

# Reduction of Plasma Levels of Soluble Tumor Necrosis Factor and Interleukin-2 Receptors by Means of a Novel Immunoabsorption Column

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**Abstract:** This non-randomized open clinical study investigated the safety and efficacy of extracorporeal fractionated plasma adsorption using the Oncosorb immune adsorption column. The column selectively bound soluble tumor necrosis factor receptors 1 and 2 (sTNF-R1 and sTNF-R2) and soluble interleukin-2 receptors (sIL2-R) by lowering plasma levels in patients with metastatic cancer. Nine patients (three men and six women; mean age 48 years, aged 41–68 years) with metastatic cancer received at least 12 immune adsorption procedures. Thrice-weekly immune adsorption separated a low molecular weight (150 000 D) plasma fraction from the patients' blood, passing the plasma fraction through the column in an extracorporeal plasma perfusion circuit. Respective plasma receptor mean concentrations before and after 167 procedures were: sTNF-R1:  $1936 \pm 788$  pg/mL before treatment

vs.  $1312 \pm 989$  pg/mL after treatment; sTNF-R2:  $3140 \pm 1173$  pg/mL before treatment vs.  $1816 \pm 1677$  pg/mL after treatment ( $P < 0.01$  for each inhibitor). Mean reductions in sTNF-R1 (48%), sTNF-R2 (55%), and sIL2-R levels (72%) were observed for treatments 3–12 ( $P < 0.001$ ). Clinical findings indicated tumor inflammation and necrosis in most patients. Side-effects were low-grade fever, flu-like symptoms; tumor pain and redness, warmth, tenderness, and edema. The column demonstrated safety and efficacy in lowering plasma sTNF-R1, sTNF-R2, and sIL2-R levels. Minor clinical adverse effects common to the use of extracorporeal devices were seen. **Key Words:** Cytokine, Immunoabsorption, Metastatic cancer, Plasma, Soluble interleukin-2 receptor, Soluble tumor necrosis factor receptor.

Immune suppression has long been recognized as a hallmark of patients with advanced malignancies (1–3). Tumor necrosis factor (TNF) and interleukin-2 (IL-2) are pro-inflammatory cytokines produced by activated mononuclear white blood cells (WBC) that bind to tumor cells via specific receptors and induce cell death by both oxidative stress and apoptosis. This process constitutes a major part of the normal response of the immune system in its constant fight against cancer cells. Overproduction and shedding by tumor cells of high levels of two soluble receptors for TNF (sTNF-R1 and sTNF-R2) and IL-2 (sIL2-R) are believed to be an effective mechanism by which tumor cells locally block the attack and their subsequent destruction by the immune system. These

soluble receptors act as competitive inhibitors with membrane receptors directly affecting the interaction of TNF and the surface receptors of cancer cells. This mechanism explains why high levels of sTNF-R1 and sTNF-R2 have been negatively associated with the prognosis of tumor patients in several clinical and epidemiological investigations (3–8). Extracorporeal removal of these inhibitors, with the goal of reducing the local tissue inhibitor concentrations below the tumor-protective threshold, has therefore been considered to be a potential therapeutic approach for cancer treatment (2,3,7–9).

The original approach used to accomplish this was a plasma fractionation method, which filtered essentially all plasma proteins below the molecular weight of IgG (150 000 D). This technology, termed Ultrafiltration, had the disadvantage of requiring large quantities of replacement fluids. The proteins filtered by Ultrafiltration are known to be distributed in extracellular water and are not significantly reduced

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by standard plasma exchange (6–9). A more specific immune adsorption procedure has been developed using a plasma separator in combination with an immuno-affinity adsorber, the Oncosorb column, that contains immobilized antibodies against only sTNF-R and sIL2-R. It obviates the need for replacement solution and selectively removes the soluble receptors to TNF and IL-2. Inserted into a standard plasma perfusion circuit, the Oncosorb immune adsorption column allows the perfusion of the required plasma fraction. The present study was conducted to assess the efficacy, safety, and tolerability of the extracorporeal immune adsorption with the Oncosorb column to reduce the plasma levels of sTNF-R1, sTNF-R2, and sIL2-R.

## PATIENTS AND METHODS

### Patients

The study was conducted in accordance with the Declaration of Helsinki Good Clinical Practice guidelines and with local ethics committee approval. All patients gave their written informed consent.

