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Review

# Optimising the radioimmunotherapy of malignant disease: the broadening choice of carrier and effector moieties

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**Abstract**

The treatment of cancer by radioimmunotherapy remains an experimental approach successful only in a limited number of selected disease conditions. One ground for optimism over the future of radioimmunotherapy lies in the fact that where cures have been obtained it has been despite the design of the immunoconjugate rather than because of it. As the choice of available functional components for conjugate construction increases, the process of evaluation and optimisation is underway. The replacement of the commonly used $^{131}I$ with radionuclides possessing radiation characteristics more suited to particular disease states, and tailored to the behaviour of the carrier vehicle, should bring improved energy deposition within tumour while reducing whole body radiation burden. Similarly, the introduction of chemically or genetically engineered targeting molecules in place of conventional antibodies may bring improved pharmacokinetic characteristics and higher tumour accumulation. Optimisation of the therapeutic and carrier moieties employed in radioimmunotherapy should bring distinct improvements in clinical efficacy.

*Keywords:* Cancer; Radioimmunotherapy; Radionuclides; Targeting

## 1. Introduction

The 'Magic bullet' approach to the selective destruction of malignant cells is attractive because of the apparent simplicity of the concept. A carrier molecule possessing a prefered affinity for the target tumour should serve as a selective delivery system for a cytotoxic agent, thereby achieving a localised cell killing while sparing normal body tissues. The field as a whole consists of numerous diverse strategies, based around the delivery of cytotoxic proteins (Wawrzynczak et al., 1991), conventional chemotherapeutics (Smyth et al., 1986) and high LET radionuclides (Langmuir, 1992). Each strategy has its own set of related problems which serve to illustrate just how deceptive the conceptual simplicity of the 'Magic bullet' is. The latter approach, termed Radioimmunotherapy (RIT), has suffered in that the targeting capacity of the carrier molecules used to date have barely been adequate to achieve therapeutic doses to tumour, whereas their relatively long half-lives in vivo together with the radiation characteristics of the commonly used radionuclide $^{131}I$, have resulted in high whole body radiation burdens and correspondingly poor therapeutic indices. Thus in clinical RIT studies performed to date a large variation in efficacy has been observed. This variation depends on many contributory factors, including radiosensitivity of the target tumour, its accessibility and the distribution characteristics of the antibody selected for use. As a result, many studies have been performed on haematological cancers which have good systemic accessibility (Scheinberg et al., 1991; DeNardo et al.,

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1990), or employing a regional therapeutic approach such as that used against ovarian tumours in which the radioimmunoconjugate can be administered direct into the peritoneum (Epenetos et al., 1987). The generally disappointing clinical results so far obtained must be seen realistically. RIT remains an experimental therapy and as a result it has often been the case that RIT could be applied only to those patients for whom conventional medicine had little more to offer, thus weighting the odds heavily against success. Despite this, in a recent clinical study using $^{131}$I-labelled Mabs against the CD20 and CD37 antigens expressed on B-cell lymphomas, Press was able to achieve complete remission in 16 of the 19 relapsed patients with poor prognosis, and which had been selected on the basis of favourable Mab distribution as determined in diagnostic studies. A further two patients showed partial remission with one showing only a minor response (Press et al., 1993). These encouraging results were obtained using large doses of $^{131}$I of between 8.6 and 28.7 GBq and all patients were given autologous bone marrow infusion to protect against myelosuppression. Similar results indicating high remission rates obtained following treatment of Hodgkins Disease were recently presented by Kaminski et al. at the Ninth Conference on Monoclonal Antibodies for Cancer in San Diego. In this case much lower levels of radioactivity were used and a clear effect of the Mab alone could be seen although an additional benefit was observed when using the $^{131}$I-labelled Mab.

The future application of RIT as an adjuvant component in 'first choice' therapies offered to newly diagnosed patients, enjoying a better prognosis, should allow a more realistic assessment of the value of this approach to be made. For the present, clinical results such as those refered to above encourage us to believe that, as the design of radioimmunoconjugates (RICs) is improved, the number of clinical situations in which a role for RIT can be identified will increase. In this review we consider the ever broadening choice of therapeutic and carrier moieties available for use in RIC construction.

## 2. The choice of therapeutic radionuclide

### *2.1. Optimal properties of therapeutic radionuclides*

In RIT, potentially therapeutic radionuclides are transported to the desired site of action by target-selective vehicles. As a consequence, any consideration of the optimal properties of such nuclides must take into account the nature and in vivo behaviour of the respective vehicle. Despite this obvious limitation, some gen-

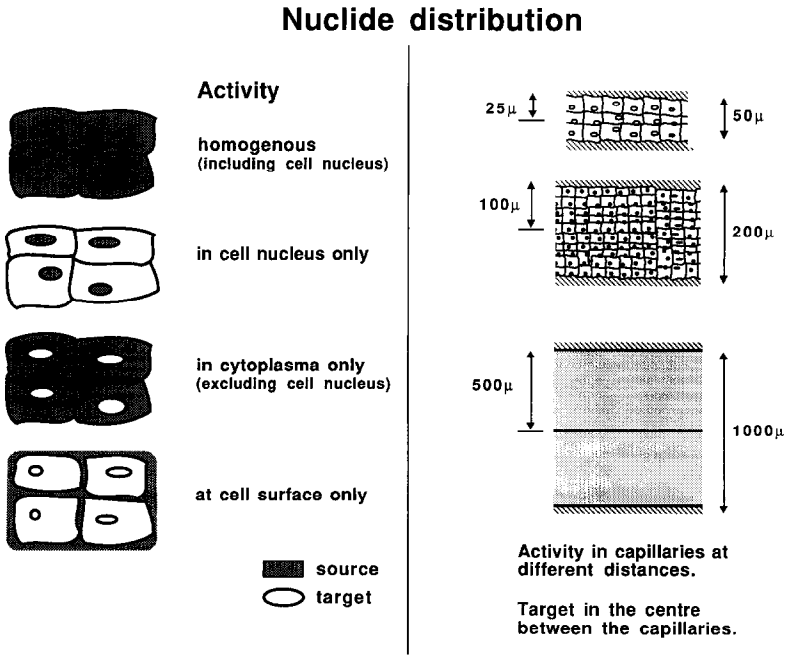



Fig. 1. Assumed tissue distribution patterns for given radionuclides as used in the calculation of doses to least irradiated cells (see Table 1).

eralisations can be made about the required physical characteristics of therapy nuclides, and these have formed the basis of numerous publications (e.g., Wessels and Rogus, 1984; Schubiger and Andres, 1987). Of primary importance, when selecting a nuclide for application with a given vehicle, are the nature of the associated radiation, the ratio of penetrating to non-penetrating radiation, the physical half-life ($t_{1/2}$) of the nuclide, the stability of any daughter nuclides formed and, of practical significance, the route of production or availability.

The killing of tumour cells by a radioimmunoconjugate is most effectively achieved by relatively high LET radiations which deposit the majority of their ionising energy within the targeted tumour. Due to the diversity of vehicles and their sites of action, the optimal range in tissue of the particulate high LET emissions varies accordingly. For illustration, some possible tissue distribution patterns are shown (Fig. 1) and for each pattern radiation doses to the cell nucleus have been calculated (Wernli, 1986), assuming the targeted nuclide to be either Auger electron emitting, $\beta$-emitting (both high and low energies) or $\alpha$-emitting. In Table 1 the resulting doses to the least irradiated cell nucleus, normalised to the doses delivered by homogeneously distributed nuclide, are given. The Auger electron emitters are shown to be quite potent but only if they can penetrate the cell membrane and come into close proximity with the nucleus. The $\alpha$ emitters are also very potent cell killing agents but $\alpha$ particles are restricted in range to around 50 $\mu$m in tissue, so that little 'crossfire' can be expected. The clear implication here is that in both cases the nuclide distribution must be highly homogeneous with respect to the tumour volume in order to uniformly irradiate all tumour cells. As pointed out by Wheldon, even very modest levels of heterogeneity involving small numbers of untargeted cells are catastrophic in terms of tumour cure (Wheldon, 1993).

$\beta$-Emitters yield a homogeneous dose distribution even when they are heterogeneously distributed within the target tissue. Depending on the $\beta$-energies, the tumour size for optimal curability will vary, for example from 3 mm for $^{131}$I to 3 cm for $^{90}$Y (Wheldon, 1993). Smaller metastases will receive a reduced dose due to deposition of energy outside the desired volume. A very detailed discussion of radiation dosimetry in RIT has been given by Humm (Humm, 1986).

The highly penetrating $\gamma$ radiation associated with many radionuclides contributes significantly to the whole body radiation burden borne by patients undergoing a programme of RIT but does not contribute significantly to the tumour dose. Dosimetric calculations for RIT based on patient data with $^{131}$I-Mab have been published (Langmuir, 1992) suggesting penetrating $\gamma$ radiation to contribute up to 50% of the dose-limiting radiation toxicity to the bone marrow. The other 50% comes from non-penetrating radiation (i.e. particulate, high LET radiation). Similar calculations based on animal data and extrapolated to man resulted in a value of 30% as the contribution of penetrating radiation to the bone marrow dose (Buchegger et al., 1991). This makes a strong case for the replacement of the most commonly used nuclide, $^{131}$I (main $\gamma = 360$ keV, 82% abundance), with nuclides displaying a lower accompanying $\gamma$ component. Ideally, however, nuclides possessing a low abundance (5–20%) of $\gamma$ radiation in the range of 100–200 keV seem to be optimal, as this allows easy measurement during experimental work-up, biodistribution studies, scintigraphy and dosimetric calculations.

