudon V. Biological basis for chemo-radiotherapy interactions. Eur J Cancer 2002;38(2):223-30.
12. Shewach DS, Lawrence TS. Antimetabolite radiosensitizers. J Clin Oncol 2007;25(26):4043-50.
13. Angioli R, Sevin BU, Perras JP, Untch M, Koechli OR, Nguyen HN, et al. In vitro potentiation of radiation cytotoxicity by recombinant interferons in cervical cancer cell lines. Cancer 1993;71(11):3717-25.
14. Chang AY, Keng PC. Potentiation of radiation cytotoxicity by recombinant interferons, a phenomenon associated with increased blockage at the G2-M phase of the cell cycle. Cancer Res 1987;47(16):4338-41.
15. Gould MN, Kakria RC, Olson S, Borden EC. Radiosensitization of human bronchogenic carcinoma cells by interferon beta. J Interferon Res 1984;4(1):123-8.
16. Hoffmann W, Blase MA, Santo-Hoeltje L, Herskind C, Bamberg M, Rodemann HP. Radiation sensitivity of human squamous cell carcinoma cells in vitro is modulated by all-trans and 13-cis-retinoic acid in combination with interferon-alpha. Int J Radiat Oncol Biol Phys 1999;45(4):991-8.

17. Schmidberger H, Rave-Frank M, Lehmann J, Schweinfurth S, Rehring E, Henckel K, et al. The combined effect of interferon beta and radiation on five human tumor cell lines and embryonal lung fibroblasts. Int J Radiat Oncol Biol Phys 1999;43(2):405-12.
18. Basu P, Biswas J, Mandal R, Choudhury P. Is interferon-alpha and retinoic acid combination along with radiation superior to chemo-radiation in the treatment of advanced carcinoma of cervix? Indian J Cancer 2006;43(2):54-9.
19. Holsti LR, Mattson K, Niiranen A, Standertskiold-Nordenstam CG, Stenman S, Sovijarvi A, et al. Enhancement of radiation effects by alpha interferon in the treatment of small cell carcinoma of the lung. Int J Radiat Oncol Biol Phys 1987;13(8):1161-6.
20. Ma JH, Patrut E, Schmidt J, Knaebel HP, Buchler MW, Marten A. Synergistic effects of interferon-alpha in combination with chemoradiation on human pancreatic adenocarcinoma. World J Gastroenterol 2005;11(10):1521-8.
21. Linehan DC, Tan MC, Strasberg SM, Drebin JA, Hawkins WG, Picus J, et al. Adjuvant interferon-based chemoradiation followed by gemcitabine for resected pancreatic adenocarcinoma: a single-institution phase II study. Ann Surg 2008;248(2):145-51.
22. Picozzi VJ, Kozarek RA, Traverso LW. Interferon-based adjuvant chemoradiation therapy after pancreaticoduodenectomy for pancreatic adenocarcinoma. Am J Surg 2003;185(5):476-80.
23. Knaebel HP, Marten A, Schmidt J, Hoffmann K, Seiler C, Lindel K, et al. Phase III trial of postoperative cisplatin, interferon alpha-2b, and 5-FU combined with external radiation treatment versus 5-FU alone for patients with resected pancreatic adenocarcinoma -- CapRI: study protocol [ISRCTN62866759]. BMC Cancer 2005;5(1):37.
24. Saidi RF, Williams F, Silberberg B, Mittal VK, ReMine SG, Jacobs MJ. Expression of interferon receptors in pancreatic cancer: identification of a novel prognostic factor. Surgery 2006;139(6):743-8.
25. Dritschilo A, Mossman K, Gray M, Sreevalsan T. Potentiation of radiation injury by interferon. Am J Clin Oncol 1982;5(1):79-82.
26. Hofland LJ, van Koetsveld PM, Lamberts SW. Percoll density gradient centrifugation of rat pituitary tumor cells: a study of functional heterogeneity within and between tumors with respect to growth rates, prolactin production and responsiveness to the somatostatin analog SMS 201-995. Eur J Cancer 1990;26(1):37-44.
27. Vitale G, van Eijck CH, van Koetsveld Ing PM, Erdmann JI, Speel EJ, van der Wansem Ing K, et al. Type I interferons in the treatment of pancreatic cancer: mechanisms of action and role of related receptors. Ann Surg 2007;246(2):259-68.
28. Pawlik TM, Keyomarsi K. Role of cell cycle in mediating sensitivity to radiotherapy. Int J Radiat Oncol Biol Phys 2004;59(4):928-42.
29. Sprong D, Janssen HL, Vens C, Begg AC. Resistance of hypoxic cells to ionizing radiation is influenced by homologous recombination status. Int J Radiat Oncol Biol Phys 2006;64(2):562-72.
30. Overgaard J. Hypoxic radiosensitization: adored and ignored. J Clin Oncol 2007;25(26):4066-74.
31. Wardman P. Chemical radiosensitizers for use in radiotherapy. Clin Oncol (R Coll Radiol) 2007;19(6):397-417.
32. Hunter A, Hendrikse A, Renan M, Abratt R. Does the tumor microenvironment influence radiation-induced apoptosis? Apoptosis 2006;11(10):1727-35.
33. Lindsay KJ, Coates PJ, Lorimore SA, Wright EG. The genetic basis of tissue responses to ionizing radiation. Br J Radiol 2007;80 Spec No 1:S2-6.
34. Gukovskaya AS, Pandol SJ. Cell death pathways in pancreatitis and pancreatic cancer. Pancreatology 2004;4(6):567-86.
35. Igney FH, Krammer PH. Death and anti-death: tumour resistance to apoptosis. Nat Rev Cancer 2002;2(4):277-88.
36. Platanias LC. Interferons: laboratory to clinic investigations. Curr Opin Oncol 1995;7(6):560-5.

