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Preclinical study

Dendrimer-platinate: a novel approach to cancer chemotherapy

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A polyamidoamine (PAMAM™) dendrimer generation 3.5 with a sodium carboxylate surface was conjugated to cisplatin giving a dendrimer-platinate (dendrimer-Pt; 20–25 wt% platinum) which was highly water soluble and released platinum slowly *in vitro*. *In vivo* the dendrimer-Pt and cisplatin were equi-active i.p. against i.p. L1210, and at high dose dendrimer-Pt given i.p. showed activity against i.p. B16F10 whereas cisplatin did not. Additionally, when administered i.v. to treat a palpable s.c. B16F10 melanoma, the dendrimer-Pt displayed antitumor activity whereas cisplatin was inactive. Measurement of platinum levels in blood and tissues after i.v. injection of cisplatin (1 mg/kg) or dendrimer-Pt (15 mg/kg)—the maximum tolerated dose (MTD) of these compounds—showed selective accumulation of the dendrimer-Pt in solid tumor tissue by the EPR effect (a 50-fold increase in area under curve compared with cisplatin). The dendrimer-Pt was also less toxic (3- to15-fold) than cisplatin and thus has potential for further investigation as a novel antitumor approach. [© 1999 Lippincott Williams & Wilkins.]

Key words: Dendrimer, EPR effect, HPMA copolymers, platinate, polymer therapeutics, tumor targeting.

Introduction

Although we continue to learn more of the genetic basis of cancer, discovery of chemotherapy effective for the treatment of the major diseases (including breast, colon, prostate and lung) remains a challenge for the next century.[1] Interesting preclinical (reviewed in by Duncan *et al.*[2–4]) and early clinical data[5–8] emerging from the development of *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-drug conjugates suggest that polymer therapeutics constitute an important new class of anticancer agents. Although still at an early stage of development they have shown antitumor activity in each of the three phase I studies reported so far and the clinical dose schedule is still to be optimized in respect of the known pharmacokinetics of these macromolecular prodrugs.

Traditionally linear, hydrophilic polymers (like HPMA copolymers) have been examined as carriers for anticancer agents. Although the only polymeric carriers to be successfully transferred to the clinic, HPMA copolymers have the disadvantage of lack of biodegradability in the main polymer chain (which limits the molecular weight range that can be safely administered to man), and they result in a polydisperse and heterogeneous product. The purpose of this study was to investigate for the first time novel hyperbranched polymers called dendrimers as potential carriers for antitumor agents. Dendrimers, sometimes also called arborols or cascade molecules, are macromolecules (typically 5000–500 000 Da) born out of innovative organic chemistry of the last decade (summarized in Newkome *et al.*[9]). They offer particular advantages compared with linear polymeric carriers; their nanoscale spherical architecture, narrow polydispersity and the possibility to tailor-make their surface chemistries.[10] They also have a relatively empty intramolecular cavity amenable to a host-molecule entrapment[11] with opportunities for subsequent controlled drug release. Dendrimers have already been synthesized in a variety of forms (reviewed in Newkome *et al.*[9]) but few are suitable for biomedical use. On finding that the anionic polyamidoamine starburst™ (PAMAM) dendrimers (see Figure 1) were non-toxic *in vitro*,[12] PAMAM generation 3.5 with a sodium carboxylate surface and molecular weight 12 931 Da was selected for reaction with cisplatin to prepare a dendrimer-platinate (dendrimer-Pt).

Cisplatin and carboplatin are important anticancer agents. They are widely used in the treatment of

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ovarian, head and neck, and testicular cancer, and cisplatin is especially effective in the combined chemotherapy against squamous cell carcinoma and small cell lung carcinoma.[13] However, the platinate drugs still have well-recognized drawbacks, including low water solubility, severe toxic side effects and the inherent or acquired resistance seen in many tumors.[14] Synthesis of polymeric platinates is an attractive means to increase platinum solubility, reduce systemic toxicity and localize more drug in the tumor via the enhanced permeability and retention effect (EPR effect)[15,16] with consequent potential to overcome the wide ranging mechanisms of resistance. We have already described the preparation and biological properties of polymer platinates prepared using natural polymers[17] (carboxymethyl dextran; poly glutamic acid; alginates), polyamidoamines[18] and HPMA copolymers,[19] and it was considered important to compare the dendrimer-Pt with these linear polymer-platinates.

First the rate of platinum release and cytotoxicity of the dendrimer-Pt was determined *in vitro*. Antitumor activity was assessed *in vivo*, initially using i.p. L1210 and B16F10 models treated by i.p. administration of drug. This model was selected to allow historical comparison with the reported activity of low molecular weight platinates and to assess the ability of the dendrimer-Pt to liberate active diaqua-platinum species. Subsequently, mice bearing palpable s.c. B16F10 (a well-characterized tumor model known to capture polymeric antitumor agents by the EPR effect[16,19,20]) were used to study the pharmacological activity after i.v. administration. The biodistribution of cisplatin (1 mg/kg) and dendrimer-Pt (15 mg/kg) was also assessed in mice bearing s.c. B16F10 using atomic absorption spectroscopy (AAS) to measure platinum levels.

Materials and methods

Materials

PAMAM dendrimer generation 3.5 was obtained from Aldrich (Poole, UK). This dendrimer was prepared according to the methods of Tomalia and colleagues as described elsewhere.[21] Cisplatin, 5-dimethylthiazol-2-yl-2,5-diphenyl tetrazolium bromide (MTT) and dimethylsulfoxide were supplied by Sigma (Poole, UK). L1210 cells were obtained from European Collection of Cell Cultures (Centre for Applied Biology, Microbiology and Research, Salisbury, Wiltshire UK) and B16F10 cells were a kind gift from Professor Ian Hart (St Thomas' Hospital, London, UK).

