Immunoscintigraphy for Therapy Decision Making and Follow-Up of Biological Therapies

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Abstract: With the availability of new biological therapies there is the need of more accurate diagnostic tools to non-invasively assess the presence of their targets. In this scenario nuclear medicine offers many radiopharmaceuticals for SPECT or PET imaging of many pathological conditions. The availability of monoclonal antibodies provides tools to target specific antigens involved in angiogenesis, cell cycle or modulation of the immune systems. The radiolabelling of such therapeutic mAbs is a promising method to evaluate the antigenic status of each cancer lesion or inflamed sites before starting the therapy. It may also allow to perform follow-up of such biological therapies. In the present review we provide an overview of the most studied radiolabelled antibodies for therapy decision making and follow-up of patients affected by cancer and other pathological conditions.

Keywords: Immunoscintigraphy, Nuclear medicine, Personalized medicine, Radiolabelled mAbs, Radiopharmaceuticals.

INTRODUCTION

In the last decade, nuclear medicine has been routinely used in the field of oncology and inflammatory diseases because of its diagnostic utility, but also for its great contribution to the evaluation of disease activity. This is relevant, since in many cases, several therapeutic options are available for different activity states and early and appropriate treatment is necessary (1). Indeed, recent advances in the development of new radiopharmaceuticals have significantly contributed to the identification of molecular pathways, responsible for the physiological, biochemical and structural changes, a step forward to personalized therapy [2, 3].

This also helped to develop several biological therapies that have rapidly entered the market and are now available for both cancer and inflammatory disease, such as somatostatin, estrogen analogues, hormone receptor monoclonal antibodies (mAbs), growth factor inhibitors, cytokines, chemokines, tyrosine kinase inhibitors, etc [4]. All of these are able to interfere with the different pathways by binding key signalling molecules. Indeed, these receptor systems and their cognate ligands, collaborate in regulating cellular programs: quiescence, cell division, terminal differentiation, activation of different intracellular processes and replacement of old cells or death cells, under normal physiologic conditions. Presence or changes in the density of the receptor/ligand systems under pathological conditions provide the basis for biological therapies and also for nuclear medicine imaging. With the availability of the novel molecular targeted drugs, it is increasingly important to select the patients that might benefit from the targeted therapy. This offers a potential role for receptor imaging with radiolabelled variants of these targeted drugs or with endogenous molecules. The use of new radio-pharmaceuticals, could not only provide information on patho-biochemical processes but also indicate which treatment to apply, when to start it, end it or modify it, reducing costs and increasing the benefits.

CAN NUCLEAR MEDICINE PROVIDE A RATIONALE FOR STARTING BIOLOGICAL THERAPIES?

The diagnosis and follow-up of the pathological process in a specific disease is an important goal in medicine. Recent advances in the understanding of the pathophysiology at molecular level have led to the development of peptides, cytokines and mAbs that selectively accumulate in target tissue and organs, via binding to specific receptors [5, 6]. Biological therapies involve the use of biologically derived agents (mAb, peptide analogues and drugs) designed to activate the patient's immune system, to treat inflammation and specific types of cancer and to control side effects caused by the treatments such as immunotherapy and chemotherapy. Radiolabelled variants of these agents can act as probes for the detection of target cells through SPECT and PET scanners. Several radiolabelled peptides, cytokines and mAb are used for

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imaging tumours and inflammation and infection, with the intention to improve the monitoring of the efficacy of new biological therapies [7]. A nuclear medicine approach will have the advantage of not being invasive respect to other techniques such as biopsy, colonoscopy and others. From this point of view, radiolabelled monoclonal antibodies have always shown a great potential, but till now only a few reached phase III clinical trials. This review provides some examples where immunoscintigraphy could give a high contribution for therapy decision making and follow-up of biological therapies.

