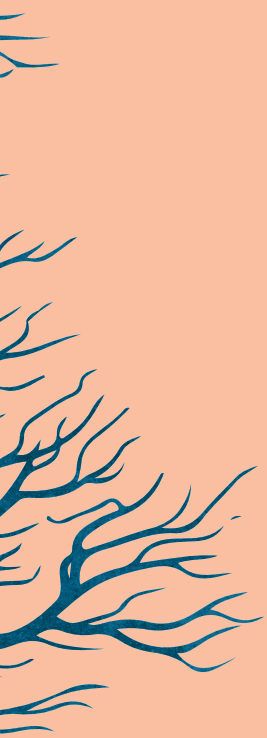
A translational approach towards perihilar cholangiocarcinoma

Roos, E.

Publication date
2019
Document Version
Other version
License
Other

1

General Introduction and Outline of the Thesis



GENERAL INTRODUCTION

"If one is truly to succeed in leading a person to a specific place, one must first and foremost take care to find him where he is and begin there. This is the secret in the entire art of helping."
Søren Kierkegaard

Perihilar cholangiocarcinoma is a rare tumor that derives from the epithelium of the biliary tract. Cholangiocarcinomas are rare in Western countries: they comprise 3% of all gastrointestinal malignancies and affect 1-2 per 100.000 people per year in the United States.[1,2] However, in some regions, especially North Thailand, China, Japan and Chili, incidence is much higher, mainly due to a higher rate of infections of the biliary tract with liver flukes.[1,3,4] Perihilar cholangiocarcinoma, as the name suggests, is a tumor located in the hilum of the liver, proximal to the cystic duct and distal to the second bifurcation of the hepatic ducts.[5,6] In 1965, the tumor was first described by Gerald Klatskin, and is therefore often called Klatskin tumor. Although Klatskin started as a surgical resident, he became fascinated with the workings of the liver, later, resulting in a switch to a medical residency. His collaborative work with other disciplines, such as hepatology, surgery and pathology led him to become one of the leading figures in hepatological science. His "multidisciplinary" way of solving scientific problems has laid the foundation for the best approach to treat the tumor that bears his name: perihilar cholangiocarcinoma.

Due to its challenging anatomical location, patients with perihilar cholangiocarcinoma are treated by a team consisting of specialists from different medical fields. The aim of this thesis was to improve the management of patients with perihilar cholangiocarcinoma by connecting several different points of view; the view of the gastroenterologist and intervention radiologist, who need to manage patients with obstructive jaundice and several of the benign diseases that mimic cholangiocarcinoma. The perspective of the surgeon and medical oncologist, who treat these patients in different stages of their disease. And, finally, the point of view of the pathologist and the molecular biologists who aid in the understanding of the disease and assess the residual disease status after a resection. I am convinced, as was Klatskin, that new solutions to old dilemmas can be discovered and integrated by translating the different 'languages' spoken by the specialists involved in diagnosis, treatment and care. The right translation potentiates a multidisciplinary approach by combining their expertise both in the field of clinical care and scientific research.

The first part of the thesis, addresses the arising dilemmas that come with the care for these patients affected by perihilar cholangiocarcinoma. In the second part, the basics of biological behavior of biliary tract tumors are assessed. In the third part, two novel techniques are tested in order to improve diagnosis of perihilar cholangiocarcinoma.

OUTLINE OF THE THESIS

Part I: Dilemmas in the management of patients with perihilar cholangiocarcinoma

"Data, data, data!" he cried impatiently "I cannot make bricks without clay." Sherlock Holmes, the adventure of the copper breaches, Arthur Conan Doyle

Patients with perihilar cholangiocarcinoma (PHC) often present with obstructive jaundice. Obstruction of the flow of bile from the liver impairs the proper functioning of the liver.[7–9] This increases the risk of complications in patients with PHC. Since major surgery, often an (extended) hemihepatectomy with extrahepatic bile duct resection and lymphadenectomy or even a liver transplantation, is the only curative treatment for patients with PHC, improving the function of the liver preoperatively is one of the corner stones of PHC management.[10,11] However, only a minority of patients, around 20%, is a candidate for surgical treatment and of these patients a further 40% is found to be unresectable preoperatively due to advanced disease. [12] Surgical treatment of PHC comes with considerable risk of complications due to the major procedure required and the impaired liver function due to obstructive jaundice. Postoperative morbidity and mortality are as high as 40-70% and 10-18% respectively.[13–17] An overview of postoperative complications is given in **CHAPTER 2**. The high risk of postoperative death is mainly caused by an impaired liver function and insufficient regeneration of the liver, possibly worsened by obstructive jaundice prior to surgery.[14,18] Then, the future remnant liver (FRL) is too impaired in its function to perform properly.[19,20] Thorough preoperative management and work-up of resectable PHC patients is therefore crucial to identify the patients benefitting from curative surgery. Several challenges are characteristic for the work-up of PHC patients: first, establishing the diagnosis and second improvement of liver function.

