

The present and future of radiolabelled antibodies in oncology

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This presentation is about the radioisotope, the epitope and the monoclonal antibody. The clinical questions that radioimmuno-scintigraphy must answer are the following. It must be able to demonstrate subclinical and subradiological disease. It must be able to demonstrate recurrences before elevation of serum markers. It must be able to show the location of the site of recurrence when serum markers are elevated especially when radiology is negative. It must be able to demonstrate that a clinical or radiologically evident mass is viable tumour and not post surgical fibrosis and it must be able to evaluate the effect of chemotherapy without second look operation.

The technical requirements for radioimmuno-scintigraphy are an antigen specific to the cancer, a monoclonal antibody against this antigen, the best radiolabel appropriate to the kinetics of uptake of the antibody by the cancer, a radiolabelling method that preserves immunoreactivity and a gamma camera imaging system optimised for the radiolabel and the site of the cancer.^{1,2} There are several classes of antigens against which monoclonal antibodies can be made and radiolabelled. Some are normal epithelial surface antigens, some are cancer derived antigens, some are oncofoetal antigens, enzyme antigens, viral antigens, idiotypic antigens and even synthetic antigens.

The choice of radionuclide label is important and there has been an evolution from ¹³¹I to ¹²³I to ¹¹¹In-dium to ^{99m}Tc. There are advantages and disadvantages to each. Compare images of a four-year old child with neuroblastoma studied with one millicurie of ¹³¹I antibody UJ13A, with the use of the same activity of ¹²³I. With 1 mCi of I-131, one would hardly see the tumour even on a three day image.

With 1 mCi I-123 labelled UJ13A, the same tumour shows uptake by four hours. This shows that there is no delay in the antibody arriving at the tumour. The visualisation of the tumour depends on the signal. A poor signal such as I-131 means late visualisation. A good signal, such as I-123 gives early visualisation. Thus, the shorter the half life, the greater the administered activity, the higher the count rate, the less the noise from the signal, the earlier the detection and the smaller the lesion detected¹. In fact, as Professor Buraggi showed, the optimal time for imaging antibody is between six and twenty-four hours.³

OVARIAN CANCER

Ovarian cancer is an important disease which presents late in women and radiological techniques are not very helpful. Many antibodies may be used against ovarian cancer. We have used, for example, HMFG2 which reacts with a surface epithelial antigen.⁴ It is the architectural disruption of cancer that exposes this antigen to blood and allows a high uptake of antibody. The patient protocol is as follows: The patients are selected by the surgeon; the test is explained to the patient; informed consent is obtained; no skin test is done as it causes sensitisation, but patients with allergy to foreign proteins or with severe allergies are excluded. The protocol for the different radiolabels is very similar. After injection of the labelled antibody, usually half a milligram only, images of the upper and lower abdomen and pelvis, anterior and posterior are taken with early images at ten minutes, further images at six hours and late images at twenty four hours. Single photon emission tomography is performed by about six hours and the next morning.

Consider a typical example of an ovarian 'cyst' imaged at ten minutes and at four hours. At first a defect is seen obscuring the aorta and iliac vessels. At four hours something that was not present on the

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earlier image becomes evident. This the specific uptake of the antibody by the cancer in the wall of the cyst.⁴ If one were to subtract the ten minute image from the four hour image, an image approximating to the operative specimen would be obtained. This is the basis of the technique that we introduced for subtracting the early image from the late image.⁵ There are no problems of attenuation because the images are obtained with the same radioisotope.

As Professor Hisada will remember, when we first came to Japan in 1982 we demonstrated a technique of tomography, the J & P tomoscanner. With this system, we undertook the first studies, as far as we are aware, of single photon emission tomography of antibody uptake in ovarian cancer.⁶ We have now refined this subtraction technique into one called Kinetic Analysis with Probability Mapping.⁷ It is fortunate that the time dependency of biological processes is one of nuclear medicine's greatest assets. If a series of images are taken over a period of time and then analysed in such a way to identify the temporal changes of the count rate distribution then sites of specific uptake will be thereby identified. Typical of non specific uptake is that after the initial distribution, the background and vascular activity in a lesion decreases with time. Typical of specific uptake is that the uptake is increasing with time over the first 24 hours, as with antibody uptake in an ovarian cancer. Thus the time relationships of uptake tell whether there is specific uptake or non specific uptake. This technique is used with the change detection algorithm and the probability mapping to demonstrate tumours in the abdomen that cannot seen on the conventional image. Half centimetre tumours confirmed at operation may be detected in this way.⁸

