Diagnostic and intraoperative targeted molecular imaging for pancreatic cancer

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Chapter | 13

General discussion and future perspectives

In the field of molecular imaging, significant progress has been made over the last years. Tumor-specific optical imaging has entered clinical trials, and safety and feasibility has been shown for several cancer types. This thesis shows the complete roadmap from preclinical development and validation to clinical translation in a phase I trial for pancreatic cancer patients. Now, next steps need to be taken by the field to move this technique from Phase I trials to more wide-spread use and larger clinical trials. To be able to achieve wide-spread use, more specific aspects of tumor biology need to be taken into account, especially the effects of neoadjuvant therapy, before tumor-specific imaging can be applicable in all patients. Also, inter- and intrapatient tumor heterogeneity is a known complicating factor in the search for optimal biomarkers for tumor detection.

The search for the ideal biomarker

In pancreatic cancer, it is known that tumor cells are selected by means of fitness and growth advantages.[1] Cells that enter the blood stream from the site of the primary tumor and lead to metastases, are probably the cells that have achieved the highest fitness in the primary site. These have the greatest chance of homing in new microenvironments, such as the liver, lung or peritoneum, which are the common sites of metastasis in pancreatic cancer.[1] Homing of tumor cells into new microenvironments will lead to novel mutations, and consequently further heterogeneity, both between the primary lesions and metastases, and between different metastases. This heterogeneity will make it almost impossible to identify a single biomarker that would allow, tumor-specific imaging for each tumor in each patient. To solve this issue, one could imagine that in the future multiple imaging agents directed against different biomarkers are available, and that prior to surgery the most optimal agent is chosen, based on biomarker expression from biopsy tissue. This would, on the other hand, not solve the problem of heterogeneity within the primary tumor, or between primary tumor and potential metastatic sites. An additional complicating factor in pancreatic cancer is that some patients do not receive a biopsy prior to surgery, and if a biopsy is taken, the material is not always representative for the suspected tumor. In those cases, it could be preferred to use a cocktail with different imaging agents, targeting a set of the most common biomarkers.[2, 3]

Another complicating factor in the identification of the ideal biomarker, is the use of (neo)adjuvant therapy. This form of therapy undoubtly influences the biomarker expression on tumor cells. This phenomena is extensively studied in breast cancer patients, but little is known of the effect in pancreatic cancer patients.[4, 5] In Chapter 4, we assessed the effect of neoadjuvant therapy on CEA and αvβ6 expression. There, we have shown the importance of a separate analysis on neoadjuvant treated tumor tissue. If neoadjuvant treated patients were not included in this IHC analysis. We would have concluded that CEA was the optimal target for pancreatic cancer, However, it was seen that CEA expression almost disappeared after neoadjuvant therapy, while tumor cells remained vital, making it impossible to target that receptor in neoadjuvant treated pancreatic cancer patients. Taking this into account, it was concluded that αvβ6 was the most ideal biomarker in all clinical relevant situations.

In this thesis we mainly focused on tumor-specific imaging directed against the cancer cell, while in chapter 2, we have also described the possibility to target the micro-environment in pancreatic cancer. It is recognized that pancreatic cancer for a large part consists out of stroma tissue compared to actual tumor cells. Since the micro-environment plays an important role in tumor development and progression, it could be of additional value and potentially more complete targeting of the tumor could be achieved, when choosing this route.[6] Since there is currently no experience with targeting only the stroma cell compartment in patients, we have chosen to focus on the tumor cells in this early phase work for pancreatic cancer. However, in the future, it could be recommended to use a cocktail of agents targeting both the tumor, and it's micro-environment.

Early detection of pancreatic cancer

As described in this thesis, pancreatic cancer has a dismal prognosis, mainly due to late onset of symptoms when disease is already spread to other organs, either distant or locally. This stresses the need for early detection. Fluorescence-guided surgery as described in this thesis will not be beneficial for early detection of pancreatic cancer, but early detection of this and other types of cancer could be performed in various ways; first, one could focus on identifying and validating a biomarker which may likely be very different compared to those of late stage disease. Identification of these kind of early biomarkers could be done in studies

