

# Combination of Three Metals for the Treatment of Cancer: Gallium, Rhenium and Platinum. 1. Determination of the Optimal Schedule of Treatment

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**Abstract.** Platinum is well known for its anticancer activity, firstly used as cis-diaminedichloroplatinum (II) (CDDP), with a wide range of activity. Its main mechanism of action involves its binding to DNA. Gallium, another metal, has also demonstrated apoptotic effects on malignant cells, but through interaction with targets other than DNA, such as the membrane, cytoskeleton and proteasome, and on enzyme activities. An antitumor synergism between CDDP and both gallium and rhenium compounds has been demonstrated. For these reasons, we proposed to combine these three metals and to determine at which doses each compound could be administered without major toxicity. CDDP, tetrakis(1-octanol) tris(5-aminosalicylate)gallium(III), and a diseleno-ether rhenium(I) complex were used in this experimental study in breast cancer MCF-7 tumor-bearing mice. CDDP was administered intraperitoneally (*i.p.*) twice a week at the dose of 3 mg/kg. Tetrakis(1-octanol) tris(5-aminosalicylate) gallium (III) and rhenium(I) diseleno-ether complexes were administered orally, daily, five days a week for three weeks, at doses ranging from 20 to 100 mg/kg for the gallium compound

and from 10 to 50 mg/kg for the rhenium compound. Doses of 10 mg/kg of rhenium(I) diseleno-ether, and 100 mg/kg of the salicylate gallium compound, in combination with CDDP induced a significant decrease of 50% of the tumor volume, by comparison with the control group. In contrast, the decrease of the tumor volume in mice treated by CDDP alone was less than 25%. Changes in the sequence of administration of the three metals will be discussed to improve the therapeutic index.

Cis-diaminedichloroplatinum (CDDP) has been studied since 1968 for its powerful anticancer activity and is still a major agent for the treatment of lung and bladder cancer. In order to improve the solubility and anticancer activity of CDDP, further platinum (Pt) compounds have been used such as carboplatin and oxaliplatin. The study of DNA binding could inspire new drug design (1). The main mechanism of action of CDDP involves covalent binding with DNA, leading to the formation of adducts and cross links, and finally to apoptosis.

A steady interest in gallium (Ga) compounds is due to the proven ability of Ga cations to inhibit tumor growth, on the one hand, and enhanced bioavailability and efficacy provided by the conversion of Ga into chelate complexes, on the other. Ga was first studied as a nitrate salt and it was observed that a continuous venous infusion was better tolerated than a single intra-venous (*i.v.*) infusion, allowing the administration of higher doses without therapy limiting renal toxicity (2, 3).

It was noted that the inhibitory effects of Ga on malignant cells were not only dependent on the doses, but also on the duration of exposure (4, 5). Gallium chloride (GaCl<sub>3</sub>) has thus been proposed as a prolonged daily oral administration

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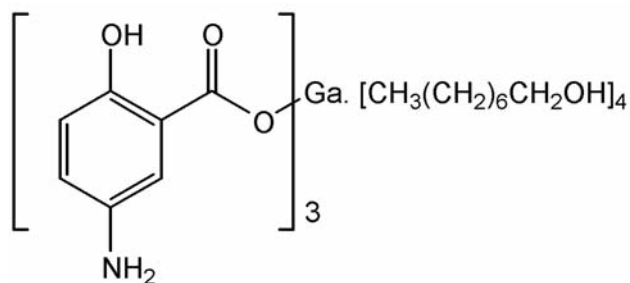


Figure 1. Structure of tetrakis(1-octanol) tris(5-aminosalicylate) gallium(III).

to favor selective uptake by malignant cells and to allow continuous exposure to the drug (6, 7). The mechanism of action of Ga does not seem to involve DNA (8), but seems to be more due to its effect on ribonucleotide reductase, with a competitive effect with iron (9-11). Many other biological effects of Ga have been observed on membrane permeability (12-14), the cytoskeleton (15), mitochondria (16), the activity of several enzymes involved in the development of cancer cells (9, 17-20) and proteasome activity (21). Ga induces the synthesis of collagen and fibronectin (22), which could explain the fibrosis of tumors observed after its prolonged administration (7, 23). Ga is implicated in intracellular oxidative stress, with a decrease in the ratio of cellular glutathion reduced form (GSH) on glutathion oxidized form (GSSG), an increase in metallothionein (MT) and in hemoxygenase-1 (HO-1) gene expression (24).

Pt and Ga can act synergistically as their mechanisms of action are different. This synergism has been observed in ovarian and colon carcinoma cells (25) and in cancer patients (26) with the combination of a Ga compound with Pt. On the other hand, it has been shown that Ga is able to potentiate other cytotoxic agents, such as paclitaxel and vinorelbine (27). Finally, Ga compounds have been demonstrated to be active against multicellular resistance in cancer cells (28). Organic Ga compounds have been also proposed, in particular, tris(8-quinolinolato) gallium(III) (29-32), gallium maltolate (33, 34) and gallium salicylate (35). Salicylates have anti-inflammatory, antitumor (36-43) and antiangiogenic properties (44, 45) as well as the capacity to inhibit tumor cell invasiveness (46). Salicylates can protect from CDDP toxicity (47, 48) and from irradiation toxicity (49), and increase chemosensitivity to anticancer drugs (50). Pt complexes of salicylate derivatives have also been proposed (51). The compound that we used for this study is tetrakis(1-octanol) tris(5-aminosalicylate) gallium(III).

Rhenium (Re) compounds present significant cytotoxicity towards breast cancer MCF-7 tumoral cells (52). When combined with rhenium, the biological effects of CDDP are improved (53). Rhenium adamantate, dichlorotetra- $\mu$ -

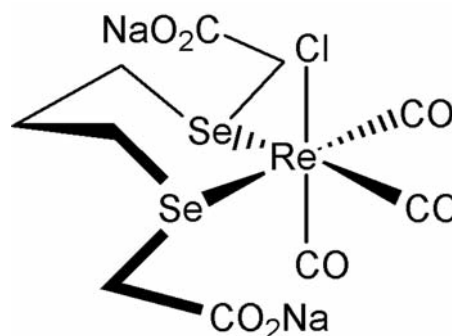


Figure 2. Structure of rhenium(I)diseleno-ether.

isobutytratodirhenium(III) and gamma-aminobutyric acid (GABA) rhenium(III), as liposomes, have antioxidant properties (54) and synergistic antitumor effects with CDDP, when administered as subcutaneous injections to Wistar rats bearing a Guerink carcinoma (55-57). Since most of these compounds are not soluble in water, use of a water-soluble rhenium(I) diseleno-ether complex has been proposed by Kermagoret *et al.* (58) and we used this compound in the current experimental study.

## Materials and Methods

*Synthesis and analytical structure of metal-based compounds.* Tetrakis(1-octanol) tris(5-aminosalicylate)gallium(III) was synthesised by Syntheval (Caen, France) with the cooperation of Abdelfattah Badawi.

*Tetrakis(1-octanol) tris(5-aminosalicylate)gallium(III)* (Figure 1): Gallium hydroxide (2 g, 16.6 mmol) was added to a solution of 5-aminosalicylic acid (7.6 g, 49.8 mmol) in 100 ml of anhydrous octanol and heated in an autoclave with stirring for 24 h at 150°C. After cooling, the brown solution was filtered. The filtrate was evaporated to dryness under vacuum and the residue was resuspended by shaking three times with 100 ml of diethyl ether and then dried. A purplish residue was obtained: mass=3.5 g, yield=20%.

The analytical structure was confirmed by IR ( $\text{cm}^{-1}$ ): 3530 (OH), 3136 (CH arom.), 2956, 2929 (CH Aliph.), 1680 (CO), 1600 (C=C arom.);  $^1\text{H}_1\text{NMR}$  (DMSO- $d_6$ ): 6.86 (1H, s, H5), 6.62 (1H, d, H4), 6.46 (1H, d, H2), 1.5 to 3.5 (56H, CH2), 0.8 (12H, t, CH3); MS:  $m/z$  526, 152, 130.

This compound is not soluble in water and was administered to mice in carboxymethylcellulose (CMC, 0.5%).

*Rhenium(I)diseleno-ether*  $\text{Re}(\text{CO})_3\text{Cl}(\text{NaO}_2\text{CCH}_2)_3\text{Se}(\text{CH}_2)_3\text{SeCH}_2\text{CO}_2\text{Na}$  (Figure 2): was synthesized by ligand exchange from pentacarbonylchlororhenium with 3,7-diselenanonanedioic acid followed, by disodium salt formation with sodium carbonate and characterized as reported earlier (58).

*Experimental design.* Human hormone-dependent breast cancer MCF-7 cells were used to induce experimental tumors in athymic nude mice. The experiment was performed at Cellvax Laboratory, Maison Alfort, France.

Table I. Doses of rhenium diselenate (Re complex) and of tetrakis(1-octanol) tris(5-aminosalicylate)gallium(III) (Ga complex) for each treated group.

	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10	Group 11
Re complex (mg/kg)	10	10	10	20	20	20	50	50	50
Ga complex (mg/kg)	20	40	100	20	40	100	20	40	100

**Animal husbandry.** The animals, athymic nu/nu mice (Balb/C nude), provided by Harlan, France, were 5 to 6 weeks old, female, of about 20 g each and specific and opportunistic pathogen free. They were acclimatized for at least seven days before the initiation of the designed study. A total of 110 mice were used for this pilot study.

Animals were housed in polyethylene cages (5/cage) measuring 36.5×20.7×14.0 cm, corresponding to a ground surface of 530 cm<sup>3</sup>, in a climate-and light-controlled environment. All animals were kept under environmentally controlled housing conditions: lights on between 7:00 AM to 7:00 PM; temperature inside of the animal facility strictly maintained at 21±1°C; relative humidity of 70% throughout the entire study period, and maintained in accordance with Cellvax approved standard operation procedures (SOP) and with local Ethical Committee approval. Animals were fed with commercially available rodent food (Safe, Les Tremblats, Augy, France). Water (sterilized water) was available *ad libitum*.

