Cancer remains one of the most fatal illnesses known. In industrial countries, cancer together with heart disease are the leading causes of death (1). After a prolonged “war on cancer” involving ever stronger anticancer drugs, radical treatment strategies and advances in our understanding of the molecular mechanisms of oncogenesis, the mortality rates from cancer continue to rise. In the USA, for example, about one million people died of cancer in 1991 (2). In Europe, 1.4 million cancers were diagnosed in 1990 resulting in close to 900,000 registered deaths (3). The number of primary cancer patients worldwide reaches several millions. By the year 2010, approximately 1.1 million Europeans are predicted to succumb to cancer (4). During recent years, cancer prevalence has not changed, despite enormous efforts to improve the diagnostic and therapeutic measures (5).

Detecting cancer when a tumour already exists is not complicated; moreover, in most cases, detection cannot increase patient survival. The most challenging task is to recognize the disease in its earliest, precancerous stages, thereby providing an opportunity for prevention strategies. Detecting cancer in its earliest stages and preventing progression of the disease are the two main aims of the new approach to monitoring cancer patients. We found that soluble cell surface tumour-associated antigens (sTAA) can successfully serve both these aims.

The soluble p51 protein in cancer detection

Vast efforts have been made to find a cancer-specific factor that can be used as a highly specific and reliable cancer marker. Unfortunately, despite the wide use of serological determinations of TAA or their antibodies for cancer detection (6, 7), almost none of the commercially utilized markers can satisfy the physicians’ requirements. An ideal tumour marker should be found only in the blood of cancer patients. In reality, cancer is indicated by the high expression of sTAA, which are also found in healthy people.
Although many tumour markers are used in clinical practice (CA125, CA50, CA15.3, CA19-9, CEA, MCA, etc.), very few of them, apart from α-fetoprotein for liver cancer (8) and, perhaps, prostate antigen (9), have a high specificity in cancer diagnosis (10). Other tumour markers should be regarded instead as having prognostic significance for therapy (11). The low tumour specificity and extremely high variability of tumour markers are the main reasons that none are used for early cancer diagnosis (12).

A new method for the isolation of low-molecular-mass proteins in their soluble form was developed in my laboratory and has been used as a serological method for cancer detection. The diagnostic procedure involves two steps: i) isolation of sTAA from the serum of cancer patients with affinity chromatography columns (13) and ii) determination of the blood levels of different components of the sTAA using high performance liquid chromatography (HPLC) (14).

Gel fibreglass membranes were used as a support for the affinity chromatography columns (15), which showed similar effectiveness to commercial BioRad P-60 columns (16). The method has enabled the isolation of two soluble proteins from cancer patients: a 66 kDa protein belonging to a group of albumins and a 51 kDa protein belonging to a p53 tumour-suppressor family. The relationship between these proteins reflects the pathological disease-connected status of the patients.

