Radioimmunotherapy for delivery of cytotoxic radioisotopes - current status and challenges

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Abstract

Introduction: Radioimmunotherapy (RIT) with monoclonal antibodies and their fragments, labeled with radionuclides emitting α-particles, β-particles or Auger electrons have been used for many years in the development of anticancer strategies. While RIT has resulted in approved radiopharmaceuticals for the treatment of hematological malignancies, its use in solid tumors still remains more challenging.

Areas covered: In this review we discuss the exciting progress towards elucidating the potential of current and novel radioimmunoconjugates and address the challenges for translation into clinical practice.

Expert opinion: There are still technical and logistical challenges associated with the use of RIT in routine clinical practice, including development of novel and more specific targeting moieties, broader access to α-emitters and better tailoring of pretargeting approaches. Moreover, improved understanding of the heterogeneous nature of solid tumors and the critical role of tumor microenvironment will help to optimize clinical response to RIT by delivering sufficient radiation dose even to more radioresistant tumor cells.

Highlight box :

- Systemic radiotherapy with radiolabeled immunoconjugates delivers a non-uniform, low dose rate irradiation over a prolonged period of time, in contrast to external beam radiotherapy
- The opportunity of theragnostics, i.e. quantitative imaging of antibodies labeled with PET or SPECT radionuclides to predict subsequent therapeutic effects of an antibody radiolabeled with therapeutic ✔ or 🚲 emitting radionuclides, significantly contributes to a personalized treatment delivery
- Radioimmunotherapy is more successful in hematological cancers than in solid tumors
- The choice of the radionuclide is of pivotal importance for therapeutic efficacy and radiation-related toxicity.
- Modification of the antibody may improve the therapeutic window when tumor targeting is preserved, while blood clearance is accelerated.
- Application of bispecific monoclonal antibodies, binding to both tumor antigens and haptens, allows faster targeting of rapidly clearing radiolabeled small molecules, thereby improving the therapeutic window of radioimmunotherapy.

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1. Introduction

Ionizing radiation is a double-edged sword since it has the mutagenic potential to promote cancer development, while also commonly used in the clinic to induce DNA damage to selectively kill tumor cells. Next to surgery, radiotherapy still remains the most effective form of cancer treatment [1]. There are two different types of ionizing radiation, electromagnetic radiation (photons, one of the types of ionizing radiation typically used for external beam radiotherapy, EBRT) and particle radiation, typically used in systemic radiotherapy with radionuclides, and in the case of protons and carbon ions also for EBRT. The different types of DNA damage induced by ionizing radiation have been widely characterized over the years [2]. Amongst the DNA insults caused by ionizing radiation, double-strand breaks (DSBs) and clustered damage are the most deleterious with the greatest mutagenic potential [3-6]. Clustered damage relates to the formation of two or more lesions within one or two helical turns of the DNA by a single radiation track [7]. The lesions that compose clustered damage can include not only DSBs, but also single-strand breaks (SSBs) in proximity to base lesions [8-11]. It has been hypothesized that clustered damage occurrence may increase with an increase in ionization potential. Approximately 30% of the DSBs induced by low linear energy transfer (LET) ionizing radiation are complex due to the presence of additional breaks. This number rises to approximately 70% when high-LET radiation is used instead [12]. The plethora and complexity of damage induced by different ionization density of radiation highlight the deleterious effects it poses to genomic DNA.

The concept of radioimmunotherapy (RIT) emerged as an alternative to EBRT when the disease burden (e.g. radiosensitive tumors such as leukaemias and lymphomas) complicates treatment-planning options [13]. In RIT cytotoxic α- or β^--particle emitters are delivered by targeting molecules (e.g. monoclonal antibodies (mAbs), small proteins) providing continuous radiation exposure specifically to tumor-associated antigens while sparing the surrounding non-targeted normal tissues. These compounds are systemically administered, permitting the radioimmunoconjugate when in contact with a tumor cell to specifically bind to a given antigen via a direct interaction with the targeting moiety. The absorbed high amounts of energy promote direct macromolecular damage as well as the generation of reactive oxygen species [14]. The delivery of radiation doses capable of inducing cellular death may also pose detrimental effects to normal tissues, highlighting the need for a targeting moiety to specifically recognize an antigen in order to maximize the dose deposition to the tumor cells, enhancing the therapeutic index [15]. Of note, the enhanced specificity attained with targeting moieties such as mAbs may also result in a delivery of irradiation doses to normal tissue due to the rather slow clearance of these molecules [16, 17].

In 1950 when protein labeling with ^{131}I was performed without any significant alterations in terms of specificity, Pressman and Korngold assessed the tumor-targeting potential of a ^{131}I-labelled BSA in osteosarcoma-bearing rats, confirming its specific uptake in the tumor [18, 19]. The first clinical trial using this radioligand in patients with metastatic melanoma showed a complete remission in one patient [20]. Kőhler and Milstein's development of the hybridoma technique permitted the production and isolation of pure human mAbs against a single epitope. This resulted in the identification of several antigens that could be targeted for cancer treatment such as surface antigen CD20 e.g. highly expressed in non-Hodgkin's lymphoma (NHL) patients and not expressed in stem cells, and carcinoembryonic antigen (CEA) a common feature of colorectal cancer [21, 22]. Since then, hematological malignancies have become favorable targets for RIT due to their sensitivity for radiation and the broad variety of expressed antigens on their cellular surface, including CD5, CD22 and CD45 in acute lymphoblastic leukemia (ALL), CD15 and CD33 in acute myeloid leukemia (AML), as well as CD19-22 in NHL [13, 23]. Moreover, the antigen CD20 highly expressed in B-cell associated malignancies (e.g. in more than 90% of B-cell lymphoma cases), but not in plasma cells or non-lymphoid normal tissues, provides the importantly required tumor specificity for RIT. So far, two radiolabelled anti-CD20 antibodies have been approved for clinical use, and proven effective in the treatment of B-cell NHL, namely ^{90}Y-ibritumomab tiuexetan (Zevalin) and ^{131}I-tositumomab (Bexxar). The latter requires pre-therapy imaging in order to establish the dose to be delivered [24], whereas ^{90}Y-ibritumomab is typically administered at a dose of 14.8 MBq/kg, being reduced to 11.1 MBq/kg if the platelet counts are below 150,000. Furthermore, to avoid severe bone marrow toxicity the use of these tracers is not recommended in patients where the bone marrow involvement is more than 25% [25]. Experimental and clinical evidence suggest that radioconjugates targeting CD20 can significantly decrease disease progression [26, 27]. Treatment of NHL patients with ^{90}Y-ibritumomab tiuexetan led to a greater absorbed dose in the tumor when compared to normal tissues such as the liver, and thus increased the therapeutic index and treatment response [28]. Moreover, the effect of ^{90}Y-ibritumomab tiuexetan (Zevalin) and ^{131}I-tositumomab (Bexxar) have been reported to improve the overall (60-80%) and complete response rates (15-40%) in relapsed NHL patients when compared to treatment with unlabeled antibodies [26, 27]. Even though encouraging results were observed with ^{131}I-tositumomab (Bexxar), this radioimmunoconjugate is no longer available in the U.S., since its production has been discontinued [29]. In the case of ^{90}Y-ibritumomab tiuexetan (Zevalin), as reviewed by Rizzieri, this radioimmunoconjugate has shown promise for the treatment of NHL patients in comparison to EBRT, with trial results showing that this radioimmunoconjugate is an efficient therapeutic option for those patients who are

resistant to chemotherapy and rituximab (anti-CD20 antibody) [26, 30, 31]. It is believed that with an increase in awareness of the therapeutic benefits of this strategy, ^{90}Y-ibritumomab tiuexetan will assume a more prominent role in the treatment options of NHL patients [30]. Janik *et al.* have also reported the clinical use of ^{90}Y-daclizumab, an anti-CD25 monoclonal antibody, which was resulted in responses in 50% of the treated patients with relapsed NHL [32].

Unfortunately, despite the success of radioconjugates targeting antigens in hematological malignancies, RIT treatment of solid tumors still remains a challenge. Their greater radioresistance and limited capacity of penetration by large molecules such as mAbs impact on the treatment efficacy. The use of RIT is thought to be better suited to treat small-volume metastatic and post-surgery residual disease rather than a stand-alone therapeutic strategy in wide-spread metastatic disease. In comparison to EBRT, RIT has the ability to treat not only residual tumor in surgical resection margins, but also systemic malignancy (e.g. bone metastases) and tumor cells in circulation.

2. Choice of the radionuclide

RIT efficacy is inherently related to the capacity of the chosen isotope to incur DNA damage to the cells beyond their repair capacity. Depending on the nature of the radionuclide, the type and severity of the induced damage is quite diverse. Damage induction is dependent on the radiation quality or linear energy transfer (LET), which refers to the amount of deposited energy per unit track length (Figure 1) [12, 33, 34]. Conventionally, the radioisotopes of choice are β^-, α or Auger electron emitters (Table 1). The β–emitters (e.g. ^{131}I, ^{90}Y, ^{177}Lu, ^{188}Re, ^{186}Re and ^{67}Cu) produce low-LET radiation of approximately 0.2 keV/μm with a range of 0.5-12 mm in tissue, and energies between 30 keV and 2.3 MeV, in the form of β^- particles, internal conversion electrons, and γ or X-rays. These forms of radiation are commonly referred to as sparsely ionizing radiation, where the long range allows for energy deposition in neighboring non-targeted cells: ‘crossfire effect’. Conversely, it must also be considered that the range in tissue will have damaging effects on the surrounding normal tissues, increasing non-targeted toxicity, thus it is imperative to consider normal tissue toxicity when determining the therapeutic radionuclide to use. Moreover, sparsely ionizing radiation typically induces less complex damage, where 70% of the insults induced to the genomic DNA of cells are a direct result of the production of OH radicals, highlighting the importance of normal oxygen conditions to enhance radiation damage [35-37]. Therefore, high levels of hypoxia within the tumor mass will dramatically reduce the level of radiation damage incurred to the cells using such radioisotopes. In addition, the tumor microenvironment has a significant influence on