In the case of high energy $\beta$-emitters such as $^{90}$Y, the bone marrow is subjected to a more uniform irradiation due to the higher penetrance of the emitted

Table 1
Doses to the least irradiated cell nuclei

| Nuclide distribution | Auger | $\beta^-$ | | | $\alpha$ |
|---|---|---|---|---|---|
| | Ga-67 (< 10 keV) | I-131 (180 keV) | Ag-111 (350 keV) | Y-90 (930 keV) | At-211 (≥ 5900 keV) |
| Homogeneous | 1 | 1 | 1 | 1 | 1 |
| Cell nucleus | ≈ 20 | ≈ 1 | ≈ 1 | ≈ 1 | ≈ 1 |
| Cytoplasm | < 0.2 | ≈ 1 | ≈ 1 | ≈ 1 | ≈ 1 |
| Cell surface (20 $\mu$m) | 0 | ≈ 1 | ≈ 1 | ≈ 1 | ≈ 0.9 |
| Capillary (50 $\mu$m) | 0 | ≈ 0.9 | ≈ 1 | ≈ 1 | ≈ 0.8 |
| Capillary (200 $\mu$m) | 0 | ≈ 0.8 | ≈ 0.9 | ≈ 1 | 0 |
| Capillary (1000 $\mu$m) | 0 | ≈ 0.3 | ≈ 0.7 | ≈ 0.9 | 0 |

particles. This suggests it may be beneficial to use $\beta$-emitters of lower energy, such as $^{67}$Cu, so as to obtain a marrow sparing effect (Langmuir, 1992). Elaborate accounts of tumour dosimetry following application of therapeutic nuclides have been made (Saenger et al., 1979; Humm, 1986; Jönsson and Strand, 1986; Langmuir, 1992) and dosimetric calculations relating to the use of Auger electrons have also been published (Booz, 1984; Humm and Cobb, 1990; Johnson et al., 1992; Sgouros, 1992, Sgouros, 1993).

In terms of the physical $t_{1/2}$ of any given radionuclide, this should approach the biological $t_{1/2}$ at the tumour of the radioimmunoconjugate. Clearly, attaining a sufficiently high integrated tumour dose to achieve sterilisation (> 50 Gy, Rösler et al., 1986) will prove problematic if a nuclide possesses a physical half-life which is inappropriately short for use with a chosen vehicle. On the other hand, a certain minimal dose rate (> 0.4 Gy/h, Andres et al., 1986) must be achieved over a long enough period so as to ensure that all cells are irradiated during the most radiation sensitive M and $G_2$ phases of the cell cycle. For these reasons a physical $t_{1/2}$ between 10 h and 10 days seems to be appropriate. Spencer has argued for the application of nuclides displaying short half-lives, of the order of a few hours, especially in the context of irradiating synovial membranes, where leakage into the lymphatic channels, nodes and distant parts of the body can be a problem (Spencer, 1987). Also, he has suggested the use of such nuclides in fractionated therapy. He noted, however, the shortcomings of this recommendation when considering applications where a significant delivery time is required (from the site of administration to the point of uptake by the lesion) and also the problem of the removal of necrotic tissue following rapid destruction.

Following the initial disintegration of a radioactive nuclide which has been chemically linked to a carrier molecule the residual atom may be released and then distribute itself in vivo according to a very different pattern to that observed for the original therapeutic agent. If the resulting daughter nuclide is unstable, this could lead to unwanted and uncontrollable irradiation of non-target tissues. For example, with the $\alpha$-emitter $^{211}$At one has to take into account the biological fate of the daughter nuclides $^{211}$Po and $^{207}$Bi (Franke et al., 1986). As a consequence, only nuclides with stable or very long-lived daughter nuclides are considered for application in RIT.

Biological vehicles take advantage of naturally occurring structures presented by the target cell. For any given vehicle, such sites of recognition are limited in number, resulting in a corresponding limitation in the number of molecules which can interact with the target cell (Spencer, 1987). In order to deliver an adequate dose to the tumour it is therefore important that therapeutic nuclides can be produced in either a carrier-free form, or at least with high specific activity. As a consequence, production may require high flux reactors to obtain sufficiently high neutron fluence, or high energy–high beam cyclotrons as a source of protons. Finally, the separation of desired therapy nuclide from precursor nuclide can require very sophisticated methods, such as that for the separation of $^{111}$Ag from the target material Pd (Alberto et al., 1992).

Table 2
Physical characteristics of potential therapeutic radionuclides

| Nuclides | $t_{1/2}$ | Radiation type (MeV) | Particle max.range | Authors |
|---|---|---|---|---|
| $^{80m}$Br | 4.42 h | Auger, $\gamma$ (0.037) | < 10 nm | d |
| $^{67}$Ga | 3.26 d | Auger, $\gamma$ (0.09) | 10 nm | b |
| $^{125}$I | 60.0 d | Auger, $\gamma$ (0.027) | 10 nm | d |
| $^{211}$At | 7.2 h | $\alpha$ (6.8) | 65 $\mu$m | b,d,e |
| $^{212}$Bi | 1.0 h | $\alpha$ (7.8), $\gamma$ (0.72) | 70 $\mu$m | d,e |
| $^{169}$Er | 9.5 d | $\beta$ (0.34) | 1.0 mm | c,d |
| $^{177}$Lu | 6.7 d | $\beta$ (0.497), $\gamma$ (0.208) | 1.5 mm | b |
| $^{161}$Tb | 6.91 d | $\beta$ (0.51), $\gamma$ (0.025) | 1.7 mm | b |
| $^{67}$Cu | 2.58 d | $\beta$ (0.54), $\gamma$ (0.185) | 1.8 mm | c,d |
| $^{105}$Rh | 1.48 d | $\beta$ (0.57), $\gamma$ (0.320) | 1.9 mm | b |
| $^{131}$I | 8.0 d | $\beta$ (0.6), $\gamma$ (0.364) | 2.0 mm | c,d,e,f |
| $^{77}$As | 1.62 d | $\beta$ (0.68), $\gamma$ (0.239) | 2.5 mm | b |
| $^{127}$Te | 9.4 h | $\beta$ (0.7) | 2.6 mm | c |
| $^{153}$Sm | 1.95 d | $\beta$ (0.8), $\gamma$ (0.103) | 3.0 mm | b,d,f |
| $^{198}$Au | 2.7 d | $\beta$ (0.97), $\gamma$ (0.411) | 4.4 mm | c,d |
| $^{111}$Ag | 7.47 d | $\beta$ (1.05), $\gamma$ (0.34) | 4.8 mm | a,b |
| $^{149}$Pm | 2.21 d | $\beta$ (1.07), $\gamma$ (0.289) | 5.0 mm | b |
| $^{186}$Re | 3.77 d | $\beta$(1.08), $\gamma$ (0.131) | 5.0 mm | c,d,e,f |
| $^{89}$Sr | 50.5 d | $\beta$ (1.49) | 8.0 mm | d,f |
| $^{32}$P | 14.3 d | $\beta$ (1.71) | 8.7 mm | b,c,d,f |
| $^{188}$Re | 16.95 h | $\beta$ (2.13), $\gamma$ (0.155) | 11.0 mm | b,c,e |
| $^{142}$Pr | 19.1 h | $\beta$ (2.16), $\gamma$ (1.6) | 11.3 mm | c |
| $^{90}$Y | 2.67 d | $\beta$ (2.28) | 12.0 mm | b,c,d,e,f |

[a] Hahn and Sheppard, 1947; [b] Schubiger and Andres, 1987; [c] Adelstein and Kassis, 1987; [d] Hoefnagel, 1991; [e] Eary, 1991; [f] Lewington, 1993.

### 2.2. Potential therapeutic nuclides

Having defined the optimal properties desirable in a therapeutic nuclide, a search can be made through the table of nuclides to find potential candidates. Depending on the precise definition and preferred properties, many lists have emerged in the literature (e.g., Schubiger and Andres, 1987; Adelstein and Kassis, 1987; Hoefnagel, 1991; Eary, 1991; Lewington, 1993). Table 2

Table 3
A selection of radionuclides with therapeutic potential

| Radiation | Nuclide ($t_{1/2}$) | Mean energy (keV) | Mean range | $E\gamma$ (KeV) | (%) |
|---|---|---|---|---|---|
| Auger | $^{67}$Ga (3.26 d) | < 10 | < 100 nm | 185 | (24) |
| $\beta$ | $^{186}$Re (3.77 d) | 350 | 1 mm | 137 | (10) |
| | $^{131}$I (8.04 d) | 180 | 0.3 mm | 364 | (82) |
| | $^{111}$Ag (7.47 d) | 350 | 1 mm | 342 | (6) |
| | $^{90}$Y (2.67 d) | 930 | 4 mm | – | |
| | $^{67}$Cu (2.58 d) | 141 | 0.2 mm | 185 | (45) |
| $\alpha$ | $^{211}$At (7.2 h) | 5900, 7500 | ≈ 65 $\mu$m | – | |

shows the physical characteristics of those nuclides proposed in the above mentioned reviews.

