PROSTATE CANCER

EXPERIMENTAL RADIOIMMUNOLOCALISATION

AND

CLINICAL STAGING WITH BONE SCINTIGRAPHY

ANDERS RYDH
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AKADEMISK AVHANDLING

Som med vederbörligt tillstånd av Rektorsämbetet vid Umeå Universitet för avläggande av doktorsexamen i medicinsk vetenskap kommer att offentligen försvaras i Röntgens föreläsningssal, Norrlands Universitetssjukhus, by 3A, 2 tr, fredagen den 18 februari 2000 kl 09.00

Av

Anders Rydh
Leg läkare

UMEÅ 2000
TO THE PATIENTS
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AIMS OF THE STUDY

The overall aims of this thesis were the following:

- to evaluate the targeting properties of a new monoclonal antiprostate antibody, MAb E4, in an experimental animal model using xenografted human prostate cancer
- to evaluate the accuracy of bone scintigraphy in clinical practice

The specific aims were the following:

- to investigate the antibody kinetics and dosimetry of radiolabelled MAb E4
- to evaluate the influence of different doses and dose intervals of $^{125}\text{I}$-labelled MAb E4 on the tumour radiation dose
- to study the therapeutic efficacy of $^{131}\text{I}$-labelled MAb E4
- to investigate the relation between different levels of S-PSA and the outcome of bone scintigraphy
- to evaluate the value of quantitative bone scintigraphy in prostate cancer
ORIGINAL PAPERS


## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AP</td>
<td>Antero-posterior</td>
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<tr>
<td>BPH</td>
<td>Benign prostatic hyperplasia</td>
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<tr>
<td>BS</td>
<td>Bone scintigraphy</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>DRE</td>
<td>Digital rectal examination</td>
</tr>
<tr>
<td>HAMA</td>
<td>Human anti-mouse antibodies</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>i.p.</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>i.v.</td>
<td>Intravenous</td>
</tr>
<tr>
<td>LUTS</td>
<td>Lower urinary tract symptoms</td>
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<tr>
<td>MAb</td>
<td>Monoclonal antibody</td>
</tr>
<tr>
<td>MIRD</td>
<td>Medical Internal Radiation Dose (Committee)</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>PA</td>
<td>Postero-anterior</td>
</tr>
<tr>
<td>PCa</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td>PSA</td>
<td>Prostate specific antigen</td>
</tr>
<tr>
<td>QBS</td>
<td>Quantitative bone scintigraphy</td>
</tr>
<tr>
<td>RIL</td>
<td>Radioimmunolocalisation</td>
</tr>
<tr>
<td>RIS</td>
<td>Radioimmunoscintigraphy</td>
</tr>
<tr>
<td>RIT</td>
<td>Radioimmunotherapy</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operating characteristic</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single photon emission computed tomography</td>
</tr>
<tr>
<td>TNM</td>
<td>T= tumour, N= (lymph) node, M= metastasis</td>
</tr>
<tr>
<td>TRUS</td>
<td>Transrectal ultrasonography</td>
</tr>
<tr>
<td>TUR</td>
<td>Trans-urethral resection</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
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INTRODUCTION

Prostate cancer

Epidemiology and Clinical Features

Prostate cancer (PCa) is the most common malignant tumour among males in the western world. The annual incidence of PCa in Sweden has increased from 0.9 per thousand (‰) to 1.4 ‰ during the period from 1960 to 1997, and was somewhat higher in the county of Västerbotten in northern Sweden i.e. 1.7 ‰. The increased incidence is mainly a consequence of improved radiological and clinical diagnostic procedures including a widespread testing for prostate specific antigen (PSA) even in asymptomatic patients. It should be pointed out that the increased incidence during the period 1974 to 1987 mostly consists of a simultaneously increased proportion of highly (+74%) and intermediately differentiated (+130%) tumours. This explains why the mortality from PCa in Sweden has not increased correspondingly. Interestingly, in a study from Netherlands, the increased incidence of PCa among men <60 years of age did not show a similar shift in the mortality rate.

The prevalence of microscopic PCa, based on autopsy materials, is high i.e. approximately 50% of patients at 50 years and over 70% at 70 years of age. Microscopic examination of material from trans-urethral resection (TUR) performed in connection to treatment of benign prostatic hyperplasia (BPH) revealed PCa in 14% of the patients. In a prospective study including 100 men with lower urinary tract symptoms (LUTS) and without suspicion of PCa on digital rectal examination (DRE), prostate biopsies revealed PCa in 14%.

PCa is traditionally regarded as a rather innocent tumour in elderly men. In its early stages PCa is asymptomatic. Most commonly LUTS and/or an elevated level of PSA lead to the suspicion of PCa. It is true that most of the patients with small, highly differentiated tumours are old with short life time expectancy and will not get any symptoms from the PCa before death from intercurrent diseases. However, 10% of all males will get clinically significant PCa and symptomatic PCa is a frightful
condition associated with severe suffering and high mortality.

In spite of the fact that the increased incidence of PCa consists mostly of small tumours confined to the prostate gland, about 20% of the patients still present with metastatic disease at the time of diagnosis. The skeleton is the primary site of metastases from PCa, but soft tissue metastases occur, most frequently in pelvic and abdominal lymph nodes. Ten to 15% of patients with PCa have soft tissue metastases, engaging primarily abdominal and pelvic lymph nodes, and rarely lungs and liver. This is typically a manifestation of poorly differentiated and aggressively growing tumours.