Imaging of the biliary tract by means of computer tomography (CT), magnetic resonance imaging (MRI) or endoscopic retrograde cholangio-pancreaticography (ERCP) is most commonly the first step to determine a biliary stricture.[21,22] However, not only PHC presents as a biliary structure, but also other (benign) inflammatory diseases of the

biliary tract such as primary sclerosing cholangitis and IgG4-associated cholangitis.[21,23] ERCP coupled with brush cytology is necessary to ensure pathology confirmation of a malignancy. However, due to the periductal growth pattern of PHC and its high stromal component, sensitivity of brush cytology is low (27-56%).[24] Because of this, the pathological diagnosis of PHC often remains unconfirmed.[25,26] Several techniques have been tested to improve sensitivity of tissue diagnosis and will be further discussed in **PART III**. In case of inconclusive pathology, resection is still considered. In approximately 50% of patients with presumed, resectable PHC, partial hepatectomy is undertaken without histopathological confirmation of malignancy.[21,26] A consequence is that 15% of patients who undergo surgery under the suspicion of PHC, do not have a malignancy at final pathological assessment of the resection specimen.

Sufficient liver function is key to a successful operation. Function and volume of the FRL can be assessed by hepatobiliary scintigraphy or CT-volumetry.[27–30] Preoperative biliary drainage of the FRL before surgery, can improve preoperative liverfunction.[31–33] When the FRL is still not sufficient enough, preoperative portal vein embolization of the liver can improve volume and function of the FRL.[7,34,35] These challenges in the preoperative work-up of PHC are elaborated in **CHAPTER 3**.

Preoperative biliary drainage has been one of the corner stones of a thorough work-up for resection of PHC. One of the benefits is that it improves liver function by reducing cholestasis in the liver.[33,36] One of the disadvantages is that biliary drainage also can cause complications, such as cholangitis.[37,38] Unfortunately cholangitis itself impairs postoperative outcome.[38] Therefore when using biliary drainage for PHC, there is a fine line between the choice to drain or not to drain. In a large retrospective cohort, Farges et al found that biliary drainage improved postoperative outcome and that it was recommended, especially when patients were jaundiced and had a small FLR.[31] A second debate concerns the best drainage technique. Preoperative biliary drainage, in Western countries, is often performed endoscopically, by a gastroenterologist or percutaneously through the liver, by an interventional radiologist. To provide an answer to the question which technique is superior, the first randomized controlled trial (DRAINAGE trial) including patients with PHC was started in 2013.[39] The primary outcome was the number of drainage related complications before surgery. Secondary outcome was postoperative morbidity and mortality. The results of this trial are presented in **CHAPTER 4**.

After resection of the tumor, the resection specimen is evaluated by a pathologist. This is an essential step to evaluate residual disease and oncological status. Residual disease and other pathology parameters are prognostic for survival in most tumors. However,

their value as a prognostic factor in cholangiocarcinoma research is not always evident from the literature and previous studies.[40] The lack of implemented standard pathology reporting combined with the complexity of the specimen (with multiple resection and dissection margins) leads to incomplete reporting.[41–43] In **CHAPTER 5** the bottlenecks in PHC pathology reporting are evaluated. Recommendations for future improvement of assessment and interpretation of the PHC resection specimen are provided as well.

