IRC-083927 is a new tubulin binder that inhibits growth of human tumor cells resistant to standard tubulin-binding agents

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Abstract

Tubulin is a validated target for antitumor drugs. However, the effectiveness of these microtubule-interacting agents is limited by the fact that they are substrates for drug efflux pumps (P-glycoprotein) and/or the acquisition of point mutations in tubulin residues important for drug-tubulin binding. To bypass these resistance systems, we have identified and characterized a novel synthetic imidazole derivative IRC-083927, which inhibits the tubulin polymerization by a binding to the colchicine site. IRC-083927 inhibits in vitro cell growth of human cancer cell lines in the low nanomolar range. More interesting, it remains highly active against cell lines resistant to microtubule-interacting agents (taxanes, Vinca alkaloids, or epothilones). Such resistances are due to the presence of efflux pumps (NCI-H69/LX4 resistant to navelbine and paclitaxel) and/or the presence of mutations on β-tubulin and on α-tubulin (A549.EpoB40/A549. EpoB480 resistant to epothilone B or paclitaxel). IRC-083927 displayed cell cycle arrest in G2-M phase in tumor cells, including in the drug-resistant cells. In addition, IRC-083927 inhibited endothelial cell proliferation in vitro and vessel formation in the low nanomolar range supporting an antiangiogenic behavior. Finally, chronic oral treatment with IRC-083927 (5 mg/kg) inhibits the growth of two human tumor xenografts in nude mice (C33-A, human cervical cancer and MDA-MB-231, human hormone-independent breast cancer). Together, the antitumor effects induced by IRC-083927 on tumor models resistant to tubulin agents support further investigations to fully evaluate its potential for the treatment of advanced cancers, particularly those resistant to current clinically available drugs. [Mol Cancer Ther 2008;7(8):2426–34]

Introduction

Microtubules, a major type of cytoskeletal filament in cells, are formed from tubulin subunits, including α-tubulin and β-tubulin. They play an important role in cellular functions, such as replication, cell movement, and organelle transport. Microtubules are the target of a diverse group of anticancer drugs derived mostly from natural products (1–5). Given the success of this class of drugs in cancer treatment, microtubules may be considered as one of the more validated therapeutic targets identified to date in patients. Tubulin-binding agents are characterized by their binding sites on tubulin and their effects on tubulin polymerization. Vinca alkaloids (vincristine, vinblastine, and vinorelbine (navelbine)) bind to the Vinca domain (6, 7) and inhibit tubulin assembly, whereas paclitaxel, docetaxel, and epothilones bind to the taxane-binding site (8–10) and stabilize microtubules. Their efficacies are well proven in clinic, but a limitation is the development of drug resistance due to the overexpression of the drug efflux pumps (11–13) and/or the appearance of mutations in tubulin genes (14–17).

A third tubulin-binding site has been described for its ability to bind a naturally occurring tricyclic alkaloid colchicine, which inhibits tubulin polymerization (18–23). Colchicine itself is not a useful anticancer agent because of its narrow therapeutic window, but compounds with diverse chemical structures that bind to this site are now in clinical developments [ABT-751 (24, 25), CA4P (26, 27), indibulin (28), and STX140 (29)].

Here, we report the identification of a structurally novel compound, IRC-083927, which binds to the colchicine-binding site and shows highly potent antiproliferative activity on human tumor cell lines, which are resistant to standard tubulin-binding agents. Furthermore, the oral administration of IRC-083927 in athymic mouse models showed a significant in vivo antitumor activity without apparent toxicity.

