Preclinical PET imaging of antibody therapies for cancer

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Chapter 7

Summary and future perspectives

SUMMARY

Monoclonal antibodies have become important in the systemic treatment of cancer. They are applied in different ways. Among antibodies, the immune checkpoint inhibitors now play a clear role by activating the immune system. Antibodies can also be charged with a cytotoxic or radioactive payload. The antibody-drug conjugates (ADCs) are obtaining a firm role in treating patients with cancer. And increasingly studies are ongoing with antibodies radiolabeled with beta- (especially lutetium-177) and alpha-emitting radionuclides. For so-called targeted alpha therapy, alpha particle emitting radionuclides, e.g., thorium-227(^{227}Th), or actinium-225 (^{225}Ac) are coupled to tumor-targeting monoclonal antibodies. As for all systemic treatments, treatment with antibodies does not benefit all patients. It may help to understand the whole-body behavior of these antibodies, shedding light on their unique target-mediated distribution and heterogeneous tumor-targeting. Positron emission tomography (PET) could visualize whole-body distribution, noninvasively, of zirconium-89 (^{89}Zr) labeled antibody therapies. Access to therapeutic antibodies is globally limited because they are expensive. The availability and use of monoclonal antibody biosimilars could potentially retain the healthcare costs of these expensive drugs. The research in this thesis focusses on monoclonal antibodies and aims to identify challenges for monoclonal antibody biosimilars in cancer and explore preclinical ^{89}Zr PET imaging of antibody therapies to evaluate their in vivo behavior, e.g., biodistribution, tumor uptake, and pharmacodynamics.

Chapter 1 provides background information and an outline of this thesis.

Monoclonal antibodies are complex, expensive, but clinically relevant molecules and with their increasing number and application, they are a major burden to the healthcare systems. Uptake and use of biosimilars, highly similar copies of already-approved off-patent originators, could increase the availability and sustainability of antibody therapies. **Chapter 2** provides a review of monoclonal antibody biosimilars in cancer. Based on approval information from bevacizumab biosimilars that we compared, monoclonal antibody variability is modest and most often occurs in glycan group patterns and charge heterogeneity. This is likely due to unpredictable post-translational modifications by the cells in which they are produced or triggered through manufacturing processes. This inevitable variability is well-tolerated in patients. The biosimilar landscape may predict an increase in biosimilars in the near future. Yet still, several challenges need to be tackled. Clinical efficacy trials are still required with additional data on switching in some countries. Recently, biosimilar approval on the robust data set of analytical assessment and patient pharmacokinetics alone, is being explored by European Medicines Agency, among others. Moreover, the long patents for monoclonal antibodies, that cause a barrier, is approached by Medicines Patent Pool. Eventually, biosimilar development could be cost-effective

on a larger scale, reaching countries with few resources. The continuous provision of knowledge and financial awareness is paramount to fully exploit biosimilars for these valuable monoclonal antibody cancer treatments.

Monoclonal antibodies which block programmed death-ligand 1 (PD-L1), activate the human's immune system by lifting this immune-evasive checkpoint. These therapies can, however, also induce immune-related toxicities in healthy PD-L1-expressing tissues. Probody CX-072 is a PD-L1-blocking monoclonal antibody designed so proteases in the tumor have to cleave its masking peptide to allow the antibody to bind to its target. The idea is that this might reduce side effects. In **chapter 3** we conjugated CX-072 to chelator N-sucDf and radiolabeled the conjugate with ^{89}Zr. We studied ^{89}Zr-CX-072 behavior in male mice with human or murine PD-L1-expressing tumors. PET imaging of BALB/c nude mice bearing an MDA-MB-231 tumor revealed 2.1-fold higher tumor-to-blood ratios of ^{89}Zr-CX-072 at day 6 post tracer injection compared to an ^{89}Zr labeled non-specific control Probody. ^{89}Zr-CX-072 uptake by lymphoid tissues of C57BL/6J mice bearing a MC38 tumor was low compared to the ^{89}Zr labeled parental antibody that lacks the masking peptides. PD-L1 specificity was confirmed by tumor autoradiography. Activated CX-072 species were detected in tumors, with 5.3-fold lower levels found in spleen. These data confirm the Probody design of CX-072, thus PD-L1 specific uptake in tumor and minimal uptake in lymphoid tissues.