Some of these proposed nuclides do not fulfil the conditions relating to length of half-life ($^{80m}$Br, 4.42 h; $^{212}$Bi, 1.0 h; $^{89}$Sr, 50.5 d). As a result, these nuclides can not be considered as realistic candidates for use in RIT. Others have a relatively high abundance of $\gamma$ radiation (e.g., $^{131}$I with 82% and $^{67}$Cu with 60%). However, as $^{131}$I has been used most extensively in RIT up to this point it must also come into consideration and serves as a benchmark for comparison. $^{67}$Cu is presently available on a limited basis and can also be used as a model system for studies on the behaviour of metal nuclides in vivo.

For more comprehensive discussion some promising nuclides of differing types have been selected. As a typical Auger-electron emitter, $^{67}$Ga is chosen, from the $\beta$-emitters $^{131}$I, $^{186}$Re, $^{111}$Ag, $^{67}$Cu and $^{90}$Y, and for the $\alpha$-emitters $^{211}$At. These nuclides are listed in Table 3 together with their physical properties.

The $\beta$-emitting nuclide $^{131}$I, by far the most commonly used nuclide in RIT, has serious disadvantages. The high abundance of $\gamma$ radiation (< 90%) contributes insignificantly to the dose to the tumour but does add heavily to whole body dose and to the dose-limiting toxicity to the bone marrow. As detailed in the preceeding section, the penetrating radiation from $^{131}$I accounts for 30 –50% of the bone marrow dose burden. In this respect, $^{67}$Cu ($\gamma$ radiation abundance ≈ 60%) would lead to a reduction of the radiation burden to the bone marrow, an effect even more significant in the cases of $^{111}$Ag ($\gamma$-abundance ≈ 7%) and $^{186}$Re ($\gamma$-abundance ≈ 16%). $^{90}$Y has no $\gamma$-radiation, but its relatively high energy $\beta$ particles together with its predilection for accumulation in the bone adds to the dose-limiting toxicity to the bone marrow. (Langmuir, 1992).

The ranges of the $\beta$ particles emitted by $^{186}$Re, $^{111}$Ag and $^{67}$Cu permit the irradiation of several layers of tumour cells, a prerequisite for successful RIT. Of course, it must be borne in mind that there is no single ideal therapy nuclide, rather a number of candidates which are suited to a greater or lesser extent to the employed vehicle and the clinical target. The development of vehicles capable of targeting tumour cell DNA may allow Auger-emitting nuclides such as $^{67}$Ga to come into consideration. In the case of the $\alpha$-emitting nuclides, their relatively short path length means that their effective application requires almost every tumour cell be targeted by the radioimmunoconjugate, as may be achievable with certain blood-borne malignancies. Additionally, their application together with $\beta$-emitting nuclides as part of a nuclide 'cocktail' approach may be feasible.

At present we consider the $\beta$-emitting nuclides $^{186}$Re, $^{111}$Ag and $^{67}$Cu to be among the most promising for application in RIT.

### 2.3. Nuclide chelators and chemistry of linkage

Methods for the production of therapeutic nuclides are not dealt with here and the interested reader is referred to the review article of Volkert et al., 1991. However, it should be mentioned that one of the most important aspects of a production method is the achievable specific activity of the desired radionuclide. Obviously therapy nuclides produced from an inactive isotope (e.g., $^{186}$Re from Re by n,$\gamma$-reaction) can hardly be carrier free, whereas with a target material different from the therapy nuclide a high specific activity can be obtained after following suitable separation procedures (e.g., $^{67}$Cu from Zn by p,n-reaction or $^{111}$Ag from Pd).

Having secured a therapy nuclide of adequate specification, one has to tackle the problem of finding a suitable chelator with a functional group allowing linkage to the carrier antibody. A general outline of these coupling procedures is given in Fig. 2. The first and sometimes difficult step is the synthesis of a nuclide-selective chelator capable of forming complexes of high in vivo stability. Such stability is determined by the kinetic inertness of the complex and can not be guaranteed by simple selection of chelators possessing high thermodynamic stability. For Re and Cu such chelators have been published and successfully tested. Fig. 3 gives their structures and references. The list does not include all known chelators but some typical examples used in preclinical and clinical work. Others are often quite similar in appearance, such as TETA (a 14-member macrocycle) and DOTA (a 12-member macrocycle) (see Mausner et al., 1993), or the MAG 3-chelators (Visser et al., 1993) with two carbon atoms less in the

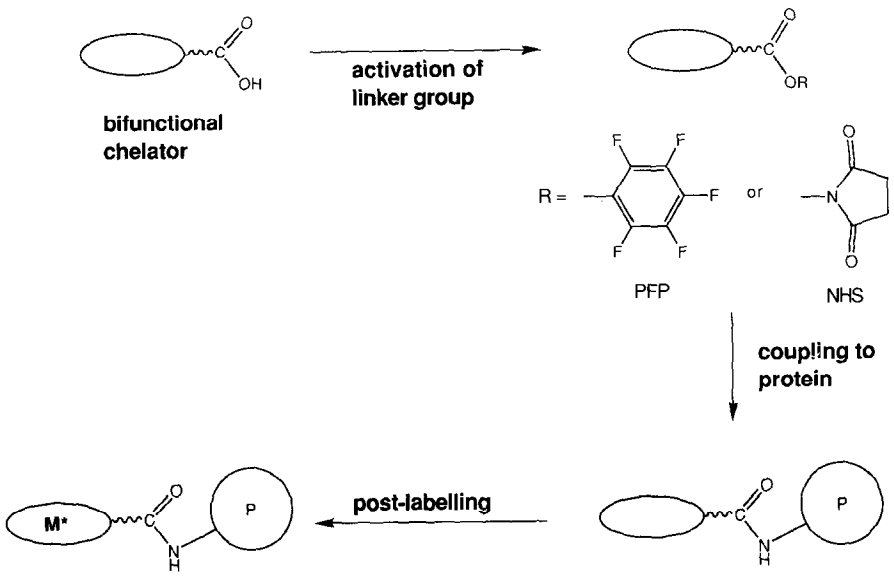
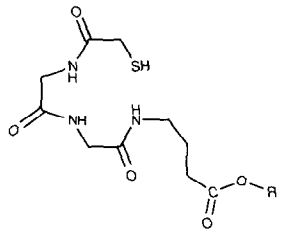
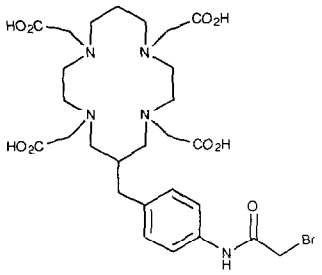


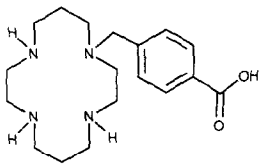

Fig. 2. The activation, coupling to antibody and subsequent radiolabelling of bifunctional ligands. In this example NHS or PFP are used to convert a carboxyl group of the chelator into an active ester which can then react with primary amino groups in protein. The radiolabelling step is then performed in an appropriate buffer system.



MAG3:
R-(N-mercaptoacetylglycylglycyl)-γ-amino-butyrate (Breitz et al., 1992).



BAT:
6-bromoacetamidobenzyl-1,4,8,11 tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid (Moi et al., 1985).



CTPA:
[1,4,8,11 tetraazacyclotetradec-1-yl]-methyl-benzoic acid (Studer et al., 1990).

Fig. 3. Representative chelators for use with the therapeutic radionuclides rhenium and copper: the open-chained MAG3 (Breitz et al., 1992) which has found application with $^{186}$Re and the 14-membered macrocycles BAT (Moi et al., 1985) and CTPA (Studer et al., 1990) which have been used extensively with $^{67}$Cu.

spacer arm of the coupling group compared to the variant published by Breitz et al., 1992 (see Fig. 3).

The next step in the coupling procedure is often the activation of the bifunctional chelator so as to allow ist reaction with the carrier moiety. A possible chelator activation is given in Fig. 2, where potential leaving groups, R, are pentafluorophenol (PFP) or *N*-hydroxy-succinimide (NHS). Esterification with these groups is aided by the participation of 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC). Finally the bifunctional chelator is reacted with an accessible primary amino group (predominantly lysine) on the antibody. An alternative strategy is to first introduce a reactive group into the antibody followed by reaction with a chelator bearing an appropriate secondary reactive group. For example the linker 2-iminothiolane has been bound to primary amino groups of an antibody and then the sulfhydryl group reacted with 6-*p*-(bromoacetamido)benzyl-1,4,8,11-tetraazacyclotetradecane-*N,N,N,N*-tetraacetic acid (BAT) (McCall et al., 1990).

A direct approach to coupling is achieved by reducing disulfide bonds in the antibody and allowing Re to react directly with the resultant sulfhydryl groups (Griffiths et al., 1991). This is directly analogous to the known direct labelling methods for $^{99m}$Tc.

If the therapy nuclide is introduced into its chelator after the chelator has been attached to the carrier antibody, this is refered to as a post-labelling. However, one could also consider a pre-labelling procedure, forming the radionuclide-chelator complex proir to ac-

tivation and substitution into the antibody. The decision as to which labelling strategy is to be adopted will be influenced by a variety of considerations. For example, if the radionuclide is not abundantly available the efficiency of each given step in the labelling procedure needs to be considered and may lead to the conclusion that a direct one-step post-labelling is most appropriate. Similarly, where complex formation between nuclide and chelator can only be achieved under non-physiological conditions (i.e., in organic solvents or with heating) then clearly a pre-labelling is indicated.