37. Ota H, Nagano H, Doki Y, Sekimoto M, Kondo M, Wada H, et al. Expression of type I interferon receptor as a predictor of clinical response to interferon-alpha therapy of gastrointestinal cancers. Oncol Rep 2006;16(2):249-55.
38. Pfeffer LM, Dinarello CA, Herberman RB, Williams BR, Borden EC, Bordens R, et al. Biological properties of recombinant alpha-interferons: 40th anniversary of the discovery of interferons. Cancer Res 1998;58(12):2489-99.
39. Platanias LC, Fish EN. Signaling pathways activated by interferons. Exp Hematol 1999;27(11):1583-92.
40. Marten A, Schmidt J, Ose J, Harig S, Abel U, Munter MW, et al. A randomized multicentre phase II trial comparing adjuvant therapy in patients with interferon alpha-2b and 5-FU alone or in combination with either external radiation treatment and cisplatin (CapRI) or radiation alone regarding event-free survival - CapRI-2. BMC Cancer 2009;9:160.

7



PART IV

Closure

CHAPTER 8

General discussion

Outcome in patients with pancreatic and periampullary cancer is poor and a curative treatment strategy with more effective drugs and individualised treatment regimens have yet to be discovered. In resectable cancer, both neo-adjuvant and adjuvant schedules were suggested including solely systemic therapy or combined systemic and local therapy. Hardly any differentiation was made between periampullary and pancreatic head cancer. In locally advanced cancer, patients with metastasised cancer were included in the same studies and based on these results, radiotherapy was declared ineffective. Over the years knowledge has evolved and studies have become more reproducible, but a breakthrough in patients' survival was not found.

PART I
RESECTABLE PANCREATIC AND PERIAMPULLARY CANCER

Patients with resectable pancreatic and periampullary cancer develop both local and distant metastases after resection. In order to prevent recurrent disease, a combined local and systemic adjuvant treatment should be considered. In part I we treated patients with intra-arterial chemotherapy. Celiac artery infusion (CAI), resulting in a higher dose delivered in the liver through the common hepatic artery, might extend survival in locally advanced nonresectable as well as in resected pancreatic cancers. This treatment reduced hepatic progression from 62% to 17% and hepatic metastases related death from 33% to 8%.[1-3] However, no reduction in local recurrence rates was found in these studies and radiotherapy might be the solution for this problem. Therefore, we combined celiac artery infusion with radiotherapy (CAI/ RT).

An important outcome of our studies is the significance of differentiating between periampullary tumours and pancreatic carcinoma. For the whole study group CAI/RT did not improve survival. Patients operated for periampullary tumours survived significantly longer compared to patients with pancreatic cancer (27 vs. 14 months; $p<0.001$) and developed less liver metastases after intra-arterial chemotherapy and radiotherapy compared to observation (log-rank $p<0.03$). Patients with pancreatic cancer developed more local recurrences compared to periampullary tumours (log-rank $p<0.01$) but CAI/RT had no significant effect on local recurrence rate (log-rank $p=0.12$) nor on the development of liver metastases compared to the observation group (log-rank $p=0.76$). Consequently, no effect of CAI/RT was found on overall survival in pancreatic cancer.

After long-term follow-up, these figures were in line with aforementioned. However, for the periampullary cancers the number of long-term survivors (>60 months) in the CAI/RT group was twofold the number of long-term survivors after surgery alone (5 vs. 10). This did not result in a long-term significant survival benefit (median actual survival of 37 vs. 28 months, $p=0.08$).

However, survival in patients with moderate/ poor differentiated periampullary cancers did benefit from CAI/RT (median 22 vs. 36 months, p<0.01 log-rank).

The overall survival in all patients (19 months after surgery combined with CAI/RT, 18 months after surgery alone) was lengthy compared to the median survival of 12-15 months after surgery alone described in literature.[4, 5] However, evidently this was not caused by the treatment itself but might be the result of our high volume expertise and/ or the patient case mix (almost 50% of our patients had a periampullary tumour which favours survival).