the delivery of the radioconjugates to the cancer cells. The combination of reduced blood flow and increased interstitial fluid pressure will increase the hypoxic levels within the tumor, ultimately resulting in a reduction in tumor uptake or a potential heterogeneous distribution of the conjugate across the tumor burden, concomitantly with an increase in radioresistance due to the lack of oxygen [38]. The most promising use for β^-–emitters in RIT lies with their ability to bypass tumor antigen heterogeneity and non-homogeneous penetration of intact mAbs. The most clinically relevant β^-–emitters that have been used so far in more than 95% of RIT trials are ^{131}I, ^{90}Y, ^{177}Lu and ^{186}Re [39-43]. These isotopes are readily available, have favorable emission characteristics, and adjustable radiochemistry facilitating conjugation with mAbs. For example, ^{131}I is inexpensive and has the advantage of being used for both single-photon emission computed tomography (SPECT) imaging and therapy, including treatment of thyroid cancer and malignancies such as NHL and AML [24, 44]. The commonly utilized radiochemistry for radioiodination has the disadvantage of leading to rapid de-iodination of the ^{131}I-labelled proteins that undergo endocytosis, being quickly degraded and released into the bloodstream as ^{131}I-tyrosin and free ^{131}I [15, 45]. Alternative chemistry can help preventing such effect [46]. Furthermore, the ^{131}I decay originates a high frequency of γ-rays, which can be toxic to surrounding tissues and which require radiation safety procedures for both patient's relatives and healthcare practitioners, potentially requiring longer hospitalization times. Alternatively, ^{90}Y has been used exclusively for therapeutic purposes, being almost a pure β^--emitter [47]. The higher energy characteristic of the β^--particles resulting from the decay of ^{90}Y leads to 70% of their energy being deposited outside small tumors, making ^{90}Y-labelled mAbs unsuitable for the treatment of small malignant lesions [48]. Moreover, even though ^{90}Y residualizes more readily than ^{131}I within the cancer cells following endocytosis, unchelated ^{90}Y has affinity for bone leading to relatively high radiation doses to the bone marrow, causing myelosuppression, and therefore increasing normal tissue toxicity [26, 49].

Given that solid tumors are typically poorly oxygenated, α–emitters represent a valid alternative for RIT treatment of such tumors. These isotopes are capable of generating high-LET radiation of 50-230 keV/μm, with energies ranging from 5 to 9 MeV (e.g. ^{225}Ac, ^{211}At, ^{212}Bi, ^{213}Bi and ^{212}Pb) [50, 51]. These particles have a much shorter range in tissue (typically 50-100 μm) when compared to β^-–particles, reducing toxicity to neighboring cells and increasing the number of ionizations per track. Ultimately, such emitters generate clustered radiation damage independently of the oxygenation status of the tumors, as highlighted by Wulbrand *et al*. [52]. Additionally, α–emitters can prove useful in the treatment of small-volume disseminated disease, which only require low numbers of

particles traversing the cell nucleus (one to three) to completely eradicate the cells [53]. Furthermore, α–emitters have a greater relative biological effectiveness (RBE) when compared to β^-–emitters, leading to greater levels of unrepaired DNA damage, which in turn results in a more prominent level of cell killing for the same delivered dose [50, 51, 54].

Moreover, Auger-electron emitters such as ^{125}I, ^{111}In, ^{67}Ga and ^{195m}Pt have also been used for RIT. These isotopes emit intermediate-LET radiation (4-26 keV/μm) with energies between 1 eV and 1 keV, and a range lower than 1 μm in tissue. This leads to an intense energy deposition in the nanometer scale, making these radionuclides ideal candidates for the treatment of single or clusters of cells, minimizing 'crossfire' toxicity [55, 56]. However, given the ultralow range of Auger-electrons, internalization and transport into the nucleus is key to achieve an effect by DNA damage induction, which may also translate into higher activities being required for treatment [57, 58]. As an example, ^{111}In-labeled anti-prostate-specific membrane antigen antibody J591 was assessed during a phase1 study in castrate metastatic prostate cancer, with the conjugate being well tolerated by the patients [59].

The choice of the optimal radionuclide for RIT is inherently dependent on the practical considerations related to its specific application. Therefore, apart from physical characteristics (e.g. half-life ($T_{1/2}$), type of emission(s), energy of the radiation(s), daughter product(s), method of production, and radionuclide purity) it is also necessary to consider the biochemical characteristics (e.g. selective concentration and prolonged retention in the tumor, minimum uptake in normal tissues, metabolism of the antigen-targeting molecule complex) that may aid or limit the anti-cancer effects of RIT. For instance, the radiation effects may be enhanced by the retention of the radionuclide within the lysosomes or storage proteins, or dramatically reduced if the radionuclide is quickly cleared from the cells, potentially also enhancing normal tissue toxicity. Antigens such as CD5, CD22 or PSMA, which are rapidly internalized, and subsequently catabolized by cancer cells, also may lead to a quick dissociation of the attached radionuclide. Therefore, molecules targeting such antigens are preferentially conjugated with residualizing radiometals such as ^{177}Lu, ^{90}Y and ^{213}Bi. These are retained within the cells leading to a continuous radiation exposure. Cells excrete radionuclides more promptly when non-residualizing radionuclides such as radioiodides are combined with fast internalizing targets. Therefore, antigens that have a prolonged retention on the cellular membrane may be better candidates for radiolabelling with non-residualizing radionuclides, promoting a prolonged exposure [15].

3. Antigens and targeting molecules

Ideally, the optimal antigen for RIT should be highly expressed (typically >100,000 sites per cell) in tumor cells but not in normal tissues, which will maximize the delivery of radiation dose specifically to the tumor [15]. Currently, the most frequently used targeting moieties for RIT are mAbs since a broad variety of therapeutic mAbs are available in the clinic [60]. Targets include the CD antigens; glycoproteins; enzymes such as prostate-specific membrane antigen (PSMA); blood vessel components like the vascular endothelial growth factor receptor (VEGFR); and cell-membrane receptors involved in the transduction of multiple signaling pathways such as the human epidermal growth factor receptor 2 (HER2) [15]. Clinical RIT trials in patients with solid tumors involved a wide variety of cancers including brain, colorectal, head and neck, renal or breast. In these trials whole antibodies (immunoglobulin G, IgG, 150 kDa) were used due to their availability and increased tumor uptake observed in preclinical models [21, 26, 31, 61-67].

A plethora of preclinical studies suggest that radioimmunoconjugate-based treatments can significantly decrease disease progression (see Table 2). For instance, Song *et al.* have studied the effect of anti-EGFR-targeted RIT in esophageal squamous cell carcinoma (OSCC) models using ^{177}Lu-cetuximab. This study has shown that animals receiving RIT treatment with ^{177}Lu-cetuximab exhibited a significant inhibition in tumor growth, followed by a reduction in [^{18}F]-FDG tumor uptake compared to the control group [68]. Timmermand *et al.* have reported the effective therapeutic use of the murine 11B6 antibody (m11B6), targeting human kallikrein-related peptidase 2 (hK2) radiolabelled with ^{177}Lu in subcutaneous prostate cancer xenografts [69]. The mice treated with 10, 19 or 36 MBq of ^{177}Lu-m11B6 survived for 88 to 120 days compared to an average of 39 days in the control group. The doses deposited in the tumor were estimated to be between 48 and 180 Gy, with bone marrow absorbed doses ranging between 4.5 and 16 Gy. Furthermore, ^{225}Ac α-particle based RIT targeting PSMA on prostate cancer cells, led to complete remission in two patients with metastatic castration-resistance prostate cancer [70]. Encouragingly, these results point towards a novel strategy for prostate cancer treatment with theoretically tolerable adverse effects. Furthermore, head and neck squamous cell carcinoma was more efficiently treated with ^{90}Y-cetuximab when compared to unlabeled cetuximab in UM-SCC-22B xenografts [71]. Impressive results have been observed when trastuzumab radiolabelled with ^{211}At promoted complete responses in SKOV-3 xenografts in comparison to unlabeled trastuzumab [72]. Moreover, several studies have reported the use of the same α-emitter coupled to MX35 F(ab')2, for the treatment of ovarian cancer, leading to a phase 1 clinical trial [73-78].Additionally, Derrien *et al.* tested the use of an anti-CD138 antibody radiolabelled with an α-emitter (^{213}Bi) to perform RIT in a mouse model of ovarian peritoneal carcinomatosis, a pathology currently lacking effective

treatment regimens. The authors demonstrated that selective irradiation of tumor cells overexpressing the CD138 antigen, increased the overall survival to approximately 70% after 90 days, compared to a median survival of 68 days in the control group [79]. These results indicate a potential therapeutic approach of using α-emitting radionuclides based RIT for the treatment of epithelial ovarian carcinoma. Chevallier *et al.* have also reported that RIT was well tolerated during a dose-escalation phase 1 study involving the use of ^{90}Y-labelled anti-CD22 epratuzumab tetraxetan in adults with refractory or relapsed B-cell acute lymphoblastic leukemia [80].

The slow blood clearance of intact IgG antibodies results in a prolonged blood circulation, leading to high tumor accumulation, concomitantly with an increased radiation exposure of the red marrow, potentially resulting in unwanted myelosuppression (reduction in platelets and white blood cells as well as red blood cells) and accumulation in critical organs such as the liver, when long-lived isotopes are used for radiolabelling [81, 82]. Therefore, antibody fragments ($F(ab')_2$, F(ab′), Fab; 110-55 kDa), single-chain variable fragments (scFv; 25 kDa) or engineered protein scaffolds including diabodies (dimers of scFv; 50 kDa) or affibody molecules (6-7 kDa) have been investigated as alternatives in animal models, intending to increase tumor penetration and to reduce the time required for blood clearance [83-85]