MONOCLONAL ANTIBODIES

Since their discovery, monoclonal antibodies have always been considered "magic bullets" that could virtually bind to any antigen with a great potential. Indeed, some of them were able to reach the market proving a high therapeutic efficacy. However, others barely concluded clinical trials or were not able to do so because of controversial results. In nuclear medicine, therapy decision making and follow-up of therapies based on the use of mAbs could be easily achievable by radiolabelling the same mAb used for treatment [8]. The followings are the most studied radiolabelled mAbs that can be used to predict therapeutic response to their unlabelled drug.

Anti-CD20

Some rheumatologic, autoimmune (AI) and lymphoproliferative diseases recognize common cellular markers. One of the most investigated, for both diagnostic and therapeutic purpose, is represented by B cells that can be identified by the expression of the antigen CD20 on their surface. The development of Rituximab, a mAb directed against CD20, has improved the management of several disease in particular in patients affected by active rheumathoid arthritis (RA) who do not respond to one or more TNF-alpha antagonist [9] or affected by non-Hodgkin's lymphomas (NHL) [10]. It has also been proposed and used for the treatment of other autoimmune diseases, such as pemphigus vulgaris, primary Sjogren Syndrome (pSS) and ANCA-associated vasculitis [11-13]. Nuclear Medicine can be potentially useful in all these pathologies for the *in vivo* imaging of B cell infiltration through the use of radiolabelled-Rituximab [14]. Physiologic biodistribution of the radiolabelled mAb is shown in Figure **1**.

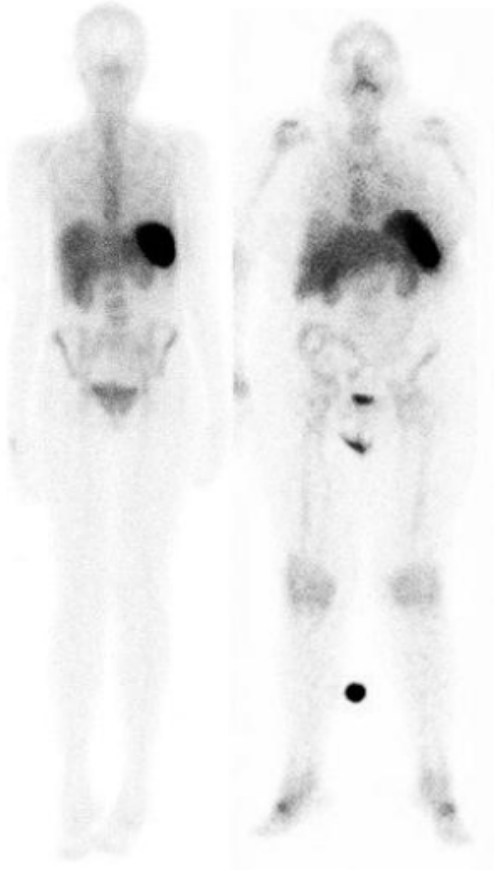

Figure 1: ^{99m}Tc-Rituximab scintigraphy in a normal subject (left) and in a patient affected by rheumatoid arthritis. In the latter there is an evident radiopharmaceutical accumulation in the knees and shoulders suggesting active inflammation in the joints.

A study by Malviya *et al*. demonstrated the utility of radiolabelled Rituximab for the assessment and for programming a tailored therapy in some AI diseases: RA, Psoriatic Arthritis, dermatopolymyositis, pSS, Behçet's disease, relapsing polychondritis and sarcoidosis [15]. Scintigrafic images were acquired at 6 and 20 hours post injection of ^{99m}Tc-Rituximab and showed increased uptake of the radiopharmaceutical in different sites according to the patient's pathology. For example in pSS patients an increased uptake was seen in salivary and lachrymal glands, in the five patients with active RA and the three patients with Psoriatic Arthritis, a mild to moderate uptake was observed in the affected joints, in Behçet's disease ^{99m}Tc-Rituximab was mainly concentrated in the oral cavity, whereas the patient with polychondritis showed an abnormal uptakes in thyroid and cricoid cartilages and in a lymph node of the neck. The scintigraphy of the patient affected by sarcoidosis showed an increased uptake in the chest, nasal cavity, lachrymal and salivary glands with a pattern of distribution that was similar the one

observed with ^{67}Ga-citrate. Interestingly the different biodistribution of the radiopharmaceutical seen in these different pathologies seems to reflect the different entity of B cell infiltration the tissues.

