**Title:** EGFR 37 KDA FRAGMENT AS CANCER MARKER

**Abstract**

The present invention relates to the presence of a 37KDa epidermal growth factor receptor (EGFR) fragment in the urine of patients with transitional cell carcinoma of the bladder. The presence of the 37KDa EGFR fragment in urine can be ascertained using an antibody. The presence of the 37KDa EGFR fragment in the urine of patients can be a test for the presence of prostate cancer and can therefore be used as a general screen for health in the genitourinary area.
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EGFR 37 KDA FRAGMENT AS CANCER MARKER

The present invention relates to a method of diagnosis of bladder cancer or prostate cancer and to a method of detecting recurrence of bladder or prostate cancer. More particularly the invention relates to an accessible marker.

Transitional cell carcinoma (TCC) of the bladder accounts for 1% of all cancers and is the fifth most common malignancy in people over the age of sixty in industrialised parts of the world (Russell et al., 1988; Gleave et al., 1993). Eighty percent of all bladder TCC is superficial at presentation; the remaining 20% is muscle invasive and 50% of patients in this category die despite treatment (Simoneau and Jones, 1994). Of those patients initially presenting with superficial tumours, 50 to 70% have recurrences within two years. These recurrences are usually superficial, although 10 to 20% progress to the muscle invasive form (Parmer et al., 1989; Fradet, 1992; Harland, 1994).

The high frequency of recurrent TCCB and the increase in disease status in a proportion of patients means
that lifetime follow-up using cystoscopy and urinary
cytology is essential. The standard procedure is an
initial check cystoscopy three months after disease
presentation; if this is clear cystoscopy should then
be carried out every six months, for one to two years
and then annually thereafter with a flexible
cystoscope. At present the recurrence rate of TCCB
means that annual lifetime cystoscopies should be
carried out for all stabilised patients.

Cystoscopy involves insertion of a cystoscope into the
bladder via the urethra to allow visualisation of the
tumour using fibre optics. It confirms clinically and
pathologically the presence of tumour within the
bladder and allows a morphological description (Hossan
and Striegel 1993). However it has the disadvantages
of being an invasive, uncomfortable procedure. The
frequent recurrences of TCCB mean that patients must
undergo lifetime follow-up using cystoscopy; this
results in the further disadvantage of a large
expenditure by the health service.

Urine cytology is used for the detection of recurrent
bladder TCC and although it offers the advantages of
being a non-invasive, inexpensive, easily accessible
procedure (Zein and Milad, 1991), it has a poor
sensitivity, especially at lower stages and grades of
disease. The result is false positive and negative
findings with reported sensitivities ranging from 37.9%
(Miyanaga et al., 1997) to 64% (Martins et al., 1997).

Numerous studies have been carried out to find the
ideal bladder cancer marker. However, none are
adequately sensitive or specific enough to fulfil a
diagnostic role at present. The most successful to
date appears to be the Bard BTA, STAT and TRAK tests
with overall sensitivities of 55% (Bard promotional information), 72% (Leyh et al., 1997) and 88% (Bard promotional information) respectively.

Bladder cancer is a frequently recurring disease; patients require lifetime monitoring using cystoscopy and urinary cytology. Cystoscopy is an invasive technique and urinary cytology while non-invasive has a low sensitivity.

It is an aim of the present invention to replace these two procedures with a sensitive, non-invasive urinary test which would allow detection of first presentation and recurrent bladder cancer.

The invention relates to the presence of a 37KDa epidermal growth factor receptor (EGFR) fragment in the urine of patients with transitional cell carcinoma of the bladder (TCCB) and in the urine of some patients with prostate cancer.

This fragment had not previously been detected and its presence permits the development of a novel and inventive diagnostic test.

The 37KDa fragment can be observed in a western blot of proteins from a urine sample from a patient with TCCB.

According to the present invention there is provided a marker for bladder cancer, the marker comprising a 37KDa EGFR fragment which is detectable in urine.

The marker may also or alternatively be used as a marker for prostate cancer.

The invention provides a test for the presence of a
37KDa EGFR fragment in urine, the test comprising
detecting the 37KDa EGFR fragment with an antibody.
The test may comprise a western blot assay.

Alternatively the test may comprise an
immunochromatographic assay, an ELISA test, latex agglutination or radioimmunoassay.

