The effect of imatinib mesylate (Glivec) on human tumor-derived cells

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Imatinib mesylate is a specific inhibitor of the Bcr–Abl protein tyrosine kinase that competes with ATP for its specific binding site in the kinase domain. It has activity against platelet-derived growth factor receptor alpha and beta (PDGFR-α and -β), and c-kit, the receptor for stem cell factor. We have used a standardized ATP-tumor chemosensitivity assay and immunohistochemistry to determine the cytotoxicity of imatinib mesylate in tumor-derived cells from cutaneous and uveal melanoma, and ovarian carcinoma. Imatinib mesylate was tested at concentrations ranging from 2.0 to 0.0625 μmol/l alone and in combination with a cytotoxic drug (cisplatin, doxorubicin, paclitaxel or treosulfan). Imatinib mesylate showed low inhibition ($Index_{SUM} > 300$) across the range of concentrations tested in this study, with few tumors exhibiting increasing inhibition with increased drug concentration. The median IC_{90} values for cutaneous and uveal melanoma and ovarian carcinoma were 13.2 μmol/l (4.0–294.3 μmol/l), 12.0 μmol/l (2.0–285.4 μmol/l) and 7.71 μmol/l (6.51–11.02 μmol/l), respectively. Imatinib mesylate potentiated the effect of different cytotoxics in 9% (5/54) of cases and had a negative effect in 13% (7/54) of cases, with no effect in the remainder. No correlation of effect was noted with c-kit, platelet-derived growth factor receptor-α or platelet-derived growth factor receptor-β expression, assessed by immunohistochemistry. The signaling pathways mediated by activation of c-kit or platelet-derived growth factor receptor may act as antiapoptotic survival signals in some cancers and inhibition of these pathways may potentiate the activity of some cytotoxic drugs by inhibiting the survival signal. Growth inhibition, however, may reduce the efficacy of cytotoxic drugs, which tend to target proliferating cells preferentially, and clinical effects are therefore difficult to predict. *Anti-Cancer Drugs* 17:649–655 © 2006 Lippincott Williams & Wilkins.

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Introduction

Imatinib mesylate (STI571, Gleevec or Glivec; Novartis, Basel, Switzerland) is a specific inhibitor of the Bcr–Abl protein tyrosine kinase and functions by competing with ATP for its specific binding site in the kinase domain. It was originally developed for the treatment of chronic myelogenous leukemia (CML) in which the molecular pathogenesis of the disease depends on the formation of the *bcr–abl* oncogene, leading to the constitutive expression of the tyrosine kinase fusion protein, Bcr–Abl [1]. It also has activity against platelet-derived growth factor receptor (PDGFR; IC_{50} 0.1 μmol/l) and c-kit (IC_{50} 0.15 μmol/l), the receptor for stem cell factor (SCF) [2]. In the UK, it is licensed for use in newly diagnosed CML, in which bone marrow transplant is not considered the first line of treatment, and also in c-kit-positive ($CD117^+$) unresectable or malignant gastrointestinal stromal tumors (GIST).

Platelet-derived growth factor (PDGF) is a powerful mitogen for cells of mesenchymal origin. It exerts its effects by binding and stimulating autophosphorylation of two structurally related protein tyrosine kinases, PDGFR-α and PDGFR-β. Following autophosphorylation, PDGFR is able to bind signal transduction molecules containing SH2 domains; this includes phospholipase C-γ, phosphatidylinositol 3′-kinase (PI3K), the Src family of tyrosine kinases, the tyrosine phosphatase SH-2, a GTPase activating protein for Ras, and members of the transcription factor signal transducers and activators of transcription (STAT) family [3]. Activation of these signal transduction molecules mediates cellular responses

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including cell proliferation, cell growth, differentiation, motility and survival. A recent study by Matei *et al.* [4] demonstrated that imatinib inhibited the growth of ovarian cancer cell lines (C272-hTert/E7, C889h/Tert, CSOC848, CSOC908 and CSOC918) in a PDGFR-specific manner at clinically relevant concentrations (IC_{50} <1 μmol/l).

