# Synergistic effects of imatinib (STI 571) in combination with chemotherapeutic drugs in head and neck cancer

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**The tyrosine kinase inhibitor imatinib (STI 571; glivec) is a potent inhibitor of bcr–abl, c-kit and platelet-derived growth factor receptors. Imatinib was evaluated both alone and in combination with established chemotherapeutic agents in adenoid cystic carcinoma (ACC) primary cultures and established cell lines representing squamous cell carcinoma of the head and neck (HNSCC). Over 90% of ACC tumors are c-kit-positive, and these primary cultures proved to be of short-term usefulness in assessing chemosensitivity. Interaction was determined over a wide range of drug combinations using a statistical three-dimensional analysis model. Both ACC short-term cultures and HNSCC cell lines were demonstrated to have a response ranging from additive to synergistic when imatinib and cisplatin were combined. The interaction of imatinib on cisplatin-induced DNA cross-linking was further investigated using the comet-X assay. In contrast, significant antagonism was observed when imatinib and gemcitabine were combined. Since gemcitabine is activated by deoxycytidine kinase (dCK), the effect of imatinib on this enzyme was investigated. A dose-dependent inhibition of dCK was observed, highlighting this kinase as a possible additional secondary molecular target for imatinib. This work demonstrates a synergistic interaction between cisplatin and imatinib, which may prove to be clinically relevant in the future management of both ACC and HNSCC. *Anti-Cancer Drugs* 16:719–726 © 2005 Lippincott Williams & Wilkins.**

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## Introduction

Imatinib (STI 571, glivec) is a 2-phenylaminopyrimidine derivative and is a known inhibitor of BCR–ABL and platelet-derived growth factor receptor (PDGFR) tyrosine kinase [1,2]. There is close homology between the kinase domains of PDGFR and a further receptor in the tyrosine kinase subclass 3 family, i.e. c-kit. c-kit is a 145-kDa transmembrane glycoprotein and is the product of the c-*kit* gene, the normal cellular homolog of the viral oncogene v-*kit*. Heinrich *et al.* demonstrated that imatinib selectively inhibits c-kit tyrosine kinase activity and downstream activation of target proteins involved in cellular proliferation and cell survival in human leukemic cell lines [3].

Overexpression of c-kit has been identified in salivary gland adenoid cystic carcinoma (ACC) [4,5]. Using immunohistochemistry, two studies have demonstrated that c-kit protein was expressed in 27 of 30 (90%) and 20 of 25 (80%) ACCs, suggesting a possible role for overexpression in the pathogenesis of this type of salivary carcinoma. ACC arises from mucus-secreting intercalated ducts in salivary gland tissue derived embryologically from the small gut, and accounts for 2–3% of head and neck malignancies [6,7]. Treatment is generally by surgical excision with post-operative radiotherapy [8,9]. Despite this combined approach, there is a marked propensity for local recurrence, which is often many years after the initial treatment [7]. The disease-free interval following initial treatment can range from 1 month to over 19 years (median 36 months). The lung is the commonest site of distant spread and although the patient may remain asymptomatic for a considerable length of time, once symptoms develop or the tumor spreads to viscera or bone, survival is less than 24 months [10].

The purpose of this study was to evaluate imatinib, both alone and in combination with established chemotherapeutic agents in a tumor possessing a known target for imatinib inhibition, i.e. ACC. Since no ACC cell lines are available, short-term primary cultures were established. We were able to maintain these primary cultures for sufficient time to evaluate growth inhibition of a number of compounds both singularly and in combination. We were, however, unable to establish a permanent cell line.

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In the case of the more common cancers of the head and neck, other tyrosine kinase inhibitors (TKIs) have been shown to be effective against cultured cells. The most widely studied agent in this class being the epidermal growth factor receptor (EGFR)-TKI gefitinib ('Iressa'). The exact mechanism of action of gefitinib on head and neck squamous cell carcinoma (HNSCC) cells remains unclear. Some authors have demonstrated that neither the EGFR expression level nor constitutive receptor activation seemed to predict sensitivity to the drug [11]. Gefitinib activity has also been shown in cell lines with low EGFR expression [12]. Consequently, if the recognized target for gefitinib inhibition is not definitely required for its action, we postulated that other TKIs might also have an action on HNSCC cell lines. Therefore further evaluation of imatinib against cell lines derived from HNSCC was undertaken. The drug panel used in the study was designed to broadly represent the most common drug classes available in the clinic. The panel was composed of DNA cross-linking agents (cisplatin, melphalan), antitubulin agents (taxol, vinblastine), 'antimetabolites' (gemcitabine, 5-fluorouracil) and an anthracycline antibiotic (adriamycin).

## Materials and methods

### Cell culture

Cells were maintained in M6 medium (50% RPMI and 50% L-15; Sigma, St Louis, Missouri, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS; Sigma), glutamine and antibiotics. Cell lines were subcultured weekly and routinely screened for mycoplasma contamination. All cell lines and primary cultures were found to be negative for mycoplasma. The human HNSCC cell lines Cal27 and FaDu were used, along with Dok, a dysplastic head and neck squamous cell line with partially transformed phenotype.