### Table I. Serum levels of p51 and p66 proteins in patients with cancer or non-cancerous diseases (After ref. 28-30).

<table>
<thead>
<tr>
<th>Groups of patients</th>
<th>n</th>
<th>p51 (mg/ml) (mean±SE)</th>
<th>p66 (mg/ml) (mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy people</td>
<td>26</td>
<td>0.22±0.15</td>
<td>1.23±0.21</td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td>66</td>
<td>0.42±0.16</td>
<td>2.84±0.46</td>
</tr>
<tr>
<td>Blood vessel disorders</td>
<td>24</td>
<td>0.66±0.28</td>
<td>2.86±0.66</td>
</tr>
<tr>
<td>Polyposis</td>
<td>38</td>
<td>1.32±0.29</td>
<td>4.55±0.68</td>
</tr>
<tr>
<td>High risk of cancer</td>
<td>28</td>
<td>1.72±0.67</td>
<td>2.35±0.61</td>
</tr>
<tr>
<td>Primary colon cancer, adenoma</td>
<td>28</td>
<td>1.82±0.46</td>
<td>3.65±0.31</td>
</tr>
<tr>
<td>Primary colon cancer, adenocarcinoma</td>
<td>70</td>
<td>3.55±1.46</td>
<td>3.96±0.72</td>
</tr>
<tr>
<td>Recurrent colon cancer</td>
<td>40</td>
<td>5.62±1.06</td>
<td>2.92±0.86</td>
</tr>
<tr>
<td>Uterine hyperplasia</td>
<td>26</td>
<td>0.82±0.26</td>
<td>n.d.</td>
</tr>
<tr>
<td>Uterine cancer</td>
<td>42</td>
<td>3.12±1.06</td>
<td>n.d.</td>
</tr>
<tr>
<td>Primary melanoma</td>
<td>35</td>
<td>2.61±0.42</td>
<td>n.d.</td>
</tr>
<tr>
<td>Melanoma with metastases</td>
<td>25</td>
<td>5.72±0.72</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

*Significantly different from healthy control (p<0.05).  
**Significantly different from non-cancer groups (p<0.05-0.01) 
†Significantly different from patients with melanoma without metastases (p<0.01).

Both proteins are present in the serum of healthy individuals, but in extremely low concentrations. A high concentration of p66 is typical for non-cancerous disorders, whereas high concentrations of both antigens are characteristic for patients with primary cancer (Table I). In patients with recurrent cancer, the serum p51 level increases significantly with a concomitant decrease in p66 concentration (15, 28).

### Table II. Serum levels of p51 protein in uterine cancer patients and expression of p53 protein in tumour cells of the same patients (After ref. 29).

<table>
<thead>
<tr>
<th>Gradation of lesions</th>
<th>Serum levels of p51 protein (mg/ml)</th>
<th>Concentration of p53 protein in tumour cell nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine hyperplasia</td>
<td>0.7±0.3†</td>
<td>0.7±0.1b</td>
</tr>
<tr>
<td>Uterine adenocarcinomas</td>
<td>3.2±1.0c</td>
<td>2.6±0.4c</td>
</tr>
</tbody>
</table>

*a* mean±SE.  
bMaximum stained cells in a field.  
*c* Significantly different from hyperplastic tissues and patients with benign tumours, p < 0.01.

The p51 gene has been described as a homolog of the p53 gene (17). The p51 protein in its nuclear fraction is considered to be a member of the p53 oncogene family related to the manifestation of different types of cancer (18-21). Soluble p51 protein was found on the plasma membrane in tumour cells (22). p66 has been described as a stress protein (23, 24), substrate of the epidermal growth factor receptor (25) and was found to be correlated with cancer development (26, 27).

Both proteins are multifunctional. The p51 and p66 proteins are used in experimental practice (CA125, CA50, CA15.3, CA19-9, CEA, MCA, etc.), very few of them, apart from α-fetoprotein for liver cancer (8) and, perhaps, prostate antigen (9), have a high specificity in cancer diagnosis (10). Other tumour markers should be regarded instead as having prognostic significance for therapy (11). The low tumour specificity and extremely high variability of tumour markers are the main reasons that none are used for early cancer diagnosis (12).

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evaluating the progress in cancer. In colon cancer patients with recurrent cancer, the blood level of p51 increased, whereas the blood level of p66 was not changed in the same patients (31). An increase in the serum level of p51 protein reflects more precisely changes in the progress of cancer than the commercial marker carcinoembryonic antigen (CEA). The correlation and regression coefficients between the serum level of p51 protein and the progress in colon cancer were 0.48 and 0.88, respectively (31). In patients with melanoma, development of metastases significantly increased the blood levels of p51 (30).

It has been found that some patients among the high-risk group can be considered "cancer suspect" due to their high serum level of sTAA and, especially, of p51 protein (31). Subsequent determination of blood levels of p51 in these patients can help to verify the earliest stages of cancer and to draw a conclusion regarding the clinical status of the patient and the results of therapy.