Materials and Methods

Materials

Paclitaxel (T7402), epothilone B (E2656), colchicine (C9315), and navelbine (vinorelbine ditartrate V2264) were purchased from Sigma. All compounds (10−5 mol/L) were...
dissolved in 100% dimethylacetamide (Aldrich) just before use. IRC-083927 was synthesized as shown in Fig. 1. The starting material, 4-nitro-3-fluoro-phenol, was hydrogenated to afford the corresponding aniline (1), which on heating with 4-fluoroacetophenone in a polar solvent gave the aryloxycetophenone (2). The aniline (2) was directly converted to the bromoketone (3) using bromine in ethanol. Compound (4) was prepared by reacting the cesium salt of the phenoxy methyl carboxylic acid with the aryl bromoketone (3) to afford an intermediate keto-ester, which on heating with an excess of ammonium acetate gave the required imidazole (4). Compound (4) was reacted with N-tert-butoxycarbonylsulfamoyl chloride (prepared in situ using chlorosulfonyl isocyanate and tert-butanol) followed by deprotection of the Boc group under acidic conditions to afford IRC-083927 as a white solid. Melting point 185°C to 187°C; 1H nuclear magnetic resonance (6 ppm, DMSO): 5.43 (s, 2H); 6.87 to 8.09 (m, 15H); 9.01 (s, 1H); 12.4 (bs, 2H). This compound was also characterized by mass spectrometry and high-performance liquid chromatography with a purity superior to 99%.

**Cell lines**

The cell lines were obtained from the American Type Culture Collection. MIA PaCa-2 was established from a tumor tissue of the pancreas. DU-145 was isolated from a tumor in the brain of a patient with widespread metastatic carcinoma of the prostate and a 3-year history of lymphocytic leukemia. MDA-MB-231 was isolated from a pleural effusion of a breast adenocarcinoma. Cells were grown in DMEM (Life Technologies) at 4.5 g/L glucose supplemented with 10% heat-inactivated FCS (Life Technologies) plus 2 mmol/L glutamine, 50 units/mL penicillin, and 50 mg/mL streptomycin (Life Technologies) at 37°C in a humidified atmosphere containing 5% CO₂. NCI-H69 was derived from the pleural fluid of a 55-year-old Caucasian male with small cell carcinoma of the lung. NCI-H69/AR cells are resistant to doxorubicin, mitoxantrone, etoposide, vincristine, and vinblastine and show MRP overexpression but are P-glycoprotein (PgP) negative. NCI-H69/Lx4 cells are PgP positive and are resistant to doxorubicin and taxanes. NCI-H69 were cultured in RPMI 1640 (Eurobio) completed with 0.4 μg/mL doxorubicin for NCI-H69/Lx4. A549 and its variants were obtained from the Department of Molecular Pharmacology, Albert Einstein College of Medicine. A549.EpoB40 cells have a mutation on β-tubulin (Gln²⁹² → Glu). A549.EpoB480 cells have a mutation of β-tubulin (Gln²⁹² → Glu; Val⁶⁰ → Phe) and a mutation of α-tubulin (Leu¹⁹⁵ → Met). A549 cells were cultured in RPMI 1640 (Eurobio) completed with epothilone B (125 nmol/L) for the variants (14).

**Tubulin Polymerization Assay**

The polymerization of purified bovine brain tubulin (99% purity) was monitored using the Tubulin Polymerization Assay Kit from CytoDYNAMIX (Screen 03, Cytoskeleton; refs. 30, 31). Briefly, tubulin polymerization was monitored spectrophotometrically by the change in absorbance at 340 nm. The absorbance was measured at 1-min intervals for 60 min using a Victor spectrophotometer.

**Tubulin Competitive Binding Assay**

The competitive colchicine-binding assay is based on a scintillation proximity assay technology using bovine brain tubulin, which has been modified so that random surface lysines contain a covalently linked, long-chain biotin derivative (CytoDYNAMIX screen 15, Cytoskeleton, Tebu-Bio). Briefly, 10 μL of each compound were added to a well of a 96-well plate (Low PB Corning Costar). Tritiated colchicine (100 μL; Perkin-Elmer specific activity, 70-80 Ci/mmol) was added to 300 μL tubulin-binding buffer and then 10 μL were added in each well. Premix tubulin-biotin-streptavidin scintillation proximity assay beads (180 μL; 4.4 mg streptavidin yttrium silicate beads are mixed into 15 mL buffer and incubated on a slow 10 rpm rotator at 4°C for

![Figure 1](MolecularCancerTherapeutics_2008_7_8_Fig1.png) Synthesis of IRC-083927 hydrochloride.
30 min; Amersham Bioscience) were added to each well for 3 h at 37°C. The plates were then read on a scintillation counter (Packard Instrument, Topcount Microplate Reader) and the percentage of inhibition was calculated (32).