The majority of patients with PHC, an estimated 70-80%, are unresectable and cannot be cured. One of the main issues for patients with (locally) advanced disease, is local obstruction of the bile ducts. Although stenting is performed, tumor growth into the stents can obstruct them again and decrease stent patency.[49] Therefore, ablative techniques in combination with systemic treatment are an attractive option since they might prolong stent patency. Options for systemic treatment alone, to elongate and improve quality of life are limited.[44] First line systemic treatment consists of chemotherapy with gemcitabine and cisplatin.[45] This increases survival from 9 to 12 months. Several new targeted treatment options are being researched at the moment.[46–48] In **CHAPTER 6**, a new treatment modality, irreversible electroporation (IRE), is introduced that might be able to help with this problem.[50]

Part II: The basics of biliary tract cancer

"Chaos is found in greatest abundance wherever order is being sought. It always defeats order, because it is better organized." Terry Pratchett

Perihilar cholangiocarcinoma is part of a diverse group of cancers that derive from the biliary tract. This group can be subdivided into four anatomical subtypes according to their localization. Intrahepatic cholangiocarcinoma (ICC) originates from the peripheral bile ducts, proximal of the second bifurcation of the hepatic ducts. Perihilar cholangiocarcinoma originates distal from the second bifurcation and proximal from the cystic duct. Gallbladder carcinoma (GBC) originates from the epithelium of the gallbladder or cystic duct and distal cholangiocarcinoma (DCC) originates from the mid-section of the common bile duct towards the ampulla. There has always been much debate on the classification of biliary tract carcinoma (BTC)[51]. From a surgical perspective BTC are often treated as different entities. Anatomical subtypes require different surgical treatments and therefore reporting is often done per subtype. Since surgical treatment differs, postoperative outcomes differ as well: probably mainly related to the extent of surgery.[52,53] However, it is not clear in what way the long-term (oncological) outcome

is influenced by anatomical subtype. In **CHAPTER 7** an overview of long and short-term outcome of patients with resected biliary tract cancers is provided. Differences and overlap in biological behavior could be explained by genomic background of tumors.[54]

Several authors have looked into the molecular background of the four different anatomical subtypes of biliary tract cancer. Interestingly, genomic profiles tend to be related to the cell of origin from which the tumor derives.[55–57] Intrahepatic tumors can derive from the stem cell niche or from dedifferentiated cells in the smaller or larger bile ducts whereas perihilar and distal tumors can derive from the progenitor cells in the peribiliary glands or from dedifferentiated mature cells in the larger bile ducts.[58–61] More insight into the overlap and differences in the genomic profile of biliary tract cancers can help with the development of new diagnostic techniques, such as liquid biopsies.[62–65] Furthermore, it could help to find targets for therapy.[47,66–68] An overview, however, of mutations of the whole biliary tract was lacking. In **CHAPTER 8** this overview is provided as a systematic review and meta-analysis of the current literature on mutations of biliary tract cancer.

Part III: Novel approaches to diagnose perihilar cholangiocarcinoma

"But I don't want to go among mad people!" Alice remarked. "Oh, you can't help that" said the Cat: "we're all mad here. I'm mad, you're mad." "How do you know I'm mad?" said Alice. "You must be" said the Cat, "or you wouldn't have come here." Alice in Wonderland, Lewis Caroll

The integration of molecular data in the daily practice of medicine is a promising field. Novel techniques, such as targeted sequencing of circulating tumor DNA and resection, biopsy and cytology specimens have been added to the repertoire of physicians in the treatment and diagnostics of for example lung- and colorectal carcinoma.[62,63,69,70] However, for many rare diseases, including CCA, the role of molecular biology in daily practice, is still in its early days. Nevertheless, it is a promising field. It can be used, not only to understand disease, but also to increase diagnostic effectiveness and to find targets for therapy. As mentioned earlier, one of the main issues in establishing a diagnosis of PHC is to differentiate it from benign disease such as IgG4-associated cholangitis or primary sclerosing cholangitis, since all these entities can cause hilar strictures. Simply measuring serum IgG4 is not an optimal method to establish IgG4-relate disease. This is mainly due to the fact that not all patients with IgG4-related disease have a high serum IgG4, and, furthermore, some 15% of patients with a malignancy of the pancreas or biliary tract have increased IgG4.[71,72] This phenomenon has been observed in other malignancies and is further elaborated in **CHAPTER 9**. ERCP with brush cytology

