Insulin-like growth factor-I receptor (IGF-IR) targeting with monoclonal antibody cixutumumab (IMC-A12) inhibits IGF-I action in endometrial cancer cells

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ARTICLE INFO
Article history:
Received 21 November 2010
Received in revised form 22 February 2011
Accepted 25 February 2011
Available online 28 March 2011

Keywords:
Insulin-like growth factor-I (IGF-I)
IGF-I receptor
Endometrial cancer
Targeted therapy
Cixutumumab

ABSTRACT
Specific insulin-like growth factor-I receptor (IGF-IR) targeting emerged in recent years as a promising therapeutic strategy in cancer. Endometrial cancer is the most common gynecological cancer in the Western world. The aim of this study was to evaluate the potential of cixutumumab (IMC-A12, ImClone Systems), a fully human monoclonal antibody against the IGF-IR, to inhibit IGF-I-mediated biological actions and cell signalling events in four endometrial carcinoma-derived cell lines (ECC-1, Ishikawa, USPC-1 and USPC-2). Our results demonstrate that cixutumumab was able to block the IGF-I-induced autophosphorylation of the IGF-IR. In addition, the PI3K and MAPK downstream signalling pathways were also inactivated by cixutumumab in part of the cell lines. Prolonged (24 h and 48 h) exposures to cixutumumab reduced IGF-IR expression. Furthermore, confocal microscopy of GFP-tagged receptors shows that cixutumumab treatment led to IGF-IR redistribution from the cell membrane to the cytoplasm. Antiapoptotic effects were evaluated by cleavage of caspase 3 and PARP, and mitogenicity and transformation by proliferation and cell cycle assays. Results obtained showed that cixutumumab abrogated the IGF-I-stimulated increase in proliferation rate, and increased caspase-3 and PARP cleavage, two markers of apoptosis. Of importance, cixutumumab had no effect neither on insulin receptor (IR) expression nor on IGF-I activation of IR. In summary, in a cellular model of endometrial cancer cixutumumab was able to inhibit the IGF-I-induced activation of intracellular cascades, apoptosis and proliferation.

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1. Introduction
Insulin-like growth factors (IGF)-I and IGF-II belong to a family of mitogenic growth factors, binding proteins and receptors that are involved in normal growth and differentiation of most tissues and organs. The IGF system is also implicated in numerous pathological states, including disrupted growth conditions and cancer.1,2 The biological actions of both IGF-I and IGF-II are mediated by the IGF-1 receptor (IGF-IR), a transmembrane heterotetramer that signals mitogenic, antiapoptotic and transforming activities.3–5 The IGF-IR harbours a tyrosine kinase domain in its cytoplasmic portion which is...
coupled to several intracellular second messenger pathways, including the ras-raf-MAPK and PI3K signalling cascades. IGF-IR is vital for cell survival, as illustrated by the lethal phenotype of mice in which the IGF-IR gene was disrupted by homologous recombination. Examination of multiple types of tumours shows an abundant expression of IGF-IR, suggesting that up-regulation of the IGF-IR gene constitutes a common paradigm in cancer development.

Endometrial cancer is the most widespread gynaecologic cancer in Western countries, accounting for 6 percent of all cancers in women. Women have an approximate 2.5 percent lifetime risk of developing endometrial cancer. The National Cancer Institute estimates that 42,160 new cases have been reported in 2009 in the United States alone. The incidence of the disease has been increasing in recent years, presumably as a result of the growing obesity epidemic. In the uterus, cyclic changes in IGF-I expression and signalling play an important role in regulating the transition of the premenopausal endometrium through proliferative, secretory and menstrual cycles, and plays a pivotal role in the process of pausal endometrium through proliferative, secretory and an important role in regulating the transition of the premenopausal endometrium through proliferative, secretory and menstrual cycles, and plays a pivotal role in the process of pausal endometrium through proliferative, secretory and menstrual cycles, and plays a pivotal role in the process of pausal endometrium through proliferative, secretory and menstrual cycles.