PET imaging could be potentially applied to AI disorders as demonstrated for example by some studies in which ^{124}I-Rituximab imaging was performed in five patients affected by RA, however its usefulness in this specific clinical setting is still not justified by the high costs [16].

As previously anticipated, anti CD20 mAbs find a large application also in hematologic disorders and in particular for immunotherapy of relapsing/refractory NHL [12, 17-21]. Ibritumomab is a murine mAb directed against CD20 similar to Rituximab and it is actually the only agent available in Europe. It can be used alone for the treatment of NHL or in association with β-emitting isotopes for radioimmunotherapy (RIT) mainly using ^{90}Y (Zevalin®) or ^{131}I and ^{177}Lu [21, 22]. A scintigraphic imaging using ^{111}In is recommended for pre-therapy assessment of CD20 expression and for prediction of subsequent ^{90}Y-Zevalin biodistribution [23, 24]. The use of ^{90}Y-Zevalin is now consolidated for the management of these kind of patients and it has been widely demonstrated that the addition of the radioisotope to the mAb increases therapeutic efficacy of the anti-CD20 in terms of overall response rate and stable responses [25-27].

Anti-EGFR

Cetuximab binds to the epidermal growth factor receptor (EGFR), which is variably expressed on cellular surface. Among the numerous natural ligands of this receptor, we must mention the epidermal growth factor (EGF) and transforming growth factor-α (TGFα) [28]. This binding initiates several signal transduction cascades that principally involve Akt and MAPK pathways, leading to DNA synthesis, cell proliferation, cell migration and adhesion [29]. Mutations that persistently activate EGFR or that cause an overexpression of this receptor on cellular surface can promote oncogenesis and sustain tumor development. These mutations have been observed in several cancers of different districts for example colo-rectal, lung, head and neck and breast [30-33]. For these tumors the development of anti-EGFR therapy could represent an important therapeutic tool for personalized therapy. Cetuximab is a recombinant, chimeric monoclonal antibody directed against the EGFR. It prevents the activation of receptor and its dimerization resulting in an inhibition of signal transduction and consequently in an antineoplastic activity. Cetuximab is approved by FDA (Erbitux®) for metastatic colo-rectal cancer and squamous cell carcinoma of head and neck but several efforts are being directed also in other types of cancers. It has been demonstrated that the presence of RAS wild type in patients affected by colo-rectal cancer is associated to a response to Cetuximab, while patients with mutated K-RAS seems to have not benefits from this therapy [32]. So it could be important to have a diagnostic tool that can predict the response to therapy in the view of a personalized and targeted treatment. Some authors explored the feasibility of ^{89}Zr-Cetuximab for PET imaging in 10 patients affected by colorectal cancer in order to assess the biodistribution of the radiopharmaceutical and to predict the success of therapy [34]. Despite an important limitation represented by the inability to detect liver lesion, in this small series ^{89}Zr-Cetuximab seems to provide important information regarding tumor expression of receptors however these results need to be confirmed by larger studies. Some studies on ^{90}Y or ^{177}Lu are also emerging in the last years aiming to explore the feasibility of radiolabelled Cetuximab as radio-immunotherapy [35] but of course they require more extensive studies.

Anti-TNF-α

Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine produced by macrophages, lymphocytes and other cells in some inflammatory chronic conditions such as rheumatoid arthritis (AR), ankylosing spondylitis, Crohn's disease or sarcoidosis. Because of high levels of TNF-α in these pathologies, it is possible to reveal it using monoclonal antibodies (mAbs) in order to do a diagnosis and a decision-making therapy.

