Antibody-based cancer treatment with ultra-short range Auger electron-emitting radionuclides: Dual receptor and DNA targeting strategies

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Hell J Nucl Med 2007; 10(3): 155-159 • Published online: 19 November 2007

Abstract

The long-heralded potential of targeted cancer treatment using monoclonal antibodies is finally being realized. Several antibodies are already used in the oncology clinic and many others are undergoing preclinical evaluation. In addition to the development of unconjugated antibodies, there is intense interest in the potential clinical use of antibodies as vehicles for targeting cytotoxic agents specifically to cancer cells. For example, radioimmunotherapy which involves the use of antibodies to deliver radionuclides to target cells is an approved treatment modality for cancer. Our laboratory is involved in developing technologies for radioimmunotherapy using a unique class of radionuclides, known as Auger electron emitters. A key feature of the Auger electrons emitted by these radionuclides is that they traverse very small ranges (molecular dimensions) in biological tissues. The emission of Auger electrons results in a gradient of energy deposition with the majority of the radiochemical damage occurring in the immediate vicinity (within a few cubic nanometers) of the decaying radionuclide. Therefore, realizing the full potential of Auger electron emitting isotopes in radioimmunotherapy requires more sophisticated approaches than directly radiolabeling anticancer antibodies. Strategies which involve targeting the radionuclide not only to cancer cells but also to the DNA of those cells are necessary. In this paper potential dual, receptor and DNA, targeting systems for radioimmunotherapy with Auger electron-emitting radionuclides are discussed.

Keywords: Monoclonal antibodies – Radioimmunotherapy – Non-Hodgkin's lymphoma – Auger electrons – Receptor-mediated endocytosis

Introduction

Paul Ehrlich's vision of a "magic bullet" for cancer therapy dates back about a century [1]. This well-known concept followed the realization that a substance in the serum, which could be transferred from one animal to another via a process known as passive serotherapy, was responsible for conferring resistance to infectious disease [2]. It required a further half-century to identify antibodies as the substance in the serum that is responsible for these effects. These initial discoveries lead to numerous immunotherapy studies using serum-derived polyclonal antibodies with diverse clinical outcomes. The development of the hybridoma technique in 1975, which allowed production of monoclonal antibodies with high specificity for a single antigen, was the breakthrough that renewed interest in antibody-based cancer therapies [3]. It resulted in a rapid expansion in the experimental and clinical evaluation of monoclonal antibodies targeting tumour-associated antigens.

However, it required more than two decades for antibody-based pharmaceuticals to emerge as the next generation of anticancer therapies. The first antibody to be approved by the United States Food and Drug Administration (FDA) for cancer therapy was Rituximab (Rituxan) [4]. The anti-CD20 IgG, was introduced into the clinic in 1996 for the treatment of non-Hodgkin's lymphoma. Currently there are five FDA approved antibodies in the oncology clinic and many others are in pharmaceutical industry pipelines and are advancing in clinical trials (Table 1).

It is worthy to mention that although these new biological therapies represent a significant addition to the anticancer arsenal, they have very significant financial implications. The cost of antibody therapies are staggering and are either out of reach for average paying patient or create a challenge to healthcare systems. A prime example is the anti-HER2 anti-

Table 1. *FDA approved antibodies for the treatment of cancer and selected antibodies in advanced clinical trials.*

FDA Approved			
Generic name (Trade name)	Target	Cancer	Approval
Rituximab (Rituxan)	CD20	B-cell lymphoma	1997
Trastuzumab (Heceptin)	HER2	Breast	1998
Alemtuzumab (Campath-1)	CD52	Chronic lymphocytic leukaemia	2001
Cetuximab (Erbitux)	EGFR	Colorectal	2004
		Head/neck	2006
Bevacizumab (Avastin)	VEGF	Colorectal	2004
Advanced clinical trials			
Epratuzumab	CD22	Non-Hodgkin's lymphoma	
Lumiliximab	CD23	Chronic lymphocytic leukaemia	
Orgegovomab	CA125	Ovarian	
Pertuzumab	HER2	Breast, prostate, ovarian	
Rencarex	G250	Kidney	
Vitaxin	avb3	Melanoma, prostate	