The choice of chelator (if there is a choice) for any given nuclide may be influenced by the metabolic fate of the free nuclide and its chelated form. Ideally, the chelated nuclide would be rapidly excreted via the urinary tract without intra-cellular retention, with intact conjugate accumulating and being retained selectively at the tumour site. This is however, clearly not always the case and reality is more complicated. For example, Anderson et al. compared the biodistribution of the anti-colon carcinoma Mab 1A3 labelled with $^{64}$Cu using BAT and CTPA. Their conclusion was that there were no significant differences between the two $^{64}$Cu-labelled intact Mab preparations in terms of in vivo biodistribution; however, the accumulation of $^{64}$Cu-CTPA-F(ab')$_2$ in the kidneys was 2.5-times greater than that of $^{64}$Cu-TETA-F(ab')$_2$ (Anderson et al., 1993).

## 3. The choice of carrier moiety

### *3.1. Monoclonal antibodies and their antigen-binding fragments*

The development of selective anti-cancer therapeutics based on the principle of antibody targeting received new impetus in 1975, following the description of innovative methodology allowing the generation of immortal murine cell lines secreting monoclonal antibodies (Mabs) of pre-determined specificity (Kohler and Milstein, 1975). This technique centres around the fusion of antibody secreting 'B' lymphocytes from immunized mice (usually spleen cells) with an immortal murine myeloma cell line. Fusion is usually assisted by polyethylene glycol although electrofusion has also been used (Ohnishi et al., 1987). After fusion, a mixture of hybrid cells, myeloma cells and spleen cells can be identified. The spleen cells can not survive in vitro and die over a period of several days. The myeloma cells, having been preselected for the absence of the HGPRT enzyme which can rescue cells from the toxic effects of aminopterin, are killed by addition of HAT

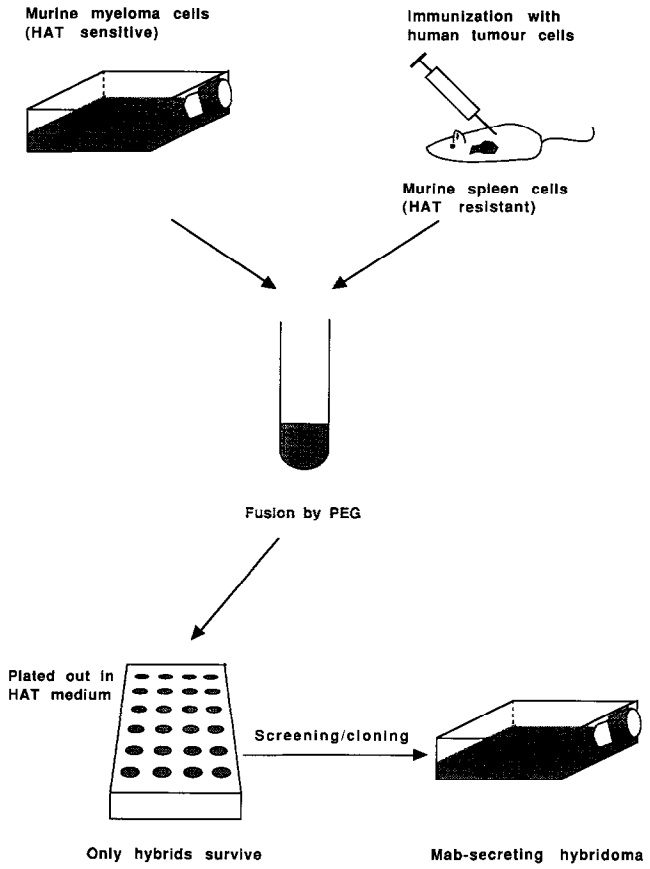



Fig. 4. The production of murine monoclonal antibodies directed against antigens asociated with human tumour cells. Immortal myeloma cells are fused with antibody secreting murine 'B' lymphocytes. The subsequent processes of screening and cloning lead to an established monoclonal cell line secreting antibody of pre-determined specificity.

medium (Hypoxanthine, Aminopterin, Thymidine). The hybridoma cells are rescued by HGPRT inherited from the spleen cells and emerge as the only surviving cell type. (Fig. 4). Identification of hybrids producing Mabs of the desired specificity is then carried out by screening of culture supernatants for reactivity against target tumour cells, usually by immunofluorescence or enzyme-linked immunosorbant assay, and then separation is achieved by a process of limiting dilution to the point of monoclonality.

This technique brought with it the possibility of generating a constant supply of a desired Mab directed against a defined tumour specific antigen (TSA). In initial in vivo studies performed in model systems consisting of mice bearing human tumour xenografts, selective uptake was modest with values around 1% of the injected dose per gram of tumour (%ID/g). The

continual search for better antibodies of higher affinity has improved these values so that xenograft accumulation of 10–35%ID/g is commonly observed (Buchegger et al., 1990; Smith et al., 1994), with even higher values in particularly well vascularised xenograft systems, although such manipulation of model test systems clearly has no relevance to clinical performance of a given RIC. Radioimmunoconjugates consisting of such murine Mabs and radiolabelled with $^{131}I$ have been used successfully to cure mice bearing a variety of xenografted human tumours such as neuroblastoma (Cheung et al., 1986) colon carcinoma (Buchegger et al., 1990) and small cell lung cancer (Smith et al., 1990). Despite intense efforts, no Mabs have yet been generated which fulfil original expectations of being truly tumour-specific. They all display reactivity to antigens which are to some extent expressed on normal tissue and thus define tumour associated antigens (TAA) rather than TSAs, the latter appearing less realistic an objective than was initially thought.

The pharmacokinetic characteristics of Mabs can be modified in that they can be subjected to selective proteolytic degradation to generate either a divalent immunoreactive fragment of 100 kDa, the $F(ab')_2$ fragment, or a monovalent immunoreactive Fab fragment of 50 kDa (Fig. 5). The most commonly used proteolytic enzymes for $F(ab')_2$ and Fab generation are pepsin and papain respectively although a number of alternative enzymes are now available (Milenic et al., 1989). Apart from alteration of the pharmacokinetic behaviour of the Mab, fragmentation may offer improvement by virtue of reduced immunogenicity and the absence of an Fc region might be expected to abrogate uptake by the Fc receptors of the reticuloendothelial system (RES).

Fab and $F(ab')_2$ fragments have been shown to possess pharmacokinetic characteristics which are distinctly different from those observed for the 150 kDa parent molecule and which may bring advantages in terms of therapeutic ratios. Both fragments display a more rapid partitioning and clearance in vivo and because of their reduced size show a more rapid and uniform penetration of tumour (Humm, 1986). Particularly in the case of the Fab, which is small enough to be cleared over the kidney by glomerular filtration, the lower levels of these fragments observed circulating in the blood and normal tissues has been shown to result in improved tumour to non-tumour localisation ratios and to allow improved radioimmunoscintigraphy for diagnostic purposes. Rapid clearance has, however, a second consequence in that absolute tumour uptake is generally lower than with intact Mab, although some exceptions have been reported (Buchegger et al., 1990). When considering the difficulty presently experienced in trying to achieve a therapeutic dose to the tumour, requiring many GBq to be injected, then this leads to the conclusion that intact antibodies are currently to be favoured over Mab fragments for therapeutic application. In addition, recent studies undertaken with radiometal-labelled $F(ab')_2$ fragments (Brown et al., 1987a; Smith et al., 1993, Smith et al., 1994; Anderson et al., 1992) have revealed altered patterns of in vivo distribution when compared with conventional radioiodine-labelled $F(ab')_2$ and, in particular, a high level of kidney uptake which appears to translate to the patient situation and may negate the use of such fragments with metal nuclides (Halpern et al., 1988). The reason for the altered patterns of distribution observed when comparing radioiodine and radiometals seems to centre around the ability of radioiodide or radioiodotyrosine to exit cells following uptake and degradation of the immunoconjugate whereas many metal nuclide-chelator complexes remain trapped within the cell (Press et al., 1990).