The patients were enrolled on the basis of specific inclusion/exclusion criteria. Inclusion criteria included the following: biopsy-proven metastatic or recurrent cancer that had failed to respond to standard systemic chemotherapy or hormone therapy; unidimensionally measurable tumor; Karnofsky status  $\geq 70\%$ ; life expectancy  $> 3$  months; age  $> 18$  years; measurable plasma sTNF-R1 and/or sTNF-R2 levels; and patients who refused conventional therapies under informed consent. Exclusion criteria included the following: patients with active tumors growing in the central nervous system (CNS); total body tumor mass  $> 1000$  g; normal renal and hepatic function; negative serology for hepatitis A, B and C viruses, and the human immunodeficiency virus. Additionally, patients were excluded from the study if they were: women who were pregnant, lactating, or practicing inadequate contraception; patients with infectious diseases requiring antibiotics 30 days prior to study; patients with congestive heart failure, myocardial infarction in the previous six months, or clinically significant hypotension; patients taking angiotensin converting enzyme (ACE) inhibitors; patients with coexisting second malignancies; or patients with a hematocrit  $< 30\%$ , WBC count  $< 2500$ /mL, or a platelet count  $< 100\,000$ /mL.

### Materials and methods

#### Study design

The study was a single-site, non-randomized open pilot study designed to evaluate the ability of the

column to remove these inhibitors, and to determine clinical side effects related to the column and to the reduction of pro-inflammatory cytokine inhibitors in patients with metastatic cancer. Any changes in tumor size were observed as a secondary objective. Investigators conducted the study at the Institute for Clinical Research and Development (Institut für klinische Forschung und Entwicklung; Mainz, Germany), from November 2003 until April 2004 on behalf of BioPheresis (Heidelberg, Germany).

#### Study device, ancillary equipment, and methods

The immune adsorption column (Oncosorb) comprises a 325 mL medical grade polycarbonate, autoclave-sterilized column (PNS-400146; Fresenius HemoCare, Redmond, WA; USA) containing a sterile matrix of Sepharose 4B beads (Amersham-Biosciences, Uppsala, Sweden) onto which rabbit antihuman polyclonal antibodies to sTNF-R1, sTNF-R2, and sIL2-R (Biopheresis, Heidelberg, Germany) are covalently coupled (Fig. 1). Columns were prepared under aseptic conditions according to good manufacturing practice, individually checked for sterility and endotoxin levels after preparation, filled with 0.1%  $\text{NaN}_3$  in phosphate-buffered saline (PBS), and stored at  $2\text{--}8^\circ\text{C}$  until clinical use. One Evaflux 4A filter (Kuraray Medical, Osaka, Japan) was used per patient per procedure. The Evaflux 4A



**FIG. 1.** The immune adsorption Oncosorb column (currently under review for a CE license).

filter was chosen because of its demonstrated sieving coefficient to sTNF-R1 of approximately 60% and sTNF-R2 of approximately 51%, and a sieving coefficient to fibrinogen, a non-globular protein, of near 0%.

Investigators regenerated the column after every 9 L of filtered plasma and at the end of a treatment. Regeneration was accomplished by elution of the column with 1 L of normal saline, then with sterile glycine buffered saline (pH 2.8) followed by 1 L of sterile saline. The elution rinse from the glycine wash was collected and analyzed by immunoassay (R&D Systems, Minneapolis, MN, USA) to determine the overall amounts of soluble TNF-R1, TNF-R2, and IL2-R removed from the plasma. At the end of each treatment day, regeneration was achieved in the same manner. For storage, the column was filled with 1 L 0.01% NaN<sub>3</sub> in PBS and stored at 2–8°C until the next use. The NaN<sub>3</sub> was removed prior to clinical use by flushing the column with 4 L of sterile saline.