The divalent constructs have shown faster blood clearance with higher tumor retention when compared to monovalent proteins [86]. Their faster blood clearance is inherently related to their smaller size and lack of the Fc portion of the IgG responsible for binding to the neonatal Fc receptor and increased blood retention [25]. Subsequently, when compared to mAbs, antibody fragments reduce the dose delivered to the red marrow, permitting an escalation of the total activity delivered to the tumor. Smaller protein scaffolds are also superior in terms of traversing the vascular channels, accelerating tumor targeting and providing more attractive tumor-to-normal tissue ratios. Faster clearance from the blood allows for a more rapid delivery of the radioactivity to the tumor cells, providing higher dose-rates for efficient cell killing [87]. However, it limits the timeframe for target interaction, leading in turn to lower overall tumor uptake when compared to IgG constructs [81]. Furthermore, the faster delivery rates are concomitant with rapid excretion rates of a large proportion of the injected dose, requiring then injection of higher amounts of radioactivity, which can in turn result in increased renal toxicity rates. Therefore, in the clinical setting, antibody fragments have not been as successful as initially anticipated, possibly due to a mismatch between the fragment of choice and the radionuclide [25]. Affibody molecules have also been recently investigated. Their high target specificity (nM-pM range) and small molecular weight make them ideal candidates for imaging agents and therapy delivery platforms, allowing for rapid blood clearance and

favorable tumor uptake when conjugated with radioisotopes. However, their predominant renal excretion and retention of the radioactive metabolites in the proximal tubular cells results in a high kidney accumulation of radioactivity over time. Interestingly, recent data suggest that the overall reduction in dose delivered to the kidney is of two-fold, which may not be sufficient to limit potential long-term renal-associated side effects in clinical studies [25, 88, 89]. On the other hand, it has been reported that pre-dosing with cationic amino acids might significantly reduce the uptake of radiolabelled Fab in the kidneys in preclinical models, allowing for an activity escalation without increasing renal toxicity [25, 88, 89]. Moreover, dosimetry estimation studies in mouse xenografts have shown that ^{188}Re-labelled affibody molecules specifically targeting the HER2 receptor can deliver 79 Gy to the tumor, without exceeding the limiting doses delivered to the kidneys or bone marrow [90]. Encouragingly, Tolmachev *et al.* also conjugated the molecule to an albumin-binding domain (ABD) and showed further reduction in renal uptake of HER2-targetting affibody molecules, whilst permitting the delivery of therapeutic doses of ^{177}Lu. Treatment of SKOV-3 microxenografts (high-HER2 expression) with 17 or 22 MBq of ^{177}Lu-CHX-A"-DTPA-ABD-$(Z_{HER2:342})_2$ prevented the formation of tumors in contrast to the mice receiving placebo or ^{177}Lu-labelled non-specific affibody molecules [91, 92]. In addition, the same group has also evaluated another affibody-based construct, ZHER2:2891-ABD035-DOTA (ABY-027), radiolabeled with ^{177}Lu in HER2-expressing cells and SKOV-3 xenografts, suggesting this radioconjugate has potential for therapeutic intervention [93]. Despite the fact that affibody molecules show promise, further investigations of the use of such targeting moieties for RIT applications are required.

Furthermore, the applicability of dual-receptor targeted RIT was assessed by Razumienko *et al.* in breast cancer xenografts using bispecific radioimmunoconjugates (bsRICs) targeting both the HER2 and EGFR receptors [94]. These bsRICs comprised of trastuzumab Fab fragments and the EGF ligand labeled with either ^{111}In or ^{177}Lu. Both radioimmunoconjugates were found to bind *in vitro* with high specificity to HER2 and EGFR, presenting higher cytotoxic effects when compared to monospecific radioconjugates. The tumor uptake of ^{177}Lu-labelled bsRICs was 2-fold greater than with monospecific radioconjugates, additionally reducing tumor growth in both trastuzumab-sensitive MDA-MB-231/H2N and trastuzumab-resistant TrR1 tumors. This therapeutic regimen could become an alternative for patients with trastuzumab-acquired resistance. Other groups have also explored the use of cell-penetrating peptides to transport the radionuclides across the cellular membrane since they might facilitate RIT delivery to molecules localized in the cell nucleus such as γH2AX, a known DNA DSB biomarker. In fact, antibodies targeting this biomarker were conjugated with a TAT peptide and radiolabelled with ^{111}In. Internalization of this radioconjugate was confirmed in a panel of

breast cancer cell lines. Moreover, the use of ^{111}In-γH2AX-TAT was reported to delay tumorigenesis in genetically engineered mice of neuT-overexpressing breast cancer; by targeting the early onset of DNA damage formation, characteristic of cancer development [95].

4. Considerations for RIT in solid tumors

One of the reasons why RIT has mainly been a successful treatment approach for hematological cancers lies with the fact these cancers are typically more radiosensitive than solid tumors. Additionally, the high cost of RIT trials, limitations involving access to such form of therapy, and issues regarding eligibility criteria, are main reasons why the majority of RIT clinical trials for the treatment of solid tumors have not progressed beyond Phase I/II trials. Many clinical trials failed due to the treatment regimen being established without taking into consideration dosimetry and radiobiology [62, 96, 97]. For example, ^{90}Y-Pentumomab administered to patients with ovarian carcinoma, led to no increase in survival rates or time to relapse compared to the standard treatment most likely because the radiation doses were too low to promote tumor cell killing [98]. In addition, it is possible that the β-particles due to their range in tissue did not deposit the majority of the dose within the tumor, contributing to normal tissue toxicity, together with the hematological toxicity caused by slow blood clearance when full IgG antibodies are utilized as targeting moieties. It is therefore essential to account for the sensitivity of tumor cells during treatment planning, which can be described by well established α:β ratios [99]. Typically, high α:β ratios characterize tissues with low repair capacity, and low ratios are representative of moderately radiosensitive tissues (e.g. solid tumors) [99]. These ratios are conventionally used in the clinic with the linear quadratic model. This mathematical model has become the model of choice for bio-effect estimation in radiotherapy since its introduction around 1980. Computed with the linear quadratic model, the α:β ratios can be used to describe the repair capacity of the different tissues, assisting in the estimation of dose prescriptions required to guarantee tumor control and prevent normal tissue complications [99].

In order to maximize the effect of RIT it is necessary to better understand the radiobiology involved in this therapeutic approach. RIT is usually characterized by a non-uniform and low-dose rate irradiation, in contrast to conventional EBRT. Low-dose rate irradiations can be compared to fractionated radiotherapy, since in both cases the irradiated cells can repair the radiation-induced damage, being therefore necessary to account for dose and fractionation-dose related effects when optimizing RIT treatment regimens. Consequently, it is imperative to determine the absorbed dose delivered to the tumor burden, in order to

achieve more prominent patient responses following RIT. In conventional EBRT, doses in the order of 50 Gy are usually necessary to achieve clinical response in multiple forms of cancer, such as breast, lung, and colorectal [100]. The doses delivered by RIT are typically in the order of 1.8 Gy to 33 Gy, and therefore not sufficient to promote cell killing capable of eradicating the disease [101]. Calculating the total dose delivered by RIT to the tumor can be quite challenging due to the formation of non-uniform energy deposits. Therefore, some cells may receive high doses while others remain unirradiated. Dose fractionation could in principle counteract such issue due to improvements in distribution of the tracer, leading to a more homogeneous absorbed dose across the tumor burden. So, to accurately estimate the required dose for particular patient it would be helpful to acquire anatomical (CT or MRI) and molecular (PET or SPECT) scans. Assessment of the distribution of the tracer within the tumor and its pharmacokinetic profile could help to estimate the delivered dose per patient when applying RIT. Recently, Schwart *et al.* have reported studies where imaging with ^{124}I-labelled antibodies strengthened a potential role of image-based dosimetry to optimize RIT treatment schedules of patients with either renal or colorectal cancer, and guaranteed the appropriate dose delivery to the tumor whilst sparing normal tissues [102].

5. Strategies to improve RIT efficacy in treating solid tumors

Several strategies and approaches have been considered to improve the delivery and efficacy of RIT when treating solid tumors, including the use of non-conventional radionuclides. For diagnostic purposes, ^{89}Zr, ^{124}I or ^{111}In are the most frequently used isotopes for antibody labeling, as the decay time is ideal for PET and SPECT imaging, respectively. For therapy however, the majority of studies rely on the use of ^{131}I, ^{177}Lu and ^{90}Y. The conventional workflow requires a radionuclide (γ or β^+ emitter) to be used to evaluate the expression of the target antigen, dosimetric estimations, metabolic and clearance rates, and a radionuclide (β^- or α-emitter) to be used for therapy. The use of a radionuclide with favorable decay characteristics allowing for both efficient therapy and imaging would be therefore ideal [103]. For example, ^{47}Sc ideally fits into such category, being a β-emitter ($T_{1/2}$: 3.35 d; Eβ^-: 162 keV; Eγ: 159 keV), permitting radionuclide imaging and tumor therapy similarly to the clinically established ^{177}Lu ($T_{1/2}$: 6.65 d; Eβ^-: 134 keV; Eγ: 113, 208 keV). More recently, efforts have been put into facilitating the availability of ^{47}Sc, and into the development of radiochemistry allowing its conjugation to targeting moieties [104-106]. Additionally, the fact that ^{212}Pb, and ^{225}Ac/^{213}Bi can be produced by generators, might justify further investments in order to facilitate their availability making these isotopes attractive alternatives for α-emitter based RIT [107-110]. Furthermore,

^{203}Pb can be used as a matched SPECT imaging partner for ^{212}Pb, minimizing the challenges associated with the preclinical evaluation of biodistribution and targeting assays performed with ^{212}Pb-radiolabeled molecules [111].

In order to improve tumor targeting, an approach known as pretargeting has also been investigated. This strategy involves the separate administration of the targeting mAb, which is allowed to accumulate in the tumor followed by injection of the radionuclide conjugated with to a small molecule that binds to the mAb (hapten). Apart from concerns regarding the dose delivered to the kidneys due to excretion of the radionuclide, several preclinical and clinical studies have highlighted the benefit of such strategy in improving tumor uptake [112]. Such therapeutic approach was assessed in prostate cancer PC3 xenografts using the trivalent bispecific antibody TF12 (anti-TROP2 × anti-HSG [histamine-succinyl-glycine]) followed by ^{177}Lu-labeled diHSG-peptide (IMP288). Mice receiving 2 or 3 cycles of pretargeted RIT presented a median survival of >150 days, compared to 76 days observed in the control mice [113, 114]. Additionally, Schoffelen *et al.* have reported the clinical results obtained using pretargeted RIT in colorectal carcinoma patients using a bispecific mAb targeting the carcinoembryonic antigen (CEA) [115]. The utilized bispecific mAb (TF2) is a humanized tri-Fab molecule, comprising two anti-CEA Fab fragments, and one Fab fragment recognizing the hapten peptide (IMP288) radiolabelled with ^{111}In (imaging) or ^{177}Lu (therapy). This study demonstrated the feasibility and safety of utilizing pretargeted RIT for rapid and specific tumor targeting in CEA-expressing CRC patients [115]. Salaun *et al*. have also assessed the utility of anti-CEA pretargeted RIT in rapidly progressing metastatic medullary thyroid carcinoma (MTC) patients through a prospective multicenter trial [116, 117]. In addition, in this case the doubling time of serum biomarkers was correlated with clinical outcome. In total, 42 patients were treated with anti-CEA mAb followed by injection of ^{131}I bivalent hapten (1.8 Gb/m^2) 4-6 days later. Overall, pretargeted RIT led to a disease control rate of 76.2% with manageable hematological toxicity in progressive MTC, and increased serum biomarker doubling time was correlated with overall survival [116]. Bodet-Milin *et al.* reported the utility of pretargeted immuno-PET with ^{68}Ga-IMP288 and the anti-CEA bispecific mAb (TF2) in medullary thyroid carcinoma (MTC), as an optimization strategy for clinical optimization of pretargeting parameters [118]. The same group utilized a similar strategy to optimize the delivery of pretargeted RIT in in CEA-expressing advanced lung cancer patients [119].

