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Review

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Application of Radionuclides and Antibody -Drug Conjugates to Target Cancer

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ABSTRACT

Radionuclide therapy and antibody-drug conjugates are used to locate and kill cancer cells by the utilisation of monoclonal antibodies. These bio-vectors are able to transport a cytotoxic drug payload and/or radiation in the form of alpha or beta particles to bind onto antigen specific cancer cells initiating apoptosis. This inaugural article aims to deliver a brief account of these targeted therapies in the treatment of oncological disease states such as leukaemia, non-Hodgkin's lymphoma, neuroendocrine tumours, breast cancer and prostate cancer bone metastases.

KEYWORDS: Targeted alpha therapy; Radionuclide therapy; Antibody-drug conjugates; Monoclonal antibody, Alpharadin®; Xofigo®; Bexxar®; Zevalin®; Adcetris®; Kadcyla®.

INTRODUCTION

A century ago, the bacteriologist Paul Ehrlich (1908 Noble Prize) a pioneer of chemotherapy and haematology, first postulated the concept of targeted therapy towards the treatment of disease causing agents.[1] This concept was to create an ideal therapeutic agent termed the 'magic bullet' which went directly to specific cellular targets in order to attack the disease. Currently, Ehrlich's vision is now being realized in the treatment of cancer with the development of targeted therapies, mainly based on monoclonal antibodies.[2]

A major breakthrough was made in 1975, by the Nobel Prize winners Milstein and Köhler, in the development of hybridoma technology. This technology platform revolutionised the production of antibodies by having a single specificity towards the cognate antigen, in the development of targeted therapies.[3] Moreover, this approach is being exploited by several biopharmaceutical companies to develop strategies for delivering radionuclides to image and destroy a variety of cancers including adequate cytotoxic drug payloads.[4]

These cytotoxic drug payloads are utilised in the development of Antibody-Drug Conjugates (ADCs) and include the anti-neoplastic agents: Mono Methyl Auristatin E (MMAE) and mertansine (DM1) to target the microtubules in cancerous cells. These other payloads include the DNA damaging agents calicheamicins and duocarmycins extending to the topoisomerase II inhibitors doxorubicins and camptothecins.[5] All of these cytotoxic drugs have demonstrated *in vitro* potency against several tumour cell lines, down to the picomolar level. This compares to first generation ADCs using nanomolar amounts of doxorubicin.[6]

Currently, other emerging drug payloads such as the sequence selective DNA alkylating agents called Pyrrolo Benzo Diazepines (PBDs) will form the basis of the next generation of ADCs.[7] These PBDs have shown to be ten thousand times more potent than systemic chemotherapeutics and nearly a thousand times more potent than other cytotoxins used in ADCs.

The ideal treatment plan for a patient is first to locate the cancerous site(s) by using a radionuclide antibody capable of imaging the tumour volume. The following imaging modalities can be applied: planar imaging; Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET). These techniques can be extended to state-of-

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the-art clinical hybrid imaging systems which combine SPECT (or PET) with Computed Tomography (CT) and more recently PET scanners with functional magnetic resonance imaging (f-MRI) instruments.[8,9]

However, if the cancer site is shown to retain an appropriate level of the antibody - through the application of these imaging techniques - based on gamma or positron emitters: it would be reasonable for the patient to receive a therapeutic dose of the same antibody - labelled with a radionuclide emitting alpha or beta radiation - which ever proves more capable of killing the cancer cells.

The limitation of utilising murine antibodies has been circumvented by the use of chimeric, humanized, or fully human monoclonal antibodies.[10] The challenge of targeting solid tumours with alpha and beta radiation is the dilemma of inducing a sufficient response on the cancerous mass without producing lethal toxic side effects. Therefore, it is paramount to analyse and control the radiation dose being delivered to tumour site(s) and compare the effects of this dose on the surrounding healthy cells.

