VP-128, a novel oestradiol-platinum(II) hybrid with selective anti-tumour activity towards hormone-dependent breast cancer cells in vivo

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Abstract
We have previously reported the synthesis of VP-128, a new 17β-oestradiol (E2)-linked platinum(II) hybrid with high affinity for oestrogen receptor α (ERα). In the present study, we have investigated the anti-tumour activity of VP-128 towards breast cancer cells in vitro and in vivo. We used human ERα-positive (MCF-7) and -negative (MDA-MB-468) cells as a model for treatment with increasing doses of VP-128, cisplatin or E2 in vitro and for xenograft experiments in nude mice in vivo. Compared with cisplatin, VP-128 showed markedly improved in vitro and in vivo anti-tumour activity towards ERα-positive MCF-7 breast cancer cells, without increased systemic toxicity. In these caspase-3-deficient cells, treatment with VP-128 overcame weak cellular sensitivity to cisplatin in vitro and in vivo. In these cells, only the hybrid induced apoptosis in an ERα-dependent manner, inactivated both X-linked inhibitor of apoptosis protein and Akt, and induced selective nuclear accumulation of ERα and the expression of ER-regulated genes c-myc and tff1, which was blocked by ERα-specific antagonist ICI 282 780. In the case of ERα-negative MDA-MB-468 cells, VP-128, but not cisplatin, induced nuclear accumulation of apoptosis-inducing factor and inhibited c-myc expression. However, VP-128 did not show enhanced in vivo anti-tumour activity compared with cisplatin. These results reveal two different modes of action for VP-128 in ERα-positive and -negative breast cancer cells, and highlight the promising therapeutic value of this unique E2-platinum hybrid for selective targeting of hormone-dependent cancers.

Endocrine-Related Cancer (2009) 16 1185–1195

Introduction
Most breast cancers are classified as hormone dependent due to the overexpression of oestrogen receptors (ERα in particular), compared to corresponding normal tissues (Sommer & Fuqua 2001). ERα has already been validated as a biological target for the treatment of hormone-dependent breast cancer, and antagonists such as tamoxifen (Nolvadex) are widely used as a treatment (MacGregor & Jordan 1998, Fisher et al. 2005). However, tamoxifen belongs to the family of selective ER modulators (SERMs) and shows adverse agonistic activity on ER in the endometrium (Jordan & O’Malley 2007): as a result, long-term use of tamoxifen is associated with increased endometrial cancer risk (Fornander et al. 1989, Fisher et al. 1994). In addition, resistance to tamoxifen is common (Clarke et al. 2001). Alternative approaches to the use of SERMs are thus needed for the targeting of ERα-overexpressing breast tumour cells.

Cisplatin (cis-diaminedichloroplatinum(II)) is already used for the treatment of several types of cancers (Muggia & Fojo 2004) and is currently under preclinical and clinical trials for breast cancer (Ott & Gust 2007). Cisplatin binds to the DNA of fast growing cells, which is a common feature of tumour cells, and stops cell proliferation leading to apoptosis (Wang &
Lippard 2005). The absence of selectivity of platinum-based drugs for cancer cells, however, results in severe side effects (Kelland 2007). The use of 17β-oestradiol (E2) as a carrier molecule for targeted delivery of cisplatin to breast cancer cells could putatively increase both the anti-neoplastic activity and the selectivity of cisplatin towards hormone-dependent breast cancer cells. Indeed, pre-treatment of hormone-dependent breast cancer cell lines with E2 has been reported to increase cellular sensitivity to cisplatin (He et al. 2000), and previous in vivo studies have shown higher anti-neoplastic activity towards ERα-overexpressing tumours, with lower systemic toxicity, when E2–cytotoxic complexes were administered compared with the cytotoxic moiety alone (Betsch 1990, Sharma et al. 2004).

We have recently reported the synthesis of a new family of E2-platinum hybrids (Perron et al. 2005, Descoteaux et al. 2008). Preliminary studies had revealed that one of these hybrids, VP-128, shows high affinity for binding to ERα (similar to that of E2) and increased growth-suppressive properties compared with cisplatin towards breast cancer cell lines in vitro (Perron et al. 2005, Descoteaux et al. 2008). In the present study, we have investigated in vivo anti-tumour activity of the hybrid, using human ERα-positive and -negative breast cancer xenografts in nude mice as a model. In addition, we have investigated the mode of action of VP-128 in breast cancer cells.