The method is highly specific in isolation of sTAA and their different components: the amount of isolated sTAA, and especially their main components the p66 and p51 proteins, was significantly higher when isolation was performed with anti-sTAA rabbit IgG compared to commercial rabbit IgG (32).

In cases with endometrial cancer, a close relationship between the serum level of p51 protein and the cellular concentration of p53 protein was found (Table II). The serum level of p51 protein increased in parallel with cellular accumulation of p53 in tumour cells: the coefficient of correlation between both these parameters was 0.42 (29). The coefficient of correlation between gradation of lesions and blood level of p51 was also 0.42. An increase in the initial concentration of TAA induced the maximum rate of tumour growth in its early stages and increased the risk of cancer (33).

Chronic diseases are accompanied by an increase in the blood concentrations of heat-shock proteins and, sometimes, TAA (34). Using our method, it is possible to monitor patients with chronic diseases and to detect transformation of these diseases into cancer by repeated increases in the serum level of p51. This transformation has been shown in diseases such as ulcerative colitis (35), gastritis (36) and cirrhosis of the liver (37). Overexpression of p53 antibodies was seen in the serum of patients with bowel inflammatory disease (38). The 51 kDa antigen also appears to be related to autoimmune diseases (39). This could explain this protein's accumulation in the serum of patients with non-cancer diseases (28).

Serological methods for cancer diagnosis with commercial markers are based on determining blood levels of antitumour antibodies. The method for isolating sTAA is also effective for the isolation of tumour-associated antibodies as markers for cancer detection (40). Both forms of the p51 protein (antigen and antibody) were isolated with high accuracy for cancer diagnosis (87% to 93%) (40).

The specificity of the method was proven using the commercial p53 antibody OD1: GFG columns with anti-sTAA IgG isolated the same proteins which had been isolated by GFG columns with commercial antibody. Moreover, the amount of isolated proteins, as either antigens or antibodies, was similar (40).

The lead-time in detection of recurrent cancer can significantly be decreased using our method of cancer detection. In the case studied, recurrent cancer was discovered by the commercial marker 6 months after the appearance of recurrent tumour. By our method, the first signs of recurrent cancer were evaluated a few months before the morphological appearance of a tumour (Zusman, unpublished).

**p51 and p66 proteins in prevention and therapy of chemically-induced cancer**

Recent insights into the identification of human tumour antigens has renewed the enthusiasm for the development of cancer vaccines. Thus, the tumour antigen has re-emerged as the focal point of tumour immunology, whether the object is detection, therapy, or prevention. Many cancer vaccines are derived from tumour cells and can stimulate a wide spectrum of immune responses directed against a variety of tumour antigens. Tumour cells, and TAA expressed by them, have been used widely in experimental and clinical studies aimed at tumour suppression (41-43).

There are many examples of experiments using such anticancer immunotherapy. Genetically-modified tumour cells have been applied successfully for the therapy of grafted syngeneic tumours in mice (44, 45). Specific immunotherapy with allogeneic cells was used for metastatic colorectal carcinoma in humans (46, 47). Vaccination of *neu* transgenic mice with a cell product from breast cancer prevented the development of mammary tumours (48). Vaccination of mice with various tumour-specific peptides enhanced the cytolytic T lymphocyte response and exhibited a tumour-suppressive effect (49, 50).