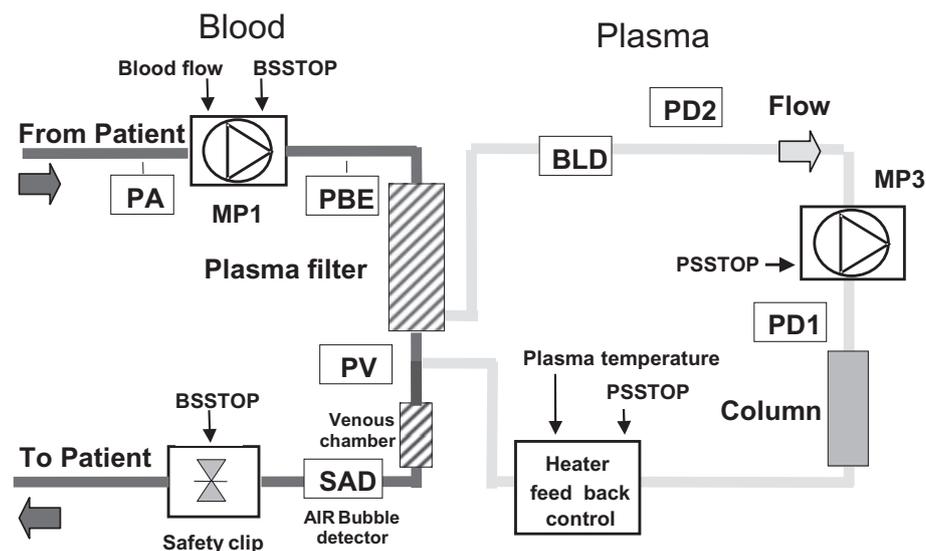
Investigators obtained vascular access using a standard dialysis catheter (Hemoaccess Type, Hospal, Hechingen, Germany). The plasma perfusion controller employed was a B Braun Diapact CRRT unit (B Braun Medizintechnologie, Melsungen, Germany), and the plasma fractionator was the Evaflex 4A plasma filter.

#### Patient treatment

The inclusion and exclusion criteria were assured by a complete medical history and physical examination, laboratory examinations, electrocardiogram, tumor assessment by computed tomography (CT) or magnetic resonance imaging (MRI), direct measure-

ment of surface tumors, and written informed consent was obtained for each patient to undergo the procedures. A catheter placement visit took place one day before the first of 12 immunopheresis treatments. Investigators performed the treatment procedures 3–4 times a week over a period of 4 weeks. Most patients had a borderline low peripheral WBC at enrollment (total WBC < 4000) and a compromised marrow reserve from prior treatment. They received granulocyte monocyte colony stimulating factor (125–250 µg subcutaneously) at bedtime the evening before treatment to keep the WBC count between 8000 and 15 000 cells/mL.

Treatment consisted of plasma perfusion of the low molecular weight fraction of the patient's plasma. This plasma fraction was perfused through the Oncosorb column to adsorb sTNF-R1, sTNF-R2, and sIL2-R. After adsorption, the plasma fraction returned to the venous air trap in the extracorporeal circuit, where it mixed with the patient's blood and returned to the patient (Fig. 2). Three patients received two cycles (24 procedures), and two patients received three cycles (36 procedures). Treatment cycles were separated by treatment rest periods of one month. On treatment days, an amount of plasma filtrate up to one calculated extracellular water volume (ECWV), estimated as 20% of total body weight (kg, expressed in liters), was perfused with the aim of reducing sTNF-R1 and sTNF-R2 to a low normal range (750 pg/mL and 1250 pg/mL in plasma, respectively) for three out of seven hours. Pre- and post-treatment receptor levels were determined for sTNF-R1 and sTNF-R2. Plasma sIL2-R levels were obtained before and after treatment on visits 1, 3, 5, 7,



**FIG. 2.** Schematic depiction of immunopheresis with the Oncosorb column. BLD, blood leak detector; BSSTOP, blood safety stop valve; MP, pump; PA, arterial pressure; PBE, blood entry pressure; PD, pressure detector; PSSTOP, plasma safety stop valve; PV, venous pressure; SAD, safety air detector.

and 9 during each cycle. The filtrate of the blood filter in this system was perfused over the Oncosorb column, which selectively removed sTNF-Rs and sIL-2R by immunoadsorption. All other plasma proteins were returned to the patient.

Laboratory assessments were performed by enzyme-linked immunosorbent assay (R&D Systems, Neu Isenburg, Germany). Blood chemistry panels and tumor assessments by physical examination were performed once weekly, if applicable, and the findings documented. At the end of the 12-procedure treatment cycle, the physical examinations and laboratory determinations were repeated and the dialysis catheter removed. All adverse events and changes in medications were recorded in the patient's diary, which was reviewed each treatment day. Patients were allowed to return to the site for a second or third treatment cycle, if appropriate.

#### Data acquisition and analysis

The medical histories were provided by the patients and their referring oncologists. Original data and laboratory results were recorded in case report forms. The data was entered into a database and analyzed using descriptive statistics. Mean values were calculated for the levels of sTNF-R1, sTNF-R2, and sIL2-R before and immediately after treatment, and compared using the Student's *t*-test. A value of  $P < 0.05$  was considered statistically significant. Nominal data (e.g. patient's characteristics and adverse events) were tabulated. Three weeks after the end of one treatment cycle, the patients underwent repeat radiological workups (e.g. CT and MRI scans). Results were entered into the source data.

