Comparison of different classes of radionuclides for potential use in radioimmunotherapy

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Abstract

Currently, β-emitting radionuclides are used almost exclusively in the clinic and in clinical radioimmunotherapy studies. The main advantage of β-emitters is the relatively long path length in biological tissue (in the mm range), which is sufficient to irradiate cancer cells that do not have bound radiolabelled antibody (cross-fire effect). This alleviates problems with inadequate uptake and heterogeneous distribution of radiolabelled antibodies in tumours. Hence, β-emitters provide a relatively uniform radiation dose to the tumour and it is generally accepted that this class of radionuclides is more appropriate for radioimmunotherapy of solid tumours and large tumour burdens (> 0.5 cm). However, the shorter-range α-emitters (50-100 mm) and the ultra-short range Auger electron-emitting radionuclides (the majority of electrons traverse a few nm), have been shown to be more efficient than β-emitters at inducing lethal lesions in single cells. It has been suggested that these classes of radionuclides may have the potential to provide a more favourable therapeutic index than β-emitters for radioimmunotherapy of single tumour cells in the circulation, micrometastases and in certain cases, minimal residual disease. The aim of this article is to discuss the different classes of radionuclides with potential for clinical use in radioimmunotherapy.

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Introduction

Radioimmunotherapy involves the use of specific anti-tumour antibodies to selectively deliver a radionuclide to tumour cells. When compared to conventional radiotherapy, the use of radiolabelled antibodies with a high degree of specificity for tumour-associated antigens offers the potential for minimising damage to normal tissues by targeting the radiation dose more specifically to the tumour. However, the majority of the clinical studies (particularly for solid tumours) do not agree well with the theoretical expectation. A notable exception to this trend is radioimmunotherapy of non-Hodgkin's lymphoma.

There are a number of limitations that are associated with radioimmunotherapy. These include, heterogeneous antigen expression on tumour cells, generation of antigen-loss tumour variants, non-absolute specificity of antibodies for tumour cell-antigens (for example, antibodies may bind to differentiation antigens on normal cells), bone marrow toxicity from the slow-blood clearance of antibodies and immunogenicity of antibodies – i.e. production of human anti-mouse and human anti-chimeric antibodies [1, 2]. However, the major clinical limitation of radioimmunotherapy, particular for treatment of solid tumours, results from inefficient uptake and non-optimal distribution of the relatively large (approximately 150 kDa) radiolabelled antibodies in the tumour [3-5].

Radiopharmaceuticals, β-emitters

To circumvent some of the problems associated with radioimmunotherapy, β-emitting radionuclides particularly yttrium-90 (^{90}Y) and iodine-131, (^{131}I) are used almost exclusively in the clinic and in clinical radioimmunotherapy studies. The range of the β-particles in biological tissues (mean range in tissue equivalent matter of 0.8 mm for ^{131}I and 2.7 mm for ^{90}Y) is sufficient to irradiate tumour cells that do not have bound radiolabelled antibody. This phenomenon commonly referred to as the cross-fire irradiation effect [6, 7]. Hence, β-emitters are used to alleviate the problems of inadequate uptake and heterogeneous distribution (related to uptake and antigen expression) of radiolabelled antibodies in tumour and therefore, to provide a relatively uniform radiation dose to the tumour [8].

Radioimmunotherapy with anti-CD20 monoclonal antibodies labelled with the β-emitters ^{90}Y or ^{131}I has recently been introduced as a therapeutic modality for B-cell non-Hodgkin's lymphoma. The US Food and Drug Administration (FDA) has approved ^{90}Y-ibritumomab

tiuxetan (Zevalin; IDEC Pharmaceuticals Corp., San Diego CA) and ^{131}I-tositumomab (Bexxar; Corixa Corp., Seattle WA), for clinical use. It should be noted that radioimmunotherapy of non-Hodgkin's lymphoma is the major exception to the general trend observed in the clinical studies (i.e. relatively low response rates to radiolabelled antibodies). In part, this is because lymphomas are more sensitive to immunotherapy and to other cytotoxic agents [9]. Furthermore, appropriate antigens (CD20 and CD22) have been identified and are targeted with monoclonal antibodies [9, 10]. The majority of studies have focused on the CD20 antigen, which is preserved and expressed ubiquitously throughout the human population [9]. This antigen is expressed on B-cells (cell-specific) and not on plasma cells (which produce immunoglobulin and are important for protection from infection) or pluripotent stem cells (which produce cell precursors). It has been shown that the CD20 antigen is important for cell cycle initiation and cell differentiation [10]. Importantly with respect to targeting, the antigen is anchored into the membrane and is not shed into the circulation [10].