body trastuzumab (Herceptin), which is indicated for the use in HER2 positive breast cancer patients [5]. The yearly cost per patient for Herceptin therapy is in the order of $50,000 US (about $70,000 in Australia and £20,000 in the UK and Europe). Following an intense media campaign by the mainstream media worldwide and public advocacy, certain countries have approved subsidization of Herceptin for the treatment of early and advanced breast cancer under their respective pharmaceutical benefit schemes. A similar economic concern is arising with cetuximab (Erbitux), an anti-epidermal growth factor receptor antibody, which is approved for the treatment of metastatic colorectal cancer as well as head and neck cancers [6]. Although economic considerations are beyond the scope this article, the prevailing aspiration of health professionals is that those cancer patients who can benefit from emerging antibody-based therapies, have the ability to access the pharmaceuticals.

Nevertheless, apart from unconjugated 'naked' antibodies, there has been intense interest in the clinical use of antibodies as carriers of cytotoxic agents. Numerous antibody-drug and antibody-toxin (immunotoxins) conjugates have been investigated in clinical trials, and an example of a drug immunoconjugate has been approved by the FDA for clinical use thus far. Gemtuzumab ozogamicin (Mylotarg), a conjugate of a humanized anti-CD33 antibody, linked to the potent antitumour drug colicheamicin for the treatment of relapsed acute myelocytic leukaemia [7]. Although not involving an antibody, a peptide-based receptor-targetted immunotoxin has been approved for clinical use. The immunotoxin denileukin diftitox (Ontak), which is a modified diphtheria toxin coupled to interleukin-2 is registered for the treatment of cutaneous T-cell lymphoma [8]. Furthermore, there has been a long-standing interest in radioimmunotherapy, i.e. in the use of monoclonal antibodies to deliver a radionuclide specifically to cancer cells. Indeed radiolabelled antibodies were the first group of immunoconjugates to be investigated, with initial positive clinical responses being reported in 1951 [9]. In this early clinical trial, complete responses were observed in advanced melanoma patients treated with ^{131}I-labelled rabbit polyclonal antibodies.

Radioimmunotherapeutic agents. Auger emitting radionuclides

Despite the early report of clinical success, it was not until more than a half-century later that radioimmunotherapy was finally inducted as new therapeutic modality for cancer. Currently the anti-CD20 antibody conjugates, yttrium-90, ^{90}Y-ibritumomab tiuxetan (Zevalin) and ^{131}I-tositumomab (Bexxar) are both approved by the FDA for the treatment of chemotherapy-refractive, follicular non-Hodgkin's lymphoma [10]. Although the longer range (can penetrate up to a few mm) β-emitting radionuclides, such as ^{90}Y and ^{131}I, are used exclusively in the clinic and in the majority of clinical trials there is also considerable interest in the potential use of α-emitters [11]. Alpha particles (helium-4 nuclei) traverse only a few cell diameters (50-100 μm), however, they are far more efficient at inducing cytotoxic lesions than β-emitters [11]. The ultra short-range Auger electron emitters are another class of radionuclides which can potentially be used in radioimmunotherapy [12-14].

Auger electron-emitting radionuclides decay by electron capture or internal conversion. They emit low energy electrons by a series of complex vacancy cascades that involve transition of electrons between orbital shells, which was first described by the French physicist, Pierre Auger in the early 1920s [15]. For example, the classical Auger emitter, ^{125}I, emits an average of 15-21 low energy Auger electrons per decay [16-18]. With respect to targetted cancer radiotherapy the key feature is that the majority of these electrons (> 90%) traverse only molecular dimensions (1-20 nm) in biological tissues [16]. Therefore, the simultaneous emission of Auger electrons results in a gradient of energy deposition with the majority of the radiochemical damage occurring in the immediate vicinity (within a few cubic nm) of the decaying isotope. Numerous molecular and cell culture based studies using DNA precursors (such as ^{125}I-deoxycytidine or ^{125}I-iododeoxyuridine [19-21]) to incorporate ^{125}I into DNA or DNA binding ligands (e.g. the minor groove binding ligand ^{125}I-iodo-Hoechst [22, 23] or the intercalator ^{125}I-iodorivanol [24]) to localize the radionuclide in close proximity to DNA, have demonstrated the intense and highly localized DNA damage and cytotoxicity induced by DNA-associated ^{125}I. In contrast, mammalian clonogenic survival assays have shown that ^{125}I is much less efficient (by a factor of approximately 8-10 compared to DNA incorporated radionuclide) at inducing cell-death when it is localized on the cell membrane or is confined in the cytoplasm [25, 26].

