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Research Article

Reaction Pattern in Vertebrate Cells (RiV): Studies of the Course of Tumor Therapy using an anti-RiV ELISA (Initial Results)

Detection of anti-RiV antibodies by ELISA can be used to follow a patient's response to treatments such as cancer surgery or RiV therapy. The initial results of the authors demonstrated that it is necessary to optimize the sensitivity of the ELISA. Known tumor markers such as prostate specific antigen (PSA), especially the ratio of free PSA (fPSA)/total PSA (tPSA) or neuron-specific enolase (NSE) detect the therapeutic effect of treatment with RiV particle preparation (RiV-PP) more rapidly and with greater sensitivity than does the anti-RiV antibody assay. However, a continuous decrease of anti-RiV-antibody titers seems to indicate a good prognosis. RiV therapy improved the quality of life and achieved an apparent prolongation of life of cancer patients. After treatment with 12 mL of RiV-PP, a patient with a prostate hyperplasia of uncertain genesis became free of symptoms. This monotherapy with an adequate dose of RiV-PP resulted in a decrease of tPSA and an increase of fPSA in the first 200 days. The general value of RiV ELISA is emphasized by the fact that it could detect RiV antigen in urinary samples offering a simple means of early diagnosis and monitoring. Since chemo- or/and radiotherapy, both of which are immunosuppressive, are frequently used to treat cancer patients, an ELISA for the detection of RiV (particles as) antigen may be more informative than one designed to detect anti RiV antibodies.

Keywords: Antibodies, Medical biotechnology, Exosomes, Tumor patient sera

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1 Introduction

The reaction pattern in vertebrate cells (RiV) is mainly represented by characteristic RiV particles with a mean diameter of 30–70 nm. After comparing the results using electron microscopy (EM) of ultrathin sections of different stressed cell cultures [1, 2], of biocomplex RiV samples – RiV particle preparations (PP) – after negative staining in the EM [3–5] and by biochemical analyses of RiV-PP [6, 7] with the corresponding studies of exosomes [8–11] it was concluded that RiV particles are exosome-like particles [4]. RiV-PP have been applied as an experimental vaccine against laryngeal papillomatosis [12, 13], foot and mouth disease of guinea pigs [3] and mammary carcinoma of mice [14]. RiV-PP treatment of BALB/c mice into which $4 \cdot 10^6$ J558L myeloma cells had been transplanted re-

duced tumor growth by $46 \pm 15.2\%$ under the chosen conditions (3×0.2 mL RiV-PP i.m. at intervals of 2 days, starting on day 1 after tumor cell transfer) [15]. This RiV vaccine had been boiled for 10 min to inactivate possible viral contaminants. In 1995, a further experimental RiV vaccine was used on the 10th, 12th, and 14th day after the tumor cell transplantation. The tumor mass was $44 \pm 20.6\%$ of the control at the end point of the experiment (day 30) for the native vaccine and $56 \pm 9.1\%$ of the control on day 30 for the boiled vaccine. The differences between boiled and native vaccine were not statistically significant [15]. The survival time of mice was not determined, but it was noted that tumors of RiV-PP treated animals were not open as in all mice of the untreated control group, but instead encapsulated and hardened [16].

RiV-PP has also been used in a general practise to treat AIDS, cancer and hepatitis C patients [2, 6, 17, 18]. Impressive results were obtained with RiV-PP treatment of a generalized psoriatic erythrodermy of a patient with progressed AIDS [19]. Before the RiV therapy there were strong inflammatory reactions, considerable scaling, a syndrome similar to Lyell-syndrome all of which were much improved after a total dose

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of 21 mL of RiV-PP (i.m.) administered over a treatment period of 12 weeks. Recently, RiV-PP was used to treat idiopathic lung fibrosis. In this case, RiV-PP was applied because corticosteroids were ineffective and chemotherapy was not tolerated. Treatment with RiV vaccine normalized the strongly increased C-reactive protein (CRP) titer and stopped the progress of fibrosis for at least 12 months. Severe disease phases were strongly diminished [20].

It has also been reported that an RiV-antibody ELISA may detect but not diagnose pathological cell proliferation [5]. This assay should use RiV-PP from a human cell culture as the permanent HeLa cell line and might be used as a screening method for diseases which are characterized by auto-antibodies against RiV-specific proteins. A great number of these proteins are tumor-associated antigens [7, 21].

In this paper, the authors investigated under which conditions an anti-RiV-antibody assay is useful for monitoring the course of cancer therapies, including monotherapy with RiV particle preparation from stressed calf kidney cell cultures. In particular, it was asked whether the RiV reactive antibody assay can be used for a prognostic evaluation of the course of diseases. Initial results indicate that an increase of the RiV reactive antibody titer is associated with a poor prognosis.