like the baseline study recently initiated by Verily Life Sciences, performed in 10.000 healthy volunteers who will be monitored daily (NCT03154346). These studies will generate invaluable data regarding early signs of disease, and the time until development of symptoms. Another method for the early detection of pancreatic cancer could be to develop a standardized bio-repository with longitudinal follow-up, established in patients with pancreatic cancer precursor lesions such as IPMN or with high risk for pancreatic cancer based on family history and established genetic risk factors. By the collection of tissues, cystic fluids, serum and plasma, captured prior to pancreatic cancer onset, predictive and early detection biomarkers can be identified. The next challenge after identification of biomarkers associated with early onset of disease, is the method to detect these markers. This can be performed in bodily fluids, such as the FOPT test in colon cancer, or by using imaging, such as breast cancer screening. In the thesis, we have focused on the improved detection of pancreatic cancer by targeted imaging. Both targeted ultrasound, in form of photoacoustic imaging, and targeted PET-CT could be used for this purpose. Photoacoustic imaging with targeted agents, such as microbubbles, could be a non-invasive way to image the pancreas for screening purposes.[7, 8] Due to the costs and radiation used for targeted PET-CT imaging, this would not be a imaging method used in the general population for screening, but could be used in high-risk patients during periodical screening.

Other novel molecular imaging techniques

In addition to fluorescence and photoacoustic imaging, other potential novel molecular imaging techniques could be beneficial during surgery. In chapter 2 of this thesis, Raman imaging is shortly addressed, together with the advantages and disadvantages. Raman and Cherenkov luminescence imaging (CLI) are molecular imaging techniques that receive tremendous attention in preclinical research, but currently, clinical applications are limited. In CLI, the visible photons emitted by Cherenkov radiation are captured, resulting in optical imaging of radiotracers, which could be used for intraoperative guidance in a way SPECT is used now in clinical trials.[9] CLI could overcome the depth limitation of fluorescence-guided surgery, since it used radiation for visualization instead of emitted light. Thorek and colleagues described the successful use of CLI in patients undergoing FDG-PET in 2014,[10] but since then no clinical studies are performed. The main advantage of Raman imaging is that it could be used

for multiplexing. The advantage of multiplexing is that it allows simultaneous detection of multiple biomarkers which is ideal in case of heterogenic tumors. By varying the Raman active layer, different Raman particles can be created, each emitting a characteristic spectrum. Since each type of particles has a unique spectral signature they can be used for multiplexing when functionalized for different targets.[11] For now, a disadvantage of Raman imaging is the duration it takes to scan a tissue surface, limiting the clinical applicability. To overcome this limitation, Garai and colleagues developed a clinical endoscopic Raman system able to rapidly scan large tissue surfaces such as colon.[11]

A standard method to assess novel imaging agents

As described in this thesis, multiple early phase clinical trials are performed in the field of fluorescence-guided surgery. In Chapter 12, we described the need for a uniform set-up of these clinical trials. Currently, most trials are designed just to show feasibility of the technique. By now, enough proof is generated showing the capability of targeting tumors in a tissue-specific manner using optical imaging agents. At this point, the field should move one step further and show the world this technique helps to improve surgical care for the patient otherwise wide-spread use will not be reached. Furthermore, to get novel imaging agents approved by regulatory bodies, large trials showing patient benefit have to be performed. To execute these large clinical trials, extensive resources are needed which are mostly only possible when supported by industry. However, if the clinical trials would be designed in a similar manner and shared endpoints are chosen, results of different trials could be put together and collective conclusions could be drawn, leading to a more cost-efficient and less time-consuming strategy as described in chapter 8.

Quantification in fluorescence imaging

Another crucial aspect which is still in its infancy in fluorescence-guided surgery is quantification of the signal. At this point, the intra-operative use of fluorescence is based on binary “fluorescence yes or no”, instead of the numerical intensity of the signal. The problem with this way of interpreting fluorescence is that most imaging systems have an incorporated method to enhance the imaging signal even when it is weak, and therefore it is not clear for the surgeon how intense the signal actually is. This is important because depending on the imaging agent, normal tissue could express the biomarker

and therefore some fluorescence signal as well. In these cases, the intensity of the signal provides information on how likely it is that the fluorescent tissue is actual tumor. Although the potential of tumor-specific optical imaging is yet to be realized, tumor-specific intraoperative optical imaging is likely to play an important role in the identification and treatment of cancer in the near future. Therefore, real-time quantification should be available to provide better understanding of the signal to the surgeon and increasing the sensitivity and specificity of this technique, to allow clinical decision-making based on this technique.