Because this regime is quite intense, necessitating hospital admissions for every cycle and visits to the outpatient clinic daily during radiotherapy we concomitantly performed a quality of life investigation. According to the EORTC QLQ-C30 principles, CAI/ RT did not result in a worse quality of life compared to the observation arm and in some scores a positive effect was found. However, it is unclear if this effect was due to the placebo effect, to the provision of hope, or to the increased medical attention associated with being in a study (although follow up was similar for both groups) or due to minor tumour shrinkage relieving some of the symptoms and a longer disease free period.

In our opinion, differentiating between periampullary and pancreatic tumours is of paramount importance for further treatment strategies; Periampullary cancer has a more favourable survival pattern, suggesting another recurrence pattern and a different response to adjuvant treatment than pancreatic head cancer as found in our randomised controlled trial. This was also found in the ESPAC-3 trial were no effect of adjuvant chemotherapy was found in patients with periampullary cancer in contrast to patients with pancreatic cancer.[6] Therefore, individualized treatment, for both periampullary tumours and pancreatic cancer is necessary. Since survival is substantially worse in patients with resectable pancreatic head tumours, neo-adjuvant treatment might be a better option, thereby selecting the better patients for resection. For patients with periampullary cancer, adjuvant CAI might be a novel option to reduce the development of liver metastasis, however local recurrence remains also in these patients a serious problem, which could not be prevented by radiotherapy.

Since differentiating between pancreatic and periampullary cancer pre-operatively by CT scanning or endoscopic ultrasound appears to be difficult if not impossible, new treatment schedules including neoadjuvant therapy for only true pancreatic cancers will be difficult to develop. Nowadays the final diagnosis is based on histopathological findings of the operative specimen, thus requiring resection and consequently eliminating the possibility of tumour specific neoadjuvant treatment. And even pathological differentiation between periampullary (distal common bile duct, ampulla carcinomas) and the true pancreatic ductal adenocarcinomas is difficult although microRNA analysis might help differentiate.[7] Due to their type-specific survival

patterns and different response to chemo(radio)therapy, differentiation should be made both for the patient (prognosis) as well as for research and further treatment options.
However future improvements in CT scanning techniques, endoscopic ultrasound experience with subsequent fine needle aspiration and DNA analysis might facilitate pre-operative differentiation and therefore facilitate individualised and targeted therapy.

PART II
UNRESECTABLE PANCREATIC CANCER

Unresectable locally advanced pancreatic cancer should be differentiated from metastasised pancreatic cancer. In metastatic disease, the survival determining lesions occur outside the pancreas and it is an illusion to be able to treat this with localised (radio)therapy.
Unresectability is based on (mainly arterial) vascular invasion. Resectability is assessed on CT scan by a dedicated radiologist. However, in our study laparoscopy nevertheless reveals CT occult metastases in 35% of the patients. A similar result was found by Shoup and Liu.[8-10] Detection of these occult lesions might lead to amendment of the offered treatment in the individual patient, but might also explain negative study results of radiotherapy in these patients due to the actual metastasised character of their disease. A thoracic CT scan should be part of the work-up in these patients to exclude pulmonary metastases.
After ruling out metastasized disease, we treated this selected group of patient with true unresectable pancreatic cancer with oral Tegafur/ Uracil (UFT) combined with leucovorin (folinic acid), celecoxib and radiotherapy in a phase II trial. Unfortunately, toxicity was substantial and compared to literature, no clear survival benefit could be found of our short intensive chemoradiotherapy schedule consisting of radiation, 50 Gy in four weeks, oral UFT, leucovorin, and celecoxib (median overall survival was 10.6 months with a median time to progression of 6.9 months) and none of the patients subsequently underwent an exploratory laparotomy.[11-15] A recent randomised controlled trial performed by Chauffert[16] revealed a significant survival benefit of gemcitabine alone, over intensive 5-FU and cisplatin based chemoradiotherapy followed by the same maintenance gemcitabine therapy (median survival 13 vs. 8.6 months; p= 0.03).
Only after careful staging including laparoscopy, study results on either locoregional therapy or systemic therapy for locally advanced pancreatic cancer can be interpreted correctly.
Chaufferts study is promising and the results should be confirmed. However, survival benefit is still only 4 months which leads the way for further research including newer agents. Based on our trial and recent literature, with promising results of the new FOLFIRINOX regimens, we will

initiate a phase II trial in patients with locally advanced pancreatic cancer. First, of all patients will be adequately staged, including diagnostic laparoscopy and will start with FOLFIRINOX. In case of stable disease or partial response, only these patients will start high dose stereotactic radiotherapy.