If a series of images are taken with a monoclonal antibody labelled with Indium-111 antibody uptake is evident already at four hours. It is not necessary to wait up to ninety six hours to see the antibody taken up although In-111 allows imaging up to this time. The question is whether you have a good enough signal to show the tumour. The main problems with Indium-111 is its cost, its high radiation dose, the high bone marrow and liver uptake and even gut uptake, and the fact that it has to be ordered. Conversely with ^{99m}Tc imaging can start shortly after the request has been received. It is cheap, gives a low radiation dose and low liver, marrow and bowel uptake.

Many attempts have been made to label antibodies with Technetium-99m. The first method of Rhodes & Burchiel⁹ was rather non specific with Technetium-99m being weakly bound. The breakthrough came with the technique of Schwarz and Steinstrasser in 1987¹⁰ using 2-mercaptoethanol as a reducing agent.

The antibody has SS bonds at the hinge region. Using the reducing agent 2 mercapto ethanol, the SS bridges are broken to make SH groups. Then a normal bone scanning kit provides tin and then pertechnetate. The Technetium-99m links to the SS groups at the hinge to create a stable bond. Using this method there is no free Pertechnetate uptake in the thyroid region.

We are presently working with a Technetium-99m labelled monoclonal antibody, a new one called SM3.^{11,12} This reacts with the mucin core protein epitope of five amino acids as compared with the HMFG2 which reacts with a three amino acid epitope. This makes SM3 more specific for cancer. SM3 has a malignant to benign ratio of 17.0 compared with HMFG1 of 9.6 and HMFG2 of 6.6 showing it is better designed for distinguishing malignant from benign tumours, such as following ultrasound of the pelvis. Our results in gynaecological tumours show that the specificity with Indium-111 antibody was poor (73%), a little better with ¹²³I antibody (85%) and best with the Technetium-99m antibody (94%), and the accuracy is best with the Technetium-99m labelled antibody. The clinical role of radio-immunoscintigraphy in ovarian cancer allows one to make diagnosis of cancer when ultrasound is positive for a pelvic mass or when serum markers are positive. We can help to stage the cancer. We can look at the effects of chemotherapy and we can detect recurrences that cannot be detected by X-ray CT or clinically. We can also use it as the prelude to therapy.

MELANOMA

Radioimmunoscintigraphy has been undertaken in melanoma, particularly by the Italian group using an antibody against a high molecular weight antigen present in melanoma cells.^{3,13} We have used this technique in ocular melanoma imaging the uptake using ^{99m}Tc labelled 225.28S.¹⁴ The sensitivity and accuracy of this technique was around 95%. Ultrasound was only able to say that an eye tumour was melanoma in 17%. Another eye tumour, negative for melanoma imaging, was positive for imaging for ovarian cancer and was shown to be a metastasis in the eye from ovarian cancer.¹⁵

COLORECTAL CANCER

There are three different sorts of antigen for colorectal cancer. There is a de-differentiation protein such as CEA with anti CEA monoclonal antibodies. There are antigens derived from the malignant cells for which B72.3 is an example of the antibody. There are antibodies derived against normal cell surface

antigens such as the one we use called PR1A3 or just 1A3.¹⁶ One problem with the anti-CEA antibody which may be shown on an image of a surgical specimen is that, as well as uptake in the tumour, there is uptake in normal lymph nodes because CEA diffuses into normal lymph nodes.¹⁷ The smallest tumour we have detected is 1.2 grams. The antigen against which 1A3 reacts is fixed and does not diffuse into the lymphatics or the blood stream so there is no uptake in the normal lymph nodes.

For rectal cancer, a squat view helps to distinguish anterior bladder activity from tumour in the presacral region. A typical liver metastasis is one where at ten minutes you have a big defect. At six hours you see the defect is getting smaller and at twenty four hours even smaller as the antibody is taken up round the edge of the metastasis. A small liver metastasis shows no uptake or defect on the early image and specific uptake on the late image. In the operating theatre a special probe sensitive for Technetium-99m is used. The patient is imaged on the morning of operation at 24 hours and then the surgeon uses the probe to detect the cancer in the abdomen. That the tumour bed is free of cancer after resection of the bowel can be confirmed in this way.