## RESULTS

### Efficacy

A total of nine adult patients (three men and six women; mean age 48 years, aged 41–68 years) with metastatic solid tumors were enrolled in the study and received at least 12 immune adsorption procedures. The types of cancers included breast cancer (six patients), prostate cancer (one patient), renal cell carcinoma (one patient), and squamous cell carcinoma of the head and neck (one patient). All nine patients completed the first cycle of 12 immunopheresis treatments ( $n = 107$  treatments, as one patient received only 11 treatments instead of 12), three received a second cycle (24 treatment procedures), and two patients completed a third treatment cycle (36 treatment procedures). A total of 167 treatments were thus performed on these nine patients and were

**TABLE 1.** Averaged patient plasma levels of soluble tumour necrosis factor receptors 1 and 2 (sTNF-R1 and sTNF-R2) and soluble interleukin-2 receptor (sIL2-R) for treatments 1–12 with the Oncosorb immune adsorption column ( $n = 167$ )

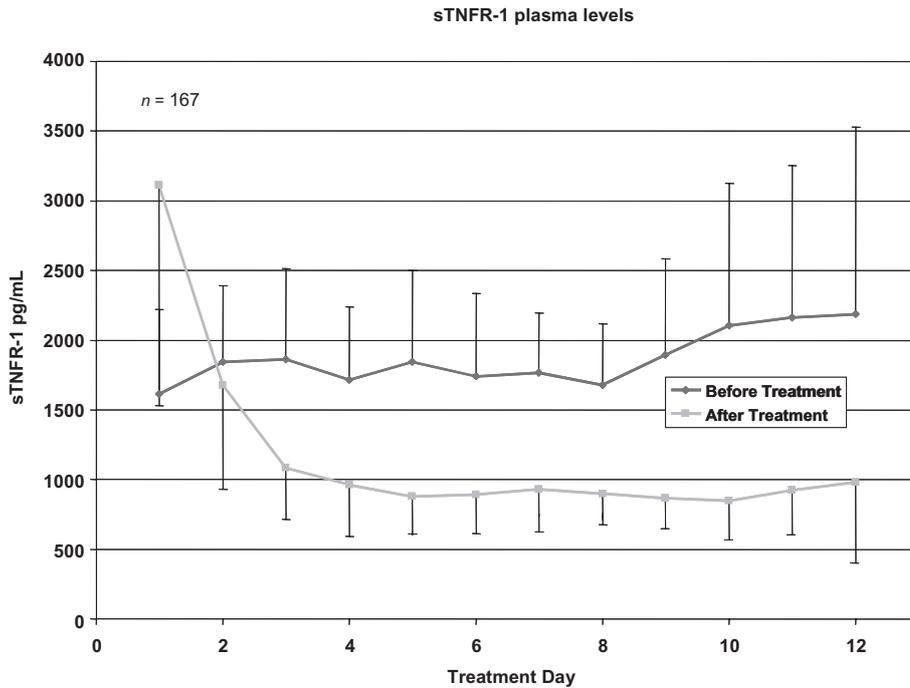
Receptor	Before treatment	After treatment	<i>P</i>
sTNF-R1 (pg/mL)	1936 ± 788	1312 ± 989	<0.001
sTNF-R2 (pg/mL)	3140 ± 1173	1816 ± 1266	<0.001
sIL2-R (pg/mL)	4095 ± 2565	1721 ± 1677	<0.001

included in the analysis of the primary efficacy, that is, the reduction of plasma levels of sTNF-R1, sTNF-R2, and sIL2-R.

The mean plasma levels of sTNF-R1, sTNF-R2, and sIL2-R measured before and after all treatments are presented in Table 1. Plasma concentrations of sTNF-R1 and sTNF-R2 showed a rebound after the first treatment, but then declined with subsequent treatments. They reached consistently lower levels from treatments 3–12 (Figs. 3–5). The mean pre- and post-treatment plasma levels and percent reductions ( $n = 149$ ) for this period of stable performance are given in Table 2.

