significance of the presence of more than two μ heavy-chain genes in childhood acute lymphoblastic leukaemia of B precursor cell origin. *Blood* 1986, **67**, 698–702.
9. Katz F, Ball L, Gibbons B, Chessells J. The use of DNA probes to monitor minimal residual disease in childhood acute lymphoblastic leukaemia. *Br J Haematol* 1989, **73**, 173–180.
10. Bird J, Galili N, Link M, Stites D, Sklar J. Continuing rearrangement but absence of somatic hypermutation in immunoglobulin genes of human B precursor leukaemia. *J Exp Med* 1988, **168**, 229–245.
11. Stankovic T, Darbyshire P, Mann J, Pomfret M, Taylor AMR. Rapid production of diversity during the progression of a mixed lineage leukaemia. *Leukemia Res* 1992, **16**, 993–1002.
12. Wasserman R, Yamada M, Yoshinori, *et al.* V_H gene rearrangement events can modify the immunoglobulin heavy chain during progression of B lineage acute lymphoblastic leukemia. *Blood* 1992, **79**, 223–228.
13. Tosaka T, Kita K, Miwa H, *et al.* Cross-lineage gene rearrangements in human leukemic B precursor cells occur frequently with V-DJ rearrangements of IgH genes. *Blood* 1989, **74**, 361–368.
14. Kitchingman G, Rovigati U, Muer AM, Melvin S, Murphy SB, Stass S. Rearrangement of immunoglobulin heavy chain genes in T cell acute lymphoblastic leukemia. *Blood* 1985, **65**, 725–729.
15. Fontenay M, Flandrin G, Baurman H, *et al.* T cell receptor δ gene rearrangements occur predominantly in immature myeloid leukaemias exhibiting lineage promiscuity. *Leukemia* 1990, **4**, 100–105.
16. Greaves MF, Chan LC, Furley AJW, Watt SM, Molgaard HV. Lineage promiscuity in hematopoietic differentiation and leukaemia. *Blood* 1986, **67**, 1–11.
17. Sambrook J, Fritsch EF, Maniatis T. *Molecular Cloning, a Laboratory Manual*, 2nd edition. Cold Spring Harbor Laboratory, 1989.
18. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 1975, **98**, 503–517.
19. Forestier E, Nordenson I, Lindstrom A, *et al.* Simultaneous immunoglobulin/T cell receptor gene rearrangement and multiclonality in childhood leukaemia. *Acta Paed* 1994, **83**, 319–326.
20. Raghavachar A, Thiel E, Bartram CR. Analyses of phenotype and genotype in acute lymphoblastic leukaemias at first presentation and in relapse. *Blood* 1987, **70**, 1079–1083.
21. Bunin NJ, Raimondi SC, Mirro JJR, Behm FG, Goorha R, Kitchingman GR. Alterations in immunoglobulin in T cell receptor gene rearrangement at relapse: involvement of 11q23 and changes in immunophenotype. *Leukemia* 1990, **4**, 727–731.
22. Biondi A, Yokota S, Hansen-Hage TE, *et al.* Minimal residual disease in childhood acute lymphoblastic leukaemia: analysis of patients in continuous complete remission with consecutive relapse. *Leukaemia* 1992, **6**, 282–288.
23. Biondi A, Rossi V, di Celle PF, *et al.* Unique genotypic features of infant acute lymphoblastic leukaemia at presentation and relapse. *Br J Haematol* 1993, **80**, 472–479.

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Feature Articles

Renal Cell Carcinoma and Interleukin-2: A Review

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CANCERS ARISING in the kidney are relatively uncommon, accounting for approximately 1.4% of all cancers in Northern Europe and 1.5% of all cancer deaths. The disease is twice as common in men as in women, and has a peak age of around 60 years [1]. Outside of paediatric practice, the dominant histology is adenocarcinoma with less than 10% being squamous or transitional carcinomas of the renal pelvis. The former histological type has also been termed hypernephroma [2] or Grawitz tumour [3].

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Renal cell carcinoma (RCC) may remain clinically occult for most of its course. The presenting clinical features of RCC have been well described and are usually indicative of advanced disease [4–6]. Although the classical triad of gross haematuria, abdominal mass and pain are only seen in 9% of patients, individual symptoms including haematuria (59%), abdominal mass (45%), pain (41%), weight loss (29%) and anaemia (21%) are common. Approximately 30% of patients have metastatic disease at presentation, another 25% have locally advanced disease, leaving 45% with localised tumour [7]. The TNM staging system provides an accurate method of classifying the extent of tumour involvement, although the staging has been simplified by dividing the patients into four groups [8]. The shortcomings of this system become obvious, however, when it is noted that the survival rate of patients with stage II is less than that of stage III (Table 1), indicating an inappropriate assignment to prognostic groups. The grouping of renal vein,

Table 1. Long term survival after nephrectomy in 309 patients with renal cell carcinoma [9]

Stage	TNM stage	5-year survival (%)	10-year survival (%)
I	T1, N0, M0	65	56
II	T2, N0, M0	47	20
III	T1, N1, M0	51	37
	T2, N1, M0		
	T3a, N0, N1, M0		
	T3b, N0, N1, M0		
IV	T4, any N, M0	8	7
	Any T, N2, N3, M0		
	Any T, Any N, M1		

vena caval and lymph node involvement in stage III causes the survival rates to be higher because, with adequate surgery, renal vein involvement is not a dire prognostic factor.

Extension of tumour to regional lymph nodes is now accepted as being of major prognostic importance with the 10-year survival of node positive patients being only 17% [9]. Almost half of these recurrences occur within the first 12 months of nephrectomy. Once metastatic disease has occurred, the prognosis without therapy is sombre. Riches [10] reported 3- and 5-year survivals of only 4.4 and 1.7%, respectively. Factors which apparently affect the outcome for patients with metastatic disease include time from initial diagnosis, weight loss, sedimentation rate and the number of metastatic sites involved [7,11–13]. It is important to bear these points in mind when considering the results presented of small patient series (phase II studies) since selection of a relatively good prognostic category of patients may be rather more important in determining the apparent results than the treatment itself.

Hormone manipulations have been used for the treatment of RCC for several decades. Bloom [14] first drew attention to progesterone, reporting a 16% response rate. Collected response rates vary between 5 and 9%, are rarely complete and are usually of short duration. When strict modern response criteria are applied, the response rate is probably only 2% [15, 16].

