Effects of the Aurora kinase inhibitor VX-680 on anaplastic thyroid cancer-derived cell lines

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Abstract

Anaplastic thyroid cancers (ATC) are aggressive tumors, which exhibit cell cycle misregulations leading to uncontrolled cellular proliferation and genomic instability. They fail to respond to chemotherapeutic agents and radiation therapy, and most patients die within a few months of diagnosis. In the present study, we evaluated the in vitro effects on ATC cells of VX-680, an inhibitor of the Aurora serine/threonine kinases involved in the regulation of multiple aspects of chromosome segregation and cytokinesis. The effects of VX-680 on proliferation, apoptosis, soft agar colony formation, cell cycle, and ploidy were tested on the ATC-derived cell lines CAL-62, 8305C, 8505C, and BHT-101. Treatment of the different ATC cells with VX-680 inhibited proliferation in a time- and dose-dependent manner, with the IC50 between 25 and 150 nM. The VX-680 significantly impaired the ability of the different cell lines to form colonies in soft agar. Analysis of caspase-3 activity showed that VX-680 induced apoptosis in the different cell lines. CAL-62 cells exposed for 12 h to VX-680 showed an accumulation of cells with ≥ 4N DNA content. Time-lapse analysis demonstrated that VX-680-treated CAL-62 cells exit metaphase without dividing. Moreover, histone H3 phosphorylation was abrogated following VX-680 treatment. In conclusion, our data demonstrated that VX-680 is effective in reducing cell growth of different ATC-derived cell lines and warrant further investigation to exploit its potential therapeutic value for ATC treatment.

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Introduction

Although derived from the same cell type, different thyroid neoplasms show specific histological features, biological behavior, and degree of differentiation, as a consequence of different genetic alterations (Fagin 2002, Sherman 2003). The majority of thyroid cancers are represented by the differentiated papillary and follicular thyroid carcinomas that, following dedifferentiation, are thought to give rise to the anaplastic thyroid carcinoma (ATC; Fagin 2002, Wreesmann et al. 2002, Are & Shaha 2006). The latter is one of the most aggressive and lethal tumors in humans, which fails to respond to all available chemotherapeutic agents and radiotherapy. Therefore, most patients die within a year of the diagnosis (Fagin 2002, Wreesmann et al. 2002, Are & Shaha 2006).

The genetic instability represents a hallmark of solid cancers including thyroid carcinomas, in which it has been suggested to underlie the progression to more aggressive phenotypes (Shahedian et al. 2001, Fagin 2002, Wreesmann et al. 2002, Are & Shaha 2006). Over the last decade, our knowledge regarding the molecular processes controlling the mitotic phase of the cell cycle has increased considerably. As a result, different mitotic proteins, expression or function of which has been found to be altered in human cancer tissues, are now thought to play a role in tumor genetic instability. These include the three Aurora kinase family members, Aurora-A, Aurora-B, and Aurora-C, implicated in the regulation of multiple aspects of chromosome segregation and cytokinesis (Katayama et al. 1999, Takahashi et al. 2000, Tanner...
et al. 2000, Miyoshi et al. 2001, Nigg 2001, Sakakura et al. 2001, Ota et al. 2002, Carmena & Earnshaw 2003, Vagnarelli & Earnshaw 2004). During the cell cycle, their expression is tightly regulated, being maximal in the G2/M phase, while their rapid degradation at the end of mitosis by the ubiquitin–proteasome pathway is required for the cell entry into a new cell cycle (Arlot-Bonnemains et al. 2001, Nigg 2001, Carmena & Earnshaw 2003, Vagnarelli & Earnshaw 2004). Aurora-A localizes onto the duplicated centrosomes and is involved in their positioning, the recruitment of components at the formation of mitotic spindle and its stability (Nigg 2001, Carmena & Earnshaw 2003, Vagnarelli & Earnshaw 2004). Aurora-B is a chromosomal passenger protein, which at the beginning of mitosis associates with chromatin where it forms a complex with proteins as inner centromere protein (INCENP), survivin, and borealin, leading to the phosphorylation of histone H3 (Nigg 2001, Carmena & Earnshaw 2003, Vagnarelli & Earnshaw 2004). Moreover, during the transition from anaphase to telophase, Aurora-B plays a role in the mitotic spindle dynamics and cleavage furrow, and can be observed in the midbody of cytokinetic cells. Aurora-C is also a chromosomal passenger protein, shown to co-localize and form complexes with Aurora-B, INCENP, and survivin in mitotic cells (Yan et al. 2005).