Animals were numbered and given a unique animal identification ear notch mark.

**Ethical manager.** A Ph.D. and Veterinary Doctor at Cellvax company assumed the function of 'Ethical Manager' within this project.

**Hormonal treatment.** All mice were treated prior to the study with estradiol sulfate (E 2S) hormone (Innovative Research of America, FL, USA). Pellets of hormone were subcutaneously administered to mice 72 h before tumor cell implantation.

**Tumor cell transplantation.** Human breast tumor MCF-7 cells, derived from the pleural effusion of mammary gland, of epithelial cell type reference number: ATCC#HTB-22YTM, obtained from the American Type Culture Collection (ATCC), Manassas, Virginia, USA were cultured with several passagings in order to ensure their viability and to reach the required number of cells. The log-growing tumor cells were then trypsinized, counted, washed and resuspended in ice-cold Matrigel (BD Biosciences, Le Pont-De-Claix, France) for injection into the right flanks of athymic nu/nu mice. With a cell viability of about 97%, 5.0×10<sup>6</sup> cells per mouse were subcutaneously (*s.c.*) injected.

**Experimental groups.** Eleven groups of 10 mice each for a total of 110 mice, were treated.

**Group 1:** negative control; mice were treated with vehicle control;  
**Group 2:** positive control; mice were treated with a standard chemotherapeutic agent, CDDP (Mylan, France) at a dose of 3 mg/kg, twice a week for three weeks, by intraperitoneal (*i.p.*) injection;

**Groups 3-11:** test groups; mice were treated with a combination of CDDP (same treatment regimen as in group 2) and the two metal complexes *per os* (*p.o.*), once a day from Monday to Friday, for a

period of three weeks (15 days). The doses of the Re complex and of the Ga complex differed according to the group, as shown in Table I.

The treatments were started when the average volume of the induced tumors reached approximately 70-100 mm<sup>3</sup>, corresponding to day 13 after the inoculation of the tumor cells.

**Toxicity evaluation.** Determination of body weight was performed twice a week for each mouse. The lethal toxicity was reported.

**Antitumor effect.** The tumor growth was measured (tumor length, width and volume) twice a week by using an external caliper. The mean tumor volumes [MTV; MTV + (SD); MTV + (SEM)] were estimated. The tumor growth data was recorded for each individually identified mouse. Tumor volume was calculated by using the following formula:  $V = \text{length} \times \text{width}^2 / 2$ .

Tumor growth inhibition (T/C%) was defined as the ratio of the mean tumor volumes of treated *versus* vehicle-treated (control) groups as a percentage.

Statistical evaluation of the antitumor effect was assessed by using a non parametric statistical analysis, the Wilcoxon signed-rank test, comparing two related groups.

## Results

**Efficacy.** The combination of CDDP, Ga and Re complexes induced a significant reduction ( $p=0.04$ , Wilcoxon test) of the volume of the tumors in group 5 (434±183 mm<sup>3</sup>) *versus* the control group (891±538 mm<sup>3</sup>) at day 32. The minimal T/C% was observed in this group 5 at day 32, with a decrease of more than 50% of the tumor volume, by comparison with the control group (T/C%=49). In comparison, T/C% was only 76% at day 32 for the CDDP treated group. In groups 3 and 4, the decrease of the tumor volume was similar to that of the CDDP group. In groups 6, 7, 8, 10 and 11, the reduction in tumor volume was not greater than in group 5 and there was an increase of the toxicity.

Results are presented in Table II showing the mean volume of the tumors, their SD and the SEM, at day 32 after the inoculation of the tumor cells for each group. The ratio of the tumor volume of the treated group to the tumor volume of the control is expressed as T/C%.

The SD observed for tumor volumes was very important in each group, with a great interindividual variability. In order to reach statistically significant results, showing a much greater reduction of the tumor volumes in mice treated

Table II. Volume of the tumors of the mice at day 32 after the inoculation of the tumor cells.

Group	Volume of the tumor (mm <sup>3</sup> )	SD	SEM	T/C (%)
1	891	538	170.26	
2	678	279	93.05	76.09
3	673	230	76.66	75.53
4	727	75	33.34	81.59
5	434	183	61.01	48.71
6	552	284	100.39	61.95
7	572	230	76.59	64.20
8	558	227	75.63	62.63
9	547	163	57.71	61.39
10	657	232	81.97	73.74
11	617	241	80.47	69.25

SD: Standard deviation; SEM: standard error of the mean; T/C%=mean tumor volume of treated group /mean tumor volume of vehicle-treated group ×100.

by Ga, Re and CDDP combined *versus* CDDP alone, a greater number of mice might be necessary.

Table III shows the mean weight of the mice of each group, (±SD), at day 32 after the inoculation of the tumor cells. The percentage of weight loss by comparison with the control group is also shown.

Figure 3 represents the tumor volumes in terms of days after the inoculation of the tumor cells.

**Toxicity.** There was one death in the control group at day 39; one death in the group treated with CDDP alone, observed at day 25; one death in group 3 at day 21; and one death in group 5 at day 28. Group 4 was considered an outlier and was excluded due to an abnormal unexplained great number of deaths. In groups 6 to 11, an increase of lethal toxicity was observed, with two deaths in groups 6, 8, 9 and 10, and five deaths in groups 7 and 11. In group 5, the loss of body weight was less than 6% in comparison with the control group.

Use of the maximal dose (100 mg/kg) of the Ga complex did not increase the toxicity by comparison with the group treated with CDDP alone (group 2), nor with the control group (group 1), when administered with 10 mg/kg of the Re complex. The same dose of the Ga complex with higher doses of the Re complex induced lethal toxicity.

Figure 4 shows the graph of the body weight of mice in terms of the days following the inoculation of the tumor cells.

We conclude from this study that the maximum tolerated doses were 10 mg/kg of Re complex in combination with 100 mg/kg of the Ga complex, when they are associated with CDDP at the dose used in this study. These doses induced a significant reduction of the tumor volume by comparison to the control groups, which was not observed in the CDDP-

Table III. Weights of the mice at day 32 after the inoculation of the tumor cells.

Groups	Weight of the mice (g)	SD	Loss of weight (%)
1	24.34	2.27	
2	22.23	3.78	8.7
3	22.00	2.07	9.6
4	19.99	2.63	17.9
5	22.98	1.62	5.6
6	22.3	2.16	8.4
7	22.84	2.41	6.2
8	21.18	2.09	13.0
9	22.58	2.72	7.2
10	20.6	3.18	15.4
11	23.11	1.3	5.1

SD: Standard deviation.

treated group. Higher doses of the Re complex did not improve the antitumour activity but did increase the toxicity.

**Discussion**

Doses of 10 mg/kg of the Re complex and 100 mg/kg of the Ga complex might be considered as the optimal therapy, but the therapeutic index could probably be improved by several approaches: the sequence of the treatment by each metal compound, the determination of the duration of each sequence of treatment, a pharmacological approach, and the choice for the ligand. The best indication for this schedule of treatment will also be discussed.

**Sequence of treatment.** In this study, the Ga and the Re complexes were given simultaneously orally and CDDP was administered twice a week during the same period. It might be more useful to administer the Ga complex first, then stop it and then only administer the CDDP treatment when gallium is selectively retained in cancer cells after a wash-out period. The time of the administration of the Re complex remains to be defined: simultaneously with the Ga complex or as a sequential administration, but in any cases also before the CDDP injection.

To propose this new sequence of treatment, we shall use the results obtained with other Ga and Re complexes.

**Rationale for a wash-out period between the administration of the Ga complex and the administration of CDDP.** The inhibitory effects of Ga are delayed and augmented after cessation of the exposure to Ga. Previous studies already showed that with GaCl<sub>3</sub>, the inhibitory effects were dependent not only on the dose but also on the duration of the exposure to the cancer cells and that this effect was

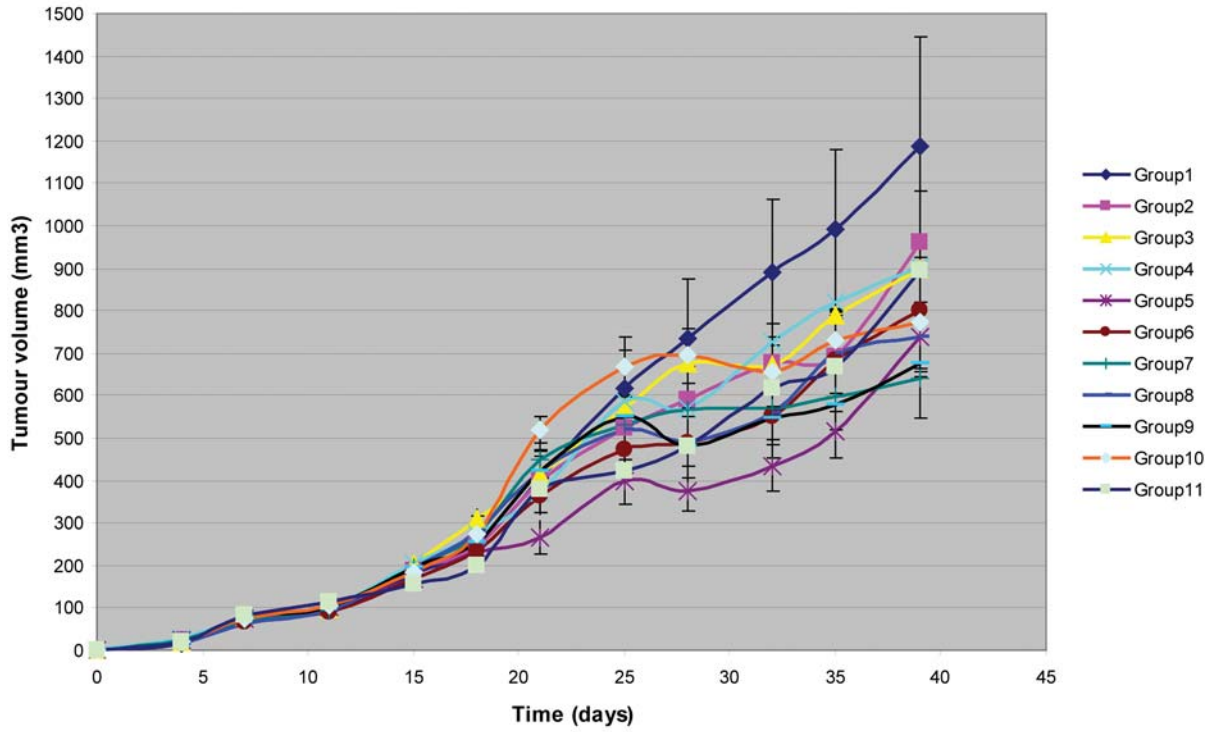


Figure 3. Tumor weight as a function of days after the inoculation of the tumor cells.