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Endometrial cancers are classified into two major categories, Type I and Type II, with Type I tumours being the most frequent (~80% of cases). Type I tumours are usually oestrogen-dependent, low-grade neoplasms, with an endometroid, well-differentiated morphology and are generally associated with a relatively good prognosis. Type II tumours, on the other hand, are usually diagnosed at an advanced age, are not associated with exposure to oestrogens, display a less differentiated phenotype and have a worst prognosis. Uterine serous papillary endometrial carcinoma (USC) constitutes the predominant histological class among Type II tumours. USC is diagnosed at an advanced stage and accounts for 50% of all endometrial cancer relapses, with a 5-year survival rate of 55%. While early-stage endometrial cancers are highly curable (mostly by surgery alone), the prognosis of invasive and metastatic endometrial cancer is poor. Invasive and metastatic endometrial cancers are highly curable (mostly by surgery alone), the prognosis of invasive and metastatic endometrial cancer is poor.

IGF-IR targeting is emerging as a very active area in cancer therapeutics. IGF-IR targeting is expected to result in: (1) inhibition of IGF-I expression; (2) blockade of ligand–receptor interaction; and/or (3) impairment of receptor activation. No targeted therapy against the IGF-IR, however, has been implemented in endometrial cancer. Cixutumumab (IMC-A12) is a fully human antibody that binds to the IGF-IR with high affinity (4.1 × 10⁻¹¹ M) and inhibits ligand binding with an IC₅₀ of 0.6–1 nM. Cixutumumab was shown to inhibit IGF-I- and IGF-II-stimulated proliferation in different cell types and xenograft tumour models with very high efficiency and specificity. Previous studies have demonstrated that cixutumumab induces apoptosis in human cancer cells by two distinct mechanisms: first, by interfering with IGF-I binding to the receptor, and second, by mediating IGF-IR internalisation and degradation. In view of the important role of the IGF-IR in endometrial cancer biology, the aim of the present study was to evaluate the antiproliferative potential of cixutumumab as a targeted therapy approach in Type I and Type II endometrial cancers.

2. Materials and methods

2.1. Anti-IGF-IR antibody

Cixutumumab (IMC-A12) (ImClone Systems, New York) is a fully human antibody antagonist to the human IGF-IR. The generation and characterisation of cixutumumab has been described.

2.2. Endometrial cancer cell lines

The ECC-1 and Ishikawa cell lines were employed as typical Type I (endometroid) endometrial cancer cell lines. The USC-1 and USC-2 cell lines were employed as typical Type II cell lines. The ECC-1 and Ishikawa cell lines were obtained from Dr. Y. Sharoni, Ben Gurion University, Beer-Sheba, Israel and the USC cell lines were provided by Dr. A.D. Santin, Yale University School of Medicine, New Haven, CT, USA. ECC-1 and Ishikawa cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 4.5 g/l D-glucose (Gibco BRL, Paisley, Scotland) and USC cells were maintained in RPMI-1640 medium (Biological Industries, Kibbutz Haemek, Israel). Both media were supplemented with 10% foetal bovine serum (FBS), 2 mM glutamine, 50 mg/ml gentamicin sulphate and 5.6 mg/l fungizone. In all of the experiments, cells were treated with IGF-I (50 ng/ml) (Cytolab Ltd., Rehovot, Israel), in the absence or presence of cixutumumab (10 µg/ml).

2.3. RT-PCR for IGF-IR mRNA expression

Total RNA was prepared from endometrial cancer cell lines using the Trizol reagent (Sigma–Aldrich). 2.5 µg of total RNA was reverse transcribed and amplified by PCR. The primers used for IGF-IR mRNA were: sense, GAA-GTG-GAA-CCC-TCC-CTC-TC; antisense, CTT-CTC-GGC-TTC-AGT-TTT-GG. The size of the band was 275 bp. For control purposes, levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA were measured using the following primers: sense, 5’-ACCACAGTCCATGCCATCAC-3’; antisense, 5’-TCCAC-CACCCTGTTGCTGTGTA-3’. The size of the amplified GAPDH mRNA band was 452 bp.