The prognosis for patients with newly diagnosed PCa is highly dependent on the stage of the disease. If metastases are present at diagnosis, median survival is only 2 to 3 years. On the other hand, if metastases are not present at diagnosis, the efficiency of treatment has been discussed. Disease specific survival rate at 5 and 10 years was 90% and 74%, respectively, in a Swedish study applying delayed treatment in 50 patients with locally advanced non-metastatic PCa. Furthermore, if the staging procedure before radical prostatectomy wrongly states that the tumour is confined to the prostate gland, and tumour specimen shows marginal tumour growth, 12-14 years mortality will be about 50%. In case of a longer life expectancy a curatively intended therapy is usually employed, even in poorly differentiated tumours with a significant risk for occult extended disease. It is therefore crucial to exclude disseminated disease before curatively intended therapy is instituted.

The overall PCa-specific mortality in northern Sweden between 1971 and 1987 was 55%.

**Histopathology, Tumour Grading and Staging**

Most malignant tumours of the prostate gland are adenocarcinomas derived from epithelial cells located in the peripheral zone of the gland. Based on the histopathologic pattern, PCa is classified as Grade 1 (highly differentiated, G1), Grade 2 (intermediately differentiated, G2), or Grade 3 (poorly differentiated, G3) according to the WHO classification. The cellular patterns of PCa were described and graded by Gleason in 1966. The Gleason score is calculated as the sum of the two most abundant of five different microscopic growth patterns of the PCa tumour, and this score is increasingly used as a prognostic factor. It has been shown that the
Gleason score is the strongest independent predictor of disease free survival following radical prostatectomy, as compared to preoperative PSA level and TNM stage. PSA determination, however, is today a mandatory diagnostic test, and, in combination with DRE and the Gleason score it correlates well with the tumour burden of the patients. TNM (T= Tumour grade, N= (lymph) Node, M= Metastasis), which is the most commonly used staging system, is usually based on DRE and transrectal ultrasound (TRUS) combined with needle biopsy and bone scintigraphy (BS).

It has been proposed that surgical staging need not be performed if PSA <4 g/l. Understaging, however, is not unusual, since up to one third of patients who have undergone radical prostatectomy get recurrent tumour growth, indicating that the initial staging was incorrect. This has increased the interest in development of new and better staging procedures. When curative therapy is intended, staging surgery with biopsies from pelvic lymph nodes is required.

**Prostate Specific Antigen**

Prostate specific antigen (PSA) is a tissue specific glycoprotein, produced by glandular epithelial cells in normal, hyperplastic and neoplastic prostate tissue. PCa is usually associated with elevated PSA. A commonly used normal limit for PSA is 4 g/l. However, 30-50% of men with histologically proven BPH have PSA >4 g/l. Additionally, according to a study on US screening programs, 30-45% of patients with PCa will have PSA <4 g/l. It has been proposed that screening with PSA should be performed in all men above 50 years of age.

Screening can reduce morbidity if used in the search for a frequent disease, where an accurate diagnostic procedure and an effective treatment are available, and where prognosis in case of diagnosis at an early stage is definitely improved. These prerequisites for screening are however not all fulfilled concerning PSA-testing for PCa. In addition, PSA screening would lead to detection of many small cancers that would else never become clinically manifest. Furthermore, it has not so far been proven that curatively intended therapy improves neither quality of life nor survival rate for small PCa tumours. This uncertain impact has limited implementation of PSA screening programs, though screening probably is appropriate in high risk groups like PCa gene carriers and men with more than one close relative with PCa.
Radiologic imaging in prostate cancer

**SCINTIGRAPHY**

Bone scintigraphy (BS) is the best choice in the search for bone metastases, due to its high sensitivity and the ease in performing surveys of the entire skeleton. The metastases can be detected before they are visible on conventional radiography. Additional information is obtained by using single photon emission computed tomography (SPECT), rendering a detailed three-dimensional mapping of the activity. The inherent drawback of BS, apart from the lack of specificity, is the fact that a visual estimation of a change in intensity of a focal lesion in repeated BSs is not sensitive enough for monitoring changes in the state of the disease. Attempts have therefore been made to perform quantitative bone scintigraphy (QBS). However, the methods applied have hitherto been too complicated, and are consequently rarely used in clinical practice.

**CONVENTIONAL RADIOGRAPHY**

The usually sclerotic metastatic bone lesions from PCa as well as the rare lytic variants are detected by radiography. Conventional radiographic examinations are therefore indispensable in evaluation of an equivocal increased focal activity in BS. If a matching degenerative disease can be shown with conventional radiography, metastases are less probable. However, the detection of the metastases might be delayed compared to BS, due to low sensitivity of the conventional radiographic evaluation.

**ULTRASONOGRAPHY**

Trans-rectal ultrasonography (TRUS) is well established for primary evaluation of suspected PCa, due to the possibility to identify non-palpable lesions and to enable guided biopsies of the prostate gland and the seminal vesicles. TRUS offers contributions in determining the local extent of the disease and the tumour volume. The positive predictive value of TRUS for PCa is 30-40%. The diagnostic accuracy of TRUS is lower in the central and transition zones compared to the peripheral zone. A drawback is that the ultrasonographic procedures are more operator-dependent than other radiologic modalities.

**COMPUTED TOMOGRAPHY**

Computed tomography (CT) has poor accuracy concerning PCa in the prostate gland, but gives further information about extension of soft tissue and skeletal metastases. In case the findings from the BS and the conventional radiography in the
diagnosis of skeletal metastasis are equivocal, CT might reveal the correct diagnosis. CT is less accurate than MRI in diagnosis of primary tumour growth and regional spread to pelvic lymph nodes, with an overall accuracy of staging of 67%\textsuperscript{50}. Due to its low sensitivity for microscopic PCa growth in normal or slightly enlarged lymph nodes, CT should be used in combination with guided biopsies\textsuperscript{51}.

