Preclinical molecular imaging to study the biodistribution of antibody derivatives in oncology

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Chapter 7

General discussion and future perspectives

Positron emission tomography (PET) imaging and drug development

The development of targeted anticancer drugs is a slow and expensive process. Generally targeted drugs are effective in a subpopulation of the patients that are included in clinical trails. Therefore, there is a growing interest in the selection of patients that most likely benefit from targeted drugs. As PET imaging gives insight into whole body distribution and tumor uptake of radiolabeled drugs, it might facilitate the selection of the patients that most likely respond. Such an enrichment of the study population may reduce the number of patients required in the later phase of clinical development. Furthermore, PET imaging may support the optimization of drug dosing and dose scheduling.[1,2] This approach is increasingly used during drug development. To optimize implementation of PET imaging in clinical trials, it is critical to take into account the need for standardized scanning methods and the costs of PET imaging.[3,4] Moreover a good collaboration between academia and industry will expand the incorporation of molecular imaging in general and PET-imaging in particular, in clinical trials.

Optical imaging of tumors

Optical imaging with fluorescent tracers that bind tumor associated antigens might facilitate real-time tumor visualization in an intra-operative setting and visualization of tumor-margins in excised tissue. Optical imaging can additionally be used to study drug distribution on a cellular level in excised or biopsied tumor tissue. In **chapter 3** we fluorescently labeled anti-human epidermal growth factor receptor (HER)2 nanobodies with a near infrared fluorescent dye: IRDye 800CW. The fluorescently labeled nanobody 800CW-11A4 enabled tumor visualization of HER2 overexpressing tumors in mice as soon as 4 hours after injection. Fast tumor visualization might enable administration and tumor visualization/resection on the same day. Given the slower pharmacokinetics of antibodies, same day resection is less likely possible for fluorescently labeled anti-HER2 antibodies. Nanobodies have a high stability and can be produced straightforward production.[5] Clinical trials with 800CW-11A4 are therefore of potential interest and comparison with 800CW-trastuzumab would give an answer what the best approach is in which setting.

Penetration of near infrared light is limited due to strong scattering of light in tissue.[6] Therefore the use of real-time optical imaging might be restricted to the visualization of tumor lesions during surgery and endoscopic examination. Optoacoustic imaging may support visualization of deeper positioned tumors. This modality allows the use of specific dyes that absorb light and generate ultrasonic waves, detectable at multiple positions. The penetration depth can therefore be enlarged up to multiple centimeters.[7] The drawback of optoacoustic imaging however, is its small field of view. Real-time visualization of tumors with molecular imaging might therefore require the combination of optoacoustic with fluorescent imaging.

Half-life extension of therapeutic proteins

Anticancer drugs that are smaller than the renal cut-off value of 60-70kDa are prone to fast renal excretion, resulting in relative low tumor exposure. In order to increase tumor exposure, these drug need to be administered frequently. For example, blanitumumab is given as a 4-week

continuous infusion. Alternatively, the circulation half-life of these anticancer drugs can be extended by introducing albumin affinity, as has been realized for MSB0010853 (**chapter 4**). Other used methods include the conjugation of drugs to polyethylene glycol (PEG), fragment crystallizable (Fc)-domains of IgG antibodies or to albumin.[8] However, conjugation to Fc-domains of IgG antibodies or to albumin does not necessarily result in half-lives similar to respectively IgG antibodies or albumin. Although half-life of $IgG_{1, 2 \text{ and } 4}$ antibodies in humans is ~21 days and that of albumin in humans is ~19 days, half-lives of protein constructs that are conjugated to Fc-domains of IgG antibodies or albumin generally do not exceed 5 days in humans.[8] Clinical studies have to demonstrate to what extent the half-life of MSB0010853 is increased in cancer patients. Incorporation of PET imaging in clinical studies with 89-Zirconium (^{89}Zr) labeled MSB0010853 could reveal whole body distribution and tissue pharmacokinetics of a nanobody construct that is able to bind serum albumin in cancer patients.

Cancer immunotherapy and bispecific T-cell engagers (BiTEs)

Immunotherapy is gaining much attention as a novel treatment of cancer patients. With immunotherapy the immune system of cancer patients is stimulated to kill cancer cells more effectively. Currently, researchers mainly focus on new strategies, effective combinations and biomarkers to assess the effectiveness of new therapeutic options.

As a novel class of immunotherapy drugs, BiTEs can potentially be used to stimulate the host immune system to attack tumor cells. To date, the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) have approved one BiTE antibody (blinatumomab) for the treatment of Philadelphia chromosome-negative relapsed and refractory acute lymphoblastic leukemia. In contrast to blinatumomab that targets CD 19, the BiTEs AMG 110 and AMG 211, studied in **chapters 5 & 6**, target respectively epithelial cell adhesion molecule (EpCAM) and carcinoembryonic antigen (CEA) positive tumor cells in solid tumors. Solid tumors present additional challenges as given heterogeneous antigen expression, accessibility and a complex microenvironment that could suppress immune responses.[9] A clinical study with AMG 110 (NCT00635596) did demonstrate signs of biological activity, namely decreased tumor markers, anti-tumor activity in biopsied tumors and decreased circulating tumor cells.[10] Clinical evaluation of AMG 211 is currently ongoing (NCT02291614 and NCT01284231). Incorporating clinical PET imaging with ^{89}Zr labeled BiTEs in early phase clinical trials may provide information about tumor uptake and distribution in cancer patients. This information could potentially be used to facilitate the search for the optimal dosing regimen for BiTEs and help to identify patients that may benefit from BiTE treatment. For that reason the biodistribution and tumor uptake of ^{89}Zr-AMG211 is currently studied in a phase one clinical study (NCT02760199).

Preclinical data suggest that it might be of interest to combine BiTEs with other anti-cancer therapies. One such strategy to maximize T-cell mediated cancer cell death is combining BiTE treatment with the inhibition of immune checkpoints.[11] To date the FDA and EMA already approved several immune checkpoints inhibitors (e.g. ipilimumab, nivolumab, pembrolizumab and atezolizumab). In addition, there is a rational to evaluate immunotherapy combined

with other anticancer treatments.[12] Unfortunately, the population of cancer patients in which combinations can be tested is limited and determining effectiveness by overall survival is time-consuming and costly. Furthermore, the response patterns of immunotherapy can be completely different from response patterns seen with conventional chemotherapy. For example, response to immunotherapy may be preceded by apparent disease progression.[13] Therefore there is a strong need for consistent biomarkers that can be used to accurately select patient that most likely benefit from cancer immunotherapy. Important predictive biomarkers might be target expression, the presence of immune cells (e.g. T-cells) in tumors and the ability of the drug to penetrate and accumulate in tumors. Molecular imaging with imaging probes, including radiolabeled drugs, might enable whole body quantification of these biomarkers. Therefore there is a real opportunity for the clinical use of molecular imaging in order to monitor treatment response.

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