

# Radioimmunotargeting: Some Recent Advances

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## ABSTRACT

Several new aspects of radioimmunotargeting for neoplastic lesions are reviewed in this paper. These include the use of single domain antibody, oncogene-coded protein and other intracellular targets so as to obtain pancarcinoma reactivity, newer methods of linking  $^{99m}\text{Tc}$  to antibodies for radioimmunoscintigraphy. The selection of candidate radionuclides for radioimmunotherapy, particularly the possible use of radiosilver and radiogold, and the strategies of enhanced tumour targeting were also reviewed.

## 1. INTRODUCTION

Over the last ten years there has been an explosion of activities in the field of radioimmunotargeting. Ehrlich's concept of 'magic bullet' selectively targeting noxious cells without harming the healthy host cells was achieved in the case of most bacteria after the epoch-making red dye-prontosil. Similar selective immunotargeting of cancer cells now appears to be possible with the use of the fine specificity of the **antigen-antibody** recognition. The advent of hybridoma technology for generating pure **monoclonal** antibodies in gram quantities, the recognition of the repertoire of tumour-associated antigen and the availability of DNA recombinant technology were some of the important links in the chain that promises to contribute to the successful immunotargeting. The amount of interest generated in this field can be gauged by the fact that at least five major companies have mounted research efforts in the development of radioimmunotherapy, and one of these was proclaiming the slogan: '**Monoclonal** Antibodies-The Vision of the Future', at the Society of Nuclear Medicine Meeting at Washington in June 1990.

The promises and problems in radioimmunotargeting have been reviewed over the last five years by several workers and our own group had reviewed the

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state-of-the-art from 1988 onward\*. The present paper will focus on some newer advances in the field of radioimmunotargeting.

## 2. SINGLE DOMAIN ANTIBODIES

The conventional dogma that antigen recognition and binding are dependent on the properties of the terminal domains of both light and heavy chains has now been challenged by the report from Cambridge<sup>3</sup> that a single domain of heavy chain is sufficient to ensure specific binding. These single domains are produced in an ingenious manner combining the two frontier areas of hybridoma technology and genetic engineering. The hybridomas are first produced in the usual **manner**<sup>4</sup>. The DNA sequences of these hybridomas are then cloned, followed by amplification by polymerase chain reaction and the use of **transfection** to place these DNA fragments by **plasmids** into *E coli* bacteria<sup>5</sup>. The bacteria then express and secrete the single heavy chain domains of desired specificity. It is much easier to maintain bacterial cultures as compared to tissue cultures of hybridoma cells. Further, these single domain antibodies may avoid the **hypersensitivity—HAMA** (human antimouse antibody) reaction, enhancing stability of the radionuclide-chelate-antibody linkage. It is also expected that these single domain antibodies will be easier to couple without distortion of antigen combining sites. The affinity of single domain antibodies is lower than that of the conventional **monoclonal** antibodies but it may be possible to overcome this by using larger quantities.

## 3. ONCOGENES AS TARGETS

As far back as 1982, Hazra, et al<sup>1</sup> and, Hazra and Saran had suggested that oncogenes, oncogene-coded receptors and mitochondria could be profitable targets for radioimmunotherapy in contrast to shed antigens or non-oncogene-coded surface targets<sup>6,7</sup>. It was suggested that this would also obviate the problems of antigenic modulation.

It was also pointed out that in view of the fewer and fewer oncogenes being **recognised** to be of importance, it should be possible to target a large number of tumours with a library of a few antibodies directed against the oncogene-coded proteins. Chan, et al have demonstrated that anti-c-myc oncogene **radioimmuno-**targeting has been successful in radioimmunoscintigraphy of lung tumours, although the possibility that the oncogenes targeted are sited in the tumour cell debris rather in the cells themselves cannot be ruled out\*. The clinical use of antibodies directed against the EGF receptors has now been reported in brain gliomas as well, where both radioimmunoscintigraphy and radioimmunotherapy have been achieved'.