Preclinical evaluation of ^{86}Y- or ^{177}Lu-DOTA-Bn binding scFv C825/GPA33 IgG bispecific immunoconjugates showed promising results in SW1222 colorectal carcinoma xenografts, with 9 out of 9 mice having a complete response following 66.6 or 111 MBq of the radioconjugate [85]. Houghton *et al.* reported the applicability of a bioorthogonal reaction

between transcyclooctene (TCO) and tetrazine (Tz), to specifically target pancreatic cancers expressing the carbohydrate antigen 19.9 (CA19.9) utilizing a fully human mAb (5B1). This antibody was modified with a TCO and used as the targeting vector, followed by administration of ^{64}Cu-NOTA-PEG7-Tz for PET imaging. This approach revealed a 25-fold lower total body dose in Capan-2 orthotopic models compared to ^{89}Zr-labelled 5B1, highlighting the potential of pretargeting [120]. The same approach also showed benefit in SW1222 human colorectal carcinoma xenografts [121].

As mentioned above, the tumor microenvironment impacts on the delivery of radioconjugates to cancer cells [38]. To overcome microenvironment-related hurdles, antiangiogenic agents targeting VEGF or its receptor have been used to normalize the tumor vasculature, enhancing the efficiency of RIT, as reported by the growth inhibition induced in SKOV-3 cells when exposed to ^{131}I-bevacizumab (anti-VEGF antibody) [122]. Contrastingly, Desar *et al.* and Muselaers *et al.* have reported that the use of agents such as sorafenib (VEGFR inhibitor) leads to increased vasculature disruption and necrosis in renal cell cancer patients, resulting in reduced tumor uptake of ^{111}In-bevacizumab and ^{111}In-girentuximab (anti-carbonic anhydrase IX mAb), without alterations in target antigen expression [123, 124]. More work is required in addressing the potential utility of VEGFR as a target for RIT. Moreover, Myiamoto *et al.* reported the benefits of mild hyperthermia in enhancing the delivery of cetuximab (EGFR mAb) in pancreatic cancer, where an increase in tumor accumulation was observed in BxPC-3, Capan-1, and in Ope-xeno xenografts, accompanied by a decrease in tumor volume [125]. Such strategy could be employed to enhance RIT delivery using cetuximab as the targeting moiety. The use of biological agents has also been equated with the purpose to modulate the expression of the target antigen, and therefore maximizing the dose delivery to cancer cells. Aquino *et al.* have reviewed the effect of drugs (e.g. 5-fluorouracil), cytokines (e.g. interferons or interleukin-6), differentiating agents (e.g. sodium butyrate) and protein kinase inhibitors (e.g. staurosporine) in up-regulating the expression of CEA [126-129].

6. Conclusions

In this review we have discussed the current status of RIT and ongoing research aiming to improve RIT delivery and the use of this therapeutic strategy to tackle pathologies lacking efficient therapeutic alternatives. Undoubtedly, after many years of intense research there are still technical and logistical challenges associated with the use of RIT in routine clinical practice, including development of novel and more specific targeting moieties, broader access to α-emitters and better tailoring of pretargeting approaches.

Tumor specificity of novel RIT approaches could be assessed through radiolabelling the targeting molecules used for RIT with PET radioisotopes. Quantitative analysis of PET

images may provide complementary information about the pharmacokinetics of the radioconjugate and help to more precisely estimate tumor dosimetry leading to better understanding of how to accurately design the RIT regimen (single vs. fractionated dose). However, we believe that the major hurdle that needs to be overcome to further enhance the clinical response to RIT is delivering sufficient radiation dose to kill more radioresistant tumor cells. Given the complicated tumor microenvironment and overall complexity of RIT, resolving these issues would be beneficial and allow for higher tumor dose delivery while sparing normal radiosensitive tissues.

In conclusion, clearly there is a need for more RIT clinical trials addressing the treatment efficacy of targeting specific antigens particularly in solid tumors, but the encouraging preclinical and clinical data highlight the potential usefulness of targeted intraperitoneal and systemic radiotherapy to treat a wide variety of different cancers.

7. Expert opinion

Radioimmunotherapy (RIT) has been successfully developed for treatment of patients with hematological malignancies, in particular non-Hodgkin's lymphoma. Using monoclonal antibodies labeled with β^--emitting radionuclides, durable clinical responses were achieved. Although the RIT approach results in clinically meaningful responses in these patients, the radiopharmaceuticals approved for this indication failed to become widely used therapies as the impact on patient survival was judged to be limited as compared to other treatment options. Financial implications are also thought to impact on the use of RIT in NHL, given the importance of reimbursement for such treatments. Combined with a lack of treatment sites, these points highlight a necessity for financially viable solutions encouraging such treatment approaches [130]. For solid tumors, RIT has been less successful and research has not resulted in an approved therapeutic radiopharmaceutical. This is due to a number of factors, of which the lower sensitivity of solid tumors to radiation is of major importance. Furthermore, in most trials several approaches to optimize the efficacy of RIT have been applied. The use of antibody fragments rather than whole IgG molecules results in faster clearance from non-target tissues, limiting the radiation dose to normal organs. Unfortunately, targeting of tumors is also generally lower, which means that there is no major effect on the therapeutic window (anti-tumor effect vs. radiation-induced side effects). Pretargeted radioimmunotherapy (PRIT) approaches hold major promise for improvement. In PRIT, the tumor is first targeted with an unlabeled multivalent antibody that has affinity for a tumor antigen as well as a small molecule. After allowing this molecule to target the tumor and clear from normal tissues, the radiolabeled small molecule targets to the antibody on the tumor, while being cleared fast from normal tissues. PRIT allows fine-tuning approach by antibody modifications, optimization of dosing regimens as well as the use of more effective radionuclides for therapy (α instead of β^--emitters). This flexibility to optimize treatment is on one side an asset, but also makes development and translation not straightforward, more complicated and more costly. Additionally, development of this technology must be performed in carefully selected patients. This is of importance as (P)RIT yields optimal results in patients with small volume disease which is not rapidly progressive. Radiation doses to bulky disease are generally not sufficient to induce durable responses. Patient selection can be improved by using the theranostic concept, exploiting the strengths of molecular imaging with immunoSPECT or immunoPET for detection, characterization and quantification of antigen expression on tumors, to depict normal organ uptake and to perform dosimetric analysis, estimating the radiation dose to the tumor lesions and normal organs. As it is apparent that patients may experience more benefit from combination of treatment

modalities, further research is needed to investigate potential synergic effects of (P)RIT with anti-cancer drugs or external beam radiotherapy.

BIBLIOGRAPHY

Papers of special note have been highlighted as either of interest **(•)** or of considerable interest **(••)** to readers.

1. James ND, Hussain SA, Hall E, Jenkins P, Tremlett J, Rawlings C, et al. Radiotherapy with or without Chemotherapy in Muscle-Invasive Bladder Cancer. N Engl J Med 2012 Apr 19;366(16):1477-88.

2. Cadet J, Ravanat JL, TavernaPorro M, Menoni H, Angelov D. Oxidatively generated complex DNA damage: Tandem and clustered lesions. Cancer Lett 2012 Dec 31;327(1-2):5-15.

3. Rothkamm K, Kruger I, Thompson LH, Lobrich M. Pathways of DNA double-strand break repair during the mammalian cell cycle. Mol Cell Biol 2003 Aug;23(16):5706-15.

4. Mladenov E, Magin S, Soni A, Iliakis G. DNA double-strand break repair as determinant of cellular radiosensitivity to killing and target in radiation therapy. Front Oncol 2013;3:113.

• Interesting overview of the important of DNA double-strand break repair in radiosensitivity.

5. Hunt CR, Ramnarain D, Horikoshi N, Iyengar P, Pandita RK, Shay JW, et al. Histone modifications and DNA double-strand break repair after exposure to ionizing radiations. Radiat Res 2013 Apr;179(4):383-92.

6. Price BD, D'Andrea AD. Chromatin remodeling at DNA double-strand breaks. Cell 2013 Mar 14;152(6):1344-54.

7. Gulston M, de Lara C, Jenner T, Davis E, O'Neill P. Processing of clustered DNA damage generates additional double-strand breaks in mammalian cells post-irradiation. Nucleic Acids Res 2004;32(4):1602-9.

8. Gulston M, Fulford J, Jenner T, de Lara C, O'Neill P. Clustered DNA damage induced by radiation in human fibroblasts (HF19), hamster (V79-4) cells and plasmid DNA is revealed as Fpg and Nth sensitive sites. Nucleic Acids Res 2002 Aug 1;30(15):3464-72.

9. Sutherland BM, Bennett PV, Sidorkina O, Laval J. Clustered DNA damages induced in isolated DNA and in human cells by low doses of ionizing radiation. Proc Natl Acad Sci USA 2000 Jan 4;97(1):103-8.

10. Sutherland BM, Bennett PV, Sutherland JC, Laval J. Clustered DNA damages induced by X rays in human cells. Radiat Res 2002 Jun;157(6):611-6.

11. Goodarzi AA, Jeggo PA. The repair and signaling responses to DNA double-strand breaks. Adv Genet 2013;82:1-45.

• Summary of the DNA damage response and DNA double-strand break repair pathways.

12. Nikjoo H, O'Neill P, Wilson WE, Goodhead DT. Computational approach for determining the spectrum of DNA damage induced by ionizing radiation. Radiat Res 2001 Nov;156(5):577-83.

13. Jurcic JG. Radioimmunotherapy for hematopoietic cell transplantation. Immunotherapy 2013 Apr;5(4):383-94.

14. Prise KM, O'Sullivan JM. Radiation-induced bystander signalling in cancer therapy. Nat Rev Cancer 2009 May;9(5):351-60.

15. Larson SM, Carrasquillo JA, Cheung NKV, Press OW. Radioimmunotherapy of human tumours. Nat Rev Cancer 2015 Jun;15(6):347-60.

•• **Overview of Radioimmunotherapy and clinical applications.**

16. Sharkey RM, Mottahennessy C, Pawlyk D, Siegel JA, Goldenberg DM. Biodistribution and Radiation-Dose Estimates for Yttrium-Labeled and Iodine-Labeled Monoclonal-Antibody Igg and Fragments in Nude-Mice Bearing Human Colonic Tumor Xenografts. Cancer research 1990 Apr 15;50(8):2330-6.