Recently, we reported that all three Aurora kinases are overexpressed in human thyroid cancer-derived cell lines and tissues (Ulisse et al. 2006). These observations may help to clarify the molecular mechanisms involved in the pathogenesis and the genetic instability of thyroid cancers. In fact, the overexpression of Aurora-A has been shown to induce centrosome amplification and to potentiate the oncogenic action of Ras. Both these events are involved in human thyrocytes transformation and chromosome instability (Miyoshi et al. 2001, Tatsuka et al. 2005). Furthermore, it has been documented that p53 is phosphorylated by Aurora-A on Ser215, inducing the inhibition of the p53 transactivating action on several genes (Liu et al. 2004). On the other hand, p53 binding has been shown to inactivate Aurora-A in certain cell types (Chen et al. 2002). Thus, alterations in the crosstalk between Aurora kinase-A and p53 could be relevant in thyroid cancer progression by compromising the faithfulness of chromosome segregation (Parry et al. 1998, Shahedian et al. 2001).

The finding that the expression of all three Aurora kinases is altered in human thyroid cancers may also have potential therapeutic implications. In fact, over the last few years, specific inhibitors of Aurora kinases have been identified, which may open a new scenario in cancer therapy, especially against those cancers that do not respond to the available chemotherapeutic agents, including the ATC (Harrington et al. 2004, Hata et al. 2005, Matthews et al. 2006, Manfredi et al. 2007). Among these, VX-680 is a potent Aurora kinase inhibitor, shown to suppress tumor growth in xenograft models, leading to regression of leukemia, colon, and pancreatic tumors at well-tolerated doses (Harrington et al. 2004).

In the present study, we evaluated the effects of VX-680 on cell cycle progression, proliferation, apoptosis, and soft agar colony formation of different ATC-derived cell lines.

Materials and methods

Cell line and materials

The anaplastic carcinoma-derived cell lines CAL-62, 8305C, 8505C, and BHT-101 were purchased from German Collection of Microorganisms and Cell Cultures (DMSZ, Braunschweig, Germany). Agar, monoclonal anti-β-tubulin, polyclonal anti-γ-tubulin, and anti-actin antibodies were from Sigma Chemical Co. Nonidet P-40 (NP-40) and Protease Inhibitor Cocktail Set III were from Bio-Rad Laboratories. The Aurora kinase inhibitor VX-680 was from KAVA Technology (San Diego, CA, USA). Bradford protein assay kit and electrophoresis reagents were from Bio-Rad Laboratories. The Aurora kinase inhibitor VX-680 was from KAVA Technology (San Diego, CA, USA). The goat polyclonal antibody against TACC-3 was from Santa Cruz Biotechnology (Heidelberg, Germany). The polyclonal anti-Aurora-C antibody was generated against a 16 amino acid peptide of the C-terminal part of Aurora-C (aa 259–275) by Eurogentec (Seraing, Belgium). The monoclonal antibodies against Aurora-A (31C1) and Aurora-B (AIM-1), and the polyclonal antibody against P-histone H3 were from Abcam (Cambridge, UK). The secondary antibodies (tetramethyl rhodamine isothiocyanate (TRITC)-conjugated anti-goat or anti-rabbit and fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG) were from Jackson Laboratories (West Grove, PA, USA). Vectashield was from Vector Laboratories (Burlingame, KS, USA).