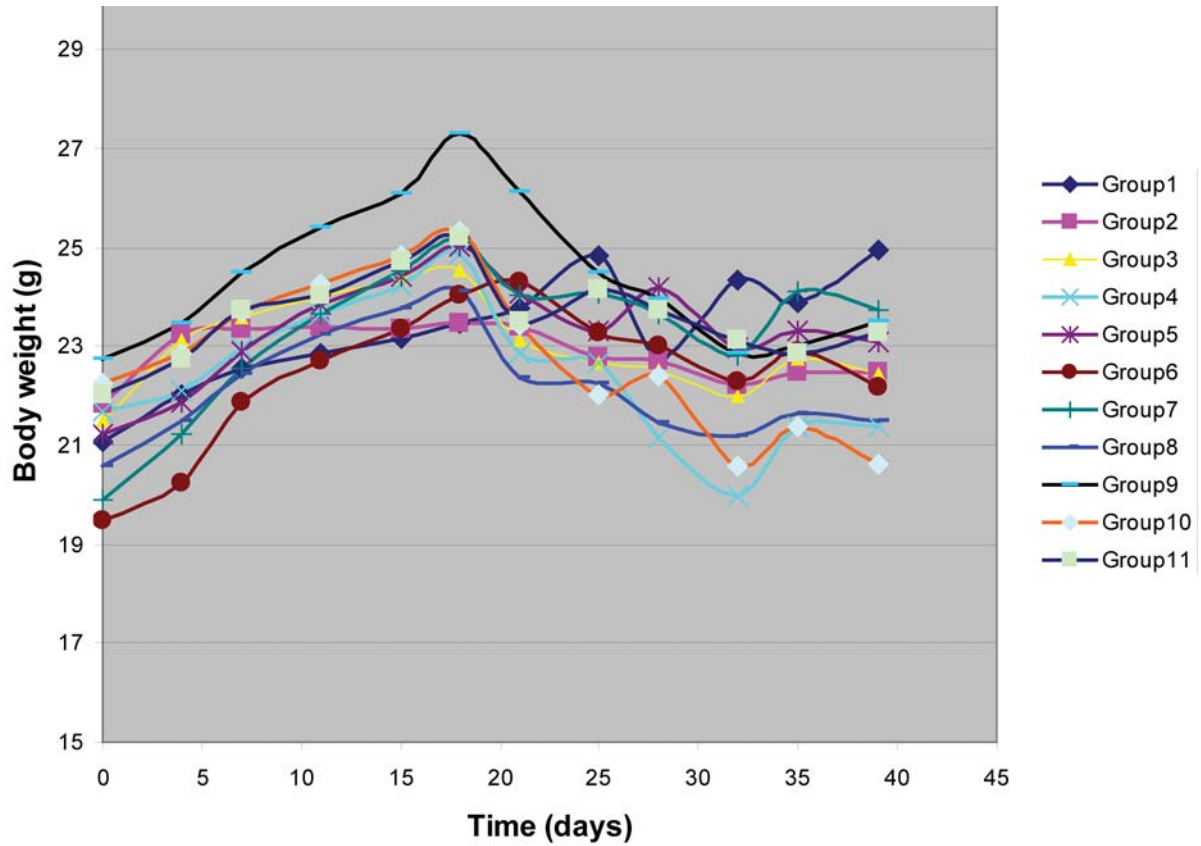


Figure 4. Representative the graph of the mice weights as a fonction of the day after the inoculation of the tumor cells.

delayed (59). This was studied with the malignant cell line U937, derived from lymphoma. The exposure time of malignant cells to GaCl<sub>3</sub> was 72 h, using doses of 50, 100 and 200 µM. After this exposure, the cells were washed, resuspended in culture medium without GaCl<sub>3</sub> and cell growth was observed for up to 76 h. Cell viability was measured by the method of Trypan blue exclusion every 24 h. The percentage of growth inhibition was determined relative to growth of control cells.

Only the concentration of 200 µM of GaCl<sub>3</sub> induced a growth inhibition of 50% of cells at the end of 72 h of exposure. However, inhibition of cell growth was prolonged even after cessation of cell exposure to GaCl<sub>3</sub>; this inhibition reached 80% at a concentration of 100 µM and was almost complete at 200 µM on day 6 after the end of exposure.

*Tissue retention of Ga after the interruption of Ga treatment.* It was observed that Ga was taken up by the tumor cells, with selective retention in the tumor and much more in metastases when the treatment was interrupted.

Tissue Ga concentrations were assayed after death in patients having received a prolonged treatment by GaCl<sub>3</sub>, but with cessation of the treatment before death.

Ga was assayed in two patients with lung cancer (60). One patient with squamous cell carcinoma, who died of cerebral, hepatic and kidney metastases, received GaCl<sub>3</sub> for a period of six months (maximum dose of 600 mg/24 h). In this patient, the Ga concentration was 5.5 µg/g (wet tissue) in the primary tumor (with large necrosis) *versus* 6.5 µg/g in the healthy pulmonary tissue; 46.3 µg/g in the liver metastasis *versus* 18.5 µg/g in the healthy liver; and 12.7 µg/g in a kidney metastasis *versus* 1.7 µg/g in the healthy kidney. The second patient had adenocarcinoma and died of adenopathies, pleural, hepatic and spleen metastases after eight months of treatment by GaCl<sub>3</sub> (maximum dose of 1200 mg/24 h). The Ga concentration was 1.5 µg/g in the primary tumor *versus* 2.8 µg/g in the lung; 13.3 µg/g in a malignant adenopathy *versus* 4.2 µg/g in a healthy node; 12.4 µg/g in a liver metastasis (also with a large necrosis and fibrosis) *versus* 25.2 µg/g in the healthy liver; 3.3 µg in a metastasis of the adrenal; 2.6 µg/g in a pleural metastasis, 2.1 µg in a metastasis of the pericardium; 43.5 µg/g in the spleen, and 2.9 µg in the kidney.

Tissue Ga concentrations were also assayed after death in two other patients (61).

The first one had a lymphoma of the oesophagus and received 600 mg per day of GaCl<sub>3</sub> for three months: the Ga concentration was 6.7 µg/g (0.096 µmol/g) inside the tumor, 1.95 µg/g (0.028 µmol/g) in the healthy tissue of the same organ and 2.0 µg/g (0.029 µmol/g) in the kidney. In the second patient, who was treated for more than 2 years, the treatment with GaCl<sub>3</sub> was stopped two months before death and the concentration of Ga was 7.8 µg/g (0.112 µmol/g) in the lung tumor (adenocarcinoma), in a metastatic brain tumor

4.8 µg/g (0.069 µmol/g), and in a metastatic tumor of the kidney 10.1 µg/g (0.145 µmol/g). In the healthy tissue of all these organs of this patient, the concentration was less than 1.0 µg/g (0.014 µmol/g) (wet tissue).

If Ga is mainly localized in cancer cells, and moreover after a period of interruption of the Ga treatment, this should increase the cytotoxicity of CDDP only in these cells, not in healthy cells. The effects should even be more important in metastatic situations than in primary tumors, with higher concentrations in metastases than in the primary tumor.

*The importance of the sequence of administration of Ga was demonstrated in an experiment with paclitaxel.* It was proven that synergism can occur *in vitro* between Ga and paclitaxel in a study comparing different modalities of exposure to Ga nitrate to paclitaxel; Ga nitrate had to be administered before the exposure to paclitaxel, and not simultaneously, in order to obtain a synergistic effect (62).

*Sequence of administration of the Ga and the Re complexes.* Re compounds have been proposed to protect the membrane of red blood cells from the toxicity of CDDP thanks to their antioxidant properties (54), and avoidance of CDDP-induced anemia (55). It was further demonstrated that rhenium compounds can increase the antitumor effects of CDDP (53, 56, 57), without increasing its toxicity, while a protective effect on CDDP-induced erythrocyte damage was confirmed.

Ga-induced anemia may be due to another mechanism of action *via* its effect on ribonucleotide reductase (9, 63, 64). Therefore, Re compounds may not protect against this decrease of hemoglobin production. Interactions between Ga and Re compounds are not known and more pharmacological and toxicological data with both Re diselenoether and Ga salicylate are necessary. Such data will indicate if Re compounds increase the toxicity of Ga compounds and if these two metals have to be administered concomitantly or as sequential treatments.

*Choice of CDDP dose to be administered after Ga and Re treatments.* It might be better to administer CDDP as a single injection, after Ga exposure, but also at the end of the Re treatment and not simultaneously. In that case, the choice of the CDDP dose should be in the range of 5-10 mg/kg, at which antitumor activity and toxicity are acceptable (65-67). The dose of 8 mg/kg of CDDP was used by Shtemenko and Coltery when they demonstrated synergism between CDDP and several Re compounds (53, 56, 57).