Therefore, realization of the full potential of Auger electron emitters in radioimmunotherapy requires targeting of the radionuclides not only to cancer cells but also to the DNA of those cells. Consequently, more sophisticated targeting approaches than simply radiolabelling internalising anticancer antibodies are needed (Fig. 1). The dual, receptor and DNA, strategy developed in our laboratory involves conjugating iodinated analogues of the DNA minor groove binding ligand, Hoechst 33258, to tumour-specific proteins or antibodies (as detailed in the following patent: Targetted therapies: PCT/AU2005/000266, Australia, 2005; Cell targeting conjugates: 05706302.6-2101-AU2005000266, European Patent Office, 2006; Cell targeting conjugates: 10/590784, US Patent Office, 2006). The conjugates are prepared in such a way that following receptor-mediated internalisation, the ^{125}I-iodo-Hoechst and protein moieties are cleaved thereby releasing the free radiolabelled drug. The lipophilic drug molecule then localizes the radionuclide in close proximity (within 5 angstroms) to the DNA (Fig. 1). To date, we have completed proof-of-concept experiments using the transferrin-mediated endocytosis cycle as a model system. In similar studies we have invoked protein and antibody conjugates of an extremely phototoxic Hoechst analogue, to demonstrate transferrin and epidermal growth factor receptor-specific UV_A-mediated

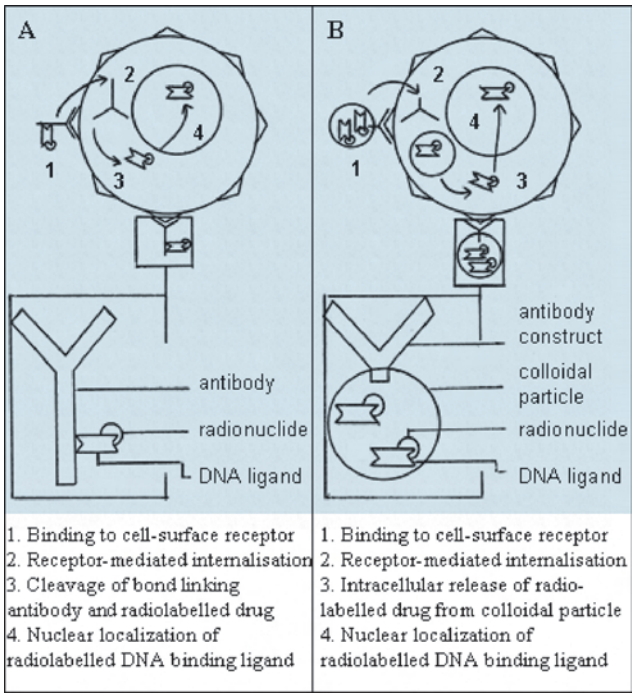

Figure 1. Receptor-mediated targeting of Auger electron-emitting radionuclides to the DNA of cancer cells. (A) A radiolabelled DNA binding drug is directly conjugated to an anticancer antibody. (B) Radiolabelled DNA binding drug molecules are incorporated into colloidal particles, which are coated with an antibody construct with an intact receptor-binding domain. The colloidal particle may be a liposome, nanocapsule or polymeric micelle. The antibody construct may be the whole immunoglobulin or a fragment (such as the $F(ab')_2$, Fab, scFv, diabody or minibody) produced by enzyme digestion or antibody engineering.

cell-death in K562 and A431 cells, respectively [27, 28].

An alternative dual, receptor and DNA, targeting system for radioimmunotherapy with Auger emitters could for example, involve packaging DNA ligands labelled with an Auger radionuclide into colloidal particles such as liposomes, nanocapsules or polymeric micelles, which in turn are coated with a tumour-specific antibody or antibody fragment (Fig. 1). Although considerably more development is required for the latter approach, it may have the advantage of delivering more radiolabelled drug per cancer cell receptor. We have determined that between 2-3 ^{125}I-iodo-Hoechst molecules can be directly conjugated per antibody without significantly affecting the biological properties of the protein. In contrast, it is anticipated that in the order of a few thousand radiolabelled drug molecules may be incorporated into colloidal particles.