We are at present doing a study to look at patients with Dukes' C cancer one year after operation, before serum markers are raised, because serum markers are poor indicators of the presence of small primary or recurrent cancer. Preliminary data shows that a patient with no symptoms and a normal CEA has detectable tumour by radioimmunosciintigraphy. Overall, we have an accuracy of 95% using RIS in the diagnosis of primary and recurrent colorectal cancer and liver metastases. We can answer the important clinical questions, demonstrating subclinical and subradiological disease, recurrences before rising serum markers and localisation if the serum markers are raised. We can demonstrate that a mass seen on CT scan or ultrasound is viable tumour and not post surgical fibrosis and demonstrate the extent of recurrence for radiotherapy planning.

People ask about human antimouse antibodies, HAMA. We have had one such reaction. With HAMA all the activity appears in the liver very quickly. The key to avoiding HAMA is to use less than one milligram of antibody. Then clinical reactions are rare, about one in a thousand, although some HAMA response is seen biochemically. Only after the third injection will about 10% of diagnostic studies be affected. However, the HAMA response is very important problem for therapy because large amounts of monoclonal antibody have to be administered. Therefore there is a move to humanised antibodies. In this technique, the CDR segments,

complementarity determinant regions, are grafted on to the human antibody. We were the first to use such antibodies against placental alkaline phosphatase labelled with ^{99m}Tc for imaging for ovarian cancer.¹⁸ Genetic engineering may be used to improve the affinity of the antibody, to reduce the antigenicity of the mouse monoclonal antibody by making it human, to develop bispecific antibodies and to incorporate a Technetium-99m specific binding site into the antibody.

For imaging, we can conclude that Technetium-99m labelled antibodies are the most cost effective of radioimmunosciintigraphy for a routine nuclear medicine department providing a service for patients suffering with cancer. Technetium-99m labelled genetically engineered antibody-like molecules will provide this service in the future.

RADIOIMMUNOTHERAPY

The treatment of malignant disease after its primary treatment by surgery is either by external beam radiotherapy which is effective but local, by chemotherapy which is effective but not selective, and by nuclear medicine techniques, either dependent on cell function, receptor binding or antigen antibody uptake. They are systemic and selective but are they effective? There are several specific requirements for radioimmunotherapy over radioimmunosciintigraphy. A much larger amount of antibody has to be taken up. The radiotherapy labelled antibody has to be able to penetrate the whole of the tumour whereas for imaging only uptake on the surface of the tumour is needed. The antibody has to be fixed to the cell for a much longer time than for imaging. It is also required that the normal tissue has a much lower uptake than the tumour tissue. This is the main problem for radioimmunotherapy.

The first approach was to use antibody therapy locoregionally to avoid the irradiation of normal tissues, for example, by the injection of radiolabelled antibody into the abdomen for the treatment of malignant ascites. The results of this approach were not at all satisfactory. We have shown that the tumour is not just in the peritoneum but spreads under the peritoneum where the antibody cannot gain access to it. One area where local antibody therapy is successful is in cerebral malignant meningitis as shown by Dr. Coakham and his colleagues in Bristol in England.¹⁹ The antibody is injected into the cerebral spinal fluid and images of the tumour are obtained. Because the antibody does not escape, they have shown this approach to malignant meningitis has improved the survival from two or three months to, in some cases, up to two years, proving that the intracavity approach can

work. Recently, direct injection of radiolabelled antibodies into brain tumours has been undertaken and also shown to be effective.²⁰

For malignant ascites, people have turned to a different radiolabel, Yttrium-90, instead of ¹³¹I, with a half life of 64 hours, a pure beta emitter with an energetic beta emission. One can image the Bremstrahlen radiation crudely to determine tumour uptake. The problem with Yttrium-90 chelated by DTPA to antibody is that it falls off the antibody and gets into the blood stream and binds to bone, irradiating bone marrow. An infusion of EDTA so as to bind the Yttrium-90 enables it to be excreted in the urine. This reduces the irradiation of the normal bone marrow. Almost twice the activity can be given into the peritoneum with a halving of the marrow toxicity. However, the results are still very poor for solid tumour, except in one circumstance, in patients with negative peritoneal washings, from a study by Epenetos.²¹ In this adjuvant situation the patients had a 5 year, 95%, survival compared with the usual 5 year survival of only 25%.

