PET imaging and in silico analyses to support personalized treatment in oncology

Moek, Kirsten

DOI:
10.33612/diss.112978295

01

General introduction

Background

Cancer is a major cause of death and its worldwide annual mortality rate is predicted to reach 11.5 million in 2030.[1] As most of these patients die of metastatic disease, there is an urgent need for better drugs to improve survival of cancer patients. With the recent advances in molecular and cellular biology, many molecules and key pathways involved in the hallmarks of cancer were identified.[2] Therefore, over the last decades next to DNA-damaging chemotherapy, targeted agents and immunotherapy have been developed. Since the mid-1990s, monoclonal antibodies (mAbs) have grown steadily into a drug category with currently 34 mAbs approved by the U.S. Food and Drug Administration and/or European Medicines Agency for oncological indications. In addition, 32 investigational antibody therapeutics are undergoing evaluation in late-stage clinical studies.[3] To increase their potency, mAbs have always been reshaped and modified, in order to try to improve their effectiveness.[4] For instance, antitumor activity has been augmented by conjugating antibodies with cytotoxic agents.[5-7] These antibody-drug conjugates are designed to improve the potency of chemotherapy by increasing the accumulation of the cytotoxic drug within tumor cells, thereby reducing systemic toxic effects.[8,9] In addition, immunotherapy has become a major research focus in oncology with already firm embedment in the clinic of the immunomodulatory mAbs called immune checkpoint inhibitors. For example, treatment with cytotoxic T-lymphocyte associated antigen-4 directed ipilimumab as well as the programmed cell death protein 1 directed antibodies pembrolizumab and nivolumab have shown overall survival benefits in stage IV melanoma.[10] To increase the effect of antibodies numerous modifications are tested. For example, bispecific T-cell dependent antibodies like bispecific T-cell engager (BiTE) antibody constructs, dual-affinity re-targeting antibodies, and full-length bispecific antibodies, have entered the clinic to exploit the immune system for cancer treatment.[11,12] These drugs are engineered to redirected T-cells to tumor cells by simultaneously binding to the cluster of differentiation 3 (CD3) subunit of the T-cell receptor complex and a tumor target antigen.[11] Since the binding is independent of antigen specificity, T-cell dependent bispecific antibodies are considered of potential interest for less immunogenic tumors lacking enough neo-antigens.

In the rapidly evolving field of targeted agents and immunotherapy there are still major questions that have yet to be solved, including which patient and which tumor type benefits from therapy. In daily patient care, most often immunohistochemistry (IHC) or quantitative polymerase chain reaction are performed to explore the presence or absence of tumor targets.[13] Limitations of these traditional biomarker analyses include procedural risks, and the accessibility of primary tumor and metastatic lesions.

Moreover, biopsies provide only static information of a small part of the tumor, while tumor heterogeneity and changes in target expression over time are not considered. It is increasingly being acknowledged that heterogeneity in tumor target expression exists, and plays an important role in efficacy of targeted therapies.[14-17] In this respect, molecular imaging – defined as the visualization, characterization, and measurement of biological processes at the molecular and cellular levels – with positron emission tomography (PET) is of interest.[18] This tool provides whole-body information about drug target expression in a non-invasive matter, but also informs about drug biodistribution. Furthermore, it may support decisions regarding dosing schedules, since it can show whether the drug reaches and accumulates in tumors and inform about tumor target saturation. Antibodies have ideal characteristics for molecular imaging because they are designed against a specific target and relatively easy to radiolabel. While antibodies have been extensively studied with molecular imaging, such an approach has not yet been used to study behavior of bispecific antibodies in patients.