Stimulation of c-kit (also known as CD117 or stem cell factor receptor) by its ligand, stem cell factor, activates many signal transduction pathways including JAK/STAT, mitogen-activated protein kinase and PI3K [5]. It is expressed in a number of tumor types including mastocytosis/mast cell leukemia, germ cell tumors, small cell lung cancer, GIST, acute myelogenous leukemia, neuroblastoma, melanoma, and ovarian and breast cancers. In all these tumor types excluding mastocytosis/mast cell leukemia and GIST, the role of c-kit expression in the pathogenesis of the tumor is poorly defined and rarely related to a mutation of c-kit [6,7]. Ning *et al.* [8] demonstrated that in leukemia cells, an activating mutation of c-kit at codon 816 (Asp816) induced constitutive activation of STAT3 and STAT1 and upregulated the downstream targets, Bcl-X_L and c-*myc*. The mutation also caused the PI3K/Akt pathway but not the Ras-mediated mitogen-activated protein kinase pathway to be constitutively activated. Evidence in melanoma suggests that c-kit may act as a growth gene [9], although activating mutations have not been identified. One study reported that c-kit expression progressively decreases during local tumor growth and invasion of human melanomas [10].

Evidence from cell line experiments suggests that imatinib mesylate may be useful as a chemosensitizing agent in combination with cytotoxic chemotherapy. In-vitro experiments by Zhang *et al.* [11] demonstrated that the lung cancer cell line, A549, was resistant to cisplatin; however, addition of imatinib to cisplatin-treated A549 cells resulted in a synergistic cell-killing effect.

Although imatinib has disappointing single-agent clinical activity in both melanoma (which commonly expresses c-kit and PDGFR) and ovarian cancer (which commonly expresses PDGFR), there have been multiple reports suggesting that it has activity against cell lines derived from these cancers [4,12–15]. We therefore wondered whether there might be activity in combination with other drugs used in these tumors. The following experiments were undertaken to determine the cytotoxicity of imatinib mesylate alone and in combination with chemotherapeutic drugs in cutaneous and uveal melanoma tumors and ovarian carcinoma tumor-derived cells as examples of tumors likely to express c-kit, PDGFR-α and PDGFR-β. We also wished to examine whether the protein level of c-kit, PDGFR-α and PDGFR-β showed any correlation with the activity of imatinib in tumor-derived cells.

Materials and Methods

Tumors

A total of 42 tumors (29 from female patients and 13 from male patients) were tested in the study and the individual tumor types are described in Table 1. Of the cutaneous melanoma samples ($n = 17$), one patient had been previously treated with vinorelbine, 15/17 had no previous treatment and for one patient there was no further clinical data available; the median age was 56 (27–83) years. Of the uveal melanoma samples ($n = 14$), all 14 patients had no previous treatment; the median age was 63 (25–81) years. Of the ovarian carcinomas ($n = 11$), 6/11 were recurrent stage 3/4 cancers and had been previously treated with either single-agent platinum therapy or platinum plus taxane therapy; 3/11 were primary tumors and had no previous treatment, and for two samples there were no clinical data available; the median age was 64 (49–78) years.

In each case, only tumor material not required for diagnosis was sent for ATP-tumor chemosensitivity assay (ATP-TCA), and in all cases consent had been obtained and permission had been granted by the local ethics committee.