PART III
IN VITRO

Based on our studies, as well as on literature, pancreatic cancer is fairly radiotherapy-resistant. Because we believe in combined treatment aimed at either preventing local recurrence or tumour growth by radiotherapy and distant progression by chemotherapy, we sought for ways to enhance the effect of radiotherapy in pancreatic cancer. In several tumours radiosensitizing properties of interferon alpha (IFNα) and interferon beta (IFNβ) have been demonstrated in vitro[17-22] as well as in vivo.[23, 24] In pancreatic cancer cell lines, IFNα has already shown to act as radiosensitizer.[25]
The exact mechanism by which IFN exerts this radiosensitising activity is unclear. They have direct anti-tumour effects including apoptosis, cell damage, upregulation of cancer antigens and a growth inhibitory effect with accumulation of cells in S phase. The radiosensitising mechanism might result from the interference of interferon on the repair of sublethal DNA damage resulting in an inability of cells to accumulate this kind of DNA damage. Furthermore alteration in oncogene expression levels might sensitize radioresistant cells to radiotherapy.
In vivo studies showed that approximately 20% of pancreatic cancers express the universal IFN receptor (IFNAR) with a higher affinity for IFNβ.[26] We decided to address these issues in colony forming assays, because besides the apoptotic effects of radiation and IFNs, the reproductive integrity of tumour cells (i.e. the capacity to produce an expanding colony of descendants, and therefore to regrow the tumour if left intact at the end of treatment) is of pivotal importance.
Our *in vitro* study shows a radiosensitizing effect of type I interferons on pancreatic cancer cell lines MiaPaca-2 and Panc-1. Miapaca-2 was 2.15 times more sensitive to radiotherapy after treatment with IFNα 3000 IU/ml and 1.72 times more sensitive after IFNβ 100 IU/ml. The sensitivity to radiotherapy of Panc-1 increased 1.90 times after treatment with IFNα 3000 IU/ml and 1.51 times after IFNβ 100 IU/ml. Radiosensitization was dose dependently, with higher doses of interferons resulting in a higher radiosensitivity, i.e. a lower surviving fraction at 2 Gray (SF2). Both overall radiosensitivity and sensitivity to interferon therapy was higher in MiaPaca-2 than in Panc-1, which does express more plasma membrane located IFN receptors than Panc-1.
Beneficial effects of IFNα combined with chemoradiotherapy were already demonstrated in phase

II studies in patients with resectable pancreatic cancer. However, treatment is associated with considerable toxicity.[27-29]

Interferon β seems the most promising drug. One of the problems for future in vivo studies is obtaining a sufficient level of IFNβ for a sufficient period in the cancerous pancreas without subsequent toxicity.

Off course, interferons are not the only radiosensitising agencies. Newer drugs and newer combinations are under research all to improve survival in this still devastating disease.

Pancreatic cancer, and to a lesser extend periampullary cancer, is such a devastating disease, only modestly repliant to regular treatment. A modestly positive effect of treatment in clinical studies only occurs in selected patients such as non-metastatic periampullary tumours and IFN receptor positive cells in the *in vitro* experiments.

Already at diagnosis, we should be able to direct patients to their tumour specific treatment, which might be neo-adjuvant therapy. In patients with locally advanced disease, a diagnostic laparoscopy should be performed for optimal staging. Since we cannot differentiate at diagnosis between pancreatic and periampullary cancer we will not be able to proceed into an individualised neo-adjuvant treatment. Until then, unnecessary toxicity and diminished quality of life without consequent survival benefit in some patients cannot be avoided. Probably, pre-operative FNA biopsies followed by tumor genetic profiling will determine which patients might benefit from either neo-adjuvant therapy or personalized systemic chemo(radio)therapy.