**MAGNETIC RESONANCE IMAGING**

MRI provides accurate information about tumour growth confined to the prostate as well as its local and distant spread\textsuperscript{52}. The accuracy of MRI for identifying prostate capsular involvement is 80\%, but is much dependent on the experience of the radiologist\textsuperscript{53}. The value of MRI for diagnosis of PCa has also been questioned depending on the difficulties in separating BPH and highly differentiated PCa\textsuperscript{54}. An MRI study dealing with staging of PCa showed a 20\% frequency of false positive diagnosis of pelvic soft tissue tumour spread\textsuperscript{55}.

MR spectroscopy of prostate tissue is a promising diagnostic alternative. Changes in the citrate/choline ratio seem to identify PCa very accurately even prior to histopathological identification\textsuperscript{56}.

**Radioimmunolocalisation**

Antibodies with specificity against tumour antigens can be labelled with radionuclides and used for scintigraphic tumour detection, i.e. radioimmunosctintigraphy (RIS). If the radionuclide is intended for therapy, the radiolabelled antibody can be used for internal tumour radiation, i.e. radioimmunotherapy (RIT). Since the introduction of the hybridoma technology for production of monoclonal antibodies (MAbs) there has been a considerable development in RIS and RIT of cancer\textsuperscript{57,58}.

The radiolabelled MAbs are usually administered intravenously (i.v.) to patients and intraperitoneally (i.p.) to experimental animals. Intraperitoneal administration is easy and safe in animal experiments, and causes rapid distribution of the radiolabelled MAb to the circulation\textsuperscript{59}.

**ANTIBODIES**

Antibodies, or immunoglobulins, are glycoproteins whose basic structure is similar in most mammals. The basic structure consists of two heavy and two light chains. The immunospecificity is determined in the variable region of the light and heavy chains. The antigen binds to the specific complementarity-determining
region (CDR), situated at the outer parts of the chains (Fig1).

Fig. 1. A schematic description of an IgG antibody and two antigen molecules.
The hybridoma technique described by Köhler and Milstein enabled the production of MAb\textsuperscript{60}. The technique utilises mice that are initially immunised with the antigen, and subsequently the lymphocytes from the immunised mice are fused with mouse myeloma cells to secrete immortal antigen-producing hybridomas. Finally, the hybridomas are separated, or cloned, by limited dilution, and each clone is subsequently producing antibodies, i.e. MAbs, with specificity exclusively against one antigen. In order to enhance the tumour uptake of antibodies, different fragments and artificial variants of antibodies have been produced. In chimeric antibody variants, a section of the human immunoglobulin is replaced by a corresponding part of mouse immunoglobulin containing the specific reaction site (CDR-region). In “humanised” antibodies, only the mouse derived CDR is inserted into a human immunoglobulin molecule. Both these variants are increasingly used\textsuperscript{61,62}.

As MAbs are most commonly of mouse origin, repetitive injections almost inevitably cause immune reactions in patients, so called human anti-mouse antibody (HAMA) reactions. HAMA reactions often remain undiagnosed but can cause symptoms as well as disturb the result of the radioimmunolocalisation (RIL), giving both false positive and false negative results. Immunosuppressive therapy is effective in reducing the HAMA response. The frequency of HAMA reactions is also reduced if chimeric or humanised antibodies are used.

Clinical RIS has been successful in detecting metastatic PCa in a group of patients where conventional staging procedures have shown equivocal results\textsuperscript{63}. Previously, \textsuperscript{111}In-CYT-356 (Prostascint\textsuperscript{®}), was produced by immunising mice with LNCaP (a human PCa cell line), and was proven effective in detecting PCa foci\textsuperscript{64,65}. In a group of PCa patients at high risk for metastatic disease, RIS with Prostascint\textsuperscript{®} detected occult metastases in over 50% of the patients\textsuperscript{63}. Using anti-PAP MAb \textsuperscript{111}In-PAY-276, PCa metastases were detected by RIS in 75% of the patients\textsuperscript{63}. Using anti-PSA MAb \textsuperscript{111}In-DTPA-PSA-399 that detected metastases in 90% of the RIS investigated patients\textsuperscript{67}. Four new IgG MAbs (J591, J533, J415, and E99) have been shown to bind to two different epitopes on the extracellular domain of prostate-specific membrane antigen and also to vascular endothelium of a variety of carcinomas\textsuperscript{68}. However, RIS has not entered routine clinical use for PCa.
**TUMOUR ANTIGENS**

An ideal tumour antigen should be synthesised only by tumour cells and preferably be situated on the cell surface. Antigens situated in poorly vascularised parts of the tumour or inside tumour cells are less ideal as targets for RIT. Intracellular antigens can however be present in necrotic parts of tumours, and are thus exposed to an extent to the radiolabelled tracer. Shedding of antigen to the circulation contributes to a higher non-tumour concentration of the antigen-antibody complex, and might therefore be disadvantageous for RIL. MAbs used for RIL in prostate cancer have been developed using circulating antigens like prostatic acid phosphatase, PSA, or common antigens from a variety of tumours. Another antigen for RIL is prostate-specific membrane antigen, which is a glycoprotein of approximately 100 kDa restricted to the prostate epithelial cell membrane.