One of the major advantages of radioimmunotherapy compared to other antibody-based therapies is the ability to kill cancer cells that are not directly labelled with the radionuclide by the cross-fire effect. Cross-fire irradiation alleviates problems associated with heterogeneous antigen expression on cancer cells and inadequate penetration of antibodies in tumours [29]. This phenomenon is mainly applicable to β-emitting radionuclides, which have an effective biological range of up to a few millimetres, but also applies to α-particles which can penetrate a few cell diameters [30]. Given the physical and radiobiological properties of Auger emitters, traditionally it has been reasonably assumed that homogeneous expression of internalising cell-surface antigens is a minimal requirement for successful radioimmunotherapy with these ultra-short range radionuclides. However, this dogma has been called into question by a seminal study in which it was demonstrated that ^{125}I induces bystander effects *in vivo* [31]. The findings from this study indicated that factors originating from cells labelled with DNA incorporated ^{125}I, resulted in inhibition of the growth of non-irradiated cells transplanted into mice [31]. Importantly, the ability of ^{125}I to kill unlabelled cells by inducing bystander effects may prove to be analogous to the cross-fire effect induced by β- and α-emitters. However, it must be cautioned that the bystander phenomenon requires further experimental clarification.

Selection of the appropriate Auger radionuclide

Selection of the appropriate Auger radionuclide for radioimmunotherapy requires some consideration. The metal radionuclides, such as ^{67}Ga and ^{111}In, have an appropriate half-life (about 3 days for both) that is compatible with radioimmunotherapy [32, 33]. However, these isotopes require an elaborate conjugate chemistry, which involves incorporating a metal chelating moiety into the molecule that is to be radiolabelled [32, 33]. On other hand, although the direct iodination of tyrosine residues in proteins is a simple and well-characterized reaction, there are issues with the stability of ^{125}I-labelled antibodies *in vivo*. Deiodination of iodine-labelled antibodies in vivo is most likely due to the fact that the ^{125}I-iodophenol group in directly iodinated tyrosine residues is analogous to that in endogenous thyroid hormones, such as 3, 5, 3' – triiodothyronine (T_3), for which deiodinases (or dehalogenases) are known to exist [34, 35]. It is generally accepted that the ultimate catabolite following intracellular processing of the iodophenol group is free radioiodine which is rapidly excluded from cells and is efficiently absorbed by the thyroid gland or is excreted in the urine. Incidentally, the ^{125}I-iodophenol group resulting from the direct iodination of Hoechst 33258 is analogous to that in ^{125}I-iodotyrosine and T_3 (Fig. 2). Therefore, to avoid issues related to in vivo dehalogenation, we synthesize stannylated Hoechst analogues without the hydroxyl group, which are iodinated in a specific position by radioiodo-destannylation. Radioiodo-destannylation of tin precursors is well known to produce labelled compounds with high radiochemical purity and yield [36]. Overall, by using an appropriate intermediate DNA binding ligand which is directed to the target cells by an antibody, both the therapeutic potency of the Auger radionuclide and *in vivo* stability may be improved simultaneously.

Unfortunately, the long half-life of ^{125}I (60 days) renders the prototype Auger radionuclide inappropriate for radioimmunotherapy. It is incompatible with antibody pharmacokinetics and tumour localization and the long half-life imposes severe limitations from a radiation safety standpoint. There-