Many small studies of chemotherapy with single or multiple agents in patients with advanced renal cell carcinoma have been reported. Response criteria, particularly for the older studies vary considerably. Vinblastine appears the most active agent, with response rates between 15 and 30%. When responses do occur, they are usually only partial, and evidence that they prolong survival is lacking [16]. Efforts to improve these results with combination chemotherapy have proven disappointing, and many physicians do not advise chemotherapy routinely.

The occurrence of spontaneous regression is a recognised feature of RCC. Possinger and colleagues have reviewed the literature [17] and several other authors have contributed to the debate [18–20]. In the collected series of 2411 patients, there were 11 spontaneous regressions (0.46%). Oliver and colleagues [21] reported a spontaneous regression rate of 8% in patients who were followed prospectively prior to the initiation of therapy with interferon. The majority of these responses occurred in lung metastases. The importance of these data lies in the fact that all patients should undergo a period of observation prior to entry into experimental protocols to ensure that progression is documented. Regression after nephrectomy has also been reported to occur. Montie and associates [22], however, observed this in only 4 of 474 (0.84%) undergoing nephrectomy.

The occurrence of spontaneous regressions suggested that the immune system may, in certain circumstances, be able to overcome the tumour. This concept formed the basis for the use of immune stimulants, such as the interferons (IFN), in metastatic RCC. The results from published studies with the interferons are summarised in Table 2. The doses of IFN-α range from 1 to 100 MU per dose with a wide variety of schedules. Perusal of the data leads one to conclude that at doses less than 3 MU/day responses were uncommon. Apart from this, there is no clear dose–response effect. In the papers presented in Table 2, it is possible to gain an idea of some prognostic factors for response to IFN. 61 of 354 (17%) who had undergone a nephrectomy responded compared with 11 of 93 (12%) who had not. 20% (93/459) of patients with lung metastases responded compared to only 11% (30/268) of those without pulmonary metastases. If the only site of metastatic disease was in the lungs, then the response rate was 34% (32/93). Appreciation of these results is necessary to put in perspective more recently developed cytokine protocols.

Interleukin-2 (IL-2) is a glycoprotein with a molecular weight of between 14,500 and 17,000 Da with the variation being due to differences in glycosylation. The recombinant protein which is in clinical use is not glycosylated but appears to have equivalent biological activity to the natural product. The IL-2 molecule consists of 133 amino acids with a disulphide bond between residues 58 and 105, which is essential for the activity of the cytokine [53]. The biological characteristics of this cytokine are such that is is unlikely to possess any direct anticancer effects against solid tumours. Any antitumour effects observed *in vivo* must be due to induction of secondary effector systems, and IL-2 can, therefore, be considered a true biological response modifier. Characteristics which make IL-2 attractive for use as an anticancer agent include:

(i) stimulation of the proliferation of helper and cytotoxic T cells primed by antigen [53–55];
(ii) induction of the secretion of lymphokines such as IFN-γ and tumour necrosis factor (TNF) [56, 57], cytokines shown to have direct antiproliferative effects;
(iii) enhancement of the cytotoxicity of T and natural killer (NK) cells [53–55, 58].

Under the influence of IL-2, a subpopulation of lymphocytes can develop "lymphokine activated killer" (LAK) cell activity. LAK activity is defined operationally as the *in vitro* lysis of both autologous and allogeneic fresh tumour cells, as well as cultured tumour cells including those that are NK-resistant [58–61]. LAK cytotoxicity is non-major histocompatibility complex restricted. The majority of these cells exhibit an NK phenotype but T lymphocytes, especially those with the gamma/delta receptor, can also show LAK cytotoxicity [58].

A large number of phase I and II studies with IL-2 given as single agent to patients with metastatic renal cell carcinoma have been published [62]. Of 384 patients reported, 16 (4.2%) achieved complete and 39 (10.2%) partial remissions. When LAK cells were included in the therapy, complete and partial remissions were observed in 8.4 and 17.8% of 309 patients, respectively [63]. Only one randomised trial has compared IL-2 with or without LAK cells in a mixed group of melanoma and renal cell carcinoma patients. Although there appeared to be more complete remissions in the LAK arm, this was of borderline statistical significance [64].

It thus remains controversial as to whether LAK cells add

Table 2. Therapy with interferon in metastatic renal cell carcinoma

Study	Dose (MU)	Schedule	Number evaluated	% Response
A: Natural leucocyte interferon				
Quesada *et al.* [23]	3	Daily	50	26
de Kernion *et al.* [24]	3	5×/week	43	10
Magnusson *et al.* [25]	4–16	Daily	7	0
Kirkwood *et al.* [26]	1	Daily	14	0
	10	Daily	16	19
Edsmyr *et al.* [27]	3	Daily	11	27
B: Recombinant interferon-α				
Krown *et al.* [28]	50	3×/week	37	14
Einzig *et al.* [29]	3–36	Daily	62	11
Quesada *et al.* [30]	2/m²	Daily	15	0
	20/m²	Daily	41	29
Kuzmits *et al.* [31]	10	Daily	8	13
Schnall *et al.* [32]	3–36	Daily	22	5
Fossa *et al.* [33]	18–36	Daily	2	20
Kempf *et al.* [34]	2/m²	3×/week	10	0
	30/m²	5×/week	10	10
Umeda & Niijima [35]	6–10	3–5 days/week	45	18
	3–36	Daily	108	14
Scheithaurer & Niijima [36]	10	Daily	18	11
Buzaid *et al.* [37]	3–36	Daily	22	23
Muss *et al.* [38]	2/m²	3×/week	51	10
Figlin *et al.* [39]	3–36	Days 1–5 weekly	19	26
Foon *et al.* [40]	2/m²	3×/week	21	5
Otto *et al.* [41]	1	3×/week	42	17
Porzsolt *et al.* [42]	2	Days 1–5 weekly	18	11
Marshall *et al.* [43]	1	Daily	16	25
Fossa *et al.* [44]	18	3×/week	53	11
C: Lymphoblastoid interferon				
Neidhart *et al.* [45]	5/m²	3×/week	33	15
Neidhart *et al.* [46]	3–20	Days 1–10 every 3 weeks	23	22
	5–50	Days 1–5 every 3 weeks	9	22
Marumo *et al.* [47]	3	Daily	18	6
Vugrim *et al.* [48]	3/m²	3×/week	21	5
Vugrim *et al.* [49]	30/m²	3×/week	16	0
Umeda & Niijima [35]	5	Daily	73	23
Trump *et al.* [50]	3–20/m²	Days 1–10 every 3 weeks	39	13
Eisenhauwer *et al.* [51]	30–100	Weekly	37	11
Fujita *et al.* [52]	3	Daily	24	25
		Total	1100	14.6%