Materials and methods

Cell lines and reagents

Human breast carcinoma cell lines MCF-7 (ERα-positive) and MDA-MB-468 (ERα-negative) were generously provided by Dr René C-Gaudreault (CHUQ Research Center, Québec, Canada) and maintained in RPMI medium containing 10% bovine growth serum containing 50 μg/ml gentamycin. All antibodies were from Cell Signaling Technology (Beverly, MA, USA) except for HRP-conjugated goat anti-rabbit secondary antibody (Bio-Rad Laboratories), ERα (Ab-16 from Neomarkers, Thermo Fisher Scientific, Fremont, CA, USA) and ERβ (Ab-24 from Neomarkers) antibodies. Cisplatin was purchased from Sigma, and E2-platinum(II) hybrid VP-128 was synthesized using our most recent methodology and purified by chromatography (Descoteaux et al. 2008). ICI 182 780 was purchased from Cedarlane Laboratories (Burlington, ON, Canada).

Drug treatments

MCF-7 and MDA-MB-468 cells were seeded in 100 mm3 Petri dishes (2.5×10⁶ MCF-7 cells and 2.0×10⁶ MDA-MB-468 cells per dish). The following day, cells were pre-treated or not with 1 μM ICI 282 780 for 1 h, followed by treatment with increasing concentrations of cisplatin, E2 or VP-128 (0, 0.1, 1 or 10 μM) for the indicated time periods. Adherent and floating cells were collected.

In vivo testing of VP-128

All animal protocols were approved by the Université du Québec à Trois-Rivières animal care committee. Subcutaneous tumour xenografts of human cancer cells were established in 6-week-old CD-1 nude mice (Charles River Laboratories, Lasalle, QC, Canada) by injection of 10×10⁶ cells in 100 μl of 2 mg/ml Matrigel (VWR, Mississauga, ON, Canada) at both flanks near the posterior legs. Starting on the day of xenografts, mice inoculated with hormone-dependent MCF-7 tumour cells received a s.c. injection of E2 (0.15 mg/animal) at 3-day interval, to prime tumour growth. For each animal, tumour size was measured twice a week using callipers. Tumour volume was calculated using the formula 0.5×length×(width)² (Euhus et al. 1986). Treatments with the drugs were initiated when the tumours derived from a given cell line reached a mean volume of 100 mm³ (day 0). Mice received i.p. injections of either VP-128 or cisplatin (0.00615 mmol/kg) or control (vehicle) solution (5% cremophor, 5% ethanol and 0.9% sodium chloride in water), at 3-day interval starting at day 0. Animals were also weighted twice a week starting at day 0. Mice were killed after 21 days; at that time, tumour size had not grown over 2500 mm³. Mice were subjected to macroscopic examination at the time of necropsy, but no detectable abnormality was noted.

Western blots analysis

The procedure described in Van Themscne et al. (2007) was followed.

Subcellular fractionation

NE-PER Nuclear and Cytoplasmic Extraction Reagent (Thermo Fisher Scientific) was used according to the manufacturer’s instructions.

RNA extraction and RT-PCR analysis

Total RNA was isolated from cells using TRIZol Reagent (Invitrogen) according to the manufacturer’s instructions. First strand cDNA was synthesized from
0.4 μg RNA using MMLV reverse transcriptase (Invitrogen). Primers for PCR amplification were: 5′-TAATTCAGCGAGGGCAGA-3′ (sense) and 5′-GGTCCCTCGAGAAGTCTG-3′ (antisense) for c-myc; 5′-GGGTTCAAGCAGACACTA-3′ (sense) and 5′-GGTCCGTGCAAGTGCTC-3′ (antisense) for c-fos; 5′-CAATGGCCACCAGGAGAC-3′ (sense) and 5′-AACGGTGTCGAAACAGC-3′ (antisense) for tff1; 5′-GTCAGTGTTGACCCTGACCT-3′ (sense) and 5′-TGAGCTTCAACAAAGTGGTG-3′ (antisense) for gapdh. PCRs were conducted in a MJ Research (Waltham, MA, USA) Thermal cycler (model PTC-100) using the following parameters: 30 s at 94°C, and 1 min at 72°C, for 35 cycles except for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (25 cycles). The reaction mixture was size separated on an agarose gel and visualized using SYBR-Safe (Invitrogen) staining upon u.v. transillumination.