**RADIONUCLIDES AND RADIOLABELLING**

For RIS, radionuclides emitting photons with an energy level suited for gamma camera detection (100-200 KeV) with a half-life corresponding to tumour accumulation of the radiolabelled MAb are required. In routine clinical scintigraphic imaging, $^{99m}$Tc is indispensable due to its availability, short half-life (6hrs), and an adequate energy spectrum (peak 149 keV) for acquisition in a gamma camera.

$I-131$ is used in clinical RIS due to its properties in radiolabelling. However, $^{131}I$ is seldom used for experimental RIS, since its energy spectrum is not ideally suited for gamma camera detection, and the half-life of 8 days might also be too short when performing kinetic studies of the MAbs.

$I-123$ is an excellent radionuclide for clinical RIS due to both the energy spectrum (peak 159 keV) and the physical half-life (13 hours). In experimental RIS, $^{123}I$ is less useful since its half-life is too short for evaluation of antibody kinetics.

$I-125$ has an energy spectrum that is suitable for scintigraphy in small animals, and has a half-life of 60 days which is an advantage when performing experimental evaluation of different antigen-MAb systems. On the other hand, $^{125}I$ has little value for clinical RIS since its radiation energy does not sufficiently penetrate the tissues of large bodies.

For RIT, the ideal radionuclide should emit particles with an energy level permitting irradiation of the entire tumour, irrespective of the distribution of the radiolabelled MAb. The half-life of the...
radionuclide should cover the entire phase of tumour accumulation of the radiolabelled MAb.

Some radionuclides used for RIT are described in Table 1.

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Half-life</th>
<th>Emitted particles</th>
<th>Mean range</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{131}$I</td>
<td>8 days</td>
<td>beta</td>
<td>0.8 mm</td>
</tr>
<tr>
<td>$^{90}$Y</td>
<td>2.7 days</td>
<td>beta</td>
<td>5.0 mm</td>
</tr>
<tr>
<td>$^{67}$Cu</td>
<td>2.5 days</td>
<td>beta</td>
<td>0.6 mm</td>
</tr>
<tr>
<td>$^{199}$Au</td>
<td>3.1 days</td>
<td>beta</td>
<td>0.3 mm</td>
</tr>
<tr>
<td>$^{211}$At</td>
<td>7 hours</td>
<td>alfa</td>
<td>0.05 mm</td>
</tr>
<tr>
<td>$^{212}$Bi</td>
<td>1 hour</td>
<td>alfa</td>
<td>0.05 mm</td>
</tr>
<tr>
<td>$^{125}$I</td>
<td>60 days</td>
<td>Auger electrons</td>
<td>~1 µm</td>
</tr>
<tr>
<td>$^{77}$Br</td>
<td>2.4 days</td>
<td>Auger electrons</td>
<td>~1 µm</td>
</tr>
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</table>

Table 1. Radionuclides used for radioimmunotherapy.$^{74}$

Radionuclides should cover the entire phase of tumour accumulation of the radiolabelled MAb.

Radionuclides used for RIT are described in Table 1.

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Radiolabelling of intact MAbs using $^{99m}$Tc frequently reduces the immunoreactivity of the MAb. Besides, the half-life of $^{99m}$Tc is usually too short when intact MAbs are used for RIS.$^{71}$ Radiolabelling using isotopes of iodine is more easily performed without loss of immunoreactivity. Radiolabelling with isotopes of iodine using the Chloramine-T method is widely used in experimental and clinical RIL, and was also used in our experiments.$^{72,73}$

**TUMOUR UPTAKE**

A high tumour uptake and a high tumour to non-tumour ratio of radioactivity are decisive of the image quality in RIS as well as for the absorbed tumour dose in RIT. The typically low tumour uptake of the radiolabelled MAb is probably the main reason why RIS and RIT
have not gained acceptance in routine clinical practice. The tumour uptake can be increased by developing MAbs with high specificity against an abundant tumour antigen and high affinity to the antigen. The non-tumour radioactivity can be reduced by clearance of circulating radiolabelled MAbs. This might be achieved by preinjection of non-labelled MAb, reducing the circulating antigens, and postinjection of an antiidiotypic MAb which usually causes a rapid clearance of the resulting MAb-complex. Plasmapheresis or extracorporeal immunoadsorption can also reduce the amount of circulating radiolabelled MAbs. The non-tumour dose can be significantly reduced by a three-step administration of MAb. First, a biotinylated MAb is administrated systemically. When blood clearance has reached maximum, an excess dose of a chase molecule (e.g. avidin, with strong affinity to biotin) is administered, which will attach to both circulating and tumour bound MAbs. When circulating MAb-avidin complex has cleared from plasma, radiolabelled biotin is administered, which will attach to the target cell bound MAbs. Using this method, a 20:1 tumour to non-tumour ratio has been achieved. There is no significant difference in tumour uptake whether the radiolabelled MAb is administered i.v. or i.p. Direct intratumoural injection of the radiolabelled MAbs renders very high tumour accumulation. Administration by i.v. or i.p. route renders significantly lower radiation doses in the target tissue compared to intratumoural installation, but is better suited for treatment of disseminated tumour spread.

**Radioimmunoscintigraphy**

A high tumour uptake of the radiolabelled MAb with a consequent high tumour to non-tumour activity ratio is needed for rendering images of good quality. However, tumour uptake is often low in RIL, and, in addition, tumour uptake is disturbed by high activity levels in liver, spleen, urinary bladder, and colon. As a consequence, SPECT with 3D-reconstruction are usually indispensable in the search for pathological uptakes. Image fusion techniques used in combination with CT or MRI provide valuable contributions in interpreting the examinations.