anything to the response rates of IL-2 alone. A number of phase I/II studies have reported the combination of IL-2 and IFN-α [65]. Some 45 of 153 (29.4%) achieved complete or partial responses in these heterogeneous protocols. Recently, Palmer and coworkers [66] constructed a prognostic model using a database of 327 renal cell carcinoma patients treated with IL-2. Patients could be divided into prognostic groups based on the following factors: Eastern Cooperative Oncology Group performance score (0 versus 1), time from diagnosis to treatment (>24 months versus ≤24 months), and number of metastatic sites (1 versus ≥2). Patients could then be classified into four groups dependent on the number of risk factors present. The median survivals of these four subgroups were 28, 17, 10 and 5 months, respectively. The model was validated by the use of an independent cohort of patients treated with subcutaneous IL-2 plus IFN-α and appeared to work well particularly with regard to the very low and very high risk groups which represented 12.8 and 20.8% of the total patients, respectively.

In Rotterdam, Stoter and colleagues have conducted a phase II study combining IL-2, IFN-α_{2a} and LAK cells [67]. Treatment consisted of IL-2 18 × 10^6 IU/m²/day, by continuous infusion on days 1–5, together with IFN-α_{2a} 5 × 10^6 IU/m²/day by intramuscular injection. Leucapheresis was carried out on days 7–9 and the *in vitro* generated LAK cells were re-infused on days 12–14 with IL-2 and IFN at the same dose and schedule on days 12–16. 40 patients were available for analysis and 5 (13%) achieved complete and 11 (28%) partial remissions. The median time to progression was 8 months and that for remission duration 14 months. The median survival was 28 months. The model described above was used to compare the survival of these patients with that predicted from the prognostic factor analysis. The data are presented in Table 3. This protocol appears highly active but is associated with substantial toxicity. If protocols such as this or that developed by Rosenberg and colleagues [64] are to be widely used outside of academic hospitals then amelioration of the toxicity becomes a high priority.

Table 3. Data from the Rotterdam Phase II study [67] analysed using the prognostic factor model of Palmer and colleagues [66]

Prognostic group	I	II	III	IV
Number of patients	5	12	11	12
Median survival (months)				
-Predicted from model	28	17	10	5
-Observed	Not reached	14	28	13

IL-2-associated toxicity is characterised by major alterations in the homeostasis of the cardiovascular system. These perturbations lead to the toxicity to a variety of vital organs [68–70]. This cycle of events is initiated by changes both in circulating cells and endothelium [71].

Treatment with IL-2 induces the secondary production of a complex network of secondary cytokines. Among them are the pro-inflammatory cytokines IL-1, IL-6, IL-8 and TNF [72]. This results in the activation of circulating immune cells (granulocytes, lymphocytes and monocytes) and activation of the endothelium. Adhesion of activated immune cells to the endothelium and the consequent excessive local production of cytokines and other inflammatory products is thought to be of central importance for the pathogenesis of IL-2-induced toxicity.

Both activated lymphocytes [73] and neutrophil granulocytes [74–76] have been implicated in the cellular damage to the endothelium. Activation of leucocytes stimulates them to produce cytokines, proteolytic enzymes, oxygen radicals and nitric oxide, and the expression of a variety of adhesion molecules. In animal studies, the IL-2 induced sequestration of granulocytes in lung vasculature suggested that these cells play a pivotal role in the pathogenesis of IL-2-associated toxicity.

IL-2 *per se* has no effect on endothelial cells, but secondary cytokines induced by IL-2 activate the endothelium. Their effects include the altered expression of adhesion molecules, the production of further cytokines, the generation of a procoagulant state and the production of vasoactive products. Both IL-1 and TNF have been demonstrated to exert key roles in these processes [77, 78].

Among the cytokines which are thought to be involved in IL-2-induced toxicity, TNF has received the most attention. IL-2 stimulates the immediate production of TNF by a variety of cell types. Furthermore, agents which inhibit the production of TNF, such as corticosteroids and pentoxiphylline, have been shown to reduce IL-2 toxicity [79, 80]. Additionally, the inhibition of TNF activity by passive immunisation has also been demonstrated to reduce IL-2 toxicity [81]. Together, these findings emphasise the importance of secondary TNF generation for IL-2 toxicity.

These initial changes in the integrity of the vascular endothelium lead to the subsequent activation of two major cascade systems, namely the complement system and the contact system of coagulation [82]. It has now been clearly demonstrated that activation of the complement system correlates with the severity of IL-2 toxicity [83], both in terms of hypotension (anaphylotoxins such as C3a & C5a) and the capillary leak syndrome (membrane attack complex can exacerbate endothelial cell damage). In addition, inhibition of complement activation by C1-esterase inhibitor (own results) and inhibition of complement activity by a recombinant human complement receptor [84] were effective in reducing IL-2 toxicity. Coagulation abnormalities may also contribute to IL-2 toxicity by inducing the release of vasoactive molecules such as kallikrein. The induction of a procoagulant state can also cause problems with in-dwelling central venous catheters [85].

Many, if not all, of the vasoactive substances produced as a consequence of IL-2 administration cause their alterations in vascular tone by the generation of nitric oxide (NO). It has been shown that human neutrophils and vascular smooth muscle cells contain the inducible form of the enzyme NO synthase, which is responsible for the sustained generation of large amounts of NO after stimulation by pro-inflammatory cytokines [86, 87]. The intimate interaction of activated neutrophils with endothelium may result in a local accumulation of supra physiological amounts of NO which might further exacerbate endothelial damage. Treatment with IL-2 induces concentrations of NO metabolites which are similar to levels attained during septic shock [88, 89]. In this condition the administration of NO synthase inhibitors has been proven to have a protective effect [90]. In addition, the finding that N^G -methyl-L-arginine (an NO synthase inhibitor) inhibits TNF-induced hypotension [91, 92], suggests that it may also ameliorate IL-2-induced hypotension.