is to date the reference standard to obtain a pathology diagnosis.[24,73] However, its sensitivity is low 27-56%.[21] One of the main problems, is the low yield of tumor cells of biliary brushes. Perihilar tumors often show periductal growth and have a high stromal content. Moreover, strictures and concomitant inflammation of the hilar ducts may cause reactive atypia of the biliary epithelium. Therefore, brush cytology is often inconclusive since it only contains stromal or atypical cells. Several auxiliary techniques have been investigated to either increase tissue yield (different brush techniques, intraluminal biopsies or cholangioscopy) or to detect molecular fingerprints of cancer in cytology samples, such as FISH or qPCR.[74–80] PCR of *KRAS* and FISH proved useful in brush cytology of patients with pancreatic cancer or distal cholangiocarcinoma.[78,79] However, *KRAS* mutations are less frequent in perihilar cholangiocarcinoma compared to pancreatic cancer.[81] Targeted sequencing of multiple genes, not *KRAS* only, could help to further determine these samples.

In **CHAPTER 10** the results of a pilot study in which inconclusive brush cytology was sequenced are presented. The lack of accuracy of diagnostic tools results in an unnecessary surgical procedure in at least 15% of all patients with presumed resectable PHC.[23] Some of these patients have IgG4-associated cholangitis, a biliary tract manifestation of the systemic IgG4-related disease.[82,83] The disease is characterized by pseudotumor formation and inflammation, and is not easily diagnosed. In 2016, Doorenspleet *et al.* developed a test that determined the IgG4/IgG RNA ratio by qPCR in blood.[84] Since IgG4-associated cholangitis is a systemic disease, it remains active, even after resection. Immunosuppression with corticosteroid therapy is often the effective treatment.[85] Therefore, it is useful to detect the disease, even after resection for presumed PHC. In **CHAPTER 11**, we determined which patients who were resected for PHC actually had IgG4-associated cholangitis and tested if they still had active disease using the IgG/IgG4 ratio.

REFERENCES

1 Khan SA, Thomas HC, Davidson BR, Taylor-Robinson SD. Cholangiocarcinoma. *Lancet* 2005; **366**: 1303–14.

2 Nathan H, Pawlik TM, Wolfgang CL, Choti MA, Cameron JL, Schulick RD. Trends in Survival after Surgery for Cholangiocarcinoma: A 30-Year Population-Based SEER Database Analysis. *J Gastrointest Surg* 2007; **11**: 1488–97.

3 Banales JM, Cardinale V, Carpino G, *et al.* Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). *Nat Rev Gastroenterol Hepatol* 2016; **13**: 261–80.

4 Chan-On W, Nairismagi ML, Ong CK, *et al.* Exome sequencing identifies distinct mutational patterns in liver fluke-related and non-infection-related bile duct cancers. *Nat Genet* 2013; **45**: 1474–8.

5 Bismuth H, Nakache R, Diamond T. Management strategies in resection for hilar cholangiocarcinoma. *Ann Surg* 1992; **Jan**: 31–8.

6 Bismuth H. Surgical anatomy and anatomical surgery of the liver. *World J Surg* 1982; **6**: 3–9.

7 de Graaf W, van Lienden KP, van den Esschert JW, Bennink RJ, van Gulik TM. Increase in future remnant liver function after preoperative portal vein embolization. *Br J Surg* 2011; **98**: 825–34.

8 Yoshida Y, Ajiki T, Ueno K, *et al.* Preoperative Bile Replacement Improves Immune Function for Jaundiced Patients Treated with External Biliary Drainage. *J Gastrointest Surg* 2014; **18**: 2095–104.

9 van Gulik TM, Kloek JJ, Ruys AT, *et al.* Multidisciplinary management of hilar cholangiocarcinoma (Klatskin tumor): Extended resection is associated with improved survival. *Eur J Surg Oncol* 2011; **37**: 65–71.

10 van Gulik TM, Ruys AT, Busch ORC, Rauws EAJ, Gouma DJ. Extent of Liver Resection for Hilar Cholangiocarcinoma (Klatskin Tumor): How Much Is Enough? *Dig Surg* 2011; **28**: 141–7.

11 Gores GJ, Darwish Murad S, Heimbach JK, Rosen CB. Liver Transplantation for Perihilar Cholangiocarcinoma. *Dig Dis* 2013; **31**: 126–9.

12 Coelen RJS, Ruys AT, Besselink MGH, Busch ORC, van Gulik TM. Diagnostic accuracy of staging laparoscopy for detecting metastasized or locally advanced perihilar cholangiocarcinoma: a systematic review and meta-analysis. *Surg Endosc Other Interv Tech* 2016; **30**: 4163–71.