ATP-tumor chemosensitivity assay

The ATP-TCA was performed as previously described [16,17]. Briefly, cells obtained from enzymatic dissociation of solid tumor material or ascites were plated in 96-well polypropylene plates (Corning Life Sciences, High Wycombe, UK) at 20 000 or 10 000 cells/well, respectively, in a serum-free complete assay medium (CAM; DCS Innovative Diagnostik Systeme, Hamburg, Germany). Drugs were added to triplicate wells at serial dilutions corresponding to 200–6.25% of a test drug concentration (TDC) estimated from pharmacokinetic data, including the degree of protein binding. All TDCs were within clinically achievable levels. Two controls were included in each plate: a no-drug (M0) control consisting of media alone and a maximum inhibitor (MI) control that killed all cells present. The plates were incubated for 6 days at 37°C with 5% CO_2. At the end of the incubation period, the remaining cells were lysed by addition of cell extraction reagent (DCS Innovative Diagnostik Systeme). An aliquot of the lysate from each well was added to the corresponding wells of a white 96-well microplate (Thermo Life Sciences, Basingstoke,

Table 1 Patient characteristics and tumor types tested in the imatinib mesylate study

Tumor type	*N*	Median age (range)	Sex
Cutaneous melanoma	17	56 (27–83)	10F:7M
Uveal melanoma	14	63 (25–81)	8F:6M
Ovarian carcinoma	11	64 (49–78)	11F:0M
Total	42	61 (25–83)	29F:13M

UK), followed by addition of luciferin–luciferase reagent. The light output corresponding to the level of ATP present was measured in a luminometer (MPLX; Berthold Diagnostic Systems, Hamburg, Germany). These data were transferred automatically to an Excel spreadsheet in which the percentage inhibition achieved at each concentration tested was calculated using the equation $1-(\text{test}-\text{MI})/(\text{M0}-\text{MI})\times 100$. To allow a comparison of results between tumors, a simple logarithmic sum index ($\text{Index}_{\text{SUM}}$) was derived by summing the percentage inhibition at each level of TDC tested as $\text{Index} = 600 - \text{Sum}[\text{Inhibition}_{6.25\ldots200}]$. Total inhibition resulted in an index of 0 and no inhibition at any concentrations produced an index of 600 [18]. In cases where negative values were obtained for percentage inhibition, the $\text{Index}_{\text{SUM}}$ value could be above 600. Area under the concentration–inhibition curve ($\text{Index}_{\text{AUC}}$) was calculated from the data by using the trapezoidal rule.

Drugs

The cytotoxic drugs used in the assay were obtained as vials for injection and made up according to the manufacturer's instructions. Treosulfan, doxorubicin and imatinib mesylate were stored in aliquots at -20°C, whereas cisplatin and paclitaxel were stored at room temperature [19]. Table 2 shows the 100% TDC for each of the drugs used. Doxorubicin was tested at three times its normal concentration (doxorubicin $\times$ 3) to represent liposomal doxorubicin. Drug combinations were tested by combining single agents, but no sequential studies were carried out. Glivec (kindly provided by Novartis) was tested at concentrations ranging from 2.0 to 0.0625 µmol/l.

Data analysis

The results were entered into an Access database for further analysis. Statistical tests were performed using non-parametric methods.

Immunohistochemistry

Tissue was available for immunohistochemical staining with c-kit (M7183; Dako, Ely, UK), PDGFR-α (MAB322; R&D Systems, Abingdon, UK) and PDGFR-β (610113; BD Biosciences, Oxford, UK) in 64% (27/42) of cases comprising seven ovarian carcinomas, eight cutaneous melanomas and 12 uveal melanomas. Paraffin-embedded sections 4-µm thick were dewaxed and rehydrated in preparation for immunohistochemical staining. Endogenous peroxidase was blocked using 3% hydrogen peroxide in methanol. For immunohistochemical studies on cutaneous and uveal melanoma tissue, the Vectastain ABC-AP protocol (AK-5200; Vector Laboratories, Peterborough, UK) and fuchsin substrate-chromagen (K0624; Dako) was used. For immunohistochemical studies on ovarian tissue, the immunoperoxidase secondary detection system (Det-HP1000; Chemicom International, Eastleigh, UK) and 3,3′-diaminobezidine chromagen (DAB; 4170; HD Supplies, Aylesbury, UK) were used. Monoclonal antibody for c-kit was used at a dilution of 1:200 and incubated with sections for 30 min at room temperature. Monoclonal antibody for PDGFR-α was used at a dilution of 1:25 and incubated with sections for 1 h at room temperature. PDGFR-β was used at a dilution of 1:75 and incubated with sections for 30 min at room temperature. Positive (GIST) and negative controls were included in each staining run.