REFERENCES

1. Beger HG, Gansauge F, Buchler MW, Link KH. Intraarterial adjuvant chemotherapy after pancreaticoduodenectomy for pancreatic cancer: significant reduction in occurrence of liver metastasis. World J Surg 1999; 23(9):946-9.
2. Hayashibe A, Kameyama M, Shinbo M, Makimoto S. Clinical results on intra-arterial adjuvant chemotherapy for prevention of liver metastasis following curative resection of pancreatic cancer. Ann Surg Oncol 2007; 14(1):190-4.
3. Ishikawa O, Ohhigashi H, Sasaki Y, et al. Extended pancreatectomy and liver perfusion chemotherapy for resectable adenocarcinoma of the pancreas. Digestion 1999; 60 Suppl 1:135-8.
4. Neoptolemos JP, Dunn JA, Stocken DD, et al. Adjuvant chemoradiotherapy and chemotherapy in resectable pancreatic cancer: a randomised controlled trial. Lancet 2001; 358(9293):1576-85.
5. Willett CG, Czito BG, Bendell JC. Adjuvant therapy of pancreatic cancer. Cancer J 2007; 13(3):185-91.
6. Neoptolemos JP, Stocken DD, Bassi C, et al. Adjuvant chemotherapy with fluorouracil plus folinic acid vs gemcitabine following pancreatic cancer resection: a randomized controlled trial. Jama; 304(10):1073-81.
7. Marsh Rde W, Alonzo M, Bajaj S, et al. Comprehensive review of the diagnosis and treatment of biliary tract cancer 2012. Part I: diagnosis-clinical staging and pathology. J Surg Oncol; 106(3):332-8.
8. Liu RC, Traverso LW. Laparoscopic staging should be used routinely for locally extensive cancer of the pancreatic head. J Gastrointest Surg 2004; 8(8):923-4.
9. Liu RC, Traverso LW. Diagnostic laparoscopy improves staging of pancreatic cancer deemed locally unresectable by computed tomography. Surg Endosc 2005; 19(5):638-42.
10. Shoup M, Winston C, Brennan MF, et al. Is there a role for staging laparoscopy in patients with locally advanced, unresectable pancreatic adenocarcinoma? J Gastrointest Surg 2004; 8(8):1068-71.
11. Isacoff WH, Bendetti JK, Barstis JJ, et al. Phase II trial of infusional fluorouracil, leucovorin, mitomycin, and dipyridamole in locally advanced unresectable pancreatic adenocarcinoma: SWOG S9700. J Clin Oncol 2007; 25(13):1665-9.
12. Park JK, Ryu JK, Lee JK, et al. Gemcitabine chemotherapy versus 5-fluorouracil-based concurrent chemoradiotherapy in locally advanced unresectable pancreatic cancer. Pancreas 2006; 33(4):397-402.
13. Goldstein D, Van Hazel G, Walpole E, et al. Gemcitabine with a specific conformal 3D 5FU radiochemotherapy technique is safe and effective in the definitive management of locally advanced pancreatic cancer. Br J Cancer 2007; 97(4):464-71.
14. Haddock MG, Swaminathan R, Foster NR, et al. Gemcitabine, cisplatin, and radiotherapy for patients with locally advanced pancreatic adenocarcinoma: results of the North Central Cancer Treatment Group Phase II Study N9942. J Clin Oncol 2007; 25(18):2567-72.
15. Jeekel J, Treurniet-Donker AD. Treatment perspectives in locally advanced unresectable pancreatic cancer. Br J Surg 1991; 78(11):1332-4.
16. Chauffert B, Mornex F, Bonnetain F, et al. Phase III trial comparing intensive induction chemoradiotherapy (60 Gy, infusional 5-FU and intermittent cisplatin) followed by maintenance gemcitabine with gemcitabine alone for locally advanced unresectable pancreatic cancer. Definitive results of the 2000-01 FFCD/SFRO study. Ann Oncol 2008; 19(9):1592-9.
17. Angioli R, Sevin BU, Perras JP, et al. In vitro potentiation of radiation cytotoxicity by recombinant interferons in cervical cancer cell lines. Cancer 1993; 71(11):3717-25.
18. Chang AY, Keng PC. Potentiation of radiation cytotoxicity by recombinant interferons, a phenomenon associated with increased blockage at the G2-M phase of the cell cycle. Cancer Res 1987; 47(16):4338-41.
19. Gould MN, Kakria RC, Olson S, Borden EC. Radiosensitization of human bronchogenic carcinoma cells by interferon beta. J Interferon Res 1984; 4(1):123-8.

20. Hoffmann W, Blase MA, Santo-Hoeltje L, et al. Radiation sensitivity of human squamous cell carcinoma cells in vitro is modulated by all-trans and 13-cis-retinoic acid in combination with interferon-alpha. Int J Radiat Oncol Biol Phys 1999; 45(4):991-8.
21. Schmidberger H, Rave-Frank M, Lehmann J, et al. Radiosensitizing effect of natural and recombinant beta-interferons in a human lung carcinoma in vitro. J Cancer Res Clin Oncol 1999; 125(6):350-6.
22. Schmidberger H, Rave-Frank M, Lehmann J, et al. The combined effect of interferon beta and radiation on five human tumor cell lines and embryonal lung fibroblasts. Int J Radiat Oncol Biol Phys 1999; 43(2):405-12.
23. Basu P, Biswas J, Mandal R, Choudhury P. Is interferon-alpha and retinoic acid combination along with radiation superior to chemo-radiation in the treatment of advanced carcinoma of cervix? Indian J Cancer 2006; 43(2):54-9.
24. Holsti LR, Mattson K, Niiranen A, et al. Enhancement of radiation effects by alpha interferon in the treatment of small cell carcinoma of the lung. Int J Radiat Oncol Biol Phys 1987; 13(8):1161-6.
25. Ma JH, Patrut E, Schmidt J, et al. Synergistic effects of interferon-alpha in combination with chemoradiation on human pancreatic adenocarcinoma. World J Gastroenterol 2005; 11(10):1521-8.
26. Saidi RF, Remine SG, Jacobs MJ. Interferon receptor alpha/beta is associated with improved survival after adjuvant therapy in resected pancreatic cancer. HPB (Oxford) 2007; 9(4):289-94.
27. Linehan DC, Tan MC, Strasberg SM, et al. Adjuvant interferon-based chemoradiation followed by gemcitabine for resected pancreatic adenocarcinoma: a single-institution phase II study. Ann Surg 2008; 248(2):145-51.
28. Marten A, Schmidt J, Ose J, et al. A randomized multicentre phase II trial comparing adjuvant therapy in patients with interferon alpha-2b and 5-FU alone or in combination with either external radiation treatment and cisplatin (CapRI) or radiation alone regarding event-free survival - CapRI-2. BMC Cancer 2009; 9:160.
29. Picozzi VJ, Kozarek RA, Traverso LW. Interferon-based adjuvant chemoradiation therapy after pancreaticoduodenectomy for pancreatic adenocarcinoma. Am J Surg 2003; 185(5):476-80.