The identification of TAA recognized by cellular or humoral effectors of the immune system has provided new perspectives for cancer therapy. Different groups of TAA have been described as targets for cytotoxic T lymphocytes *in vitro* and *in vivo*: cancer-testis antigens, which are expressed in different tumours and normal testes, melanocyte-differentiation antigens, point mutations of normal genes, antigens that are overexpressed in malignant tissues, and viral antigens (51). Commercial TAA are useful for the therapy of different types of cancer and many of them are not cancer-specific. CEA has been used for the therapy of colorectal cancer and non-small cell lung cancer (52, 53). Breast cancer antigen CA15-3, mucus antigen and
It has been suggested that vaccination stimulates the synthesis of new sTAA in the host and helps them to recognize and destroy tumour cells, as has been described for the effect of antibodies (71). In vaccinated rats, the number of proliferate Ki67+ cells decreased, and the number of CD8+ lymphocytes and macrophages, as well as AI, increased (72). In combining treatments with CPA, sTAA sharply increased AI compared to the effect of antibodies (71). In vaccinated rats, the number of Ki67+ cells decreased, compared to controls, the yield of tumours and also the total area of tumours (68). Both parameters showed a further significant decrease after treatment with 5-Fu and, especially, after the combined treatment with sTAA and 5-Fu.

Vaccination with sTAA enhanced the tumour-suppressive effects of a hormone-related drug tamoxifen (69). The number of regressed tumours increased to 10% among rats treated with sTAA and to 14.3% among rats treated with both sTAA and the drug (Table IV). The 50% survival rate of tumour-bearing rats increased up to 18 weeks after the combined effect of tamoxifen and sTAA, compared to 13 weeks in controls or in tamoxifen-treated rats.

No significant differences were found between the anti-tumour effects of the p53 gene, sTAA and anti-sTAA polyclonal antibodies. Systemic p53 gene therapy increased the number of tumour-free nude mice up to 60% (70). Fifty-six % of tumour-free animals were found among DMH-induced colon cancer rats vaccinated with IgG generated against the sTAA (56, 57). Fifty-four % of rats remained tumour-free of DMBA-induced mammary cancer when they were vaccinated with sTAA (59, 60).

It has been found that immunization of mammary tumour-bearing rats with sTAA promoted a tumour-suppressive effect of CPA (67). The number of malignant tumours decreased from 75% in controls to 50% in drug-treated rats and to 30% in rats treated with a drug and sTAA. The number of regressed tumours increased from 3% in controls to 25% in drug-treated rats and to 38% in rats treated with a drug and sTAA. Vaccination decreased significantly the sizes of tumours and, in combination with CPA, stopped their further growth.

High doses of carcinogen caused a high rate of tumorigenesis. The treatment with sTAA alone significantly decreased, compared to controls, the yield of tumours and also the total area of tumours (68). Both parameters showed a significant decrease after treatment with 5-FU and, especially, after the combined treatment with sTAA and 5-Fu. Vaccination with sTAA enhanced the tumour-suppressive effects of a hormone-related drug tamoxifen (69). The number of regressed tumours increased to 10% among rats treated with sTAA and to 14.3% among rats treated with both sTAA and the drug (Table IV). The 50% survival rate of tumour-bearing rats increased up to 18 weeks after the combined effect of tamoxifen and sTAA, compared to 13 weeks in controls or in tamoxifen-treated rats.

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The tumour-preventive effect of vaccination is connected with the activation of the host immune system (70). Vaccination with sTAA increased the areas of B- and T-cell

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Table III. Expression of different tumorigenic parameters in tumours obtained from vaccinated and non-vaccinated rats (After ref. 59, 61).

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>Apoptotic index</th>
<th>p53</th>
<th>PCNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(% positive cells)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-vaccinated</td>
<td>6.8±0.3</td>
<td>20.1±1.2</td>
<td>11.8±0.8</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>14.4±0.8*</td>
<td>26.3±1.5a</td>
<td>8.7±0.4a</td>
</tr>
</tbody>
</table>

*aSignificantly different from non-vaccinated rats, p<0.01
PCNA, proliferate cells nuclear antigen.