FUTURE PERSPECTIVES

The future of cancer treatment

This thesis shows the great potential of tumor-specific imaging and it is expected that it's use both in the diagnostic process and for intra-operative use will increase in the future. In the past, treatments in general, but specifically in oncology were based on a "one-size fits-all"-strategy undervaluing all the different characteristics disease and patients can have. Later on, this was optimized by stratification of patients by disease subtypes, clinical features and demographics. This method still did not lead to the preferred results in patient outcome, and therefore at this point there is a need to further specialize care based on precision medicine. Here, a doctor needs to look at the individual patient and has to take patient preferences, demographics, medication history, clinical features of the disease, biomarkers and behavior into account when deciding on the most optimal treatment. The call for precision medicine will require the need to search for treatment strategies that are focused on these specific characteristics of the tumor and the patient. Since the results of early phase clinical trials in the field of tumor-specific imaging are promising, this modality could help to fill part of that need.

The future of surgery

Besides the need for precision medicine, the increasing use of minimally-invasive surgery (MIS), such as robotic-assisted and laparoscopic surgery, requires a method to substitute the lack of tactile information for the surgeon. During open surgery the consistency of tissue gives the surgeon tremendous

information regarding the potential benign or malignant nature of the tissue. To substitute this information in MIS, novel methods are needed providing visual feedback to the surgeon regarding the tissue status. This visual feedback could come from a fluorescent imaging signal in case of tumor-specific imaging. For photoacoustic imaging, the second intraoperative optical imaging modality discussed in this thesis, this becomes more challenging. Photoacoustic imaging requires an ultrasound probe able to enter the abdominal cavity through laparoscopic trocars. At this point, such photoacoustic imaging probes are not available yet, however certain groups are working on this feature. Also, the photoacoustic signal cannot be projected on the screen already used by the surgeon to perform the surgery, so an additional device and screen are needed in the operating room.

The future of pancreatic cancer diagnostics

Precision medicine will not only change the way surgery is performed nowadays, also the diagnostic process needs to implement this phenomena. In this thesis the first steps of this transition are shown in chapter 9 using tumor-specific PET-CT imaging to diagnose pancreatic cancer. In the case of pancreatic cancer, tissue-specific diagnostics could help differentiating between tumor and inflammation prior to resection and/or to determine resectability. Also, this method could potentially be used to monitor response to neoadjuvant therapy. A large clinical trial is about to start in the LUMC investigating this specific aspect. Another area of development for both diagnostic and intra-operative tissue-specific imaging is hybrid imaging were one agent is conjugated to both a radioactive label for diagnostics and a fluorescent label for intra-operative use. The main benefits of this method are the fact that a patient has to undergo only one injection with the agent, the high probability that the same lesions are found both on the diagnostic modality as during surgery, and the possibility to perform radioguided surgery together with fluorescence. In this case, both the fluorescent and the radioactive signal can be used during the surgery with a Gamma counter and a fluorescent camera. The radioactive signal can be used to direct the surgeon to the area of interest and the high sensitivity of the fluorescence for the actual resection or identification of the tumor.[12] Besides these important benefits of a hybrid agent, the use of two separate agents should be preferred looking at the logistics in a hospital. In case of a hybrid agent, the diagnostic imaging and the surgery should be performed

within a few days to be able to use both the radioactive and the fluorescent signal since both will decay after a certain amount of time. In addition, the radioactive signal cannot be too high during surgery, exposing the surgeon and the OR team to radioactivity. Therefore, a hybrid agent is an interesting idea for research purposes, but it remains questionable how feasible this is for actual patient care.