Table 2 Drug concentrations used in the ATP-tumor chemosensitivity assay

Drug	Test drug concentration (µmol/l)
Cisplatin	10.00
Doxorubicin × 3	2.59
Imatinib mesylate	1.00
Paclitaxel	15.93
Treosulfan	71.9

Samples were assessed by a pathologist using the H-score. Intensity was graded on a scale ranging between 0, +, + + and + + + (where + = weak staining, + + = moderate and + + + = intense), and the proportion of cells stained at the highest intensity. The two values were then multiplied together to give the final value between 0 and 300.

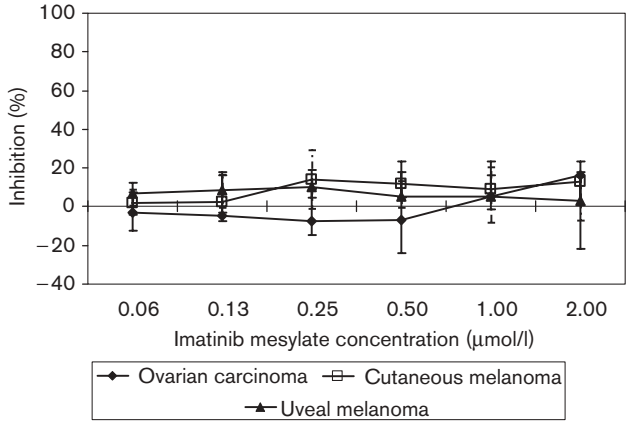
Results

Imatinib mesylate showed low inhibition ($\text{Index}_{\text{SUM}} > 300$) across the range of concentrations tested in this study, with few tumors exhibiting increasing inhibition with increased drug concentration. Twelve percent (2/17) of the cutaneous melanoma samples and 7% (1/14) of the uveal melanoma samples exhibited considerable inhibition (above 50% inhibition at 100% TDC), with the majority exhibiting low inhibition (Fig. 1). The response to imatinib mesylate was heterogeneous between the tumor types tested and within the same tumor type, as shown in Fig. 2. To compare sensitivity and resistance of imatinib mesylate between different tumor types, an $\text{Index}_{\text{SUM}} < 300$ corresponding to 50% inhibition across the range of concentrations tested was taken to indicate sensitivity and was used to compare results. On the basis of the $\text{Index}_{\text{SUM}}$ value, only one tumor, a uveal melanoma, exhibited sensitivity ($\text{Index}_{\text{SUM}} = 70$). The median IC_{90} and $\text{Index}_{\text{SUM}}$ values for each tumor type are summarized in Table 3.

In 88% (37/42) of samples (16 cutaneous melanoma, 11 uveal melanoma and 10 ovarian carcinomas), there was sufficient material available to test imatinib mesylate in combination with different cytotoxic drugs; some were tested with more than one combination.

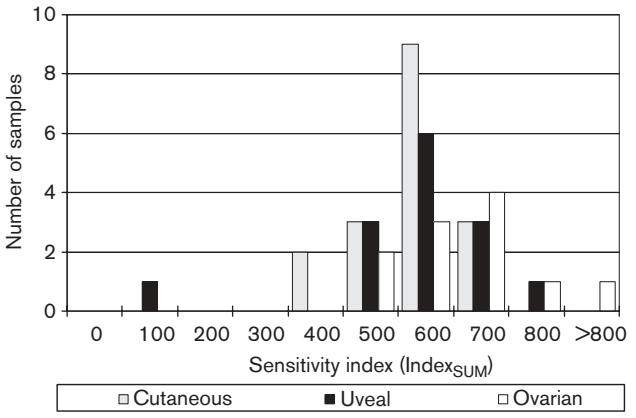
Both decreases and increases were observed in $\text{Index}_{\text{SUM}}$ values when cytotoxic drugs were combined with

Fig. 1



Median effect of imatinib mesylate on tumor-derived cells from ovarian carcinoma ($n=11$), cutaneous melanoma ($n=17$) and uveal melanoma ($n=14$) samples. Error bars show 25th and 75th interquartile range.