### Primary cell culture

Tumor material for the ACC short-term cultures was obtained routinely as part of the patients' planned surgical procedures. This material was obtained with full informed consent of the patient and approval of the local ethics committee. The tissue samples were collected and transported in M6 medium. After removal of necrotic material the tumor was minced to a fine slurry using 'crossed scalpels'. The slurry was then passed through filters of decreasing diameter (100 and 40 μm) and added to tissue culture flasks containing M6 medium. The medium was changed regularly and once the tumor cells had proliferated to near confluence the cells were subcultured for up to four passages. The three ACC primary cultures included were referred to as HN1, HN3 and HN5.

### Growth-inhibition assay

Growth inhibition was measured using a modification of the sulforhodamine B (SRB) assay for quantifying cellular protein content [13]. The SRB assay does not discriminate between growth inhibition and cell killing. However, the cells were seeded at low density and growth was confirmed microscopically in the control wells after the incubation period. Briefly, cells were plated into 96 well plates (Greiner, Gloucestershire, UK) at 1000 cells/well in 100 μl of medium. The cells were then allowed to adhere for 24 h prior to the addition of the drug. A 2-fold serial dilution of imatinib (Novartis, Basel, Switzerland) was added in triplicate and the plates were incubated for 5–7 days, depending upon the previously determined growth characteristics of each individual cell culture. The cells were fixed with 100 μl of pre-chilled 10% trichloroacetic acid after removal of the medium. The plates were then chilled at 4°C for 1 h. Following this the plates were washed twice with water and stained with 50 μl of 0.4% SRB in 1% acetic acid for 10 min at room temperature. The unbound dye was removed by sequential washing with 1% acetic acid. The plates were then air-dried and the bound dye solubilized in 100 μl of unbuffered 10 mM Tris base (pH 8.0) and fluorescence determined using a FL500 fluorescence spectrophotometer (Labsystems, Witchford, UK) with wavelength settings of excitation = 520 nm and emission = 590 nm. Growth inhibition curves were constructed and $IC_{50}$ values determined.

### Synergy assay

The interactions of imatinib in combination were evaluated based upon the technique described by Prichard and Shipman [14]. Briefly, the vertical drug was added in eight serial 2-fold dilutions across the plates and the horizontal drug in five serial dilutions plated at 90° to the first drug. This resulted in a matrix combination of 40 doses in duplicate. In this study the vertical drug was always imatinib. The $IC_{50}$ value for each cell culture as determined previously for each drug was used as a guide to selecting the initial concentrations of each drug combination. After incubation the plates were fixed, stained and the fluorescence read as described previously. A three-dimensional spreadsheet model (a kind gift from the National Cancer Institute Drug Screening Laboratory) was used to process the data generated from two 96-well plates. The data from the experimental drug combination assay was then represented graphically as a three-dimensional surface plot representing additive, synergistic or antagonistic interaction. Synergy or antagonism was quantified by the volume of the area above (positive volume) or below (negative volume) the additive plane. Combinations producing a flat surface plot were deemed additive. These volumes were calculated at the 95% confidence interval and from these a value representing the degree of synergy or antagonism was derived.

### Deoxycytidine kinase (dCK) assay

The effect of imatinib on dCK activity was measured using a modified method of Singhal *et al.* [15]. Briefly, cells were pelleted by centrifugation and lysed in

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Cytobuster (Novagen, Nottingham UK). The cytosolic fraction was removed following further centrifugation. The standard reaction mixture contained in a total volume of 150 µl: 50 µl of cytosol fraction, 3.5 mM ATP, 60 nmol of [5-$^3$H]deoxycytidine, 20 mM Tris–HCl (pH 8.0), 3.5 mM $MgCl_2$, 0.2 mM dithioerythritol and 1 mM KCl. This reaction mixture was incubated for 30 min at 37°C with increasing concentrations of imatinib. Boiling for 3 min stopped the reaction and the precipitated protein was sedimented by microcentrifugation for 60 s. Aliquots of 50 µl of supernatant were subsequently applied to disks of DEA 81 cellulose paper. Unphosphorylated deoxycitidine was removed by three successive 5-min washes with 1 mM ammonium formate. Finally, the disks were dehydrated in ethanol for a further 5 min. The disks where then allowed to air dry, placed in scintillation vials and counted in 5 ml of xylene-based scintillant.

Thymidine kinase may also phosphorylate deoxycytidine [16] and therefore cold thymidine was added to each reaction mixture to a final concentration of 50 mM in order to saturate thymidine kinase activity (50 mM thymidine was not itself inhibitory—data not shown).