In summary, the adhesion of activated neutrophil granulocytes to the endothelium and the local accumulation of inflammatory mediators, notably proteases, complement and coagulation factors, oxygen and nitrogen radicals seem to be responsible for IL-2-induced toxicity. These data suggest a number of possible targets whereby IL-2-induced side-effects may be reduced.

TOXICITY OF IL-2 THERAPY

Recently, we have treated 6 patients with a combination of IL-2 (8×10^6 IU/m^2/day for 5 days by continuous infusion) plus IFN-α_{2b} (5×10^6 IU/m^2/day for 5 days by i.m. injection) either with or without LAK cells. These patients illustrate the problems involved with intensive IL-2 therapy since all six patients had to have the IL-2 suspended because of toxicity. The reasons for stopping were: atrial flutter and cardiac ischaemia (1), hypotension and adult respiratory distress syndrome (1), oliguria and rising creatinine (4). One of the patients stopping therapy because of oliguria and rising serum creatinine is depicted in Figure 1. Of note is the fact that mean blood pressure fell by a maximum of 30% which represents WHO grade 3 toxicity. Urine output is re-established rapidly after cessation of IL-2 but several days are required before the serum creatinine begins to fall. These changes occurred despite the use of dopamine at 5 μg/kg/min and the use of plasma expanders.

Between March 1987 and December 1993 more than 100 patients have been entered into clinical trials with IL-2 in the Free University Hospital. Systematic blood sampling has been performed in these patients which has allowed the study of the pathogenesis of IL-2-induced toxicity. The results of these studies are summarised below.

IL-2-induced production of secondary cytokines

Following a bolus administration of IL-2, TNF, IL-6 and IL-8 are generated in this sequence with their peak values being at 2, 3 and 4 h, respectively (Figure 2, [93]). These cytokines are responsible for activating both circulating cells and endothelium. Furthermore, IL-6 is the major cytokine involved in generating an acute phase response and also acts as an endogenous pyrogen. IL-8, also known as neutrophil activating peptide, is responsible for activating neutrophils.

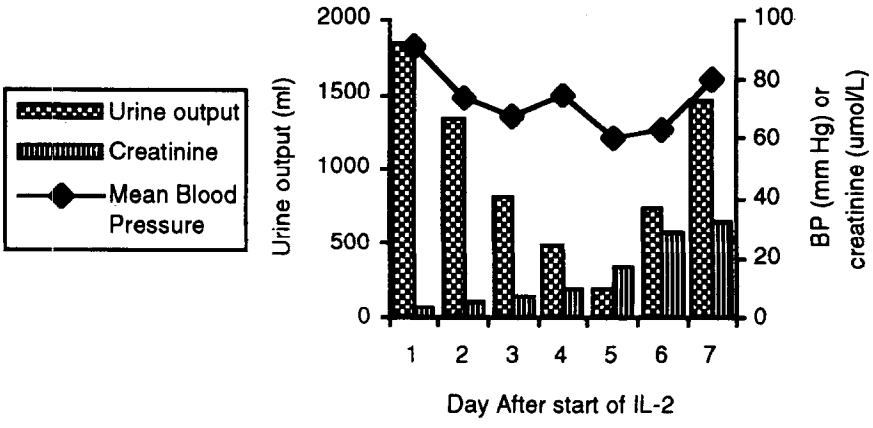

Figure 1. Patient number 2 treated with IL-2 + IFN from days 1–5.

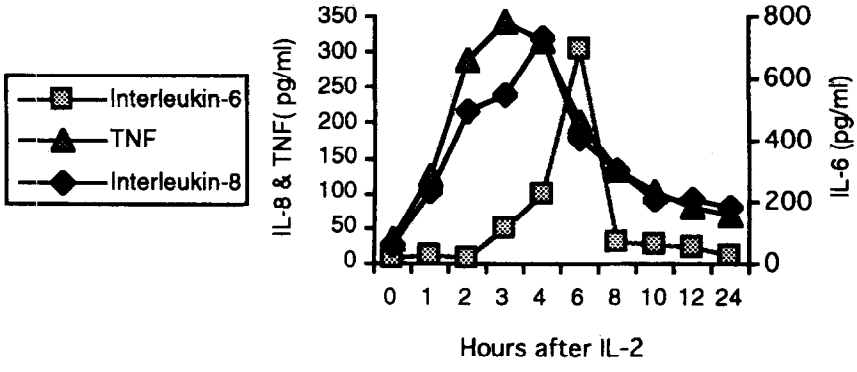

Figure 2. IL-6, IL-8 and TNF levels after a 15 min infusion of 18 MIU/m² IL-2.

IL-2-induced activation of polymorphonuclear neutrophils

We have shown that IL-2 stimulates the activation of polymorphonuclear neutrophils [94]. This was monitored by measuring elastase/α_1-antitrypsin and lactoferrin concentrations in the circulation of IL-2-treated patients. Increasing the dose of IL-2 resulted in higher levels of these markers of neutrophil activation. These events occur early after the administration of IL-2, and emphasise the important role that neutrophils play in initiating endothelial damage during IL-2 therapy.

IL-2-induced complement activation

IL-2 has been clearly demonstrated to induce the activation of the complement system. The C3a circulatory levels were significantly correlated with the degree of hypotension and with parameters of capillary leakage, such as weight gain and fall in serum albumin [94, 95]. In addition, a pilot study has demonstrated that inhibition of complement activation by the use of C1 esterase inhibitor has some ameliorating effect on IL-2-induced toxicity [96, 97].

IL-2-induced activation of coagulation and fibrinolysis

IL-2 therapy stimulates activation of the intrinsic pathway of coagulation [98, 99]. Notably, the decrease of factor XII and prekallikrein, corrected for protein leakage, correlated significantly with parameters of vascular leakage, suggesting the involvement of this system in IL-2 toxicity.

IL-2-induced vascular leakage

The degree of capillary leakage can be evaluated indirectly by such parameters as weight gain and fall in serum albumin. These are, however, rather insensitive tools. Because of this, the changes in intra- and extracellular fluid volumes during IL-2 therapy were evaluated using conductivity measurements [100]. In addition, the efficacies of two radio-isotope probes (^{67}Gallium and ^{99m}Tc-labelled red blood cells) in measuring pulmonary vascular leakage after cardiopulmonary bypass surgery have been assessed in this institute [101]. Both methods are being used to quantitate vascular leakage during IL-2 therapy, and should prove valuable tools for comparing the relative efficacies of the various planned interventional strategies.