As discussed, for radioimmunotherapy of non-Hodgkin's lymphoma, the β-particle emitting radionuclides, ^{131}I and ^{90}Y are used. The choice of ^{131}I (β: E_{max} 608 keV, γ: E_{max} 384 keV; $t_{1/2}$ 8.01 days) has been motivated in part because of the extensive clinical experience with this radionuclide in the treatment of thyroid disorders. Furthermore, the availability, the convenient half-life and the simple radiolabelling chemistry of ^{131}I are favourable properties of this radionuclide. Although, the high-energy photon emitted by ^{131}I is imageable and is used to derive patient specific doses based on the uptake of tracer-^{131}I-labelled antibody, it results in undesirable non-specific irradiation of the whole body [10]. In contrast, ^{90}Y (β: E_{max} 2.4 MeV; $t_{1/2}$ 64 hours) is a pure β-emitter. Unlike ^{131}I, which is incorporated into antibodies by direct iodination of tyrosine residues, ^{90}Y is conjugated to antibodies via a chelator (MX-DTPA) in a process requiring more elaborate chemistry [10-12]. Due to the absence of an imageable g-emission, autologous indium-111, ^{111}In-labelled antibody is used for imaging and dosimetry calculations prior to administration of therapeutic doses of the ^{90}Y-labelled antibody [10, 11].

Overall, the main advantage of using β-emitters is the relatively long-range of the β-particles in biological tissues, which is sufficient to irradiate tumour cells (by cross-fire) that do not have bound radiolabelled antibody [6-8]. Paradoxically, the effective range of the β-particles in tissue is sufficient to irradiate normal neighbouring cells, resulting in toxicity. Irradiation of normal bone marrow (the dose limiting organ in radioimmunotherapy) has been noted in clinical radioimmunotherapy using ^{90}Y-ibritumomab tiuxetan and ^{131}I-tositumomab [10, 13]. Nevertheless, the findings from the clinical studies have demonstrated that toxicity is reversible and that both radiolabelled antibodies are more effective than chemotherapy and immunotherapy with unlabelled anti-CD20 antibody (rituximab) with respect to producing clinically meaningful responses [9, 12].

Another medium-energy β-emitter, lutetium-177, ^{177}Lu (β: 800 keV; $t_{1/2}$ 6.7 days), which has similar physical properties to ^{131}I, also appears to have potential for use in radioimmunotherapy [14, 15]. In a recent study it was demonstrated that ^{177}Lu-antibodies had a higher specificity index (i.e. less non-specific cell killing) than analogous antibodies labelled with ^{90}Y in Raji B lymphoma cells. This is not unexpected given the much higher energy of the ^{90}Y β-particle (2.4 MeV), which results in higher levels of non-specific irradiation of the medium [15]. Similarly, ^{177}Lu-LL1 antibody resulted in less non-specific toxicity than ^{90}Y-LL1 in a human Raji B-cell lymphoma xenograft model in mice. Interestingly, in this initial comparative study it appeared that ^{177}Lu-antibodies were slightly less potent than ^{131}I-labelled antibodies on a per decay basis [15]. However, it was concluded that this minor difference would not be an overriding factor in the selection of the optimal radionuclide for clinical use [15]. Indeed, further research is required to establish the efficacy of ^{177}Lu-antibodies. In particular it would be important to compare the *in vivo* stability of antibodies labelled with ^{177}Lu and ^{131}I, which are known to be prone to extensive dehalogenation *in vivo*. Experimental radioimmunotherapy with ^{177}Lu-labelled antibodies has generally been insufficient due to the limited availability of the radionuclide. Until recently, ^{177}Lu was only available from a reactor with a radioactive abundance of approximately 25%. Higher purity ^{177}Lu (approximately 50%) is now available and anticipated improvements in the production and purification of ^{177}Lu will allow further investigation of the potential clinical utility of this radionuclide [15].