**Cell Proliferation Assay**

At day 0, cells were seeded in 96-well plate (1,500 cells for MDA-MB-231, DU-145, and NCI-H69; 1,200 cells for MIA PaCa-2; and 750 cells for A549) in 90 μL complete medium per well (tissue culture grade, TPP). At day 1, cells were treated for 72 h (except for 96 h with NCI-H69 and A549 cell lines) with increasing concentrations of drugs. The inhibition was determined using a colorimetric WST1 assay (Roche Diagnostic). These experiments were done twice with eight determinations per tested concentration. For each compound, values falling in the linear part of the sigmoid curve were included in a linear regression analysis to calculate the IC_{50} values (33).

**Assessment of Cell Cycle Phase Distribution**

After cell seeding (24 h), the cells were treated for 24 h and then detached by adding 300 μL trypsin-EDTA (Lonza France). The adherent and floating cells were centrifuged at 200 × g for 5 min at room temperature and the pellet was resuspended and fixed in 1 mL of 70% ice-cold ethanol (Sigma) and stored at −20°C for 48 h. After fixation, cells were suspended in 100 μL phosphate-citrate buffer [0.19 mol/L Na_2HPO_4 and 4 mmol/L citric acid (both from Sigma)] and incubated for 30 min at room temperature. Cells were then resuspended in 1 mL PBS (Lonza France) containing 10 μg/mL propidium iodide (Sigma) and 10 μg/mL RNase A (Sigma). The propidium iodide–stained cell samples were analyzed with a flow cytometer (FACSCalibur, Becton Dickinson).

**Angiogenes Assay**

*In vitro* vessel formation was assessed using an angiogenesis kit (TCS Cellworks). Human umbilical vein endothelial cells were cultured in a 24-well plate within a matrix of human diploid fibroblasts of dermal origin in optimized medium (TCS Cellworks). The cocultured cells were incubated throughout the experiment at 37°C under 5% CO_2 in a humidified incubator. On day 1, the culture medium was removed and replaced with medium containing IRC-083927. On days 4, 7, and 9, the medium was replaced with fresh medium containing IRC-083927. IRC-083927 was tested in triplicate. On day 11, the cells were washed (PBS) and 70% ethanol (1 mL) was added to each well for 30 min to fix the cells. After fixation the cells were washed with blocking buffer (1 mL, PBS + 1% BSA; Sigma) and stained for CD31 in accordance with the manufacturer’s instructions (TCS Cellworks). The extent of vessel formation was then quantified using a variation of a previously described technique (34). Briefly, using a high-resolution transmissive scanner (ScanMaker 9800, Microtek) each well was scanned and saved as a tagged image format file in Photoshop (Adobe). The image was then converted to a black-and-white image using the photocopy filter in Photoshop (2 ×, 10 Detail, 25 Darkness) and saved as an uncompressed tagged image format file. The files were transferred to the AngioSys software (TCS Cellworks), all background and non-tubule-like structures were removed using the erode (1 ×) and clean (100 pixels) functions, and the number of pixels representing vessels was counted.

**Preliminary Pharmacokinetic Studies Assessing IRC-083927 in Mice**

MF-1 nude mice were dosed with 10 mg/kg IRC-083927 in 3% dimethylacetamide-2% Montanox-95% saline by oral administration. Blood samples were taken from 30 to 1,440 min in vena cava. IRC-083927 levels in plasma were quantified by liquid chromatography-tandem mass spectrometry.

**Human Tumor Xenograft Models**

Human cancer cells (10^6) were s.c. injected into the flanks of female athymic NCr-nu/nu 4- to 6-week-old mice. Once the tumors were established (50-100 mm^3), treatment was carried out per os as indicated in the legends, with 6 to 8 animals per group. Tumor volume and animal body weight were monitored two to three times weekly. Tumor volumes were calculated as described previously and animal care was in accordance with institutional guidelines (35). Statistical analyses of the results were done at the end of the treatment period and at the end of the follow-up period using the Mann-Whitney’s test (two-tailed).