Two monoclonal antibodies are available against both soluble and membrane-bound TNF-α, Infliximab (Remicade) and Adalimumab (Humira).

Infliximab, approved by Food and Drug Administration (FDA) in 1998 for the treatment of rheumatoid arthritis, is a chimeric monoclonal antibody that was directly labelled with ^{99m}Tc for imaging diseases [36].

Indeed Conti *et al*. [37] described a successful case report of a man with undifferentiated spondylarthro-pathy, performing a scintigraphy at 6 and 24 hours post injection of ^{99m}Tc-Infliximab. Scintigraphic images

showed an accumulation of the radiopharmaceutical in the site of lesion. The patient was treated with Infliximab and was re-evaluated up to 8 months of follow up, showing a complete remission of knee arthritis because of absence of TNF-α.

Another study focused the attention to evaluate the production of TNF-α by T helper 1 cells (Th1), in the active phase of Crohn's disease (CD). After *in vitro* and preclinical studies, ten patients underwent ^{99m}Tc-Infliximab scintigraphy to detect TNF-α expression in the small bowel. Results showed that scintigraphic analysis cannot be considered predictive of clinical outcome and it needs further investigations about the pathological and molecular mechanisms of anti- TNF-α monoclonal antibodies [38].

Recently, Vis and colleagues [39] performed SPECT/CT imaging at 6 and 20 hours after the injection of ^{99m}Tc-Infliximab in ten patients with pulmonary sarcoidosis. Scintigraphic analysis showed an accumulation of the radiopharmaceutical in the pulmonary tissue, especially in the hilar lymph nodes, confirmed by laboratory and clinical parameters too, despite of some opposite results between patients. Thus, other studies are required to clarify the role of ^{99m}Tc-Infliximab in the anti-TNF-α therapy response.

Adalimumab is another monoclonal antibody directed against both forms of TNF-α, approved by FDA in 2002 for the treatment of active rheumatoid arthritis and psoriatic arthritis. It is the first fully-human mAb and for this reason it is less immunogenic than Infliximab. It can be labelled with ^{99m}Tc through direct, using 2-mercaptoethanol, or indirect method, using the bifunctional chelator S-HYNIC [40, 41].

^{99m}Tc-Adalimumab was investigated by Barrera *et al*. [41] in patients with active rheumatoid arthritis, who underwent two scintigraphies, the first at 5 minutes, 4 and 24 hours post radiopharmaceutical micro-dose injection to evaluate the biodistribution and the second after two weeks for the specificity and sensitivity for inflammation changing. Indeed the first scintigraphy revealed a good uptake in inflamed joints compared to the absence of uptake in normal joints. Instead, before the second scintigraphy, one group was treated with a therapeutic dose of unlabelled mAb, the other received an intramuscular dose of corticosteroid to reduce inflammation. Through this study, authors demonstrated the specificity and sensitivity of ^{99m}Tc-Adalimumab to visualize inflammation in patients with AR and it could be considered predictive to relevant clinical changes.

In 2008, ^{99m}Tc-Infliximab and ^{99m}Tc-Adalimumab were investigated in a study for therapy-decision making and follow up in patients with active AR. Patients underwent scintigraphy before and after 3 months of intra-articular therapy with Infliximab (n=9) or systemic therapy with Adalimumab (n=12) [42]. Results showed the same biodistribution of two anti-TNF-α mAbs, a variable degree of joint uptake has been observed, which was not always correlated with joint pain or swelling. After the therapy, a reduction of uptake of ^{99m}Tc-anti-TNF mAb correlated with the reduction of clinical symptoms and more benefits were observed in patients with higher uptake. However, the complete mechanism of uptake of the compound was not still clear. Therefore further diagnostic methods could help to understand the mechanism.