**Radioimmunotherapy**

The radiation effects of RIT depend on radiation energy, dose rate, and tumour to non-tumour dose ratio. The radiobiological effect on tumour cells thus depend on tumour cell proliferation rate, biological
half life of the radiolabelled MAb-antigen complex in the target cells, the cellular localisation of the antigens, energy level, and dose rate of the radionuclide. Long range ?-emitters may be inappropriate in small tumours due to inefficient absorption of radionuclide disintegration energy in small volumes. On the other hand, short-range emitters may not reach all parts of tumours. 

I-131 is a ?-emitter with a mean range of 0.8 mm, which is well suited for treatment of tumours <1.5 cm diameter. However, 131I also emits high-energy ?-radiation (365 keV), which increases the total body radiation and involves radiation risk to individuals close to the patient. 

I-125 emits Auger electrons, which are effective in RIT only if the isotope is located in the cell nucleus. Thus, the internal distribution of the radionuclide in the tumour is of great importance. When localised to vital tumour tissue, a short-range emitter is preferred because it emits less radiation to surrounding non-tumour tissues. The administered activity is however usually heterogeneously distributed in the tumour, demanding a radionuclide with a longer range in order to irradiate the entire tumour. 

The dose rate in RIT is low, which means there is a requirement for a higher total dose compared to external beam therapy in order to reach an equivalent therapeutic effect. Thus, a curatively intended therapy of metastases requiring 60-70 Gy with external radiation would instead require 70-84 Gy at the lower dose rates available from RIT. Since the tumour cells in RIT are exposed to radiation during the entire cell cycle, including the radiosensitive mitose phase, this demand of higher radiation dose can however be reduced. This is called the inverse dose-rate effect. 

So far, the most promising results of RIT have been achieved in leukaemias, lymphomas, and small solid tumours (<300 µm in diameter), probably due to easy access for the radiolabelled MAb to the antigens. In larger solid tumours, the high pressure in the interstitial tumour tissue and heterogeneous blood circulation delay antibody access. Therefore, biological degradation of the radiolabelled MAbs occurs before binding to the tumour cells, thus reducing the radiation effect.

In a study including thirteen patients with advanced malignancies, with tumours less than 3 cm in diameter, disease stabilisation was seen in six patients following RIT using an 131I-labelled anti-CEA antibody. In another study, twelve patients with hormone-refractory prostate cancer were treated with 90Y-CYT-356.
MAb, and three of them had transient subjective improvement. A phase-II study using $^{131}$I-labelled MAb CC49 in combination with adjuvant interferon led to pain relief for five of six patients, and minor tumour regression in two of the six.

In spite of these promising results, RIT has so far not gained acceptance in routine clinical patient care. There are still problems regarding the radionuclide-MAb-complex in use. Inadvertent high radiation to non-tumour tissues is also concerning.

The maximal level of administered radiolabelled MAb is primarily restricted by inadvertent irradiation to the bone marrow, as well as to other organs, e.g. liver, spleen, and kidneys. Bone marrow depletion per se is usually not life threatening due to the availability of bone marrow transplantation. A high tumour to non-tumour dose ratio admits a high tumour dose with less risk for damage to other organs. If an additional radiation dose is required, repeated injections of radiolabelled MAb may be used, usually without any loss of properties of the target antigen. Absorbed radiation dose in the tumour, other organs, and circulation should be carefully assessed in order to evaluate the efficacy of the MAb and to perform accurate dose planning.

MATERIAL AND METHODS

Experimental model
The experimental PCa cell line DU-145 from a brain metastasis of human prostate cancer was initially described in 1978. DU-145 xenografts grow fast, and nude mice die in about two months after inoculation of tumour cells if untreated. In our experiments, $2.5 \times 10^6$ DU-145 cells were inoculated subcutaneously in front of one or both hindlegs in each animal. The tumour size was measured with a slide-calliper in two diameters every second day. An average tumour diameter of 5 mm was achieved after a period of 14 days. The tumour volume was calculated using the formula for an ellipsoid ($\frac{4}{3} \pi \times$ length x width$^2$). The density of the tumour was set to 1.0 g/cm$^3$.

MAb E4 was produced using the hybridoma technique. Immunisation was first performed in female mice using dispersed cells from human BPH. Following immunisation, lymphocytes derived from regional lymph nodes were used for fusion. The selected E4 hybridoma was then injected i.p. in mice for antibody production. The immunoreactivity of the MAb from the hybridomas with DU-145 cells was proven by immunohistochemistry.
Immunoreactivity tests on DU-145 cells cytosponed onto cover slips were also performed. The testing was performed using Multi-link kit (BioGenex, CA, USA), and showed immunoreactivity.

The MAb E4 is an IgG2α kappa antibody, directed against an antigen (a polypeptide with a molecular weight of 70 kDa) located on the surface of prostate epithelial cells⁹⁴. An endocytosis assay on DU-145 cells revealed a rapid release of radioactivity from the cells within the first two hours after antibody incubation. However, a substantial part was found to be internalised with 30% of the radioactivity retained in the cells after 48 h. This indicates a slow antibody processing in the cells, which is favourable when a MAb is used for RIT⁹⁶.

**Experimental methods**

**RADIOLABELLING**

The MAb E4 was, as in earlier studies, labelled with ¹²⁵I (IMS 30, Amersham, Little Chalfont, UK) to a specific activity of 50-70 MBq/mg, using the Chloramine T method. Following radiolabelling, free iodine was removed by gel filtration on a Sephadex G50 (Pharmacia Biotech, Uppsala, Sweden) column. The remaining specific activity after the labelling procedures was 50-70 MBq/mg⁷²,⁷³.