## 4. PANCARCINOMA REACTIVITY

It had always seemed an obstacle that each particular tumour might need a custom-tailored antibody or a set of antibodies to target it because of endless diversity of tumour antigens. One solution suggested, as pointed out above, was the use of antibodies directed against oncogene-coded proteins or receptors. The recent work from Epstein's group<sup>8</sup> indicates another approach where antibodies were directed against nuclear **histone** antigens present in the necrotic centres of tumours. **Using**

such an antibody to the intracellular insoluble antigen may differentiate rapidly growing malignant cell tumours with high cell death rate from normal tissues with low cell death rate. However, such antibodies would also target other necrotic non-malignant lesions such as myocardial infarction or abscess, etc. However, these relatively non-specific antihistone antibodies could complement the localization of tumour-specific antibodies in the better perfused areas of the tumour, thereby, providing a double-pronged attack on the tumour. A snowball effect has also been postulated<sup>7</sup>. The antibody **labelled** with the lethal warhead will target **tumour** and kill cells exposing more targets.,

In any case, the interest now being shown in intracellular targets has borne out the prediction made eight years ago that these were the most profitable **targets**<sup>6,7</sup>. Apart from histones, intracellular fragments such as keratin and nuclear steroid receptors are also being targeted. We are engaged in an effort to develop antibodies specific to tumour mitochondria since we believe that with their differential sources of energy. they are likely to have distinctive antigens. It has also been pointed **out** recently that because of their poor rate of repair, the mitochondria of cells are much more likely to show mutations as compared to the nucleus, and the mitochondrial DNA fixes mutation 10 to 12 times faster than nuclear DNA. Time alone will tell whether this approach is going to bear fruit.

## 5. INTERNALIZATION OF AN ANTIBODY INTO A TUMOUR

This can be enhanced by the increased permeability of the malignant cells as well as by selective **irradiation**<sup>7</sup>. Matzku from Heidelberg in 1989 found on autoradiography that antibodies binding to distinct epitopes of an antigen internalize to different degrees suggesting that one could influence the internalization of the given antibody through the coordinate action of a companion antibody. The use of external **irradiation**<sup>7</sup>, localized hyperthermia, or lymphokines injected systematically or in the regional circulation to enhance the permeability of the **tumour** cell membrane differentially (i.e., more than that of the normal cell), are other approaches being explored.

## 6. STRATEGIES FOR ENHANCING SPECIFICITY

One of the major problems of radioimmunotargeting is non-specific localization in the reticuloendothelial system, bones and kidneys. Several approaches to this problem are the subject of research at various centres. Various enhancement strategies have been devised to improve radioimmunotargeting as shown below:

- (a) Radionuclidic-using <sup>123</sup>I rather than <sup>99m</sup>Tc for **radioimmunosciintigraphy**.
- (b) Radiochemical-using bifunctional chelates and macrocyclic reagents, spacers, and linkers.
- (c) Manipulating the antibody using mouse human **chimaera**, humanised antibodies, bispecific antibodies, antibody fragments, and perhaps in the future, single domain antibodies.
- (d) The two-punch approaches in radioimmunotargeting is a phrase that was used by Hazra, *et al*<sup>12</sup> for **categorising** a set of approaches that involve the primary injection of anti-tumour antibody followed by a second step in

which a warhead is conveyed to those targets where after a period of time, the primary antibody is still present in sufficient quantity while having cleared from non-specific areas of localization. Such measures include the use of a heterologous anti-IgG directed against the first antibody to enhance blood clearance or to convey a warhead; the use of radiolabelled streptavidin or biotin to link with biotin or **avidin** on primary antibody. **Paganelli**<sup>13</sup> has reported the use of 2 mg biotinylated antibody intraperitoneally followed 3 to 5 days later by **100-150  $\mu\text{g}$   $^{111}\text{In}$ -labelled** streptavidin. He has also described the reverse approach where one mg of the biotinylated antibody is followed after three days by 4 mg cold **avidin** and two days later by **200-300  $\mu\text{g}$  of biotin labelled with  $^{111}\text{In}$** . In the latter approach, pure streptavidin is not required. The former approach was suitable for intraperitoneal regional localization of the tumour whereas the latter method was suitable for intravenous systemic administration. Other approaches are the use of radiolabelled protein **A** and the use of a bifunctional antibody one arm of which looks at a tumour antigen while the other arm looks at a warhead transporting molecule. Somewhat analogous to this two-punch approach is the use of boron-labelled primary antibodies which are later irradiated by thermal neutrons for local conversion into lithium and release of alpha particles.

- (e) Manipulating the tumour by irradiation or lymphokines or increasing vascularity .