Preparation of the dendrimer-Pt

Initially experiments were conducted to optimize the synthesis by varying the dendrimer:platinum (cisplatin) molar ratio (in the range 1:10 to 1:100). A ratio of 1:35 was selected for all further syntheses. Typically, cisplatin (845 mg) was dissolved in 422 ml double-deionized distilled water (DDW) with gentle heating (30°C). PAMAM dendrimer generation 3.5 (10% w/v solution in methanol diluted in 10 ml of DDW) was then added dropwise under stirring. This solution was kept at room temperature and stirred for 4 h, during which time chloride ion release was monitored using a chloride meter (Jenway PCLM 3). The reaction mixture was purified using either Centriprep concentrators (cut-off 3000 Da; Amicon, Watford, UK) by centrifugation in three steps at 4000 *g* over 90, 45 and 15 min steps (15 ml per concentrator) or by dialysis against DDW (Spectropor; 3500 Da cut-off). At each step the total volume in the concentrator was made back upto 15 ml. The resultant sample was checked for purity using thin layer chromatography and then dried by lyophilization. Total platinum was determined either by AAS or the *o*-phenylenediamine (*o*-PDA) (colorimetric) assay.[22]

Release of platinum *in vitro*

The dendrimer-Pt (5 mg/ml) was dissolved in phosphate-buffered saline (pH 7.4) or citrate phosphate buffer (0.05 M, pH 5.5) and samples were dialyzed against the respective buffers at 37°C for 72 h. The dialyzate was examined for platinum content (AAS) over time.

In vitro cytotoxicity

Cisplatin or the dendrimer-Pt were incubated with three cell lines: B16F10, COR-L23 or L1210. B16F10 cells were seeded at a starting cell density of 1×10^4 cells/well and Cor L23 and L1210 cells were seeded at 5×10^3 cells/well; in a 96-well microtiter plates (RPMI 1640 media with serum, 5% CO_2 at 37°C) and left to recover for 24 h. Cisplatin or dendrimer-Pt were added in fresh tissue culture medium, and after 72 h cell viability was assessed using the tetrazolium-based colorimetric MTT assay.[23] Results are expressed as a percentage of the viability of cells grown in the absence of drug.

Evaluation of antitumor activity *in vivo*

All animal experiments were carried out according to criteria laid down in the UKCCCR guidelines for the welfare of animals in experimental neoplasia. To establish the i.p. tumor models, B16F10 murine melanoma or L1210 (10^6 cells) were injected into C57 male mice (6–8 weeks old) or DBA_2 male mice (6–8 weeks old) (Banton & Kingman, Hull, UK), respectively (five to 10 animals per experimental group). Cisplatin (0.5–3 mg/kg) or dendrimer-Pt (5–40 mg/kg; note, all doses shown are given in platinum-equivalent doses unless otherwise stated) were administered i.p. (see Table 2). The B16F10 tumor was treated by a single dose on day 1; for L1210, treatment was administered on days 1, 2 and 3 after tumor inoculation.

To provide a solid tumor model, B16F10 cells (10^5 cells) were injected s.c.into C57 mice. Once the tumor had developed to palpable size, about 50–70 mm^2 (as measured by the product of two orthogonal diameters), the animals were treated by a single i.v. injection of cisplatin (0.5–1.0 mg/kg) or dendrimer-Pt (5–15 mg/kg).

In all experiments animals were monitored for general health, weight loss, tumor progression and at termination they were subject to post-mortem examination. Where possible tumor weights were also measured at termination.

Biodistribution of dendrimer-Pt

C57 mice bearing a s.c. B16F10 tumor (as above) were injected i.v. with either cisplatin 1 mg/kg (its maximum tolerated dose) or the dendrimer-Pt (1 and 15 mg/kg). After 0, 1, 5 and 24 h mice (five per group) were culled and a blood sample taken, and the tumor, liver and kidneys were removed. The organs were dissolved in a known volume of nitric acid (2 days, 10 M) and sufficient hydrogen peroxide (30% v/v) subsequently added to a give colorless solution. Each sample was made up to a known volume with water and analyzed using an atomic absorption (flameless graphite furnace) spectrometer. Cisplatin was used as a standard with matrix matching.

Statistical methods

All the *in vitro* data are expressed as the mean ± SD. All the *in vivo* data are expressed as the mean ± SE. Statistical analysis of the mice survival time, tumor and blood data were performed using the Student's *t*-test. $p < 0.05$ was considered statistically significant.

Results

Synthesis and characterization of dendrimer-Pt

The reaction of PAMAM dendrimer generation 3.5 with cisplatin in aqueous solution lead to faster chloride release than seen for cisplatin alone (results not shown). Chloride release was essentially complete within 4 h. The resultant dendrimer-Pt preparations reproducibly displayed a platinum loading of 20–25 wt% with a mean of 23.6 ± 1.8 wt% (Table 1) and were 10-fold more soluble in water than cisplatin. ^{1}H- and ^{13}C-NMR of the product showed distinct alterations in the spectra indicative of platinum conjugation at the dendrimer surface via carboxy groups. Size exclusion chromatography (GPC) and particle sizing by photon correlation spectroscopy (results not shown) revealed that the platinate conjugate was comprised of a number of species with dendrimer cross-linked via platinum bridges giving an increase in size from 3–4 nm in the parent dendrimer to the 30–40 nm diameter of the dendrimer-Pt. The proposed structure of the dendrimer-platinate is shown in Figure 1. Free platinum species were not detectable in the conjugate by thin layer chromatography (TLC). As reaction of cispatin PAMAM dendrimer generation 4 (of molecular weight 14 215 Da and containing surface amine groups) gave no detectable platinum association this suggests inability of cisplatin to react with or become sequestered within the dendrimer core.

During incubation of the dendrimer-Pt in buffers at pH 7.4 and 5.5, selected to mimic the environment that would be encountered in plasma and intracellularly in the secondary lysosomes, no platinum release

Table 1. Characterization of several batches of dendrimer-Pt

Batch	Pt content (wt%)	Method of analysis
1	23.0	*o*-PDA
2	22.0	*o*-PDA
3	23.2	AAS
4	23.8	AAS
5	24.5	AAS
6	21.8	AAS
7	26.9	AAS

Mean=23.6 ± 1.8 wt%.