On the basis of dosimetry calculations it is generally accepted that β-emitters are the most appropriate radionuclides for the management solid tumours and large tumour burdens. The prevailing view is that β-emitters are optimal for treatment of tumour lesions > 0.5 cm (given the inaccuracies and complexity of theoretical dosimetry calculations, this is controversial, for example, in a theoretical study it was concluded that ^{90}Y and ^{131}I would be optimal for the treatment of metastases with diameters of 28-42 mm and 2.6-5 mm, respectively [16]). In contrast, on the basis of *in vitro* cytotoxicity findings, *in vivo* studies and upon theoretical dosimetry calculations, it has been suggested that shorter-range α-emitting radionuclides (effective ranges of only a few cell diameters) and Auger electron-emitting radionuclides (effective ranges of molecular dimensions) have the potential to provide a more favourable therapeutic index than β-emitters for radioimmunotherapy of single tumour cells in the circulation, micrometastases and in certain cases, minimal residual disease (small clusters of a few tumour cells [8, 17-22]). In addition, a potential role for the shorter-range radionuclides has been suggested for the treatment of cancers, such as neoplastic meningitis and ovarian cancer, which are characterized by thin sheets of tumour cells on body cavities (Table 1 and Fig. 1) [19].

Radiopharmaceuticals, α-emitters

It is well established that α-particles (monoenergetic helium-4 nuclei) are more efficient in inducing cytotoxic lesions in single cells than β-emitters [19, 23-26]. Due to their short-range (mean range of approximately 50-100 mm in tissue equivalent

Table 1. *Characteristics of selected radionuclides with potential for clinical use in radioimmunotherapy*

Radionuclide	Physical half-life	Max range in tissue	Clinical use or animal model studies/Key features*
β-emitters			
^{90}Yttrium (^{90}Y)	64.1 h	11.3 mm	^{90}Y-ibritumomab tiuxetan (Zevalin) FDA approved for C20 positive non-Hodgkin's lymphoma
^{131}Iodine (^{131}I)	8.0 d	2.3 mm	^{131}I-tositumomab (Bexxar) FDA approved for C20 positive non-Hodgkin's lymphoma
^{177}Lutetium (^{177}Lu)	6.7 d	1.8 mm	^{177}Lu-LL1 antibody investigated in mice bearing B-cell lymphoma xenografts; limited availability
α-emitters			
^{211}Astatine (^{211}At)	7.2 h	60 μm	^{211}At-Mov18 antibody investigated in mice bearing human ovarian cancer; limited availability
^{212}Bismuth (^{212}Bi)	60.6 m	90 μm	^{212}Bi-B72.3 used in a murine model of human colon carcinoma; short half life may limit to locoregional applications
^{213}Bismuth (^{213}Bi)	45.6 m	84 μm	^{213}Bi-HuM 195 in clinical trial for CD33 positive acute or chronic myeloid leukemia; short half life may limit to locoregional applications
Auger emitters			
^{125}Iodine (^{125}I)	60.2 d	< 100 nm	^{125}I-A33 antibody used in phase I/II clinical trials in patients with advanced colon cancer; long half life may limit clinical utility
^{123}Iodine (^{123}I)	13.2 h	< 100 nm	DNA-associated decay of ^{123}I shown to be effective at inducing DNA damage and cytotoxicity due to Auger component; relatively high energy γ-emission used for diagnostic imaging
^{111}Indium (^{111}In)	8.0 d	< 100 nm	^{111}In-anti HER2 antibodies shown to specifically induce cytotoxicity in human breast and ovarian cancer cell lines; mainly used for imaging and dosimetry prior to therapeutic administration of Zevalin

*The key properties and potential applications of the radionuclides in radioimmunotherapy are discussed in more detail in the text

matter) and their high energy (e.g. E_{max} 7.45 MeV for astatine-211, ^{211}At), α-particles have a high linear energy transfer (LET, mean approximately 100 keV/mm compared to a mean of approximately 0.2 keV/mm for β-particles [19]). High LET radiations offer a number of distinct advantages for radioimmunotherapy. A greater relative biological effectiveness is associated with high LET radiation (largely due to a higher probability of inducing double strand breaks), the cytotoxic effectiveness of high LET radiation is only marginally dependent on the dose rate (related to kinetics of repair of sublethal damage and repopulation) and the cell cycle (cells in S-phase are more resistant to the effects of low LET radiation [19]). Furthermore, the oxygen enhancement ratio for high LET radiation is approximately one, hence hypoxic as well as euoxic tumour cells may be treated with use of high LET radiations [19].