### Comet assay

DNA cross-linking was determined using the comet-X assay [17]. Drug-treated and control Cal27 cells were trypsinized, transferred to plastic bijou tubes and immediately chilled on ice. Both cisplatin-treated and cisplatin + imatinib-treated samples were then subjected to 20 Gy irradiation using a $^{137}$Cs source (0.4 Gy/min). The samples were irradiated in order to introduce a fixed number of random DNA strand breaks. The retardation of migration of these strand breaks, which form a comet tail, is used as an indication of DNA cross-linking in response to treatment with drugs. Samples were maintained on ice to prevent repair of any DNA strand breaks prior to immediate processing in the comet assay. Control irradiated cells and imatinib-alone-treated cells were maintained on ice in the same manner as treated samples. Approximately 0.5 ml of cell suspension was mixed with low-melting-point agarose and pipetted onto a pre-coated microscope slide and allowed to gel for 1–2 min on ice. The slides were then immersed in ice-cold lysing solution (100 mM EDTA, 10 mM Tris–HCl, 1% Triton X-100, 1% DMSO, 2.5 M NaCl) for 1 h, washed 3 times in distilled water then placed onto a flat-bed electrophoresis tank and left for 45 min in alkali DNA unwinding solution (50 mM NaOH, 1 mM EDTA buffered to pH 12.5). The slides were then subjected to electrophoresis at 0.6 V/cm for 25 min, followed by neutralization with 1 ml of 0.4 M Tris–HCl, pH 8.0 and air drying. The dried slides were subsequently rehydrated in double-distilled water and stained for 30 min with SYBr gold solution (FMC, Rockland, Maine, USA). The slides were examined at × 250 magnification under an epifluorescent microscope (Zeiss-Jenamed, Berlin, Germany). Twenty-five images from each of two duplicate slides were captured and analyzed using Komet version 5 software (Kinetic Imaging, Liverpool, UK). Both DNA damage and percent DNA cross-linked were calculated.

The experiment was designed to evaluate the effect of imatinib (20 µM) on DNA cross-linking following a single (15 µM) 6-h exposure to cisplatin. Imatinib was added either simultaneously for 6 h with cisplatin, for 24 h before cisplatin or for 24 h following cisplatin treatment.

### Receptor status

The c-kit receptor status of the primary tumors was established as positive on diagnosis. The c-kit receptor status of explanted primary tumor cultures and established cell lines were carried out using immunohistochemistry by the Histology Department of the Christie Hospital.

## Results

### Growth inhibition

The $IC_{50}$ values for the panel of drugs tested are shown in Table 1. The response of the cell lines and ACC primary cultures to imatinib was relatively uniform with the $IC_{50}$ in the range 17–33 µM. A similar level of response (micromolar) was also seen with the two cross-linking agents cisplatin and melphalan; however, the two ACC primary cultures HN1 and HN3 were much more resistant to melphalan than the established cell lines or HN5. Response to tubulin-binding agents was uniform across the panel, with slight resistance being observed in HN1 and HN3 compared to other lines in the panel.

### Synergy

Although the volumes representing the degree of synergy or antagonism were calculated at the 95% confidence

**Table 1** $IC_{50}$ values (SRB assay) for both the primary cultures and the cell lines (repeated at least twice on two separate occasions)

| | Imatinib (µM) | Cisplatin (µM) | Adriamycin (µM) | Taxol (ng/ml) | Melphalan (µM) | 5-Fluorouracil (µM) | Vinblastine (ng/ml) | Gemcitabine (nM) |
|---|---|---|---|---|---|---|---|---|
| Dok | 30.1 | 3.7 | 0.02 | 2.7 | 12.7 | 22.0 | 0.3 | 7.1 |
| FaDu | 33.7 | 2.4 | 0.04 | 2.1 | 6.4 | 3.7 | 0.1 | 4.1 |
| Cal27 | 18.1 | 7.4 | 0.02 | 3.8 | 11.3 | 4.4 | 0.2 | 7.0 |
| HN1 | 33.2 | 5.0 | 0.08 | 6.1 | 66.6 | 4.8 | 1.3 | NR |
| HN3 | 28.1 | 2.8 | NR | 5.1 | 27.5 | NR | 0.9 | 0.3 |
| HN5 | 16.9 | 3.3 | 0.08 | 2.4 | 7.3 | 1.2 | NR | NR |
| Mean SD[a] | 4.1 | 2.1 | 0.02 | 0.5 | 2.9 | 0.7 | 0.03 | 2.1 |

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interval, it was decided that an arbitrary range of + 25 to – 25 would essentially represent a potentially synergistic or antagonistic interaction whilst values higher than this represented true synergy or antagonism. The interaction for each drug combination is recorded in Table 2. Synergy was clearly observed in both the Dok and Cal27 cell lines when imatinib and cisplatin were combined (synergy values + 47.3 and + 85.7, respectively) (Fig. 1). Interestingly, the FaDu cell line showed less synergy with the imatinib/cisplatin combination despite having a response to both drugs similar to that of the Dok cell line. The imatinib and cisplatin combination was demonstrated to be potentially synergistic against two of the ACC primary cultures.

### Antagonism

Response to imatinib in combination with gemcitabine was uniformly significantly antagonistic in both cell lines and primary cultures (mean antagonism values ranging from – 86.5 to – 223.3; Table 2 and Fig. 2). Since gemcitabine requires activation by dCK [18], this antagonism could be the result of an interaction between imatinib and dCK. The effect of imatinib on this enzyme was investigated using cytosol extracts from Cal27 cells and a dose-dependant inhibition of dCK activity was observed with an $IC_{50}$ of 110 μM (Fig. 3).