**Determination of apoptosis using Hoechst nuclear staining**

Treated cells were collected, washed twice in PBS, resuspended at an approximate density of 2×10⁵ cells/ml in PBS containing 1 μg/ml Hoechst 33258 (Sigma) and incubated for 24 h at 4°C before fluorescence microscopy analysis of apoptotic cells. At least, 200 cells were counted for each sample, and a percentage of apoptotic cells was calculated as the ratio of apoptotic cells (with characteristic apoptotic morphology such as nuclear shrinkage and condensation) to total cell count.

**Immunofluorescence analysis**

Cells were prepared as described in Van Themsche et al. (2007) and observed under a fluorescence microscope (magnification: 1000×).

**Statistical analyses**

Data were subjected to one-way ANOVA (PRISM software version 3.03; GraphPad, San Diego, CA, USA). Differences between experimental groups were determined by the Tukey’s test. Statistical significance was accepted when \( P < 0.05 \).

**Results**

**VP-128 shows enhanced anti-tumour activity compared with cisplatin towards hormone-dependent breast cancer xenografts in vivo**

We used a classical xenograft model where human breast cancer cells MCF-7 (ERα-positive) and MDA-MB-468 (ERα-negative) were grown subcutaneously in nude mice to evaluate the biological activity of VP-128 (Fig. 1A) in vivo. We found that administration of VP-128 to mice efficiently suppressed the growth of hormone-dependent (derived from MCF-7 cells, Fig. 1B) as well as -independent (derived from MDA-MB-468 cells, Fig. 1C) tumours in vivo, indicating that VP-128 can exert potent anti-tumour activity over the two types of breast cancer cells in vivo. Noteworthy, VP-128 was more potent than cisplatin to suppress tumour growth only in the case of ERα-positive MCF-7 cells (Fig. 1B): this indicates that in vivo VP-128 selectively targets hormone-dependent breast cancer cells. At the concentrations used, animals treated with VP-128 or cisplatin did not suffer any weight loss (Fig. 1B and C) and showed no visible sign of toxic side effects, both during the treatment and upon examination at the time of necropsy.

**VP-128 is more efficient than cisplatin to induce apoptosis in breast cancer cells**

We have conducted microscopic analysis of Hoechst staining to compare the apoptotic indices induced by VP-128 and cisplatin in model breast cancer cell lines. As reported by others (Blanc et al. 2000), we observed that cisplatin only weakly induces apoptosis in caspase-3-deficient MCF-7 cells (Fig. 2A). However, at equimolar concentration, VP-128 induced a higher extent of apoptosis in these cells, as early as 24 h post-treatment (Fig. 2A). This indicates that the E2-Pt(II) hybrid VP-128 is able to overcome weak cisplatin sensitivity in ERα-positive cells. VP-128 also rapidly induced apoptosis in ERα-negative MDA-MB-468 cells (Fig. 2B), indicating that the hybrid can trigger apoptosis in an ERα-independent manner in breast cancer cells. VP-128 was more efficient than cisplatin to induce apoptosis in both MDA-MB-468 cells and MCF-7 cells (Fig. 2A and B); however, VP-128 induced two times more apoptotic cells than cisplatin in ERα-negative MDA-MB-468 cells, whereas it induced almost five times more apoptosis than cisplatin in ERα-positive MCF-7 cells (Table 1). Thus, the toxicity of VP-128 compared with cisplatin towards breast cancer cells in vitro is enhanced in ERα-positive cells.

**VP-128 differentially activates caspase activity depending on ER status**

Cisplatin has been shown to activate caspase-9-mediated apoptosis (Kuwahara et al. 2000), and our results showed that cisplatin as well as E2-Pt(II) complex, VP-128, induced caspase-9 cleavage/activation in ERα-positive MCF-7 cells (Fig. 2A) as...
VP-128 also induced caspase-3 cleavage in caspase-3-wild-type MDA-MB-468 cells (Fig. 2B). In the two model cell lines, both cisplatin and VP-128 could induce PARP cleavage/degradation, consistent with the induction of apoptosis by both drugs (Fig. 2A and B). In MCF-7 cells, cleavage of poly (ADP-ribose) polymerase (PARP) by the two drugs was proportional to their efficiency to induce apoptosis (Fig. 2A). In MDA-MB-468 cells however, VP-128 induced less caspase-3 and PARP fragments than cisplatin, even though it induced a higher extent of apoptosis than cisplatin (Fig. 2B). This suggests that VP-128 can activate caspase-independent apoptosis in ERα-negative cells.