IL-2-induced NO synthesis

IL-2 therapy has also been shown to induce an increase in the circulatory levels of the NO metabolites NO_2- and NO_3- (unpublished results). Increasing the dose of IL-2 resulted in higher concentrations of NO metabolites in the blood. The increase in the concentration of NO metabolites correlated with the development of hypotension in these patients during the first 24 h of therapy (Figure 3).

Furthermore, over the whole 5-day treatment period, the concentration of NO metabolites increased parallel to the rise in C3a. Inhibition of IL-2-induced complement activation by the use of C_1-esterase inhibitor resulted in a reduced increase in NO metabolites. These data suggest that NO plays an important final common pathway role in generating IL-2-induced haemodynamic changes and also the capillary leak syndrome. NO activity is, therefore, an important target to modify in an attempt to reduce IL-2 toxicity. NO has also been shown to be an important mediator of the haemodynamic changes associated with septic shock syndrome. The intracellular effects of NO are

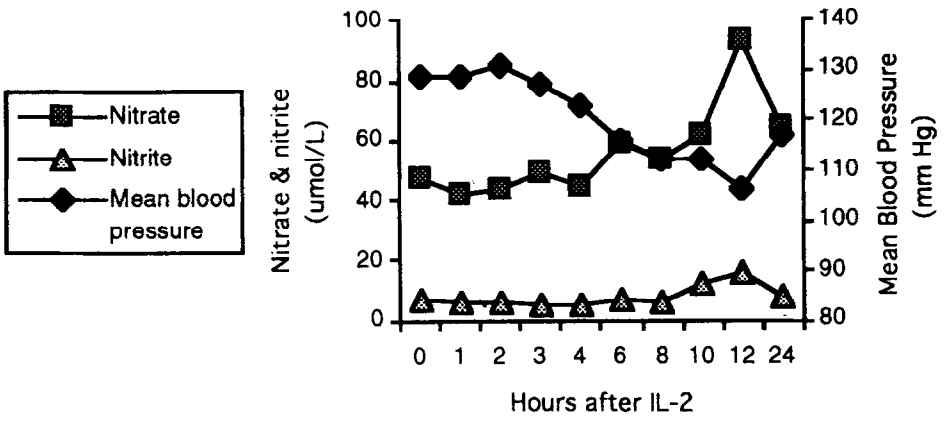

Figure 3. N0 metabolites following a 15 min IL-2 infusion.

mediated via the generation of cyclic GMP by the enzyme guanylate cyclase. The activity of this enzyme can be inhibited by methylene blue. In a recent study it has been demonstrated that the haemodynamic changes associated with the septic shock syndrome could be attenuated by the administration of methylene blue [102].

In conclusion, the extensive data which have been generated over the last few years have provided a rational basis for conducting studies to determine whether it is possible to reduce the toxicity of IL-2 and other cytokines without diminishing their antitumour effects. As a spin-off, these studies may also point to interventions which could potentially improve the prognosis of patients with septic shock. We therefore believe this work to be of considerable importance.

1. Doll R, Peto R. Epidemiology of cancer. In Weatherall DJ, Ledingham GG, Warrell DA, eds. *Oxford Textbook of Medicine*. Oxford, 1987, 4.95–4.123.
2. Grawitz P. Die sogenannten Lipome der niere. *Virchows Arch Pathol Anat* 1883, **93**, 39–63.
3. Doderlein A, Birch-Hirschfeld FV. Embryonale Drusengeschwulst der Nierengegend im Kindesater. *Sex Organe* 1894, **3**, 88–99.
4. Ritchie AWS, de Kernion JB. The natural history and clinical features of renal cell carcinoma. *Sem Nephr* 1987, **7**, 131–139.
5. Skinner DG, Colvin RB, Vermillion CD. Diagnosis and management of renal cell carcinoma. A clinical and pathologic study of 309 cases. *Cancer* 1971, **28**, 1165–1177.
6. Haertig A, Kuss R. Clinical signs in renal neoplasia. A comparison of two series of three hundred cases. In Kuss R, Murphy GP, Khoury S, *et al.*, eds. *Renal Tumours: Proceedings of the First International Symposium on Kidney Tumours*. New York, A.R. Liss, 1982, 337–340.
7. Golimbu M, Joshi P, Sperber A. Renal cell carcinoma: survival and prognostic factors. *Urology* 1986, **27**, 291–301.
8. Beahrs OH, Henson DE, Hutter RVP, Myers MH. *Manual for Staging of Cancer*. Philadelphia, J.B. Lippincott, 1988, 199–201.
9. Skinner DG, Vermillion CD, Colvin RB. The surgical management of renal cell carcinoma. *J Urol* 1972, **107**, 705–710.
10. Riches E. In *Tumours of the Kidneys and Ureters*. Edinburgh, E.S. Livingston, 1964.
11. Elson PJ, Witte RS, Trump DL. Prognostic factors for survival in patients with recurrent or metastatic renal cell carcinoma. *Cancer Res* 1988, **48**, 7310–7313.
12. de Forges A, Rey A, Klink M, Ghosh M, Kramer A, Droz JP. Prognostic factors of adult metastatic renal carcinoma: a multivariate analysis. *Semin Surg Oncol* 1988, **4**, 149–154.
13. Rafla S. Renal cell carcinoma: natural history and results of treatment. *Cancer* 1970, **28**, 26–40.
14. Bloom HJG. Medroxyprogesterone acetate (Provera) in the treatment of metastatic renal cell cancer. *Br J Cancer* 1971, **25**, 250–265.
15. Linehan WM, Shipley WJ, Longo DL. Cancer of the kidney and ureter. In De Vita VT, Hellman S, Rosenberg SA, eds. *Principles and Practice of Oncology*. Philadelphia, J.B. Lippincott, 1989, 279–1005.
16. Harris DT. Hormonal therapy and chemotherapy of renal carcinoma. *Semin Oncol* 1983, **10**, 422–430.
17. Possinger K, Wagner H, Beck R, *et al.* Renal cell carcinoma. *Controlled Oncol* 1988, **30**, 195–207.
18. McNichols DW, Segura JW, De Weerd JH. Renal cell carcinoma: long-term survival and late recurrence. *J Urol* 1981, **126**, 17–23.
19. Patel NP, Lavengood RW. Renal cell carcinoma: natural history and results of treatment. *J Urol* 1978, **119**, 722–726.
20. Waters WB, Richie JP. Aggressive surgical approach to renal cell carcinoma: review of 130 cases. *J Urol* 1979, **122**, 306–309.
21. Oliver RTD, Nethersell AB, Bottomley JM. Unexplained spontaneous regression and alpha-interferon as treatment for metastatic renal cell carcinoma. *Br J Urol* 1989, **63**, 128–131.
22. Montie JE, Stewart BH, Straffon RA. The role of adjunctive nephrectomy in patients with metastatic renal cell carcinoma. *J Urol* 1977, **117**, 272–275.
23. Quesada JR, Swanson D, Gutterman JU. Renal cell carcinoma: antitumor effects of leukocyte interferon. *Cancer Res* 1983, **42**, 940–947.
24. de Kernion B, Sarna G, Trinidada A. The treatment of renal cell carcinoma with human leukocyte alpha interferon. *J Urol* 1983, **130**, 1063–1066.
25. Magnusson K, Christopherson IS, Jordel R. Interferon therapy in recurrent renal cell carcinoma. *Acta Medica Scand* 1983, **213**, 221–224.
26. Kirkwood JM, Harris JE, Vera R, *et al.* A randomised study of low and high doses of leukocyte interferon in metastatic renal cell carcinoma: The American Cancer Society collaborative trial. *Cancer Res* 1985, **45**, 863–871.
27. Edsmyr F, Eposti PL, Andersson L, Steineck G, Lagergren C, Strander H. Interferon therapy in disseminated renal cell carcinoma. *Radiother Oncol* 1985, **4**, 21–26.
28. Krown SE, Einzig AI, Abramson JD. Treatment of advanced renal cell cancer (RCC) with recombinant leukocyte A interferon (rlFN-A). *Proc Am Soc Clin Oncol* 1983, **2**, 58.
29. Einzig AI, Krown SE, Oettegen HF. Recombinant leukocyte A interferon (rlFN-A) in renal cell carcinoma. *Proc Am Soc Clin Oncol* 1984, **3**, 227.
30. Quesada JR, Rios A, Swanson D, Trown P, Gutterman JK. Antitumor activity of recombinant derived interferon-alpha in metastatic renal cell carcinoma. *J Clin Oncol* 1985, **3**, 1522–1528.
31. Kuzmits R, Kokoschka EM, Micksche M, Ludwig H, Flener R. Phase II results with recombinant interferons: renal cell carcinoma and malignant melanoma. *Oncology* 1985, **42** (suppl.), 26–30.
32. Schnall SF, Davis C, Kirkwood JM. Treatment of metastatic renal cell carcinoma with intramuscular (IM) recombinant alpha A (IFN, Hoffman-La-Roche). *Proc Am Soc Clin Oncol* 1986, **5**, 227.
33. Fossa SD, DeGaris ST, Heier MS, *et al.* Recombinant interferon-alpha-2a with or without vinblastine in metastatic renal cell carcinoma. *Cancer* 1986, **57**, 1700–1704.
34. Kempf RA, Grunberg SM, Daniels JR, *et al.* Recombinant interferon-alpha-2a (Intron A) in a phase II study of renal cell carcinoma. *J Biol Resp Mod* 1986, **5**, 27–35.