**Table 2 Table summarizing the predominant interaction for imatinib in combination with cisplatin and gemcitabine**

| Cells | Cisplatin | Gemcitabine |
|---|---|---|
| HN1 | 6 (8.7) | – 96.7 (83.9) |
| HN3 | 0 | – 86.5 (25.7) |
| HN5 | 6 (5.7) | – 170 (33.3) |
| Dok | 47.3 (28.3) | – 158 (46.7) |
| Cal27 | 87.5 (51.3) | – 223 (141) |
| FaDu | 6.7 (10.2) | – 160 (36) |

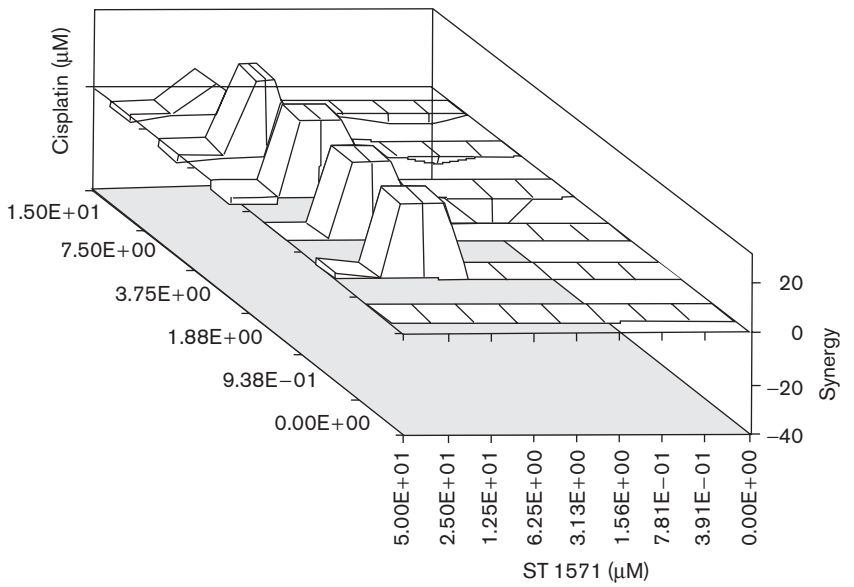
### Comet-X assay

Irradiation of Cal27 cells alone resulted in some 40% of DNA migrating from the nucleus to form a comet tail. The degree of retardation of this migration in drug-treated cells is taken as evidence of DNA interstrand cross-linking and is expressed as percent DNA cross-linked. The effect of differing schedules of imatinib on cisplatin cross-linking is shown in Figure 4. A 6-h cisplatin treatment resulted in a significant amount (68%, $p < 0.005$) of DNA cross-linked compared to irradiation alone (Fig. 4A). Subsequent incubation in drug-free medium (Fig. 4B) for 24 h resulted in only 48% of DNA cross-linked (28% repair). Imatinib treatment for 24 h either preceding or following cisplatin treatment resulted in significantly higher ($p < 0.005$) levels of DNA cross-linking (69.6 and 77.2%, respectively; Fig. 4G and C), representing 3.8 and 0% repair. Simultaneous treatment of cells with both cisplatin and imatinib for 6 h resulted in 62% DNA cross-linked (Fig. 4D), which decreased to 41.8% on incubation in drug-free medium (33% repair) (Fig. 4E).

### Receptor status

The morphology of the explanted tumor tissue was observed as epithelial; however, the c-kit receptor status was found to be very low and was regarded as negative. Since the c-kit receptor status of the original tumor was

**Fig. 1**




Three-dimensional synergy plot of the response of Cal27 cells to various combinations of imatinib and cisplatin (volume profiles shown are at the 95% confidence interval).

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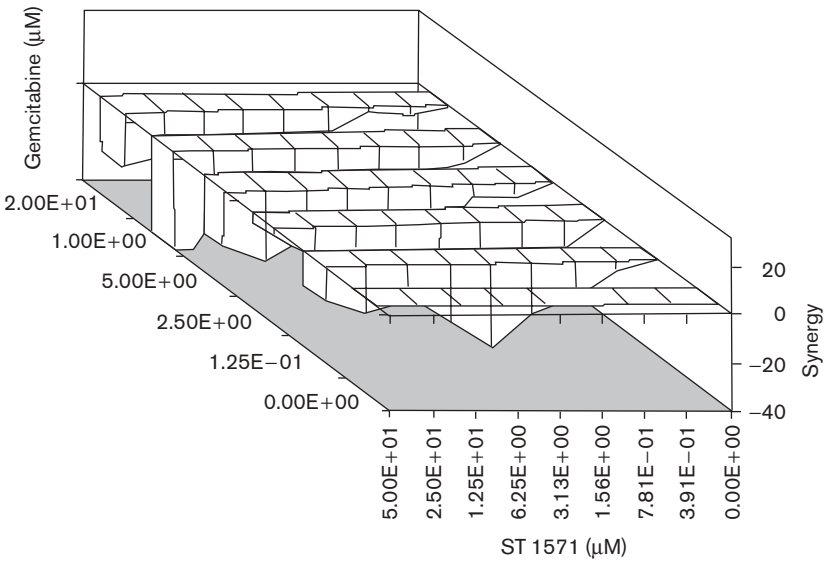
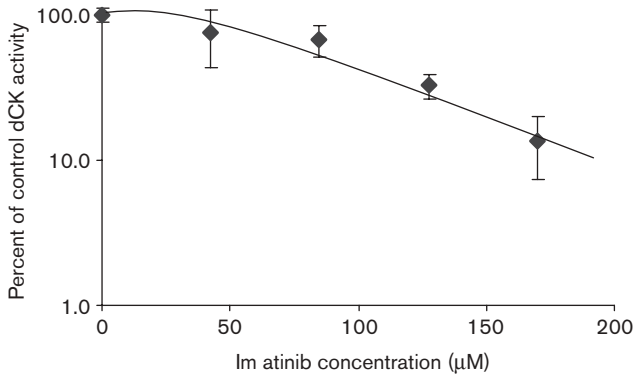
**Fig. 2**