2 Materials and Methods

2.1 Cells and Preparation Procedure for the RiV-PP as an Antigen for ELISA

Primary calf kidney cells were cultivated and the RiV-PP (RiV sample) was prepared as previously described [4, 22]. Briefly, the cells were grown in roller bottles in alphaMEM Eagle medium (Lonza) with 10 % fetal calf serum (FCS). Once the cultures reached confluence, they were further cultured for 3 to 4 days in a medium without serum. Cells were then disrupted by freeze-thaw cycles followed by sonication. The RiV particles were purified and concentrated by low and high speed centrifugations/ultracentrifugation and a short treatment with an organic solvent [4]. If necessary, residual organic solvent was removed using a nitrogen stream at room temperature. The RiV-PP used as an antigen for the ELISA was produced under GMP conditions as was the RiV vaccine.

2.2 RiV Vaccine

The RiV-PP complex used here as a therapeutic vaccine was prepared in the same way as the RiV-PP used for the ELISA. The material was also isolated from calf kidney cell cultures. The cell culture was tested both for cytopathogenic and for haemadsorbing viruses. The isolation of RiV-PP was carried out under GMP conditions. The RiV-PP preparations were characterized by electron microscopy, protein determination, SDS-PAGE, immunoblot (using anti-annexin I, anti-annexin V and a standard RiV-specific antiserum from rabbits), UV spectroscopy and ELISA (using also a standard anti-RiV-PP antiserum from rabbits). Tests for sterility were performed in ac-

cordance with the European pharmacopoeia standards. Safety was tested using mice.

2.3 Sera

RiV-PP-Specific Antiserum: A polyclonal antiserum pool against the RiV-PP was produced by subcutaneous and intramuscular injections into rabbits as previously described [4] and was used as a standard RiV-specific antiserum (1:500) for RiV antigen titration. *Patient sera* were obtained from a doctor's practice.

2.4 Protein Quantification

The protein content of the samples was determined by a modified method of Lowry et al. [23], using a micro-variant.

2.5 UV Spectroscopy

For the measurement of the ultraviolet absorption spectrum of RiV samples, a universal spectral photometer (Shimadzu, Japan) was used. The calculation of protein concentration was performed as described previously [4].

2.6 ELISA

ARIVA

In the examination of human sera by ARIVA (anti-RiV antibody assay) the assay was performed analogously to an ELISA described earlier [24]. Polystyrene microplates (MP) with high adsorption capacity (Greiner, Frickenhausen, Germany) were coated with a defined dilution of RiV antigen in PBS (pH 7.4 for 2 h at 37 °C). An RiV-PP sample prepared from calf kidney cell cultures was used as a standard antigen (SA). After each step of the ELISA, the plates were washed 4 times with Immuno Wash (Bio-Rad, Hercules, CA, USA). Normally, two wells of the plates were incubated with a 1:50 dilution of a human serum for 2 h at 37 °C. A pool of normal sera (negative sera pool) and a pool of positive sera were used as low and high controls, respectively. After incubation with horseradish peroxidase(HRP)-conjugated secondary antibodies (anti-human-IgG-HRP and anti-human-IgM-HRP, respectively) for 45 min at 37 °C, bound antibodies in the test sera were detected using *o*-phenylenediamine. Optical density (OD) was determined with an ELISA reader (anthos 2020, Anthos Labtec Instruments, Krefeld) at 492 nm. The OD value in the ELISAs of the positive sera pool at a dilution of 1:50 must be always $> \times 2$ the cut-off value.

The index values of the sera were then determined (ratio of OD at 492 nm to cut-off). A temporary cut-off for the RiV-reactive IgG and IgM values was obtained from the mean value of the normal (negative) sera pool plus 0.1 OD. A gray area for the index was defined with 1.0 ± 0.1 . Sera with index values < 0.9 were normal (or "negative") and sera with index values

>1.1 were positive. Sera with index values ≥ 2.5 were considered strongly positive.

RiV Antigen Titration

The assay for the RiV antigen titration was carried out as an indirect ELISA with horseradish-conjugated secondary anti-rabbit antibodies and *o*-phenylenediamine as a substrate as previously described, using a rabbit RiV-specific antiserum as a standard in a dilution of 1:500 [4].

2.7 Electron Microscopy

Negatively stained RiV samples were prepared on Pioloform-carbon-coated copper grids (400 mesh). After glow-discharging the grids, samples were adsorbed for 10 min, followed by two washes with double-distilled water and negative staining with 1 % uranyl acetate for 30 s.

2.8 ELISA for the Detection of RiV Antigen in Urine

The urine samples were first freeze-thawed (3 or 4 \times) and then clarified by low speed centrifugation. No ultracentrifugation concentration was carried out here. The ELISA was performed essentially as described for the RiV-PP antigen titration, using rabbit anti-RiV-PP serum.

3 Results and Discussion

3.1 Analysis of RiV-PP Antigen or/and RiV Vaccine by ELISA, SDS-PAGE, Immunoblot, UV Spectrometry and Electron Microscopy

Analyses of RiV-PP samples were performed using ELISA, SDS-PAGE, immunoblot and UV spectroscopy as previously described [4].

After the titration of the “standard antigen” SA in dilutions from 1:20 to 1:1280, a dilution of 1:80 was normally used for this SA in the ELISA of patient sera.