CHAPTER 9

Dutch summary (Samenvatting)

Alvleesklierkanker is de acht meest voorkomende vorm van kanker in Europa, met zo'n 96.000 nieuw gediagnosticeerde patiënten per jaar. Het is een agressieve vorm van kanker waarbij het incidentie cijfer nagenoeg gelijk is aan het jaarlijkse mortaliteitscijfer. De 5 jaars overleving voor de gehele groep ten tijde van de diagnose ligt rond de 5%, met een mediane overleving van 2-8 maanden. Bij diagnose wordt onderscheid gemaakt tussen patiënten met resectabele, irresectabele en gemetastaseerde tumoren. Slechts in 15-20% van de patiënten betreft het een operabele tumor op het moment van diagnose en alleen deze patiënten zijn mogelijk curatief te behandelen. Het merendeel van de patiënten presenteert zich met lokaal doorgegroeide (irresectabel) of uitgezaaide (gemetastaseerde) tumoren waarvoor resectie geen optie meer is. In deze groep van patiënten is curatie niet mogelijk en zal een eventuele behandeling verlenging van het leven beogen, waarbij de kwaliteit van leven voor deze patiënten van groot belang is.. Het spreekt voor zich dat patiënten na een succesvolle operatie een betere levensverwachting hebben dan patiënten met irresectabele of gemetastaseerde tumoren.
In het pancreas (alvleesklier) komen verschillende maligne (kwaadaardige) tumoren voor welke verschillen in biologisch gedrag. Meest voorkomend is het ductaal adenocarcinoom uitgaand van de ductale cellen (bekleding van de afvoergang). Dit is ook de meest agressieve vorm van pancreaskanker met de kortste overleving (5 jaars overleving 5% to 25%, mediaan 12-15 maanden). De tweede vorm van kanker in de pancreaskop is het periampullair carcinoom. Deze tumoren zijn gelegen rond de uitmonding van de ductus pancreaticus en choledochus in het duodenum, de ampulla van Vater genaamd, en betreft zowel tumoren uitgaand van het laatste deel van de galgang, de ampul zelf of het duodenum. De overleving van deze patiënten na operatie is gunstiger dan voor patiënten met een ductaal adenocarcinoom (5 jaars overleving 40% to 70%, mediaan 25- 42 maanden).

RESECTABELE TUMOREN (DEEL I)

Ondanks dat de overleving van patiënten met resectabele pancreastumoren na resectie beter is dan voor patiënten met irresectabele of gemetastaseerde tumoren, is er met een mediane overleving van 12-15 maanden voldoende ruimte voor verbetering. De teleurstellende overleving wordt veroorzaakt door het ontstaan van lokale recidieven en metastasen (uitzaaiingen) bij 90% van de patiënten. Juist om deze recidieven en metastasering terug te dringen is er in het verleden reeds gezocht naar een manier om naast lokale controle middels chirurgie in combinatie met radiotherapie, ook systemische controle middels aanvullende chemotherapie te verkrijgen. Verscheidene combinaties zijn in het verleden onderzocht, zonder eenduidig overlevingsvoordeel.

Een veelbelovende therapie is onderzocht in patiënten met resectabele en irresectabele tumoren waarbij chemotherapie gegeven wordt via een intra arteriële lijn gelegen in de truncus coeliacus (directe aflevering van de chemotherapie in de slagader naar de lever) waarbij een hoge dosis in de lever wordt bereikt. Dit heeft tot doel levermetastasen te voorkomen, maar heeft geen effect op het lokale recidief. Door radiotherapie aan dit schema toe te voegen, wat met name een lokaal effect heeft, wordt beoogd zowel lokaal als op afstand het recidief te voorkomen en daarmee de overleving na resectie in patiënten met ductaal adenocarcinoom en periampullaire tumoren te verlengen.
De resultaten van een gerandomiseerde studie waarin deze aanvullende behandeling werd aangeboden aan patiënten na operatie worden beschreven in **hoofdstuk 2.** In de gecombineerde groep patiënten met ductaal adenocarcinoom of periampullaire tumor heeft deze intensieve vorm van nabehandeling geen effect op de overleving. In de gehele groep is er wel minder progressie van de ziekte, hetgeen met name bepaald wordt doordat de patiënten met periampullaire tumoren minder levermetastasen ontwikkelen. In deze selecte patiënten is er echter geen verlengde overleving, ook niet na lange termijn analyse (**hoofdstuk 4**). Het effect van deze langdurige en invasieve nabehandeling op de kwaliteit van leven is beschreven in **hoofdstuk 3** waarbij opvalt dat patiënten geen nadelige gevolgen ervaren op het gebied van lichamelijk, emotioneel en sociaal functioneren. Op het gebied van pijn, misselijkheid en braken werd zelfs een betere kwaliteit van leven vermeld over een periode van 24 maanden.