The transfer of murine Mabs from predominantly mouse model systems into the clinical situation is not without consequence, so that only limited conclusions can be drawn from preclinical work. For example,

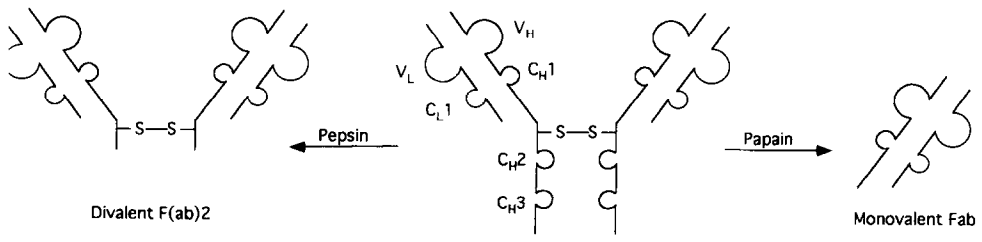



Fig. 5. The generation of Mab fragments using the proteolytic enzymes pepsin and papain. The basic immunoglobulin structure is shown schematically to consist of two light (L) and two heavy (H) chains linked by disulfide bridging. The constant (C) and variable (V) domains are indicated. Depending on the primary site of action of the enzyme, either the 50 kDa monovalent Fab or the 100 kDa divalent $F(ab')_2$ fragment is generated.

metal nuclide-labelled murine Mabs show little evidence of liver accumulation in mouse model systems but can show very high levels in patient studies (Pochon et al., 1989; Ryser et al., 1992), possibly as a result of uptake by the reticuloendothelial system. In contrast, the high accumulation of metal nuclides in the kidney, following administration of radiolabelled Mab fragments, is an effect which is observed both in animal

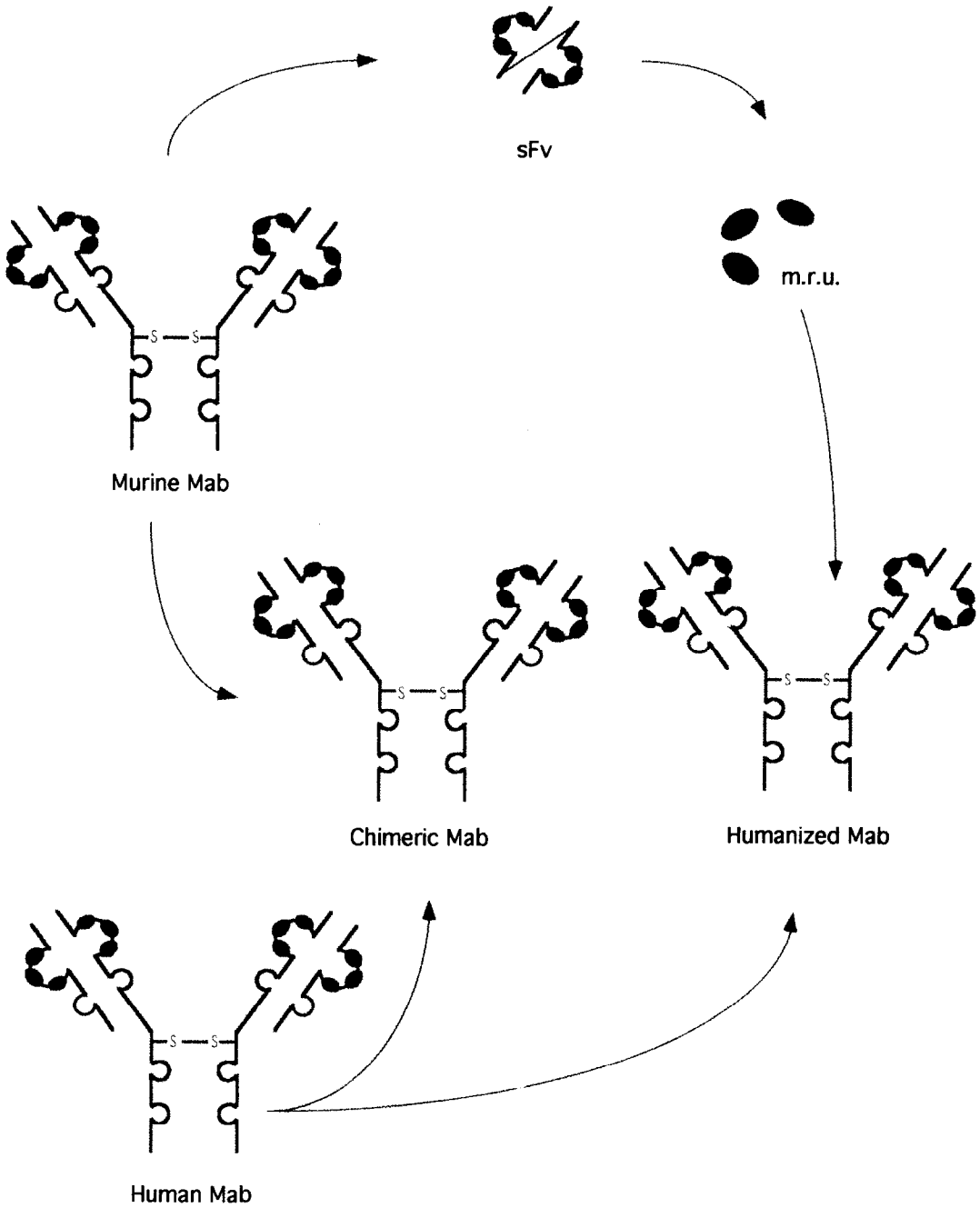



Fig. 6. The genetic engineering of vehicles for use in RIT. Using these techniques a range of possible constructs can be generated such as chimeric antibodies consisting of murine variable and human constant regions. Humanized antibodies can also be made in which murine CDRs are grafted into a human immunoglobulin so that the variable framework and constant regions are human. Smaller antigen-binding fragments can also be generated, such as single chain Fvs and minimum recognition units consisting of isolated CDRs.

model studies (Brown et al., 1987a; Anderson et al., 1992) and in patient studies (Halpern et al., 1988).

Human Mabs would be expected to offer a number of advantages over murine Mabs. For example, immunisation of mice with human tumour cells tends to ellicit responses against very obvious antigenic determinants, such as the HLA system. Patients clearly will not respond to such antigens which determine 'self' and are therefore more likely to respond to the more subtle and obscure TSAs (Sikora et al., 1983). Also, as a protein of human origin, any patient immune response to RICs constructed using such Mabs should be restricted to anti-idiotypic responses directed against the complementarity determining regions (CDR) of the antibody or against the chelator. This would be expected to help in circumvention of the human anti-mouse antibody (HAMA) response associated with clinical studies involving murine Mabs and which is considered to be a hindrance to fractionated application of RIT. The most frequently used approach to the generation of human Mabs has been the fusion of B cells from the draining lymph nodes or from the peripheral blood of cancer patients with established murine myeloma cell lines. Initial studies on human Mabs, in which human antibody secreting cells were fused with murine myeloma cells, were unfortunately plagued with problems in that a preferential deletion of human chromosomes by the resultant hybridomas was observed together with instability in culture (Glassy et al., 1987). Substituting human myeloma cells so that human/human hybridomas were produced resolved the problem of instability but yielded only low amounts of Mab. Another approach to the immortalisation of human cells which secrete antibody has been direct transformation using the Epstein–Barr virus, although this has also been found to lead to low-secreting cell lines requiring frequent re-cloning to ensure stability and often producing Mabs of the IgM class (Steinitz et al., 1977; Kozbor and Roder, 1981). These approaches seem now to have been largely surpassed by the techniques of genetic molecular engineering.

### *3.2. Genetic engineering of antibody constructs*

The introduction of genetic engineering to the field of immunotargeting may well prove to be an even more significant event than the introduction of the original monoclonal antibody technology which revolutionised the field in the late 1970s. Perhaps the most obvious advantage to be gained from this methodolgy is the capacity to 'humanize' Mabs so as to make them appear less foreign, and therefore less immunogenic, when used in clinical studies and, in some instances, to enhance recruitment of human effector mechanisms.

The first application of this approach was to construct chimeric Mabs in which the entire murine constant region was replaced with its human counterpart. For example, murine variable region genes from the B6.2 anti-cancer Mab and human constant region exons were fused to yield chimeric immunoglobulin genes which were cotransfected into murine myeloma cells which then secreted the intact chimeric antibody, cB6.2 (Brown et al., 1987b). However, this approach did not completely resolve the problem of immunogenicity of the murine variable region, although HAMA responses were markedly reduced and occassionally absent. It appears that each chimeric Mab is unique in its immunogenicity (Khazaeli et al., 1991).

Recognising the degree of conformational similarity between the framework regions of the majority of antibodies, Jones (Jones et al., 1986) suggested that the specificity of antibody binding may be independant of the framework regions, thereby opening up the possibility of further minimising the murine nature of the antibody by grafting the rodent hypervariable CDRs into a human variable region framework. The resultant three dimensional configuration of such constructs, and accordingly the affinity, have proven sometimes to be detrimentally effected, although the selection of human framework regions bearing strong similarity to the original rodent regions was found to reduce this risk (Queen et al., 1989). Additionally, molecular modelling can be used to identify, and subsequently preserve, critical amino acids outside the CDRs which may influence conformation.

Apart from the potential advantages to be gained by humanizing a given Mab genetic engineering brings the possibility of altering pharmacokinetic behaviour by a variety of means. For example, the same logic which was applied to conventional Mabs has also been applied to genetically engineered carriers. On the assumption that smaller molecules should partition more rapidly throughout the body, a series of genetically engineered Mab fragments have been generated (Fig. 6). Antigen-binding Fv fragments are non-covalently bound heterodimers consisting of a variable light-chain ($V_L$) and variable heavy-chain ($V_H$). These have proven to be prone to dissociation, although the extent to which this occurs varies from construct to construct. This tendency to dissociate can be overcome through insertion of a peptide sequence linking the two chains to give a single-chain Fv, or sFv. The first in vivo studies involving an sFv were performed by Colcher. $V_L$ and $V_H$ regions taken from the murine B6.2 anti-

cancer Mab, were linked via a synthetic peptide bridge and the biological properties of the resulting sFv compared with the original B6.2 Fab′ fragment (Colcher et al., 1990). In vitro cell binding studies revealed the affinity constant of the sFv to be not significantly different from the B6.2 Fab′ fragment. Pharmacokinetic differences were however observed in vivo, in a mouse model system, where the reduced molecular weight of the sFv at 25 kDa led to more rapid clearance than was seen with the 50 kDa Fab′. Despite this, tumour xenograft accumulation of the two materials was found to be comparable. These initial encouraging results were found, unfortunately, to be the exception rather than the rule and subsequent studies have revealed very low absolute accumulation of sFv fragments in tumour xenograft model systems (Schott et al., 1992).