*Duration of each sequence of treatment.* The duration of the Ga treatment, in order to obtain an antitumor effect could be a more important parameter than the dose. Concentrations of Ga were assayed in healthy rats receiving an oral administration of GaCl<sub>3</sub> of 200 mg/kg per day for 20 and 30 days (68).

In the rats receiving oral 200 mg/kg/24 h of GaCl<sub>3</sub> for 20 days, the highest concentrations of Ga were found in the bone (29.9±5.7 mg/g) and then in the lungs (11.5±15.3 mg/g). Concentrations were comparable in kidneys (5.9±1.4 mg/g), spleen (5.8±2.5 mg/g), adrenal glands (4.6±2 µg/g), liver (4.3±2.3 mg/g) and ovary (2.9±1.3 mg/g). Concentrations were much lower in muscle (1.4±1.5 mg/g), heart (0.6±0.3 mg/g) and brain (0.3±0.5 mg/g). Plasma concentrations reached values as high as 1 mg/L (68).

Ga concentrations increased with the duration of administration of GaCl<sub>3</sub> for 30 days in the bones, spleen, adrenals and heart, while the plasma concentrations of Ga remained steady, indicating tissue accumulation of Ga.

Ga tissue retention was noted after stopping the administration of GaCl<sub>3</sub>. After a period of five days without treatment, Ga concentrations decreased significantly in plasma and kidney, but did not vary in other tissues, indicating tissue retention of Ga. Retention of Ga in the bone, liver and spleen was observed, while it was rapidly cleared from plasma and kidney.

Increasing of the dose of GaCl<sub>3</sub> was deleterious concerning the selective uptake of Ga by the tumor (69). After an administration of 200 mg/kg GaCl<sub>3</sub> per day for 30 days, the Ga concentrations in mice bearing C3H-BA carcinoma were of 2.16±0.93 µg/g in the tumor and 2.03±0.80 µg/g in the kidney. Higher doses of 400 mg/kg GaCl<sub>3</sub> per day increased the Ga concentrations in the kidney (3.27±0.88 µg/g) much more than in the tumor (2.60±0.88 µg/g).

*Biological effects depend on the duration of the Ga treatment.* With the oral dose of 200 mg/kg GaCl<sub>3</sub> per day, the tumor Ga concentrations depended on the duration of the treatment: 38.4±30 nmol/g after 21 days of administration and 13.4±7.3 nmol/g after 42 days of oral administration. To interpret these results, it is necessary to consider that the GaCl<sub>3</sub> treatment induced fibrosis inside the tumors after 42 days of treatment, which was not observed after 21 days (23). This tumor fibrosis was also observed in female dogs with spontaneous mammary carcinoma after a prolonged treatment of GaCl<sub>3</sub> (7).

*Rhenium.* There is no study comparing the effects of Re compounds with different durations of treatment. In studies demonstrating the synergistic effects of Re compounds with CDDP, the duration of the treatment with the Re compound was of 18 days (53, 56, 57), but as subcutaneous injections every two days and not as an oral administration. Further studies are required with the Re diselenoether compound, either after oral or parenteral administrations.

*CDDP.* CDDP is usually administered every 21 days or 28 days in patients.

*Adaptation of the doses according to pharmacological data.* The synergism between CDDP and Ga was observed in lung cancer patients receiving either CDDP-etoposide or CDDP-etoposide and an oral daily administration of 400 mg/kg of GaCl<sub>3</sub>. In this study, the cohort was small but very significant: the four patients receiving GaCl<sub>3</sub> had a major response of more than 50%, while the four patients without GaCl<sub>3</sub> experienced disease progression (26). This effect was observed after three cycles of chemotherapy with CDDP-etoposide. However, severe toxicity appeared during the subsequent cycles in patients receiving GaCl<sub>3</sub>, thus requiring an interruption of the treatment.

To avoid this cumulative toxicity which could mainly be due to CDDP, an adaptation of the dose of CDDP has been proposed to maintain the same area under the curve (AUC) of the plasma Pt concentrations during the five days of each continuous platinum infusion (AUC<sub>0-120</sub>). It is possible to maintain an AUC<sub>0-120</sub> between 80 000 and 100 000 µg/(l h), thanks to an adaptation of the dose of CDDP at 72 hours of each infusion as a function of the Pt concentrations observed during the first 48 h (70-72).

In these conditions, the treatment with CDDP with a targeted AUC<sub>0-120</sub> of 80 000 µg/(lh), etoposide and a daily oral administration of 400 mg/kg GaCl<sub>3</sub> was administered to nine patients with a non-small cell lung cancer (NSCLC) and to three patients with small cell lung cancer (SCLC). An objective response was observed in five of the NSCLC patients and in the three SCLC patients after three cycles of the combined therapy. In six responders, three additional courses were given without major toxicity, allowing for a much more important decrease in the tumor volume in four of them. The maximal plasma Ga concentrations were statistically higher in the responders (244±34 µg/l in NSCLC; 243±132 µg/l in SCLC) than in the non-responders (112±57 µg/l).

However, if the dose of GaCl<sub>3</sub> was raised to 800 mg/kg and to 1200 mg/kg, there was no benefit but only the appearance of signs of toxicity (70-72).

The results of a multicentric French clinical trial with 1200 mg/kg GaCl<sub>3</sub> and CDDP without adaptation of the doses, completely failed to demonstrate any benefit in lung cancer patients (73).

When administered concomitantly, the optimal schedule of treatment should then be an adaptation of CDDP with a targeted AUC and an oral dose of GaCl<sub>3</sub> of 400 mg/24 h, allowing for objective responses to be obtained and for this therapy to be maintained without cumulative toxicity.

In a pilot study, 30 cancer patients were treated with GaCl<sub>3</sub>. The dose was gradually increased from 300 to 800 mg/day (76). The median time of treatment was 4.5 months. Serum Ga concentrations were assayed by atomic absorption spectrophotometry (AAS) in 10 patients. A relationship was observed between the serum Ga concentrations and the clinical

response: the mean value was  $415 \pm 167$   $\mu\text{g/l}$  in five patients with progressive disease versus  $771 \pm 205$   $\mu\text{g/l}$  for 5 others with a non-evolutive disease. All patients were evaluable for toxicity: no renal toxicity was observed even in patients treated daily for more than one year with 800 mg  $\text{GaCl}_3$ . Microcytosis was common, as well as a Mg deficiency, with a more frequent decrease in red blood cells (RBC) than in plasma. Twenty-three patients were evaluable for efficiency. Two partial responses were observed, one in a patient previously treated for locoregional recurrence of an ovarian adenocarcinoma and another in a case treated for a peritoneal carcinosis of an ovarian adenocarcinoma. According to this study,  $\text{GaCl}_3$  dose should be adjusted to obtain serum Ga levels higher than 600  $\mu\text{g/l}$ .

This serum Ga concentration was not achievable in a pharmacoclinical trial in 45 lung cancer patients. Doses of 400 mg/kg per day of  $\text{GaCl}_3$  were determined as the optimal dose to be delivered to lung cancer patients. With this dose, the plasma Ga concentration at the steady state was of  $371 \pm 142$   $\mu\text{g/l}$  (60). Higher doses of  $\text{GaCl}_3$  did not significantly increase the plasma Ga concentration, but a very high interindividual variability was observed.

Plasma Ga concentrations depend on the tumor mass (74), the plasma transferrin concentration, the presence or not of metastases (75), the type of the primary tumor and the type of metastases (76), but also on the Ga ligand (30, 33). It does not appear possible to manage plasma Ga concentration by monitoring the dose of the  $\text{GaCl}_3$  treatment, but rather to use the plasma Ga concentration as a marker of the efficacy of therapy.

*Choice of the ligand. Gallium:* Several type of Ga complexes have been proposed; *tris*(8-quinolinolato)gallium(III), gallium maltolate and gallium salicylate, with the objective of increasing the bioavailability of Ga.

*Tris(8-quinolinolato)gallium(III):* Tris(8-quinolinolato)gallium(III) is lipophilic, with a higher bioavailability than  $\text{GaCl}_3$ , with a high uptake by bone (30). It was ten fold more effective against cancer cells in culture than was  $\text{GaCl}_3$  (77). With doses of 48 mg/kg, a significant decrease of the tumor volume of Walker 256 carcinosarcoma in Fisher rats was observed after 10 days of an oral administration by comparison with the control. The maximum dose inducing no lethal toxicity was 62.5 mg/kg after daily oral administration for 14 days (30). The therapeutic index thus seems low, probably due to a poor selective uptake by the tumor cells (78). A phase I clinical trial has been performed (31) but no further phase II trial has yet been conducted.

*Gallium maltolate:* Ga maltolate was proposed by Bernstein *et al.* in 2000 due to better bioavailability than gallium nitrate (33), and the ability to circumvent lymphoma cell resistance to gallium nitrate (79). It has been mainly proposed for the

treatment of hepatocellular carcinoma (34), and for lymphoma in combination with bortezomib (80). Pharmacokinetic studies have been performed (81, 82), but the therapeutic index of this compound has not yet been defined.

*Gallium salicylate:* Gallium salicylate has the advantage of combining Ga and an active ligand, salicylate. The growth arrest induced by salicylate is associated with its action on signaling factors such as mitogen activated protein kinases (83, 84), *cmyc*, cyclin D1, cyclin A, proliferating cell nuclear antigen (37), p21 *WAF-1/CIP1* (36, 39), (ERK1/2) (40), nuclear factor kappaB (85), (TNF) alpha (41) and (ICAM-1) (42). Salicylate compounds inhibit angiogenesis (44, 45, 86), cell migration and invasion (42, 46), increase chemosensitivity to anticancer drugs (50), modulate the production of reactive oxygen species (40, 87, 88), and reduce the side-effects of CDDP (47, 48). Copper salicylate (49, 89), platinum (II) complexes of salicylate derivatives (51), and gallium ethanol salicylates (35) have been synthesized.