The invention further provides a method of diagnosing bladder cancer or prostate cancer or detecting recurrence of these, the method comprising the steps of reacting a urine sample from an individual to be tested with means to detect a 37KDa EGFR fragment and analysing results.

Herein the term "diagnosing" relates to first presentation diagnosis and detection of recurrence.

In one embodiment the means to detect the 37KDa EGFR fragment is an antibody.

Preferably the antibody is raised against a peptide corresponding to amino acid residues 1005 to 1016 of EGFR or binds to such a peptide or a peptide substantially similar thereto.

A substantially similar peptide is 60% homologous to the amino acid sequence along at least 50% of the length of the 37KDa peptide.

In a particular embodiment of the invention the antibody is Ab4 EGFR antibody available from Oncogene Science, Inc.

The invention further provides the use of antibody Ab4 EGFR in a test to detect the present of 34KDa EGFR
fragment in urine.

The invention also encompasses the use of specific antibodies raised to the 37KDa fragment of EGFR.

In one embodiment the test is in the form of a dip stick.

The test can be used in conjunction with other appropriate tests to diagnose TCCB, prostate cancer and urinary infection.

**Experiment 1**

A 37KDa EGFR fragment has been detected in urine from patients with bladder cancer. First morning urine samples were collected from 24 TCC patients, 6 patients who had bladder cancer previously but who were now disease free and 13 healthy volunteers. 10mls of urine from each was freeze dried and the powdered residue reconstituted in LaemmlI lysis buffer. After heating at 110°C for 20 minutes, all samples were stored at -70°C until required for analysis. Samples were then probed with the Ab4 EGFR antibody (Oncogene Sciences) to the internal domain of the receptor by western blot analysis.

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<tr>
<th>Disease Status</th>
<th>No</th>
<th>Presence of the 37KDA Fragment</th>
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<td>Healthy</td>
<td>13</td>
<td>1</td>
<td>12</td>
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<tr>
<td>TCC</td>
<td>24</td>
<td>21</td>
<td>3</td>
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<td>Remission (disease free)</td>
<td>6</td>
<td>4</td>
<td>2</td>
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A 37KDa fragment was detected in 88% (21/24) of TCC patients, 66% (4/6) of disease free patients and 7% (1/13) of healthy volunteer urine samples. There was
an overall significant association between detection of
the 37KDa fragment and presence of bladder cancer.
Although four out of six patients who were though to be
disease free tested positively, two had frank low grade
tumours and two had bladder inflammation at the time
the urine sample was taken. This 37KDa fragment
therefore appears to be of diagnostic importance. It
has a much higher sensitivity than urinary cytology and
the Bard BTA and STAT tests, and it appears to be
comparable to the Bard TRAK test.

Experiment 2

<table>
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<th>Disease Status</th>
<th>Number†</th>
<th>Presence of the 37KDa Fragment</th>
<th>Absence of the 37KDa Fragment</th>
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<td>Healthy</td>
<td>25(13)</td>
<td>1(4%)</td>
<td>24(96%)</td>
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<tr>
<td>Urinary Infection</td>
<td>16(12)</td>
<td>10(62.5%)</td>
<td>6(37.5%)</td>
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<td>Remission (disease free)</td>
<td>6(2)†</td>
<td>0</td>
<td>6(100%)</td>
<td>46.17*</td>
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<td>TCC</td>
<td>32(24)</td>
<td>28(87.5%)</td>
<td>4(12.5%)</td>
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<tr>
<td>Prostate Cancer</td>
<td>10(0)</td>
<td>5(50%)</td>
<td>5(50%)</td>
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</table>

Sensitivity levels for the detection of a 37KDa EGFR
fragment in urine.

* denotes significant (p<0.001); † number in brackets is
the number originally reported.

† This is somewhat different from Experiment 1 - the 6
so called remission patents were in fact all in
remission when the notes were checked.

In fact: two were in remission, BUT two had
inflammation and two frank low grade tumour - and have been reassigned. Four more patients who are definitely in remission at the time of the test were added and there are now 6 confirmed remission patients with no marker.

Overall the second study has increased the number by a small amount and the data is holding up well. A group of prostate cancer patients has been added in since males often have undiagnosed prostate cancer. This could be a confounding factor as 50% are positive. However there is a blood test for prostate cancer so this would have to be carried out on positive patients along with a check for infection.

It is possible that the 37KDa protein could be used to distinguish between stage or grade in prostate cancer. The biology of prostate should be clarified and then collated with the patients tested. The test could be used as a general screen for health in the genitourinary area since it might pick up silent bladder and prostate tumours and infection - a positive test could lead to other tests to rule these possibilities out.