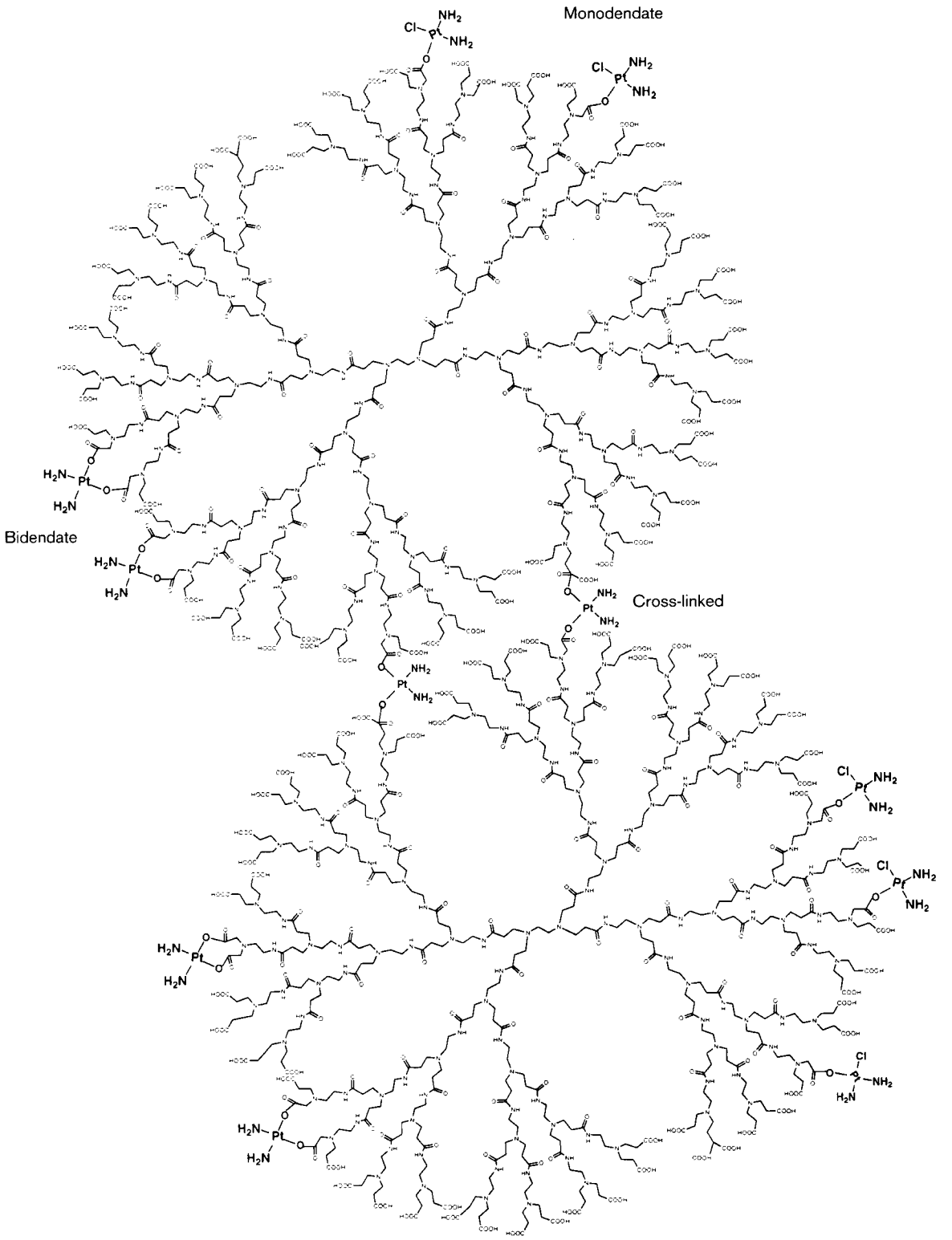

Figure 1. Proposed structure of generation 3.5 PAMAM dendrimer-Pt with the three possible different Pt binding structures: monodendate, bidendate and cross-linked.

(less than 1% of the total platinum) was detected over 72 h (Figure 2).

Cytotoxicity of dendrimer-Pt *in vitro*

The dendrimer-Pt (IC_{50} values from 500 to >2000 μg/ml Pt equivalent) was much less toxic (200- to 550-fold) than cisplatin (IC_{50} values 1-10 μg/ml) when incubated with CCRF, COR L23 and B16F10 cells (Figure 3). Indeed, against the B16F10 line the conjugate was completely inactive over the concentration range used (Figure 3c). Macromolecular drug conjugates are typically poorly active *in vitro* compared to conventional antitumor agents,[2] as unlike small molecules which rapidly (within minutes) penetrate the cell membrane, they enter the cell relatively slowly by endocytosis and the active species are liberated very slowly thereafter. However, *in vitro* screening cannot be used as a predictor of therapeutic activity of macromolecular conjugates *in vivo*.[2]

In vivo pharmacology and pharmacokinetics

Initially, the dendrimer-Pt was evaluated against i.p. mouse tumor models followed by i.p. treatment. Mice bearing i.p. L1210 leukemia treated with the dendrimer-Pt displayed a T/C of >191% (one long-term

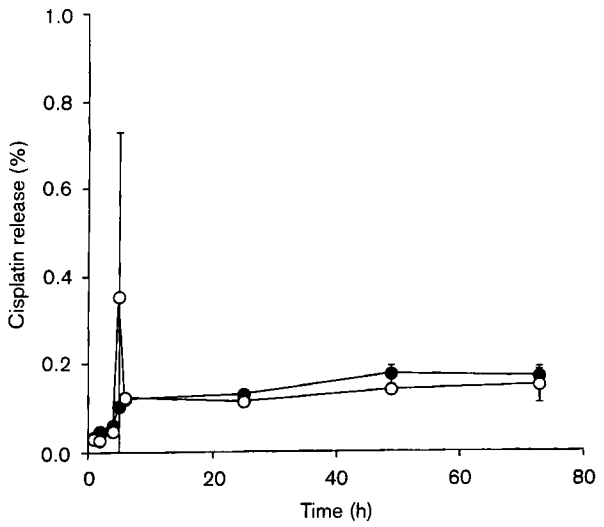

Figure 2. *In vitro* release of platinum from generation 3.5 PAMAM dendrimer-Pt. During incubation in phosphate-buffered saline at pH 7.4 (●) and in citrate phosphate buffer at pH 5.5 (○). Data represent mean ± SD; n=3.