Although annexin I and V could be readily detected by SDS-PAGE as the main components in the SA, the RiV-PP and immunoblot, an annexin ELISA was insensitive. The insensitivity of the ELISA might be due to the fact that the annexin-specific antibodies used reacted well with continuous (linear) epitopes, but weakly with conformation epitopes and/or many epitopes of annexins were hidden in the native RiV sample.

The protein content of the SA used was 77 ± 17 $\mu\text{g}/\text{mL}$, determined by UV measurement and 105 ± 14 $\mu\text{g}/\text{mL}$ determined according to the method of Lowry et al. For the comparison of the antigen concentration of other RiV particle preparations, both the protein concentration, as well as an ELISA of the RiV antigen sample and the standard RiV antigen performed as previously described were applied using a standard batch of rabbit anti-RiV-PP antiserum [4]. The RiV vaccine, also prepared from calf kidney cell cultures and used in

the RiV therapy studies, had a protein content of 90 ± 10 $\mu\text{g}/\text{mL}$ determined colorimetrically and in ELISA (antigen titration) a factor *F* of 1.16 ± 0.19 (see [5]). Electron microscopy was used as a qualitative control of RiV-PP and, by comparison with an internal standard, for an estimation of the quantity and purity of RiV particles, but compare [25]. Fig. 1A displays more details of the sample, and the data shown in Fig. 1B allowed an estimation of the quantity of RiV particles.

3.2 ARIVA: Therapy Course Studies

Monitoring studies following tumor surgery or during a chemo-, radio-immuno- or RiV-therapy are important for an early detection of tumor recurrence or growth of metastases. Currently, a number of tumor markers are available for such tests. Such monitoring studies were started using the ARIVA assay which may be applicable to cancers for which specific tumor markers are not available.

Fig. 2 displays the development of RiV-PP reactive antibodies (IgG) of a 66 years old patient after surgical removal of a tonsil carcinoma and radio-chemotherapy. The patient was also treated with RiV-PP (sample 030602) in the intervals between the radio-chemotherapy (from February 2003 to the end of July 2003 25 mL were administered). The course of IgG-index values showed no significant changes in the first 190 days which suggests that the ARIVA is not a suitable monitoring procedure under the immunosuppressive conditions of radio-chemotherapy. The significant increase of RiV-PP-reactive antibody values in April 2004 indicated a poor prognosis and was followed by the death of the patient in December 2004.

Fig. 3A shows the course of RiV-PP reacting antibody development in a 45-year-old female patient, with an inoperable rectal carcinoma and 52 lung metastasis. Prior to RiV therapy, the patient was in a poor nutritional and psychological condition and the tumor had perforated the wall of vagina. RiV-PP therapy was started in October 2002. Following application of 16 mL of RiV-PP in the intervals between a neoadjuvant radio- and chemotherapy which was started in September 2002, the blood cell count normalized more rapidly than in untreated patients at a similar stage of the disease. The RiV-PP therapy may thus have enabled an increased frequency of the chemotherapy cycles. After applying 30 to 38 mL RiV-PP in conjunction with the radio- and chemotherapy, the primary tumor was operable and was removed surgically, lung metastasis became smaller and no new metastasis were found. The patient was in a good general condition, and body weight increased. Further RiV treatment was continued with lower RiV-PP dosage, see Fig 3B. Because of many lung metastases the patient remained under continuously oncological care with chemotherapy in between. This patient returned to work.

As seen in Fig. 3, the antibody values (IgG index) were in the normal range, only the last value was in the gray sector. The RiV reactive IgM index values became strongly positive after the 50th day from the first blood sample and decreased again into the gray zone at the 160th day (not shown). No further serum samples of this patient were obtained after the 278th day. The slow increase of the IgG-antibody values in the