The future of fluorescence guided surgery

When looking at the future of fluorescence guided surgery, not only the NIR window from 700-900 nm has become of interest to the field, also the wavelengths in the second near-infrared window (NIR II) of 900 nm and above seem promising.[13, 14] Preclinical research using these wavelengths show diminished tissue autofluorescence, reduced photon scattering, and low levels of photon absorption allowing centimeters imaging depth at low resolution and micron-scale resolution at low depths.[13] Until recent, all NIR-II fluorophores were inorganic nanomaterials, which are excreted slowly and are largely retained within the reticuloendothelial system, making clinical translation nearly impossible due to unknown long-term toxicity concerns.[15] In 2015, Antaris and colleagues published a study developing a small molecule dye for the NIR-II window of 8.9 kDa with renal excretion.[16] The agent outperformed ICG on several levels in mouse models. The disadvantage of this small molecule dye is the labor-intensive and expensive synthesis, making large scale synthesis for human use impossible. Thus far, no clinical results using NIR-II imaging are shown and no clinical imaging systems are available. As seen in the past, it is hard to translate successful preclinical results into the human situation, therefore the potential benefit of NIR-II imaging for patient care with clinically available agents is still something that needs to be investigated in the future.

The future of organ-sparing strategies in cancer treatment

Especially in rectal and breast cancer, another aspect that becomes increasingly important in the way medicine is practiced today, is the trend towards organ-sparing treatment. For pancreatic cancer this is not a feasible treatment option for now, since successful neoadjuvant regimens have just been initiated in this disease and long term effects still need to be investigated. Studies have been suggesting that “organ preservation” strategies might be appropriate alternatives to standard resection in patients with a clinical complete response (cCR) on

neoadjuvant therapy.[17, 18] In a so-called "Watch-and-Wait" (W&W) strategy, surgery is omitted and instead, patients are closely monitored with intensive clinical and imaging follow-up. Although several successful small cohort studies have been conducted in the last years, W&W is not widely adopted yet. One of the main reasons is because the accuracy of conventional imaging modalities is insufficient, resulting in reported 2-year local tumor regrowth rates of 15-33%.[19] With tumor-specific targeted imaging, a non-invasive imaging approach can be implemented to help improve the response monitoring during diagnostic imaging in these patients. This specific use of tumor-specific imaging also increases the need to identify a biomarker which is present on viable tumor cells, but not on chemotherapy-induced necrosis and fibrosis.

In the future, molecular imaging could not only be used to diagnose and treat patients directly. Also for the development of novel drugs molecular imaging could play an important role. Imaging could be used to inform physicians and researcher and lead to better understanding for the most optimal implementation of novel drugs. In drug development, imaging could provide information on biodistribution, the difference between individuals on pharmacodynamic and -kinetic level, and it could determine disease progression to verify for example an indication.[20]

GENERAL CONCLUSIONS

Pancreatic cancer remains an extremely challenging disease for a surgeon to tackle. Since most of the patients will not benefit from surgical resection, it is essential to be able the select only those patients that will benefit. Implementing tumor-specific imaging, both in the diagnostic process and during surgery will improve the detection of small lesions, assess resection margins, and help to identify a non-curative resection. In this thesis, we have identified integrin αvβ6 as the most optimal target for pancreatic cancer, to distinguish cancer from normal pancreatic tissue and inflammation. Also, expression is retained after neoadjuvant therapy in vital tumor cells compared to chemotherapy-induced necrosis and fibrosis. In preclinical mouse models, we have shown the potential to target αvβ6 using a fluorescent agent, and in healthy volunteers and patients the possibility is shown to diagnose pancreatic cancer targeting αvβ6 with

PET-CT imaging. In addition, we have proven the safety and feasibility of tumor-specific fluorescence imaging using cetuximab-IRDye800 for the intraoperative detection of pancreatic cancer, even in the case of subclinical disease.

Now that practicing medicine has changed to a more precision-type approach, tailored for each patient, tumor-specific optical imaging is likely to get an important role. Before this role can be claimed by the field, we have stressed the need to proof of patient benefit by showing improved survival and reduced morbidity. This can be achieved by performing large, randomized controlled, clinical trials, by developing a standardized way clinical trials are performed, and by choosing relevant clinical endpoints. Hopefully this can lead to wide-spread implementation of this technique in the near future, and eventually to improved prognosis for patients with pancreatic cancer.