If radiolabelled antibodies are to be used for therapy, the lessons of nuclear medicine from the past must be remembered.²² Thyroid cancer is our model and there are six lessons. We confirm the *in vivo* tumour uptake before therapy. We avoid interfering drugs. We use a long lived beta emitting radionuclide. We give a high activity and repeat it. We have a rapid excretion pathway to reduce the normal tissue irradiation. We avoid immunosuppression which allows a normal immunological response to the radiodamaged cells. The success of Strontium-89 therapy in prostate cancer again teaches us that we can use a very long lived beta emitter with a half life of 50.5 days and get a good therapeutic response, and this can be done as an outpatient. This shows us that we can use long lived beta emitters for cancer therapy. Using radionuclides, we spare the immunological competent cells. We also damage the cancer cell surface with our irradiation creating a targeted host response and we also create antimouse or antihuman antibodies giving an idiotypic-1 and an idiotypic-2 reaction. It is the combination of targeted radiotherapy and host response that is the reason for nuclear medicine's success in therapy.

Which radionuclide shall we choose? For the intracavity approach we want a high energy, short lived radionuclide so that it delivers its radiation before it escapes to the blood stream. We will use then for that: ⁹⁰Yttrium or ⁶⁷Copper or ¹⁵³Samarium or ¹⁸⁶Rhenium. If we want to give long lived therapy for solid tumours, we want to have a long lived radionuclide such as Indium-114m or ³²P Phosphate with a fourteen day half life.

We need to rethink the radiobiology of radioimmunotherapy. Traditionally, external beam therapy is extrapolated from a high dose, short blast to long lived low dose effects and as a result there is an extrapolation of the concepts of repair from a high dose to those from a low dose. External beam therapy causes double strand breaks in DNA but our slow internal irradiation disrupts the transfer of information from the cell surface to the nucleus and the repair process is quite different. It is the subversion of cellular repair not breaking the DNA strands that is the way to successful radioimmunotherapy. The higher the frequency of the elemental doses and the longer the repetition through a long half radionuclide, the more the effectiveness in preventing the repair process. Lastly, long lived radiation means that the cells of the tumour are likely to enter the radiosensitive G2 phase during the irradiation period so that we will have a better radiosensitivity with a long half life radionuclide than with a short half life one.

With external beam radiotherapy it has been shown that fractionation of the dose is more effective. Even hyperfractionation two doses per day is the best giving twice the effect for the same radiation as a single dose. With radionuclide therapy we are giving continuous fractionation so we are more effective than external beam therapy dose for dose. Lastly, the calculation of radiation effect by MIRD assumes a uniform distribution of dose as with external beam therapy but for radioimmunotherapy we have selective uptake at the clonogenic cancer sites and so again we are twice as effective dose for dose as external beam therapy. When we look at the choice of radionuclide, Auger electrons could be used but the radioactivity must be internalised which may be difficult. You could use alpha particles but antibody labelling is difficult and their half life is usually less than a day, and so our best choice is to use beta rays with a long half life, high energy and no gamma ray emission.

To summarise, the key to radioimmunotherapy is time.²³ For any given antibody, the residence time in the blood determines the supply to the tumour and the radiation dose to the critical organ. For any activity taken up, the residence time in the tumour determines the radiation dose to the tumour. For any given uptake, the ratio of the residence times in the tumour to the residence time in the critical organ will determine the therapeutic ratio. Therefore, a long lived radiotherapy nuclide with a half life in weeks may be more effective than a short lived one whose therapeutic ratio depends primarily on relative uptake. So long lived resident radioimmunotherapy requires a two or three stage technique. It requires that we give an antibody that is unlabelled which is