17. Seidl C, Zockler C, Beck R, Quintanilla-Martinez L, Bruchertseifer F, Senekowitsch-Schmidtke R. Lu-177-immunotherapy of experimental peritoneal carcinomatosis shows comparable effectiveness to Bi-213-immunotherapy, but causes toxicity not observed with Bi-213. Eur J Nucl Med Mol I 2011 Feb;38(2):312-22.

18. Eisen HN, Keston AS. The immunologic reactivity of bovine serum albumin labeled with trace-amounts of radioactive iodine (I131). J Immunol 1949 Sep;63(1):71-80.

19. Pressman D, Korngold L. The in vivo localization of anti-Wagner-osteogenic-sarcoma antibodies. Cancer 1953 May;6(3):619-23.

20. Beierwaltes W. Radioiodine-labelled compounds previously or currently used for tumour localization. *Tumour localization with radioactive agents* 1976.

21. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975 Aug 7;256(5517):495-7.

22. Tiernan JP, Perry SL, Verghese ET, West NP, Yeluri S, Jayne DG, et al. Carcinoembryonic antigen is the preferred biomarker for in vivo colorectal cancer targeting. Br J Cancer 2013 Feb 19;108(3):662-7.

23. Ng D. Radioimmunotherapy: a brief overview. Biomedical Imaging and Intervention Journal 2006;2(3):e23-e.

24. Rajendran JG, Gopal AK, Fisher DR, Durack LD, Gooley TA, Press OW. Myeloablative 131I-tositumomab radioimmunotherapy in treating non-Hodgkin's lymphoma: comparison of dosimetry based on whole-body retention and dose to critical organ receiving the highest dose. J Nucl Med 2008 May;49(5):837-44.

25. Sharkey RM, Goldenberg DM. Cancer radioimmunotherapy. Immunotherapy 2011 Mar;3(3):349-70.

26. Witzig TE, Gordon LI, Cabanillas F, Czuczman MS, Emmanouilides C, Joyce R, et al. Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or

refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. J Clin Oncol 2002 May 15;20(10):2453-63.

27. Davis TA, Kaminski MS, Leonard JP, Hsu FJ, Wilkinson M, Zelenetz A, et al. The radioisotope contributes significantly to the activity of radioimmunotherapy. Clin Cancer Res 2004 Dec 1;10(23):7792-8.

28. Postema EJ, Oyen WJ, Boerman OC, Corstens FH. Administration guidelines for radioimmunotherapy of non-Hodgkin's lymphoma with 90Y-labeled anti-CD20 monoclonal antibody. J Nucl Med 2003 May;44(5):853.

29. Shimoni A, Zwas ST. Radioimmunotherapy and Autologous Stem-Cell Transplantation in the Treatment of B-Cell Non-Hodgkin Lymphoma. Semin Nucl Med 2016 Mar;46(2):119-25.

30. Rizzieri D. Zevalin((R)) (ibritumomab tiuxetan): After more than a decade of treatment experience, what have we learned? Crit Rev Oncol Hematol 2016 Sep;105:5-17.

•• Overview of the use of ^{90}Y-ibritumomab tiuexetan for RIT treatment in the clinic.

31. Witzig TE, Flinn IW, Gordon LI, Emmanouilides C, Czuczman MS, Saleh MN, et al. Treatment with ibritumomab tiuxetan radioimmunotherapy in patients with rituximab-refractory follicular non-Hodgkin's lymphoma. J Clin Oncol 2002 Aug 01;20(15):3262-9.

•• Interesting report on the use of ^{90}Y-ibritumomab tiuexetan for RIT in follicular NHL who do not respond to rituximab.

32. Janik JE, Morris JC, O'Mahony D, Pittaluga S, Jaffe ES, Redon CE, et al. 90Y-daclizumab, an anti-CD25 monoclonal antibody, provided responses in 50% of patients with relapsed Hodgkin's lymphoma. Proc Natl Acad Sci U S A 2015 Oct 20;112(42):13045-50.

33. Lehnert S. Biomolecular action of ionizing radiation. Biomolecular Action of Ionizing Radiation Series: Series in Medical Physics and Biomedical Engineering, ISBN: 978-0-7503-0824-3 Taylor & Francis, Edited by Shirley Lehnert 2007;1.

34. Goodhead DT. The Initial Physical Damage Produced by Ionizing-Radiations. Int J Radiat Biol 1989 Nov;56(5):623-34.

35. Delara CM, Jenner TJ, Townsend KMS, Marsden SJ, Oneill P. The Effect of Dimethyl-Sulfoxide on the Induction of DNA Double-Strand Breaks in V79-4 Mammalian-Cells by Alpha-Particles. Radiat Res 1995 Oct;144(1):43-9.

36. Roots R, Okada S. Estimation of Life Times and Diffusion Distances of Radicals Involved in X-Ray-Induced DNA Strand Breaks or Killing of Mammalian-Cells. Radiat Res 1975;64(2):306-20.

37. Hirayama R, Ito A, Noguchi M, Matsumoto Y, Uzawa A, Kobashi G, et al. OH Radicals from the Indirect Actions of X-Rays Induce Cell Lethality and Mediate the Majority of the Oxygen Enhancement Effect. Radiat Res 2013 Nov;180(5):514-23.

38. Jain RK. Barriers to Drug-Delivery in Solid Tumors. Sci Am 1994 Jul;271(1):58-65.

39. Press OW. Radioimmunotherapy for non-Hodgkin's lymphomas: A historical perspective. Semin Oncol 2003 Apr;30(2):10-21.

40. Press OW, Rasey J. Principles of radioimmunotherapy for hematologists and oncologists. Semin Oncol 2000 Dec;27(6):62-73.

41. Barbet J, Bardies M, Bourgeois M, Chatal JF, Cherel M, Davodeau F, et al. Radiolabeled antibodies for cancer imaging and therapy. Methods Mol Biol 2012;907:681-97.

42. Navarro-Teulon I, Lozza C, Pelegrin A, Vives E, Pouget JP. General overview of radioimmunotherapy of solid tumors. Immunotherapy 2013 May;5(5):467-87.

43. Pouget JP, Lozza C, Deshayes E, Boudousq V, Navarro-Teulon I. Introduction to radiobiology of targeted radionuclide therapy. Front Med (Lausanne) 2015;2:12.

44. Pagel JM, Gooley TA, Rajendran J, Fisher DR, Wilson WA, Sandmaier BM, et al. Allogeneic hematopoietic cell transplantation after conditioning with 131I-anti-CD45 antibody plus fludarabine and low-dose total body irradiation for elderly patients with advanced acute myeloid leukemia or high-risk myelodysplastic syndrome. Blood 2009 Dec 24;114(27):5444-53.

45. Geissler F, Anderson SK, Press O. Intracellular Catabolism of Radiolabeled Anti-Cd3 Antibodies by Leukemic T-Cells. Cell Immunol 1991 Oct 1;137(1):96-110.

46. Vaidyanathan G, Zalutsky MR. Preparation of N-succinimidyl 3-[*I] iodobenzoate: an agent for the indirect radioiodination of proteins. Nat Protoc 2006;1(2):707-13.

47. Milenic DE, Brady ED, Brechbiel MW. Antibody-targeted radiation cancer therapy. Nat Rev Drug Discov 2004 Jun;3(6):488-99.

48. Koppe MJ, Postema EJ, Aarts F, Oyen WJ, Bleichrodt RP, Boerman OC. Antibody-guided radiation therapy of cancer. Cancer Met Rev 2005 Dec;24(4):539-67.

49. Kaminski MS, Zelenetz AD, Press OW, Saleh M, Leonard J, Fehrenbacher L, et al. Pivotal study of iodine I 131 Tositumomab for chemotherapy-refractory low-grade or transformed low-grade B-cell non-Hodgkin's lymphomas. J Clin Oncol 2001 Oct 1;19(19):3918-28.

50. Seidl C. Radioimmunotherapy with alpha-particle-emitting radionuclides. Immunotherapy 2014;6(4):431-58.

51. Seidl C, Essler M. Radioimmunotherapy for peritoneal cancers. Immunotherapy 2013 Apr;5(4):395-405.

52. Wulbrand C, Seidl C, Gaertner FC, Bruchertseifer F, Morgenstern A, Essler M, et al. Alpha-particle emitting 213Bi-anti-EGFR immunoconjugates eradicate tumor cells independent of oxygenation. PLoS One 2013;8(5):e64730.

53. Pouget JP, Navarro-Teulon I, Bardies M, Chouin N, Cartron G, Pelegrin A, et al. Clinical radioimmunotherapy-the role of radiobiology. Nat Rev Clin Oncol 2011 Dec;8(12):720-34.

•• Interesting report comprising the principles of RIT and the implications of radiobiology for efficient RIT treatment.

54. Kim YS, Brechbiel MW. An overview of targeted alpha therapy. Tumor Biol 2012 Jun;33(3):573-90.

55. Jackson MR, Falzone N, Vallis KA. Advances in Anticancer Radiopharmaceuticals. Clin Oncol 2013 Oct;25(10):604-9.

56. Kassis AI. Molecular and Cellular Radiobiological Effects of Auger Emitting Radionuclides. Radiat Prot Dosim 2011 Feb;143(2-4):241-7.

57. Reilly RM. Monoclonal antibody and peptide-targeted radiotherapy of cancer: John Wiley & Sons, 2010.

58. Li HK, Morokoshi Y, Daino K, Furukawa T, Kamada T, Saga T, et al. Transcriptomic Signatures of Auger Electron Radioimmunotherapy Using Nuclear Targeting (111)In-Trastuzumab for Potential Combination Therapies. Cancer Biother Radiopharm 2015 Oct;30(8):349-58.

59. Morris MJ, Divgi CR, Pandit-Taskar N, Batraki M, Warren N, Nacca A, et al. Pilot trial of unlabeled and indium-111-labeled anti-prostate-specific membrane antigen antibody J591 for castrate metastatic prostate cancer. Clin Cancer Res 2005 Oct 15;11(20):7454-61.

•• Report on the use of the Auger emitter ^{111}In-J591 targeting the prostate-specific membrane antigen.

60. Scott AM, Wolchok JD, Old LJ. Antibody therapy of cancer. Nat Rev Cancer 2012 Apr;12(4):278-87.

61. DeNardo SJ, OGrady LF, Richman CM, Goldstein DS, ODonnell RT, DeNardo DA, et al. Radioimmunotherapy for advanced breast cancer using I-131-ChL6 antibody. Anticancer Res 1997 May-Jun;17(3b):1745-51.

62. Navarro-Teulon I, Lozza C, Pèlegrin A, Vivès E, Pouget J-P. General overview of radioimmunotherapy of solid tumors. Immunotherapy 2013;5(5):467-87.