35. Umeda T, Niijima N. Phase 2 study of alpha interferon on renal cell carcinoma: summary of three collaborative trials. *Cancer* 1986, **58**, 1231–1235.
36. Scheithaurer W, Kuerer I, Kutzmits R. Phase 2 trial of recombinant interferon-alpha-2a in metastatic renal cell carcinoma. *J Biol Reg Homeo Agents* 1987, **1**, 81–86.
37. Buzaid AC, Robertone A, Kisala C, Salmon SE. Phase 2 study of interferon-alpha-2a recombinant (Roferon A) in metastatic renal cell carcinoma. *J Clin Oncol* 1987, **5**, 1083–1089.
38. Muss HB, Costanzi JJ, Leavitt R, *et al.* Recombinant alpha interferon in renal cell carcinoma: a randomised trial of two routes of administration. *J Clin Oncol* 1987, **5**, 286–291.
39. Figlin RA, de Kernion JB, Mukamel E, Palleroni AV, Itri LM, Sarna GP. Recombinant interferon-alpha-2a in metastatic renal cell carcinoma: assessment of antitumor activity and anti-interferon antibody formation. *J Clin Oncol* 1988, **6**, 1604–1610.
40. Foon K, Doroshaw J, Bonnem E, *et al.* A prospective randomised trial of α-2b-interferon, gamma-interferon or the combination in advanced metastatic renal cell carcinoma. *J Biol Resp Mod* 1988, **7**, 540–545.
41. Otto U, Bauer HW, Jager N. Alpha-2 recombinant interferon treatment of metastatic renal cell carcinoma. *Semin Surg Oncol* 1988, **4**, 184–191.
42. Porzsolt F, Messere D, Hartman R, *et al.* Treatment of advanced renal cell cancer with recombinant interferon-alpha as a single agent and in combination with medroxyprogesterone acetate. A randomised multicentre trial. *J Cancer Res Clin Oncol* 1988, **114**, 95–100.
43. Marshall ME, Simpson W, Butler K, Fried A, Fer M. Treatment of renal cell carcinoma with low dose alpha interferon. *J Biol Resp Mod* 1989, **8**, 453–461.
44. Fossa S, Martinelli G, Otto U. Recombinant interferon-alpha-2a with or without vinblastine in metastatic renal cell carcinoma: results of a European multicenter phase III study. *Ann Oncol* 1992, **3**, 301–305.
45. Neidhart J, Gagen M, Kisner R. Therapy of renal cell cancer with low (LD), intermediate (ID) and high (HD) dose regimens of human lymphoblastoid inteferon (HBLI, Wellferon). *Proc Am Soc Clin Oncol* 1984, **3**, 60.
46. Neidhart JA, Gagen NM, Young D, *et al.* Interferon-alpha therapy of renal cell cancer. *Cancer Res* 1984, **44**, 4140–4143.
47. Marumo K, Murai M, Hayakawa M, Tazaki H. Human lymphoblastoid interferon therapy for advanced renal cell carcinoma. *Urology* 1984, **24**, 567–571.
48. Vugrim D, Hood L, Taylor W, Laszlo J. Phase 2 study of human lymphoblastoid interferon in patients with advanced renal cell carcinoma. *Cancer Treat Rep* 1985, **68**, 817–820.
49. Vugrim D, Hood L, Laszlo J. A phase 2 trial of high dose human lymphoblastoid inteferon in patients with advanced renal cell carcinoma. *J Biol Resp Mod* 1986, **5**, 309–314.
50. Trump DL, Elson PJ, Borden EC, *et al.* High dose lymphoblastoid interferon in advanced renal cell carcinoma: An Eastern Cooperative Oncology Group Study. *Cancer Treat Rep* 1987, **71**, 165–169.
51. Eisenhauwer EA, Silver HK, Venner PM, Thirell MP, Weinerman B, Coppin CM. Phase 2 study of high dose weekly intravenous human lymphoblastoid interferon in renal cell carcinoma. A study of the National Cancer Institute of Canada Clinical Trials Group. *Br J Cancer* 1987, **55**, 541–543.
52. Fujita T, Haruyosi A, Naide Y. Antitumor effects of human lymphoblastoid interferon on advanced renal cell carcinoma. *J Urol* 1988, **139**, 256–258.
53. Smith KA. Interleukin-2: inception, impact and implications. *Science* 1988, **240**, 1169–1176.
54. Ruscetti FW. Interleukin-2. In Oppenheim JJ, Shevach EM, eds. Immunophysiology. *The Role of Cells and Cytokines in Immunity and Inflammation*. Oxford, Oxford University Press, 1991, 46–49.
55. Taniguchi T, Matsui H, Fujita T, *et al.* Structure and expression of cloned cDNA for human interleukin-2. *Nature* 1983, **302**, 305–310.
56. Meir JW, Vachino G, van der Meer JW. Induction of circulating tumor necrosis factor (TNF alpha) as the mechanism for the febrile response to interleukin-2 (IL-2) in cancer patients. *J Clin Immunol* 1988, **8**, 426–436.
57. Gemlo BT, Pallafino MA, Jaffe HS, Espevik TP, Rayner AA. Circulating cytokines in patients with metastatic cancer treated with recombinant interleukin-2 and lymphokine activated killer cells. *Cancer Res* 1988, **48**, 5864–5867.