**Figure 1** Selective improvement of anti-tumour activity of oestradiol-platinum hybrid VP-128 compared with unlinked cisplatin towards ERα-positive breast cancer cells in vivo. (A) Chemical structure of the 17β-oestradiol-Pt(II) hybrid VP-128 used in this study. (B and C) ERα-positive breast cancer cell line MCF-7 (B) and ERα-negative breast cancer cell line MDA-MB-468 (C) were inoculated subcutaneously in the flanks of nude mice. When tumours reached a mean volume of 100 mm³ (day 0), mice received i.p. injections of either VP-128 or cisplatin (0.00615 mmol/kg) or control (mock treatment), at 3-day interval. Tumour volume was measured routinely using callipers; tumour size at day 0 of treatment corresponds to 100%. Mice weight was determined twice a week; weight at day 0 corresponds to 100%. Results are mean ± S.E.M. of the indicated number of mice per group. *P < 0.05 compared with cisplatin-treated tumours.

**VP-128 and cisplatin differentially target Akt activity and X-linked inhibitor of apoptosis protein in breast cancer cells**

Both Akt kinase and X-linked inhibitor of apoptosis protein (XIAP) can protect cancer cells against cisplatin-induced apoptosis (Gagnon et al. 2008, Lee et al. 2008). We found that VP-128 decreased P-Akt and XIAP levels in MCF-7 (Fig. 2A) as well as MDA-MB-468 (Fig. 2B) cells. In MDA-MB-468 cells, VP-128 decreased total Akt levels (Fig. 2B), suggesting that decrease of P-Akt levels by VP-128 results from a decrease of total Akt content in these cells. Contrary to VP-128, cisplatin only reduced P-Akt levels in MDA-MB-468 cells (Fig. 2B).
Altogether, these results indicate that unlike cisplatin, VP-128 targets both P-Akt and XIAP in breast cancer cells.

VP-128 induces translocation of apoptosis-inducing factor to the nucleus in ERα-negative MDA-MB-468 cells

Cisplatin can activate caspase-independent apoptosis in cancer cells (Havelka et al. 2007), which is characterized by the translocation of apoptosis-inducing factor (AIF) from the mitochondria to the nucleus where it induces large-scale DNA fragmentation and peripheral chromatin condensation (Susin et al. 2000). VP-128 but not cisplatin induced a decrease of total AIF levels in both MCF-7 (Fig. 3A) and MDA-MB-468 (Fig. 3B) cells. However, VP-128 induced a translocation of AIF to the nucleus in MDA-MB-468 (Fig. 3B) but not in MCF-7 (Fig. 3A) cells, strongly suggesting that in ERα-negative MDA-MB-468 cells, VP-128 induces caspase-independent apoptosis.

### Table 1 Comparison between apoptosis induced by VP-128 and cisplatin in breast cancer cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>VP-128 (µM)</th>
<th>Cisplatin (µM)</th>
<th>Ratio VP-128/cisplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td>38.6 ± 4.6</td>
<td>8.25 ± 1.4</td>
<td>4.7</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>80.4 ± 9.21</td>
<td>41.1 ± 6.1</td>
<td>2.0</td>
</tr>
</tbody>
</table>
VP-128 specifically targets ERα to the nucleus in breast cancer cells

We observed that VP-128 specifically increased ERα protein levels in MCF-7 cells (Fig. 4A). In addition, treatment with VP-128 induced specific translocation of ERα from the cytosol to the nucleus in these cells, similar to E2 (Fig. 4A). Localization of ERβ, on the other hand, did not change in response to VP-128 or E2, in both ERα-positive MCF-7 cells (Fig. 4A) and ERα-negative MDA-MB-468 cells (Fig. 4B). This indicates that VP-128, which we have previously shown to bind ERα with high affinity in a cell-free assay (Descoteaux et al. 2008), selectively targets ERα to the nucleus in breast cancer cells.

VP-128 regulates ER-mediated gene expression in breast cancer cells

VP-128 induces the translocation of ERα to the nucleus in breast cancer cells (Fig. 4A). RT-PCR analysis showed that in ERα-positive MCF-7 cells, VP-128 induced the expression of ER-regulated genes tff1 (Nunez et al. 1987) and c-myc (Dubik & Shiu 1992), similar to E2 (Fig. 5A). VP-128-induced gene expression was blocked by ERα antagonist ICI 182 780 (ICI; Fig. 5A), indicating that VP-128 induces gene expression in an ERα-dependent manner. Another early response gene, c-fos (Loose-Mitchell et al. 1988), was induced by E2 but not by VP-128 (Fig. 5A). In ERα-negative MDA-MB-468 cells, both VP-128 and E2 did not modify the expression of ERα-regulated gene c-fos, but repressed the expression of c-myc in an ERα-independent manner (Fig. 5B). Altogether, these results indicate that in breast cancer cells, VP-128 regulates ER-mediated gene expression, in a distinct manner depending on ER status.