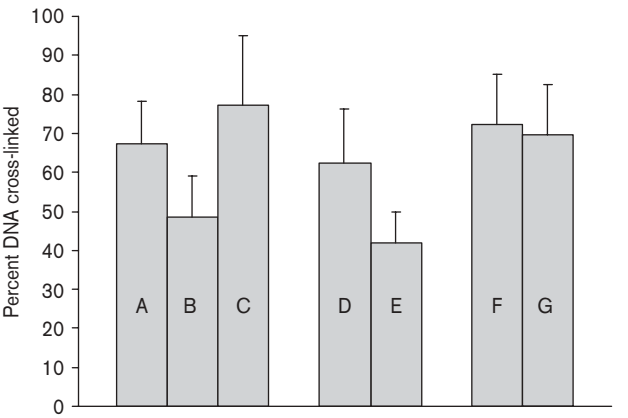

Three-dimensional synergy plot of the response of Cal27 cells to various combinations of imatinib and gemcitabine (volume profiles shown are at the 95% confidence interval).

**Fig. 3**




The effect of imatinib on dCK activity in Cal27 cytosol extracts. Error bars represent SD.

**Fig. 4**




The effect of Imatinib on cisplatin-induced DNA cross-linking measured using the comet-X assay. (A) Cisplatin alone. (B) Cisplatin followed by repair in drug-free medium for 24 h. (C) Cisplatin followed by 24 h repair in the presence of imatinib. (D) Cisplatin and imatinib simultaneously administered. (E) Simultaneous cisplatin and imatinib followed by repair in drug-free medium. (F) Imatinib administered 24 h prior to and during cisplatin treatment. (G) Imatinib administered 24 h prior to and during cisplatin treatment followed by repair in drug-free medium. The experiment was performed in duplicate with each value representing the mean of two slides with 25 comets being scored per slide. Error bars represent SD.

verified at diagnosis as positive, downregulation of the receptor must have occurred as a response to culture conditions. The established HNSCC cell lines were EGFR-positive, but c-kit receptor-negative.

## Discussion

### Growth inhibitory effects

Initial experience with cytotoxic chemotherapy led some clinicians to advocate no role for chemotherapy in ACC other than for symptomatic recurrent and metastatic disease [19]. Published single-agent response rates are low, with the most active agents appearing to be cisplatin, 5-fluorouracil and the anthracyclines [20–22]. The original ACC tumor biopsies were positive for c-kit receptor expression; clearly this expression appears to have been downregulated on adaptation to short-term culture. In this study we have confirmed growth

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inhibition in primary ACC cultures following treatment with imatinib at doses similar to those observed to inhibit the established HNSCC cell lines. The $IC_{50}$ values obtained for imatinib against the ACC short-term cultures (17–33 μM) were high when compared to the c-kit-expressing myeloid leukemia cell line, M-07e (100 nmol/l) [3]. However, the values were comparable to those shown to potently inhibit the proliferation of cultured cells transfected with the various c-kit mutations found in gastrointestinal stromal tumors (GIST) (1–10 μM) [23]. GIST tumors have also been shown to respond to imatinib despite low expression of c-kit [24]. Frequently, *in vitro* studies using medium containing serum tend to result in receptor saturation by endogenous ligands. As a consequence, response to TKIs *in vitro* often requires high dose ranges [12,25]. Nonetheless, responses are often seen and result from a number of unrelated factors of which receptor density/activity is only one. Clinically, there is conflicting evidence for the effectiveness of imatinib against ACCs. A study on the use of imatinib in ACC primary tumors has suggested that this class of head and neck tumor is indeed unresponsive to this drug when administered as a single agent [26]. However, a recent case report documents the cases of two patients with unresectable ACC who responded well to treatment with imatinib. In one of these cases the tumor regression was significant enough to facilitate salvage surgical resection and, although a similar result was found in the other case, the patient refused further surgical intervention [27].

Since no established ACC cell lines are available, further in-depth studies on the effects of imatinib in ACC were not possible. However, we have clearly shown imatinib to have growth-inhibitory effects on ACC primary cultures (despite the downregulation of c-kit). A panel of cell lines derived from the commonest head and neck malignancy (HNSCC) were used to facilitate further evaluation of the drug. Previous reports have shown other TKIs to have a growth inhibitory effect in HNSCC cells. However, such TKIs targeted the EGFR receptor often expressed in HNSCC [28,29]. Evaluation of the most studied EGFR-TKI, i.e. gefitinib, did not demonstrate a clear mechanism of action for this drug. Indeed, in several studies, neither EGFR expression nor constitutive receptor activation predicted sensitivity [11,12,30]. Consequently, the importance of the defined molecular targets of agents such as gefitinib is questionable. With regard to HNSCC, the possibility of the existence of other, as yet unidentified, secondary targets for TKIs was considered. Therefore, it was of interest to evaluate imatinib in the absence of a known target in HNSCC cell lines.