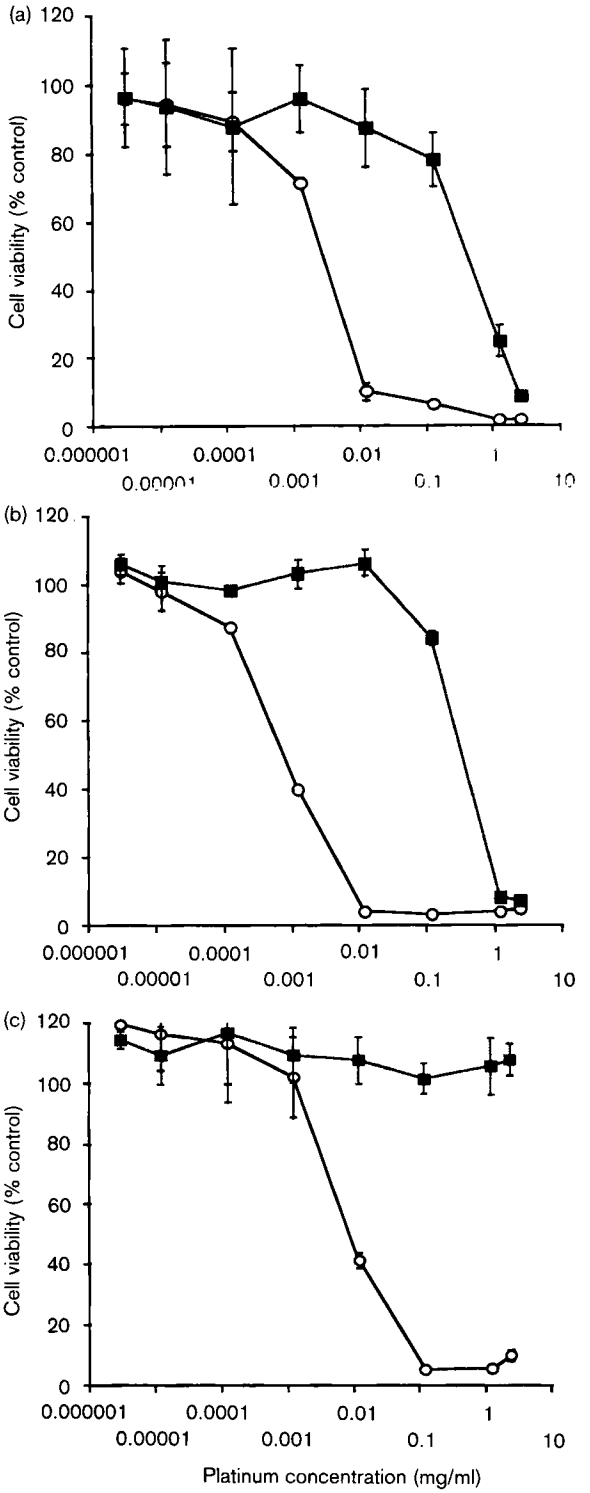

Figure 3. Cytotoxicity of cisplatin and the generation 3.5 PAMAM dendrimer-Pt. (a) CCRF cells. (b) COR L23 cells. (c) B16F10 cells. In all cases the cytotoxicty of cisplatin (○) and generation 3.5 PAMAM dendrimer-Pt (■) are shown. Concentrations are expressed in platinum equivalents. Data represent mean ± SD; n=3.

survivor). This was better then the T/C seen for cisplatin at its MTD (171%). These observations confirm the ability of the dendrimer-Pt to release a biologically active diaqua-platinum species. The dendrimer itself displayed neither antitumor activity nor toxicity when given at 95 mg/kg × 3 (the equivalent dose to that used in the highest platinum conjugate) (Table 2 and Figure 4a). The dendrimer-Pt was approximately 8-fold less toxic than cisplatin (Table 2 and Figure 4b).

Cisplatin was inactive against the i.p. B16F10 tumor model at its MTD and although the dendrimer-Pt was also poorly active, at the highest dose (15 mg/kg) it displayed a significant increase in T/C (129). In addition, the B16F10 tumors recovered at termination after treatment with dendrimer-Pt at 15 mg/kg had a significantly lower weight than those recovered from the control and cisplatin groups (Figure 5). Indeed the animals treated with dendrimer-Pt at 15 mg/kg were removed from the experiment due to late onset toxicity rather than tumor growth.