Indeed, another mAb, Certolizumab pegol, was revealed effective to detect TNF-α by immuno-scintigraphy in patients with AR and spondyloarthritis. Certolizumab pegol is an engineered humanised mAb Fc-free Fab fragment with specificity for human TNF-α and it was labelled with ^{99m}Tc, using S-HYNIC as bifunctional chelator. Scintigraphic imaging was performed in 20 patients at 1, 4, 6 and 24 hours after the radiopharmaceutical injection, before and after 12 and 24 weeks treatment with Certolizumab pegol. Results showed a good uptake in involved joints, especially at 4-6 hours, but also up to 24 hours, with a good correlation with clinical evaluation and conventional imaging. This study was only a proof-of-concept, which did not include a negative control to observe the presence of unspecific phenomena. So, if further studies confirmed these results, ^{99m}Tc-Certolizumab pegol could be a crucial step for biological therapy [43].

Anti-PD-L1

One of the newest and most promising pathways that can be targeted by immunotherapies is the programmed (PD-1) and its ligands, especially PD-L1, that are involved in autoimmunity mechanisms. PD-L1 is overexpressed in the majority of solid tumors (including melanoma, non-small cell lung cancer, Merkel cell carcinoma, breast cancer, and squamous cell carcinomas) and its binding to PD-1 inhibits local T cells response to cancer cells. [44] Preclinical studies demonstrated that blocking this pathway by using anti-PD-L1 antibodies could activate autoimmune response to tumor, fueling important clinical application for cancer therapy. [45] Consequently, the expression of PD-L1 by tumor microenvironment can be an essential

biomarker to predict response to targeted immunotherapies. Tumor with low expression of PD-L1 will not respond to these drugs. Developing radiolabelled anti-PD-L1 tracers can noninvasively provide accurate informations about tumor PD-L1 expression that can otherwise be assessed only through biopsy and immunohistochemical staining. *In vivo* imaging of PD-L1 can be very useful both for patient selection for targeted immunotherapy and for treatment monitoring [46]. It also can be critical for clinical development of these drugs, providing pharmacokinetics information about whole-body biodistribution, tumor uptake and blood half-life. In the past few years new tracers have been developed for imaging the PD-L1 tumor expression. Josefsson *et al*. [47] developed a murine anti-PD-L1 antibody conjugated to Indium-111 for SPECT imaging and biodistribution studies in an immune-intact mouse model of breast cancer. Also Heskamp *et al*. [48] used anti–PD-L1 antibody PD-L1.3.1 radiolabelled with Indium-111; they analyzed tumor targeting by SPECT/CT imaging in mice bearing subcutaneous cancer xenografts with different PD-L1 expression levels. Lesniak *et al*. [49] assessed ^{64}Cu-Atezolizumab for detecting of PD-L1 expression in tumors. Atezolizumab is a humanized, human and mouse cross-reactive, therapeutic PDL1 antibody that was conjugated with DOTAGA, and radiolabelled with copper. The resulting ^{64}Cu-Atezolizumab was assessed for *in vitro* and *in vivo* specificity in multiple cell lines and tumors of variable PD-L1 expression. They performed PET/CT imaging, biodistribution, and blocking studies in NSG mice bearing tumors with constitutive PD-L1 expression and in controls. Other radiotracers developed for PD-L1 PET imaging and biodistribution studies in mice are ^{64}Cu–DOTA–HAC by Maute *et al*. [50] and ^{64}Cu-NOTA-PD-L1 mAb by Hettich *et al*. [46].