In the era of personalized medicine, identification of novel drugable targets is of high priority. Broad knowledge concerning frequency of target overexpression across tumor types is warranted to fully exploit therapeutic possibilities. However, performing IHC analyses on such a large scale is time-consuming and demands many resources. Moreover, standardized protocols for IHC staining are seldom available and it has clearly been demonstrated that lack of standardization has a strong impact on IHC results.[19] To overcome these IHC disadvantages, *in silico* functional genomic mRNA profiling (FGmRNA profiling) can be used to predict target overexpression at the protein level across a broad range of tumors. This technique can be applied to genetic expression datasets and enables an enhanced view on the downstream effects of genomic alterations on gene expression levels in tumors.[20]

Aim of the thesis

The aim of this thesis is to gain insight into the behavior of antibodies in patients, thereby focusing on novel bispecific T-cell engager antibody constructs, via early clinical studies and molecular PET imaging. Moreover, we aimed to contribute to a more personalized anti-cancer treatment approach by predicting overexpression rates of drugable targets across a plethora of tumor types using functional genomic mRNA profiling.

Outline of the thesis

When targeted compounds are used to determine a treatment strategy by combining diagnostics and therapeutics this is called “theranostics”. **Chapter 2** contains a review about theranostics using radiolabeled antibodies and antibody-related therapeutics in the oncological field. In addition to describing our own experience, literature was reviewed by searching PubMed for relevant articles which are summarized and discussed. Moreover, we explored ongoing clinical trials via a search of ClinicalTrials.gov and summarized our findings.

While immune checkpoint inhibitors have proven to be a powerful treatment approach for several tumor types, patients with advanced gastrointestinal tumors derived only little benefit from these agents except for patients with microsatellite instability-high or mismatch repair-deficient tumors. This has stimulated the search for new drugs to induce an anti-cancer immune response and improve survival of patients with gastrointestinal cancers. One novel approach is the use of BiTE antibody constructs like AMG 211. AMG 211 is directed against carcinoembryonic antigen (CEA) on tumor cells and CD3 on T-cells. A first-in-human study with 3 hour infusion once a day on day 1 through 5 in 28-days treatment cycles, showed linear and dose proportional pharmacokinetics but no tumor responses.[21] To achieve sustained target coverage, AMG 211 continuous intravenous infusion for 24 hours per day up to 28 days was subsequently explored in a multicenter phase I study, which is described in **chapter 3**. In this dose-escalation dose-expansion study the safety, tolerability, immunogenicity, pharmacokinetics, and preliminary signs of clinical efficacy of single-agent AMG 211 are determined in patients with advanced and heavily pre-treated gastrointestinal adenocarcinomas. Moreover, as exploratory objectives, pharmacodynamics like plasma inflammatory cytokines, and tumor CEA expression were studied. AMG 211 was administered as continuous intravenous infusion via central venous access for either 7 or 14 consecutive days in 28-days cycles, or 28 consecutive days in 42-days cycles. At the start of each cycle, patients were hospitalized for a minimum of 48 hours before treatment continuation in the outpatient setting. Outpatient clinic visits were scheduled at least once a week (twice during cycle 1) for safety monitoring and blood was collected for regular laboratory assessments (e.g. hematology, chemistry, coagulation) and to study antibody-drug antibodies, pharmacokinetics, and pharmacodynamics. The National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.03 were used for grading of adverse events.[22] Response evaluation with diagnostic CT was performed repeatedly after every 2 treatment cycles and assessed according to the immune-related response criteria.[23] Mandatory pre- and optional during-treatment biopsies were used to study CEA expression on tumor cells.