2.4. Western blots

Cells were serum starved overnight and then incubated with cixutumumab (10 mg/ml), in the presence or absence of IGF-1. After incubation, cells were harvested and whole cell lysates were prepared. Samples were subjected to 10% SDS-PAGE, followed by electrophotoretic transfer of the proteins to nitrocellulose membranes. After blocking with 3% bovine serum albumin or fat-free milk in 20 mM Tris–HCl (pH 7.5), 155 mM...
ECC-1 and USPC-2 cells were plated on cover slips in 6-well plates for cell cycle analysis, cells were seeded in 6-well plates (2 x 10^5 cells/well) for 24 h. Cells were transfected using the JetPEI reagent (Polyplus Transfection, Illkirch, France), according to manufacturer’s recommendations, with a plasmid containing an IGF-IR cDNA fused to a green fluorescence protein (GFP) (1 µg) marker. The IGF-IR-GFP cDNA plasmid was provided by Prof. Rosemary O’Connor (University of Cork, Ireland). After 48 h cells were treated with cixutumumab for 60 min and washed with PBS. Fixation was performed with 100% methanol for 20 min at 4 °C and washed with PBS. Cells were dyed with DAPI (5 µg/ml) (Sigma–Aldrich), for 5 min and cover slips were mounted on microscope slides. Imaging was done using a Leica SP5 confocal microscope (Wetzlar, Germany).

2.9. Statistical analysis

The statistical significance of the differences observed between groups was assessed using the t-test (two samples, equal variance). p < 0.05 was considered statistically significant.

3. Results

3.1. IGF-IR is expressed in cell lines of endometrial carcinoma

To evaluate the expression of IGF-IR in different types of endometrial carcinoma, protein levels were assessed in four different cell lines. The ECC-1 and Ishikawa cell lines are Type I endometrial carcinoma cells whereas the USPC-1 and USPC-2 cell lines are Type II uterine serous papillary carcinoma. Results of Western blots show that IGF-IR is robustly expressed in all endometrial cancer cell lines (Fig. 1). Protein levels are higher in ECC-1 and USPC-1 as compared to Ishikawa and USPC-2 cell lines. Results of RT-PCR assays show that IGF-IR mRNA levels correlated with the protein levels (data not shown).

3.2. Cixutumumab blocks phosphorylation of the IGF-IR and associated downstream signalling mediators

To evaluate the ability of cixutumumab to block activation of the IGF-IR as well as the associated downstream signalling pathways, cells were incubated with IGF-1, in the presence or absence of cixutumumab. Western blots were probed with antibodies against phosphorylated and total IGF-IR as well as the associated downstream signalling mediators.

Fig. 1 – IGF-IR expression in endometrial cancer cell lines. Western blot analysis of IGF-IR levels in endometrial cancer cell lines ECC-1, Ishikawa, USPC-1 and USPC-2. Cells were lysed and extracts were electrophoresed through SDS-PAGE, followed by transfer and incubation with an anti-IGF-IR β-subunit.
downstream signalling mediators (ERK and AKT, lower four rows) was variable. Thus, in ECC-1 and USPC-1 cells baseline phosphorylation levels were low, while treatment with IGF-I caused a pronounced phosphorylation that was abrogated by cixutumumab. In Ishikawa and USPC-2 cells baseline phosphorylation was high and IGF-I treatment did not cause any further increase in phosphorylation nor did cixutumumab reduce it. Given that cixutumumab is a monoclonal IgG1 antibody we next examined the specificity of IGF-IR blockade. To this end, cells were incubated with IGF-I as described above and cixutumumab was replaced by polyclonal IgG1 treatment. Results obtained showed that IGF-IR and its downstream signalling pathways were not blocked by non-specific IgG1 (Fig. 2B). Given that IGF-IR levels are more robust in the ECC-1 and USPC-1 cell lines, most of our next experiments were carried out in these two cell lines.