Both, *in vitro* cell survival studies and *in vivo* studies in mice have confirmed that α-emitting radionuclides are more potent at inducing lethal lesions in single cells and at treating human xenograft microtumours in mice, than β-emitting radionuclides [17, 19, 24, 26, 27]. It has been demonstrated that β-emitters such as ^{131}I and ^{90}Y are unable to exert efficient and specific cytotoxicity in single tumour cells and that the majority of the radiotoxicity results from cross-fire radiation from the radiolabelled antibodies in the medium rather than from cell-associated radionuclide [23, 25]. In one particular study it was found that using ^{131}I-labelled OC125 antibody which binds to the cell surface antigen, CA125, a cell surviving fraction less than 0.01 could not be obtained in two ovarian cell lines, OVC-433 ($5x10^6$ antigens per cell) and OVCAR-3 ($6x10^6$ antigens per cell [26]). In contrast, *in vitro* cell survival studies indicate that α-emitting radionuclides are extremely potent in inducing cytotoxicity [17]. Based on the cytotoxicity studies and on dosimetry calculation it has been calculated that approximately 300 and 600 α-particle decays on the cell-surface are sufficient to inactivate 99% and 99.99% of the cell population, respectively [24]. Furthermore, it has been estimated that 1-3 α-particle tracks through the cell nucleus are sufficient to induce a cytotoxic lesion in the target cell [28].

Among the α-emitting radionuclides, ^{211}At (α: 5.87 and 7.45 MeV; $t_{1/2}$ 7.2 hours), bismuth-213, ^{213}Bi (α: E_{max} 8.4 MeV; $t_{1/2}$ 45.6 minutes) and bismuth-212, ^{212}Bi (α: E_{mean} 7.8 MeV; $t_{1/2}$ 60.6 minutes) have received the most serious consideration for use in radioimmunotherapy [19, 28]. In a series of studies, a greater therapeutic efficacy of antibodies radiolabelled with ^{211}At rather than with β-emitting radionuclides (^{131}I or ^{90}Y) has been observed *in vivo*, using models of neoplastic meningitis in rats and models of ovarian cancer in mice [19, 29]. Although complete findings have yet to be published, ^{211}At-labelled chimeric antitenascin antibody has been used in a clinical radioimmunotherapy trial in patients with recurrent malignant glioma [30]. Similarly, significant anticancer effects and improvement in survival times with accept-

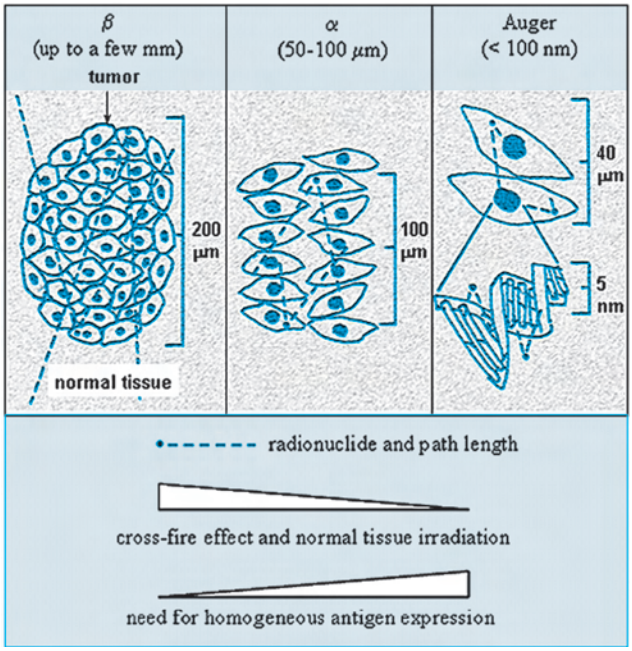

Figure 1. Schematic representation of the path length of β-, α- and Auger emitting radionuclides. The paradoxical nature of β-emitting radionuclides is highlighted. The relatively long path length of β-emitters is sufficient to irradiate cancer cells that do not have bound radiolabelled antibody. However, the cross-fire effect may also result in significant irradiation of normal neighbouring tissues. The shorter path length of α-particles results in cross-fire irradiation, but only in the range of a few cell diameters. In contrast, the ultra short-range Auger electron emitting radionuclides deposit the majority of the radiation dose within molecular dimensions (a few nm) from the site of decay. A consequence of the minimal cross-fire effect induced by α-particles and in particular Auger emitters, is the critical need for homogeneous antigen expression on cancer cells for successful radioimmunotherapy with the shorter range radionuclides.