In case of modified bispecific antibodies, the potentially different binding affinity for the target of each of the arms might affect biodistribution. However, very limited information is available regarding whole-body distribution of bispecific antibodies in patients and regarding BiTE antibody constructs.[24,25] Improved understanding of biodistribution of these bispecific antibodies might help to guide drug dosing schedules and inform about potential target-related drug impact *in vivo*. By using zirconium-89 (^{89}Zr)-labeled AMG 211 as tracer for PET imaging, important information concerning AMG 211 tumor uptake, whole-body biodistribution, and organ pharmacokinetics can be gathered. In **chapter 4** we therefore report a two-center, first-in-human molecular PET imaging study with ^{89}Zr-AMG 211. Our primary aim was to gain insight into biodistribution of ^{89}Zr-AMG 211 in healthy tissues and tumor lesions both before and during AMG 211 treatment in the parallel running phase I trial **(chapter 3)**. ^{89}Zr-AMG 211 was developed according to good manufacturing practice.[26,27] Patients eligible for the phase I study were also asked for participation in the imaging study, which was performed before and/or immediately after the end of the second AMG 211 treatment period of 28 days. A fixed dose of 37 MBq ~200 µg ^{89}Zr-AMG 211 with or without cold ("unlabeled") AMG 211 was intravenously administered over 3 hours, followed by PET scans at 3, 6, and 24 hours after completion of the injection. After tracer infusion, patients were observed in the hospital for 24 hours to detect any side effects, which were graded according to NCI CTCAE v4.03.[22] Standardized uptake values were calculated for healthy tissues and tumor lesions and were compared within and between patients to study heterogeneity. Blood samples were collected at each PET scan time point to study tracer pharmacokinetics, tracer integrity using gel electrophoresis, and tracer binding to immune cells via counting blood fractions.

Identification of novel drugable targets is of great value with anticancer drug development focusing on personalized approaches. While mAbs were initially directed against oncogenic "driver" pathways, antigen targets for novel compounds like bispecific antibodies, antibody-drug conjugates and chimeric antigen receptors, do not have to be drivers of tumor growth because their main task is to serve as an anchor to bind the compounds. This clearly increases the total number of available antigen targets in cancer. In this context, glypican 3, a membrane-bound heparan sulfate proteoglycan without a clear role in tumorigenesis, is a new target of interest for anticancer immunotherapy because it is overexpressed by various tumor types, while expression in healthy tissues in uncommon. Several glypican 3 targeting therapies are in early phase clinical development. In **chapter 5**, we aimed to gain insight into the presence of the glypican 3 protein across a broad spectrum of tumor types using FGmRNA profiling. This technique was applied to expression profiles of 18,055 patient-derived tumor samples to predict glypican 3 overexpression at the protein level, using

healthy tissues as reference. Moreover, we compared our predictions with results obtained with IHC staining of a breast cancer tissue microarray, containing 391 tumor samples with on average 2.74 assessable cores per tumor, and historical IHC data in literature derived from a systematic search on PubMed.

In **chapter 6**, we performed a systematic search on PubMed and ClinicalTrials.gov to identify targets of marketed antibody-drug conjugates or antibody-drug conjugates in various phases of clinical development to explore if these targets can potentially also be used in other tumor types than initially planned. In addition, we collected from the public domain, gene expression profiles of 18,055 patient-derived tumor samples representing 60 tumor types and 3,520 samples representing 22 healthy tissue types. Next, we applied FGmRNA profiling to predict per tumor type the overexpression rate at the protein level of the identified antibody-drug conjugate targets with healthy tissue samples as a reference. With this data we aimed to support clinicians and drug developers in deciding which antibody-drug conjugate should be considered for clinical evaluation in which tumor type. This might help to guide the design of clinical trials in a broad spectrum of tumor types.

Finally, a summary of the obtained results in this thesis is described in **chapter 7** and future perspectives are discussed. **Chapter 8** provides a summary of the thesis in Dutch.

References

1 Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006;3:e442.

2 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646-674.

3 Kaplon H, Reichert JM. Antibodies to watch in 2019. MAbs. 2019;11:219-238.

4 Jain M, Kamal N, Batra SK. Engineering antibodies for clinical applications. Trends Biotechnol. 2007;25:307-316.

5 Younes A, Gopal AK, Smith SE, et al. Results of a pivotal phase II study of brentuximab vedotin in relapsed or refractory Hodgkin lymphoma. J Clin Oncol. 2012;30:2183-2189.