Fig. 2



Frequency histogram showing heterogeneity of the Index$_{SUM}$ for single-agent imatinib mesylate in ovarian carcinoma ($n=11$), cutaneous melanoma ($n=17$) and uveal melanoma ($n=14$).

Table 3 Median IC_{90} (μmol/l) and Index$_{SUM}$ values for single-agent imatinib mesylate in individual tumors ($n=42$)

Tumor type	*N*	IC_{90} (μmol/l)	Index$_{SUM}$
Cutaneous melanoma	17	13.2 (4.0–294.3)	567 (361–638)
Uveal melanoma	14	12.0 (2.0–285.4)	556 (70–716)
Ovarian carcinoma	11	7.71 (6.51–11.02)	615 (404–826)
Overall values	42	10.9 (2.0–294.3)	572 (70–826)

imatinib mesylate. All the samples tested with cisplatin plus imatinib mesylate were ovarian carcinomas; 40% (4/10) exhibited a decrease in their Index$_{SUM}$ compared with cisplatin alone; this was usually modest and only in one case constituted a change from resistance to sensitivity, with a decrease in Index$_{SUM}$ from 654 to 229 (below 300 indicates *ex vivo* sensitivity). The remaining 60% (6/10) of samples showed an increase in their Index$_{SUM}$ value, representing a change from sensitivity to resistance in one case (Index$_{SUM}$ increased from 298 to 634).

Eight ovarian carcinoma samples were tested with doxorubicin × 3 plus imatinib mesylate and 50% (4/8) showed an Index$_{SUM}$ decrease when doxorubicin × 3 was used in combination. Of the samples exhibiting a decrease in Index$_{SUM}$, three cases showed a change from resistance to sensitivity and the Index$_{SUM}$ decreased to below 300. The remaining 50% (4/8) when combined with imatinib exhibited an increase in Index$_{SUM}$ and of these, 75% (3/4) exhibited a decrease in sensitivity to doxorubicin × 3 amounting to resistance (Index$_{SUM}$ > 300).

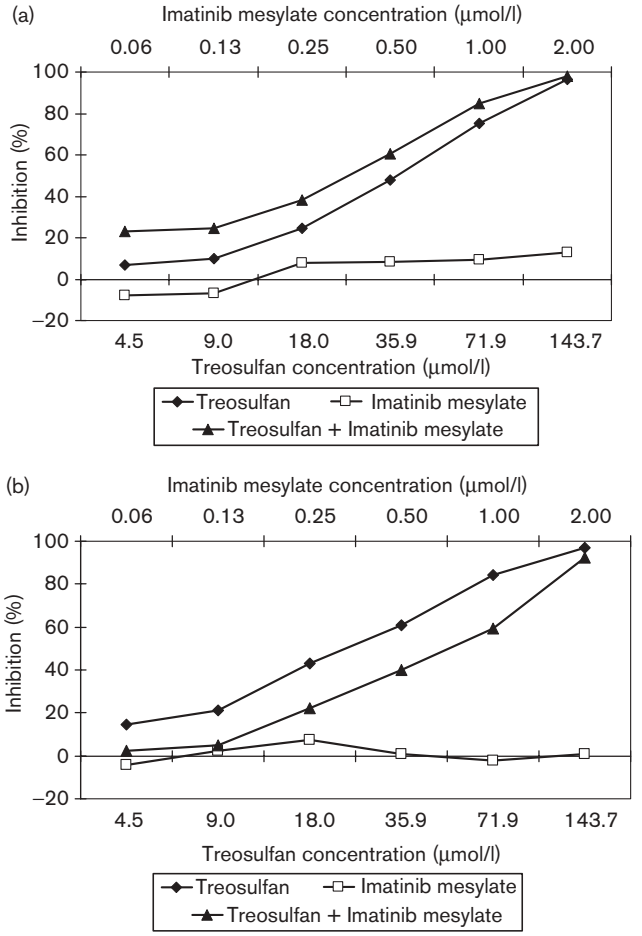
When paclitaxel was combined with imatinib mesylate in ovarian carcinomas, 56% (5/9) of samples tested exhibited a decrease in their Index$_{SUM}$; however, none of these showed a change from resistance to sensitivity to paclitaxel. The remaining 44% (4/9) showed an increase in their Index$_{SUM}$ values with one case showing resistance to paclitaxel when combined with imatinib.