able toxicity was observed using ^{212}Bi-B72.3 targeting a human colon carcinoma in a murine model [31]. The α-emitter ^{213}Bi has also been investigated in numerous preclinical *in vitro* and animal studies. In a notable endeavour aimed at preparing and guiding clinical trials, the murine antibody, M195 directed against the CD33 antigen expressed in myeloid leukaemia cells was developed. Importantly, a humanised version of the mouse anti-CD33 monoclonal antibody (designated HuM195) labelled with ^{213}Bi has been shown to have favourable pharmakokinetic and biodistribution properties and a good toxicity profile in mouse models [33]. Furthermore, ^{213}Bi-HuM195 was used in the first proof-of-concept study for radioimmunotherapy in patients with myeloid leukaemia [32].

Although *in vitro* (survival assays) and *in vivo* (radioimmunotherapy of human tumour xenografts in mice and the clinical trial in leukaemia patients) studies have demonstrated the therapeutic potential of these radionuclides, there are a number of limitations associated with their use in clinical radioimmunotherapy trials. The general disadvantage is related to the extreme cytotoxic potency of α-particles, which could create problems with respect to irradiation of normal healthy tissue [28]. To avoid unacceptable irradiation of normal tissue it necessary to use antibodies with very high affinity and specificity for the target cancer cells and the α-emitter-monoclonal antibody conjugate must be very stable *in vivo* to minimize release of the free radionuclide. There are also various radionuclide-specific problems associated with α-emitters. For example, a major limitation of ^{211}At is availability, since an accelerator for production of the radionuclide is required in close proximity to the place of application [28]. Although the bismuth radionuclides are produced by long-lived parent nuclides and they can be obtained from generators, their extremely short half-life is expected to limit their use to locoregional applications (for example, intralesional injections for radioimmunotherapy of melanoma or intraperitoneal injections for the treatment of micrometastases from ovarian cancer [19, 28]).

An alternative α-emitting radionuclide, Actinium-225 (^{225}Ac, α: 8.38 MeV, β: 1.42 MeV; $t_{1/2}$ 10 days) with a compatible half-life for radioimmunotherapy, has recently been shown to act as an atomic nanogenerator, emitting five α- and three β-particles as it decays [28]. It has been investigated in various preclinical radioimmunotherapy model systems. For example the humanised monoclonal antibody trastuzumab (Herceptin), which recognizes the Her-2 receptor has been radiolabelled with ^{225}Ac has been shown to inhibit the growth of breast cancer spheroids [34]. Furthermore, ^{225}Ac-trastuzumab was not toxic and was shown to extend survival time in mice transplanted with ovarian cancer (SKOV3) cells [35]. Interestingly, the toxicity profile of ^{225}Ac-HuM195 has been investigated in non-human primates (cynomolgus monkeys) to provide a starting point for calculating doses for human clinical trials [36]. Phase I trials of ^{225}Ac-HuM195 for radioimmunotherapy in patients with CD33 positive advanced myeloid leukaemia have been heralded [30], however clinical findings have not appeared in a publication to date.

Auger electron emitters

The Auger electron emitters represent another class of radionuclides that has potential for use in radioimmunotherapy [37-39]. Auger electron-emitting radionuclides decay by electron capture and/or internal conversion resulting in the emission of low energy Auger electrons. The Auger electrons traverse very small distances (majority within a few nm) in biological tissue. Hence, emission of Auger electrons results in a highly localized energy deposition in the immediate site of the decaying radionuclide.