6 Verma S, Miles D, Gianni L, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med. 2012;367:1783-1791.

7 Kantarjian HM, DeAngelo DJ, Stelljes M, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. N Engl J Med. 2016;375:740-753.

8 Thomas A, Teicher BA, Hassan R. Antibody-drug conjugates for cancer therapy. Lancet Oncol. 2016;17:e254-262.

9 Tolcher AW. Antibody drug conjugates: lessons from 20 years of clinical experience. Ann Oncol. 2016;27:2168-2172.

10 Weiss SA, Wolchok JD, Sznol M. Immunotherapy of melanoma: facts and hopes. Clin Cancer Res. 2019. DOI: 10.1158/1078-0432.

11 Carter PJ, Lazar GA. Next generation antibody drugs: pursuit of the 'high-hanging fruit'. Nat Rev Drug Discov. 2017;17:197-223.

12 Brinkmann U, Kontermann RE. The making of bispecific antibodies. MAbs. 2017;9:182-212.

13 Sawyers CL. The cancer biomarker problem. Nature. 2008;452:548-552.

14 Tan DS, Thomas GV, Garrett MD, et al. Biomarker-driven early clinical trials in oncology: a paradigm shift in drug development. Cancer J. 2009;15:406-420.

15 Amir E, Clemons M, Purdie CA, et al. Tissue confirmation of disease recurrence in breast cancer patients: pooled analysis of multi-centre, multi-disciplinary prospective studies. Cancer Treat Rev. 2012;38:708-714.

16 Curigliano G, Bagnardi V, Viale G, et al. Should liver metastases of breast cancer be biopsied to improve treatment choice? Ann Oncol. 2011;22:2227-2233.

17 Amir E, Miller N, Geddie W, et al. Prospective study evaluating the impact of tissue confirmation of metastatic disease in patients with breast cancer. J Clin Oncol. 2012;30:587-592.

18 Mankoff DA. A definition of molecular imaging. J Nucl Med. 2007;48:18N-21N.

19 Gaber R, Watermann I, Kugler C, et al. Correlation of EGFR expression, gene copy number and clinicopathological status in NSCLC. Diagn Pathol. 2014;9:165.

20 Fehrmann RS, Karjalainen JM, Krajewska M, et al. Gene expression analysis identifies global gene dosage sensitivity in cancer. Nat Genet. 2015;47:115-125.

21 Pishvaian M, Morse MA, McDevitt J, et al. Phase 1 dose escalation study of MEDI-565, a bispecific T

T-cell engager that targets human carcinoembryonic antigen, in patients with advanced gastrointestinal adenocarcinomas. Clin Colorectal Cancer. 2016;15:345-351.

22 National Cancer Institute. NCI common terminology criteria for adverse events (CTCAE) v4.0. Bethesda, MD: National Cancer Institute, 2014.

23 Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res. 2009;15:7412-7420.

24 Tibben JG, Boerman OC, Massuger LFAG, et al. Pharmacokinetics, biodistribution and biological effects of intravenously administered bispecific monoclonal antibody OC/TR F(ab')2 in ovarian carcinoma patients. Int J Cancer. 1996;66:477-483.

25 van Brummelen EMJ, Huisman MC, de Wit-van der Veen LJ, et al. ^{89}Zr-labeled CEA-targeted IL-2 variant immunocytokine in patients with solid tumors: CEA-mediated tumor accumulation and role of IL-2 receptor-binding. Oncotarget. 2018;9:24737-24749.

26 Verel I, Visser GW, Boellaard R, et al. ^{89}Zr immune-PET: comprehensive procedures for the production of ^{89}Zr-labeled monoclonal antibodies. J Nucl Med. 2003;44:1271-1281.

27 Waaijer SJH, Warnders FJ, Stienen S, et al. Molecular imaging of radiolabeled bispecific T-cell engager ^{89}Zr-AMG211 targeting CEA-positive tumors. Clin Cancer Res. 2018;24:4988-4996.