Finally, 27 samples consisting of 16 cutaneous and 11 uveal melanomas were tested with imatinib mesylate plus treosulfan. Of the cutaneous melanomas, 50% (8/16) exhibited a decrease in their Index$_{SUM}$, but none of these samples showed a change from resistance to sensitivity to treosulfan when treated concurrently with imatinib. The remaining 50% exhibited an increase in Index$_{SUM}$, but none of these showed a change from sensitivity to resistance to treosulfan when used in combination with imatinib. Figure 3(a and b) shows both the positive and negative effects of imatinib mesylate in combination with treosulfan in two different cutaneous melanoma samples.

Of the 11 uveal melanomas tested with treosulfan plus imatinib, 45% (5/11) showed a decrease in their Index$_{SUM}$ with one case showing a change from resistance to sensitivity to treosulfan. The remaining 55% (6/11) showed an increase in Index$_{SUM}$, with 33% (2/6) showing a change from resistance to sensitivity toward treosulfan when combined with imatinib.

Overall, in 9% (5/54) of tumors tested, imatinib mesylate showed considerable potentiation of the effect of the cytotoxic drug, while in 13% (7/54) of tumors, the addition of imatinib mesylate resulted in a considerable decrease in sensitivity to the cytotoxic agent compared with single cytotoxic agent exposure, based on the Index$_{SUM}$ = 300 threshold.

Fig. 3



Tumor growth inhibition by imatinib mesylate versus treosulfan plus imatinib mesylate in two cutaneous melanoma samples. (a) Positive effect of combining treosulfan plus imatinib mesylate, compared with (b) where treosulfan plus imatinib mesylate has a negative effect.

Of the samples tested with single-agent imatinib mesylate, 64% (27/42) had material available for immunohistochemical staining with c-kit, PDGFR-α and PDGFR-β, consisting of seven ovarian carcinomas, eight cutaneous melanomas and 12 uveal melanomas. Three samples (one ovarian carcinoma, one cutaneous melanoma and one uveal melanoma) had no tumor in the sample selected for immunohistochemistry (IHC), resulting in an 89% (24/27) evaluability rate. Of the 24 samples, 29% (7/24) stained positive for c-kit as determined by an H-score ≥ 100, consisting of one cutaneous melanoma and six uveal melanoma samples, the remaining 71% (17/24) were negative. No ovarian carcinomas were c-kit$^+$ in this series. No correlation exists between the IC_{50}, IC_{90} or $Index_{SUM}$ values and c-kit expression (Spearman's rank correlation: $P > 0.1$ for all three parameters). Of the samples positive for c-kit, none were sensitive to single-agent imatinib mesylate as determined by an $Index_{SUM} < 300$. An increased sensitivity to treosulfan was, however, observed when this was combined with imatinib compared with treosulfan alone in 57% (4/7) of the positively stained samples and three of these had an $Index_{SUM}$, which decreased to below 300.