The classical Auger electron-emitting radionuclide is ^{125}I. Decay of ^{125}I by electron capture (100%) and internal conversion (93%) results in the emission of numerous low energy Auger electrons (average 21) the majority (90%) of which have effective ranges of only molecular dimensions in tissue equivalent matter [40]. It has been demonstrated that decay of DNA incorporated or DNA bound ^{125}I results in an intense focus of radiochemical damage in the immediate site of decay. Sequencing gel studies indicated that the majority of DNA damage (single and double DNA strand breaks) occurs within 4-5 bases from the decaying atom [41-43]. In general, studies have demonstrated that decay of ^{125}I that is incorporated in-

to DNA induces a double strand break with a probability of 1 [41-43]. Furthermore, the DNA damage induced by DNA incorporated and DNA bound ^{125}I is only minimally modified by radical scavengers (such a dimethyl sulphoxide) indicating a high LET mode of damage [38-40]. In contrast, a low LET mode of DNA damage that is scavengeable by dimethyl sulphoxide for decay of free ^{125}I-iodide in solution, has been observed [44, 45].

Furthermore, cell culture studies have shown that nuclear localization of the radionuclide is a requirement for the induction of high LET type cytotoxicity in mammalian cells [45-47]. Briefly, results from the radiobiological clonogenic survival studies using ^{125}I-iododeoxyuridine have indicated that only 30-60 DNA incorporated ^{125}I-decays are required to induce a lethal lesion in a variety of cell-lines [45-47].

Unfortunately, it is not possible to deliver ^{125}I to the nucleus using directly radiolabelled antibodies which bind to cell surface antigens, despite the few claims of nuclear localization reported [25, 48]. Therefore, in previous *in vitro* and *in vivo* studies, it has been attempted to enhance the radiation dose to the nucleus and therefore, to exert high levels of specific cytotoxicity with the use of ^{125}I-labelled monoclonal antibodies that are internalized into the cell following specific cell-surface antigen binding and are accumulated in various intracellular compartments [21, 22, 49-51]. Furthermore, since the internalized ^{125}I-labelled monoclonal antibodies are catabolised in lysosomes ultimately yielding free ^{125}I-iodide which is rapidly and efficiently released from the cells [52, 53], lysosomal residualizing forms of ^{125}I, such as ^{125}I-dilactitol-tyramine [54] or ^{125}I-IMP-R2 [21, 55] have been conjugated to the monoclonal antibodies. Although these studies have achieved some enhancement of the nuclear dose and consequently of antigen-specific cytotoxicity, compared to the nuclear radiation dose and cytotoxicity achieved in studies involving targeting ^{125}I-labelled antibodies to cell-surface antigens that are not internalized [25, 56], this strategy did not realize the high levels of cytotoxicity which results from the decay of DNA-associated ^{125}I [47, 57]. Nevertheless, ^{125}I-labelled monoclonal antibody A33 which recognizes an organ-specific internalizing antigen (A33 in the colon and small bowel), has been used in phase I/II clinical trials in patients with advanced colon cancer [51]. This trial indicated favourable biodistribution of the radiolabelled antibody. However, only modest therapeutic responses were observed. Importantly, bone marrow toxicity was not observed after administration of activities as high as 1.295 GBq/m^2 (350 mCi/m^2) [51].

Although high LET type radiobiological effects have not been observed using internalizing ^{125}I-labelled antibodies, it has been suggested on the basis of experimental findings and theoretical calculations that ^{125}I-labelled antibodies, that are internalized and accumulated in intracytoplasmic vesicles, are more efficient than ^{131}I-labelled antibodies in inducing lethal lesions in cells *in vitro* [21, 23, 25, 50]. In addition, it has been shown that internalising ^{125}I-labelled monoclonal antibodies provide greater therapeutic effects than autologous antibodies labelled with β-emitting radionuclides (^{131}I and ^{90}Y), in human cancer xenograft models in mice [22, 58]. These studies indicate that ^{125}I has the potential to provide a favourable therapeutic index for radioimmunotherapy. However, the minimal requirements for radioimmunotherapy with ^{125}I are that the antigen being targeted is expressed homogeneously on the cancer cells and that following binding of the ^{125}I-labelled antibody/antigen complex, is internalized into the cancer cells.