Overall, the perfusion of a mean plasma volume of  $12.22 \pm 4.02$  L was achieved. The quantity of inhibitor removed from the patient was determined by measuring the total quantity of material in the eluate on column regeneration. The column removed mean quantities per patient/procedure of  $6204 \pm 1202$  ng of sTNF-R1,  $2623 \pm 577$  ng of sTNF-R2, and  $10\,170 \pm 2186$  ng of sIL2-R. The column's performance was found to be stable over 12 procedures and regenerations, which required 24 regenerations per column, as demonstrated in Table 3.

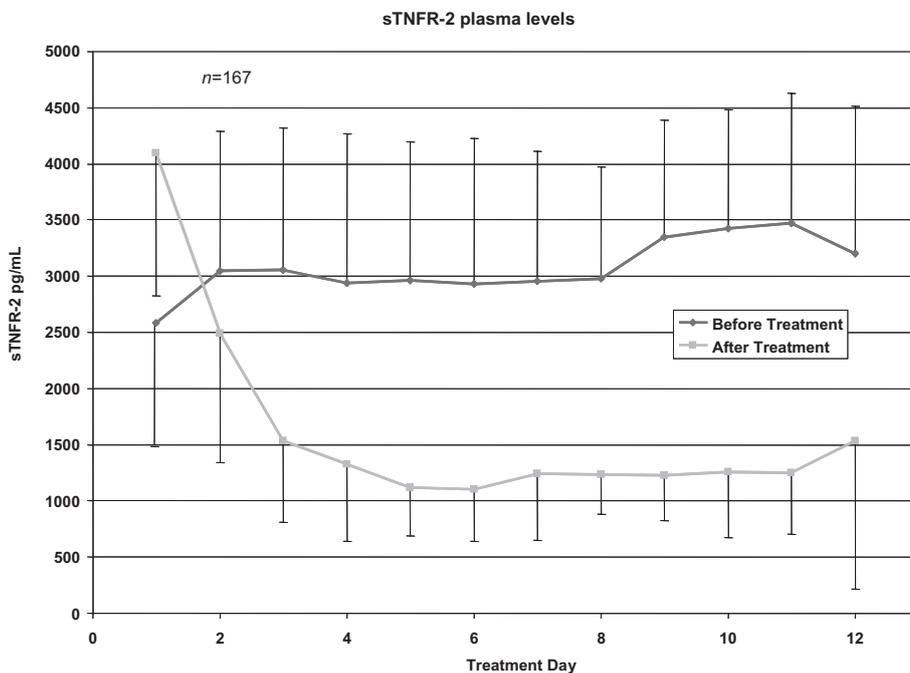
Although the clinical response was not the primary objective in this study, signs and symptoms of tumor inflammation were observed. Tumor regression following inflammation was documented. Patients with visible or palpable tumors demonstrated signs and symptoms of tumor inflammation in response to the procedure. Of the six patients with metastatic breast cancer, three had visceral or osseous metastases only. The three patients with cutaneous, subcutaneous, or nodal disease developed redness, swelling, tenderness, and warmth in response to each procedure. The patient with squamous cell carcinoma of the head and neck and massive right cervical adenopathy developed redness, swelling, tenderness, and heat in the palpable node in response to 30 of 36 procedures. The patient with renal cell carcinoma developed redness, swelling, tenderness, and heat in his subcutaneous



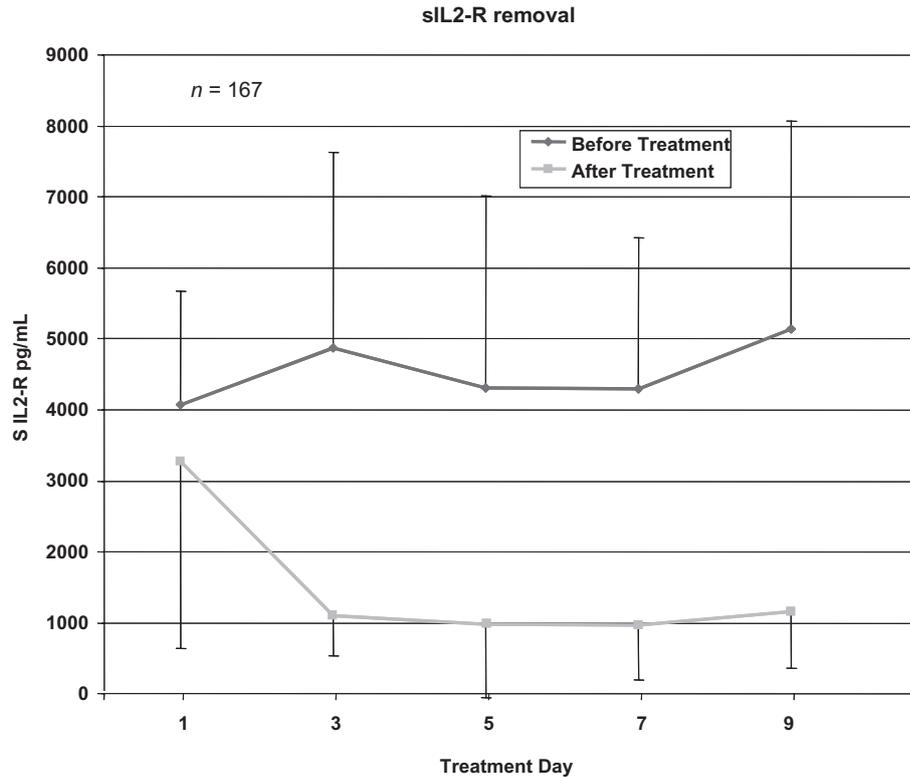
**FIG. 3.** Mean pre- and post-treatment plasma levels of soluble tumour necrosis factor receptor 1 (sTNF-R1) during repetitive treatments.