Of the samples stained for PDGFR-α, 8% (2/24) were not evaluable. Of the remaining 22 samples, 55% (12/22) were positive, determined by an H-score ≥ 50; of these, 14% (3/22) were weakly positive (H-score 50–100) and consisted of one cutaneous and two uveal melanomas, and 41% (9/22) were moderately positive (H-score 100–200) and consisted of one uveal and four cutaneous melanomas and four ovarian carcinomas. The remaining 45% (10/22) of samples were negative. No correlation was found between the IC_{50}, IC_{90} or $Index_{SUM}$ values and PDGFR-α expression; however, there was a significant correlation between the staining of PDGFR-α and PDGFR-β (Spearman's rank correlation: $P < 0.05$).

Of the samples stained for PDGFR-β, 67% (16/24) were positive as determined by an H-score ≥ 50. Of these, 38% (6/16) were weakly positive (H-score 50–100) and consisted of four uveal and two cutaneous melanomas; 31% (5/16) were moderately positive (H-score 100–200) and consisted of one uveal and three cutaneous melanomas and one ovarian carcinoma; and 31% (5/16) were strongly positive (H-score > 200; one cutaneous melanoma and four ovarian carcinoma samples). The remaining 33% (8/24) of samples were negative. No correlation was found between the IC_{50}, IC_{90} or $Index_{SUM}$ values and PDGFR-β expression. A significant difference was, however, observed between the staining of PDGFR-β between tumor types (Kruskal–Wallis non-parametric test: $P < 0.005$) with stronger staining observed in the ovarian carcinoma samples.

Discussion

The ATP-TCA has previously been used to test TKIs alone and in combination with cytotoxic drugs in different tumor types, despite the fact that these agents are usually cytostatic and rarely induce cell death within the 6-day assay period in tumor-derived cells [20].

Imatinib mesylate was designed specifically to act against the *bcr–abl* gene expressed in patients with CML; however, it also has activity against the PDGFR and c-kit [2]. Cytotoxic effects were not expected. Although we have observed some dose-dependent activity of single-agent imatinib mesylate against uveal melanoma, the majority of samples tested showed a flat or shallow concentration–activity curve. The observation of imatinib activity in uveal melanoma-derived cells is consistent with a previous study on uveal melanoma cell lines by Lefevre *et al.* [13], which concluded that imatinib mesylate might be useful in treating uveal melanoma,

although no clinical effects have been reported. Although this particular study is focused on melanoma and ovarian cancer, the ATP-TCA has been used to test single-agent imatinib mesylate on other tumor types, including a GIST. The particular tumor tested was strongly positive for c-kit by IHC and after exposure to imatinib mesylate a dose–response curve was observed with an $Index_{SUM}$ value of 296.

When imatinib mesylate was tested in combination with cytotoxic agents, the results were similar to those observed in the previous work with another TKI, gefitinib [20]; there were both increases and decreases in sensitivity to the cytotoxic agent it was combined with. Overall, the addition of imatinib mesylate to cytotoxic agents caused a decrease in $Index_{SUM}$ value in 48% (26/54) of cases and potentiated the effect of the cytotoxic it was combined with in 9% (5/54) of these. It, however, caused an increase in $Index_{SUM}$ in 52% (28/54) of cases and decreased sensitivity to the cytotoxic agent in 13% (7/54) of these. This suggests that it is may be important to identify those patients who will benefit from combining imatinib with a cytotoxic agent. The effects of combining imatinib with cytotoxic agents may be similar to those seen when tamoxifen is combined with cytotoxic chemotherapy, in which no significant increase in survival time is observed [21]. Similar results have been observed in the National Surgical Adjuvant Breast and Bowel Project trials [22,23].