13 Otsuji H, Yokoyama Y, Ebata T, *et al.* Preoperative Sarcopenia Negatively Impacts Postoperative Outcomes Following Major Hepatectomy with Extrahepatic Bile Duct Resection. *World J Surg* 2015; **39**: 2368–9.

14 Olthof PB, Wiggers JK, Groot Koerkamp B, *et al.* Postoperative Liver Failure Risk Score: Identifying Patients with Resectable Perihilar Cholangiocarcinoma Who Can Benefit from Portal Vein Embolization. *J Am Coll Surg* 2017; **225**: 387–94.

15 Wiggers JK, Groot Koerkamp B, Cieslak KP, *et al.* Postoperative Mortality after Liver Resection for Perihilar Cholangiocarcinoma: Development of a Risk Score and Importance of Biliary Drainage of the Future Liver Remnant. *J Am Coll Surg* 2016; **223**: 321–331.e1.

16 Jarnagin WR, Fong Y, DeMatteo RP, *et al.* Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. *Ann Surg* 2001; **234**: 507-17; discussion 517-9.
17 van der Gaag NA, Kloek JJ, de Bakker JK, *et al.* Survival analysis and prognostic nomogram for patients undergoing resection of extrahepatic cholangiocarcinoma. *Ann Oncol* 2012; **23**: 2642–9.
18 Rahbari NN, Garden OJ, Padbury R, Brooke-smith M. Posthepatectomy liver failure : A definition and grading by the International Study Group of Liver Surgery (ISGLS). 2011. DOI:10.1016/j.surg.2010.10.001.
19 Baharvand H, Aghdami N. Acute Liver Failure. *Regen Med Cell Ther* 2012; **1st**: 210–211,212,213.
20 Hammond JS, Guha IN, Beckingham IJ, Lobo DN. Prediction, prevention and management of postresection liver failure. *Br J Surg* 2011; **98**: 1188–200.
21 Kloek JJ, Delden OM van, Erdogan D, *et al.* Differentiation of malignant and benign proximal bile duct strictures: The diagnostic dilemma. *World J Gastroenterol* 2008; **14**: 5032.
22 Engelbrecht MR, Katz SS, van Gulik TM, Laméris JS, van Delden OM. Imaging of Perihilar Cholangiocarcinoma. *Am J Roentgenol* 2015; **204**: 782–91.
23 Erdogan D, Kloek JJ, ten Kate FJW, *et al.* Immunoglobulin G4-related sclerosing cholangitis in patients resected for presumed malignant bile duct strictures. *Br J Surg* 2008; **95**: 727–34.
24 Navaneethan U, Njei B, Lourdusamy V, Konjeti R, John V, Mansour Parsi A. Comparative effectiveness of biliary brush cytology and intraductal biopsy for detection of malignant biliary strictures: a systematic review and meta-analysis. 2016; **1**: 23–30.
25 Esnaola NF, Meyer JE, Karachristos A, Maranki JL, Camp ER, Denlinger CS. Evaluation and management of intrahepatic and extrahepatic cholangiocarcinoma. *Cancer* 2016; **122**: 1349–69.
26 Zaydfudim VM, Wang AY, De Lange EE, *et al.* IgG4-associated cholangitis can mimic hilar cholangiocarcinoma. *Gut Liver* 2015; **9**: 556–60.
27 Bennink RJ, Dinant S, Erdogan D, *et al.* Preoperative Assessment of Postoperative Remnant Liver Function Using Hepatobiliary Scintigraphy. *J Nucl Med* 2004; **45**: 965–71.
28 Cieslak KP, Runge JH, Heger M, Stoker J, Bennink RJ, van Gulik TM. New Perspectives in the Assessment of Future Remnant Liver. *Dig Surg* 2014; **31**: 255–68.
29 Martel G, Cieslak KP, Huang R, *et al.* Comparison of techniques for volumetric analysis of the future liver remnant: implications for major hepatic resections. *HPB* 2015; **17**: 1051–7.
30 Cieslak KP, Bennink RJ, de Graaf W, *et al.* Measurement of liver function using hepatobiliary scintigraphy improves risk assessment in patients undergoing major liver resection. *HPB* 2016; **18**: 773–80.
31 Farges O, Regimbeau JM, Fuks D, *et al.* Multicentre European study of preoperative biliary drainage for hilar cholangiocarcinoma. *Br J Surg* 2013; **100**: 274–83.
32 Wiggers J, Koerkamp B, Coelen R, *et al.* Preoperative biliary drainage in perihilar cholangiocarcinoma: identifying patients who require percutaneous drainage after failed endoscopic drainage. *Endoscopy* 2015; **47**: 1124–31.
33 Kennedy TJ, Yopp A, Qin Y, *et al.* Role of preoperative biliary drainage of liver remnant prior to extended liver resection for hilar cholangiocarcinoma. *HPB* 2009; **11**: 445–51.