Although these i.p. models have traditionally been used to screen the activity of low molecular weight platinates,[24] they do not allow for preferential tumor accumulation by the EPR effect. Using the s.c. B16F10 tumor model the dendrimer-Pt showed significant antitumor activity whereas cisplatin was still not active at its MTD (Figure 6). Evaluation of the biodistribution of cisplatin (mg/kg) and dendrimer-Pt (1 or 15 mg/kg) after i.v. administration to mice bearing s.c. B16F10 showed that cisplatin and dendrimer-Pt had a similar

Table 2. Antitumor activity of generation 3.5 dendrimer-Pt after i.p. administration to treat i.p. tumor models

Treatment	Dose Pt (mg/kg)	T/C[a] (%)	Toxic deaths
B16 Melanoma[b]			
cisplatin	5	89[NS]	2/5
dendrimer-Pt	5	105[NS]	0/5
	10	108[NS]	0/5
	15	129***	5/5
L1210[c]			
cisplatin	2	171**	0/10
cisplatin	3	64[NS]	9/10
dendrimer-Pt	2	>191 (1/5LTS[d])**	0/5
	5	118*	1/5
	10	144**	0/5
	15	146**	0/5
	25	40[NS]	5/5
	50	40[NS]	5/5
dendrimer generation 3.5 (no platinum)	95 mg/kg (polymer alone)	100[NS]	0/5

[a]Mean survival of the treated group/mean survival of the untreated control group × 100.
[b]Cells inoculated i.p. on day 0, animals treated i.p. on day 1, single dose.
[c]Cells inoculated on day 0, animals treated on days 1, 2, 3, three doses.
[d]LTS, long-term survivors after 30 days.
Statistical analyses were carried out using the Student's *t*-test (small samples): NS, not significant; $*p<0.05$; $**p<0.01$; $***p<0.001$.

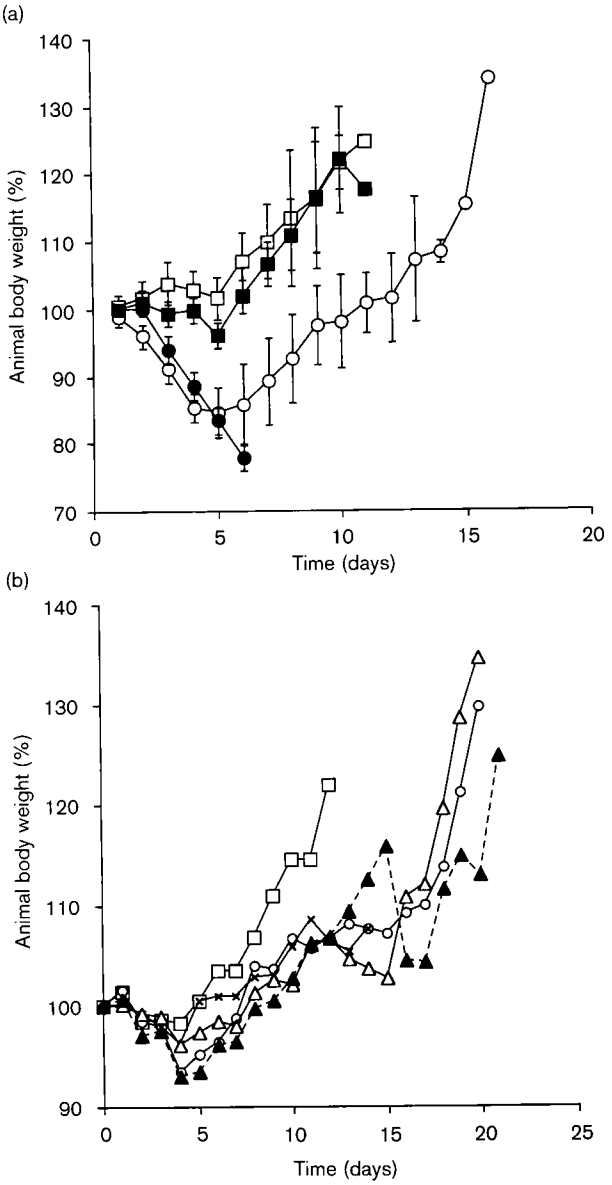

Figure 4. Effect of i.p. treatment on the weight of mice bearing L1210 tumors i.p. (a) Weight loss after treatment with saline (□); cisplatin 3 mg/kg × 3 (●); cisplatin 2 mg/kg × 3 (○) and PAMAM generation 3.5 alone (95 mg/kg × 3) (■). (b) Weight loss after treatment with saline (□); cisplatin 2 mg/kg × 3 (○); dendrimer-Pt 5 mg/kg (X); dendrimer-Pt 10 mg/kg (△) and dendrimer-Pt 15 mg/kg (▲). In all cases data represent mean ± SD; $n=5$.

blood clearance at a dose of 1 mg/kg, but the higher dendrimer-Pt dose (15 mg/kg) showed a much slower blood clearance rate (Figure 7a). The platinum levels measured in s.c. B16F10 tumors showed that administration of the dendrimer-Pt produced a 5- to 50-fold higher platinum concentration (depending on dose) in tumor tissue than can be achieved for cisplatin at its MTD (Figure 7b). The platinum levels detected in kidney and liver (Figure 7c and d) showed that administration of the dendrimer-Pt did not cause an equivalent increase in AUC in these normal tissues (Table 3) even at an equi-dose of dendrimer-Pt.

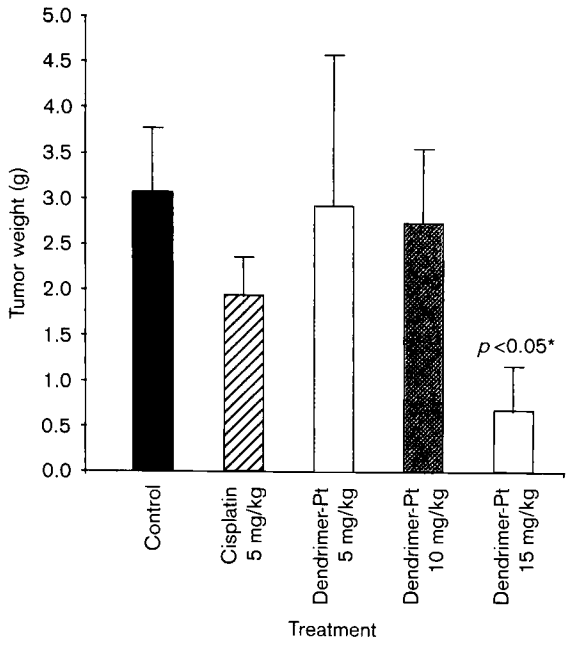

Figure 5. The weight of i.p. innoculated B16F10 tumors taken at termination from C57 mice. Data represent the mean ± SD; n=5.