Consequently, it is important to calculate the risk to other normal and/or non-neoplastic sites, capable of concentrating radioactivity, especially in the excretory organs (e.g. kidneys).[11] To help to define the risk, it is important to obtain pharmacokinetic data about the therapeutic radionuclide. This will enable a calculation of the percentage of injected dose per gram tissue therefore limiting normal tissue damage.[12-14]

The majority of precedents set by Radio-Immuno Therapy (RIT) were made by antibodies labelled with beta emitters (e.g. iodine-131). Today, after extensive research and clinical trials, RIT therapy against various cancers has now been accepted.[15] A continuation of research in the application of alpha emitters to treat cancer has been proposed for radiolabelling of many molecules, transported by various bio-vectors such as monoclonal antibodies.[16]

The main emphasis is the utilisation of radiolabelled antibodies as agents for Radio-Immuno Therapy (RIT). Following labelling with alpha emitters, Radionuclide Antibody-Conjugates (RACs) became the prototype for Targeted Alpha Therapy (TAT) using other targets and bullets, as in the case of peptides[17] and somatostatin receptors.[18]

Ongoing clinical trials have shown that somatostatin receptor peptides labelled with the beta emitters yttrium-90 and lutetium-77 have been effective in the treatment of neuroendocrine tumours.[19]

RADIONUCLIDES TARGETING CANCER

Currently, there are around 100 radionuclides that emit alpha radiation; the majority of them produced in nuclear reactors. Only a few are considered useful as therapeutics agents. These include bismuth-213 (generator produced),[20] astatine-211 (cyclotron produced),[21] actinium-225 (generator produced)[22] and thorium-227 (generator produced).[23] These radiolabelled therapeutic agents transported by bio-vectors such as monoclonal antibodies can be utilized in the treatment of a variety of cancers such as lymphomas, leukaemia and melanomas.[24] This is demonstrated further on by the ability of Xofigo® to form complexes within the area of bone metastases.[25] This is due to theactive moiety radium-223, in the form of radium-223 dichloride, to mimic calcium and the ability to complex with the bone mineral hydroxyapatite.[26]

A clinical precedent, on the practice of using alpha therapy towards bone metastases, with radium-223 dichloride (half-life=11.4 days) marketed as Alpharadin® became a first-in-class therapeutic.[27] In May 2013, Alpharadin® now called Xofigo®, was given FDA approval to treat patients with castration-resistant prostate cancer, symptomatic bone metastases with no known visceral metastatic disease.[28]

Xofigo® is the first and only alpha particle-emitting radioactive therapeutic agent approved by the FDA that has demonstrated improvement in overall survival rates.[29] To date, the most promising advance in cancer therapy is connected to the evolvement of using Radiolabelled Antibody-Conjugates (RACs) to deliver alpha particles.[30]

The basic principle of the TAT technique relies on the emission of alpha particles in which the radionuclide (e.g. actinium-225, bismuth-213, astatine-211) is held in a crown shaped chelate (e.g. derivatives of DTPA, DOTA), connected preferably to a low molecular weight drug (Figure 1). Alternatively, it may be more frequently linked to a monoclonal antibody, antibody fragments or peptide *via* a linker-chelate.[31,32] Therefore, it is paramount to get the right combination of radionuclide, linker-chelate and/or peptide, drug substrate or antibody for a particular cancer.[33-35] This is to ensure that an adequate amount of radiation is delivered to the cancer site by targeting the specific antigen (e.g. CD20) to annihilate it.

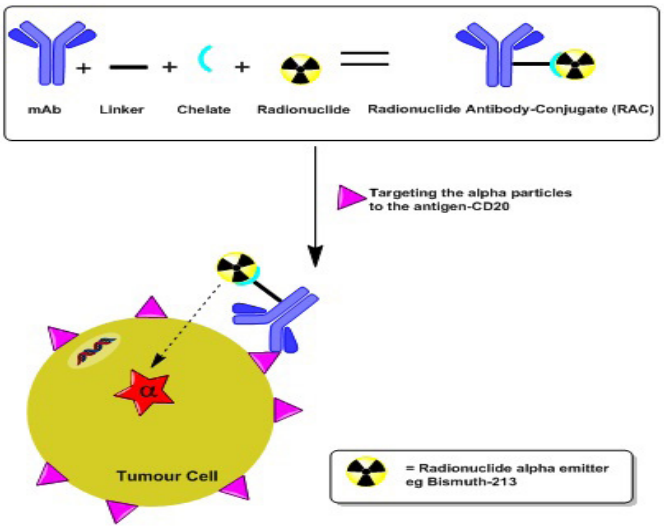

Figure 1: TAT targetingCD20 antigens on tumour cells

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Several TAT research groups have shown that the ideal radionuclide for this approach to be effective must have the following basic parameters.[36-38]:

- The radionuclide must emit an energy lower than 40 keV;
- Alpha particles have a short pathlength (50-80 microm) and high linear energy transfer of approximately 100 keV/mi crom;
- The radionuclide should have an ideal half-life of 30 min utes to 10 days to allow for logistics and treatment plan for the patient;
- For the generation of 'medical' radionuclides, the daugter radionuclide must be stable with a half-life greater than 60 days;
- The radiopharmaceutical in the form of kits and/or synthsis must be able to incorporate the radioactive label into carrier substrates as rapidly as possible for patient use.