Nardo (personal communication) has pointed out that the use of linkers between the antibody and the chelate, which are subjected to intracellular attacks by enzymes in hepatocytes, can release the chelates so that non-specific **reticulo-endothelial** system hepatic accumulation is reduced. **Paik**<sup>14</sup> has described how, using **labelled** disulphide and diester linkages between the antibody and  **$^{111}\text{In}$ -DTPA**, it was possible to enhance the target to blood ratio by a factor of 15. This **may** also be **important in the visualization** of hepatic metastasis of a tumour where the specific localization of the **antibody** in the metastatic lesion must be differentiated from non-specific localization in the normal liver. The disulphide linkage is easily metabolised by the healthy liver. The diester linkage is similarly easily metabolised in the kidney and this could be of value in reducing the kidney activity of  **$^{111}\text{In}$ -labelled** Fab fragments.

In order to reduce the bone localization the same group has described the use of macrocyclic chelating agents such as **p-bromoacetamidobenzyl-DOTA** (BAD) whose capacity to stably link to  **$^{90}\text{Y}$**  is far greater than that of older bifunctional chelates such as DTPA cyclic anhydride. **12- and 14-membered** rings have been developed for this purpose. 2-imidothalene is used as a spacer between the antibody and the chelate.

In order to prepare a radiopharmaceutical free of non-specificity bound yttrium, the BAD  **$^{90}\text{Y}$**  chelate is prepared, separated from unchelated yttrium and then conjugated with the antibody instead of using the pharmaceutical prepared by conjugating BAD with the antibody and then post-labelling with the radiometal. The spacer permits labelling avoiding steric hindrance.

## 7. ISOTOPIC VS NON-ISOTOPIC TUMOUR IMAGING

Eckelman **has listed** the advantage of radio-labelled antibodies over CT and NMR modalities of tumour imaging". With radiopharmaceuticals, the concentration of the image enhancing agent is less than 0.1  $\mu$  mole/kilo while the figures for iodinated and paramagnetic contrasts are 1000-10,000 and 10-1000  $\mu$  mole/kilo respectively. Since the latter two need contrast agents at relatively high concentrations, they are 'not' suited for measuring tissue perfusion changes and specially not well suited for measuring biochemical processes: Despite the poor resolution of planar gamma cameras as compared to CT and NMR, useful images can be obtained as it measures the changes in tissue perfusion or biochemical parameters. Isotopic diagnosis is also superior for whole body survey for extensive metastases, for predicting distribution of the subsequent therapeutic dose of the antibody and for staging and monitoring of therapeutic response.

**SPECT** studies have higher resolution than planar gamma camera but it requires 5-10 per cent of the injected dose in the target organ which is a far cry from 0.1 per cent antibody localization currently achieved.

## 8. TECHNETIUM'S COMEBACK FOR RADIOLABELLING ANTIBODIES

About a decade ago it was considered difficult to label antibodies stably with  $^{99m}\text{Tc}$  and therefore  $^{123}\text{I}$ -labelled antibodies came into use for radioimmunoscintigraphy. However,  $^{123}\text{I}$  is only available from high energy cyclotrons, and therefore is not a very practical agent for many centres as compared to  $^{99m}\text{Tc}$  available everywhere from generators.  $^{131}\text{I}$  is not very suitable for radioimmunoscintigraphy as contrasted to therapy. Better methods for making  $^{99m}\text{Tc}$ -labelled antibodies have now been developed. The **Schwarz** technique describes the method of protein modification by reducing with a thiol reagent (**2-mercaptoethanol** or **2-aminoethanethiol** hydrochloride) which essentially facilitates  $^{99m}\text{Tc}$  labelling using a standard bone imaging kit, and provides radiolabelled monoclonal antibody suitable for routine clinical use<sup>16</sup>. The approach has been adopted by Granowska, *et al* (London) using **PRIA3** antibody for imaging coldrectal carcinoma. The identification of tumour deposits as early as 4 hours after injection, **confirms** the viability of early imaging with a  $^{99m}\text{Tc}$  product. Similarly,  $^{99m}\text{Tc}$  labelling has been used with **BW431/26** monoclonal antibody for imaging liver metastases from colorectal cancer (Göttingen), **BW575/9** for neuroblastoma (Frankfurt), **BW494/32** for small bowel and ovarian tumours (Aachen) and 225.285 for imaging melanoma (Leiden). Clearly, the availability- of technetium antibodies will open up immunoscintigraphy to the majority of nuclear medicine departments. D.M. Goldenberg (CMMI, Newark, USA) has reported his initial clinical results with a new  $^{99m}\text{Tc}$  antibody kit developed by Immunomedics, Inc. (Warren, USA) which is a one vial, one step, five-minute labelling method that involves direct conjugation of the radionuclide to the Fab segment of the antibody achieving planar and SPECT imaging of tumour within two to five hours without background radioactivity being present, and lesions as small as 3 mm were **revealed**<sup>17</sup>.