tumors in response to 90% of his procedures. Visceral tumors not subject to direct examination were accessed by X-ray, CT, or MRI two weeks after completing the treatment. Tumor reduction measured two weeks after completion of treatment was seen in patients who had received two or more cycles of treatment, regardless of tumor type. The pre- and post-

treatment photographs and CT scans of three patients in the study document the reductions in tumor size experienced (Fig. 6A–C). The photograph in Figure 6A(i) depicts the mass of patient A, a 59-year-old male with a squamous cell carcinoma of the head and neck and metastases to the right cervical nodes. The mass measured 9.0 cm × 10.5 cm × 2 cm on



**FIG. 4.** Mean pre- and post-treatment plasma levels of soluble tumour necrosis factor receptor 2 (sTNF-R2) determined on repetitive treatments.



**FIG. 5.** Mean pre- and post-treatment plasma levels of soluble interleukin-2 receptor (sIL2-R) determined on repetitive treatments.

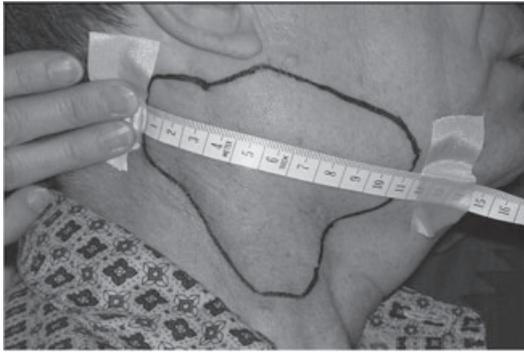
8 December 2003. The photograph in Figure 6A(ii) shows the same patient on 4 March 2004; the mass had shrunk to 6 cm × 6 cm × 1 cm post-treatment. The CT scan (Fig. 6A(iii)) shows the cervical mass pre-treatment on 26 November 2003. The CT scan shown in Figure 6A(iv), taken on 2 March 2004, shows extensive necrosis in the right cervical mass after 24 treatments.

Figure 6B presents two photographs of B, a 46-year-old patient with widespread renal cell carcinoma. Adenopathy included palpable skin metastases, which are demonstrated on the pictures. A right posterior thorax mass shrank from 12 cm × 8 cm × 4 cm on 11 November 2003 (Fig. 6B(i)) to 6 cm × 7.5 cm × 1.5 cm after 12 treatments were completed by 11 December 2003 (Fig. 6B(ii)).

**TABLE 2.** Averaged patient plasma levels of soluble tumour necrosis factor receptors 1 and 2 (sTNF-R1 and sTNF-R2) and soluble interleukin-2 receptor (sIL2-R) for treatments 3–12 with the Oncosorb immune adsorption column (n = 149)

Receptor	Before treatment	After treatment	P	%
sTNF-R1 (pg/mL)	1944 ± 794	1003 ± 431	<0.001	-48.4
sTNF-R2 (pg/mL)	3187 ± 1161	1424 ± 830	<0.001	-55.3
sIL2-R (pg/mL)	4978 ± 2637	1406 ± 1176	<0.001	-71.8