REFERENCES

1. Makohon-Moore, A. and C.A. Iacobuzio-Donahue, Pancreatic cancer biology and genetics from an evolutionary perspective. Nat Rev Cancer, 2016. 16(9): p. 553-65.
2. Campbell, P.J., S. Yachida, L.J. Mudie, et al., The patterns and dynamics of genomic instability in metastatic pancreatic cancer. Nature, 2010. 467(7319): p. 1109-13.
3. Yachida, S., S. Jones, I. Bozic, et al., Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature, 2010. 467(7319): p. 1114-7.
4. Zhou, X., J. Zhang, H. Yun, et al., Alterations of biomarker profiles after neoadjuvant chemotherapy in breast cancer: tumor heterogeneity should be taken into consideration. Oncotarget, 2015. 6(34): p. 36894-902.
5. Gahlaut, R., A. Bennett, H. Fatayer, et al., Effect of neoadjuvant chemotherapy on breast cancer phenotype, ER/PR and HER2 expression - Implications for the practising oncologist. Eur J Cancer, 2016. 60: p. 40-8.
6. Boonstra, M.C., J. Prakash, C.J. Van De Velde, et al., Stromal Targets for Fluorescent-Guided Oncologic Surgery. Front Oncol, 2015. 5: p. 254.
7. Abou-Elkacem, L., H. Wang, S.M. Chowdhury, et al., Thy1-Targeted Microbubbles for Ultrasound Molecular Imaging of Pancreatic Ductal Adenocarcinoma. Clin Cancer Res, 2018. 24(7): p. 1574-1585.
8. Valluru, K.S., K.E. Wilson, and J.K. Willmann, Photoacoustic Imaging in Oncology: Translational Preclinical and Early Clinical Experience. Radiology, 2016. 280(2): p. 332-49.
9. Bugby, S.L., J.E. Lees, and A.C. Perkins, Hybrid intraoperative imaging techniques in radioguided surgery: present clinical applications and future outlook. Clin Transl Imaging, 2017. 5(4): p. 323-341.
10. Thorek, D.L., C.C. Riedl, and J. Grimm, Clinical Cerenkov luminescence imaging of (18)F-FDG. J Nucl Med, 2014. 55(1): p. 95-8.
11. Garai, E., S. Sensarn, C.L. Zavaleta, et al., A real-time clinical endoscopic system for intraluminal, multiplexed imaging of surface-enhanced Raman scattering nanoparticles. PLoS One, 2015. 10(4): p. e0123185.
12. Hekman, M.C.H., M. Rijpkema, D.L. Bos, et al., Detection of Micrometastases Using SPECT/Fluorescence Dual-Modality Imaging in a CEA-Expressing Tumor Model. J Nucl Med, 2017. 58(5): p. 706-710.
13. Hong, G., J.C. Lee, J.T. Robinson, et al., Multifunctional in vivo vascular imaging using near-infrared II fluorescence. Nat Med, 2012. 18(12): p. 1841-6.
14. Hong, G., J.T. Robinson, Y. Zhang, et al., In vivo fluorescence imaging with Ag2S quantum dots in the second near-infrared region. Angew Chem Int Ed Engl, 2012. 51(39): p. 9818-21.
15. Choi, H.S., W. Liu, P. Misra, et al., Renal clearance of quantum dots. Nat Biotechnol, 2007. 25(10): p. 1165-70.
16. Antaris, A.L., H. Chen, K. Cheng, et al., A small-molecule dye for NIR-II imaging. Nat Mater, 2016. 15(2): p. 235-42.
17. Glynne-Jones, R., M. Wallace, J.I. Livingstone, et al., Complete clinical response after preoperative chemoradiation in rectal cancer: is a "wait and see" policy justified? Dis Colon Rectum, 2008. 51(1): p. 10-9; discussion 19-20.
18. Appelt, A.L., J. Ploen, H. Harling, et al., High-dose chemoradiotherapy and watchful waiting for distal rectal cancer: a prospective observational study. Lancet Oncol, 2015. 16(8): p. 919-27.
19. Habr-Gama, A., J. Gama-Rodrigues, G.P. Sao Juliao, et al., Local recurrence after complete clinical response and watch and wait in rectal cancer after neoadjuvant chemoradiation: impact of salvage therapy on local disease control. Int J Radiat Oncol Biol Phys, 2014. 88(4): p. 822-8.
20. Willmann, J.K., N. van Bruggen, L.M. Dinkelborg, et al., Molecular imaging in drug development. Nat Rev Drug Discov, 2008. 7(7): p. 591-607.