Fig. 2 – Effect of cixutumumab on the IGF-I-stimulated IGF-IR, AKT and ERK phosphorylation. (A) ECC-1, Ishikawa, USPC-1 and USPC-2 cells were treated with cixutumumab (IMC-A12) for 2 h, in the presence of IGF-I during the last 10 min of the incubation period. At the end of the incubation period, cells were lysed and the levels of phosphorylated and total proteins were measured by Western blot analysis. For phospho-IGF-IR measurement, cell extracts were immunoprecipitated with an IGF-IR antibody, electrophoresed through SDS-PAGE, and immunoblotted with anti-phosphotyrosine. AKT and ERK phosphorylation were measured using specific anti-phospho antibodies. The figure shows the results of a typical experiment, repeated four times with similar results. (B) Cells were incubated with IgG1 for 1 h, in the presence of IGF-I during the last 10 min of the incubation period. At the end of the incubation period, cells were lysed and processed as described above.
3.3. Cixutumumab induces IGF-IR internalisation

To elucidate the mechanism responsible for cixutumumab abrogation of IGF-IR phosphorylation, cells were transfected with a GFP tagged IGF-IR vector. Cells were visualised in the presence or absence of cixutumumab using confocal microscopy. Results obtained show that in the absence of cixutumumab IGF-IR is expressed mainly in the cell membrane (Fig. 3, left panel, representative pictures from two cell lines, ECC-1 and USPC-2). Treatment with cixutumumab caused translocation of the IGF-IR to the cytoplasm. Taken together, these findings suggest that cixutumumab binds directly to the receptor causing it to change its conformation/cellular localisation.

3.4. Cixutumumab does not block IGF-I activation of the insulin receptor

The insulin receptor (IR) closely resembles the IGF-IR in structure and function. Furthermore, both ligands (insulin and IGF-I) are able to activate both the IR and the IGF-IR, with variable affinities. To evaluate the ability of cixutumumab to block IR phosphorylation, cells were treated with IGF-I and cixutumumab as described above. Western blots of phosphorylated and total IR show that IGF-I stimulated IR phosphorylation, however, this effect was not abrogated by cixutumumab treatment (Fig. 4).

3.5. Cixutumumab abrogates IGF-I action on cell proliferation, apoptosis and cell cycle progression

The IGF-IR plays a key role in regulating growth, resistance to apoptosis and differentiation. To address the potential impact of cixutumumab treatment on IGF-I-stimulated cell proliferation ECC-1 and USPC-1 cells were plated in serum-containing media, and after 24 h media was changed to serum-free (starvation) media, with or without IGF-I and cixutumumab. Cell proliferation was examined after 72 h by MTT assays. Addition of IGF-I to ECC-1 cells significantly enhanced proliferation and cell number almost doubled after 24 h (Fig. 5A, upper panel). Addition of the antibody on top of IGF-I completely abolished the stimulatory effect of IGF-I and cell proliferation returned to baseline values (dotted line). USPC-1 cells grew slower than ECC-1 cells (Fig. 5A, lower panel). IGF-I treatment caused a slight increase in proliferation rate and addition of cixutumumab had a marked influence on proliferation (dotted line), lowering proliferation rate to below baseline levels.

To evaluate the effect of cixutumumab on IGF-I-induced protection from apoptosis, we examined the cleavage of caspase-3.
Fig. 5 – Effect of cixutumumab on endometrial cancer cells proliferation and apoptosis. (A) ECC-1 and USPC-1 cells were plated in 24-well plates at a density of $2 \times 10^4$ cells/well. After 24 h the medium was changed to serum-free medium, including or lacking IGF-I and cixutumumab. The number of cells at time 0 was assigned a value of 100%. The bars represent the mean ± S.E.M. of four independent experiments. *$p < 0.05$ versus time 0. (B) Endometrial cancer cell lines were serum starved for 24 h, after which they were treated with IGF-I (50 ng/ml) for 24 h in the absence or presence of cixutumumab. At the end of the incubation period cells were lysed and levels of poly (ADP-ribose) polymerase (PARP) (upper panel) and caspase-3 cleavage (lower panel) were measured by Western blots. The figure shows the results of a typical experiment repeated three times with similar results.
pase-3 and PARP. Baseline levels of cleaved PARP and caspase-3 were higher in ECC-1 than in USPC-1 (Fig. 5B, left column in each group). IGF-I treatment (middle columns) caused a reduction in the levels of the cleaved protein. Treatment with cixutumumab significantly increased the cleavage of caspase-3 and PARP. This effect was slightly more pronounced in ECC-1 than in USPC-1 cells.