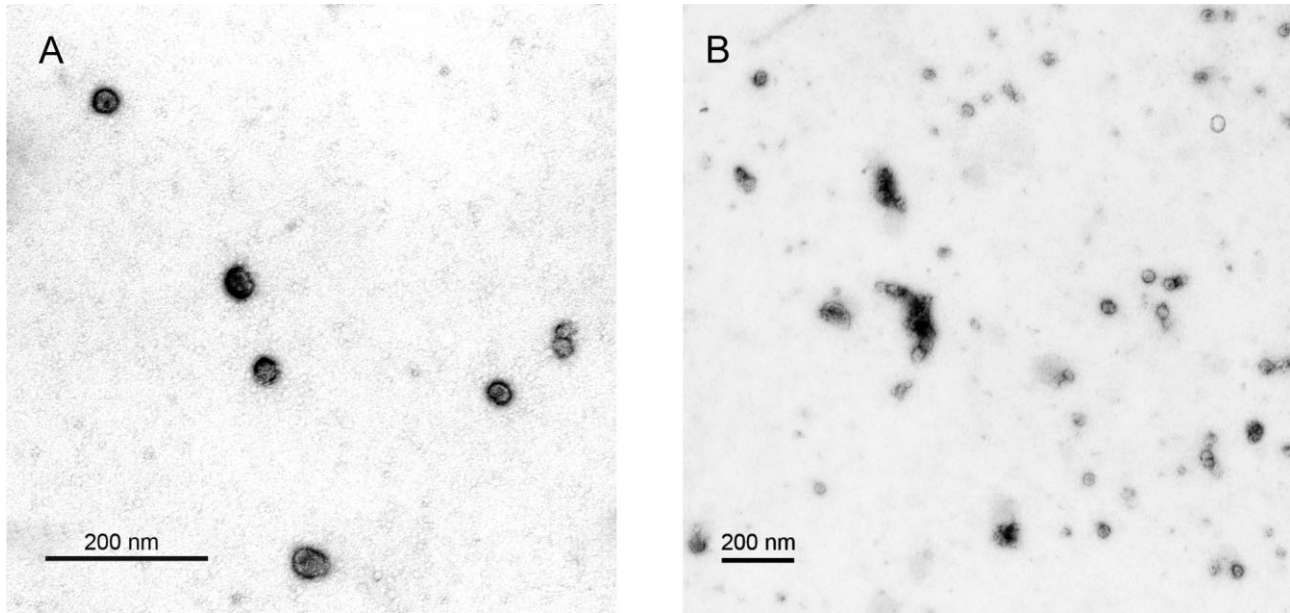


Figure 1. Electron microscopy of RiV particle preparations (RiV-PP). (A): RiV-PP, produced from calf kidney cells. The image was recorded at an instrumental magnification of 60 000. (B): the sample at an instrumental magnification of 27 800. The sample was purified as described in *Materials and Methods*. The diameter of RiV particles in this figures vary from 30 nm to 51 nm.

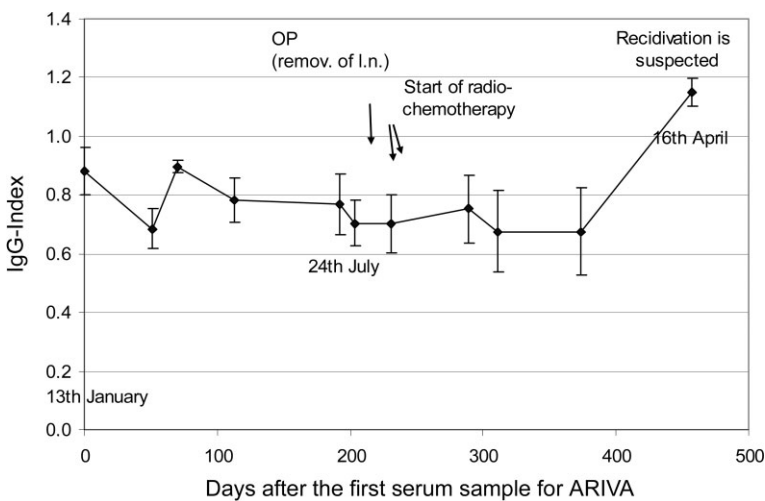


Figure 2. Time course of RiV-PP reactive antibody titers (IgG-index values) (mean values of 3 to 5 independent measurements and the corresponding standard deviations) of a male patient, aged 66 years with an operated tonsillar carcinoma. *OP (remov. of l.n.): (second) operation, removal of lymph nodes.*

first two month of measurements may be associated with the growth of metastases, see Fig. 3A. The index values in Fig. 3A are not significantly different between the 50th and 160th day, indicating that the course of RiV-PP-reactive IgG-antibody values is not a useful parameter in this patient. Only the general increase of the index values in Fig. 3A, which mirrored the increase in tumor mass, suggested a poor prognosis. In Sep-

tember 2003 pulmonary metastases were diagnosed by computer tomography (CT), accompanied by a partial atelectasis of the medial lobe of the lung.

After a growth of lung metastases and metastases in the brain and bone marrow, new chemotherapies were started and further 36-mL RiV-PP were applied up to March 2005. The patient died in June 2005.

The RiV-PP treatment may have contributed to an improvement of the quality of life in this patient who continued to work until 3 months before death. However, the time course of the titer of RiV reacting antibodies did not provide useful diagnostic data in this case (under the immunosuppressive conditions of radio-chemotherapy).

Fig. 4 shows the course of RiV-reactive IgG antibody values of a 35-year-old male patient with a grade IV gliosarcoma. After surgery in March 2001, an RiV-PP treatment was started as a monotherapy at the end of Mai 2001. 5 mL was applied i.m. on 2–3 days, followed by 5 mL on each day on which a serum sample was obtained. Thus the RiV-PP treatment was performed over a period of 10 weeks. A significant increase of antibody titer values was registered during this time. The difference of the index values of the first and of the 5th value was obtained with a probability of 95%. (The confidence intervals of the index values of these data were not overlapping or the difference $[(x_a - SD t_{n-1}\sqrt{n}) - (x_b + SD t_{n-1}\sqrt{n})]$ was greater than 0; where x_a and x_b are the mean values of the groups, (n is the number of single