IRRESECTABELE TUMOREN (DEEL II)

Indien er geen sprake is van resectabele ziekte, is het van belang onderscheid te maken tussen lokale doorgroei (lokaal irresectabel) en gemetastaseerde (uitgezaaide) ziekte. Om te spreken van een lokaal irresectabel pancreascarcinoom, moeten metastasen uitgesloten worden. Hiertoe wordt in de dagelijkse praktijk een CT scan verricht en een röntgenfoto van de thorax (borstkas). Echter, in de literatuur is reeds bewezen dat kleine metastasen in de buikholte (peritoneaal metastasen) middels CT scan slecht gedetecteerd worden in patiënten met resectabele tumoren. Om een uniforme groep te creëren van patiënten met irresectabele, niet-gemetastaseerde ziekte, hebben we in **hoofdstuk 5** bij alle patiënten met irresectabele tumoren een laparoscopie (kijkoperatie) uitgevoerd om peritoneaal metastasen uit te sluiten. In 35% van de patiënten werden alsnog peritoneaal metastasen aangetoond, hetgeen een verandering in het behandelschema teweeg bracht.

Vervolgens werden deze patiënten in een multicentrisch onderzoek in samenwerking met het Academisch Medisch Centrum, Amsterdam behandeld met radiotherapie, gecombineerd met middelen om het effect van de radiotherapie te versterken (1 chemotherapeuticum, foliumzuur en een ontstekingsremmer). Deze resultaten zijn beschreven in **hoofdstuk 6**. De mediane overleving na deze behandeling was 10.6 maanden en ernstige toxiciteit was aanwezig in 28% van de patiënten. Vergeleken met de overleving zonder behandeling (12- 15 maanden) wordt er geen overlevingswinst waargenomen en adviseren wij deze behandeling dus niet meer voor patiënten met een lokaal irresctabel pancreascarcinoom.

IN HET LAB (DEEL III)

Pancreascarcinoom blijkt vrij resistent tegen zowel chemo- als radiotherapeutische behandeling. Om de gevoeligheid voor radiotherapie te vergroten kunnen radiosensitizers toegevoegd worden aan de behandeling. Deze middelen zijn vooral van invloed op de celcyclus, met als doel om het merendeel van de cellen in dat deel van de cyclus te brengen dat het meest gevoelig is voor bestraling.
Interferonen staan bekend om hun effect op de celcyclus en als radiosensitizers in andere tumorsoorten. Een deel van de pancreascarcinomen hebben interferon receptoren op hun membraan (buitenkant van de cel). Om deze reden, hebben we in verschillende pancreas cellijnen, met verschillende hoeveelheid interferonreceptoren op hun celmembraan, het effect van voorbehandeling met interferon op de gevoeligheid voor straling bepaald (**hoofdstuk 7**). Zowel interferon-α als interferon-β verhoogd het effect van radiotherapie, waarbij interferon-β met krachtigste effect. Dit zal in de toekomst vertaald moeten gaan worden naar een studie in patiënten. Bij voorkeur in die patienten waarbij bekend is dat er een grote hoeveelheid interferon receptoren op het celmembraan aanwezig is.

Zoals beschreven in dit proefschrift, blijft het ductaal adenocarcinoom van het pancreas en in mindere mate ook de periampullaire tumoren, een levensbedreigende aandoening welke slechts matig respondeert op behandeling.
Meer en meer zal gestreeft moeten worden naar een geindividualiseerde behandeling en verdient mogelijk voorbehandeling van deze tumoren de voorkeur. Echter zolang ten tijde van de diagnose geen onderscheid gemaakt kan worden tussen peri ampullaire en ductale pancreascarcinomen,is er nog een lange weg te gaan. Om de resultaten van nieuwe behandelingen juist te kunnen interpreteren is een zorgvuldige stagering van groot belang en dient dan ook bij patienten

met een lokaal irresectabele tumor een laparoscopie te worden uitgevoerd. Voor een betere individuele behandeling is mogelijk het nemen van preoperatieve biopten voor tumor genetic profiling een hoopvolle nieuwe ontwikkeling.