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The tumour-preventive effect of vaccination is connected with the activation of the host immune system (70). Vaccination with sTAA increased the areas of B- and T-cell
related zones in the spleen (59). The number of CD4+ and CD8+ thymocytes significantly increased in rats treated with CPA alone or in combination with sTAA (75,76). Toxic effects of the CPA treatment manifested as a sharp decrease in areas with cellular components in bone marrow and in an increase in areas with fatty tissue compared to controls. Combined treatment with CPA and sTAA decreased the toxic effects of CPA and, as a result, the areas with cellular components increased compared to those in the CPA-treated group (77).

The efficacy of chemotherapy and vaccination with sTAA suggests that sTAA probably strengthen interactions between antigen-containing cells and T cells during antigen presentation, which leads to curative responses of the host’s immune organs (60). Clinical observations of spontaneous regression in size of carcinomas, and even their disappearance, as well as host response to various immunotherapeutic procedures, strongly suggest a role for the host immune system in tumorigenesis. In order to avoid the side-effects of immunotherapy with heterologous proteins, autoimmunotherapy has been studied recently as an alternative tool. It is anticipated that individual immunomodulatory compounds are not synergized with anticancer drugs, owing to differential immunosuppressive effects.

Autologous tumor cell vaccines or their lysates, known as TAA, have demonstrated beneficial activity in some types of carcinomas, such as breast cancer (78), renal cancer (79, 80), cancer of the pancreas (81) and ovarian cancer (82). Tumour-lysate-loaded autologous melanoma TAA have been used as a vaccine for melanoma patients (83). An autologous cytokine-rich serum, prepared from activated autologous peripheral blood lymphocytes, has been used for cancer autoimmunotherapy (84). Autologous sTAA also showed good therapeutic effect in a few cases of human cancer (85, 86).

**Conclusion**

The soluble p51 protein alone, or in combination with the soluble p66 (as a preparation of the sTAA), was shown to be useful in monitoring cancer patients. Determination of the serum level of p51 protein can be useful in detecting an early cancerous state, either as primary illness or as a recurrent disorder. In treated cancer patients, such determination can be used as a follow-up procedure to evaluate the results of therapy. The high therapeutic effect of sTAA and good safety profile of combining sTAA with chemotherapy should direct our efforts towards the development of strategies for clinical application of the experimental data. Screening of high-risk patients and vaccination of those who show high blood levels of p51 with their own (autologous) sTAA may prevent the development of primary cancer. The first attempts to use such an approach for humans have shown great promise.

**References**


<table>
<thead>
<tr>
<th>Rat groupsa</th>
<th>Tumour yield at the start of experiment</th>
<th>Tumour yield at the end of experiment</th>
<th>Total number of tumours</th>
<th>Number of new tumours</th>
<th>Number of regressed tumours (%)</th>
<th>Areas of tumours/ rat (cm²) (mean±SD)</th>
<th>Number of rats without malignant tumours (%)</th>
<th>Duration of experiment (weeks)</th>
<th>Time of 50% survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.6</td>
<td>4.4</td>
<td>81</td>
<td>52</td>
<td>0</td>
<td>29.7±4.5</td>
<td>5.6</td>
<td>10.4±0.8</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>1.7</td>
<td>3.3</td>
<td>69</td>
<td>32</td>
<td>7 (10.1)b</td>
<td>18.0±2.7</td>
<td>5.6</td>
<td>14.3±0.9b</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>1.8</td>
<td>4.4</td>
<td>73</td>
<td>39</td>
<td>1 (1.4)</td>
<td>21.4±2.4</td>
<td>27.3b</td>
<td>10.1±0.8</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>1.7</td>
<td>3.0</td>
<td>70</td>
<td>30</td>
<td>10 (14.3)b</td>
<td>13.5±2.0b</td>
<td>22.2b</td>
<td>16.4±0.5b</td>
<td>18</td>
</tr>
</tbody>
</table>

aRat groups: 1, control rats treated with saline; 2, treated with sTAA; 3, treated with tamoxifen; 4, treated with both sTAA and tamoxifen. Tumor yield: the number of tumours per tumour-bearing rats.

bSignificantly different from values in the group 1, p<0.05.


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