34 Nagino M, Ebata T, Yokoyama Y, *et al.* Evolution of surgical treatment for perihilar cholangiocarcinoma: a single-center 34-year review of 574 consecutive resections. *Ann Surg* 2013; **258**: 129–40.

35 Glantzounis GK, Tokidis E, Basourakos S-P, Ntzani EE, Lianos GD, Pentheroudakis G. The role of portal vein embolization in the surgical management of primary hepatobiliary cancers. A systematic review. *Eur J Surg Oncol* 2017; **43**: 32–41.

36 Kawakami H, Kuwatani M, Onodera M, *et al.* Endoscopic nasobiliary drainage is the most suitable preoperative biliary drainage method in the management of patients with hilar cholangiocarcinoma. *J Gastroenterol* 2011; **46**: 242–8.

37 Nimura Y. Preoperative biliary drainage before resection for cholangiocarcinoma (Pro). *Hpb* 2008; **10**: 130–3.

38 Ribero D, Zimmitti G, Aloia TA, *et al.* Preoperative Cholangitis and Future Liver Remnant Volume Determine the Risk of Liver Failure in Patients Undergoing Resection for Hilar Cholangiocarcinoma. *J Am Coll Surg* 2016; **223**: 87–97.

39 Wiggers JK, Coelen RJS, Rauws EAJ, *et al.* Preoperative endoscopic versus percutaneous transhepatic biliary drainage in potentially resectable perihilar cholangiocarcinoma (DRAINAGE trial): design and rationale of a randomized controlled trial. *BMC Gastroenterol* 2015; **15**: 20.

40 Komaya K, Ebata T, Yokoyama Y, *et al.* Recurrence after curative-intent resection of perihilar cholangiocarcinoma: analysis of a large cohort with a close postoperative follow-up approach. *Surg (United States)* 2018; **163**: 732–8.

41 Chatelain D, Farges O, Fuks D, Trouillet N, Pruvot FR, Regimbeau J-M. Assessment of pathology reports on hilar cholangiocarcinoma: The results of a nationwide, multicenter survey performed by the AFC-HC-2009 study group. *J Hepatol* 2012; **56**: 1121–8.

42 Westgaard A, Larønningen S, Mellem C, *et al.* Are survival predictions reliable? Hospital volume versus standardisation of histopathologic reporting for accuracy of survival estimates after pancreatoduodenectomy for adenocarcinoma. *Eur J Cancer* 2009; **45**: 2850–9.

43 Westgaard A, Clausen OPF, Gladhaug IP. Survival estimates after pancreatoduodenectomy skewed by non-standardized histopathology reports. *Apmis* 2011; **119**: 689–700.

44 Skipworth JR a, Olde Damink SWM, Imber C, Bridgewater J, Pereira SP, Malagó M. Review article: Surgical, neo-adjuvant and adjuvant management strategies in biliary tract cancer. *Aliment Pharmacol Ther* 2011; **34**: 1063–78.

45 Valle J, Wasan H, Palmer DH, *et al.* Cisplatin plus Gemcitabine versus Gemcitabine for Biliary Tract Cancer. *N Engl J Med* 2010; **362**: 1273–81.

46 Molenaar RJ, Coelen RJ, Khurshed M, *et al.* Study protocol of a phase IB/II clinical trial of metformin and chloroquine in patients with IDH1 -mutated or IDH2 -mutated solid tumours. *BMJ Open* 2017; **7**: e014961.

47 Borad MJ, Gores GJ, Roberts LR. Fibroblast growth factor receptor 2 fusions as a target for treating cholangiocarcinoma. *Curr Opin Gastroenterol* 2015; **31**: 264–8.