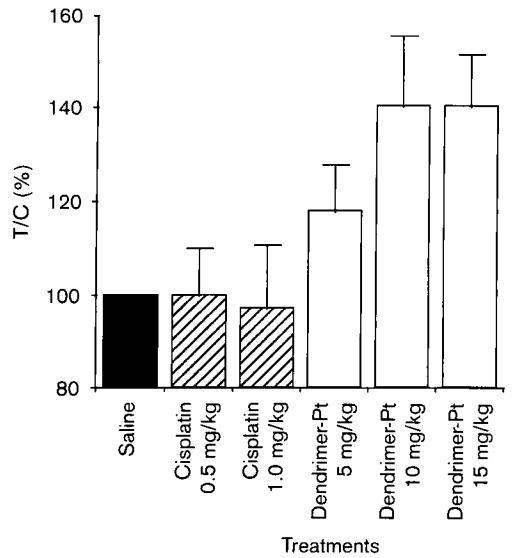

Figure 6. Mean survival of mice bearing a solid B16F10 tumor (s.c.) treated i.v. with cisplatin or generation 3.5 PAMAM dendrimer-Pt. All doses are expressed as platinum equivalents and T/C is the mean survival time of the treated group/mean survival time of the untreated control group as a percentage.

Discussion

In a recent phase I study,[5] the HPMA copolymer conjugate PK1 (FCE 28068), which contains doxorubicin linked to the polymer via a Gly-Phe-Leu-Gly linker designed for cleavage by the lysosomal thiol-dependent proteases, showed an MTD of 320 mg/m^2 (dox-equivalent), no polymer-related toxicity and antitumor activity in several chemotherapy refractory patients. These early clinical observations have verified our approach for the design of novel polymer-drug conjugates with reduced non-specific toxicity and the ability to display antitumor activity in chemotherapy-refractory patients. In addition there is evidence to support the concept of solid tumor targeting via the EPR effect using polymer therapeutics in man.[25]

Here we have shown, for the first time, that hyperbranched dendritic polymers may also be developed as polymer therapeutics for improved delivery of antitumor agents. The PAMAM generation 3.5 dendrimer had a high platinum carrying capacity (20–25 wt%), much higher than seen for HPMA copolymer platinates (3–8 wt%)[19] and a linear polyamidoamine platinate (generally 5–10 wt%).[18] Like HPMA copolymer-doxorubicin[16] and HPMA copolymer-platinates,[19] the dendrimer-Pt accumulated selectively in s.c. B16F10 tumors by the EPR effect (Figure 7b). Exposure of tumor tissue to a much higher platinum concentration than can be achieved using cisplatin at its MTD resulted in antitumor activity in this cisplatin refractory tumor (Figure 6). It should be noted that the exact proportion of the dendrimer-Pt made available as the active diaqua-species intratumorally is not yet known and indeed the time course of platinum liberation from the conjugate must be determined. However, the improved activity in the s.c. solid tumor model versus the i.p. ascites is indicative of the importance of the EPR effect in tumor targeting. Clinically, platinum resistance typically exhibits a 1- to 5-fold resistance index.[26] The observation that the dendrimer-Pt displays a dramatic increase in tumor platinum concentration may have important implica-

tions for the design of new polymer therapeutics which should be able to overcome the plethora of platinum-resistance mechanisms described to date. The dendrimer-Pt should be tested against normal and resistant tumors to verify this hypothesis. It is noteworthy that the PAMAM platinate described here had a markedly reduced toxicity in all experiments irrespective of the route of administration (Table 2 and Figure 6).

HPMA copolymer molecular weight has a marked effect on the magnitude of the EPR effect.[27] Polymers of molecular weight greater then the renal threshold