In this study we proposed the use of a 4-aminosalicylic acid Ga salt, yet many studies are still required with this new compound to determine its bioviability and its toxicity. New salicylate Ga compounds could also be investigated, with the aim of improving the solubility.

*Rhenium compounds.* Shtemenko *et al.* showed that Re compounds had a synergistic effect with CDDP (53, 56, 57). This was observed after 18 days of treatment with three rhenium compounds, administered as liposomes, at a dose of 7  $\mu\text{mol/kg}$ , after a subcutaneous injection every two days, in Wistar rats bearing Guerink carcinoma. These three Re compounds were rhenium adamantate, dichlorotetra- $\mu$ -isobutyratodirhenium(III) and (GABA) rhenium (III). The weight of the tumors were  $44.87 \pm 25.19$  g in controls. There was a decrease, but not statistically significant after the administration of the Re compounds alone:  $38.27 \pm 16.77$  g, with rhenium adamantate;  $36.88 \pm 15.60$  g with dichlorotetra- $\mu$ -isobutyratodirhenium(III);  $25.30 \pm 5.30$  g with GABA rhenium (III). In rats treated with CDDP alone, the weight of the tumor was significantly reduced ( $9.88 \pm 9.90$  g), compared to controls. The combination of CDDP with the Re compounds was superior to the effect of CDDP alone, with a significant decrease of the tumor volume:  $3.92 \pm 2.55$  g with CDDP plus rhenium adamantate;  $0.28 \pm 0.30$  g with CDDP plus dichlorotetra- $\mu$ -isobutyratodirhenium(III);  $5.90 \pm 1.20$  g with CDDP plus GABA rhenium (III).

A folate conjugate of Re(I) was screened against an adriamycin- and cisplatin-resistant human ovarian cancer cell line (A2780/AD) that overexpresses the folate receptor (FR). This Re compound was found to be cytotoxic toward the FR-positive cell line (90).

Organometallic Re thymidine complexes  $[\text{Re}(\text{CO})_3]^+$  and  $[\text{Re}(\text{CO})_2(\text{NO})]_2^+$  have been synthesized with the aim of



targeting human cytosolic thymidine kinase (hTK1), a key enzyme in cancer cell proliferation (91, 92). Their cytotoxicity was assessed against the A549 lung carcinoma cell line. Moderate toxicity was observed for conjugates carrying the rhenium moiety at position C5' or N3. No toxicity was observed for complexes modified at C2' or C5. Complex 53, with a dodecylene spacer at C5', exhibited remarkable toxicity and was more potent than cisplatin. In competitive inhibition experiments with A549 cell lysates and purified recombinant hTK-1, enzyme inhibition was observed for complexes modified at either N3 or C5' (93).

A series of five long chain Re glucosamine conjugates were tested as substrates of hexokinase: two of them were found to be competent inhibitors of hexokinase but cytotoxicity studies found that they were non toxic to concentrations as high as 1 mM (94).

Octahedral Re cluster compounds have been synthesized but did not exhibit acute cytotoxic effects up to 50  $\mu$ M (95).

A series of mononuclear Re (IV) compounds displayed potent *in vitro* antiproliferative activity against selected cancer cells (96). Re (V) mixed ligand oxorhenium complexes have been shown to be inhibitors of cysteine proteases (97).

Oximine Re (I) complexes were assessed *in vitro* in human metastatic melanoma A375 and human chronic myelogenous leukemia K562 cells. Chloride complexes were more efficient than bromide compounds in inducing apoptotic cell death in both types of cancer cell (98).

The rhenium(I) diseleno-ether that we chose has the advantage of being soluble in water. We found that the recommended doses of rhenium(I) diseleno-ether were 10 mg/kg with a daily oral administration of five days a week for three weeks, when administered with Ga and Pt.

*Choice of the best indication for treatment: bone metastases of solid tumors.* The action of Ga on bone metabolism is particularly important (27). The action of Ga on bone metabolism was studied primarily because it reduces the hypercalcemia associated with cancer. Ga inhibits osteoclastic activity. In addition, there is an increase of collagen synthesis related to the bone concentration of Ga and an increase of bone tissue formation *in vitro*. It was also noted that Ga nitrate was able to increase type I collagen, fibronectin mRNA and collagen protein levels in bone and fibroblast cells. There is a high affinity of Ga for bones, as shown with GaCl<sub>3</sub> (68) and with *tris* quinolinolato gallium (III) (30).

For all these reasons, the best indication for Ga treatment might be bone metastases of solid tumors, mainly from breast, lung and prostatic cancer.

Re as <sup>186</sup>Re-hydroxyethylidene diphosphate (HEDP) and <sup>188</sup>Re-dimercaptosuccinic acid (99) are effective in the treatment of bone tumors (100-103). An animal model of bone metastases of prostate cancer demonstrated a selective uptake of <sup>188</sup>Re-HEDP by bone metastases (104).

Patients with bone metastases could thus represent a good indication for therapy with Ga and Re. The combination of Pt with Ga and Re could be proposed for lung cancer patients, while for patients with prostatic or breast cancer, an alternative could be proposed, replacing platinum with a taxane. In patients with breast metastatic cancer, platinum could be used but only in the case of triple-negative cancer (105, 106).

## Conclusion

The oral administration of Ga and Re compounds allows for continuous cell exposure to these metals. The optimal doses of 100 mg/kg of gallium salicylate octanol 1 and 10 mg/kg of rhenium (I) diselenoether have been shown to be active in combination with CDDP, without increasing toxicity by comparison with controls.

The schedule of administration of these two metal-based anticancer drugs could be improved, either with CDDP or with other chemotherapeutic agents.

We propose to determine the period of interruption of the Ga treatment before the administration of CDDP, or of an other chemotherapeutic agent, in order to allow the elimination of Ga from the healthy cells and its selective retention in the cancer cells. Under these conditions, there will be no cumulative toxicity between Ga and CDDP or the other chemotherapeutic agent in healthy cells, but only a synergistic effect against the tumor cells.

Concerning the administration of the Re compound, it will be necessary to define if its administration should be concomitant with the Ga compound, or sequential. Additional pharmacological and toxicological data will be necessary to achieve this objective.

A long period of administration for this combined therapy by Ga and Re is required to take into account the delayed antitumor effects, as well as the induction of tumor fibrosis, and therefore to improve the survival time.

The best indication for the Ga and Re treatments should be bone metastases, either combined with Pt for patients with lung cancer, or with other chemotherapeutic agents, such as a taxane, in the case of metastases from breast or prostatic cancer.