48 Sahu S, Sun W. Targeted therapy in biliary tract cancers—current limitations and potentials in the future. *J Gastrointest Oncol* 2017; **8**: 324–36.
49 Gaspersz MP, Buettner S, van Vugt JLA, *et al.* Conditional survival in patients with unresectable perihilar cholangiocarcinoma. *HPB* 2017; **19**: 966–71.
50 Coelen RJS, Vogel JA, Vroomen LGPH, *et al.* Ablation with irreversible electroporation in patients with advanced perihilar cholangiocarcinoma (ALPACA): a multicentre phase I/II feasibility study protocol. *BMJ Open* 2017; **7**: e015810.
51 Cardinale V, Bragazzi MC, Carpino G, *et al.* Cholangiocarcinoma: increasing burden of classifications. *Hepatobiliary Surg Nutr* 2013; **2**: 272–80.
52 Ercolani G, Dazzi A, Giovinazzo F, *et al.* Intrahepatic, peri-hilar and distal cholangiocarcinoma: Three different locations of the same tumor or three different tumors? *Eur J Surg Oncol* 2015; **41**: 1162–9.
53 David W, Patel T. Intrahepatic, perihilar and distal cholangiocarcinoma: management and outcomes. *Ann Hepatol* 2017; **16**: 133–9.
54 Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW. Cancer Genome Landscapes. *Science (80-)* 2013; **339**: 1546–58.
55 Bijlsma MF, Sadanandam A, Tan P, Vermeulen L. Molecular subtypes in cancers of the gastrointestinal tract. Nat. Rev. Gastroenterol. Hepatol. 2017. DOI:10.1038/nrgastro.2017.33.
56 Aloia L, McKie MA, Huch M. Cellular plasticity in the adult liver and stomach. *J Physiol* 2016; **594**: 4815–25.
57 Bragazzi MC, Ridola L, Safarikia S, *et al.* New insights into cholangiocarcinoma: Multiple stems and related cell lineages of origin. *Ann Gastroenterol* 2018; **31**: 42–55.
58 Cardinale V, Wang Y, Carpino G, *et al.* The biliary tree—a reservoir of multipotent stem cells. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 231–40.
59 Lanzoni G, Cardinale V, Carpino G. The hepatic, biliary, and pancreatic network of stem/progenitor cell niches in humans: A new reference frame for disease and regeneration. *Hepatology* 2016; **64**: 277–86.
60 Komuta M, Govaere O, Vandecaveye V, *et al.* Histological diversity in cholangiocellular carcinoma reflects the different cholangiocyte phenotypes. *Hepatology* 2012; **55**: 1876–88.
61 Liau JY, Tsai JH, Yuan RH, Chang CN, Lee HJ, Jeng YM. Morphological subclassification of intrahepatic cholangiocarcinoma: Etiological, clinicopathological, and molecular features. *Mod Pathol* 2014; **27**: 1163–73.
62 Kinde I, Bettegowda C, Wang Y, *et al.* Evaluation of DNA from the Papanicolaou test to detect ovarian and endometrial cancers. *Sci Transl Med* 2013; **5**: 167ra4.
63 Bettegowda C, Sausen M, Leary RJ, *et al.* Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies. *Sci Transl Med* 2014; **6**: 224ra24-224ra24.
64 Phallen J, Sausen M, Adleff V, *et al.* Direct detection of early-stage cancers using circulating tumor DNA. *Sci Transl Med* 2017; **9**: eaan2415.
65 Sausen M, Phallen J, Adleff V, *et al.* Clinical implications of genomic alterations in the tumour and circulation of pancreatic cancer patients. *Nat Commun* 2015; **6**: 7686.

66 Giovannetti E, Funel N, Peters GJ, *et al.* MicroRNA-21 in Pancreatic Cancer: Correlation with Clinical Outcome and Pharmacologic Aspects Underlying Its Role in the Modulation of Gemcitabine Activity. *Cancer Res* 2010; **70**: 4528–38.

67 Marks EI, Yee NS. Molecular genetics and targeted therapeutics in biliary tract carcinoma. *World J Gastroenterol* 2016; **22**: 1335–47.

68 Yoshikawa D, Ojima H, Kokubu A, *et al.* Vandetanib (ZD6474), an inhibitor of VEGFR and EGFR signalling, as a novel molecular-targeted therapy against cholangiocarcinoma. *Br J Cancer* 2009; **100**: 1257–66.