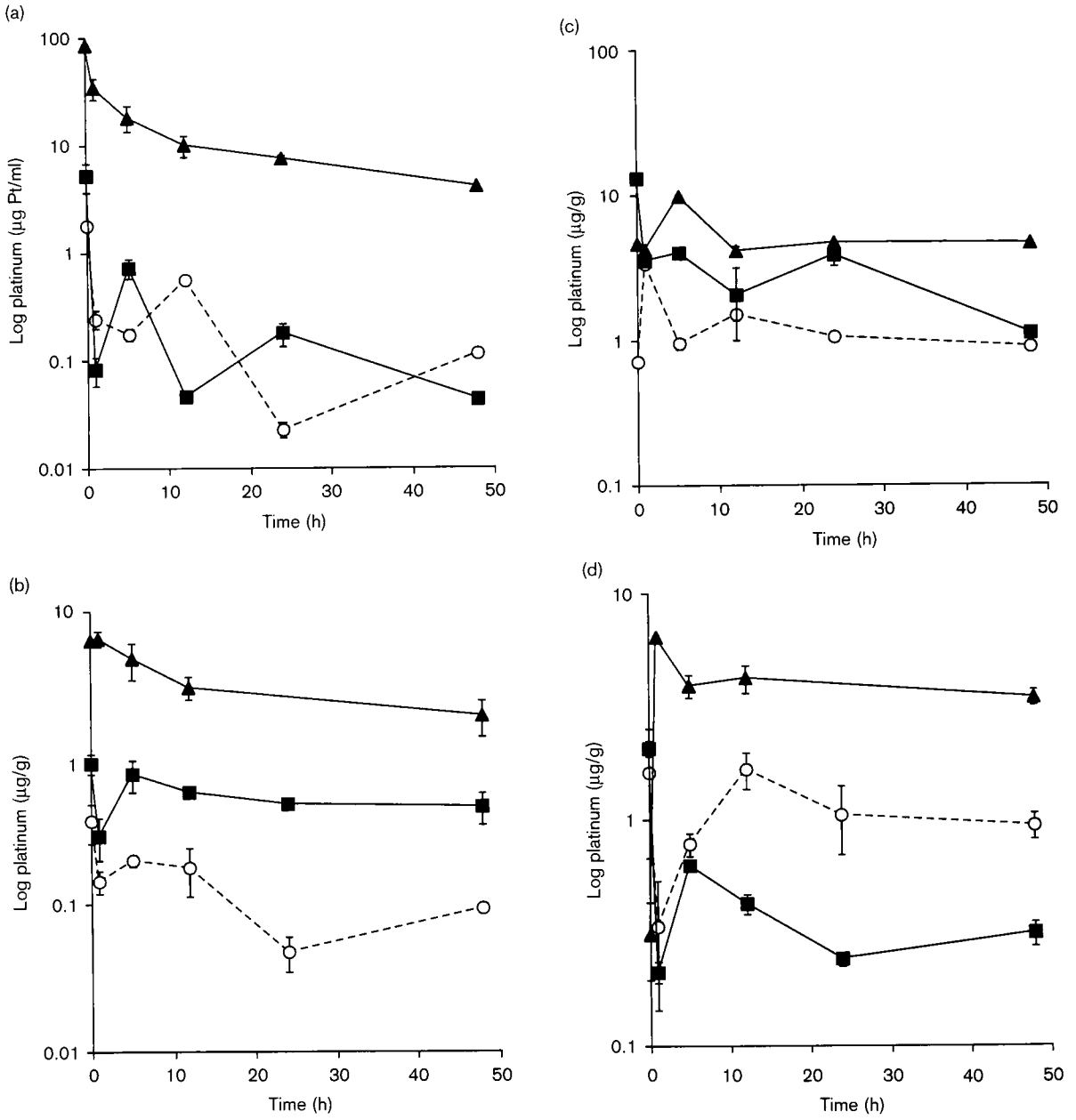

Figure 7. Biodistribution of cisplatin and generation 3.5 PAMAM dendrimer-Pt after i.v. administration to mice bearing s.c. B16F10 tumors, platinum levels were measured by AAS and in each case data represent mean ± SD; n=3. (a) Blood. (b) Tumour. (c) Kidney. (d) Liver. Key in each case: cisplatin 1 mg/kg (○) and the generation 3.5 PAMAM dendrimer-Pt at doses of 1 mg/kg (■) or 15 mg/kg (▲).

Table 3. Area under curve (μg/g/h) for platinum levels in various tissues taken from C57 mice bearing s.c. B16F10 tumors after administration of cisplatin and dendrimer-Pt (i.v.) measured over the time period of 5 min to 48 h

Organ	Cisplatin (1 mg/kg)	Dendrimer-Pt (1 mg/kg)	Dendrimer-Pt (15 mg/kg)
Blood	9.5	10.7	502.0
Liver	51.6	17.0	193.2
Kidney	57.6	138.1	244.2
Tumor	5.3	25.4	264.8

(>40 000 Da) and thus not subject to rapid renal elimination, display prolonged blood circulation times leading to continued tumor accumulation as long as the blood concentrations are high. Unfortunately it is not possible to capitalize on the benefit afforded by high molecular weight using HPMA copolymers as they are not biodegradable in the main chain and therefore unsuitable for repeated clinical administration with the risk of prolonged tissue accumulation. Although here we used PAMAM dendrimer generation 3.5 of molecular weight 12 419 Da, the platinate formed probably contains intermolecular cross-links which result in a conjugate of increased molecular size (Figure 1). Optimal tumor targeting may therefore take place before degradation releases the active platinum species and liberates the parent dendrimer molecules that are small enough to be eliminated. Undoubtedly, dendrimer characteristics such as size, surface functionality and molecular flexibility will all influence the extent of tumor capture by the EPR effect, and further studies are underway to gain a better awareness of the factors important for engineering the dendrimer structure to maximize tumor capture. PAMAM dendrimer platinates have been prepared using generation 4.5 (26 247 Da) and 5.5 (50 865 Da) to allow systematic study.

Modification of the dendrimer surface may also provide the opportunity to introduce ligands that can facilitate receptor-mediated tumor targeting. Boronated PAMAM dendrimers have been linked to antitumor antibodies[28] and epidermal growth factor (EGF)[29] as a means to improved boron neutron tumor therapy. It is questionable whether receptor-mediated targeting can augment tumor drug concentration significantly better than the EPR effect. Using Fischer rats bearing a C6-EGF transfected glioma it was found that i.v. injection of ^{131}I-labeled boronated PAMAM generation 4 containing EGF resulted in relatively low levels of tumor localization: 0.01 and 0.006% dose/g at 24 and 48 h, respectively.[29] Concomitantly 5-12% dose/g of the radioactivity was localized in liver and spleen. EGF receptors in liver or inherent propensity of the amine surface-modified generation 4 dendrimer to localize there could be responsible. More than a decade of experience has shown that even the most promising immunoconjugates designed for the treatment of solid tumors[30] have failed to realize their potential clinically due to non-selective toxicity.[31] Greater understanding of whole body and cellular pharmacokinetics of naked and modified hyperbranched molecules is needed for optimal design of organ- and cell-specific structures, but these first experiments provide considerable optimism for future use of dendrimers as tumoritropic drug carriers.