63. Reardon DA, Akabani G, Coleman RE, Friedman AH, Friedman HS, Herndon JE, 2nd, et al. Salvage radioimmunotherapy with murine iodine-131-labeled antitenascin monoclonal antibody 81C6 for patients with recurrent primary and metastatic malignant brain tumors: phase II study results. J Clin Oncol 2006 Jan 1;24(1):115-22.

64. Akabani G, Reardon DA, Coleman RE, Wong TZ, Metzler SD, Bowsher JE, et al. Dosimetry and Radiographic Analysis of 131I-Labeled Anti− Tenascin 81C6 Murine Monoclonal Antibody in Newly Diagnosed Patients with Malignant Gliomas: A Phase II Study. J Nucl Med 2005;46(6):1042-51.

65. Divgi CR, O'Donoghue JA, Welt S, O'Neel J, Finn R, Motzer RJ, et al. Phase I clinical trial with fractionated radioimmunotherapy using I-13-labeled chimeric G250 in metastatic renal cancer. J Nucl Med 2004 Aug;45(8):1412-21.

66. Vallabhajosula S, Nikolopoulou A, S Jhanwar Y, Kaur G, T Tagawa S, M Nanus D, et al. Radioimmunotherapy of Metastatic Prostate Cancer with; 177Lu-DOTAhuJ591 Anti Prostate Specific Membrane Antigen Specific Monoclonal Antibody. Curr Radiopharm 2016;9(1):44-53.

67. Kang CS, Song HA, Milenic DE, Baidoo KE, Brechbiel MW, Chong HS. Preclinical evaluation of NETA-based bifunctional ligand for radioimmunotherapy applications using 212Bi and 213Bi: radiolabeling, serum stability, and biodistribution and tumor uptake studies. Nucl Med Biol 2013 Jul;40(5):600-5.

68. Song IH, Lee TS, Park JH, Kim KI, Lee YJ, Kang JH, et al. Immuno-PET imaging and radioimmunotherapy of 64Cu/177Lu labeled anti-EGFR antibody in esophageal squamous cell carcinoma. J Nucl Med 2015;56(supplement 3):1216-.

69. Timmermand OV, Larsson E, Ulmert D, Tran T, Strand S. Radioimmunotherapy of prostate cancer targeting human kallikrein-related peptidase 2. Eur J Nucl Med Mol Imaging Res 2016;6(1):1.

70. Kratochwil C, Bruchertseifer F, Giesel FL, Weis M, Verburg FA, Mottaghy F, et al. 225Ac-PSMA-617 for PSMA-Targeted alpha-Radiation Therapy of Metastatic Castration-Resistant Prostate Cancer. J Nucl Med 2016 Dec;57(12):1941-4.

71. Niu G, Sun XL, Cao QZ, Courter D, Koong A, Le QT, et al. Cetuximab-Based Immunotherapy and Radioimmunotherapy of Head and Neck Squamous Cell Carcinoma. Clin Cancer Res 2010 Apr 1;16(7):2095-105.

•• Interesting report on the comparison of EGFR targeted RIT in head and neck cancer patients.

72. Palm S, Back T, Claesson I, Danielsson A, Elgqvist J, Frost S, et al. Therapeutic efficacy of astatine-211-labeled trastuzumab on radioresistant SKOV-3 tumors in nude mice. Int J Radiat Oncol Biol Phys 2007 Oct 1;69(2):572-9.

73. Cederkrantz E, Andersson H, Bernhardt P, Back T, Hultborn R, Jacobsson L, et al. Absorbed Doses and Risk Estimates of (211)At-MX35 F(ab')2 in Intraperitoneal Therapy of Ovarian Cancer Patients. Int J Radiat Oncol Biol Phys 2015 Nov 01;93(3):569-76.

74. Lindegren S, Andrade LN, Back T, Machado CM, Horta BB, Buchpiguel C, et al. Binding Affinity, Specificity and Comparative Biodistribution of the Parental Murine Monoclonal Antibody MX35 (Anti-NaPi2b) and Its Humanized Version Rebmab200. PLoS One 2015;10(5):e0126298.

75. Frost SH, Back T, Chouin N, Hultborn R, Jacobsson L, Elgqvist J, et al. Comparison of 211At-PRIT and 211At-RIT of ovarian microtumors in a nude mouse model. Cancer Biother Radiopharm 2013 Mar;28(2):108-14.

76. Cederkrantz E, Angenete E, Back T, Falk P, Haraldsson B, Ivarsson ML, et al. Evaluation of effects on the peritoneum after intraperitoneal alpha-radioimmunotherapy with (211)At. Cancer Biother Radiopharm 2012 Aug;27(6):353-64.

77. Gustafsson AM, Back T, Elgqvist J, Jacobsson L, Hultborn R, Albertsson P, et al. Comparison of therapeutic efficacy and biodistribution of 213Bi- and 211At-labeled monoclonal antibody MX35 in an ovarian cancer model. Nucl Med Biol 2012 Jan;39(1):15-22.

78. Andersson H, Cederkrantz E, Back T, Divgi C, Elgqvist J, Himmelman J, et al. Intraperitoneal alpha-particle radioimmunotherapy of ovarian cancer patients: pharmacokinetics and dosimetry of (211)At-MX35 F(ab')2--a phase I study. J Nucl Med 2009 Jul;50(7):1153-60.

79. Derrien A, Gouard S, Maurel C, Gaugler M-H, Bruchertseifer F, Morgenstern A, et al. Therapeutic efficacy of alpha-RIT using a 213Bi-anti-hCD138 antibody in a mouse model of ovarian peritoneal carcinomatosis. Front Med 2015;2.

80. Chevallier P, Eugene T, Robillard N, Isnard F, Nicolini F, Escoffre-Barbe M, et al. (90)Y-labelled anti-CD22 epratuzumab tetraxetan in adults with refractory or relapsed

CD22-positive B-cell acute lymphoblastic leukaemia: a phase 1 dose-escalation study. Lancet Haematol 2015 Mar;2(3):e108-17.

•• Excellent report on ^{90}Y-labelled anti-CD22 epratuzumab tetraxetan dose escalation phase 1 study in patients with refractory or relapsed lymphoblastic leukaemia.

81. Sharkey RM, Mottahennessy C, Pawlyk D, Siegel JA, Goldenberg DM. Biodistribution and Radiation-Dose Estimates for Yttrium-Labeled and Iodine-Labeled Monoclonal-Antibody Igg and Fragments in Nude-Mice Bearing Human Colonic Tumor Xenografts. Cancer Res 1990 Apr 15;50(8):2330-6.

82. Seidl C, Zockler C, Beck R, Quintanilla-Martinez L, Bruchertseifer F, Senekowitsch-Schmidtke R. 177Lu-immunotherapy of experimental peritoneal carcinomatosis shows comparable effectiveness to 213Bi-immunotherapy, but causes toxicity not observed with 213Bi. Eur J Nucl Med Mol Imaging 2011 Feb;38(2):312-22.

83. Zacchetti A, Martin F, Luison E, Coliva A, Bombardieri E, Allegretti M, et al. Antitumor effects of a human dimeric antibody fragment 131I-AFRA-DFM5.3 in a mouse model for ovarian cancer. J Nucl Med 2011 Dec;52(12):1938-46.

84. Cederkrantz E, Andersson H, Bernhardt P, Back T, Hultborn R, Jacobsson L, et al. Absorbed Doses and Risk Estimates of (211)At-MX35 F(ab')2 in Intraperitoneal Therapy of Ovarian Cancer Patients. Int J Radiat Oncol Biol Phys 2015 Nov 1;93(3):569-76.

85. Cheal SM, Xu H, Guo H-f, Lee S-g, Punzalan B, Chalasani S, et al. Theranostic pretargeted radioimmunotherapy of colorectal cancer xenografts in mice using picomolar affinity 86Y-or 177Lu-DOTA-Bn binding scFv C825/GPA33 IgG bispecific immunoconjugates. Eur J Nucl Med Mol Imaging Res 2015:1-13.

86. Olafsen T, Wu AM. Antibody Vectors for Imaging. Semin Nucl Med 2010 May;40(3):167-81.

87. Behr TM, Memtsoudis S, Sharkey RM, Blumenthal RD, Dunn RM, Gratz S, et al. Experimental studies on the role of antibody fragments in cancer radio-immunotherapy: Influence of radiation dose and dose rate on toxicity and anti-tumor efficacy. Int J Cancer 1998 Aug 31;77(5):787-95.

88. Behr TM, Sharkey RM, Sgouros G, Blumenthal RD, Dunn RM, Kolbert K, et al. Overcoming the nephrotoxicity of radiometal-labeled immunoconjugates - Improved cancer therapy administered to a nude mouse model in relation to the internal radiation dosimetry. Cancer 1997 Dec 15;80(12):2591-610.

89. Vegt E, de Jong M, Wetzels JFM, Masereeuw R, Melis M, Oyen WJG, et al. Renal Toxicity of Radiolabeled Peptides and Antibody Fragments: Mechanisms, Impact on Radionuclide Therapy, and Strategies for Prevention. J Nucl Med 2010 Jul 1;51(7):1049-58.

90. Altai M, Wallberg H, Honarvar H, Strand J, Orlova A, Varasteh Z, et al. Re-188-Z(HER2:V2), a Promising Affibody-Based Targeting Agent Against HER2-Expressing Tumors: Preclinical Assessment. J Nucl Med 2014 Nov;55(11):1842-8.

• Interesting report on the use of affibody molecules targeting the HER2 receptor as targeting moieties for RIT treatment of HER2 positive tumors.

91. Tolmachev V, Orlova A, Pehrson R, Galli J, Baastrup B, Andersson K, et al. Radionuclide therapy of HER2-positive microxenografts using a Lu-177-labeled HER2-specific affibody molecule. Cancer Res 2007 Mar 15;67(6):2773-82.

92. Tolmachev V, Wallberg H, Andersson K, Wennborg A, Lundqvist H, Orlova A. The influence of Bz-DOTA and CHX-A''-DTPA on the biodistribution of ABD-fused anti-HER2 Affibody molecules: implications for (114m)In-mediated targeting therapy. Eur J Nucl Med Mol Imaging 2009 Sep;36(9):1460-8.

93. Orlova A, Jonsson A, Rosik D, Lundqvist H, Lindborg M, Abrahmsen L, et al. Site-specific radiometal labeling and improved biodistribution using ABY-027, a novel HER2-targeting affibody molecule-albumin-binding domain fusion protein. J Nucl Med 2013 Jun;54(6):961-8.