Brentuximab-vedotin is an antibody drug conjugate (ADC) targeting cellular CD30, overexpressed by lymphomas. The drug is registered to treat Hodgkin's lymphoma, cutaneous T cell lymphoma, and systemic anaplastic large T cell lymphoma. We conjugated the naked antibody brentuximab with N-sucDf and radiolabeled the conjugate with ^{89}Zr to evaluate brentuximab's lymphoma targeting in a clinical PET imaging study of patients with a CD30-positive lymphoma eligible for brentuximab-vedotin therapy. Tumor uptake might provide predictive information for tumor response to brentuximab-vedotin. For the execution of the trial in patients, as part of the clinical trial application package, an investigational medicinal product dossier (IMPD) for ^{89}Zr-brentuximab is required to secure drug quality. **Chapter 4** describes the development trajectory towards the clinical use of ^{89}Zr-brentuximab. It also contains the IMPD, summarizing all available data regarding ^{89}Zr-brentuximab's quality, stability, and safety. ^{89}Zr-brentuximab contains a protein dose of 0.5 mg, equivalent to approximately 37 MBq, with a radiochemical purity of > 95% in 10 mL NaCl 0.9%. The product is a clear and colorless radioactive fluid, sterile and free of endotoxins, with a pH of 5.0-8.0, and a shelf life of 96 h. The immunoreactive fraction of ^{89}Zr-brentuximab is > 0.7. Based on all data ^{89}Zr-brentuximab applies to the mandatory quality standards. With recent approval by the regulatory authorities, the ^{89}Zr-brentuximab PET clinical imaging study in patients with lymphoma could start.

Targeted alpha therapy is an investigational radionuclide therapy that has the potential to inflict radiation-induced lethal DNA damage to tumor cells, while sparing healthy tissues because of high energy radiation on a short range. A mesothelin-targeted 3,2-HOPO-thorium-227 antibody conjugate (^{227}Th-MSLN) is a novel targeted alpha therapy developed to treat mesothelin overexpressing cancers. To support its clinical development, it would be valuable to have insight into whole-body distribution and tumor targeting. In **chapter 5,** we described how we radiolabeled the same 3,2-HOPO-antibody conjugate with zirconium-89 (^{89}Zr-MSLN) instead of ^{227}Th and evaluated biodistribution and tumor targeting with PET imaging in human tumor-bearing female nude mice. ^{89}Zr-MSLN PET imaging of NMRI nude mice showed mean standardized uptake value (SUV_{mean}) in high mesothelin-expressing HT29-MSLN tumors of 2.2 ± 0.5. Ex vivo tumor uptake was 10.6% ± 2.4% injected dose per gram (%ID/g) at 168 h. ^{89}Zr-MSLN tumor uptake was higher than uptake of ^{89}Zr-control (*P* = 0.0043). Moreover, ^{89}Zr-MSLN and ^{227}Th-MSLN showed comparable tumor uptake and biodistribution in Balb/c nude mice with medium mesothelin-expressing OVCAR3 tumors or HT29-MSLN tumors. Lastly, pre-treatment PET scan of NMRI nude mice revealed SUV_{mean} of 2.2 ± 0.2 in HT29-MSLN tumors, that decreased in volume upon ^{227}Th-MSLN treatment, whereas low mesothelin-expressing BxPc3 tumors showed SUV_{mean} of 1.2 ± 0.3 and remained similar in size after ^{227}Th-MSLN treatment. We concluded that the results of the ^{89}Zr-MSLN PET imaging matched with the ^{227}Th-MSLN tumor uptake, supporting clinical exploration of ^{89}Zr-MSLN together with ^{227}Th-MSLN therapy, both using the same antibody-chelator conjugate.