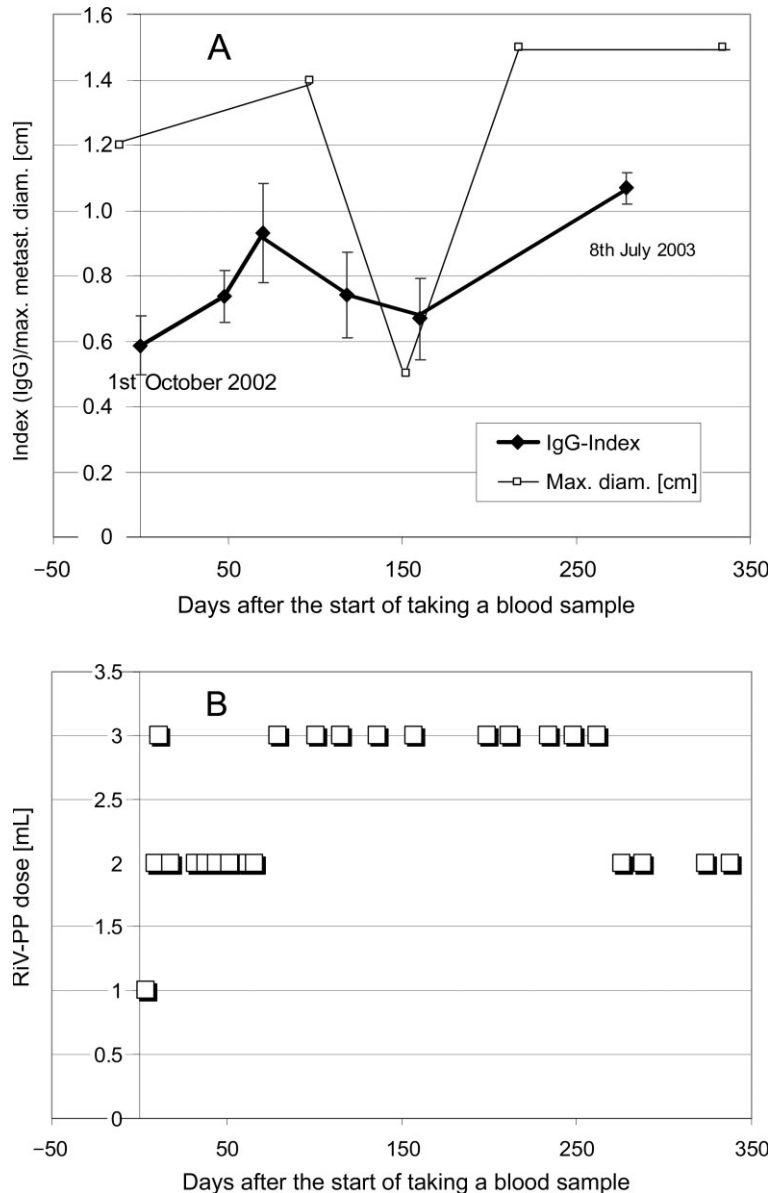


Figure 3. Time course of RiV reacting antibody titers (IgG index mean values and standard deviation of 2 to 5 independent determinations) and of maximum dimension/diameter of lung metastases of a 45-year-old female patient with rectal cancer and 52 lung metastases (The values of the max. diameters of lung metastases were from the Charité, Berlin, Arbeitsbereich Computertomography, B. Hamm, P. Rogalla).

values and t is a function of $n-1$, according to Student). A recurrence of the tumor was detected by CT in April 2002. After surgical removal in August 2002 and a third surgical intervention in December 2002, the patient died in February 2003. The antibody development between the second and third surgery did not give a clear indication of tumor recurrence. After the third surgery, the clear increase of antibody values suggests a rapid growth of the tumor residue, which was clinically confirmed. Life expectancy after the first surgery is normally 9 to 12 month [26].

Five patients who failed to respond to all standard therapies were also treated. These included rectal carcinoma with liver metastases, kidney carcinoma with brain metastases and pancreas carcinoma. An increase of RiV-reactive IgG-antibody values within the normal range (and in one case to the positive range) and RiV-reactive IgM-antibody values partially from the normal to the strong positive region was determined over a time interval of 6 to 9 weeks (some weeks before death). In comparison, the values of a blood donor remained constant within the upper normal sector over a period of 299 days.

The RiV-PP reactive antibody values (IgG-specific) of a patient with prostate hyperplasia were strongly positive at the start of RiV-PP treatment which in this case was used as a monotherapy. After a brief period, in which the values increased, the titers decreased to normal values over the course of approx. 460 days of the therapy, see Fig. 5A. Values beyond this point may have been influenced by the biopsy procedure.

High RiV-reactive IgG antibody values were seen in a second patient with a prostate hyperplasia [18]. At that time, the RiV-PP-IgM antibody values were normal. In comparison, the high total prostate specific antigen (tPSA) values (norm: <4 ng/mL) displayed a significant decrease and the ratio of free PSA to tPSA increased following the RiV-PP treatment in the first 200 days of treatment (Fig. 5B). After the application of 12 mL RiV-PP, the patient could urinate well again. But the PSA values rose again after approximately 200 days – suggesting that a higher dosage or more frequent application of RiV-PP might be indicated. The clinical state of the patient remained very satisfactory. A biopsy with 13 aspirations on day 680 (Fig. 5), did not provide evidence of prostate cancer (PC). Since sometimes after the 2nd to the 6th biopsy tumor cells are found, the formulation “prostate hyperplasia of uncertain genesis” was selected.