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References

1. Connors TA. New anti-cancer agents. *Anti-Cancer Drugs* 1996; **7**: 179-85.
2. Duncan R. Drug-polymer conjugates: potential for improved chemotherapy. *Anti-Cancer Drugs* 1992; **3**: 175-210.
3. Duncan R, Dimitrijevic S, Evagororou EG. The role of polymer conjugates in the diagnosis and treatment of cancer. *STP Pharma Sci* 1996; **6**: 237-63.
4. Brocchini S, Duncan R. Polymer drug conjugates: drug release from pendent linkers. In: Mathiowitz E, ed. *Encyclopedia of controlled release*. New York: Wiley 1999: 787-816.
5. Vasey P, Twelves C, Kaye S, *et al.* Phase I clinical and pharmacokinetic study of PKI (HPMA copolymer doxorubicin): first member of a new class of chemotherapeutic agents: drug-polymer conjugates. *Clin Cancer Res* 1999; **5**: 83-94.
6. Caiolfa VR, Zamai M, Fiorino A, *et al.* Novel perspectives in topoisomerase-1 inhibitor-based chemotherapy of solid tumours: MAG-camptothecin conjugates. In: *Proc 9th Int Symp on Recent Advances in Drug Delivery Systems* 1999: 46-9.
7. Kerr D, Seymour L, Boivin C, *et al.* Phase I clinical trial of HPMA copolymers bearing doxorubicin and galactosamine. In: *Proc 3rd Int Symp on Polymer Therapeutics*, London 1998: 23.
8. Ten Bokkel Huniunk WW, Terwogt JM, Dubbelman R, *et al.* Phase I study of PNU166945, a polymer formulated paclitaxel. In: *Proc 3rd Int Symp on Polymer Therapeutics*, London 1998: 12.
9. Newkome GR, Moorefield CN, Vögtle F. *Dendritic molecules*. New York: VCH 1996: 1-261.
10. Roy R. Recent developments in the rational design of multivalent glycoconjugates. *Top Curr Chem* 1997; **187**: 241-74.

11. Jansen JFGA, de Brabander-van den Berg EMM, Meijer EW. Encapsulation of guest molecules into a dendritic box. *Science* 1994; **266**: 1226–9.
12. Malik N, Wiwattanapatapee R, Klopsch R, *et al.* Dendrimers: relationship between structure and biocompatibility *in vitro*, and preliminary studies on the biodistribution of ^{125}I-labelled PAMAM dendrimers *in vivo*. *J Cont Rel* 1999; in press.
13. Gordon M, Hollander S. Review of platinum anticancer compounds. *J Med* 1993; **24**: 209–65.
14. Eastmen A, Schulte N, Sheibani N, Sorenson CN. Mechanisms of resistance to platinum drugs. In: Nicolini M, ed. *Platinum and other metal coordination compounds in cancer chemotherapy*. Boston, MA: Nijhoff 1988: 178–217.
15. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy; mechanism of tumoritropic accumulation of proteins and the antitumour agent SMANCS. *Cancer Res* 1986; **6**: 6387–92.
16. Seymour LW, Ulbrich K, Styger PS, *et al.* Tumouritropism and anticancer efficacy of polymer-based doxorubicin prodrugs in the treatment of subcutaneous murine B16F10 melanoma. *Br J Cancer* 1994; **70**: 636–41.
17. Duncan R, Evagorou EG, Buckley RG, Gianasi E, Wasil M, Wilson G. Comparative evaluation of novel polymer platinates. I. Synthesis, characterisation and *in vitro* properties. In: *Proc Int Symp on Controlled Release of Bioactive Materials* 1997; **24**: 775–6.
18. Ferruti P, Bignotti F, Ranucci E, *et al.* Synthesis and antitumour activity of platinum(II) complexes of novel functionalised poly(amidoamine)s. *Macromol Chem Phys* 1999; in press.
19. Gianasi E, Wasil M, Evagorou EG, Keddle A, Wilson G. Duncan R. HPMA copolymer platinates as novel antitumour agents: *in vitro* properties, pharmacokinetics and antitumour activity. *Eur J Cancer* 1999; in press.
20. Duncan R, Sat YN. Tumour targeting by enhanced permeability and retention (EPR) effect. *Ann Oncol* 1998; **9** (suppl 2): 39.
21. Tomalia DA, Baker H, Dewald JR, *et al.* A new class of polymers—starburst-dendritic macromolecules. *Polym J* 1985; **17**: 117–32.
22. Schechter B, Pauzner R, Arnon R, Wilchek M. *Cis*-platinum(II) complexes of carboxymethyl-dextran as potential antitumour agents I. Preparation and characterization. *Cancer Biochem Biophys* 1986; **8**: 277–87.
23. Sgouras D, Duncan R. Methods for the evaluation of biocompatability of soluble synthetic polymers which have potential biomedical use: 1—Use of the tetrazolium-based colorimetric assay (MTT) as a preliminary screen for evaluation of *in vitro* cytotoxicity. *J Mater Sci: Mater Med* 1990; **1**: 67–8.
24. Rose WC, Schurig JE, Huftalen JB, Bradner WT. Antitumor activity and toxicity of cisplatin analogs. *Cancer Treat Rep* 1982; **66**: 135–46.
25. Muggia FM, Doxorubicin-polymer conjugates: further demonstration of the concept of enhanced permeability and retention. *Clin Cancer Res* 1999; **5**: 7–8.
26. Harrap KR. Discovery of improved platinum analogues. In: Workman P, ed. *New approaches in cancer pharmacology: drug design and development, vol II.* European School of Oncology Monographs. Berlin: Springer-Verlag 1994: 23–37.
27. Seymour LW, Miyamoto Y, Brereton M, *et al.* Influence of molecular size on passive tumour-accumulation of soluble macromolecular drug carriers. *Eur J Cancer* 1995; **5**: 766–70.
28. Barth RF, Adams DM, Soloway AH, Alam F, Darby MV. Boronated starburst-monoclonal antibody immunoconjugates: evaluation as a potential delivery system for neutron capture therapy. *Bioconj Chem* 1994; **5**: 58–66.
29. Yang WL, Barth RF, Adams DM, Soloway AH. Intratumoural delivery of boronated epidermal growth factor for neutron capture therapy of brain tumours. *Cancer Res* 1997; **57**: 4333–9.
30. Trail P, Willner D, Hellestrom KE. Site-directed delivery of anthracyclines for the treatment of cancer. *Drug Dev Res* 1995; **34**: 196–209.
31. Onetto N, LoBuglio A, Bookman M, *et al.* Tumour targeting therapy directed to the ley antigen. In: Pavlidis N, ed. *Proc EORTC Early Drug Development Meeting.* Greece: National Library of Greece 1995; **59**: 7.

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