Cell cultures

The normal strain of human thyrocytes (HTU5) was cultured as previously described (Curcio et al. 1994). These diploid and non-tumorogenic cells retain in culture the functional features of normal human thyrocytes. The different ATC-derived cells, CAL-62, 8305C, 8505C, and BHT-101 have been shown to possess an abnormal karyotype and predicted loss-of-function mutations of the p53 gene (Gioanni et al. 1991, Olivier et al. 2002). The different cell lines were cultured in the appropriate media at 37 °C in 5% CO₂ humidified atmosphere.
Western blot

The cells were lysed in RIPA buffer (50 mM Tris–HCl (pH 7.4), 1% NP-40, 0.5% sodium deoxycholate, 150 mM sodium chloride, 1 mM EDTA, 1× Protease Inhibitor Cocktail Set III), sonicated, and then centrifuged at 15,000 g for 20 min. Protein concentrations were determined by the Bradford assay. Aliquots of 50 µg cell protein extracts were electrophoresed on a 12.5% polyacrylamide gel and transferred onto nitrocellulose membranes. The membranes were then washed with TBST (50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 0.05% Tween-20), saturated with 5% low-fat milk in TBST and then incubated at 4 °C overnight with antibodies against Aurora-A (1:500), Aurora-B (1:500), Aurora-C (1:500), or actin (1:1000) in TBS-T. After washing, the membranes were incubated with appropriate horseradish peroxidase-conjugated secondary antibodies against mouse or rabbit IgG (1:20,000) in TBST and developed using the chemiluminescence Super Signal kit (Pierce, Rockford, IL, USA). Aurora kinases and actin immunoreactive bands were quantified by scanning densitometry, using Molecular Analyst PC software for the Bio-Rad model 670 scanning densitometer. The different Aurora kinases/actin ratios were calculated and the values obtained for VX-680-treated cells were normalized against those found in control cells and reported as a fold of variation.

Proliferation assay

The CAL-62 cells were cultured in the absence (dimethyl sulfoxide, DMSO) or the presence of 500 nM VX-680 for different periods of time (1–5 days). The dose-dependent effects of VX-680 on cell proliferation were evaluated by treating the different ATC cells for 4 days with different concentrations of the Aurora inhibitor (5–500 nM). The cells were pulse labeled with 30 mM BrdU for 2 h before the end of the incubation time. The BrdU incorporation was analyzed by means of a colorimetric immunoassay using the cell proliferation ELISA kit (Roche Applied Science), according to the manufacturer’s instructions. The results from VX-680-treated cells were compared with those observed in control cells and expressed as a fold of variation versus control.

FACS analysis

The CAL-62 cells were cultured in the absence (DMSO) or the presence of 250 nM VX-680 for 72 h. Following treatment, the cells were rinsed with PBS and collected by scraping in PBS. The cells were then used to evaluate caspase-3 activity using the caspase-3/CPP32 fluorimetric assay kit (Biovision, Mountain View, CA, USA).

Caspase-3 assay

The different ATC cells were cultured in the absence (DMSO) or presence of 250 nM VX-680 for 72 h. Following treatment, the cells were rinsed with PBS and collected by scraping in PBS. The cells were then used to evaluate caspase-3 activity using the caspase-3/CPP32 fluorimetric assay kit (Biovision, Mountain View, CA, USA).

Time-lapse analysis

The CAL-62 cells were cultured in the absence (DMSO) or presence of 250 nM VX-680 and observed for 24 h under a microscope (Leica DM-IRBE) equipped with an incubation chamber at 37 °C and 5% CO2. The cell pictures were performed every 5 min using the MetaVue software.