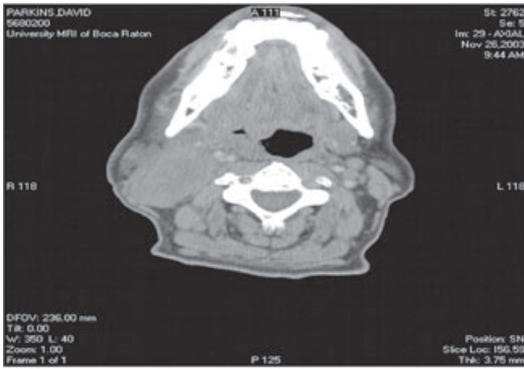
**FIG. 6.** Images showing the clinical responses to the immunoabsorption procedures. (A (i)) Patient A with a squamous cell carcinoma of head and neck showing pre-treatment adenopathy measuring 9.0 cm × 10.5 cm × 2 cm on 8 December 2003. (A(ii)) Patient A on 4 March 2004; after 24 immunoabsorption procedures the mass had shrunk to 6 cm × 6 cm × 1 cm. (A(iii)) CT scan of patient A's mass, pre-treatment, on 26 November 2003. (A(iv)) CT scan of patient A's mass on 2 March 2004 shows extensive necrosis in the right cervical mass after 24 treatments. (B(i)) Patient B with metastatic renal cell carcinoma presenting as palpable skin metastases on the right posterior thorax measuring 12 cm × 8 cm × 4 cm on 14 November 2003, before treatment. (B(ii)) Patient B on 11 December 2003; after 12 treatments the mass had shrunk to 6 cm × 7.5 cm × 1.5 cm. (C(i)) CT scan taken on 29 October 2003 of patient C with metastatic breast carcinoma, pre-treatment, showing right internal mammary nodes measuring 2.3 cm × 1.5 cm and pericardial involvement. (C(ii)) CT scan of patient C taken on 9 January 2004, three weeks after 12 treatments, showing right internal mammary nodes of a normal size and no pericardial involvement. CT scans were taken on different machines, therefore no identical images are available.



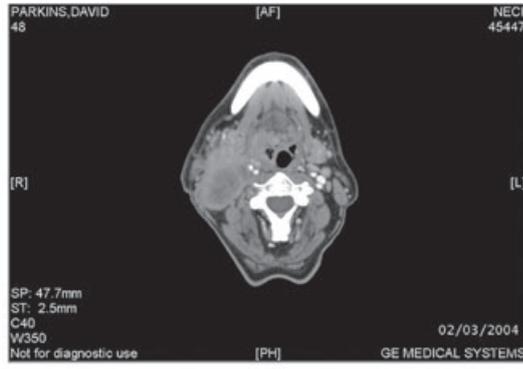
A(i)



A(ii)



A(iii)



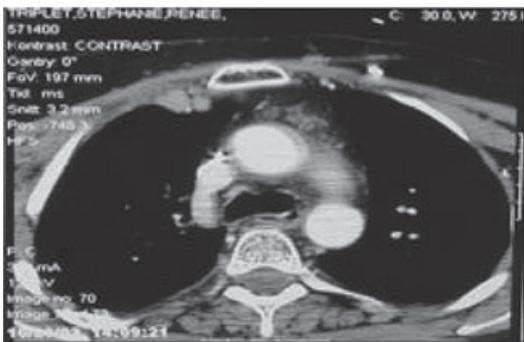
A(iv)



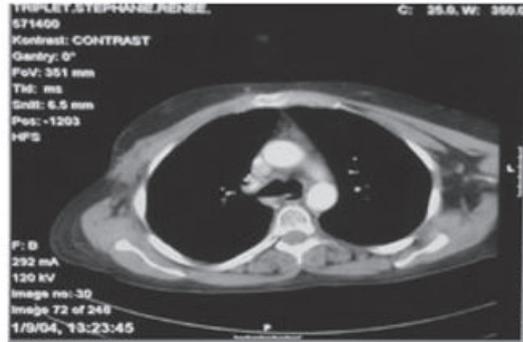
B(i)



B(ii)



C(i)



C(ii)

**TABLE 3.** Amount of receptor removed by treatment day and plasma volume treated

Procedure number	Plasma volume (L)	Removed sTNF-R1 (ng)	Removed sTNF-R2 (ng)	Removed sIL2-R (ng)
1	4.12 ± 5.31	2950	1335	6 997
2	4.95 ± 3.20	6755	2044	—
3	9.59 ± 5.97	6453	2811	12 058
4	11.68 ± 5.35	6403	3099	—
5	12.56 ± 5.19	6436	2786	10 927
6	13.11 ± 4.88	5714	2400	—
7	14.56 ± 3.61	7439	3009	10 499
8	15.31 ± 3.19	7314	3683	—
9	15.47 ± 3.24	5894	2810	10 368
10	15.64 ± 3.12	5474	2273	—
11	15.11 ± 4.54	6807	2368	—
12	14.56 ± 4.45	6815	2864	—
Mean ± SD	12.22 ± 4.02	6204 ± 1184	2623 ± 594	10 170 ± 1984