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## References

- 1 Roy S, Westmaas JA, Hagen KD, van Wezel GP and Reedijk J: Platinum(II) compounds with chelating ligands based on pyridine and pyrimidine: DNA and protein binding studies. *J Inorg Biochem* 103: 1288-1297, 2009.
- 2 Leyland-Jones B, Bhalla RB, Farag F, Williams L, Coonley CJ and Warrell RP: Administration of gallium nitrate by continuous infusion: lack of chronic nephrotoxicity confirmed by studies of enzymuria and  $\beta$ 2 microglobulinuria. *Cancer Treat Rep* 67: 941-942, 1983.
- 3 Warrell RP, Coonley CJ, Strauss DJ and Young CW: Treatment of patients with advanced malignant lymphoma using gallium nitrate administered as a seven-day continuous infusion. *Cancer* 51: 1982-1987, 1983.
- 4 Rasey JS, Nelson NJ and Larson SM: Relationship of iron metabolism to tumor cell toxicity of stable gallium salts. *Int. J. Nucl Med Biol* 8: 303-313, 1981.
- 5 Rasey JS, Nelson NJ and Larson SM: Tumor cell toxicity of stable gallium nitrate: enhancement by transferrin and protection by iron. *Eur J Cancer Clin Oncol* 18, 661-668, 1982.
- 6 Collery P, Millart H, Simoneau JP, Pluot M, Halpern S, Vistelle R, Lamiable D, Choisy H, Coudoux P and Etienne JC: Selective uptake of gallium administered orally, as chloride, by tumor cells. *New Concepts In Cancer Chemotherapy*. Spitz KH, Karrer K (eds.). Proc 13th International Congress of Chemotherapy, Vienna part 284: PS 12-11, 35-43, 1983.
- 7 Collery P, Millart H, Simoneau JP, Pluot M, Halpern S, Pechery C, Choisy H and Etienne JC: Experimental treatment of mammary carcinomas by gallium chloride after oral administration: intratumor dosages of gallium, anatomopathologic study and intracellular micro-analysis. *Trace Elem Med* 1: 159-161, 1984.
- 8 Tajmir-Rihai HA, Naoui M and Ahmad RA: comparative study of calf-thymus DNA binding trivalent Al, Ga, Cr and Fe ions in aqueous solution. *Metal Ions in Biology and Medicine*. Anastassopoulou J, Collery P, Etienne JC, Theophanides T (eds.). John Libbey Eurotext, Paris 2: 98-101, 1992.
- 9 Chitambar CR, Matthaeus WG, Antholine WE, Graff K and O'Brien WJ: Inhibition of leukemic HL60 cell growth by transferrin-gallium: effects on ribonucleotide reductase and demonstration of drug synergy with hydroxyurea. *Blood* 72: 1930-1936, 1988.
- 10 Chitambar CR, Narasimhan J, Guy J, Sem DS and O'Brien WJ: Inhibition of ribonucleotide reductase by gallium in murine leukemic L1210 cells. *Cancer Res* 51: 6199-6201, 1991.
- 11 Narasimhan J, Antholine WE and Chitambar CR: Effect of gallium on the tyrosyl radical of the iron-dependent M2 subunit of ribonucleotide reductase. *Biochem Pharmacol* 44: 2403-2408, 1992.
- 12 Bara A, Guet-Bara A, Durlach J and Collery P: Comparative effects of a carcinogenic (As) and an anticancer (Ga) metals on the transfer through the human amnion. Relationship with Mg. Ultrastructural and electrophysiological studies. *Metal Ions in Biology and Medicine*. Collery P, Poirier LA, Manfait M, Etienne JC (eds.). John Libbey Eurotext, Paris 1: 570-572, 1990.
- 13 Bara M, Guet-Bara A, Collery P and Durlach J: Gallium action on the ionic transfer through the isolated human amnion. I. Effect on the amnion as a whole and interaction between gallium and magnesium: *Trace Elem Med* 2: 99-102, 1985.
- 14 Bara M, Guet-Bara A, Collery P and Durlach J: Gallium action on the ionic transfer through the isolated human amnion. II. Effect on cellular and paracellular pathways. *Trace Elem Med* 9: 117-122, 1992.
- 15 Perchellet EM, Ladesich JB, Collery P and Perchellet JP: Microtubule-disrupting effects of gallium chloride *in vitro*. *Anticancer Drugs* 10: 477-488, 1999.
- 16 Gogvadze V, Zhukova A, Ivanov A, Khassanova L, Khassanova Z and Collery P: The effect of gallium on the calcium retention capacity of rat liver mitochondria. *Metal Ions in Biology and Medicine*, Collery P, Corbella J, Domingo JL, Etienne JC, Llobet JM (eds.). John Libbey Eurotext, Paris 4: 249-252, 1996.
- 17 Anghileri LJ and Robert J: Isomorphous ionic replacement: experimental evidence for the hypothesis proposed to explain Gallium-67 accumulation. *J Nucl Med All Sc* 26: 113-115, 1982.
- 18 Berggren MM, Burns LA, Abraham RT and Powis G: Inhibition of protein tyrosine phosphatase by the antitumor agent gallium nitrate. *Cancer Res* 53: 1862-1866, 1993.
- 19 Farzami B, Pournaki A, Collery P and Goliaei B: Effect of Gallium on the growth rate of U937 cell culture and the activity of tyrosine kinase. *Metal Ions in Biology and Medicine*, Khassanova LK, Collery P, Maynard I, Khassanova Z, Etienne JC (eds.). John Libbey Eurotext, Paris 7: 625-628, 2002.
- 20 Panagakos FS, Kumar E, Venescar C and Guidon P: The effect of gallium nitrate on synoviocyte MMP activity. *Biochimie* 82: 147-151, 2000.
- 21 Chen D, Frezza M, Shakya R, Cui QC, Milacic V, Verani CN and Dou QP: Inhibition of the proteasome activity by gallium(III) complexes contributes to their anti prostate tumor effects. *Cancer Res* 67: 9258-9265, 2007.
- 22 Bockman RS, Guidon PT, Pan LC, Salvatori R and Kawaguchi A: Gallium nitrate increases type I collagen and fibronectin mRNA and collagen protein levels in bone and fibroblast cells. *J Cell Biochem* 52: 396-403, 1993.
- 23 Collery P, Millart H, Pluot M and Anghileri LJ: Effects of gallium chloride oral administration on transplanted C3HBA mammary adenocarcinoma: Ga, Mg, Ca and Fe concentration and anatomopathological characteristics. *Anticancer Res* 6: 1085-1087, 1986.
- 24 Yang M and Chitambar CR: Role of oxidative stress in the induction of metallothionein-2A and heme oxygenase-1 gene expression by the antineoplastic agent gallium nitrate in human lymphoma cells. *Free Radic Biol Med* 45: 763-772, 2008.
- 25 Jakupec MA, Collery P and Keppler B: Synergistic antiproliferative effects of *tris*(8-quinolinolato) gallium(III) in combination with platinum drugs in ovarian and colon carcinoma cells. *Metal Ions in Biology and Medicine*. Collery P, Maynard I, Theophanides T, Khassanova L, Collery T, (eds.). John Libbey Eurotext, Paris 10: 110-115, 2008.
- 26 Collery P, Morel M, Millart H, Desoize B, Cossart C, Perdu D, Vallerand H, Bouana JC, Pechery C, Etienne JC, Choisy H and Dubois de Montreynaud JM: Oral administration of gallium in conjunction with platinum in lung cancer treatment. *Metal Ions in Biology and Medicine*. Collery P, Poirier LA, Manfait M, Etienne JC (eds.). John Libbey Eurotext, Paris 1: 437-442, 1990.
- 27 Collery P, Keppler B, Madoulet C and Desoize B: Gallium in cancer treatment. *Crit Rev Oncol Hematol* 42: 283-296, 2002.

- 28 Desoize B, Collery P, Akeli MG, Etienne JC and Keppler B: Tris(8-quinolinolato) Ga(III) is active against unicellular and multicellular resistance. *Metal Ions in Biology and Medicine*. Centeno J, Collery P, Vernet G, Finkelman RB, Gibb H, Etienne JC (eds.). John Libbey Eurotext, Paris 6: 573-576, 2000.
- 29 Collery P, Lechenault F, Cazabat A, Juvin E, Khassanova L, Evangelou A and Keppler B: Inhibitory effects of gallium chloride and tris(8-quinolinolato) gallium(III) on the proliferation of A 549 malignant cell lines. *Anticancer Res* 20: 955-958, 2000.
- 30 Collery P, Domingo JL and Keppler BK: Preclinical toxicology and gallium distribution of a new antitumor gallium compound: tris(8-quinolinolato) gallium (III). *Anticancer Res* 16: 687-692, 1996.
- 31 Collery P, Jakupec MA, Kynast B and Keppler BK: Preclinical and early clinical development of the oral Gallium complex KP 46 (FFC11). *Metal Ions in Biology and Medicine*. Alpoim MC, Morais PV, Santos MA, Cristovao A, Centeno JA, Collery P (eds.). John Libbey Eurotext, Paris 9: 521-524, 2006.
- 32 Valiahdi SM, Jakupec MA, Marculescu R and Keppler BK: Tris(8-quinolinolato)gallium(III) exerts strong antiproliferative effects in melanoma cells. Alpoim M, Morais PV, Santos MA, Cristovao AJ, Centeno JA, Collery P (eds.). John Libbey Eurotext, Paris 9: 282-286, 2006.
- 33 Bernstein LR, Tanner T, Godfrey C and Noll B: Chemistry and pharmacokinetics of gallium maltolate, a compound with high oral gallium bioavailability. *Metal Based Drugs* 7: 33-47, 2000.
- 34 Chua MS, Bernstein LR, Li R and So SK: Gallium maltolate is a promising chemotherapeutic agent for the treatment of hepatocellular carcinoma. *Anticancer Res* 26: 1739-1743, 2006.
- 35 Ismail DA, Badawi AM, Collery P, Zakhary NI and Raouf AR: Antitumor effects of gallium(III) tris(salicylate)-ethanol on malignant cell lines and tumor bearing swiss albino-mice. *Metal Ions in Biology and Medicine*. Alpoim MC, Santos MA, Cristovao AJ, Centeno JA, Collery P, Morais PV (eds.). John Libbey Eurotext, Paris 9: 358-361, 2006.
- 36 Patrignani P, Santini G, Sciulli MG, Marinacci R, Fusco O, Spoletini L, Natoli C, Procopio A and Maclouf J: Cyclooxygenase-independent induction of p21<sup>WAF-1/CIP1</sup>, apoptosis and differentiation by L-745,337 and salicylate in HT-29 colon cancer cells. *Adv Exp Med Biol* 469: 555-561, 1999.
- 37 Law BK, Waltner-Law ME, Entingh AJ, Chytil A, Aakre ME, Norgaard P and Moses HL: Salicylate-induced growth arrest is associated with inhibition of p70s6k and down-regulation of c-myc, cyclin D1, cyclin A, and proliferating cell nuclear antigen. *J Biol Chem* 275: 38261-38267, 2000.
- 38 Nunez L, Valero RA, Senovilla L, Sanz-Blasco S, Garcia-Sancho J and Villalobos C: Cell proliferation depends on mitochondrial Ca<sup>2+</sup> uptake: inhibition by salicylate. *J Physiol* 571: 57-73, 2006.
- 39 Santini G, Sciulli MG, Marinacci R, Fusco O, Spoletini L, Pace A, Ricciardulli A, Natoli C, Procopio A, Maclouf J and Patrignani P: Cyclooxygenase-independent induction of p21<sup>WAF-1/CIP1</sup>, apoptosis and differentiation by L-745,337, a selective PGH synthase-2 inhibitor, and salicylate in HT-29 cells. *Apoptosis* 4: 151-162, 1999.
- 40 Seo MS, Oh SY, Park MJ, Kim SM, Kim MY, Han SI, Park HG and Kang HS: Implication of reactive oxygen species, ERK1/2, and p38MAPK in sodium salicylate-induced heat shock protein 72 expression in C6 glioma cells. *Int J Mol Med* 16: 841-849, 2005.
- 41 McDade TP, Perugini RA, Vittimberga FJ Jr., Carrigan RC and Callery MP: Salicylates inhibit NF-kappaB activation and enhance TNF-alpha-induced apoptosis in human pancreatic cancer cells. *J Surg Res* 83: 56-61, 1999.
- 42 Katerinaki E, Haycock J W, Lalla R, Carlson KE, Yang Y, Hill RP, Lorigan PC and MacNeil S: Sodium salicylate inhibits TNF-alpha-induced NF-kappaB activation, cell migration, invasion and ICAM-1 expression in human melanoma cells. *Melanoma Res* 16: 11-22, 2006.
- 43 Perugini RA, McDade TP, Vittimberga FJ Jr., Duffy AJ and Callery MP: Sodium salicylate inhibits proliferation and induces G1 cell cycle arrest in human pancreatic cancer cell lines. *J Gastrointest Surg* 4: 24-32, discussion 32-33, 2000.
- 44 Shtivelband MI, Juneja HS, Lee S and Wu KK: Aspirin and salicylate inhibit colon cancer medium and VEGF-induced endothelial tube formation: correlation with suppression of cyclooxygenase-2 expression. *J Thromb Haemost* 1: 2225-2233, 2003.
- 45 Borthwick GM, Johnson AS, Partington M, Burn J, Wilson R and Arthur HM: Therapeutic levels of aspirin and salicylate directly inhibit a model of angiogenesis through a Cox-independent mechanism. *FASEB J* 20: 2009-2016, 2006.
- 46 Muroso S, Yoshizaki T, Sato H, Takeshita H, Furukawa M and Pagano JS: Aspirin inhibits tumor cell invasiveness induced by Epstein-Barr virus latent membrane protein 1 through suppression of matrix metalloproteinase-9 expression. *Cancer Res* 60: 2555-2561, 2000.
- 47 Hyppolito MA, de Oliveira JA and Rossato M: Cisplatin ototoxicity and otoprotection with sodium salicylate. *Eur Arch Otorhinolaryngol* 263: 798-803, 2006.
- 48 Li G, Sha SH, Zotova E, Arezzo J, Van de Water T and Schacht J: Salicylate protects hearing and kidney function from cisplatin toxicity without compromising its oncolytic action. *Lab Invest* 82: 585-596, 2002.
- 49 Soderberg LS, Barnett JB, Baker ML, Chang LW, Salari H and Sorenson JR: Copper(II)2(3,5-di-isopropylsalicylate)4 stimulates hemopoiesis in normal and irradiated mice. *Exp Hematol* 16: 577-580, 1988.
- 50 McCarty MF, and Block KI: Preadministration of high-dose salicylates, suppressors of NF-kappaB activation, may increase the chemosensitivity of many cancers: an example of proapoptotic signal modulation therapy. *Integr Cancer Ther* 5: 252-268, 2006.
- 51 Ye QS, Lou LG, Liu WP, Yu Y, Chen XZ, Hou SQ, Gao WQ and Liu Y: Synthesis and *in vitro* cytotoxicity of novel lipophilic (diamine) platinum (II) complexes of salicylate derivatives. *Bioorg Med Chem Lett* 17: 2146-2149, 2007.
- 52 Illan-Cabeza NA, Garcia-Garcia AR, Moreno-Carretero MN, Martinez-Martos JM and Ramirez-Exposito MJ: Synthesis, characterization and antiproliferative behavior of tricarbonyl complexes of rhenium(I) with some 6-amino-5-nitrosouracil derivatives: crystal structure of fac-[ReCl(CO)<sub>3</sub>(DANU-N<sup>5</sup>,O<sup>4</sup>)] (DANU=6-amino-1,3-dimethyl-5-nitrosouracil). *J Inorg Biochem* 99: 1637-1645, 2005.
- 53 Shtemenko AV, Collery P, Shtemenko NI, Domasevitch KV, Zabitkaya ED and Golichenko AA: Synthesis, characterization, *in vivo* antitumor properties of the cluster rhenium compound with GABA ligands and its synergism with cisplatin. *Dalton Trans* 26: 5132-5136, 2009.