Immunofluorescence (IF)

The CAL-62 cells cultured on glass cover slips were treated with 250 nM VX-680 or vehicle (DMSO) for 6 h. The cells were fixed in cold methanol for 2 min, washed, and preincubated with 3% BSA in PBS for 1 h at room temperature. After three washes with PBS, the cells were incubated with the antibodies anti-TACC3 (1:100), anti-Aurora-A (1:200), anti-Aurora-B (1:200), anti-Aurora-C (1:200), anti-P-histone H3 (1:1000), anti-γ-tubulin, and fixed in ice-cold ethanol. The cell samples were analyzed for DNA content (propidium iodide) and/or BrdU content (FITC) as previously described (30) using an EPICS Elite Flow cytometer (Coultronics, Hialeah, FL, USA).
(1:200), or anti-β-tubulin (1:200) for 2 h at room temperature. After washing, the cover slips were incubated with TRITC-conjugated anti-goat or anti-rabbit (1:100) and FITC-conjugated anti-mouse (1:100) antibodies for 1 h at room temperature and then mounted in Vectashield containing 1 μg/ml DAPI. The cover slips were observed with a microscope Leica DMRXA.

**Statistical analysis**

The results were expressed as the mean ± S.E.M. of three independent experiments. Statistical significance of data was evaluated by the Student t-test using the Epistat computer program. The results were considered significantly different if P values were lower than 0.05.

**Results**

**Effects of VX-680 on CAL-62, 8305C, 8505C, and BHT-101 cell proliferation**

The expression of the Aurora kinase family members (Aurora-A, Aurora-B, and Aurora-C) in the different ATC-derived cell lines was evaluated by western blot analysis in comparison with the normal strain of human thyrocyte HTU5. Similarly to that previously observed in the 8305C cells (Ulisse et al. 2006), a significant augmentation of all three proteins in the cancer cells was observed (data not shown). The effect of the Aurora kinase inhibitor VX-680 on the CAL-62 cell proliferation was evaluated on cells cultured from 1 to 5 days, in the presence of 500 nM VX-680 or the vehicle (DMSO) as control. The dose of 500 nM was adopted in these initial experiments since it was shown to elicit maximal response on different tumor cell types in vitro (Harrington et al. 2004). Our results demonstrated that VX-680 inhibits the CAL-62 proliferation in a time-dependent manner (Fig. 1A). In particular, significant (P < 0.01) inhibition of BrdU incorporation was detected after only 24 h of treatment. The anti-proliferative effect became maximal at 4–5 days of treatment, when BrdU incorporation was significantly reduced by more than 90%. Similar inhibition of proliferation was also observed on the 8305C, 8505C, and BHT-101 cells following VX-680 (500 nM) treatment for 4 days (Fig. 1B). We then evaluated the dose-dependent effects of VX-680 on the different ATC-derived cell lines proliferation by treating the cells for 4 days in the presence of increasing concentrations of the inhibitor (5–500 nM). The results of these experiments, reported in Fig. 1C, showed that the inhibition of the different ATC cells proliferation was dose dependent with half-maximal inhibitory concentrations (IC50) comprised between 25 and 150 nM.

**Effects of VX-680 on ATC cells colony formation in soft agar**

We evaluated the effects of the Aurora kinase inhibitor on the ability of the different ATC cell lines to form colonies in soft agar. In these experiments, the cells were cultured in the absence or presence of 250 nM VX-680 for 2 weeks. Control cells started to form noticeable colonies after 6–8 days of culture. As reported in Fig. 2 and Table 1, VX-680 treatment for 14 days significantly reduced the number and size of colonies by ~70% in the 8305C and 90% in the CAL-62, 8505C, and BHT-101.

**Effects of VX-680 on CAL-62 cell ploidy and cell cycle progression**

Inhibition of Aurora kinase activity by VX-680 has been demonstrated to generate polyploid cells as a
result of multiple rounds of DNA synthesis in the absence of cytokinesis. We therefore evaluated cell ploidy by FACS analysis and cell cycle by time-lapse experiments in control and VX-680-treated CAL-62 cells. The cell cultures exposed for 6 h to 250 nM VX-680 showed an accumulation of cells with 4N DNA content. Longer exposure to VX-680 (12 h) resulted in a population of cells with \( R_4N \) DNA, and a clear accumulation of cells with 4N/8N DNA content (Fig. 3A). IF experiments showed that control CAL-62 cells are characterized by large nuclei and a small amount of cytoplasm, while after treatment with 250 nM VX-680 for 24 h the cells displayed a remarkable increase in cell size and contained multiple nuclei (Fig. 3B). Time-lapse analysis showed that control cells completed their mitosis in about 200 min (Fig. 3C). By contrast, twice this time was necessary for VX-680-treated cells to process mitosis without any cytokinesis.