94. Razumienko EJ, Chen JC, Cai ZL, Chan C, Reilly RM. Dual-Receptor-Targeted Radioimmunotherapy of Human Breast Cancer Xenografts in Athymic Mice Coexpressing HER2 and EGFR Using Lu-177- or In-111-Labeled Bispecific Radioimmunoconjugates. J Nucl Med 2016 Mar;57(3):444-52.

95. Murray PJ, Cornelissen B, Vallis KA, Chapman SJ. DNA double-strand break repair: a theoretical framework and its application. J R Soc Interface 2016 Jan;13(114):20150679.

96. Boswell CA, Brechbiel MW. Development of radioimmunotherapeutic and diagnostic antibodies: an inside-out view. Nucl Med Biol 2007 Oct;34(7):757-78.

97. Schaefer NG, Huang P, Buchanan JW, Wahl RL. Radioimmunotherapy in Non-Hodgkin Lymphoma: Opinions of Nuclear Medicine Physicians and Radiation Oncologists. J Nucl Med 2011 May;52(5):830-8.

98. Verheijen RH, Massuger LF, Benigno BB, Epenetos AA, Lopes A, Soper JT, et al. Phase III trial of intraperitoneal therapy with yttrium-90-labeled HMFG1 murine monoclonal antibody in patients with epithelial ovarian cancer after a surgically defined complete remission. J Clin Oncol 2006 Feb 1;24(4):571-8.

99. Joiner MC, Van der Kogel A. Basic Clinical Radiobiology: CRC Press, 2016.

100. Gunderson LL, Tepper JE. Clinical Radiation Oncology: Elsevier Health Sciences, 2015.

101. Tempero M, Leichner P, Baranowska-Kortylewicz J, Harrison K, Augustine S, Schlom J, et al. High-dose therapy with (90)yttrium-labeled monoclonal antibody CC49: A phase I trial. Clin Cancer Res 2000 Aug;6(8):3095-102.

102. Schwartz J, Humm JL, Divgi CR, Larson SM, O' Donoghue JA. Bone Marrow Dosimetry Using I-124-PET. J Nucl Med 2012 Apr;53(4):615-21.

103. Rösch F, Baum RP. Generator-based PET radiopharmaceuticals for molecular imaging of tumours: on the way to THERANOSTICS. Dalton Transactions 2011;40(23):6104-11.

104. Kolsky K, Joshi V, Mausner L, Srivastava S. Radiochemical purification of no-carrier-added scandium-47 for radioimmunotherapy. Appl Radiat Isot 1998;49(12):1541-9.

105. Müller C, Bunka M, Haller S, Köster U, Groehn V, Bernhardt P, et al. Promising prospects for 44Sc-/47Sc-based theragnostics: application of 47Sc for radionuclide tumor therapy in mice. J Nucl Med 2014;55(10):1658-64.

106. Połosak M, Piotrowska A, Krajewski S, Bilewicz A. Stability of 47Sc-complexes with acyclic polyamino-polycarboxylate ligands. J Radioanal Nucl Chem 2013;295(3):1867-72.

107. Su FM, Beaumier P, Axworthy D, Atcher R, Fritzberg A. Pretargeted radioimmunotherapy in tumored mice using an in vivo Pb-212/Bi-212 generator. Nucl Med Biol 2005 Oct;32(7):741-7.

108. Yong KJ, Milenic DE, Baidoo KE, Brechbiel MW. (212)Pb-radioimmunotherapy induces G(2) cell-cycle arrest and delays DNA damage repair in tumor xenografts in a model for disseminated intraperitoneal disease. Mol Cancer Ther 2012 Mar;11(3):639-48.

109. Ma D, McDevitt MR, Finn RD, Scheinberg DA. Breakthrough of 225Ac and its radionuclide daughters from an 225Ac/213Bi generator: development of new methods, quantitative characterization, and implications for clinical use. Appl Radiat Isot 2001 Nov;55(5):667-78.

110. Boll RA, Malkemus D, Mirzadeh S. Production of actinium-225 for alpha particle mediated radioimmunotherapy. Appl Radiat Isot 2005 May;62(5):667-79.

111. Yong K, Brechbiel MW. Towards translation of 212Pb as a clinical therapeutic; getting the lead in! Dalton Trans 2011 Jun 21;40(23):6068-76.

112. Boerman OC, van Schaijk FG, Oyen WJG, Corstens FHM. Pretargeted radioimmunotherapy of cancer: Progress step by step. J Nucl Med 2003 Mar;44(3):400-11.

113. van Rij CM, Frielink C, Goldenberg DM, Sharkey RM, Lütje S, McBride WJ, et al. Pretargeted Radioimmunotherapy of Prostate Cancer with an Anti-TROP-2× Anti-HSG Bispecific Antibody and a 177Lu-Labeled Peptide. Cancer Biother Radiopharm 2014;29(8):323-9.

•• Excellent report on pretargeted RIT in prostate cancer with an anti-TROP-2× Anti-HSG bispecific antibody and a ^{177}Lu-labeled Peptide.

114. van Rij C, Franssen G, Sharkey R, Frielink C, McBride B, Oyen W, et al. Pretargeted immunoPET imaging and radioimmunotherapy (RIT) of prostate cancer with an anti-EGP1 x anti-HSG bispecific antibody (bsMAb). J Nucl Med 2010;51(supplement 2):501-.

115. Schoffelen R, Boerman OC, Goldenberg DM, Sharkey RM, van Herpen CML, Franssen GM, et al. Development of an imaging-guided CEA-pretargeted radionuclide treatment of advanced colorectal cancer: first clinical results. Br J Cancer 2013 Aug 20;109(4):934-42.

•• Excellent report on the clinical results obtained using pretargeted RIT in colorectal carcinoma patients with a bispecific mAb CEA.

116. Salaun PY, Campion L, Bournaud C, Faivre-Chauvet A, Vuillez JP, Taieb D, et al. Phase II Trial of Anticarcinoembryonic Antigen Pretargeted Radioimmunotherapy in Progressive Metastatic Medullary Thyroid Carcinoma: Biomarker Response and Survival Improvement. J Nucl Med 2012 Aug;53(8):1185-92.

117. Kraeber-Bodere F, Salaun PY, Ansquer C, Drui D, Mirallie E, Faivre-Chauvet A, et al. Pretargeted radioimmunotherapy (pRAIT) in medullary thyroid cancer (MTC). Tumour Biol 2012 Jun;33(3):601-6.

118. Bodet-Milin C, Faivre-Chauvet A, Carlier T, Rauscher A, Bourgeois M, Cerato E, et al. Immuno-PET Using Anticarcinoembryonic Antigen Bispecific Antibody and 68Ga-Labeled Peptide in Metastatic Medullary Thyroid Carcinoma: Clinical Optimization of the Pretargeting Parameters in a First-in-Human Trial. J Nucl Med 2016 Oct;57(10):1505-11.

119. Bodet-Milin C, Ferrer L, Rauscher A, Masson D, Rbah-Vidal L, Faivre-Chauvet A, et al. Pharmacokinetics and Dosimetry Studies for Optimization of Pretargeted Radioimmunotherapy in CEA-Expressing Advanced Lung Cancer Patients. Front Med (Lausanne) 2015;2:84.

120. Houghton JL, Zeglis BM, Abdel-Atti D, Sawada R, Scholz WW, Lewis JS. Pretargeted Immuno-PET of Pancreatic Cancer: Overcoming Circulating Antigen and Internalized Antibody to Reduce Radiation Doses. J Nucl Med 2016 Mar;57(3):453-9.

121. Zeglis BM, Brand C, Abdel-Atti D, Carnazza KE, Cook BE, Carlin S, et al. Optimization of a Pretargeted Strategy for the PET Imaging of Colorectal Carcinoma via the Modulation of Radioligand Pharmacokinetics. Mol Pharm 2015 Oct 5;12(10):3575-87.

122. Ashrafi SA, Hosseinimehr SJ, Varmira K, Abedi SM. Radioimmunotherapy with 131I-bevacizumab as a specific molecule for cells with overexpression of the vascular endothelial growth factor. Cancer Biother Radiopharm 2012;27(7):420-5.

123. Desar IM, Stillebroer AB, Oosterwijk E, Leenders WP, van Herpen CM, van der Graaf WT, et al. 111In-bevacizumab imaging of renal cell cancer and evaluation of neoadjuvant treatment with the vascular endothelial growth factor receptor inhibitor sorafenib. J Nucl Med 2010 Nov;51(11):1707-15.

124. Muselaers CH, Stillebroer AB, Desar IM, Boers-Sonderen MJ, van Herpen CM, de Weijert MC, et al. Tyrosine kinase inhibitor sorafenib decreases 111In-girentuximab uptake in patients with clear cell renal cell carcinoma. J Nucl Med 2014 Feb;55(2):242-7.

125. Miyamoto R, Oda T, Hashimoto S, Kurokawa T, Inagaki Y, Shimomura O, et al. Cetuximab delivery and antitumor effects are enhanced by mild hyperthermia in a xenograft mouse model of pancreatic cancer. Cancer Sci 2016 Apr;107(4):514-20.

126. Aquino A, Formica V, Prete SP, Correale PP, Massara MC, Turriziani M, et al. Drug-induced increase of carcinoembryonic antigen expression in cancer cells. Pharmacol Res 2004 May;49(5):383-96.

127. Eftekhar E, Naghibalhossaini F. Carcinoembryonic antigen expression level as a predictive factor for response to 5-fluorouracil in colorectal cancer. Mol Biol Rep 2014 Jan;41(1):459-66.

128. Lee HC, Ling QD, Yu WC, Hung CM, Kao TC, Huang YW, et al. Drug-resistant colon cancer cells produce high carcinoembryonic antigen and might not be cancer-initiating cells. Drug Des Dev Ther 2013;7:491-502.

129. Brouwers AH, Frielink C, Oosterwijk E, Oyen WJG, Corstens FHM, Boerman OC. Interferons can upregulate the expression of the tumor associated antigen G250-MN/CA IX, a potential target for (radio)immunotherapy of renal cell carcinoma. Cancer Biother Radiopharm 2003 Aug;18(4):539-47.

130. Rizzieri D. Zevalin (R) (ibritumomab tiuxetan): After more than a decade of treatment experience, what have we learned? Crit Rev Oncol Hemat 2016 Sep;105:5-17.

131. Cheal SM, Xu H, Guo HF, Lee SG, Punzalan B, Chalasani S, et al. Theranostic pretargeted radioimmunotherapy of colorectal cancer xenografts in mice using picomolar affinity (86)Y- or (177)Lu-DOTA-Bn binding scFv C825/GPA33 IgG bispecific immunoconjugates. Eur J Nucl Med Mol Imaging Res 2016 May;43(5):925-37.