CHAPTER 10

Appendices

List of Publications

Acknowledgements (Dankwoord)

Curriculum Vitae Auctoris

LIST OF PUBLICATIONS

Overcoming the Unexpected.
M.J.M. Morak, S. ten Raa, F. Bastos Goncalves and H.J.M. Verhagen.
Endovascular Today 2012 Sept; 80-84

Type I Interferons as Radiosensitizers for Pancreatic Cancer.
M.J.M. Morak, P.M. van Koetsveld, R. Kanaar, L.J. Hofland and C.H.J. van Eijck.
Eur J Cancer. 2011 Sep;47(13):1938-45.

Afgaans "Wild Life"
O.J.F. van Waes, M.J.M. Morak.
Nederlands Tijdschrift voor Heelkunde 2011 jun;20(5): 191- 192

Phase II trial of Uracil/Tegafur plus leucovorin and celecoxib combined with radiotherapy in locally advanced pancreatic cancer.
M.J.M. Morak, D.J. Richel, C.H.J. van Eijck, J.J. Nuyttens, A. van der Gaast, W.L. Vervenne, E.E. Padmos, E.E. Schaake, O.R. Busch and G.J. van Tienhoven.
Radiother Oncol. 2011 Feb;98(2):261-4.

Quality of life after adjuvant intra-arterial chemotherapy and radiotherapy vs. surgery alone in resectable pancreatic and periampullary cancer.
M.J.M. Morak, C.J. Pek, W.C. Hop, G. Kazemier and C.H.J. van Eijck.
Cancer

Staging for suspected locally advanced pancreatic cancer.
M.J.M. Morak, H.G. Smeenk, W.M. Renders, J.J.M.E. Nuyttens, J.J. Hermans, G. Kazemier and C.H.J. van Eijck.
Eur J Surg Oncol. 2009 Sep;35(9):963-8.

A single lumen central venous catheter for continuous and direct intra-abdominal pressure measurement.
O. van Waes, J. Jaquet, J.N.M. IJzermans, M.J.M. Morak, W.C. Hop and J. Koning.
European Journal of Trauma and Emergency Surgery 2009 Jan: 1-6.

Detectie van maagstompcarcinomen na benigne partiële maagresectie. Een pleidooi voor screening.
M.J.M. Morak, T. Hagenaars and G.W.M. Tetteroo.
Nederlands Tijdschrift voor Heelkunde 2009 jan;18(1): 25-27

Ischemic acute necrotizing pancreatitis in a marathon runner.
J. Mast, M.J.M. Morak, B.T. Brett and C.H.J. van Eijck.
JOP. 2009 Jan 8;10(1):53-4.

Adjuvant intra-arterial chemotherapy and radiotherapy vs. surgery alone in resectable pancreatic and periampullary cancer. A prospective randomized controlled trial.
M.J.M. Morak, A. van der Gaast, L. Incrocci, H. van Dekken, J.J. Hermans, J. Jeekel, W.C. Hop, G. Kazemier and C.H.J. van Eijck.
Ann Surg. 2008 Dec;248(6):1031-41.

ACKNOWLEDGEMENTS (DANKWOORD)

"Ik heb geen geheim van succes. Gewoon je best doen, iets leuk vinden
en goede mensen om je heen verzamelen.
Want alleen kan je niks en met zijn allen kun je alles."

Johan Cruijff

CURRICULUM VITAE AUCTORIS

Marjolein J.M. Morak werd geboren op 16 december 1979 te Rotterdam. Zij behaalde het eindexamen aan het Erasmiaans Gymnasium in Rotterdam in 1998. Haar Geneeskunde opleiding ving zij aan in Antwerpen waar zij haar propedeuse *met onderscheiding* haalde, waarna deze werd voortgezet aan de Erasmus Universiteit in Rotterdam. Tijdens haar co- schappen ontwikkelde zij haar interesse in de chirurgie, hetgeen resulteerde in een oudste co- schap op de afdeling heelkunde in het IJsselland Ziekenhuis. Na haar artsexamen welke zij in 2006 *cum laude* behaalde, werkte zij als arts assistent op de afdeling heelkunde van het Erasmus Medisch Centrum. In 2007 begon zij aan haar promotieonderzoek onder leiding van Prof. Dr. C.H.J. van Eijck wat uiteindelijk geresulteerd heeft in dit proefschrift. Na twee jaar full time onderzoek, begon zij op 1 januari 2009 aan haar opleiding tot chirurg in het IJsselland Ziekenhuis in Capelle a/d IJssel (opleider Dr. I. Dawson). Op 1 juli 2012 begon zij aan haar differentiatie tot vaatchirurg in het Erasmus Medisch Centrum (opleider Prof. Dr. H.J.M. Verhagen) welke zij in december 2014 afrondde in het Maasstad Ziekenhuis (opleider Dr. A.A.E.A. de Smet). Momenteel is zij gedurende 2 jaar fellow Vaatchirurgie in het Radboud Universitair Medisch Centrum en het Jeroen Bosch Ziekenhuis.
Marjolein woont samen met Martijn en hun zoontje Sepp in Rotterdam.