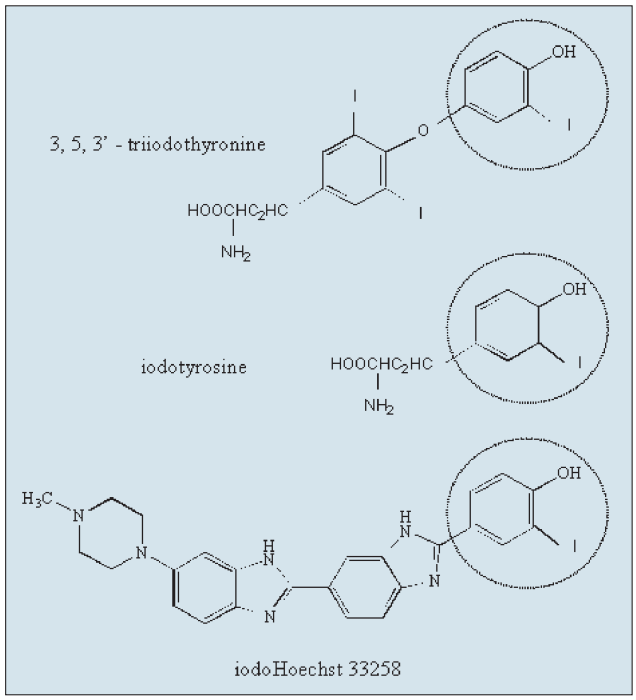

Figure 2. Molecular structures of 3, 5, 3' – triodothyronine, iodotyrosine and iodo-Hoechst 33258. Direct iodination of tyrosine residues in proteins and antibodies produces an iodophenol moiety (circled), which is analogous to that in endogenous thyroid hormones such as 3, 5, 3' – triodothyronine. Similarly, direct iodination of the phenyl ring of the DNA minor groove binding ligand, Hoechst 33258, yields the iodophenol group. To alleviate problems with *in vivo* dehalogenation, we prepare ^{125}I-iodo-Hoechst analogues without the hydroxyl group (OH) on the terminal phenyl ring, by radioiodo-destannylation of the relevant tin precursor.

fore, there is interest in the shorter-lived iodine radionuclides ^{123}I (13.2 hours) and ^{124}I (4 days). Iodine-123 is a weaker Auger emitter with an average emission of 8-11 electrons per decay compared to 15-21 for ^{125}I [16-18, 37]. However, molecular and cell culture studies suggest only a modest reduction in the DNA breakage and cytotoxic potency of DNA-associated ^{123}I compared to ^{125}I [38-40]. Given its short half-life, ^{123}I may potentially be more suited to treating cancers of the blood and for clearing metastatic cells from the circulation. Furthermore, ^{123}I could be appropriate for cancers that are amenable to loco-regional applications such as melanoma and malignant glioma.

Ironically ^{124}I which until recently was only considered as a nuisance in the preparation of ^{123}I, is emerging as a useful radionuclide for both therapy and diagnostic imaging. This is due to its convenient half-life and decay profile, which includes the emission of positrons (23%) as well as Auger electrons [41]. The potential of ^{124}I-labelled peptides and antibodies for diagnosis using positron emission tomography (PET) has already been widely investigated [42, 43]. However, the clinical utility of the radionuclide in radioimmunotherapy due to its Auger emissions has not yet been studied. Although the DNA breakage efficiency of DNA-associated ^{124}I is currently undergoing investigation with promising preliminary results, the findings have not been reported to date. The lack of studies with ^{124}I is predominantly due to the extremely limited availability of the radionuclide. It is anticipated that as ^{124}I becomes more widely available, investigation of its therapeutic efficacy due to the Auger emissions will become a priority. It should be noted however, that a component of its complex decay scheme also results in the emission of high-energy γ-rays. Therefore, whole-body irradiation is a concern with this radionuclide.

In conclusion, the long-heralded potential of targetted cancer therapy with specific anticancer antibodies is finally being realized. With further progress in molecular biology techniques, particularly microarray technology, it is expected that superior receptor targets will be identified on cancer cells. Together with improvements in antibody engineering it is anticipated that antibody-based pharmaceuticals will continue to grow as the next generation of anticancer therapeutics. The intense focus of radiochemical damage and cytotoxicity induced by Auger electron emitters, provides a basis for their potential use in radioimmunotherapy. However, Auger emitting radionuclides require targeting specifically to the DNA of cancer cells to provide a distinct dosimetric advantage compared to localizing the isotope on the cell membrane or in cytoplasmic compartments. In this context the dual, receptor and DNA, targeting strategies presented can be considered as platform technologies, which are well positioned to utilize the imminent immunological advances.

Acknowledgements: The support of the Australian Institute of Nuclear Science and Engineering is acknowledged. The author was the recipient of AINSE awards. The Molecular Radiation Biology Laboratory is supported by the National Health and Medical Research Council of Australia (350359).

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