58. Bolhuis RLH, Sturm E, Braakman E. T cell targeting in cancer therapy. *Cancer Immunol Immunother* 1991, **24**, 1–8.
59. Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA. The lymphokine-activated killer cell phenomenon: lysis of NK resistant fresh solid tumour cells by IL-2 activated autologous human peripheral blood lymphocytes. *J Exp Med* 1982, **155**, 1823–1841.
60. Grimm EA, Ramsey KM, Mazumder A, Wilson DJ, Dieu DY, Rosenberg SA. Lymphokine activated killer phenomenon II. The precursor phenotype is serologically distinct from peripheral T lymphocytes, memory CTL, and NK cells. *J Exp Med* 1983, **157**, 884–897.
61. Grimm EA, Robb RJ, Roth JA, *et al.* Lymphokine-activated killer cell (LAK) phenomenon III. Evidence that IL-2 alone is sufficient for direct activation of PBL into LAK. *J Exp Med* 1983, **158**, 1356–1361.
62. Jost LM, Stahel RA. Interleukin-2 as single agent: dose, scheduling and optimum immunomodulatory dose. In Wagstaff J, ed. *The Role of Interleukin-2 in the Treatment of Cancer Patients*. The Netherlands, Kluwer, 1993, 7–28.
63. Negrier S, Philip TO. Interleukin-2 and lymphokine activated killer (LAK) cells. In Wagstaff J, ed. *The Role of Interleukin-2 in the Treatment of Cancer Patients*. 1993, 29–43.
64. Rosenberg SA. The development of new immunotherapies for the treatment of cancer using interleukin-2. *Ann Surg* 1988, **208**, 121–135.
65. Atzpodien J, Shomberg A, Kirchner H, Poliwoda H. Interleukin-2 and other cytokines. In Wagstaff J, ed. *The Role of Interleukin-2 in the Treatment of Cancer Patients*. 1993, 141–167.
66. Palmer PA, Vinke J, Philip T, *et al.* Prognostic factors for survival in patients with advanced renal cell carcinoma treated with recombinant interleukin-2. *Ann Oncol* 1992, **3**, 475–480.
67. Stoter G, Bolhuis RLH, Goey SH, Eggermont AMM. Adoptive immunotherapy with interleukin-2 (IL-2) + α-interferon (αIFN) + IL-2 activated lymphocytes (LAK) in metastatic renal cell cancer. *Proc 8th NCI-EORTC Symposium on new drugs in cancer therapy*. 1994, 105.
68. Rosenstein M, Ettinghausen SE, Rosenberg SA. Extravasation of intravascular fluid mediated by systemic administration of recombinant interleukin-2. *J Immunol* 1986, **137**, 1735–1742.
69. Siegel JP, Puri RK. Interleukin-2 toxicity. *J Clin Oncol* 1991, **9**, 694–704.
70. Lee RE, Lotze MT, Slibber JM, *et al.* Cardiorespiratory effects of immunotherapy with interleukin-2. *J Clin Oncol* 1989, **7**, 7–20.
71. Cotran RS, Pober JS, Gimbrone MS, *et al.* Endothelial activation during interleukin-2 (IL-2) immunotherapy: a possible mechanism for the vascular leak syndrome. *J Immunol* 1988, **140**, 1883–1888.
72. Gemlo BT, Palladino MA, Jaffe HS, Espevik TP, Rayner AA. Circulating cytokines in patients with metastatic cancer treated with recombinant interleukin-2 and lymphokine-activated killer cells. *Cancer Res* 1988, **48**, 5864–5867.
73. Damle NK, Doyle LV, Bender JR, *et al.* Interleukin-2 activated human lymphocytes exhibit enhanced adhesion to normal endothelial vascular cells and cause their lysis. *J Immunol* 1987, **138**, 1779–1785.
74. Welbourne R, Goldman G, Kobitz L, Paterson I, Shepro D, Hechtman HB. Interleukin-2 induces early multisystem edema by neutrophils. *Ann Surg* 1991, **214**, 181–186.
75. Edwards MJ, Miller FN, Sims DE, Abney DL, Schuske DA, Corey TS. Interleukin-2 acutely induces platelet and neutrophil-endothelial adherence and macro molecular leakage. *Cancer Res* 1992, **52**, 3425–3431.
76. Abe Y, Sekiya S, Yamasita T, Sendo F. Vascular hyperpermeability induced by tumor necrosis factor and its augmentation by IL-1 and IFN-γ is inhibited by selective depletion of neutrophils with a monoclonal antibody. *J Immunol* 1990, **145**, 2902–2907.
77. Pober JS, Cotran RS. Cytokines and endothelial cell biology. *Phys Rev* 1990, **70**, 427–452.
78. Mantovani A, Bussolino F, Dejana E. Cytokine regulation of endothelial function. *FASEB J* 1992, **6**, 2591–2599.
79. Thompson JA, Benyunes MC, Bianco JA, Fefer A. Treatment with pentoxiphylline and ciprofloxacin reduces the toxicity of high-dose interleukin-2 and lymphokine-activated cells. *Semin Oncol* 1993, **20**, 46–51.
80. Mier JW, Vachino G, Klempner M, *et al.* Inhibition of interleukin-2 induced tumor necrosis factor release by dexamethasone: preven-