It is anticipated that the long half-life of ^{125}I (60.2 days) may impose limitations (from a radioprotection standpoint and with respect to therapeutic efficacy due to a slow dose rate) for clinical use of this radionuclide in radioimmunotherapy. Hence, with a view to in vivo and eventually clinical studies Auger electron-emitting radionuclides with shorter half-lives may be more appropriate. The metal Auger emitting radionuclides gallium-67, ^{67}Ga and ^{111}In have a half-life (about 3 days for both) that is congruent with the pharmakokinetic and biodistribution (tumour localization) profile of monoclonal antibodies in humans [14]. However, these radionuclides require more elaborate conjugation chemistry than iodine atoms – incorporation of a metal chelating moiety in the antibody is necessary. To minimize the modifications required in the radiolabelling protocols, the Auger electron-emitting radionuclide that has been considered is ^{123}I ($t_{1/2}$ 13.2 hours). The decay of another iodine atom, ^{124}I ($t_{1/2}$ 4.2 days), includes an Auger component, however, due to limited availability the potential of this radionuclide in radioimmunotherapy has not yet been investigated.

Iodine-123 decays by electron capture (100%) resulting in a metastable tellurium-123, ^{123}Te atom, which in turn decays to the ground state by g-emission (84%) or by internal conversion (16%). Given the short half-life and the emission of a 159 keV photon which is suitable for imaging, ^{123}I is routinely in diagnostic nuclear medicine. For therapeutic use the Auger electron cascades resulting from the electron capture and internal conversion processes are of interest. It has been calculated that on average approximately 6-12 [59-61] Auger electrons are emitted in the condensed phase per decay of ^{123}I (approximately 2-3 -fold less than the average number of electrons emitted by ^{125}I [40]). The results of theoretical studies have indicated that the probability of induction of a double strand break by decay of ^{123}I in DNA is approximately 0.4 compared to a probability of 1 for induction of a double strand break by ^{125}I-decay in the same model [59]. Experimentally, it has been demonstrated that DNA-associated ^{123}I produces a double stand break with a probability of 0.62 compared to 0.82 for ^{125}I by investigating the plasmid breakage efficiency of radioiodinated analogues of the DNA minor groove binding ligand, Hoechst 33258 [62]. Furthermore, the radiotoxicity of ^{123}I has been compared to that of ^{125}I using experimental *in vitro* cell survival assays [63]. In these studies ^{123}I and ^{125}I-iododeoxyuridine was used to incorporate the radionuclide into the DNA of Chinese hamster V79 lung fibroblasts. The results indicated that approximately 2.2 times more ^{123}I decays than ^{125}I decays were required for D_{37} [63]. Overall, these findings indicate that although somewhat less potent than ^{125}I

on a per decay basis, ^{123}I is sufficiently efficient at inducing DNA damage and cytotoxicity due to the Auger emissions. Therefore, ^{123}I may represent a more suitable choice than ^{125}I for potential use in radioimmunotherapy given its much shorter half-life.

Conclusion and future perspectives

Radioimmunotherapy of B-cell non-Hodgkin's lymphoma is an important additional therapeutic modality to the radiation oncology clinic. Despite the enthousiasm generated by the FDA approval of Zevalin and Bexxar and the research efforts over the past decade, the clinical success has not yet translated to other cancers. Nevertheless, there have been significant advances and a number of radiolabelled antibodies have undergone preclinical evaluation using *in vitro* and animal model systems, with encouraging results. Although the findings are not comprehensive, the initial clinical trials using antibodies labelled with α- and Auger emitting radionuclides represents a significant achievement.

Issues related to the affinity and specificity of the monoclonal antibody, to antigen expression and to the tumour type and size are all important determinants of the therapeutic efficacy of radioimmunotherapy. Here the factors involved in the selection of the optimal radionuclide for different treatment scenarios were considered. The current dogma suggests that the relatively long-range β-emitting radionuclides are more well suited to the treatment of solid tumours and larger tumour burdens due to the cross-fire effect. In contrast the shorter range α-emitters and the ultra-short range Auger emitters, which exhibit greater specific cytotoxic potency than β-emitters, are better suited for locoregional applications and for treatment of individual cancer cells in the circulation, micrometastases and small clusters of cancer cells left after surgery. However, there are currently no definitive guidelines regarding radionuclide selection and much further research is required to delineate the criteria. Generally, research has been hampered the limited availability of certain radionuclides. Together with the expected advances in antibody engineering and in the identification of better cancer cell targets, the improvements in production and consequent growing availability of radionuclides including ^{177}Lu (β), ^{225}Ac (α) and ^{124}I (Auger), is generating excitement relating to the clinical potential of radiolabelled antibodies.

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