**RADIOIMMUNOSCINTIGRAPHY**

In experiment I the RIS images were recorded using a Portacamera II C (General Electric, Milwaukee, Wis.) equipped with a pinhole collimator and connected to a Star 400 evaluation system. In experiment II a GE 400 Maxi gamma camera (General Electric, Milwaukee, Wis.) equipped with a pinhole collimator and connected to a Hermes (Nuclear Diagnostics, Stockholm, Sweden) evaluation system was used. The gamma camera systems were calibrated using a defined activity of ¹²⁵I. The mice were examined during general anaesthesia, induced by i.p. injection of 0.1-0.2 ml of a 1:1:2 cocktail of Dormicum® (Roche, Midazolam 5 mg/ml), Hypnorm® (Janssen Pharmaceutica, Fluanisonum 10 mg/ml and Fentanylum 0.02 mg/ml) and sterile water. A special holder was used in order to achieve equal geometry at each scintigraphic recording. The holder kept the anaesthetised mouse at a distance of 10 cm from the pinhole collimator. Each acquisition was performed using a 128 x 128 matrix with a pre-set count of 200 000 scintillations. In case the activity was low and the examination therefore lasted too long, the acquisition was stopped manually when the animal showed signs of waking up, which usually occurred after 20-25 minutes.
DOSIMETRY

The Medical Internal Radiation Dose Committee of the Society of Nuclear Medicine (MIRD) has provided guidance on methods for calculating radiation doses since 1968\textsuperscript{97}. Dose calculation according to MIRD formalism for small volumes has been shown to render activity values of significant accuracy\textsuperscript{98,99}. Based on quantitative scintigrams, regions of interest (ROI) were drawn manually around each depicted tumour. The counts in each ROI were then registered. For background subtraction, either a narrow region was drawn around the tumour ROI (paper I), or a mirrored ROI set on the contralateral side of the image (paper II). The tumour volume was taken into account when calculating the amount of activity in the mirrored ROI. Based on these procedures the total numbers of decays in each tumour were estimated, assuming a spherically shaped tumour with a homogeneously distributed radioactivity (Fig 2).

![Fig 2](image-url)

**Fig 2.** Outline of the two methods used on mice for background subtraction. A and B= PA view, C and D= trans-axial sections. Unbroken line= tumour ROI, dotted line= background ROI. V= part of background displaced by tumour\textsuperscript{99}. 
**Radioimmunotherapy**

The anti-tumour effect of the $^{131}$I-labelled antiprostate MAb E4 was studied in an experimental model with 41 nude mice subcutaneously xenografted with a human prostate cancer cell line (DU-145). The mice were divided into four study groups i.e. one receiving single and another two consecutive injections (each 14-19 MBq $^{131}$I-E4). A third group was injected with non-labelled MAb E4, and the fourth served as an untreated control group.

**Histopathologic Examination**

Following the localisation experiments, the tumours were sectioned into two halves after activity measurement. One half of each tumour was immediately fixed in 4% formaldehyde, dehydrated and embedded in paraffin. Six µm thick slices were cut and stained with hematoxylin-eosin and examined by light microscopy. The volume density of viable tumour tissue (part of tumour composed of intact tumour cells) was determined using stereological methods. Using a square-lattice mounted in the eyepiece of a light microscope, grid intersections over viable tumour tissue compared to total tumour tissue were counted in several random sections from each tumour at 100 X magnification. Mitotic activity was quantified by immunostaining with a polyclonal antibody against human Ki-67 (diluted 1/100) as earlier described. The percentage of immunostained tumour epithelial cell nuclei was determined by counting in each tumour section with light microscope.

**Autoradiography**

The remaining half of each tumour was fixed in Bouin’s solution (15 ml 1.2% picric acid, 5 ml 40% formaldehyde, and 1 ml concentrated acetic acid). The fixed tumour was embedded in paraffin, sectioned into 20 µm thick slices, mounted on gelatinised glass slides after removal of paraffin, and dipped in the MTB2-emulsion (KODAK) at a temperature of 40° C. The slides were exposed in the dark at 4° C for 6 weeks, followed by development in D19B-solution (KODAK) and fixation in F-24-solution (KODAK). The autoradiographies were then examined by light microscopy.

**Immunoreactivity**

The specificity of E4 was evaluated by immunohistochemistry on cytospin preparations of DU-145 cells. The slides were incubated overnight at 4° C with the antibody (0.93 µg/ml). The immunoreaction
was visualised using the supersensitive Multi-link kit (BioGenex, CA, USA) which employs alkaline phosphatase/fast red dye as the detection system.

**STATISTICS**

In paper IV, the association between tumour grade, tumour stage, and BS was tested using the $X^2$-test. Receiver operating characteristic (ROC) curves were produced in order to establish the relationship between PSA level and the probability of positive BS\textsuperscript{102}.

In paper III, the differences in mitosis frequency between treated and untreated groups were tested using the Mann-Whitney U-Test.

In paper II, differences in tumour growth were tested using the Kruskall-Wallis test.

**CLINICAL MATERIAL**

**INCLUSION CRITERIA**

In paper IV, patient records, including PSA values, tumour grades, and tumour stages were extracted from the regional cancer care register administered by the Oncology Centre at Umeå University Hospital. This register records all new cases of PCa in our catchment district.

In paper V, 11 randomly chosen patients with recently diagnosed PCa, PSA >20 and a positive BS were included in a study aimed to evaluate a method for QBS.