Comment on the table:
- shows 87.5% of TCC patients tested positive for the protein, whereas in contrast only 4% of the healthy controls expressed this protein in urine
- those patients in disease free (in remission), 100% tested negative
- the urinary infection group, 62.5% of the patients tested positive and 37.5% tested negative
50% of the prostate cancer patients test positive
to date, the overall sensitivity of the 37KDa protein is 87% and the specificity is 96%.
statistical analysis shows that detection of the 37KDa fragment is dependent on the presence of disease ($\chi^2=46.17 \ p<0.001$).

Detection of the 37KDR EGFR fragment in urine

From the investigations carried out on the detection of the 37KDa EGFR fragment, it has been statistically established that the detection of the protein is dependent on disease presence. The fact that all remission patients analysed, tested negative for the 37KDa fragment is very encouraging. To date the overall sensitivity of the fragment protein is 87% and the specificity is 96%. Both these figures are superior to those of the BTA stat and the NMP22 tests which are commercially available. The sensitivities for the NMP22 and the BTA stat are 48% and 57% respectively, with specificites of 70% and 68% respectively (Weiner et al, 1998). However, the 37KDa EGFR fragment test is not 100% sensitive or specific. The test did not pick up 4 patients who had bladder tumours at the time of analysis. It may therefore be suggested that the 37KDa test could be used in tandem with both the NMP22 and the BTA stat test to reach 100% sensitivity and specificity. If 2 out of 3 of the tests gave positive results for a particular patient, it could be predicted that the patient had a bladder tumour. However, this hypothesis needs to be researched further, in order for this statement to be confirmed.
The test of the present invention may be used alone or together with any other suitable test.

Of the prostate patients analysed, 50% tested positive for the 37KDa fragment. The medical records of these patients will have to be researched further to confirm if they also had a undetected bladder tumour at the time of urine analysis. If it is found that these patients did not have bladder cancer, they could be ruled out by performing the prostate-specific antigen (PSA) test.

From the data obtained it was also found that 57% of urinary infection patients tested positive for the 37KDa fragment. This was to be expected, as EGFR over expression has been associated with inflammation and chronic irritation (Uhlman et al., 1996). The urinary infection patients would have to be treated with a course of antibiotics before the 37KDa test could be carried out. The 37KDa fragment test has a number of clinical uses. Firstly, the test could be used to determine whether or not a patient requires cystoscopy. This would cut down on the number of cystoscopies presently carried out and would save the National Health Service considerable expense. The test would also be less traumatic for the patient than having cystoscopy, which is an uncomfortable, time consuming procedure. As males are becoming more aware of their own health, the test could also be used to screen males over 50 years, as this is the group most at risk from bladder cancer. It is hoped that a urinary dip-stick will allow quick detection of the presence of a bladder tumour.

The high frequency of recurrent TCC in the bladder and the progression to a more malignant phenotype in a
proportion of patients means that lifetime follow-up
using cystoscopy and urinary cytology is essential.
Cystoscopy is an invasive procedure and urinary
cytology while non-invasive is relatively insensitive.
At present the Bard BTA and STAT tests are the only
commercially available detectors for bladder cancer.
Their sensitivity means that at best they will only act
in conjunction with cystoscopy. The Bard TRAK test
while more sensitive has yet to be marketed and in fact
the results from the present study indicate that the
37KDa EGFR fragment is at least comparable. Further
work is required to investigate the significance of
this fragment in the detection of first presentation
and recurrent bladder TCC and to determine whether
making it into a quantitative test will offer some
insight into prognosis. Appropriate applications are
detailed below.

The 37KDa EGFR fragment may be used as a detector for
first presentation bladder and recurrent bladder TCC.
Detection of the 37KDa EGFR fragment may be carried out
by other methods of investigation as well as western
blot analysis. These methods may include
immunochromatography, ELISA, latex agglutination or
radioimmunoassay. There is currently available a one-
step immunochromatographic assay which qualitatively
detects bladder tumour antigen in urine in five
minutes. Detection of the 37KDa EGFR fragment may be
detected by a similar method. Patient urine would be
added to the small chamber where it mixes with a
colloidal gold-conjugated antibody. If the 37KDa
fragment is present, a 37KDa fragment conjugate complex
would form. The reaction mixture would flow through
the membrane which contains zones of immobilised
capture antibodies. In the test zone, the 37KDa
fragment conjugate complexes would be captured by a
second antigen-specific antibody, forming a visible
line. If the 37KDa fragment is not present in the
urine, no visible line would form.