Imatinib produced growth inhibition in the HNSCC cell lines used in this study ($IC_{50}$ values 18–34 μM) similar to those reported for gefitinib against a panel of seven HNSCC cell lines (range 6–31 μM) [12]. Therefore, despite the lack of a recognized target, imatinib treatment resulted in a similar growth-inhibitory effect to that of the most widely studied TKI in HNSCC.

### Synergy

Since imatinib clearly demonstrated an antiproliferative effect on cells in culture, it was subsequently evaluated in combination with existing clinical drugs. Two interesting drug combinations were highlighted. Imatinib was found to be synergistic with cisplatin when tested against the HNSCC cell panel (three of three cell lines). In contrast, the predominant interaction when imatinib and cisplatin were combined in the ACC cultures was weakly synergistic/additive. These results are similar to those reported by Zhang *et al.*, where simultaneous treatment with both cisplatin and imatinib resulted in an enhanced toxicity in the A549 NSCLC cell line [25]. Whilst these authors claim synergy, they do not, as we do in HNSCC cell lines, demonstrate a statistically significant interaction between these two drugs. In a separate study using the c-kit-positive small cell lung cancer line NCI-H526, combinations of the multitargeted TKI SU11248 and cisplatin resulted in enhanced tumor growth delay *in vivo*. Interestingly, these authors compared imatinib to SU11248 as single agents, but failed to combine imatinib with cisplatin [31].

The data presented here in HNSCC cells is consistent with the fact that the c-kit status of the cell line is not essential for synergy with cisplatin. This suggested an interaction between imatinib and possibly other kinases pertinent to cisplatin sensitivity. The EGFR status of many head and neck cell lines, including the ones used in this study, is often positive [32]. It is possible, therefore, that the observed synergy may be through interaction of imatinib with the EGFR receptor tyrosine kinase. Synergy has been described in A431 cells, which overexpress EGFR when exposed to the irreversible EGFR inhibitor CI-1033 and cisplatin *in vitro*. However, this synergy did not appear to involve the repair of DNA–cisplatin adducts [33]. Conversely, in a separate study using the comet assay, a reduction in the repair of cisplatin-induced DNA inter-strand cross-links was observed following cotreatment with gefitinib. This reduced ability to repair cross-links appeared to be associated with the binding of EGFR to DNA protein kinase C [34]. In the study reported here we have attempted to determine the effect of imatinib on the production and subsequent repair of cisplatin-induced DNA interstrand cross-links using the comet-X assay. We were able to clearly demonstrate that imatinib does delay the repair of cisplatin-induced DNA cross-links when administered either before or after cisplatin treatment. However, when added simultaneously for the duration of the cisplatin treatment (6 h) no delay in repair of cross-links was observed. Wozniak *et al.* have previously

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reported an increase in cisplatin-induced DNA damage following simultaneous treatment with imatinib [35]. In that study, DNA damage was assessed as strand breaks using a conventional alkaline comet assay. However, the increase in DNA damage reported could have been due to the unrepaired lesions following excision of cisplatin adducts. We believe this is the first report of a synergistic effect of imatinib on cisplatin-induced cross-links. Clearly, from the results presented here and from others, TKIs may well have a secondary effect on DNA damage/repair pathways hitherto unobserved. The precise mechanism by which imatinib and other TKIs are synergistic in combination with cisplatin remains unclear. It is possible that changes in the apoptotic threshold could produce the observed cytotoxic effect. It is known, for example, that the p53 status of HNSCC dramatically affects cisplatin sensitivity [36]. HNSCC cell lines with wild-type p53 are less sensitive to cisplatin than those with overexpression of mutant p53. In this study the HNSCC lines used are p53 mutant. Similarly, imatinib has been reported to promote the pro-apoptotic balance of the BCL-2 family of apoptosis regulators [37]. As a result, the observed synergy may well lie in the affect of imatinib on the repair of DNA damage and consequently maintaining or enhancing the molecular triggers that initiate cell death pathways.

### Antagonism

Imatinib when used in combination with gemcitabine was highly antagonistic in both the HNSCC cell lines and ACC primary cultures. We hypothesized that this observed antagonism could well be due to an inhibition of dCK activity. A direct inhibitor of dCK activity would prevent the activation of gemcitabine and thus reduce its effectiveness. Indeed, this was found to be the case. Whilst the dose of imatinib required to inhibit dCK activity is high *in vitro* (110 μM), it is less than 5-fold of the observed $IC_{50}$ of this drug in these cell lines. Since imatinib binds to the ATP-binding site of the abl protein kinase [1], it is likely that other ATP-dependent kinases may be affected. The data presented here is consistent with the hypothesis that this antagonism results, at least in part, from dCK inhibition by imatinib. Indeed, this may also help to explain the observed growth inhibition in cell lines lacking a recognized receptor kinase target for this drug. In addition, prolonged exposure to imatinib could, through its effect on dCK, affect repair by depletion of intracellular deoxynucleotide pools. Inhibition of dCK may also contribute to the observed effect of imatinib on the repair of cisplatin adducts.