Numerous clinical trials have utilised a wide range of bio-vectors to target cancer and include: HuM195 for acute myelogenous leukaemia;[39] Astatinated MX35-F(ab')2 monoclonal antibodies for ovarian cancer;[40] Radium-223 dichloride for bone metastases;[41] Murine 9.2.27 to target the Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP) antigen on melanoma;[42] CD20 antigen for lymphoma[43] and human IgG2/mouse chimeric anti-tenascin 81C6 forglioblastoma multiforme.[44]

Currently several preclinical trails include the bio-vectors: Monoclonal antibody C595 labelled with bismuth-213 to target MUC1 gene expressed by the prostate;[45] PA12 human recombinant protein to target urokinase-type Plasminogen Activator (uPA) system which is expressed in several types of cancer (e.g. breast cancer);[46] Monoclonal antibody J591 to target the Prostate Specific Membrane Antigen (PSMA)[47] and Bevacizumab (Avastin®) in the treatment of recurrent glioblastoma.[48]

Currently, other approaches to target cancer include the following FDA approved radiopharmaceuticals:

This targeted approach continues with Bexxar®, which contains the antibody tositumomab, radiolabelled with iodine-131 to target the CD20 antigen.[49] The patient first receives tositumomab, followed by the infusion of tositumomab radiolabelled with iodine-131. This is the same antibody covalently bound to the radionuclide iodine-131. The iodine-131 emits both beta and gamma radiation and decays with a half-life of 8 days (Figure 2). A successful clinical study involving 40 patients led to the approval in 2003 of Bexxar® for the treatment of rituximab-refractory, low-grade, follicular non-Hodgkin's lymphoma.[50]

The unique feature of Zevalin® is that it can be used to target the CD20 antigen on B-cell non-Hodgkin's lympho

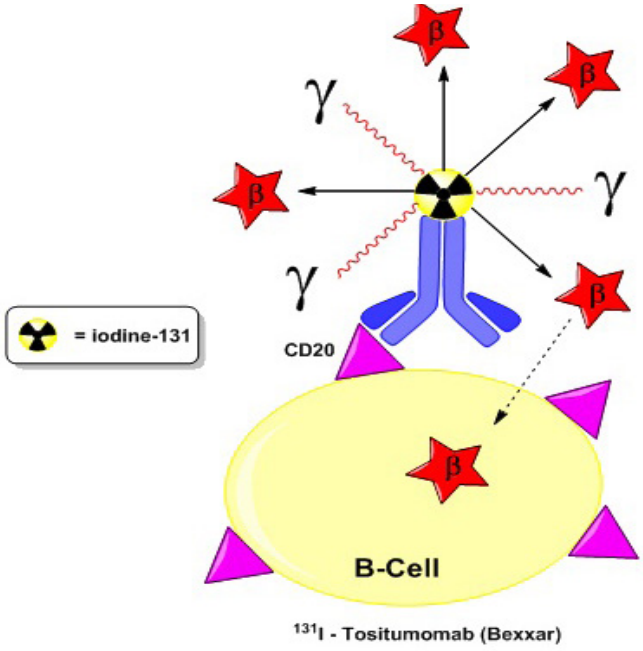

Figure 2: Bexxar® firing beta and gamma radiation to kill B-cells (Adaptation from Kitson et al. [51])

-ma to allow imaging and in therapy to destroy it. The radiopharmaceutical is first labelled with the radiometal indium-111, using tiuxetan chelation. This gamma-emitter is transported to the lymphoma sites by the monoclonal antibody ibritumomab which detects B-cells. SPECT imaging then can be used to verify that the antibody is properly distributed within the body.[52] The indium-111 is swapped with radionuclide yttrium-90, to transport beta particles to kill B-cells (Figure 3).