EGF-Receptor (Ab-4) is available from Oncogene Science,
Inc. as catalogue no. HCS16. There is no suggestion
that the antibody could be used to diagnose the
presence of the 37KDa EGFR fragment in urine or that
the presence of this fragment is indicative of bladder
or prostate cancer.

Other antibodies can be developed which are specific to
the 37KDa fragment. This may increase sensitivity of
the test.

A dip-stick test may be developed. This may require
using methods such as latex agglutination,
immunochromatography, ELISA and radioimmunoassay.

Bladder cancer prognosis has been correlated with a
number of factors, the single most important of which
is depth of invasion of the bladder wall
(Gospodarowicz, 1995); this is followed by grade of
tumour (Heney et al., 1983). Other less important
factors which influence patient outcome include tumour
size (Gospodarowicz, 1995), age of patient at diagnosis
(Fitzpatrick and Reda, 1986) and health status
(Thrasher et al, 1994). None of these factors can
predict prognosis in 100% of patients and so the 37KDa
fragment may have some use prognostically. The EGFR
fragment may be detected quantitatively using
densitometry following western blot analysis and used
to predict whether increased levels indicate a better
or worse prognosis. Other quantitative methods may be
developed to allow easier performance e.g. ELISA or
radioimmunoassay techniques.
EGF and EGFR have been implicated in the pathogenesis of solid tumours such as those of the breast. This simple test developed for urine of patients with suspected TCCB might also be used to identify the diagnostic prognostic role of serum EGFR in other tumour types.
CLAIMS

1. A marker for bladder cancer, prostate cancer or urinary infection, the marker comprising a 37KDa fragment of EGFR.

2. A method for the diagnosis of first presentation or recurrence of bladder cancer, the method comprising the detection of a 37KDa fragment of EGFR in a urine sample.

3. A method as claimed in claim 2 wherein the presence of the 37KDa EGFR fragment is detected using an antibody.

4. A method as claimed in claim 2 or claim 3 wherein the presence of 37KDa EGFR fragment is detected using antibody Ab4 EGFR available from Oncogene Science, Inc.

5. The use of antibody Ab4 EGFR in a test to detect the presence of 37KDa EGFR fragment in urine as a diagnostic test for bladder cancer.

6. A method for the diagnosis of prostate cancer, the method comprising the detection of a 37KDa fragment of EGFR in a urine sample.

7. A method as claimed in claim 6 wherein the presence of the 37KDa EGFR fragment is detected using an antibody.

8. A method as claimed in claim 6 or claim 7 wherein the presence of 37KDa EGFR fragment is detected using antibody Ab4 EGFR available from Oncogene Science, Inc.
9. The use of antibody Ab4 EGFR in a test to detect the presence of 37KDa EGFR fragment in urine as a diagnostic test for prostate cancer.

10. A method for the diagnosis of bladder cancer, and/or prostate cancer and/or urinary infection, the method comprising a test for the presence of a 37KDa fragment of EGFR in a urine sample.

11. A method as claimed in any of claims 2 to 4 and 7 to 10 in the form of a dip-stick test.

12. The use of antibodies to the 37KDa fragment of EGFR in the diagnosis of urinary infection, bladder cancer and prostate cancer.
### INTERNATIONAL SEARCH REPORT

#### A. CLASSIFICATION OF SUBJECT MATTER

| IPC  | G01N33/68 | G01N33/574 |

According to International Patent Classification (IPC) or to both national classification and IPC.

#### B. FIELDS SEARCHED

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched.

Electronic data base consulted during the international search (name of data base and, where practical, search terms used).

#### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>WO 92 21771 A (TRITON DIAGNOSTICS INC) 10 December 1992 (1992-12-10) claim 1</td>
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<td>A</td>
<td>US 4 933 294 A (WATERFIELD MICHAEL D ET AL) 12 June 1990 (1990-06-12) abstract claims</td>
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Further documents are listed in the continuation of box C.

**Patent family members are listed in annex.**

* Special categories of cited documents:
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  - "A" document member of the same patent family.

Date of the actual completion of the international search: 21 January 2000

Date of mailing of the international search report: 31/01/2000

Name and mailing address of the ISA:
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