This study has demonstrated imatinib to have a growth-inhibitory effect on both ACC primary cultures and HNSCC cell lines *in vitro*. Two clinically significant interactions between imatinib and conventional chemotherapeutic compounds have also been demonstrated; imatinib is synergistic in combination with cisplatin and antagonistic when combined with gemcitabine. However, as demonstrated by both this study and the literature, care must be taken when interpreting the results of such combination assays. By their nature they are *in vitro* and therefore susceptible to small changes in the experimental environment as well as often being conducted over only a few dose combinations. The advantage of the three-dimensional surface volume approach to synergy as proposed by Shipman *et al.* compared to isobolograms or the CI index is that it demonstrates the result of all drug interactions in a single plot. Indeed, subtle changes in combinations can be detected over the whole dose matrix. Ultimately, it is the tumor response to the drug combination *in vivo* that is important. However, *in vitro* assays do have a role to play in screening drug combinations, highlighting those suitable for further evaluation in *in vivo* models or ultimately clinical trials.

A phase II study evaluating the effect of imatinib in patients with recurrent and/or metastatic ACC is presently underway, including evaluation of imatinib and cisplatin in combination. To date the imatinib/cisplatin combination has shown useful activity [38].

## Acknowledgment

Histology Department, Paterson Institute for Cancer Research. A patent covering the use of imatinib in head and neck cancer has been applied for by Novartis (patent application PCT/EP 2004/002769)

## References

1 Buchdunger E, Zimmerman J, Mett H, Meyer T, Muller M, Druker B, *et al.* Inhibition of the abl protein-tyrosine kinase *in vitro* and *in vivo* by a 2-phenylaminopyrimidine derivative. *Cancer Res* 1996; **56**:100–104.
2 Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, *et al.* Effects of a selective inhibitor of the ABL tyrosine kinase on the growth of bcr–abl positive cells. *Nat Med* 1996; **2**:561–566.
3 Heinrich MC, Griffith DJ, Druker BJ, Wait CL, Ott KA, Zigler AJ. Inhibition of c-kit receptor tyrosine kinase activity by STI 571, a selective tyrosine kinase inhibitor. *Blood* 2000; **96**:925–932.
4 Jeng YM, Lin CY, Hsu HC. Expression of the c-kit protein is associated with certain subtypes of salivary gland carcinoma. *Cancer Lett* 2000; **154**: 107–111.
5 Holst VA, Marshall CE, Moskaluk CA, Frierson HF. Kit protein expression and analysis of c-*kit* gene mutation in adenoid cystic carcinoma. *Mod Pathol* 1999; **12**:956–960.
6 Azzopardi JG, Smith OD. Salivary gland tumours and their mucins. *J Pathol Bacteriol* 1959; **77**:131–140.
7 Jones AS, Hamilton JW, Rowley H, Husband D, Helliwell TR. Adenoid cystic carcinoma of the head and neck. *Clin Otol* 1997; **22**:434–443.
8 Renehan AG, Gleave EN, Slevin NJ, McGurk M. Clinicopathological and treatment related factors influencing survival in parotid cancer. *Br J Cancer* 1999; **80**:1296–1300.
9 Spiro RH. Distant metastasis in adenoid cystic carcinoma of salivary origin. *Am J Surg* 1997; **174**:495–498.
10 Spiro RH. The controversial adenoid cystic carcinoma. In: McGurk M, Renehan A (editors): *Controversies in the Management of Salivary Gland Disease*. Oxford: Oxford University Press; 2001, pp. 207–211.
11 Suzuki T, Nakagawa T, Endo H, Mitsudomi T, Masuda A, Yatabe Y, *et al.* The sensitivity of lung cancer cell lines to the EGFR-selective tyrosine kinase inhibitor ZD1839 ('Iressa') is not related to the expression of Egfr or HER-2 or to K-*ras* gene status. *Lung Cancer* 2003; **42**:35–41.
12 Magne N, Fischel JL, Dubreuil A, Formento P, Poupon MF, Laurent-Puig P, *et al.* Influence of epidermal growth factor receptor (EGFR),