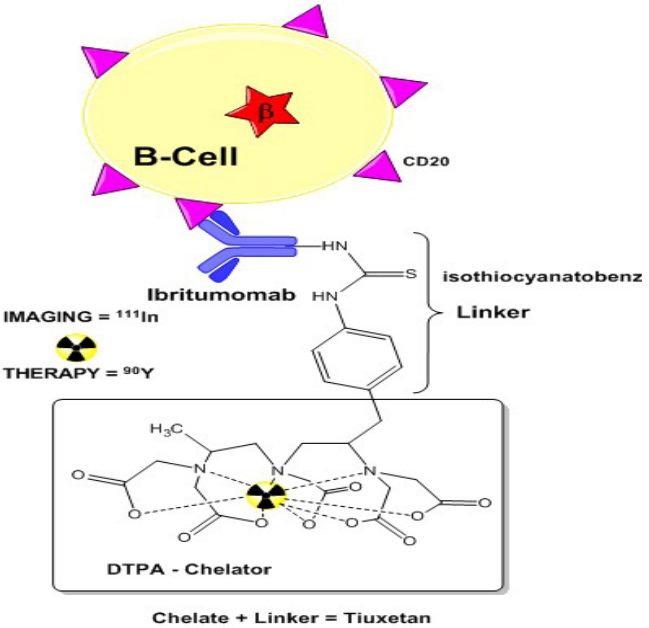

Figure 3: Zevalin® firing beta-particles at B-cells(Adaptation from Kitson et al. [51])

Zevalin® therapy has useful indications for relapsed or refractory, low grade or follicular, B-cell non-Hodgkin's

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lymphoma.[53] In 2002, the FDA gave approval for Zevalin® to be used in treatment of relapsed or refractory low-grade follicular or transformed B-cell non-Hodgkin's lymphoma; including patients with rituximab refractory follicular non-Hodgkin's lymphoma. In 2008, Zevalin® was approved as the first-line consideration for follicular lymphoma in the European Union.[54]

ANTIBODY-DRUG CONJUGATES (ADCs) TARGETING CANCER

These immunotherapeutic agents called Antibody Drug Conjugates (ADCs), target specific antigens particularly on cancer B-cellssuch as CD19, CD20, CD21, CD22, CD40, CD72, CD79b and CD180.[55] In 2011, the FDA gave approval to Adcetris® to treat Hodgkin's lymphoma and systematic anaplastic large-cell lymphoma.[56] The continued success of this therapeutic agent arrived in February 2013, when the FDA announced the approval of Kadcyla®, for the treatment of metastatic breast cancer.[57]

Adcetris® consists of the bio-vector brentuximab (IgG1 cAC10), which is a chimeric monoclonal antibody, to target the human CD30 antigen on B-cells.[58] The antibody is attached to a combination linker, *via* the Cysteine Sulfhydryl (Cys-SH) groups. These are generated from the mild reduction of the inter-chain hinge disulfide bonds of the antibody.[59] This linker combination is made up of a thiol-reactive maleimidocaproyl (mc) spacer, the dipeptide Valine-Citrulline (Val-Cit) linker and a 4-aminobenzylcarbamate (PABC) self-immolative spacer.[60] This set-up facilitates the conjugation of on average four drug Molecules of Monomethyl Auristatin E (MMAE) on the antibody (Figure 4).[61] MMAE is so toxic to healthy cells that it cannot be used as a stand-alone chemotherapeutic.[62]

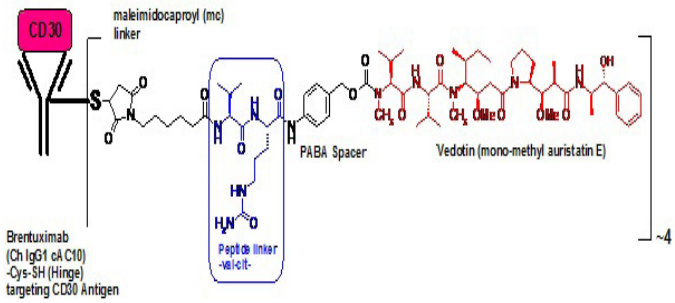

Figure 4: Structure of Adcetris®

In the mechamism of action ADC binds to the antigen on the B-cell to from an ADC-antigen complex (Figure 5). The ADC-antigen complex in the case of Adcetris® is internalized by clathrin-mediated endocytosis and transported to the intracellular lysosome compartment. The ADC-antigen complex fuses with the lysosome and the action of cathepsin-B proteases initiates a spontaneous intramolecular [1,6]-elimination of PABC to release the free-drug MMAE (picomolar potency) into the cytoplasm.[63] This drug then inhibits microtubule assembly, causing depolymerization, leading to cell cycle arrest which results in cell death.[64]