The following are the three general approaches now available for linking technetium to antibodies:

- (a) Direct labelling using the aminoacids of **IgG** to complex technetium. Such labelling was earlier reported to be weak and unstable. High affinity labelling is dependent on the reduced disulphide groups which can be

achieved in the presence of reducing agents such as stannous ions, and more recently sodium dithionite and ascorbic acid. The latter appears to be the most promising<sup>18</sup>. Low affinity binding of  $^{99m}\text{Tc}$  to other sites of antibody can be prevented by the presence of excess of DTPA, and therefore it has been suggested that direct radiolabelling of  $^{99m}\text{Tc}$  on the immunoglobulin should always be done in the presence of DTPA so that there is no loosely bound  $^{99m}\text{Tc}$ .

- (b) The second approach involves the use of bifunctional chelates linking the protein to the radiometal. Unfortunately there can be a competition between binding of the  $^{99m}\text{Tc}$  by the covalently bound chelating agents and the strong or weak direct reaction of the  $^{99m}\text{Tc}$  with the antibody. It is therefore difficult to achieve high radiochemical purity.
- (c) In the pre-labelled ligands-approach, the bifunctional **chelate** is first **labelled** with the radiometal and later the pre-labelled ligand is bound with the antibody. This eliminates any direct labelling of the protein by the  $^{99m}\text{Tc}$ . This yields the most stable bonds, but only 4 per cent of the  $^{99m}\text{Tc}$  bonds to **IgG**. The pre-labelled ligand approach thus reduces the chances of  $^{99m}\text{Tc}$  leaching off from target homed antibody which would have enhanced background.

The strong direct binding and also some chelation methods are dependent on the presence of sulphhydryl (**SH**) groups. A number of methods have been described using 2-iminothiolate to introduce a **SH**group in chelates or in the antibody for this purpose.

## 9. NEWER RADIOISOTOPES AS CANDIDATE RADIONUCLIDES FOR THERAPY

The oldest workhorse for radioisotopic therapy in thyroid carcinoma has been  $^{131}\text{I}$  and I remember being fascinated by Brig. Mazumdar dispensing therapy doses. In 'view of the ease of incorporating iodine in antibodies,  $^{131}\text{I}$  was used for radioimmunotherapy 'as well. However,  $^{131}\text{I}$  has several disadvantages-notably its gamma radiation which contributes to whole body dose and non-target dose. Several other isotopes are therefore under investigation in the West for radioimmunotherapy, notably  $^{90}\text{Y}$  and  $^{153}\text{Sm}$ . Ganso (NDI, Bethesda, USA) has been looking at the use of bismuth and lead. Apart from these beta emitters, the alpha emitter  $^{211}\text{At}$  has also attracted attention. Unfortunately many of these isotopes are unsuitable in our opinion for radioimmunotherapy. A list of candidate radionuclides for radioimmunotherapy has been prepared and classified according to half-life, and suitability for use in India (Tables 1 and 2).

Since the accumulation of antibody in the tumour takes a finite time of the order of three days, short-lived isotopes such as bismuth are of no value. We feel that radionuclides produced from the same isotopes cannot be carrier-free and would therefore pose problems in linking to chelates. However, despite these problems,  $^{188}\text{Re}$  and  $^{153}\text{Sm}$  are being advocated in certain western centres as the specific activity obtained is high enough to confer the desired dose.

For therapy in India, there are additional problems because of non-availability of a cyclotron for medical purposes, and the large distances between the production facility and the potential users throughout the country. Hazra, et al therefore considered radiogold and radiosilver as being particularly suitable for our country".