VP-128 induces apoptosis in an ERα-dependent manner in ERα-positive breast cancer cells

Since VP-128 binds ERα with high affinity (Descoteaux et al. 2008) and shows enhanced anti-tumour activity compared with cisplatin towards ERα-positive cells in vitro (Table 1) and in vivo (Fig. 1B), we hypothesized that binding of VP-128 to ERα increases the ability of VP-128 to induce apoptosis in breast cancer cells. In agreement with this hypothesis, we observed that in ERα-positive MCF-7 cells, ERα-specific antagonist ICI 182 780 (ICI; Fig. 5A), blocks its nuclear import (Dauvois et al. 1993), blocked VP-128-induced caspase-9 and PARP cleavage and apoptosis (Fig. 5A). In ERα-negative MDA-MB-468 cells on the other hand, ICI 282 780 had no impact on VP-128-induced caspase-9 and PARP cleavage and apoptosis (Fig. 5B).
cleavage or apoptosis (Fig. 5B). Altogether, these results indicate that apoptosis induced by VP-128 necessitates binding to ERα only in ERα-positive breast cancer cells.

**Discussion**

We have investigated the *in vivo* anti-tumour activity of VP-128, an E2-Pt(II) hybrid molecule with high affinity for ERα that we recently synthesized (Descoteaux *et al.* 2008). The hybrid was able to stop the growth of xenografts from human ERα-negative breast cancer cells, and achieved tumour regression in the case of ERα-positive cells. VP-128 and cisplatin showed similar activity towards ERα-negative cells, but the hybrid showed improved biological activity compared with cisplatin towards ERα-positive cells, without causing more side effects. This demonstrates that VP-128 can selectively target hormone-dependent cancers and highlights the therapeutic value of the compound.

Unlike the *in vivo* situation, VP-128 was more efficient than cisplatin in inducing apoptosis in ERα-negative MDA-MB-468 cells *in vitro*. Others have also reported a discrepancy between the *in vitro* and *in vivo* cytotoxicity of platinum compounds (Otto *et al.* 1991). In the present case, the molecular profiles and associated phenotypes may be differently modulated in the two cell lines upon their growth in the mouse microenvironment, leading to the observed difference between *in vitro* and *in vivo* responses to VP-128. Nonetheless, increased pro-apoptotic activity of VP-128 compared with cisplatin was enhanced in ERα-positive cells, which reflects selectivity towards ERα-positive cells.

Strikingly, VP-128 was able to overcome the weak sensitivity of caspase-3-deficient MCF-7 breast cancer cells to cisplatin *in vitro* and *in vivo*. This reflects the differential modes of action of the two platinum compounds. Indeed, *in vitro* experiments showed that, whereas both VP-128 and cisplatin reduce XIAP levels in ERα-positive breast cell lines, only VP-128 reduces P-Akt levels in these cells. We have previously showed that Akt could be directly cleaved by caspase-3 (Asselin *et al.* 2001), and since VP-128 reduced total Akt levels in MDA-MB-468 cells and not in caspase-3-deficient MCF-7 cells, VP-128 may target AKT in a caspase-3-dependent manner in breast cancer cells.
In addition to targeting Akt activity in MCF-7 cells in a different manner, VP-128 and cisplatin also exert a different mode of action in ERa-negative MDA-MB-468 cells, where only VP-128 induced nuclear accumulation of AIF, a marker of caspase-independent apoptosis (Susin et al. 2000). VP-128 can bind both ERa and ERb with good affinity (Descoteaux et al. 2008), and the ratio of ERa to ERb influences cellular response to ER-binding molecules (Paruthiyil et al. 2004, Lee et al. 2005). Since E2 induces release of AIF from the mitochondria only when ERb levels are higher than ERa levels (Zeng et al. 2008), the ability of VP-128 to activate features of caspase-independent apoptosis in ERa-negative MDA-MB-468 cells may result from the higher level of ERb compared with ERa in these cells. It may be for the same reason that VP-128 only repressed c-myc expression, a feature of ERb-mediated response to E2 (Paruthiyil et al. 2004), in MDA-MB-468 cells.