After the biopsy, the tPSA values rose by the biopsy and apparently as a result of inflammation. A treatment with antibiotics followed. The tPSA decreased after that, but the ratio free PSA/tPSA remained nearly constant and low after an increased RiV-PP dose. The tPSA values decreased further continuously to 4.4 in October 2007, and the fPSA/tPSA values stayed nearly below and above the cut-off value at very low RiV-PP doses of 2 mL per 3 month since March 2007.

The decrease of tPSA and the increase of fPSA/tPSA in the first 200 days of the RiV therapy suggests that RiV-PP may have been effective in this case.

Generally it would be better to determine the complexed (c) PSA. This cPSA has a greater part of t-PSA as the fPSA. The fPSA corresponds simplified with the benigne part and at a PC the cPSA increases. The measurement of fPSA should be

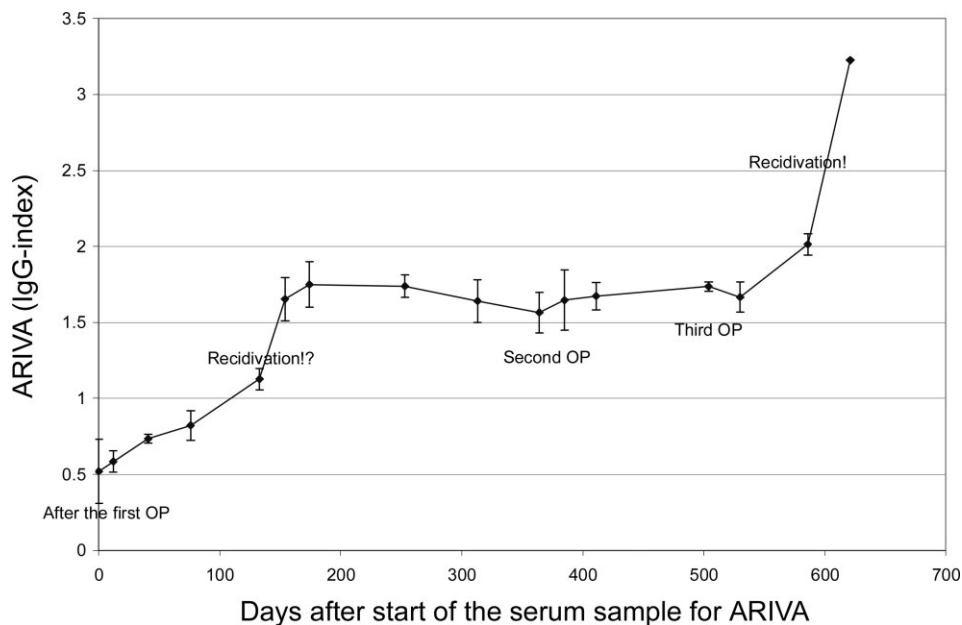


Figure 4. Time course of RiV reacting antibody titers (IgG index mean values and standard deviation of 2 to 6 independent determinations) of a 35-year-old male patient (at the start of the treatment) after the operation of a gliosarcoma grade IV. RiV-PP applications (i.m.) were on the same days as blood samples were taken. (Day 0: 22nd May 2001).

not exact, that is why cPSA should be measured, [27] (and others).

An 84-year-old female patient with a non-small-cell lung cancer (NSCLC) (adenocarcinoma) was treated by i.m. application of 8 mL of RiV-PP over 8 days, the neurone specific enolase (NSE) values were reduced from 54 to 16 and 30 ng/mL, measured after 16 and 23 days, respectively. However, the anti-RiV-PP antibody values (IgG and IgM) were normal and did not change during this time. For this patient, 13 mL of RiV-PP was applied as a monotherapy for the stabilization of the general conditions before surgery. The lung capacity was increased from ca. 50 to 85 %.

3.3 BC-RiV Antigen Detection in Urine by ELISA

An RiV-antigen detection in body fluids was proposed by [28] and this test has an advantage in comparison to antibody detection in that in immunosuppressed patients only small amounts of antibodies can be induced. A sandwich-ELISA and a source of purified RiV-specific antibodies are required for RiV-PP detection in serum samples.

Preliminary experiments with urinary samples have been carried out using freeze-thawed and clarified urine samples as antigens. As shown in Fig. 6, two of four samples were positive. For this assay using RiV-PP-specific and pre-immune rabbit sera, the limit of detection was approximately 180 ng/mL RiV protein.

The sensitivity and also the specificity for the detection of RiV particles in urinary samples can be optimized by a concentration step, i.e. by ultracentrifugation of the urine sample (1 h at 100000×g) or by ultrafiltration (i.e. with a 100 kDalton

NMW). The ultracentrifugation step is also possible for serum samples.