Table 1 Effects of the VX-680 on soft agar colony formation of different anaplastic thyroid carcinoma (ATC)-derived cell lines

<table>
<thead>
<tr>
<th>ATC cells</th>
<th>Control</th>
<th>VX-680</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL-62</td>
<td>34.8±1.7</td>
<td>2.2±0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8305C</td>
<td>37.8±2.2</td>
<td>12.7±2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8505C</td>
<td>11.7±2.0</td>
<td>0.33±0.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BHT-101</td>
<td>23.6±3.6</td>
<td>2.1±0.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The different ATC cells were cultured in soft agar in 3.5 cm Petri dishes in the absence (DMSO) or presence of 250 nM VX-680 for 2 weeks. Treated and non-treated plates were then photographed and number and size the colonies was measured by MetaVue software, scoring those larger than 50 \( \mu m \).

Effects of the VX-680 on Aurora kinases subcellular localization, TACC3 localization, and histone H3 phosphorylation in CAL-62 cells

We next investigated the effects of VX-680 on CAL-62 mitotic structures and proteins. To ascertain that VX-680 effects were due to the inhibition of Aurora kinase activities and not to changes in their protein levels, we performed western blot experiments on cell protein extracts from control and the CAL-62 cells treated with 250 nM VX-680 for 6 h (Fig. 4A). Densitometric analysis of western blot results of three independent experiments showed no significant variations between control and treated cells in Aurora-A (1.17±0.2-fold), Aurora-B (0.94±0.11-fold), or Aurora-C (0.97±0.09-fold) proteins level. The IF experiments showed that centrosomal Aurora-A localization was maintained in cells exposed to VX-680 (250 nM) for 6 h (Fig. 4B). However, the mitotic cells showed aberrant spindles characterized by shorter microtubules. In addition, the localization on spindle microtubules of the transforming acidic coiled-coil 3 (TACC3) protein, a substrate of Aurora-A which plays a major role in spindle microtubules growth and stability, was completely abrogated in VX-680-treated cells (Fig. 4B). Aurora-B localization onto the condensing chromatin during prophase was also maintained in treated cells, but, as shown in Fig. 4C, histone H3 phosphorylation was no longer detectable. In control cells, Aurora-C was solely observed localized onto the midbody of cytokinetic cells (Fig. 4D), but following VX-680 treatment no cells in telophase could be identified.
Effects of the VX-680 in inducing ATC cell apoptosis

In order to assess whether the reduced proliferation and the profound alterations in mitosis were associated with an induction of apoptosis, the accumulation of sub-G0 nuclei in the CAL-62 cells following a treatment with VX-680 (250 nM) for 72 h was investigated. As reported in Fig. 5A, the percentage of sub-G0 nuclei in control cells was 0.6 ± 0.2 and significantly ($P < 0.01$) increased to 25.3 ± 2.6 in VX-680-treated polyploid cells. Moreover, a significant ($P < 0.01$) increase of caspase-3 activity was found in the different ATC-derived cells following VX-680 (250 nM) treatment for 72 h with respect to control cells, as reported in Fig. 5B.

Discussion

Over the last decade, our knowledge regarding the molecular players controlling the mitotic process has considerably increased leading to the identification of new potential oncogenes. These include the three members of the Aurora kinase family, Aurora-A, Aurora-B, and Aurora-C, which regulate many aspects of the mitotic process (Nigg 2001, Carmena & Earnshaw 2003, Vagnarelli & Earnshaw 2004). The findings that their overexpression may cause cell transformation and that they are overexpressed in several types of human malignancies, including thyroid cancers, have lead to their evaluation as a potential target for anticancer therapy (Katayama et al. 1999, Takahashi et al. 2000, Tanner et al. 2000, Miyoshi et al. 2001, Sakakura et al. 2001, Ota et al. 2002, Mortlock et al. 2005, Sorrentino et al. 2005, Ulisse et al. 2006).