**Clinical methods**

**PSA ANALYSIS**

Serum PSA levels were assayed using AxSYM PSA\textsuperscript{®} (Abbott Laboratories, Abbott Park, Ill.). The normal limit for PSA was set to 4 ?g/l.

**BONE SCINTIGRAPHY**

BS was performed using a Giga Camera (General Electric, Milwaukee, Wis, US) with a low energy general-purpose collimator and a 256 x 256 matrix. Static acquisitions of the whole body were performed 2-3 hours post injection of 550 MBq $^{99m}$Tc-methylendiphosphonate (MDP\textsuperscript{2} Solco, Basle, Switzerland) in three anteroposterior (AP) and three posteroanterior (PA) views. QBS was performed using a STAR-3000 gamma camera (General Electric, Milwaukee, Wis, US). A matrix of 256 x 256 and a low energy general-purpose collimator was used. Acquisitions were made in AP, PA and lateral views. The thickness of the patients at the level of the focal activity uptake was measured by the gamma camera using radioactive markers fitted on the bodies. The accuracy of the measurements was tested by direct measurement using a graded forceps.
**Calculations**

Calculations of the total number of decays in each tumour were made based upon serial quantitative scintigraphies. Irregular regions of interest (ROI) were drawn manually around each tumour. The tumour mass was estimated according to the method described earlier (Paper I). Using these results, the tumour radiation doses were calculated according to MIRD formalism\(^98,99\). In Paper I, irregular regions of interest (ROI) were drawn covering the tumour, and background subtraction was made from a narrow region surrounding the tumour ROI (Fig. 2 left). In paper II, an irregular ROI was drawn covering the tumour and a mirrored ROI placed on the opposite side. The tumour volume was taken into account when calculating the amount of activity in the mirrored ROI (Fig. 2 right).

**Results**

**Paper I**

This experiment was a pilot *in vivo* investigation with MAb E4. Nude mice were inoculated subcutaneously with cells from the PCA cell line DU-145. The intact MAb E4 and an intact anticytokeratin-8 MAb TS1, which was used for comparison, were labelled with \(^{125}\)I and injected i.p. Repetitive quantitative scintigraphic recordings were performed during one month. The mice were sacrificed at day 29 after injection of the radiolabelled MABs. The tumours and the organs were dissected and weighed. The remaining activity was measured in a gamma well counter. One part of the tumour was immediately fixed in Bouin’s solution for autoradiography and the other in formaldehyde for microscopy. The study demonstrated significant radioimmunolocalisation of the MAb E4 into the DU-145 prostate tumour tissue in the animal model, with an average radiation dose of 0.08 Gy/MBq in the tumour. TS1 accumulated preferentially in necrotic parts of the tumour, yielding a tumour dose of 0.02 Gy/MBq.

The MAb E4 was shown to be a promising radiotracer for PCA. As in earlier studies, TS1 showed significant RIL into necrotic tumour tissue, occurring in PCA.

**Paper II**

The kinetics of the \(^{125}\)I-labelled monoclonal antibody (MAb) E4 in nude mice xenografted with DU-145 tumours was tested, as well as different strategies to improve the tumour uptake. Thus, the effects from a single injection of the \(^{125}\)I-labelled MAb E4, the same total amount of radiolabelled MAb divided into three repeated injections, as well as the
effect of pre-targeting with non-
labelled idiotypic antibody used to
reduce the amount of shed antigen
were investigated. For each strategy,
the tumour radiation dose delivered
from the $^{125}$I-nuclide was calculated.

The peak tumour uptake was
registered four to five days after the
injections. The single injection
strategy without pretargeting rendered
the highest mean tumour radiation
doses, i.e. 0.23 Gy/MBq. Pretargeting
with unlabeled idiotypic antibody
gave a slightly lower mean tumour
dose compared to the single injection
alone, i.e. 0.19 Gy/MBq. An even
lower mean tumour dose was
obtained when the same total
administered activity was divided into
three repeated injections, i.e. 0.12
Gy/MBq.

**Paper III**

The anti-tumoural effect of the $^{131}$I-
labelled antiprostate MAb E4 was
studied in 41 nude mice which had
been subcutaneously xenografted
with the PCa cell line DU-145. The
mice were divided into four study
groups, i.e. one receiving single and
another repeated injections of the
radiolabelled MAb. A third group
was injected with non-labelled
MAb, and the fourth served as an
untreated control group. The tumour
volumes increased similarly in all
groups during the study.

Macroscopic evaluation showed that
the treated tumours contained fluid
filled cysts. However, the tumour
volume did not decrease, evidently
due to tumour tissue replacement
with fluid as a result of the cystic
degeneration in combination with
osmosis. The cysts were lined with
tumour epithelium showing marked
cellular and nuclear polymorphism.
There was also a loss of the cellular
architecture and vacuolisation, but
no difference in mitotic frequency.

The percentage of the total tumour
volume that was composed of viable
tumour cells (the volume density)
was significantly reduced compared
to non-treated controls. However,
when the cell proliferation activity in
the remaining viable tumour cells
was examined with Ki-67-staining,
no difference was found compared to
non-treated controls. The non-treated
control tumours and single therapy
tumours remained solid. The use of
$^{131}$I-labelled E4 MAb thus
demonstrated promising therapeutic
potential.

**Paper IV**

In order to evaluate the predictive
value of PSA for the outcome of BS,
a retrospective study was performed
in a patient material from the Umeå
region in Northern Sweden. The
study also evaluated whether
different grades and local extensions
of the tumours might influence this
predictive value.
Four hundred forty-six patients newly diagnosed with PCa were included. Different levels of PSA, tumour grade, tumour stage, and combinations of these parameters were analysed concerning their ability to positively predict the BS result.