Increasingly, the immune-stimulatory effect of antibody therapies is being evaluated with molecular imaging. The anti-tumor immune response, and which markers are best suited as immune activation read-out, still need to be unraveled. In **chapter 6,** we evaluated PET imaging with ^{89}Zr-DFO-F(ab')$_2$ fragments targeting $CD4^+$ or $CD8^+$ T cells (^{89}Zr-CD4, ^{89}Zr-CD8, ^{89}Zr-control, 30 µg, 3 MBq). PET imaging of healthy DBA/2 mice showed that ^{89}Zr-CD4 and ^{89}Zr-CD8 accumulated in spleen (SUV_{mean} 2.7 ± 0.3 and 1.3 ± 0.4), in mesenteric lymph nodes (SUV_{mean} 2.2 ± 0.3 and 1.1 ± 0.1), and in cervical lymph nodes (SUV_{mean} 1.6 ± 0.1 and 0.9 ± 0.1) at 24 h, whereas ^{89}Zr-control was only visible in kidneys (SUV_{mean} 5.8 ± 0.4). For ^{89}Zr-CD4 PET, we show the feasibility of injecting the tracer a second time on day 7, with PET 24 h pi, revealing the same biodistribution. Autoradiographic analyses of lymph nodes confirmed ^{89}Zr-CD4 uptake at sites of CD4 expression. ^{89}Zr-CD8 repeated PET was not feasible, likely due to antibody formation against the tracer. In KLN205 tumor-bearing DBA/2 mice, we observed unchanged ^{89}Zr-CD4 distribution after treatment with 250 kBq/kg, 0.75 mg/kg actinium-225 labeled murine tumor-targeting antibody (^{225}Ac-mAb), compared to vehicle, with PET at day 5. These data encourage further exploration of T cell imaging in combination with targeted therapies.

FUTURE PERSPECTIVES

Sustainability of antibody therapies

The rapidly growing territory of antibody cancer therapy urges tools to support sustainability. Their global market size is growing at a fast pace (1). With antibodies losing market exclusivity, the low number of biosimilars will also increase. It is paramount that biosimilars become available as efficiently as possible with limited development costs while preserving drug quality and clinical behavior similar to the originator. This will not only potentially retain therapy costs but will make them more accessible to all but especially low- and middle-income countries. Despite progression, several bridges still need to be crossed concerning, e.g., biosimilar prices and interchangeability regulations. In addition to classical monoclonal antibodies, there are now even more advanced therefore expensive therapies becoming available, such as cell therapy and chimeric antigen T cell receptor therapy. The rich content pipeline will reach the market soon. An inter-stakeholder collaboration between governments, drug developers, pharmacists, care providers, and patients could assure access to these valuable medications, enabling cost-effectiveness. Molecular imaging for antibody therapies is a rapidly expanding field of research. The development of antibody treatments is expensive, and information about their tumor-targeting capabilities and whole-body distribution at an early stage might save valuable time and costs. Several clinical studies have proven reliable predictions of biodistribution and tumor-targeting by PET isotope-labeled therapeutic antibodies. More recently, studies also showed the relation between ^{89}Zr labeled PD-L1 and PD-1 antibody tracer tumor uptake and tumor response (3, 4). Therefore, PET may potentially identify eligible patients for these antibodies to personalize treatment and reduce healthcare costs. Moreover, technology advancements enable PET scanners with higher sensitivity and resolution to provide a more accurate PET isotope location and require lower radiation doses (5). Together with expanding knowledge, molecular imaging for antibody therapies is likely to grow further, enabling additional research to prove its role in clinical decision-making.