Still smaller than the sFvs, but still possessing antigen binding activity, are single $V_H$ domains (dAb) and even single CDRs, known as minimal recognition units or m.r.u. In relation to such fragments it must be borne in mind that there is a clear conflict between the need for rapid clearance from the body to reduce whole body toxicity and the need for higher tumour accumulation. It would seem that, at least at the moment, the two are mutually exclusive.

### *3.3. Biochemical engineering of antibody constructs*

The powerful techniques of genetic engineering are not so widely available that they can be employed at will. A more direct and accessible approach to the generation of antigen binding constructs, possessing binding and pharmacokinetic characteristics other than those displayed by intact Mabs and their conventionally derived fragments, is to use chemical linkage methodology to build novel constructs from naturally occurring Mab sub-units. Using this approach a number of fundamental questions relating to the modification of the in vivo behaviour of carrier molecules can be addressed. For example, what is the effect on tumour localisation of an increase in the valency of a Mab from divalent to trivalent? Or is a chemically stable form of an $F(ab')_2$ fragment less likely to find its way into the kidney and thereby reduce the problem of nuclide uptake in this organ?

Schott has produced synthetically cross-linked multivalent constructs generated from anti-tumour antibodies by chemical engineering (Schott et al., 1993). Starting with a Mab directed against the TAG-72 TAA, Fab fragments were first made by enzymic digestion and then reassembled using the cross-linking agents tris-and tetramaleimide to give tri-and tetravalent constructs respectively. The biodistribution patterns of these constructs, following labelling with the radionuclide $^{105}Rh$, were evaluated in a nude mouse tumour xenograft model system. The tetravalent construct, $F(ab')_4$, showed blood clearance similar to that of normal $F(ab')_2$ fragment but accumulation in the liver, spleen and whole body were markedly higher. Only kidney accumulation was reduced, in line with the higher molecular weight and stability of the $F(ab')_4$ construct. The high liver accumulation observed in these studies prevented further in vivo evaluation. The trivalent construct, $F(ab')_3$, revealed a very different pattern of distribution. The residence time in the circulation of this construct was found to be longer than that of the $F(ab')_2$ and a decreased level of kidney accumulation was seen. As a consequence of the longer circulating half-life, higher accumulation was seen in the tumour with the $F(ab')_3$ fragment than with the $F(ab')_2$. However, significantly higher levels were also found in the liver, spleen and whole body, with values between those of the $F(ab')_2$ and the intact Mab.

In an analogous study Quadri (Quadri et al., 1993) used commercially available cross-linking agents based on bis-maleimide to covalently cross-link the heavy chains of a Mab $F(ab')_2$ fragment (Fig. 7). The agents used were bis-maleimidomethyl ether (BMME), N,N′p-phenylene dimaleimide (PDM) and N,N′bismaleimidohexane (BMH). The stabilised $F(ab')_2$ fragments generated using there linkers were identical to the original $F(ab')_2$ in terms of immunoreactivity and serum stability, suggesting differences in in vivo distribution to be due to differences in linkage chemistry. Following labelling with the metallic nuclide $^{111}In$, all three constructs behaved similarly in vivo in a nude mouse xenograft model, showing very altered pharmacokinetics and distribution when compared with the conventional $F(ab')_2$ fragment. Tumour uptake in this system at 24 hours post-injection was 2-2.5 times higher for the constructs than for normal $F(ab')_2$, and similar to that observed with the intact Mab. This improved accumulation was presumably the consequence of the extended circulating half-lives of the constructs. In addition liver uptake for the three constructs was only around 40% of that observed with the intact Mab, offering hope of circumvention of the excessive liver uptake seen in the clinical situation when employing metallic radionuclides in conjunction with intact Mabs (Ryser et al., 1992; Bischof-Delaloye et al., 1994). This approach also seems to offer grounds for optimism in relation to a second significant problem, that of the nuclide accumulation normally seen in the kidneys

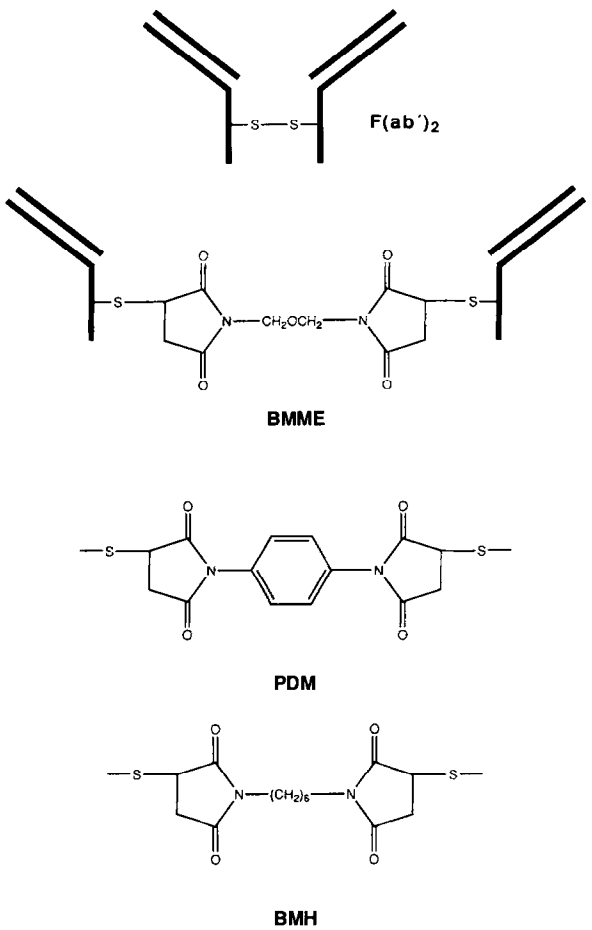



Fig. 7. Chemical stabilisation of murine $F(ab')_2$ fragments as described by Quadri et al. Following reduction of the native fragment cross-linking over the available –SH groups was performed using the commercially available reagents BMME, PDM and BMH.

when using $F(ab')_2$ fragments labelled with metallic nuclides. Much reduced levels of radiolabel were observed in the kidney in the case of the three constructs. This implies that, at least in some instances, instability of $F(ab')_2$ fragments results in reduction to the Fab form which, with a molecular weight of only 50 kDa, is subject to glomerular filtration and subsequent renal absorption. The application of such stabilised $F(ab')_2$ fragments in a clinical setting will hopefully yield interesting results.

## 4. Conclusion

The initial over-optimism in relation to the potential of RIT which accompanied the widespread introduction of monoclonal antibody technology has long-since faded. Following it came a period of consolidation, during which several key problems restricting the broad application of RIT could been identified. The various distinct strategies which have been used to try and circumvent the dose-limiting toxicity to the bone marrow have brought with them a new set of considerations. The use of alternative radionuclides to replace $^{131}I$ certainly offers advantages in terms of better radiation characteristics and more appropriate half-life. However, as shown in the case of $^{67}Cu$, $^{111}In$, $^{67}Ga$ and $^{177}Lu$, there is often a clear problem in relation to intra-cellular retention of metallic radionuclides. This reveals itself as high liver accumulation following administration of intact Mabs and as high kidney levels observed following administration of Mab fragments. The extent to which these effects occur seems also to be related to the nature of the chelator employed, with the degree of hydrophobicity being critical. Although such accumulation in non-target organs is problematic, and efforts are now being aimed at its reduction, there is a positive aspect in that nuclide accumulation within target tumour cells tends also to be raised as a result of this effect should the carrier moiety be an internalising antibody (Novak-Hofer et al., 1995). A strategy achieving a differential 'wash-out' of nuclide from non-target tissue while preserving levels within tumour is currently difficult to envisage although the application of Mabs which target non-internalising tumour antigens may offer advantages.

If a successful RIT is currently to be achieved it is clear that a real effort must be made to choose only clinical targets which lend themselves to this approach. This implies of course the selection of easily accessable and highly radiosensitive small cellular deposits. On these grounds the blood-borne malignancies have long been considered suitable targets for RIT. Additionally, disease states in which an intracavitary administration of RIC can be attempted are seen as particularly attractive. In such cases their exists the possibility of introducing the RIC into direct contact with the target tissue without significant systemic leakage and thereby avoiding adverse toxicity. A good example of such an approach would perhaps be the treatment of superficial carcinoma of the urinary bladder and towards this end studies have already been undertaken using an $^{111}In$-labelled NCRC-48 Mab which was found to give a mean tumour: normal tissue ratio of 12:1 at 2 hours post-instillation. Encouragingly, no radioactivity could be detected in blood taken 24 hours post-instillation and no HAMA response could be identified in any of the twelve patients (Kunkler et al., 1994).

Crucial to the future of systemic RIT is an improvement in the absolute amount of radioactivity which can be delivered to the target tumour without corresponding increases in dose-limiting toxicities. With this as our goal, the alteration of the pharmacokinetics of a given radioimmunoconjugate by modification of the carrier moiety appears now to hold the key to successful RIT. Whether this will best be achieved by genetic engineering or by chemical modification remains open. However, the encouraging results described involving chemically engineered Mab fragments suggest this direction may act as a useful evaluation step prior to production by genetic engineering. Whatever the nature of the constructs chosen for future application in RIT, the availablity of a panel of appropriate radionuclides, matched in terms of half-life, radiation characteristics and in vivo behaviour, will hopefully serve to enhance efficacy and improve therapeutic indices.