Among 214 patients with PSA <20 µg/l, 9 showed a positive BS. When tumours of grade 2 and 3 were excluded, the number of positive BS predictions decreased to 6. For 350 of these 446 patients, a classification according to TNM was available. Of these 350, 162 had a PSA value <20 µg/l. Within this PSA <20 µg/l group, 81 patients had small and well differentiated tumours (T1-2, G1), and only one of these 81 had a pathologic BS.

It was concluded that PSA has high predictive value (p <0.001) for the outcome of BS. In most patients with small and well differentiated tumours (T1-2, G1) and PSA <20, BS need not be carried out.

**Paper V**

The aim of the study was to evaluate the value of QBS in identifying bone metastases in PCa, when applying Fleming’s method\(^{103}\). Phantom studies were performed for calibration of the gamma camera used. QBS was performed in eleven men with recently diagnosed PCa, where routine BS showed involvement of the skeleton. Following endocrine therapy for 4 to 8 months, another QBS was performed. The results from the QBS were compared with clinical findings, and to serum level of PSA. A positive correlation was seen between the change in PSA and QBS. The overall mean error was calculated to 15%, and to 10% in repetitive examinations on the same patient.

**DISCUSSION**

Diagnostic tests for PCa like PSA and MRI with spectroscopy have improved substantially, making it possible to disclose tumours even before they are visible in the light microscope\(^{56}\). However, science has so far failed in developing efficient remedies for the large group of patients presenting with spread PCa at diagnosis. During the last decades, considerable research effort has been put into the field of radioimmunolocalisation, and this might be the field where eventually effective therapies for PCa will be produced.

In a RIS experiment using anti-prostate MAb CYT-356 labelled with \(^{111}\)In in nude mice xenografted with PCa, a maximal tumour uptake of 30% of I.D./g was achieved two to three days post injection\(^{104}\). In another study using PCa xenografts in nude mice treated with the \(^{125}\)I-
labelled MAb L6, a maximal tumour uptake of 10% of I.D./g was registered four days post injection. Similar, but lower tumour uptakes were registered in our experiments with a peak of 6.5% of I.D./g, 4-6 days post injection (Paper II). The difference in absorbed tumour dose might be explained by differences in the methods used for dosimetry. However, the image quality from our scintigraphies seems to be equal to those published from the experiments mentioned above. It should be noted that there was a four-fold higher radiation efficiency when comparing our experiments presented in paper II with those in paper I. These diverging results might depend on differences regarding the methods for background correction, and variability between the animals used. The observations are in accordance with previous findings showing that the background correction used in paper I renders too low values for radiation efficiency. This might be due to an overestimation of the background activity. The findings from paper I convinced us that the MAb E4 fulfilled the prerequisites for further evaluation with RIS or RIT. The in vivo kinetics of MAb E4 (paper II) showed that the peak tumour uptake was registered four to five days after the injections. This is a drawback when used for RIS, but might be advantageous for RIT. It was somewhat remarkable that there were no benefits for the radiation efficiency when comparing repeated to a single injection. Corresponding as well as conflicting results in this context have been presented. Consequently, it should be stated that the kinetics for each MAb needs to be evaluated separately. In accordance with previous studies our observations in paper III showed cell damage with loss of cellular architecture, tumour cell nucleus destruction, and macroscopic cystic degeneration in the tumour. This indicates that our antigen–MAb system has therapeutic possibilities although the pattern of tumour growth between treated and untreated animals was almost similar. The therapeutic applications of MAb E4 should be further evaluated. Future clinical studies seem very promising.

In the study in paper IV, only 9 of 214 patients with PCa and PSA <20 µg/l, showed positive BS. Thus, it appears that the incidence of skeletal metastases is low in these patients. Furthermore, if only patients with PCa in T1-2 and G2 are considered, only one of 81 had pathologic BS findings, supporting the reasoning to not perform BS. Previously, several investigations have proposed that BS need not be performed at PSA values <10 µg/l. It appears that the same is true for PSA values <20
μg/l. However, it should be noted that the incidence of bone metastases was found to be 2 – 5% in PCa patients with PSA <10 μg/l\textsuperscript{109,110}. Furthermore, a pathological BS frequency of <5% was found even with PSA values up to 40-45 μg/l\textsuperscript{111}.

Evidently, besides a low PSA, other parameters such as tumour grade or stage need to be considered before excluding bone metastases in PCa. Use of a combination of PSA and serum bone alkaline phosphatase test, which has been proposed recently, might lead to even better prediction of BS outcome\textsuperscript{112}.

As has been pointed out, visual evaluation of BS does not permit a valid comparison of findings from repeated examinations. Therefore, a quantitation of the skeletal uptakes would have great clinical utility. QBS originally described by Fleming\textsuperscript{103} and modified in this study (paper V) turned out to be more applicable in the clinical situation, and showed positive correlation with changes in the clinical status of PCa-patients. However, further refinements are needed in order to make the method useful in future routine clinical medicine.

**SUMMARY AND CONCLUSIONS**

The MAb E4 has shown promising results for RIL in our experimental model. Thus, it has been possible to achieve good imaging quality in RIS, and our RIT experiments have shown significant radiation effects on tumour cells.

Our results advocate BS as an important tool for staging and monitoring patients with PCa, due to its feasibility, reasonable costs, and high sensitivity. In addition, it was concluded that in most patients with small and well differentiated tumours (T1-2, G1) and PSA <20, BS need not be carried out. The diagnostic yield in follow up of PCa can be improved using a complementary QBS.
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