In the present study, we evaluated the in vitro effects of VX-680 on different well-characterized ATC-derived cell lines. We first demonstrated that treatment of these cells with VX-680 lead to a time- and dose-dependent inhibition of proliferation, with IC$_{50}$ between 25 and 150 nM, in agreement with the IC$_{50}$ reported for this inhibitor on other cancer cell types (Harrington et al. 2004). Furthermore, VX-680 induced apoptosis in all the ATC-derived cell lines tested. Finally, we showed that the VX-680 treatment significantly reduced the ability of the different ATC cells to form colonies in soft agar. In this context, it is worthwhile to note that the selective inhibition of Aurora-B expression in the ATC-derived
cell line ARO (by RNA interference or inhibition of the kinase activity by the quinazoline derivative \(N-[4-(6,7\)-dimethoxy-quinazolin-4-ylamino)-phenyl]-benzamide) has been shown to reduce cell proliferation and colony formation in soft agar by only 50%, while no activation of the apoptotic cascade was observed (Sorrentino et al. 2005). This would suggest that the sole inhibition of Aurora-B may not have the expected therapeutic value in ATC treatment (Nikiforow 2005). Moreover, in a recent report Aurora-A, but not Aurora-B or Aurora-C, was found among the most frequently and most strongly overexpressed genes in ATC (Wiseman et al. 2007). This is also consistent with the finding that gain of chromosome 20q, where the Aurora-A gene is located (20q13.2–q13.3), is frequently reported in ATC and suggested to play a role in committing differentiated thyroid cancer cells to anaplasia (Rodrigues et al. 2004).

The VX-680 has been demonstrated to inhibit the three Aurora kinases with inhibition constant \((K_i)\) ranging between 0.6 and 18 nM, showing more than 100-fold selectivity with respect to other kinases tested (Harrington et al. 2004). The only exceptions are the Fms-related tyrosine kinase-3 (FLT-3) and the c-ABL tyrosine kinase, which are inhibited by the VX-680 with \(K_i\) of 30 and 20 nM respectively (Harrington et al. 2004, Carter et al. 2005). Both tyrosine kinases have been detected in thyroid cancer cells and proposed to play a role in malignant transformation (Kung et al. 2006, Kurebayashi et al. 2006). However, the inhibition of c-ABL by Imatinib mesylate had negligible effects in suppressing human ATC cell growth (Dziba & Ain 2004, Kurebayashi et al. 2006). Similarly, the SU5416 (3-substituted indolin-2-one), reported to inhibit FLT-3, RET (rearranged upon transformation), vascular endothelial growth factor receptor, and c-kit with similar potency, causes proliferation arrest only in papillary thyroid cancer cells expressing the RET/PTC1 oncogene (Mologni et al. 2006). Therefore, the inhibitory effects of VX-680 on ATC cell growth reported here are likely to be consequent to the specific inhibition of the Aurora kinases. Consistent

**Figure 4** Effects of the VX-680 on Aurora kinase protein levels and subcellular localization, centrosome maturation, TACC3 localization, and histone H3 phosphorylation in the CAL-62 cells. The CAL-62 cells were incubated for 6 h with 250 nM VX-680 or DMSO as control. Following treatment, the cells were used for the subsequent experiments. (A) Western blot analysis of Aurora kinase protein levels. (B) Subcellular localization of Aurora-A and TACC3. Following treatment, the cells were fixed and stained for Aurora-A and \(\gamma\)-tubulin or TACC3 and \(\beta\)-tubulin. (C) Subcellular localization of Aurora-B and phosphorylated histone H3. Following treatment, the cells were fixed and stained for Aurora-B and P-histone H3. (D) Subcellular localization of Aurora-C in control CAL-62 cells. The cells were fixed and stained for Aurora-C and \(\beta\)-tubulin as a marker for microtubules. Scale bar, 10 \(\mu\)m.
with this, we found that the treatment of CAL-62 cells with VX-680 causes a major alteration in centrosome functions with abnormal spindle formation characterized by the presence of short microtubules. We and others demonstrated that Aurora-A kinase activity is required for the phosphorylation and localization of the TACC3 protein on the spindle microtubules. TACC3, in complex with the Ch-Tog protein, is essential in spindle microtubule growth and stability (Mori et al. 2007, Ulisse et al. 2007); hence, the complete abrogation of TACC3 localization following the VX-680 treatment could explain the aberrant spindle formation in the CAL-62 cells.