4 Discussion and Conclusions

Preliminary conclusions from the monitoring studies of the RiV-PP reactive antibody development during RiV-PP treatment are:

- I. In radio-chemotherapy, the ARIVA does not yield useful diagnostic data for the course of the disease.
- II. Using a monotherapy with RiV-PP, a sharp rise in the RiV-reactive IgG antibody titer may indicate recurrence or metastases growth and shows that RiV-PP therapy is ineffective against this cancer or that the dose must be increased.
- III. An increase of RiV-reactive IgG antibody values during the course of therapy indicates a poor prognosis and the continuous decrease of RiV-reactive IgG antibody titers seems to be an indicator for a good therapy effect and for a good prognosis, as already assumed [29].
- IV. Markers, such as tPSA, freePSA/tPSA [27, 30] or NSE, are more sensitive and react faster to changes than the ARIVA. However, the NSE tumor marker is reported to have a sensitivity of only 22 % and NSE did not show a clear relationship with tumor stage and histology in patients with NSCLC [31]. A more sensitive ARIVA could be helpful, in particular for monitoring studies of tumors for which no suitable molecular markers are available.
- V. The RiV-PP vaccine is a weak immunogen with regard to the induction of antibodies under the conditions described (protein concentration of the vaccine only approx.

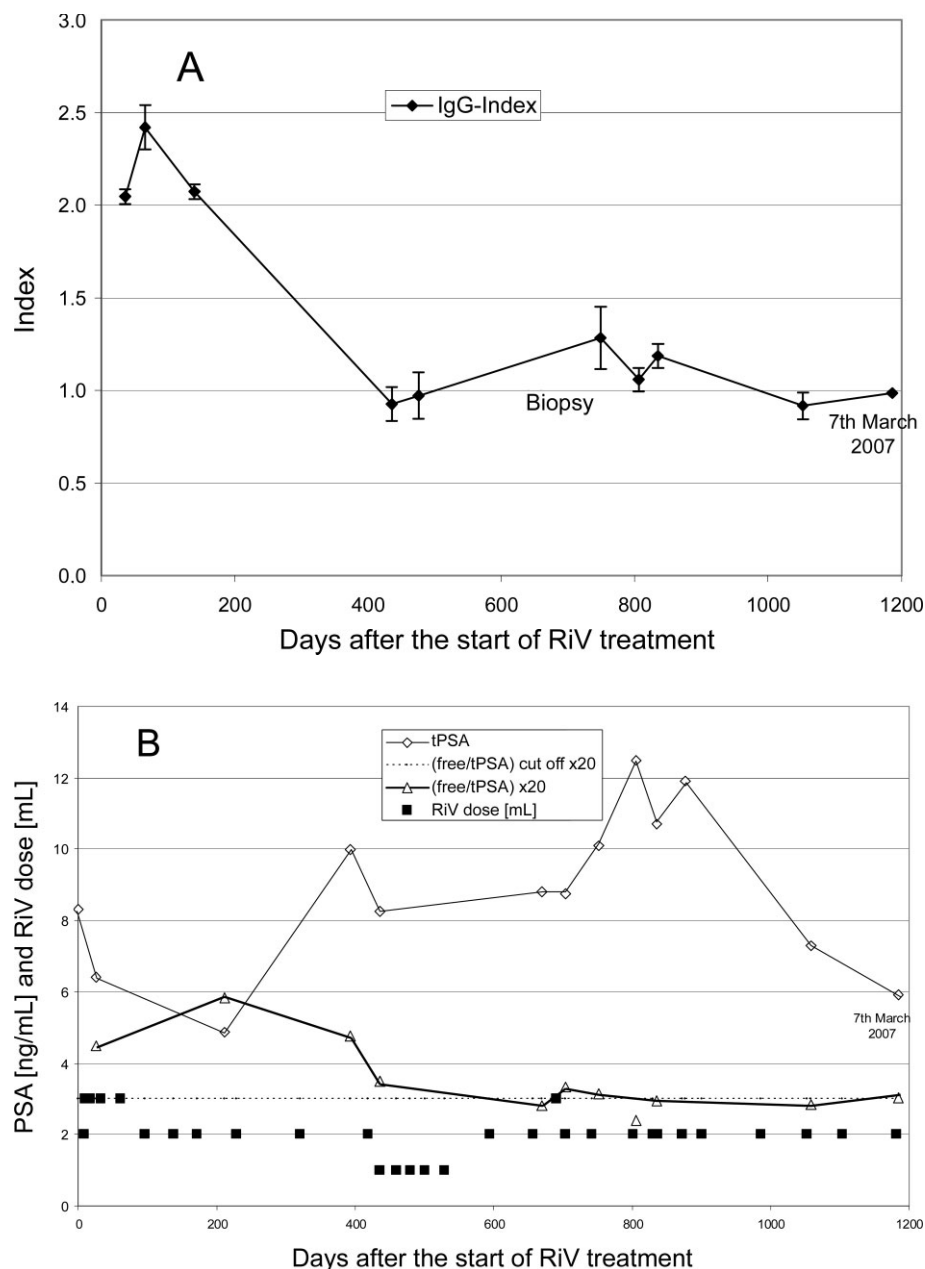


Figure 5. BC-RiV (RiV-PP) treatment of a 77-year-old patient with considerable problems in urinating (at start of treatment) (prostate hyperplasia).