- 54 Shtemenko A, Shtemenko N, Oliynyk SA and Zelenuk MA: Liposome forms of rhenium cluster compounds in models of haemolytic anemia. *Metal Ions in Biology and Medicine*. Khassanova L, Collery P, Maynard I, Khassanova Z, Etienne JC (eds.). John Libbey Eurotext, Paris 7: 558-561, 2002.
- 55 Collery P, Shtemenko N, Shtemenko A, Bourleaud M, Etienne JC, Maynard I and Loriquet P: Supplementation by rhenium compounds instead of iron compounds during the treatment by erythropoietin of anemia in cancer patients. *Metal Ions in Biology and Medicine*. Cser MA, Sziklai-Laszlo I, Etienne JC, Maynard I, Centeno J, Khassanova L, Collery P (eds.). John Libbey Eurotext, Paris 8: 534-537, 2004.
- 56 Shtemenko N, Collery P, and Shtemenko A: Synergistic effect of cisplatin and cis-rhenium(III) diadamantate on tumor growth. *Metal Ions in Biology and Medicine*. Alpoim MC, Morais PV, Santos MA, Cristovao AJ, Centeno JA, Collery P (eds.). John Libbey Eurotext, Paris 9: 374-381, 2006.
- 57 Shtemenko N, Collery P and Shtemenko A: Dichlorotetra-mu-Isobutytratodirhenium (III): enhancement of cisplatin action and RBC-stabilizing properties. *Anticancer Research* 27: 2487-2492, 2007.
- 58 Kermagoret A, Morgant G, D'Angelo J, Tomas A, Roussel P, Bastian G, Collery P and Desmaële D: Synthesis, structural characterization and biological activity against several human tumor cell lines of four rhenium(I) diseleno-ethers complexes:  $\text{Re}(\text{CO})_3\text{Cl}(\text{PhSe}(\text{CH}_2)_2\text{SePh})$ ,  $\text{Re}(\text{CO})_3\text{Cl}(\text{PhSe}(\text{CH}_2)_3\text{SePh})$ ,  $\text{Re}(\text{CO})_3\text{Cl}(\text{HO}_2\text{C}-\text{CH}_2\text{Se}(\text{CH}_2)_2\text{SeCH}_2-\text{CO}_2\text{H})$  and  $\text{Re}(\text{CO})_3\text{Cl}(\text{HO}_2\text{C}-\text{CH}_2\text{Se}(\text{CH}_2)_3\text{SeCH}_2-\text{CO}_2\text{H})$ . *Polyhedron* 30: 347-354, 2011.
- 59 Collery P, Lechenault F, Juvin E, Khassanova L, and Cazabat A: Delayed inhibitory effects of gallium chloride on U937 malignant cell lines. *Anticancer Res* 17: 4072, 1997.
- 60 Collery P, Millart H, Lamiable D, Vistelle R, Rinjard P, Tran G, Gourdiere B, Cossart C, Bouana JC and Pechery C: Clinical pharmacology of gallium chloride after oral administration in lung cancer patients. *Anticancer Res* 9: 353-356, 1989.
- 61 Collery P, Millart H, Pourny C, Choisy H and Etienne JC: Pharmacokinetics of gallium after oral administration. *Proc. IUPHAR 9th International Congress of Pharmacology*, London, abstract 501, 1984.
- 62 Hata Y, Sandler A, Loehrer PJ, Sledge GW Jr. and Weber G: Synergism of taxol and gallium nitrate in human breast carcinoma cells: schedule dependency. *Oncol Res* 6: 19-24, 1994.
- 63 Chitambar C, Zivkovic Z and Abrams R: Effect of gallium on hemoglobin production. *Proc ASCO* 5: 44, 1986.
- 64 Chitambar CR and Narasimhan J: Inhibition of ribonucleotide reductase by gallium in murine leukemic L1210 cells. *Cancer Res* 51: 6199-6201, 1991.
- 65 Johnsson A, Olsson C, Nygren O, Nilsson M, Seiving B and Cavallin-Stahl E: Pharmacokinetics and tissue distribution of cisplatin in nude mice: platinum levels and cisplatin-DNA adducts. *Cancer Chemother Pharmacol* 37: 23-31, 1995.
- 66 Fulco RA, Vannozzi M, Collecchi P, Merlo F, Parodi B, Civalleri D and Esposito M: Effect of normal saline on cisplatin pharmacokinetics and antitumor activity in mice bearing P388 leukemia. *Anticancer Res* 10: 1603-1610, 1990.
- 67 Prasad SB and Khyriam D: Cisplatin-induced changes in tissue glutathione level and glutathione-related enzymes in Dalton's lymphoma-bearing mice. *Metal Ions in Biology and Medicine*. Cser MA, Sziklai-Laszlo I, Etienne JC, Maynard I, Centeno J, Khassanova L, Collery P (eds.). John Libbey Eurotext, Paris 8: 368-372, 2004.
- 68 Vistelle R, Collery P and Millart H: *In vivo* distribution of gallium in healthy rats after oral administration and interactions with iron, magnesium and calcium. *Trace Elem Med* 6: 27-32, 1989.
- 69 Collery P, Millart H, Choisy H, Blanchot G, Leroy A and Anghileri LJ: Dose-effect of  $\text{GaCl}_3$ , orally administered, on gallium, magnesium, calcium and iron concentrations in tumour and kidneys of mammary adenocarcinoma-bearing mice. *Anticancer Res* 12: 1920-1921, 1992.
- 70 Collery P, Kleisbauer JP, Robinet G, Paillot D, Arnaud A, Balmes P, Poirier R, Claeysens S, Prevost A, Millart H, Morel M, Blanchot G and Leroy A: Management of platinum and gallium therapies in lung cancer patients. *Metal Ions in Biology and Medicine*, Collery P, Poirier L, Littlefield N, Etienne JC (eds.). John Libbey Eurotext, Paris 3: 335-342, 1994.
- 71 Collery P, Millart H, Morel M, Desoize B, Choisy H and Pechery C: Gallium-potential of cancer chemotherapy. *Handbook on Metal-Ligand Interactions in Biological Fluids 2: Part 5, Chapter 4, Section E*, 1-8, 1994.
- 72 Collery P, Morel M, Desoize B, Millart H, Perdu D, Prevost A, Vallerand H, Pechery C, Choisy H, Etienne JC and Dubois De Montreynaud JM: Combination chemotherapy with cisplatin, etoposide and gallium chloride for lung cancer: individual adaptation of doses. *Anticancer Res* 11: 1529-1531, 1991.
- 73 Collery P, Millart H, Kleisbauer JP, Paillot D, Robinet G, Durand A, Clayessen S, Legendre JM, Leroy A, Rousseau A, Pechery C and Kochmans S: Dose optimization of gallium chloride, orally administered, in combination with platinum compounds. *Anticancer Res* 14: 2299-2306, 1994.
- 74 Collery P, Morel M, Millart H, Perdu D, Lavaud F, Anghileri LJ, Pluot M, Choisy H and Pechery C: Relationship between intratumoral gallium concentration and tumor mass, after oral administration of gallium chloride. *Metal Ions in Biology and Medicine*. Collery P, Poirier LA, Manfait M, Etienne JC (eds.). John Libbey Eurotext, Paris 3: 331-334, 1994.
- 75 Collery P, Millart H, Lamiable D, Vistelle R, Berthier A, Betbeze P, Cossart C, Mulette T, Masure F, Gourdiere B *et al*: Magnesium alterations and pharmacokinetic data in gallium-treated lung cancer patients. *Magnesium* 8: 56-64, 1989.
- 76 Collery P, Millart H, Ferrand O, Jouet JB, Dubois JP, Barthes G, Pourny C, Pechery C, Choisy H, Cattan A, Dubois de Montreynaud JM and Etienne JC: Gallium chloride treatment of cancer patients after oral administration: a pilot study. *Chemotherapy* 4: 1165-1166, 1985.
- 77 Collery P, Lechenault F, Cazabat A, Juvin E, Khassanova L, Evangelou A and Keppler B: Inhibitory effects of gallium chloride and *tris*(8-quinolinolato)gallium(III) on A549 human malignant cell line. *Anticancer Res* 20: 955-958, 2000.
- 78 Thiel M, Schilling T, Gey DC, Ziegler R, Collery P and Keppler BK: *tris*(8-quinolinolato)gallium(III), a novel orally applied antitumor gallium compound. Relevance of tumor models for anticancer drug development. *Contributions to Oncology*. Fiebig HH, Burger AM (eds.). Karger, Basel 54: 439-443, 1999.
- 79 Chitambar CR, Purpi DP, Woodliff J, Yang M and Wereley JP: Development of gallium compounds for treatment of lymphoma: gallium maltolate, a novel hydroxypyron gallium compound, induces apoptosis and circumvents lymphoma cell resistance to gallium nitrate. *J Pharmacol Exp Ther* 322: 1228-1236, 2007.