Histone H3 is also a well-recognized target of Aurora-B kinase and its phosphorylation is thought to mediate chromosome condensation during prophase (Crosio et al. 2002). In agreement with a previous study (Harrington et al. 2004), we showed that the VX-680 treatment of the CAL-62 cells inhibits histone H3 phosphorylation.

Finally, the inhibition of Aurora kinase activity has been demonstrated to generate polyploid cells as a result of multiple rounds of DNA synthesis in the absence of cytokinesis (Kawasaki et al. 2001). Recently, endoreduplication and apoptosis in response to the VX-680 have been shown to be conditioned by the p53–p21-dependent post-mitotic checkpoint. In fact, the cells with intact checkpoint function arrest with 4N DNA content, while those with compromised p53-dependent pathway undergo endoreduplication and apoptosis (Gizatullin et al. 2006). All the ATC cell lines employed in the present study are characterized by predicted loss-of-function mutations of the p53 gene (Gioanni et al. 1991, Olivier et al. 2002). In agreement with the above report, we observed that VX-680 induced endoreduplication in the CAL-62 cells and apoptosis in all the ATC cell lines tested. Both IF and time-lapse experiments suggest the ability of treated cells to enter the mitotic process. However, IF experiments showed that in treated cells all mitoses were arrested in prophase. On the other hand, time-lapse analysis demonstrated that VX-680-treated cells escape mitosis without dividing.

Over the last few years, a number of different inhibitors of the Aurora kinases have been developed and some of them were reported to enter in Phase I clinical trial (Mortlock et al. 2005, Matthews et al. 2006). These include VX-680, which efficiently inhibits tumor growth in a variety of in vivo xenograft models, inducing regression of leukemia, colon, and pancreatic tumors at well-tolerated doses (Harrington et al. 2004). This inhibitor recently entered in Phase II clinical trial on patients with chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia (Vertex Pharmaceuticals Reports 2007

![Figure 5](566.png)

**Figure 5** Effects of the VX-680 on different anaplastic thyroid carcinoma (ATC)-derived cell lines apoptosis. (A) The CAL-62 cells were incubated for 72 h with 250 nM VX-680 or the vehicle (DMSO). The cells were then scraped in PBS, fixed, and analyzed by FACS. The M1 bar identified the sub-G0/apoptotic cell population scored. (B) Caspase-3 activity was measured in extracts prepared from the different ATC-derived cell lines exposed for 72 h to 250 nM VX-680 or to the vehicle alone (DMSO). Data reported represent the mean ± S.E.M. of three independent experiments. Statistical significance of data was assessed by the Student t-test. *P<0.01, **P<0.05.
Pipeline Progress). It has to be mentioned, however, that Merck has recently suspended the enrolment in clinical trials of the Aurora kinase inhibitor, MK-0457 (VX-680), pending a full analysis of all safety data for the drug. The decision was based on preliminary safety data, in which a QTc prolongation was observed in one patient. Patients currently enrolled in these trials may continue to be treated with MK-0457, with additional monitoring for QTc prolongation.

In conclusion, we demonstrated that VX-680 inhibits proliferation and induces apoptosis in different ATC-derived cell lines. In addition, the inhibitor strongly reduced the ability of these cell lines to form colonies in soft agar. These findings warrant further investigations to exploit the potential therapeutic value of Aurora kinase inhibition in the treatment of ATC.

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