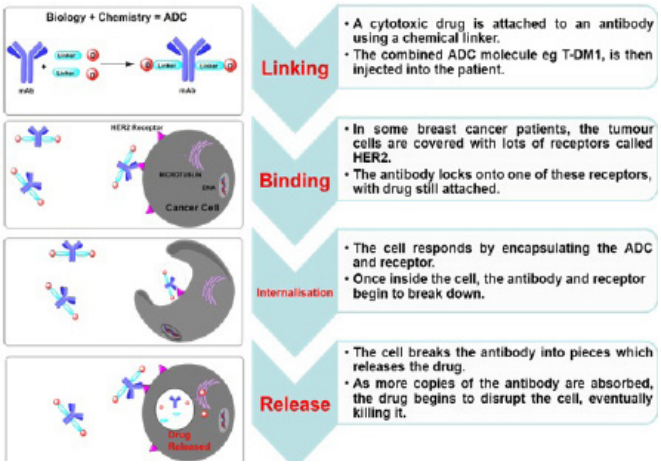

Figure 5: Mechanism of Action of ADCs

Kadcyla® consists of three components (Figure 6): the humanized MAb (IgG1) trastuzumab (Herceptin®) to target HER2 tumour antigens; the microtubule polymerization inhibitormaytansinoid DM1 drug and the (N-Maleimidomethyl) Cyclohexane-1-Carboxylate (MCC) non-cleavable thioether linker. Once the ADC is internalised into the cancer cell it undergoes catabolic metabolism releasing the cytotoxic drug DM1 from the antibody.[65]

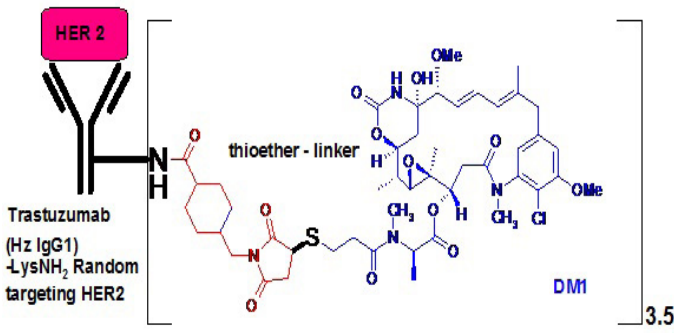

Figure 6: Structure of Kadcyla®

The majority of ADCs contain a number of the same drug attached to the monoclonal antibody, thereby producing heterogeneous mixtures. Kadcyla®, exists in such a heterogeneous form, ranging from 0-9 DM1 drug-molecules on each monoclonal antibody, with an average of 3.5 DM1 molecules per monoclonal antibody.[66] The tumour killing action of DM1, is in the inhibition of cell division, by binding tubulin, arresting the target cell in the G2/M stage of the cell cycle which results in apoptosis.[67]

Currently, strategies are being developed to produce ADCs with a greater degree of homogeneity. This is particularly directed to the Drug to Antibody Ratio (DAR), to circumvent regulatory issues.[68] The majority of ADCs typically contain a binomial distribution of cytotoxic drugs per monoclonal

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antibody, typically varying from 0-8 drugs moieties per ADC molecule. These emerging technologies are able to influence and aid the homogeneity of the DAR ratio.[69]

FUTURE PROSPECTS

Targeting cancer cells with specific monoclonal antibodies which carry cytotoxic drugs and radionuclide payloads is now a reality. This was first envisaged by Paul Ehrlich over 100 years ago. The main aim of this approach is to limit the damage to surrounding healthy cells in the vicinity of tumour cells. Currently, over 130 patients have received this experimental cancer treatment which is called Targeted Alpha Therapy (TAT). Information gathered from these first clinical trials will contribute to future safety profiles for the administration of alpha emitters in future patients.

The real successes have come from Bexxar® used in the treatment of non-Hodgkin's lymphoma, by delivering beta and gamma radiation from iodine-131. Conversely, the radiopharmaceutical Zevalin® is used both as an SPECT imaging agent and also as a therapeutic bullet. The destruction of the cancer is achieved by the usage of the beta emitter yttrium-90 to target and destroy B-cell non-Hodgkin's lymphoma.

At present, the biopharmaceutical industry is excited by the FDA approvals of Adcetris® to treat Hodgkin's lymphoma and Kadcyla® for the treatment of metastatic breast cancer. The only alpha particle emitting radioactive therapeutic agent approved by the FDA is Xofigo®, for the treatment of castration-resistant prostate cancer. This advancement of medical imaging techniques will deliver greater success to the targeted therapy approach in the management and treatment of oncological disease states.

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