- 80 Chitambar CR and Purpi DP: A novel gallium compound synergistically enhances bortezomib-induced apoptosis in mantle cell lymphoma cells. *Leuk Res* 34: 950-953, 2010.
- 81 Arnold C, Chaffin MK, Cohen N, Fajt VR, Taylor RJ and Bernstein LR: Pharmacokinetics of gallium maltolate after intragastric administration in adult horses. *Am J Vet Res* 71: 1371-1376, 2010.
- 82 Martens RJ, Mealey K, Cohen ND, Harrington JR, Chaffin MK, Taylor RJ and Bernstein LR: Pharmacokinetics of gallium maltolate after intragastric administration in neonatal foals. *Am J Vet Res* 68: 1041-1044, 2007.
- 83 Lee EJ, Park HG and Kang HS: Sodium salicylate induces apoptosis in HCT116 colorectal cancer cells through activation of p38MAPK. *Int J Oncol* 23: 503-508, 2003.
- 84 Ordan O, Rotem R, Jaspers I and Flescher E: Stress-responsive JNK mitogen-activated protein kinase mediates aspirin-induced suppression of B16 melanoma cellular proliferation. *Br J Pharmacol* 138: 1156-1162, 2003.
- 85 Hernandez M, de Arriba AF, Merlos M, Fuentes L, Crespo MS and Nieto ML: Effect of 4-trifluoromethyl derivatives of salicylate on nuclear factor kappaB-dependent transcription in human astrocytoma cells. *Br J Pharmacol* 132: 547-555, 2001.
- 86 Ghezzi F, Cesano L, Mognetti B, Pesce E, Pirro E, Corvetti G, Berta GN, Zingaro B and Di Carlo F: Salicylate inhibition of rat mammary carcinogenesis and angiogenesis in female rat compatible with misoprostol administration. *Int J Oncol* 26: 697-702, 2005.
- 87 Drew JE, Padidar S, Horgan G, Duthie GG, Russell WR, Reid M, Duncan G and Rucklidge GJ: Salicylate modulates oxidative stress in the rat colon: a proteomic approach. *Biochem Pharmacol* 72: 204-216, 2006.
- 88 Chung YM, Bae YS and Lee SY: Molecular ordering of ROS production, mitochondrial changes, and caspase activation during sodium salicylate-induced apoptosis. *Free Radic Biol Med* 34: 434-442, 2003.
- 89 Blincoe C, Chidambaram M, Salari H, Bond KB, Evans DT, Gray-Kaufman RA, Griffey HB, Tranby SG, Garner-Johnson B, Lee T and Sorenson JR: Pharmacokinetic distribution of  $^{67}\text{Cu}(\text{II})_2[3,5\text{-di-isopropyl}(\text{carboxy-}^{14}\text{C})\text{salicylate}]_4$  among murine tissues. *Curr Med Chem* 11: 3007-3015, 2004.
- 90 Viola-Villegas N, Rabideau AE, Cesnavicious J, Zubieta J and Doyle RP: Targeting the folate receptor (FR): imaging and cytotoxicity of ReI conjugates in FR-overexpressing cancer cells. *Chem Med Chem* 3: 1387-1394, 2008.
- 91 Desbouis D, Struthers H, Spiwok V, Kuster T and Schibli R: Synthesis, *in vitro*, and *in silico* evaluation of organometallic technetium and rhenium thymidine complexes with retained substrate activity toward human thymidine kinase type I. *J Med Chem* 51: 6689-6698, 2008.
- 92 Struthers H, Hagenbach A, Abram U and Schibli R: Organometallic  $[\text{Re}(\text{CO})_3]^+$  and  $[\text{Re}(\text{CO})_2(\text{NO})]^{2+}$  labeled substrates for human thymidine kinase I. *Inorg Chem* 48: 5154-5163, 2009.
- 93 Bartholoma MD, Vortherms AR, Hillier S, Ploier B, Joyal J, Babich J, Doyle RP and Zubieta J: Synthesis, cytotoxicity, and insight into the mode of action of  $\text{Re}(\text{CO})_3$  thymidine complexes. *Chem Med Chem* 5: 1513-1529, 2010.
- 94 Bowen ML, Chen ZF, Roos AM, Misri R, Hafeli U, Adam MJ and Orvig C: Long-chain rhenium and technetium glucosamine conjugates. *Dalton Trans* 42: 9228-9236, 2009.
- 95 Choi SJ, Brylev KA, Xu JZ, Mironov YV, Fedorov VE, Sohn YS, Kim SJ and Choy HJ: Cellular uptake and cytotoxicity of octahedral rhenium cluster complexes. *J Inorg Biochem* 102: 1991-1996, 2008.
- 96 Martinez-Lillo J, Mastropietro TF, Lappano R, Madeo A, Alberto ME, Russo N, Maggiolini M and De Munno G: Rhenium(IV) compounds inducing apoptosis in cancer cells. *Chem Commun (Camb)* 47: 5283-5285, 2011.
- 97 Fricker SP: Cysteine proteases as targets for metal-based drugs. *Metallomics* 2: 366-377, 2010.
- 98 Wirth S, Wallek AU, Zernickel A, Feil F, Sztiller-Sikorska M, Lesiak-Mieczkowska K, Brauchle C, Lorenz IP and Czyz M: Tautomerization of 2-nitroso-*N*-arylanilines by coordination as *N,N'*-chelate ligands to rhenium(I) complexes and the anticancer activity of newly synthesized oximine rhenium(I) complexes against human melanoma and leukemia cells *in vitro*. *J Inorg Biochem* 104: 774-789, 2010.
- 99 Vucina J and Han R: Production and therapeutic use of rhenium-186, 188, the future of radionuclides. *Med Pregl* 56: 362-365, 2003.
- 100 Scheffler J, Derejko M, Bandurski T and Romanowicz G: Application of rhenium-188 HEDP in bone metastases therapy. *Nucl Med Rev Cent East Eur* 6: 55-57, 2003.
- 101 Hindorf C, Flux GD, Ibisch C, and F, Kraeber Bodere: Clinical dosimetry in the treatment of bone tumors: old and new agents. *Q J Nucl Med Mol Imaging* 55: 198-204, 2011.
- 102 Cheng A, Chen S, Zhang Y, Yin D, and Dong M: The tolerance and therapeutic efficacy of rhenium-188 hydroxyethylidene diphosphonate in advanced cancer patients with painful osseous metastases. *Cancer Biother Radiopharm* 26: 237-244, 2011.
- 103 Li S, Liu J, Zhang H, Tian M, Wang J and Zheng X: Rhenium-188 HEDP to treat painful bone metastases. *Clin Nucl Med* 26: 919-922, 2001.
- 104 Liepe K, Geidel HH, Bergmann R, Haase M, Runge R and Kotzerke J: Autoradiographic studies of rhenium-188-hydroxyethylidene diphosphonate in normal skeleton and osteoblastic bone metastases in a rat model of metastatic prostate cancer. *Nucl Med Commun* 30: 693-699, 2009.
- 105 Isakoff SJ: Triple-negative breast cancer: role of specific chemotherapy agents. *Cancer J* 16: 53-61, 2010.
- 106 Gluz O, Liedtke C, Gottschalk N, Pusztai L, Nitz U and Harbeck N: Triple-negative breast cancer current status and future directions. *Ann Oncol* 20: 1913-1927, 2009.

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