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## References

Adelstein, S.J. and Kassis, A.I. (1987) Radiobiologic implications of the microscopic distribution of energy from radionuclides. Nucl. Med. Biol. 14, No.3, 165–169.

Alberto, R., Bläuenstein, P., Novak-Hofer, I., Smith, A. and Schubiger, P.A. (1992) An improved method for the separation of $^{111}$Ag from irradiated natural palladium. Appl. Radiat. Isot., 43, 869–872.

Andres, R.Y., Blattmann, H., Pfeiffer, G., Schubiger, P.A., Vogt, M., Weinreich, R. and Wernli, C. (1986) Radionuclides for therapy: a review. In Schubiger, P.A. and Hasler, P.H. (Eds), Radionuclides for therapy. Hoffman-LaRoche, Basel, pp. 11–20.

Anderson, C.J., Connett, J.M., Schwarz, S.W., Rocque, P.A., Guo, L.W., Philpott, G.W., Zinn, K.R., Meares, C.F. and Welch, M.J. (1992) Copper-64-labeled antibodies for PET imaging. J. Nucl. Med. 33, 1685–1691.

Anderson, C.J., Rogers, B.E., Connett, J.M., Guo, L.W., Schwarz, S.W., Zinn, K.R. and Welch, M.J. (1993) Comparison of two bifunctional chelates for labeling $^{64}$Cu to Mab 1A3 and 1A3-F(ab')$_2$: Chemistry and animal biodistribution. Tenth International Symposium on Radiopharm. Chem. Abstracts 313.

Bischof-Delaloye, A., Buchegger, F., Delaloye, B., Smith, A., Poncioni, M., Mach, J.-P. and Schubiger, P.A. Clinical evaluation of a Cu-67 labeled anti-CEA Mab. In preparation.

Booz, J. (1984) Microdosimetric basis of individualized cancer radiotherapy by radionuclides including Auger effect and the photon activation approach. Strahlentherapie 160, 745–754.

Breitz, H.B., Weiden, P.L., Vanderheyden, J.L., Appelbaum, J.W., Bjorn, M.J., Fer, M.F., Wolf, S.B., Ratliff, B.A., Seiler, C.A., Foisie, D.C., Fisher, D.R., Schroff, R.W., Fritzberg, A.R. and Abrams, P.G. (1992) Clinical experience with rhenium-186-labeled monoclonal antibodies for radioimmunotherapy: results of Phase 1 trials. J. Nucl. Med. 33, 1099–1112.

Brown, B.A., Comeau, R.D., Jones, P.L., Liberatore, F.A., Neacy, W.P., Sands, H. and Gallagher, B.M. (1987a) Pharmacokinetics of monoclonal antibody B72.3 and its fragments labeled with either $^{125}$I or $^{111}$In. Cancer Res. 47, 1149–1154.

Brown, B.A., Davis, G.L., Saltzgaber-Muller, J., Simon, P., Ho, M.K., Shaw, P.S., Stone, B.A., Sands, H. and Moore, G.P. (1987b) Tumor-specific genetically engineered murine/human chimeric monoclonal antibody. Cancer Res. 47, 3577–3583.

Buchegger, F., Pelegrin, A., Delaloye, B., Bischof-Delaloye, A. and Mach, J.P. (1990) Iodine-131-labeled Mab F(ab')$_2$ fragments are more efficient and less toxic than intact anti-CEA antibodies in radioimmunotherapy of large human colon carcinoma grafted in nude mice. J. Nucl. Med. 31, 1035–1044.

Buchegger, F., Chalandon, Y., Pelegrin, A., Hardman, N. and Mach, J.P. (1991) Bone marrow dosimetry in rats using direct tissue counting after injection of radioiodinated intact monoclonal antibodies or F(ab')$_2$ fragments. J. Nucl. Med. 32, 1414–1421.

Cheung, N.-K.V., Landmeier, B., Neeley, J., Nelson, A.D., Abramowsky, C., Ellery, S., Adams, R.B. and Miraldi, F. (1986) Complete tumour ablation with iodine 131-radiolabeled disialoganglioside GD2-specific monoclonal antibody against human neuroblastoma xenografted in nude mice. J. Natl. Cancer Inst. 77, 739–745.

Colcher, D., Bird, R., Roselli, M., Hardman, K.D., Johnson, S., Pope, S., Dodd, S.W., Pantoliano, M.W., Milenic, D.E. and Schlom, J. (1990) In vivo tumor targeting of a recombinant single-chain antigen-binding protein. J. Natl. Cancer Inst. 28, 1191–1197.

DeNardo, G.L., DeNardo, S.J., O'Grady, L.F., Mills, S.L., Lewis, J.P. and Macey, D.J. (1990) Radiation treatment of B cell malignancies with immunoconjugates. In Vaeth, J.M. (Ed.), Frontiers of Radiation Therapy and Oncology: The Present and Future Role of Monoclonal Antibodies in The Management of Cancer. Karger, Basel. Vol. 24, pp. 194–201.

Eary, J. (1991) Fundamentals of radioimmunotherapy. Nucl. Med. Biol. 18(1), 105–108.

Epenetos, A.A., Munro, A.J., Stewart, S., Rampling, R., Lambert, H.E., McKenzie, C.G., Soutter, P., Rahemtulla, A., Hooker, G., Sivolapenko, G.B., Snook, D., Courtney-Luck, N., Dhokia, B., Krausz, T., Taylor-Papadimitriou, Durbin, H. and Bodmer, W.F. (1987) Antibody-guided irradiation of advanced ovarian cancer with intraperitoneally administered radiolabeled monoclonal antibodies. J. Clin. Oncol. 5, 1890–1899.

Franke, W.G., Wunderlich, G., Dreyer, R. and Fischer, S. (1986) Astatine 211: a powerful $\alpha$-emitter for potential application in metabolic and contact therapy. In Schubiger, P.A. and Hasler, P.H. (Eds), Radionuclides for Therapy. Hoffman-LaRoche, Basel, pp. 111–124.

Glassy, M.C., Handley, H.H., Surh, C.S. and Royston, I. (1987) Genetically stable human hybridomas secreting tumor reactive human monoclonal IgM. Cancer Invest. 447–455.

Griffiths, G.L., Goldenberg, D.M., Knapp, F.F., Callahan, A.P., Chang, C.H. and Hansen, H.J. (1991) Direct radiolabeling of monoclonal antibodies with generator-produced rhenium-188 for

radioimmunotherapy: labeling and animan biodistribution studies. Cancer Res. 51, 4594–4602.

Hahn, P.F. and Sheppard, C.W. (1947) The therapeutic use of radioactive elements in malignancy. Ann. Intern. Med. 28, 98–606.

Halpern, S., Haindl, W., Beauregard, R.N., Hagan, P., Clutter, M., Amox, D., Merchant, B., Unger, M., Mongovi, C., Bartholomew, R., Jue, R., Carlo, D. and Dillman, R. (1988) Scintigraphy with In-111-labeled monoclonal antibodies: kinetics, biodistribution and tumour detection. Radiology 168, 529–536.

Hoefnagel, C.A. (1991) Radionuclide therapy revisited. Eur. J. Nucl. Med. 18, 408–431.

Humm, J.L. (1986) Dosimetric aspects of radiolabeled antibodies for tumor therapy. J. Nucl. Med. 27, 1490–1497.

Humm, J.L. and Cobb, L.M. (1990) Nonuniformity of tumor dose in radioimmunotherapy. J. Nucl. Med. 31, 75–83.

Jones, P.T., Dear, P.H., Foote, J., Neuberger, M.S. and Winter, G. (1986) Replacing the complementarity determining regions in a human antibody with those from a mouse. Nature 321, 522–525.

Jönsson, B.A. and Strand, S.E. (1986) Auger electron emitters as therapeutic agents or sources of hazard in diagnosis — a review. In: Schubiger, P.A. and Hasler, P.H. (Eds). Radionuclides for therapy. Hoffman-LaRoche, Basel, pp. 91–99.

Johnson, J.C., Langhorst, S.M., Loyalka, S.K., Volkert, W.A. and Ketring, A.R. (1992) Calculation of radiation dose at a bone-to-marrow interface using Monte-Carlo modeling techniques (EGS4). J. Nucl. Med. 33, 623–628.

Khazaeli, M.B., Saleh, M.N., Liu, T.P., Merideth, R.F., Wheeler, R.H., Baker, T.S., King, D., Secher, D., Allen, L., Rogers, K., Colcher, D., Schlom, J., Shochat, D. and LoBuglio, A.F. (1991) Pharmacokinetics and immune response of $^{131}$I-chimaeric mouse/human B72-3 (human $\gamma$4) monoclonal antibody in humans. Cancer Res. 51, 5461–5466.

Kohler, G. and Milstein, C. (1975) Continuous cultures of fused cells secreting antibodies of predefined specificity. Nature 256, 495–497.

Kozbor, D. and Roder, J.C. (1981) Requirements for the establishment of high-titered human monoclonal antibodies against tetanus toxoid using the Epstein–Barr virus technique. J. Immunol. 127, 1275–1280.

Kunkler, R.B., Bishop, M.C., Green, D.J., Pimm, M.V. and Price, M.R. (1994) Biodistribution and imaging of 111-Indium labelled monoclonal antibody (Mab) NCRC-48 following intravesical administration for bladder cancer. Br. J. Cancer 70, Suppl. XXII, 24.