(A): Time course of RiV-PP reacting IgG-antibody titers. The ELISA was performed as described in *Material and Methods*; cut-off is 1.0. The mean index values with a standard deviation of two to four independent experiments are shown. (B): Course of total prostate specific antigen (tPSA) and of the ratio of free PSA to tPSA. The 20 times value of cut-off for this ratio and the RiV-PP doses and time intervals of the treatment are also displayed.

100 $\mu\text{g}/\text{mL}$; vaccine administered without an adjuvant). On the other hand, it has previously been demonstrated that high RiV-specific antibody titers could be induced in rabbits by RiV-PP together with adjuvants, see [4]. This suggests that the RiV-PP treatment of these cancer patients was performed at an antigen dose in the range of “low

zone tolerance” [32]. On the other hand, this provides a hint that the vaccine is well tolerated during an extended time of treatment. RiV-PP, applied to BALB/c mice transplanted with mouse myeloma cells reduced the tumor growth in comparison to the untreated controls [15]. A similar experiment using athymic nude mice resulted in

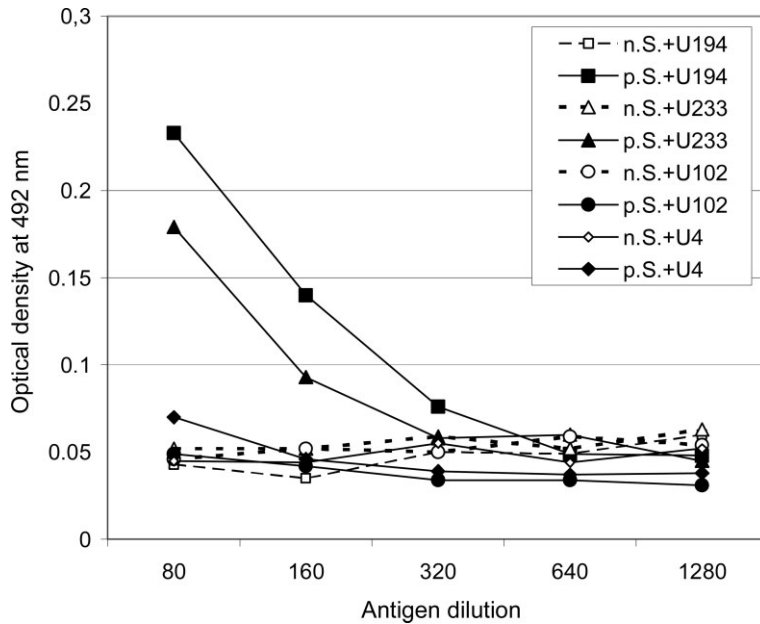


Figure 6. Detection of RiV-PP antigen in urinary samples, using rabbit anti-RiV-PP serum and rabbit pre-immune serum. The sera were used in a dilution of 1:250. Urine samples U194 and U233 were positive. The urine samples were received from clinically healthy individuals. (n.S. and p.S. stand for pre-immune and rabbit antiserum, respectively).

0% tumor growth reduction by RiV-PP treatment [33], which suggests that T-cells may be essential for the RiV-induced anti-tumor reaction.

Further studies are necessary to find out, which RiV-specific proteins react in this ELISA (ARIVA) and in immunoelectron microscopy (IEM). It is possible that a great part of the different proteins is hidden in the RiV particles. In this case, greater differences in the results of ELISA and immunogold electron microscopy, on the one hand, and an immunoblot, on the other, could be expected and the sensitivity of the ELISA with the native RiV antigen complex could be different from one using a denatured antigen complex. An RiV-specific rabbit antiserum reacted well with the annexins of RiV-PP in immunoblots, as previously shown [4]. First tests with patient sera with high antibody titers against RiV-PP in ELISA displayed also reactions in immunoblots (not shown). Further immunoblots are in preparation to evaluate, which proteins of RiV at which disease react with the patient serum. The more sensitive enhanced chemiluminescence (ECL), as already been used in [34], will be applied.

RiV Antigen Detection in Body Fluids by ELISA: During the present study, RiV antigen has been detected in urinary samples by ELISA, but the electron microscopy of these samples was not performed. Exosomes have already been reported in urinary samples by electron microscopy [35,36]. RiV antigen can be detected in urine samples with the simple indirect ELISA described here which has a sensitivity of 180 ng/mL. For an RiV antigen detection in sera, a sandwich ELISA, i. e. with purified rabbit RiV-specific antibodies, would

be better. Another variant is a concentration step by ultracentrifugation or ultrafiltration of the urine sample before the simple ELISA. Since chemo- or/and radiotherapy of cancer patients are normally used and since they both act immunosuppressively, an RiV antigen detection ELISA will probably be better than an RiV-reactive antibody detection.

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