Figure 6C presents two photographs of C, a 41-year-old female with widespread metastatic breast carcinoma with internal mammary adenopathy and pericardial metastases. The pretreatment photograph, taken on 29 October 2003 shows a mass 2.3 cm × 1.5 cm in size (Fig. 6C(i)); the photograph taken on 9 January 2004, three weeks post-treatment, shows resolution of the metastases (Fig. 6C(ii)).

### Safety and tolerability

The most frequent adverse events encountered during the study that were not expected for an extracorporeal device and dialysis catheter, but also were sometimes exacerbations of the underlying disease, included the following: tumor pain increased over baseline pain in 56 of 167 procedures (37.3%), transient nausea in 34 of 167 procedures (22.7%), fever in 12 of 167 procedures (8.0%), flu-like symptoms in 84 of 167 procedures (50%), and vomiting in 10 of 167 procedures (6.7%). It was observed that nausea and vomiting were seen shortly after the start of the procedure and were associated with transient mild hypotension consistent with a first use phenomenon. Patients did not experience any bleeding. Increasing the saline priming of the Diapact unit and the Kuraray filter from 2 to 4 liters of saline eliminated these adverse effects. Increased tumor pain, flu-like symptoms, and low-grade fever appeared to be caused by tumor-specific immune activation. One patient developed a catheter infection, requiring one day of hospitalization. This significant adverse effect was related to the catheter and not to the study device. It resolved without sequelae on appropriate antibiotic treatment, and the patient remained in the study.

### DISCUSSION

The results of this clinical study in patients with various metastatic solid tumors and elevated con-

centrations of soluble TNF and IL-2 receptors in blood reveal that the Oncosorb column significantly reduces the plasma concentrations of sTNF-R1, sTNF-R2, and sIL2-R. The column is easily regenerated and shows stable performance for at least 12 consecutive clinical treatments and 24 regenerations.

The initial increase in the levels of soluble receptors for TNF observed after the first treatment can be interpreted as a sign that the tumor is responding to decreased levels of the tumor-protective soluble receptors by increasing the release of these immunosuppressants into the tumor's microenvironment. Post-treatment levels were not increased after the second treatment and were consistently reduced from the third treatment onwards.

Inhibitor levels recovered to baseline between individual treatments, with pretreatment levels tending to be similar throughout. Each patient who subsequently developed tumor inflammation had a decrease in baseline inhibitor levels.

The observed clinical effects were consistent with a treatment-induced immune response against the tumor, consisting of tumor pain with tenderness of palpable tumors, tumor swelling, palpable heat over tumors, and low-grade fever. Longer observation showed that patients who received two treatment cycles and demonstrated clinical signs and symptoms of tumor inflammation subsequently appeared to have signs of tumor necrosis and tumor reduction, as evidenced by X-ray imaging and CT scans. The literature and clinical observations made in this study suggest that lowering the plasma levels of sTNF-R1, sTNF-R2, and sIL2-R is beneficial to patients with metastatic cancer. Administered as a consistent therapy, reduction of the soluble TNF and interleukin-2 receptor inhibitors to near-normal levels might enable the immune system to mount a successful inflammatory response against cancer.

Inflammatory signs and symptoms and procedure-related hazards associated with all extracorporeal treatments are acceptable medical risks and are manageable.

### CONCLUSION

The study data support the conclusion that the immunoabsorption column Oncosorb can achieve reliable and efficient removal of soluble TNF receptors/inhibitors types 1 and 2 and the soluble IL-2 receptors/inhibitors from a low molecular weight fraction of human plasma that is normally distributed in extracellular water. These shed receptors act as inhibitors of TNF- $\alpha$  and  $\beta$ , and IL-2 by competitively inhibiting these cytokines before they can engage membrane receptors on the tumor cells. The consequent lowering of blood levels, and hence tissue levels, of the soluble receptors can improve the normal immune response against the tumor cells. Formal clinical trials with this column are indicated to evaluate the clinical response on defined tumor types.

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