Anti-HER2

Trastuzumab is a humanized monoclonal antibody against the human epidermal growth factor receptor-2 with cytotoxic activity on target cells. HER2 is expressed in adenocarcinoma and particularly overexpressed in breast cancer. The beneficial effects on humans of Trastuzumab have been extensively reported in the literature, but the HER2 status in different lesions may vary due to tumor heterogeneity [51]. Therefore, it is important to evaluate the HER2 status, which is actually done by immunohistochemistry on biopsies. Unfortunately, some lesions are not accessible and nuclear medicine imaging with radiolabelled Trastuzumab may offer an accurate and non-invasive evaluation of the receptor expression. Indeed, radiolabelled Trastuzumab has been studied both in mice and humans with controversial results. In particular, in the study by Dijkers *et al*., in 50% of patients, almost 40% of known metastases were not detected by PET imaging with ^{89}Zr-Trastuzumab [52]. In particular, mainly lesions in the liver were not detected because of the high background in the organ, typical for radiolabelled mAbs. In other organs, this has been explained by the presence of soluble HER2 or extensive tumor load, suggesting the need of an individualized dosing schedule [53]. However, in a study by Gaykema *et al*. a correlation was observed between tumor uptake of radiolabelled Trastuzumab and reduction in size of lesions assessed by CT [54].

Promising results were also obtained when using ^{89}Zr-Trastuzumab in combination with ^{18}F-FDG-PET to predict the response to ADC trastuzumab-emtansine in patients already treated with unlabelled Trastuzumab [55].

Anti-VEGF

Bevacizumab is a commercially available mAb that binds the vascular endothelial growth factor (VEGF), which is involved in neoangiogenesis of many cancer types promoting metastatization. Therefore, Bevacizumab has been proposed as an anti-angiogenic treatment for aggressive breast tumors, ductal carcinoma and other cancer types. Whereas HER2 is bound on the plasma membrane, VEGF is a soluble factor, although some isoforms may be bound to extracellular matrix. However, it has been reported that VEGF concentration in metastases is greatly increased and this allows the use of radiolabelled Bevacizumab to evaluate the ligand expressions into metastatic lesions. The mAb has been radiolabelled with ^{99m}Tc, ^{111}In or ^{89}Zr and tested in animal models and humans with controversial results. In melanoma and primary breast cancer, it was able to visualize all known lesions, but, when focusing on lymph nodes, only 4 out of 14 were detected [54, 56]. In addition, uptake of radiolabelled Bevacizumab and concentration of VEGF into lesions not always correlated and it was dependent on the analysed isoform. Variable uptake was observed in patients affected by neuroendocrine tumors and renal cell carcinoma. In addition, no correlation between baseline tumor uptake and change of tumor size as assessed by CT was observed. In lung cancer, the correlation between radiolabelled mAb uptake and response to treatment was investigated in 7

patients with unsatisfactory results [57]. The authors hypothesised that it was not possible to distinguish between the therapeutic contribution of Bevacizumab or chemotherapy, limiting the study. The use of radiolabelled Bevacizumab for evaluation of therapeutic efficacy of anti-angiogenic drugs was also explored in renal carcinoma by Oosting *et al*. Again, it was not possible to correlate imaging results with time to progression [58]. In general, Bevacizumab uptake strongly depended on tumor type, reflecting different expression of VEGF by different cancer. In addition, inter- and intra-patient tumor heterogeneity was observed.

CONCLUSIONS

Because of the increasing availability of biological therapies based on the use of mAbs, immunoscintigraphy with the same radiolabelled drugs may help physicians in therapy decision making and follow-up. A wide range of mAbs has been radiolabelled and tested in different diseases, but till now none has entered in clinical practice. This is because many pathological conditions and antigen status have not been completely elucidated yet. This is in accordance with results from clinical trials with the unlabelled drugs that led physicians to use them in combination with other therapies rather than alone. This could be also the cause of the lack of correlation between imaging results and response to therapy, since the combination with chemotherapy or secondary effects on non-target cells (like enhanced permeability effects) may alter the biodistribution and uptake of the radiopharmaceuticals. This is crucial, since the retained biodistribution is one of the requirements that a radiolabelled mAb should meet to evaluate response to therapy with the unlabelled molecule. Intra- and inter-patient heterogeneity is another challenge, since some lesions may be undetected and combination with other imaging modalities like CT or MRI could be helpful. Once these issues will be solved, immunoscintigraphy will certainly become a reliable non-invasive technique to safely predict therapy outcome and follow-up treatment.

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