Table 1. Classification of radionuclides on the basis of half-life

Half-life	Groups								
	0	I	II	III	IV	v	VI	VII	VIII
Less than 24 hrs				$^{255}\text{Fm}$	$^{212}\text{Pb}$	$^{212}\text{Bi}$		$^{188}\text{Re}$ $^{211}\text{At}$	$^{109}\text{Pd}$
24 to 48 hrs			$^{140}\text{La}$ $^{153}\text{Sm}$	$^{143}\text{Ce}$ $^{166}\text{Ho}$	$^{121}\text{Sn}$	$^{72}\text{As}$ $^{76}\text{As}$ $^{77}\text{As}$		$^{82}\text{Br}$	$^{105}\text{Rh}$
48 hrs to 1 week	$^{133}\text{Xe}$	$^{67}\text{Cu}$ $^{198}\text{Au}$ $^{199}\text{Au}$	$^{115}\text{Cd}$	$^{47}\text{Sc}$ $^{149}\text{Pm}$ $^{175}\text{Yb}$	$^{90}\text{Y}$ $^{161}\text{Tb}$ $^{177}\text{Lu}$ $^{238}\text{Np}$		$^{127}\text{Sb}$ $^{210\text{m}}\text{Bi}$	$^{99}\text{Mo}$ $^{132}\text{Te}$	$^{186}\text{Re}$ $^{66}\text{Ni}$ $^{97}\text{Ru}$
1 to 2 weeks		$^{111}\text{Ag}$		$^{143}\text{Pr}$	$^{225}\text{Ac}$ $^{169}\text{Er}$	$^{117\text{m}}\text{Sn}$			$^{131}\text{I}$
More than 2 weeks				$^{114\text{m}}\text{In}$			$^{32}\text{P}$		$^{125}\text{I}$

Table 2. Classification of isotopes on the basis of suitability

Cyclotron production	Suitable			Unsuitable					
	Same isotope production	Bone seeker	High gamma	Chemically-toxic	Very short $T_{1/2}$	Gaseous state			
$^{111}\text{Ag}$ $^{67}\text{Cu}$	$^{82}\text{Br}$ $^{90}\text{Y}$ $^{99}\text{Mo}$	$^{32}\text{P}$ $^{47}\text{Sc}$	“sc	$^{72}\text{As}$ $^{76}\text{As}$	$^{188}\text{Re}$	$^{133}\text{Xe}$			
$^{199}\text{Au}$ $^{72}\text{As}$	$^{97}\text{Ru}$ $^{109}\text{Pd}$ $^{115}\text{Cd}$ $^{117\text{m}}\text{Sn}$ $^{121}\text{Sn}$ $^{140}\text{La}$ $^{143}\text{Ce}$ $^{153}\text{Sm}$ $^{169}\text{Er}$ $^{173}\text{Yb}$ $^{177}\text{Lu}$ $^{186}\text{Re}$ $^{188}\text{Re}$	$^{90}\text{Y}$ $^{149}\text{Pm}$ $^{151}\text{Pm}$ $^{153}\text{Sm}$ $^{161}\text{Tb}$ $^{225}\text{Ac}$ $^{143}\text{Pr}$	$^{132}\text{Te}$ $^{198}\text{Au}$	$^{77}\text{As}$ $^{109}\text{Pd}$ $^{127}\text{Sb}$	$^{212}\text{Bi}$	$^{211}\text{At}$			

$^{111}\text{Ag}$  was prepared from  $^{110}\text{Pd}$  by n-gamma process and separated by Dowex anion exchange chromatography to yield a carrier-free product.  $^{199}\text{Au}$  was similarly prepared from  $^{198}\text{Pt}$  and purified by solvent extraction. Incorporation of  $^{111}\text{Ag}$  in monoclonal antibodies poses problems as it is monovalent and therefore not suitable for conventional bifunctional chelates. Certain cryptate (crown ethers) macrocyclic agents have been suggested for monovalent cations. Certain other hetero-bifunctional agents such as para-aminobenzoic acid (PABA) and N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) are being explored for this linkage utilising the affinity of silver for sulphur, this being a soft-base-soft-acid interaction. Methods for the stable linkage of gold to immunoglobulin are available for in vitro use and their adaptation for in vivo purposes is under study.

In June 1990, Srivastava from Brookhaven has presented a poster at the Princeton Radiopharmaceutical Meeting displaying great interest in radiogold and its preparation

from platinum. He has also suggested the creation of the gold **adduct** of 11 gold atoms for linkage to antibodies. If this succeeds, a very large dose can be delivered per antibody molecule.

## 10. CONCLUSION

A birds eye view of several new facets in the exciting area of radioimmunotargeting has been provided in this paper. It is clear that the problems are great and the path is strewn with obstacles, but the sun of successful radioimmunotherapy for carcinoma is glimmering on the horizon.

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