Langmuir, V.K., (1992) Radioimmunotherapy: clinical results and dosimetric considerations. Nucl. Med. Biol. 19(2), 213–225.

Lewington, V.J. (1993) Targeted radionuclide therapy for bone metastases. Eur. J. Nucl. Med. 20, 66–74.

Mausner, L.F., Srivastava, S.C., Kolsky, K.L., Mease, R.C., Joshi, V., Meinken, G.E., Kurczak, S., Chatal, J.F. and Steplewski, Z. (1993) Development and evaluation of copper-67 labeled DOTA and TETA immunoconjugates for radioimmunotherapy. Tenth International Symposium on Radiopharm. Chem. Abstracts 374.

McCall, M.J., Diril, H. and Meares, C.F. (1990) Simplified method for conjugating macrocyclic bifunctional chelating agents to antibodies via 2-iminothiolane. Bioconjugate Chem. 2, 222–226.

Milenic, D.E., Esteban, J.M. and Colcher, D. (1989) Comparison of methods for the generation of immunoreactive fragments of a monoclonal antibody (B72.3) reactive with human carcinomas. J. Immunol. Methods 120, 71–83.

Moi, M.K., Meares, C.F., McCall, M.J., Cole, W.C. and DeNardo, S.J. (1985) Copper chelates as probes of biological systems: stable copper complexes with a macrocyclic bifunctional chelating agent. Anal. Biochem. 148, 249–253.

Novak-Hofer, I., Amstutz, H.P., Maecke, H.R., Schwarzbach, R., Zimmerman, K., Morgenthaler, J.J. and Schubiger, P.A. (1995) Cellular processing of copper-67-labeled monoclonal antibody chCE7 by human neuroblastoma cells. Cancer Res. 55, 46–50.

Ohnishi, K., Chiba, J., Goto, Y. and Tokunaga, T. (1987) Improvement in the basic technology of electrofusion for generation of antibody-producing hybridomas. J. Immunol. Methods 100, 181–189.

Pochon, S., Buchegger, F., Pelegrin, A., Mach, J.-P., Offord, R.E., Ryser, J.E. and Rose, K. (1989) A novel derivative of the chelon desferrioxamine for site-specific conjugation to antibodies. Int. J. Cancer 43, 1188–1194.

Press, O.W., DeSantes, K., Anderson, S.K. and Geissler, F. (1990) Inhibition of catabolism of radiolabeled antibodies by tumour cells using lysosomotropic amines and carboxylic ionophores. Cancer Res. 50, 1243–1250.

Press, O.W., Eary, J.F., Appelbaum, F.R., Martin, P.J., Badger, C.C., Nelp, W.B., Glenn, S., Butchko, G., Fisher, D., Porter, B., Matthews, D.C., Fisher, L.D. and Bernstein, I.D. (1993) Radiolabeled-antibody therapy of B-cell lymphoma with autologous bone marrow support. N. Engl. J. Med. 329, 1219–1224.

Quadri, S.M., Lai, J., Mohammadpour, H., Vriesendorp, H.M. and Williams, J.R. (1993) Assessment of radiolabeled stabilized $F(ab')_2$ fragments of monoclonal antiferritin in nude mouse model. J. Nucl. Med. 34, 2152–2159.

Queen, C., Schneider, W.P., Selick, H.E., Payne, P.W., Landlfi, N.F., Duncan, J.F., Avdalovic, N.M., Levitt, M., Junghans, R.P. and Waldman, T.A. (1989) A humanized antibody that binds to the interleukin 2 receptor. Proc. Natl. Acad. Sci. USA 86, 10029–10033.

Rösler, H., Noelpp, U., Bläuenstein, P., Triller, K.J. and Steffen, R. (1986) Endoarterial radionuclide embolizing tumor therapy. In: Schubiger, P.A. and Hasler, P.H. (Eds). Radionuclides for Therapy. Hoffman-LaRoche, Basel, pp. 227–244.

Ryser, J.E., Jones, R.M.L., Egeli, R., Pelegrin, A., Rose, K., Kurt, A.M., Perin, M., Broquet, P.E., Ambrosetti, P., Fisch, I., Pochon, S., Donath, A., Mach, J-P. and Offord, R.E. (1992) Colon carcinoma immunoscintigraphy by monoclonal anti-CEA antibody labeled with gallium-67-aminooxyacetyldeferroxamine. J. Nucl. Med. 33, 1766–1773.

Saenger, E.L., Kereiakes, J.G., Sodd, V.J. and David, R. (1979) Radiotherapeutic agents: properties, dosimetry and radiobiologic cosiderations. Sem. Nucl. Med. 9, No. 2, 72–84.

Scheinberg, D.A., Lovett, D., Divgi, C.R., Graham, M.C., Berman, E., Pentlow, K., Feirt, N., Finn, R.D., Clarkson, B.D., Gee, T.S., Larson, S.M., Oettgen, H.F. and Old, L.J. (1991) A Phase 1 trial of monoclonal antibody M195 in acute myelogenous leukemia: specific bone marrow targeting and internalization of radionuclide. J. Clin. Oncol. 9, 478–490.

Schott, M.E., Milenic, D.E., Yokota, T., Whitlow, M., Wood, J.F., Fordyce, W.A., Cheng, R.C. and Schlom, J. (1992) Differential metabolic patterns of iodinated versus radiometal chelated anti-carcinoma single-chain Fv molecules. Cancer Res. 52, 6413–6417.

Schott, M.E., Frazier, K.A., Pollock, D.K. and Verbanac, K.M. (1993) Preparation, characterization, and in vivo biodistribution properties of synthetically cross-linked multivalent anti-tumor antibody fragments. Bioconjugate Chem. 4, 153–165.

Schubiger, P.A. and Andres, R.Y. (1987) Radionuclides for therapy: a review. In Klinische Anforderungen an die Nuklearmedizin. Schattauer Verlag, Stuttgart, pp. 15–19.

Sgouros, G. (1992) Plasmaphoresis in radioimmunotherapy of micrometastases: a mathematical modeling and dosimetric analysis. J. Nucl. Med. 33, 2167–2179.

Sguoros, G. (1993). Bone marrow dosimetry for radioimmunotherapy: theoretical considerations. J. Nucl. Med. 34, 689–694.

Sikora, K., Alderson, T., Ellis, J., Phillips, J. and Watson, J. (1983) Human hybridomas from patients with malignant disease. Br. J. Cancer, 47, 135–145.

Smith, A., Waibel, R. and Stahel, R.A. (1990) Selective immunotherapy of small cell cancer xenografts using $^{131}$I-labelled SWA11 antibody. Br. J. Cancer 64, 263–266.

Smith, A., Alberto, R., Blaeuenstein, P., Novak-Hofer, I., Maecke, H. and Schubiger, P.A. (1993) Preclinical evaluation of $^{67}$Cu-labeled intact and fragmented anti-colon carcinoma monoclonal antibody MAb35. Cancer Res. 53, 5727–5733.

Smith, A., Zangemeister-Wittke, U., Waibel, R., Schenker, T., Schubiger, P.A. and Stahel, R.A. (1994) A comparison of $^{67}$Cu and $^{131}$I-labelled forms of monoclonal antibodies SEN7 and SWA20 directed against small cell lung cancer. Int. J. Cancer 8, 43–48.

Smyth, M.J., Pietersz, G.A., Glasson, M.J. and McKenzie, I.F.C. (1986) The specific targeting of chlorambucil to tumours. J. Natl. Cancer Inst. 76, 503–510.

Spencer, R.P. (1987) Short lived radionuclides in therapy. Nucl. Med. Biol. 14(6), 537–538.

Steinitz, M., Klein, G., Koskimies, S. and Makela, O. (1977) EB virus-induced B lymphocyte cell lines producing specific antibody. Nature 269, 420–422.

Studer, M., Kaden, T.A. and Maecke, H.R. (1990) Reactivity studies of the pendant carboxylic group in a macrocyclic $Cu^{2+}$ complex towards amide formation and its use as a protein-labelling agent. Helv. Chim. Acta 73, 149–153.

Visser, G.W.M., Gerretson, M., Herscheid, J.D.M., Snow, G.B. and van Dongen, G. (1993) Labeling of monoclonal antibodies with Rhenium-186 using the MAG3 chelate for radioimmunotherapy of cancer: a technical protocol. J. Nucl. Med. 34(11), 1953–1963.

Volkert, W.A., Goeckeler, W.F., Ehrhardt, G.J. and Ketring, A.R. (1991) Therapeutic radionuclides: production and decay property considerations. J. Nucl. Med. 32, 174–185.

Wawrzynczak, E.J. (1991) Systemic immunotoxin therapy of cancer: advances and prospects. Br. J. Cancer 64, 624–630.

Wernli, C. (1986) Effective range of different radiation types: a comparative study. In: Schubiger, P.A. and Hasler, P.H. (Eds), Radionuclides for therapy. Hoffman-LaRoche, Basel, pp. 105–110.

Wessels, B.W. and Rogus, R.D. (1984) Radionuclide selection and model absorbed dose calculations for radiolabeled tumor associated antibodies. Med. Phys. 11, 638–645.

Wheldon, T.E. (1993) Radionuclide therapy of cancer: particle range and therapeutic effectiveness. Nucl. Med. Commun. 14, 408–410