To examine the effect of cixutumumab on cell cycle progression, cells were incubated in the presence or absence of IGF-I and cixutumumab, stained with propidium iodide and measured by flow cytometry. The table shows the percentage of cells at the different cell cycle phases. The results represent the values of a typical experiment, repeated three times.

<table>
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<th>IGF-I (50 ng/ml)</th>
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<td>49</td>
<td>58</td>
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<tr>
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<tr>
<td>G2/M</td>
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<td>42.7</td>
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<tr>
<td>USPC-1 cells</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>G0/G1</td>
<td>51.9</td>
<td>50.1</td>
<td>52.5</td>
</tr>
<tr>
<td>S</td>
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<td>5.6</td>
<td>3.1</td>
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<tr>
<td>G2/M</td>
<td>43.8</td>
<td>44.3</td>
<td>44.4</td>
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Fig. 6 – Effect of cixutumumab on IGF-IR and IR expression. Endometrial cancer cell lines were treated with cixutumumab for 24 h and 48 h. At the end of the incubation period cells were lysed and levels of IGF-IR (A) and IR (B) were measured by Western blots.
analysed using a flow cytometer. Results obtained showed that in ECC-1 cells treatment with IGF-I caused the cells to advance from G<sub>0</sub> to S phase (2.7% versus 8.3% in S phase), without any change in the proportion of cells at G<sub>2</sub>M. Addition of cixutumumab caused an increase in the proportion of cells that are halted at G<sub>1</sub> phase (49.1% versus 57.5% in IGF-I treated cells in the absence versus presence of cixutumumab) (Table 1). The same trend was observed in USPC-1 cells. In these cells, however, there were not statistically significant differences between IGF-I and IGF-I + cixutumumab treatments.

### 3.6. Cixutumumab treatment downregulates IGF-IR expression

Finally, we tested the hypothesis that prolonged treatment with cixutumumab leads to downregulation of IGF-IR expression. Western blots show that after exposure to cixutumumab for 24 h and 48 h IGF-IR expression was largely reduced in antibody-treated cells as compared to untreated cells (Fig. 6a). In contrast, IR levels were not altered by cixutumumab treatment (Fig. 6b).

### 4. Discussion

Extensive evidence has suggested that excessive cellular signalling induced by the IGF-IR is linked to cancer development. Accordingly, pharmaceutical targeting of this signalling pathway could be beneficial for the treatment of cancer. Recently, a phase II study showed that addition of anti-IGF-IR antibodies to chemotherapy in advanced non-small-cell lung cancer is safe and more efficacious than chemotherapy alone. At least a dozen drugs targeting the IGF-IR axis are now under clinical investigation. The human anti-IGF-IR antibody cixutumumab (A12) was generated through screening of a Fab phage library. Cixutumumab binds to the IGF-IR with high affinity and inhibits ligand binding. Phase I trials assessing cixutumumab are being conducted in patients with refractory tumours. To our knowledge, endometrial carcinoma, the most common gynaecological cancer, has not yet been investigated as a possible target for IGF-IR inhibitors. Our results demonstrate that cixutumumab is able to block the IGF-I-induced autophosphorylation of the IGF-IR. Furthermore the PI3K and MAPK downstream signalling pathways are also inactivated by cixutumumab. We tested four different cell lines: ECC-1 and Ishikawa (Type I endometroid carcinoma) and USPC-1 and USPC-2 (Type II endometroid carcinoma). All of the cell lines expressed IGF-IR on their surface, however, ECC-1 and USPC-1 expressed higher levels of the receptor than Ishikawa and USPC-2. Historically, it was shown that tumours that do not express receptors on their surface are not reactive to treatment with antagonists (as is the case with tamoxifen and oestrogen receptor negative breast tumours).