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p53 and intrinsic MAP kinase pathway status of tumour cells on the antiproliferative effect of ZD1839 ('Iressa'). *Br J Cancer* 2002; **86**: 1518–1523.
13 Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, *et al.* New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst* 1990; **82**:1107–1112.
14 Prichard MN, Shipman C. A three-dimensional model to analyse drug–drug interactions. *Antiviral Res* 1990; **14**:181–206.
15 Singhal RL, Yeh YA, Szekeres T, Weber G. Increased deoxycytidine kinase activity in cancer cells and inhibition by difluorodeoxycytidine. *Oncol Res* 1992; **4**:517–522.
16 Arner ESJ, Eriksson S. Mammalian deoxyribonucleoside kinases. *Pharmacol Ther* 1995; **67**:155–186.
17 Ward TH, Butler J, Shahbakhti H, Richards JT. Comet assay studies on the activation of two diaziridinylbenzoquinones in K562 cells. *Biochem Pharmacol* 1997; **53**:1115–1121.
18 Kroep JR, Loves WJ, van der Wilt CL, Alvarez E, Talianidis L, Boven E, *et al.* Pretreatment deoxycytidine kinase levels predict *in vivo* gemcitabine sensitivity. *Mol Cancer Ther* 2002; **1**:371–376.
19 Eby LS, Johnson DS, Baker HW. Adenoid cystic carcinoma of the head and neck. *Cancer* 1972; **29**:1160–1168.
20 Schramm VL, Srodes DS, Baker HW. Cisplatin therapy for adenoid cystic carcinoma. *Arch Otolaryngol* 1981; **107**:739–741.
21 Tannock IF, Sutherland DJ. Chemotherapy for adenoid cystic carcinoma. *Cancer* 1980; **46**:452–454.
22 Vermorken JB, Verweij J, de Mulder PH, Cognetti F, Clavel M, Rodenhuis S. Epirubicin in patients with advanced of recurrent adenoid cystic carcinoma of the head and neck. *Ann Oncol* 1993; **33**:275–278.
23 Chen H, Isoizhaki K, Kinoshita K, Ohashi A, Matsuzawa Y, Kitmarua Y, *et al.* Imatinib inhibits various types of activating mutant kit found in gastrointestinal stromal tumors. *Int J Cancer* 2003; **105**:130–135.
24 Bauer S, Corless CL, Heinrich MC, Dirsch, Antoch G, Kanja, *et al.* Response to imatinib mesylate of a gastrointestinal stromal tumour with very low expression of KIT. *Cancer Chemother Pharmacol* 2003; **51**: 261–265.
25 Zhang P, Gao WY, Turner S, Ducatman B. Gleevec (STI 571) inhibits lung cancer cell growth (A549) and potentiates the cisplatin effect *in vitro*. *Mol Cancer* 2003; **2**:1–9.
26 Corless CL, Heinrich MC, Dimitijevic S, Dirnhofer S, Nikolova Z, Fletcher JA. Correlation of imatinib response with activation of Kit and PDGF receptors in a variety of cancers: results of the CSTIB2225 trial. *Proc Am Soc Clin Oncol* 2003; abstr 783.
27 Alcedo JC, Fabrega JM, Arosemena JR, Urrutia A. Imatinib mesylate as treatment for adenoid cystic carcinoma of the salivary glands: report of two successfully treated cases. *Head Neck* 2004; **26**:829–831.
28 Santini J, Formento JL, Francoual M, Milano G, Scneider M, Dassonville O, *et al.* Characterization, quantification and potential clinical value of the epidermal growth factor receptor in head and neck squamous cell carcinomas. *Head Neck* 1991; **13**:132–139.
29 Dassonville O, Formento JL, Francoual M, Ramaioli A, Santini J, Schneider M, *et al.* Expression of epidermal growth factor receptor and survival in upper aerodigestive tract cancer. *J Clin Oncol* 1993; **11**:1873–1878.
30 Campiglio M, Locatelli A, Olgiati C, Normanno N, Somenzi G, Vigano L, *et al.* Inhibition of proliferation and induction of apoptosis in breast cancer cells by the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor ZD1839 ('Iressa') is independent of EGFR expression level. *J Cell Physiol* 2004; **198**:259–268.
31 Abrams TJ, Lee LB, Murray LJ, Pryer NK, Cherrington JM. SU11248 inhibits KIT and platelet-derived growth factor receptor beta in preclinical models of human small cell lung cancer. *Mol Cancer Ther* 2003; **2**:471–478.
32 Bujia J, Wustrow TP. Expression of epidermal growth factor receptor in squamous carcinoma cell lines of the larynx and hypopharynx. *Allergol Immunopathol* 1992; **20**:152–156.
33 Gieseg MA, de Bock C, Ferguson LR, Denny WA. Evidence for epidermal growth factor receptor-enhanced chemosensitivity in combinations of cisplatin and the new irreversible tyrosine kinase inhibitor CI-1033. *Anticancer Drugs* 2001; **12**:683–690.
34 Friedmann B, Caplin M, Hartley JA, Hochhauser D. Modulation of DNA repair *in vitro* after treatment with chemotherapeutic agents by the epidermal growth factor receptor inhibitor gefitinib (ZD1839). *Clin Cancer Res* 2004; **10**:6467–6486.
35 Wozniak K, Czechowska A, Blasiak J. Cisplatin-evoked DNA fragmentation in normal and cancer cells and its modulation by free radical scavengers and the tyrosine kinase inhibitor STI 571. *Chem-Biol Interact* 2004; **147**:309–318.
36 Bradford CR, Zhu S, Ogawa H, Ogawa T, Ubell M, Narayan A, *et al.* P53 mutation correlates with cisplatin sensitivity in head and neck squamous cell carcinoma lines. *Head Neck* 2003; **25**:654–661.
37 Bellone G, Ferrero D, Carbone A, De Quadros MR, Gramigni C, Pra A, *et al.* Inhibition of cell survival and invasive potential of colorectal carcinoma cells by the tyrosine kinase inhibitor STI 571. *Cancer Biol Ther* 2004; **3**: 385–392.
38 Slevin NJ, Mais KL, Bruce I, Ward T, Carrington B, Hulse P, *et al.* Imatinib with cisplatin in recurrent and/or metastatic salivary adenoid cystic carcinoma–response assessed by FDG-PET scanning. *J Clin Oncol* 2004; **22**(**14S**):5604.

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