132. Orlova A, Jonsson A, Rosik D, Lundqvist H, Lindborg M, Abrahmsen L, et al. Site-Specific Radiometal Labeling and Improved Biodistribution Using ABY-027, A Novel HER2-Targeting Affibody Molecule-Albumin-Binding Domain Fusion Protein. J Nucl Med 2013 Jun 1;54(6):961-8.

133. Rousseau C, Ruellan AL, Bernardeau K, Kraeber-Bodere F, Gouard S, Loussouarn D, et al. Syndecan-1 antigen, a promising new target for triple-negative breast cancer immuno-PET and radioimmunotherapy. A preclinical study on MDA-MB-468 xenograft tumors. Eur J Nucl Med Mol Imaging Res 2011;1.

134. Abbas N, Heyerdahl H, Bruland ØS, Borrebæk J, Nesland J, Dahle J. Experimental α-particle radioimmunotherapy of breast cancer using 227Th-labeled p-benzyl-DOTA-trastuzumab. Eur J Nucl Med Mol Imaging Res 2011;1(1):1-12.

135. Vera DR, Eigner S, Henke KE, Lebeda O, Melichar F, Beran M. Preparation and preclinical evaluation of 177Lu-nimotuzumab targeting epidermal growth factor receptor overexpressing tumors. Nucl Med Biol 2012 Jan;39(1):3-13.

136. Salouti M, Babaei MH, Rajabi H, Rasaee M. Preparation and biological evaluation of (177)Lu conjugated PR81 for radioimmunotherapy of breast cancer. Nucl Med Biol 2011 Aug;38(6):849-55.

137. Rasaneh S, Rajabi H, Babaei MH, Daha FJ. 177 Lu labeling of Herceptin and preclinical validation as a new radiopharmaceutical for radioimmunotherapy of breast cancer. Nucl Med Biol 2010;37(8):949-55.

138. Josefsson A, Nedrow JR, Park S, Banerjee SR, Rittenbach A, Jammes F, et al. Imaging, Biodistribution, and Dosimetry of Radionuclide-Labeled PD-L1 Antibody in an Immunocompetent Mouse Model of Breast Cancer. Cancer Res 2016 Jan 15;76(2):472-9.

•• Excellent report on the role of PD-L1 imaging and RIT with ^{111}In- DTPA-anti-PD-L1 in the optimization and efficacy monitoring of immune checkpoint inhibition therapy.

139. Fazel J, Rotzer S, Seidl C, Feuerecker B, Autenrieth M, Weirich G, et al. Fractionated intravesical radioimmunotherapy with Bi-213-anti-EGFR-MAb is effective without toxic side-effects in a nude mouse model of advanced human bladder carcinoma. Cancer Biol Ther 2015 Oct;16(10):1526-34.

140. Lindenblatt D, Fischer E, Cohrs S, Schibli R, Grunberg J. Paclitaxel improved anti-L1CAM lutetium-177 radioimmunotherapy in an ovarian cancer xenograft model. Eur J Nucl Med Mol Imaging Res 2014 Oct 3;4.

141. Fujiwara K, Koyama K, Suga K, Ikemura M, Saito Y, Hino A, et al. 90Y-Labeled Anti-ROBO1 Monoclonal Antibody Exhibits Antitumor Activity against Small Cell Lung Cancer Xenografts. PLoS One 2015;10(5):e0125468.

142. Sugyo A, Tsuji AB, Sudo H, Okada M, Koizumi M, Satoh H, et al. Evaluation of Efficacy of Radioimmunotherapy with 90Y-Labeled Fully Human Anti-Transferrin Receptor Monoclonal Antibody in Pancreatic Cancer Mouse Models. PLoS One 2015;10(4):e0123761.

143. Elgstrom E, Eriksson SE, Ohlsson TG, Nilsson R, Tennvall J. Role of CD8 positive cells in radioimmunotherapy utilizing 177Lu-mAb in a syngeneic rat colon carcinoma model. Eur J Nucl Med Mol Imaging 2014 Oct;41:S533-S.

TABLES

Isotope	Half-life ($T_{1/2}$)	Maximum energy (keV)	Maximum range (μm)	Emission type
β^-–emitters (LET: 0.2 keV/μm)				
^{90}Y	2.67 d	2280.0	11300	β^-
^{131}I	8.02 d	606.31	2300	β^-, γ
^{177}Lu	6.65 d	498.3	1800	β^-, γ
^{67}Cu	61.83 h	577.0	2100	β^-, γ
^{186}Re	3.72 d	1069.5	4800	β^-, γ
^{188}Re	17.01 h	2120.4	10400	β^-, γ
Auger emitters (LET: 4-26 keV/μm)				
^{111}In	2.80 d	26	17	Auger, γ
^{67}Ga	3.26 d	9.6	3	Auger, β^-, γ
^{195m}Pt	4.02 d	64	76	Auger
^{125}I	59.41 d	31.7	20	Auger, γ
α–emitters (LET: 50-230 keV/μm)				
^{213}Bi	45.59 min	8400	90	α, β^-, γ
^{212}Bi	60.54 min	7800	100	α, β^-, γ
^{211}At	7.21 h	7500	80	α, EC
^{212}Pb§	10.64 h	7800	100	α, β^-, γ
^{225}Ac	9.92 d	8400	90	α, β^-, γ
^{227}Th	18.7 d	7400	70	α, β^-, γ

Table 1. Radioisotopes used in RIT (§: ^{212}Pb is not a direct α-emitter but it decays to the α-emitter ^{212}Bi). EC: Electron capture. Adapted with permission from [53].

Target Antigen	Targeting Moiety	Radionuclide	Model	Reference
hK2	murine Ab	^{177}Lu	Prostate cancer	[69]
CD138	mAb	^{213}Bi	Ovarian carcinoma	[79]
EGFR	mAb	^{177}Lu	OSCC	[68]
TROP-2	mAb	^{177}Lu	Prostate cancer	[113, 114]
GPA33	mAb	^{177}Lu/^{86}Y	Colorectal cancer	[131]
NaPi2b	F(ab')$_2$	^{211}At	Ovarian cancer	[84]
PSMA	mAb	^{177}Lu	Prostate cancer	[66]
HER2	mAb	$^{212/213}$Bi	Colon adenocarcinoma	[67]
HER2	Affibody	^{177}Lu	Ovarian carcinoma	[90, 132]
HER2	mAb	^{212}Pb	Colon adenocarcinoma	[108]
CD138	mAb	^{131}I	Breast carcinoma	[133]
HER2	mAb	^{227}Th	Breast carcinoma	[134]
FR	F(ab')$_2$	^{131}I	Ovarian cancer	[83]
EGFR	mAb	^{177}Lu	Epidermoid carcinoma	[135]
MUC1	mAb	^{177}Lu	Breast carcinoma	[136]
HER2	mAb	^{177}Lu	Breast carcinoma	[137]
PD-L1	mAb	^{111}In	Breast carcinoma	[138]
HER2/EGF	bsRICs	^{177}Lu/^{111}In	Breast carcinoma	[94]
EGFR	mAb	^{213}Bi	Bladder carcinoma	[139]
L1CAM	mAb	^{177}Lu	Ovarian cancer	[140]
ROBO1	mAb	^{90}Y	Small cell lung cancer	[141]
TfR	mAb	^{90}Y	Pancreatic cancer	[142]
Lewis Y	mAb	^{177}Lu	Colon carcinoma	[143]

Table 2. Examples of preclinical RIT studies in solid tumors since 2010.

FIGURES

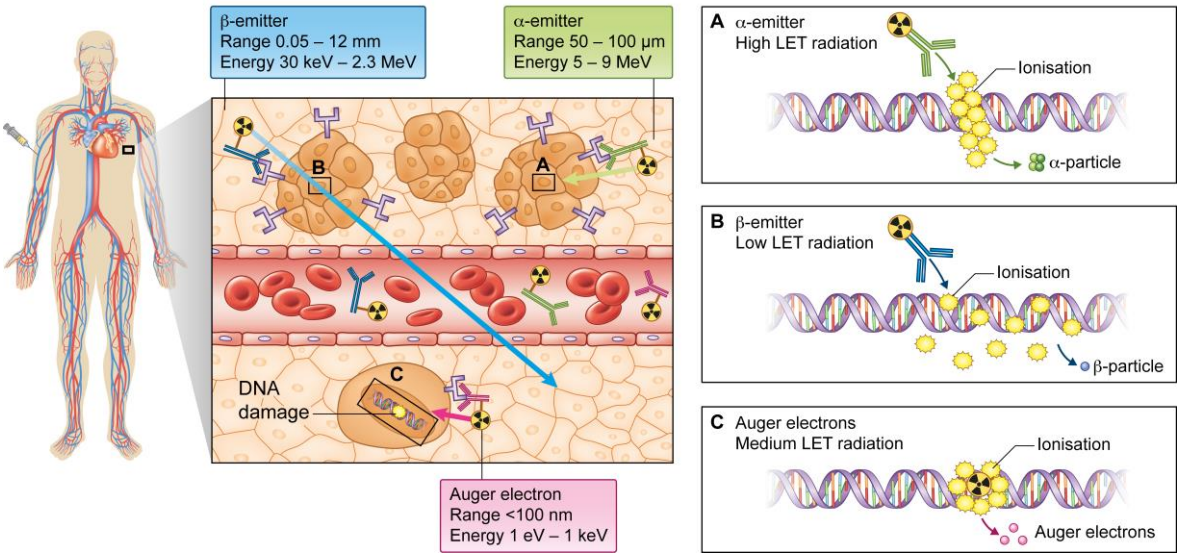

Figure 1: The mAb (targeting moiety) conjugated with a radionuclide (DNA damaging agent) is injected into the blood stream recognizing the cells expressing the target antigen. The characteristic decay of the radionuclide will generate radiation with different energies and ranges in tissue. α-emitters (**A**) produce densely ionizing high-LET radiation, with MeV energies and μm range in tissue, causing complex DNA damage leading to prominent cell killing due to unrepaired damage. β$^-$-emitters (**B**) generate low-LET radiation with keV-MeV energies and mm range in tissue (potential 'crossfire' toxicity), generally referred to as sparsely ionizing (few ionizations per track), leading to low-complexity DNA damage, more readily repaired by the DNA repair machinery. Auger-emitters (**C**) produce intermediate-LET radiation with energies between 1 eV and 1 keV, and sub-μm range in tissue, with an intense energy deposition over a short range, challenging the cellular repair capacity.