69 Valle JW, Wasan H, Lopes A, *et al.* Cediranib or placebo in combination with cisplatin and gemcitabine chemotherapy for patients with advanced biliary tract cancer (ABC-03): a randomised phase 2 trial. *Lancet Oncol* 2015; **16**: 967–78.

70 Bertotti A, Papp E, Jones S, *et al.* The genomic landscape of response to EGFR blockade in colorectal cancer. *Nature* 2015; **526**: 263–7.

71 Masaki Y, Kurose N, Yamamoto M, *et al.* Cutoff Values of Serum IgG4 and Histopathological IgG4+ Plasma Cells for Diagnosis of Patients with IgG4-Related Disease. *Int J Rheumatol* 2012; **2012**: 1–5.

72 Oseini AM, Chaiteerakij R, Shire AM, *et al.* Utility of serum immunoglobulin G4 in distinguishing immunoglobulin G4-associated cholangitis from cholangiocarcinoma. *Hepatology* 2011; **54**: 940–8.

73 Trikudanathan G, Navaneethan U, Njei B, Vargo JJ, Parsi MA. Diagnostic yield of bile duct brushings for cholangiocarcinoma in primary sclerosing cholangitis: a systematic review and meta-analysis. *Gastrointest Endosc* 2014; **79**: 783–9.

74 Malhotra N, Jackson SA, Freed LL, *et al.* The added value of using mutational profiling in addition to cytology in diagnosing aggressive pancreaticobiliary disease: review of clinical cases at a single center. *BMC Gastroenterol* 2014; **14**: 135.

75 Fogel EL, DeBellis M, McHenry L, *et al.* Effectiveness of a new long cytology brush in the evaluation of malignant biliary obstruction: a prospective study. *Gastrointest Endosc* 2006; **63**: 71–7.

76 de Bellis M, Fogel EL, Sherman S, *et al.* Influence of stricture dilation and repeat brushing on the cancer detection rate of brush cytology in the evaluation of malignant biliary obstruction. *Gastrointest Endosc* 2003; **58**: 176–82.

77 Timmer MR, Lau CT, Meijer SL, *et al.* Genetic Abnormalities in Biliary Brush Samples for Distinguishing Cholangiocarcinoma from Benign Strictures in Primary Sclerosing Cholangitis. *Gastroenterol Res Pract* 2016; **2016**: 1–9.

78 Gonda TA, Viterbo D, Gausman V, *et al.* Mutation Profile and Fluorescence In Situ Hybridization Analyses Increase Detection of Malignancies in Biliary Strictures. *Clin Gastroenterol Hepatol* 2017; **15**: 913–919.e1.

79 Kipp BR, Barr Fritcher EG, Clayton AC, *et al.* Comparison of KRAS mutation analysis and FISH for detecting pancreatobiliary tract cancer in cytology specimens collected during endoscopic retrograde cholangiopancreatography. *J Mol Diagnostics* 2010; **12**: 780–6.

80 Barr Fritcher EG, Kipp BR, Halling KC, Clayton AC. FISHing for pancreatobiliary tract malignancy in endoscopic brushings enhances the sensitivity of routine cytology. *Cytopathology* 2014; **25**: 288–301.
81 Jusakul A, Cutcutache I, Yong CH, *et al.* Whole-Genome and Epigenomic Landscapes of Etiologically Distinct Subtypes of Cholangiocarcinoma. *Cancer Discov* 2017; **7**: 1116–35.
82 Hubers LM, Maillette de Buy Wenniger LJ, Doorenspleet ME, *et al.* IgG4-Associated Cholangitis: A Comprehensive Review. *Clin Rev Allergy Immunol* 2015; **48**: 198–206.
83 Beuers U, Buy JM De, Doorenspleet M. IgG4-Associated Cholangitis. *Dig Dis* 2014; : 605–8.
84 Doorenspleet ME, Hubers LM, Culver EL, *et al.* Immunoglobulin G4 + B-cell receptor clones distinguish immunoglobulin G 4-related disease from primary sclerosing cholangitis and biliary/pancreatic malignancies. *Hepatology* 2016; **64**: 501–7.
85 Hubers LM, Beuers U. How to Diagnose Immunoglobulin G4-Associated Cholangitis: The Jack-of-All-Trades in the Biliary Tract. *Viszeralmedizin* 2015; **31**: 185–8.