Molecular imaging of immune response

The anti-tumor immune response is not yet fully understood, and suitable immune markers to predict response to immune-stimulatory therapies are therefore explored. Unraveling the underlying immune mechanisms might support response evaluation and patient stratification. Molecular imaging is a tool to provide such information. T cells play a central role in the adaptive anti-tumor immune response. Therefore, preclinical and clinical studies evaluate several tracers targeting T cells or T cell subsets. Examples of T cell markers are CD3, CD8, CD103, 41BB, lymphocyte-activation gene 3, T cell immunoglobulin, and mucin-domain containing-3. Recently, pre- and posttreatment molecular imaging of

increased cytotoxic $CD8^+$ T cells in the tumor correlated with response to immunotherapy in mice (6). So far, $CD4^+$ helper T cells have been less characterized. Yet, they are emerging as essential partners in tumor specific $CD8^+$ T cell cytotoxicity (7), and their abundance in murine tumors correlated with response to immune checkpoint inhibition (8). Therefore, CD4 might be a valuable clinical immune marker in the future. Other immune cell markers, e.g., interleukin receptor 2, PD-1, PD-L1, and markers on B cells, are evaluated with PET too. Hopefully, these studies will lead to a better understanding, evaluation, and prediction of the anti-tumor immune response.

Targeted alpha therapy and molecular imaging

Targeted alpha therapy is still in its infancy. This therapy has the potential to be effective with potentially fewer side effects than other radionuclide therapies. However, this has yet to be proven and considering its novelty, insight into whole-body distribution, pharmacokinetics, tumor-targeting, and dosimetry in an early development stage can support its development. Ideally, a targeted alpha therapy should serve as a theranostic, possessing both antitumor activity and molecular imaging properties. Gammas and positrons produced during the decay of most clinically applicable alpha particle emitting radionuclides are often too weak for quantitative molecular imaging and require the development of novel technologies with high sensitivity (9). An example of a solution is the PET isotope ^{89}Zr, which is a diagnostic surrogate for the alpha particle emitting radionuclide with therapeutic intent, to predict tumor-targeting and biodistribution (**chapter 5**). The field of theranostics is overcoming challenges, such as, finding the perfect radionuclide pair, for stable chelator complexation, while behaving similar *in vivo*. Molecular imaging of alpha-particle emitting radionuclides and surrogate isotopes such as ^{89}Zr may become part of the growing theranostics' platform.

REFERENCES

1. The Business Research Company. Summary of: Cancer monoclonal antibodies global market report 2022. Market Size, Trends, And Global Forecast 2022-2026. Accessed 28-03-2022 webpage: https://www.thebusinessresearchcompany.com/report/cancer-monoclonal-antibodie-global-market-report
2. Azuz S, Newton M, Bartels D, Poulsen BK. Uptake of biosimilar trastuzumab in Denmark compared with other European countries: a comparative study and discussion of factors influencing implementation and uptake of biosimilars. Eur J Clin Pharmacol. 2021;77:1495–1501.
3. Bensch F, van der Veen EL, Lub-de Hooge MN, et al. ^{89}Zr-atezolizumab imaging as a non-invasive approach to assess clinical response to PD-L1 blockade in cancer. Nat Med. 2018;24:1852–8.
4. Kok IC, Hooiveld JA, van de Donk PP, Giesen D, et al. ^{89}Zr-pembrolizumab imaging as a non-invasive approach to assess clinical response to PD-1 blockade in cancer. Ann Oncol. Ann Oncol. 2022;33:80–8.
5. Cherry SR, Jones T, Karp JS, et al. Total-body PET: Maximizing sensitivity to create new opportunities for clinical research and patient care. J Nucl Med. 2018;59:3–12.
6. Ortiz-Muñoz G, Brown M, Carbone CB, Pechuan-Jorge J, et al. Surveillance of in situ tumor arrays reveals early environmental control of cancer immunity. *IN PRESS* https://www.biorxiv.org/content/10.1101/2021.05.27.445482v1.full.pdf+html
7. Borst J, Ahrends T, Bąbała N, et al. $CD4^+$ T cell help in cancer immunology and immunotherapy. Nat Rev Immunol. 2018;18:635–47.
8. Kristensen LK, Fröhlich C, Christensen C, et al. CD4+ and CD8a+ PET imaging predicts response to novel PD-1 checkpoint inhibitor: studies of Sym021 in syngeneic mouse cancer models. Theranostics. 2019;9:8221–38.
9. Seo Y. Quantitative imaging of alpha-emitting therapeutic radiopharmaceuticals. Nucl Med Mol Imaging. 2019;53:182–8.