In addition to a reduced IGF-IR expression, treatment with cixutumumab did not affect the phosphorylation of ERK and AKT in USPC-2 and Ishikawa cells. PTEN is often mutated in Type I endometrial carcinoma while p53 overexpression and mutation are found in advanced disease and Type II endometrial cancer. Ishikawa cells are known to have a PTEN mutation that causes constitutive phosphorylation of AKT. It is possible that the reduced levels of IGF-IR in Ishikawa and USPC-2 ‘pushed’ the cells to develop alternative mechanisms to activate the MAPK and PI3K pathways that could not be blocked at the level of the IGF-IR.

Results of prolonged (24 h and 48 h) exposures to cixutumumab suggest that the inhibitor not only blocks IGF-IR function but also reduces IGF-IR expression. Confocal microscopy of GFP-tagged receptors shows that after treatment with cixutumumab, IGF-IRs are redistributed from the cell membrane to the cytoplasm. It is possible that by binding to the receptor, cixutumumab causes internalisation of the receptor complex and thus targets it for degradation. This may lead to an augmentation of the therapeutic effects. Longer exposure periods in an in vivo model are needed to determine if these findings have clinical ramifications. Clinically, some of the patients receiving cixutumumab developed hyperglycaemia and, therefore, concerns were raised regarding the specificity of cixutumumab to IGF-IR. We did not observe any effect of cixutumumab neither on IGF-I-induced IR phosphorylation nor on IRS-1 expression.

IGF-I is responsible for a variety of cell-specific functions, including regulation of hormone synthesis and secretion, chemoattractant migration and neuromodulation. IGF-I also participates in cell recognition by the immune response. All of these effects could potentially be influenced by IGF-IR antagonism in the clinical setting. In our model we tested effects we believe to be of paramount importance to a potential therapeutic agent. Antiapoptotic effects were evaluated by cleavage of caspase 3 and PARP, and mitogenicity and transformation by proliferation and cell cycle assays. Interestingly, cixutumumab affects ECC-1 cells (Type I) to a different degree than USPC-1 cells (Type II). These differences may be linked to other factors and/or pathways that interact with the IGF system. Oestrogen exposure is a known risk factor for development of Type I, but not Type II, endometrial cancer. Activation of the oestrogen receptor causes internalisation of the receptor complex and association with DNA. The oestrogen receptor complex recruits transcription factors and regulates the expression of genes involved in cell proliferation and differentiation, mainly up-regulating the expression of cyclin D1 which, in turn, regulates the activity of cyclin-dependent kinases leading to hypophosphorylation of Rb and arrest in the G1 phase of the cell cycle. Oestrogen and IGF-I are synergistic in their action as they differentially regulate c-myc and cyclin D1 to cooperatively stimulate proliferation. In the absence of oestrogen, IGF-I can activate the oestrogen receptor and increase the expression of its target genes. Furthermore IGF-IR gene transcription is controlled by oestrogen receptor activation. It is possible that the effect of cixutumumab on ECC-1 (oestrogen dependent) is not as pronounced as it is on proliferation and cell cycle progression on USPC-1 cells, because of the cross-talk of the IGF and oestrogen pathways. A better understanding of the oestrogen-IGF system interaction could be beneficial in development of novel therapies. In addition, a recent study has shown that the ability of a selective IGF-IR inhibitor (NVP-AEW541, Novartis) to arrest cell cycle progression and reduce in vitro migration of breast cancer cells was largely diminished in cells with reduced insulin receptor substrate-1 (IRS-1) expression.
In summary, our results suggest that in a cell line model of endometrial cancer, cixutumumab is able to bind specifically and cause internalisation of IGF-IR, thus inhibiting the IGF-1-induced activation of intracellular cascades, apoptosis and proliferation. Taken together, these results suggest that cixutumumab may be of benefit in the treatment of endometrial cancer.

Conflict of interest statement

None declared.

Acknowledgements

This work was performed in partial fulfilment of the requirements for a Ph.D. degree by Zohar Attias-Geva in the Sackler Faculty of Medicine, Tel Aviv University. We thank Drs. A.D. Santin, Y. Sharoni and R. O’Connor for cell lines and reagents. The authors wish to thank the Israel Cancer Research Fund (ICRF, Montreal, Canada) for their generous support.

REFERENCES