then taken up by the tumour and cleared from the body by normal metabolism and then this is followed by a therapy ligand which finds the antibody on the tumour. This two stage approach can be made using bifunctional monoclonal antibodies or by using the biotin streptavidin biotin approach. The avidin biotin system has a very strong binding affinity and the technique then is to prepare a biotinylated antibody which reacts with the tumour, labelled with Technetium-99m to prove tumour uptake. Then avidin is injected alone which binds to the antibody on the tumour and helps to clear unbound antibody into the liver and reticuloendothelial system for metabolism. Then a small radiotherapy biotin ligand is injected which binds the antibody streptavidin complex on the tumour to deliver its therapy.²⁴ Alternatively, we can use a bifunctional antibody where one part of the antibody binds with the cancer antigen the other part binds with the radiotherapy ligand. Bispecific antibodies may be made using the quadroma approach, by chemical linkage of Fab fragments or by linked single chain antibodies or by the direct synthesis of molecular that mimic antibodies.

We think that the advantages of ³²P for radioimmunotherapy are overwhelming. It has a reasonably long half life of fourteen days, a pure beta emission which is moderately energetic and it has a prodrug effect. If ³²P antibodies are metabolised on the tumour site, ³²P Phosphorus will be liberated which will be able to diffuse into the cell and be incorporated into the RNA or DNA of the cancer cell to continue irradiating the tumour. It is more effective than Yttrium-90 and there is a long established use of ³²P in Polycythaemia which makes the regulatory problems much less. It is suitable for two or three stage radioimmunotherapy. The Kemptide sequence, which is seven amino acids, can be used to bind ³²P to an antibody²⁵ and it can be genetically engineered. For the two stage approach, a bifunctional genetically engineered antibody is required, one Fv reacting with the cancer antigen and the other Fv reacting with the ³²P Ligand. The protocol would be to prepare the bifunctional antibody and label it with Technetium-99m to prove uptake by the tumour. One would wait three days, inject the ³²P ligand which would target the bifunctional antibody on the tumour to give uptake of the radiotherapy ligand. The ³²P ligand that was not bound, being a small molecule, would be excreted rapidly through the kidney thereby giving a high therapeutic ratio. ³²P that is released locally on metabolism of the antibody would enter the tumour cells and continue the irradiation process.

The genetic engineering process as described by Milstein and Winter²⁶ shows two directions—one

towards the CDR grafted human reshaped antibody and one towards smaller and smaller binding fragments of the antibody, the smallest being the **molecular recognition unit** of only a few amino acids. One may genetically engineer a Technetium-99m binding site using the amino acid sequence KCTTCA which will hold Technetium-99m. This has been derived from Metallothionein. That tumours may be imaged with small molecules is shown by the use of ¹²³I Octreotide, a somatostatin receptor binding agent. We have imaged an insulinoma within one minute of injecting ¹²³I Octreotide and yet this molecule only has eight amino acids.²⁷

It may be appreciated that in the electron clouds of molecular interaction in three dimensions there is a convergence: the enzyme and its substrate, the hormone and its receptor and the antibody and its epitope. In three dimensions, these are just a binder-bindee relationship, common to hormones and receptors, enzymes and substrates, antigens and antibodies, biologically active peptides and receptors, all just binders and bindees. The future then, is in these designer molecules.

One example of an alternative future molecule is the **antisense oligonucleotide**. It has already been shown that the c-myc protooncogene is important in smooth cell proliferation, for example in causing atheroma. This growth can be suppressed by using an antisense oligonucleotide. There is the potential for using radiolabelled antisense oligonucleotides both in diagnosis of oncogene proliferation which is a sign of cancer and in therapy using small internalised radionuclide therapy antisense agents.

In conclusion, in the present state of radioimmunotherapy, the percent of the injected dose that is taken up is still too low, the therapeutic ratio is much too low and therefore intracavity therapy is used in some special cases. We predict that we will not use ¹³¹I or alpha emitters in the future and that we will not improve the accumulation of antibody in tumour very much, for example, by using cytokines. It has already been shown by Paganelli²⁴ that the sandwich technique works in vivo, so we can perform two or three stage radioimmunotherapy using a long lived pure beta emitting radionuclide which will give us the correct therapeutic ratio. Genetically engineered bispecific CDR grafted designer molecules incorporating a specific radiolabelling site for imaging, a therapy ligand binding site and a tumour specific binding site of improved affinity will be successful in the future.

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