In ERa-positive MCF-7 cells, VP-128 activated the expression of two E2 early-response genes, c-myc and tff1, in an ERa-dependent manner. This suggests that the VP-128 induces E2-like genomic effects through ERa. By inducing the expression of genes promoting cellular proliferation such as c-myc, the E2 moiety of the VP-128 hybrid could thus sensitize ERa-positive cells to the cytotoxicity of the DNA-targeting moiety, explaining its improved anti-tumour activity compared with cisplatin towards ERa-positive cells in vitro and in vivo. In agreement, apoptosis induction by VP-128 was blocked by ERa antagonist ICI 282 780, which also blocked VP-128-induced expression of c-myc and tff1. The expression of another early-response gene, c-fos, was induced by E2 but not by VP-128. Induction of c-fos expression by E2 was shown to be mediated by GPR30 (Maggiolini et al. 2004), suggesting that in ERa-positive breast cancer cells, VP-128 does not activate GPR30 but selectively activates ERa. VP-128 did not modify the expression of c-fos in MDA-MB-468 cells either, and did not increase P-Akt levels in both model cell lines, suggesting that in breast cancer cells in general, VP-128 does not activate membrane-associated, non-genomic E2-activated pathways.

The results obtained in the present study suggest that the selectivity for ERa-expressing cells would result from the induction of ERa-mediated expression of proliferation-promoting genes by the E2 moiety of the hybrid, thereby sensitizing the cells to apoptosis induced by the cisplatin moiety. It should be emphasized that the two model cell lines that we used differ not only with respects to ER status and origin, but also in their global molecular profile. Genome-wide transcriptional profiling of a larger numbers of human breast cancer cell lines has confirmed the existence of a few subgroups of cell lines, termed luminal (generally ERa-positive), basal A (with mixed basal and luminal features) and basal B (with enhanced invasive properties and a predominantly mesenchymal gene expression signature; Neve et al. 2006, Blick et al. 2008). Based on their transcriptional profile, MCF-7 and MDA-MB-468 cell lines have been classified in two different subgroups,
namely luminal and basal A respectively. This reflects a molecular heterogeneity between the two model cell lines. In this regard, the expression of several genes, notably those involved in the response to apoptosis-inducing cisplatin, differs between the two cell lines. This should be taken into account when comparing the response of the two cell lines to VP-128.

BRCA2-related tumours, with a mutated BRCA2 gene (Collins et al. 1995), exhibit a distinguishing phenotype based on morphology and molecular profiles from tissue microarrays (Bane et al. 2007). They express high levels of ERα (even higher than tumours from luminal subtype; Brekelmans et al. 2007), which has been associated with better post-recurrence survival (Hubert et al. 2009). In addition, although BRCA2-related tumours are resistant to neoadjuvant chemotherapy (Hubert et al. 2009), they are sensitive to DNA damage-inducing platinum compounds cisplatin and carboplatin (Tutt et al. 2005), probably due to the mutation in DNA repair gene BRCA2. On this basis, a clinical trial evaluating carboplatin in patients with BRCA2-related tumours has been launched (Tutt et al. 2005). In patients with ERα(high)/cisplatin-sensitive tumours, one would expect VP-128 to efficiently induce apoptosis in an ERα-dependent manner, and to induce an even better clinical response than unconjugated cisplatin or carboplatin.

In conclusion, the present study highlights the ability of an E2-platinum(II) hybrid, VP-128, to selectively target hormone-dependent and cisplatin-resistant hormone-dependent breast cancers in vivo. The fact that enhanced biological activity of VP-128 compared with cisplatin is not associated with increased toxic side effects further highlights the therapeutic value of the compound. The use of VP-128 has the potential to be extended to other hormone-dependent feminine cancers, and we are currently evaluating VP-128 for the treatment of cisplatin-resistant hormone-dependent ovarian cancers (Piccart et al. 2001).

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by a team grant from the Fonds québécois de la recherche sur la nature et les technologies (FQRNT) and a grant from the Canadian Institute of Health Research (CIHR). E Asselin is chairholder of the Canada Research Chair in Molecular Gyneco-Oncology.

Acknowledgements

C Van Themsche was holder of post-doctoral fellowships from the Cancer Research Society (CRS) and the Fonds de la recherche en santé du Québec (FRSQ). C Descoteaux holds a Canada Graduate Doctoral Scholarship from NSERC.

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