tion of an acquired neutrophil chemotaxis defect and differential suppression of interleukin-2 associated side effects. *Blood* 1990, **76**, 1933–1940.
81. Fraker DL, Langstein HN, Norton JA. Passive immunization against tumor necrosis factor partially abrogates interleukin-2 toxicity. *J Exp Med* 1989, **170**, 1015–1020.
82. Vachino G, Gelfand JA, Atkins MB, Tamerius JD, Demchak P, Mier JW. Complement activation in cancer patients undergoing immunotherapy with interleukin-2 (IL-2): binding of complement and C-reactive protein by IL-2-activated lymphocytes. *Blood* 1991, **78**, 2505–2513.
83. Rabinovici R, Sofronski MD, Borboruglu P, *et al.* Interleukin-2-induced lung injury: the role of complement. *Circulation Res* 1994, **74**, 329–335.
84. Fleischmann JD, Shingleton WB, Gallagher C, Ratnoff OD, Chahine A. Fibrinolysis, thrombocytopenia and coagulation abnormalities complicating high-dose interleukin-2 immunotherapy. *J Lab Clin Med* 1991, **117**, 76–82.
85. Strack van Schijndel RJM, Voerman HJ, Golding RP, Wagstaff J. Interleukin-2 therapy and blockage of double-lumen catheters. *Lancet* 1989, **1**, 962.
86. van der Poll T, Levi M, Büller HR, *et al.* Fibrinolytic response to tumor necrosis factor in healthy subjects. *J Exp Med* 1991, **174**, 729–732.
87. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *New Engl J Med* 1993, **329**, 2002–2012.
88. Kilbourn RG, Belloni P. Endothelial production of nitrogen oxides in response to interferon-gamma in combination with tumor necrosis factor, interleukin-1, or endotoxin. *J Natl Cancer Inst* 1990, **82**, 772–776.
89. Hibbs JB, Westenfelder C, Taintor R, *et al.* Evidence after cytokine-inducible nitric oxide synthesis from L-arginine in patients receiving interleukin-2 therapy. *J Clin Invest* 1992, **89**, 867–877.
90. Kilbourn RG, Griffith OW. Overproduction of nitric oxide in cytokine-mediated and septic shock. *J Natl Cancer Inst* 1992, **84**, 827–831.
91. Petros A, Bennett D, Vallance P. Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock. *Lancet* 1991, **338**, 1557–1558.
92. Kilbourn RG, Gross SS, Jubran A, *et al.* N^G- Methyl-L-arginine inhibits tumor necrosis factor-induced hypotension: implications for the involvement of nitric oxide. *Proc Natl Acad Sci USA* 1990, **87**, 3629–3632.
93. Baars JW, Wolbink GJ, Hart MHL, *et al.* Release of interleukin-8 during intravenous bolus treatment with interleukin-2. *Ann Oncol* 1994, 5, in press.
94. Baars JW, Hack CE, Wagstaff J, *et al.* The activation of polymorphonuclear neutrophils and the complement system during immunotherapy with recombinant interleukin-2. *Br J Cancer* 1992, **65**, 96–101.
95. Thijs LG, Hack CE, Strack van Schijndel RJM, *et al.* Activation of the complement system during immunotherapy with interleukin-2. Relation to the development of side effects. *J Immunol* 1990, **144**, 2419–2424.
96. Hack CE, Ogilvie AC, Eisele B, Eerenberg AJM, Wagstaff J, Thijs LG. C1-inhibitor substitution therapy in septic shock and in the vascular leak syndrome induced by high doses of interleukin-2. *Intensive Care Med* 1993, **19**, S19–S23.
97. Ogilvie AC, Baars JW, Eerenberg AJM, *et al.* Pilot study to evaluate the effects of C1 esterase inhibitor on the toxicity of high-dose interleukin-2. *Br J Cancer* 1994, **69**, 596–598.
98. Hack CE, Wagstaff J, Strack van Schijndel RJM, *et al.* Studies on the contact system of coagulation during therapy with high doses of recombinant IL-2: implications for septic shock. *Thromb Haemost* 1991, **65**, 497–504.
99. Baars J, Boer JP. de, Wagstaff J, *et al.* Interleukin-2 induces activation coagulation and fibrinolysis: resemblance to the changes seen during experimental endotoxaemia. *Br J Haemat* 1992, **82**, 295–301.
100. Olthof CG, Baars JW, Wagstaff J, Donker AJM, Schneider H, de Vries PMJM. Determination of capillary leakage due to recombinant interleukin-2 by means of non invasive conductivity measurements. *Eur J Appl Physiol* 1993, **67**, 168–173.
101. Raijmakers PGHM, Groeneveld ABJ, Schneider AJ, *et al.* Transvascular transport ^{67}Ga in the lung after cardiopulmonary bypass surgery. *Chest* 1993, **104**, 1825–1832.
102. Daemen-Gubbels CRGM, Groeneveld PHP, Groeneveld ABJ, van Kamp GJ, Bronsveld W, Thijs LG. Methylene blue increases myocardial performance in septic shock. *Intensive Care Med* 1995, in press.

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Revisions of General Guidelines for the Preclinical Toxicology of New Cytotoxic Anticancer Agents in Europe

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INTRODUCTION

IN 1980, the Cancer Research Campaign (CRC) defined minimal toxicology requirements for the testing of novel anticancer drugs prior to Phase I clinical trials. These protocols were adopted soon afterwards by the European Organization for Research and Treatment of Cancer (EORTC). Through experience of working