Although heterogeneity was observed between tumors in response to single-agent imatinib, no correlation was found between the IC_{50}, IC_{90} or $Index_{SUM}$ values and expression of c-kit, PDGF-α or PDGFR-β. Of the samples that were available for IHC, 12 were uveal melanomas and, of these, 50% (6/12) were positive for c-kit expression; this compares to another study that found c-kit expression in 63% (84/134) of uveal melanomas tested [12]. In the same study, treatment of uveal melanoma cell lines with imatinib resulted in cell death with an $IC_{50} < 2.5$ μmol/l for c-kit phosphorylation and cell proliferation [21]. The median IC_{50} for uveal melanoma tumor-derived cells in this study was 6.65 μmol/l (0.04–158.6 μmol/l). The achievable C_{max} from oral imatinib ranges between 0.27 and 0.30 μmol/l [24] depending on the formulation of the drug. It therefore seems unlikely that many uveal melanoma patients would benefit from imatinib monotherapy. It has been reported that c-kit expression decreases in melanoma during local tumour growth and invasion [25], but of the eight cutaneous melanoma samples available for IHC, only one stained positively for c-kit. It may be possible that the remaining samples that were negative for c-kit expression were from patients with more advanced disease, but this requires further study. Alternatively, as a study by Gutman *et al.* [26] demonstrated, there was heterogeneity of expression of five different tyrosine kinase receptors, including c-kit expression within human melanoma cell lines, and therefore differences in c-kit expression in the cutaneous melanoma samples may be due to heterogeneity between patients.

Of the seven ovarian carcinoma samples available for IHC, one sample had no tumor present and the remaining six samples were negative for c-kit expression. Effects, however, were seen in this group when imatinib mesylate was used alone and in combination; in fact, in comparison with the two melanoma groups, the ovarian carcinoma group had lower median IC_{50} and IC_{90} values of 4.29 μmol/l (3.62–6.12 μmol/l) and 7.71 μmol/l (6.51–11.02 μmol/l), respectively. As none of the ovarian carcinoma samples were positive for c-kit, the activity observed may be explained by inhibition of the PDGFR.

Of the ovarian carcinoma samples stained for PDGFR-α and PDGFR-β, 80% (4/5) and 83% (5/6) were positive for PDGFR-α and PDGFR-β expression, respectively. The activity of imatinib in the ovarian carcinoma group is not consistent with previous findings by Matei *et al.* [4] in which imatinib inhibited the growth of ovarian cancer cells at clinically relevant concentrations ($IC_{50} < 1$ μmol/l). The differences between IC_{50} values in the Matei *et al.* [4] study and this study may be explained using ovarian cancer cell lines with high PDGFRα expression compared with the use of unselected ovarian carcinoma-derived cells in this study.

Conclusion

One conclusion from this study, and the lack of clinical activity of imatinib against melanoma and ovarian cancer, may be that cell lines are poor predictors of the effectiveness of anticancer agents, whether cytotoxic [27] or molecularly targeted agents. We have not examined the tumors studied here for the presence of activated c-kit or PDGFR, which could explain the apparent differences in activity of imatinib between cell lines and tumor-derived cells. The misleading activity of imatinib in cell lines may also be attributed to the use of serum-free medium and polypropylene plates in the ATP-TCA; these conditions are selective for neoplastic cells, but do not remove the normal cells immediately, thereby providing an environment that more closely matches the *in vivo* one [17,27].

The signaling pathways mediated by activation of c-kit or PDGFR may act as survival signals (antiapoptotic) in some cancer patients, and inhibition of these pathways may potentiate the activity of some cytotoxic drugs by inhibiting the survival signal. Growth inhibition, however, may adversely affect the response to the cytotoxic drug, leading to the negative effects observed in some of the samples when imatinib was combined with a cytotoxic drug. It is therefore important to develop suitable

methods for determining sensitivity to single-agent imatinib mesylate and imatinib in combination with other drugs in individual patients, and to take these effects into consideration when clinical trials of imatinib combinations are planned. The concentration of imatinib required to achieve inhibition of both melanoma and ovarian cancer cells is greater than that clinically achievable, suggesting that clinical benefit from single-agent imatinib treatment is unlikely in these patients.

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