**Results**

**IRC-083927 Inhibits Tubulin Polymerization by Binding to the Colchicine Site**

IRC-083927 is a small molecule issued from phenoxy aryl imidazole series optimized to improve the antiproliferative activity. The synthesis of [N-(2-fluoro-4-[4-[2-(phenoxy)methyl]-1H-imidazol-4-yl)phenoxy]phenyl)sulfamide] hydrochloride (IRC-083927) was prepared in five steps (Fig. 1). To investigate whether IRC-083927 affects microtubule stability in a cell free system, we have used purified bovine brain tubulin, which polymerizes in *vitro*. As expected, addition of colchicine in a concentration-dependent manner decreased the formation of new polymers with a maximal effect at 5 μmol/L (ref. 36; Fig. 2A), whereas addition of paclitaxel strongly increased it (data not shown). IRC-083927 inhibits tubulin polymerization with similar kinetics and potency compared with colchicines with a maximal effect at 5 μmol/L. No drug preincubation at 37°C has been requested to observe this polymerization inhibition. It is generally observed that drugs affecting tubulin polymerization interact with tubulin either at the taxane-binding, *Vinca*-binding, or colchicine-binding sites. Competition-binding scintillation proximity assay (Fig. 2B) shows that IRC-083927 competitively inhibited [^3]H]colchicine binding on the purified brain bovine tubulin, suggesting that IRC-083927 directly interacted with tubulin by binding to the colchicine-binding domain. Used as negative controls, neither paclitaxel nor vinblastine were able to displace labeled colchicine. In addition to these experiments using isolated materials, to visualize the microtubule changes in whole...
cells, we used an automated image acquisition and analysis system (IN Cell Analyzer 1000) to visualize the tubulin organization in human prostate tumor cells DU-145. Increasing concentrations of IRC-083927 showed a drastic disorganization of the cytoskeleton at 20 and 100 μmol/L comparable with the effects induced by colchicine (100 μmol/L; Fig. 2C).  

**IRC-083927 Inhibits the Proliferation of Human Tumor Cell Lines**

The antiproliferative effects of IRC-083927 have been compared with those of major representatives of the different classes of tubulin-binding agents: colchicine, ABT-751, paclitaxel, and navelbine on a panel of human tumor cell lines (Table 1). All the cell lines examined were highly sensitive to IRC-083927 in a concentration-dependent manner with IC_{50} values in the low nanomolar range (7-15 μmol/L). The range of activity is similar to that observed with the other agents: colchicine (13-26 μmol/L), paclitaxel (2-13 μmol/L), and navelbine (3-6 μmol/L). However, ABT-751 (426-1,721 μmol/L) seems to be less potent *in vitro* on this panel of tested cell lines.

IRC-083927 Inhibits Growth Human Tumor Cells Independently of Their MDR Status

The clinically approved tubulin-binding drugs are sensitive to the PgP efflux pump; in consequence, we have investigated whether IRC-083927 remains active against drug-resistant human lung cancer cell line expressing PgP: NCI-H69/Lx4 and compared with its parental cell line NCI-H69s (Table 1). As expected, NCI-H69s cells were much more sensitive to paclitaxel and navelbine (6 and 5 μmol/L) than the variant NCI-H69/Lx4 cells (PgP⁺; >125 and 325 μmol/L, respectively). In contrast, IRC-083927 inhibited the proliferation of both cell lines whatever their PgP status. In addition, the cytotoxic activity of IRC-083927 was investigated against another drug-resistant human lung cancer cell variant NCI-H69/AR expressing a different drug efflux pumps: MRP and compared with its parental cell line NCI-H69s (Table 1). NCI-H69s cells were highly sensitive to the clinically approved Adriamycin, whereas NCI-H69/AR cells (MRP⁺, PgP⁻) were much less sensitive. In contrast, IRC-083927 inhibited both cell lines displaying similar profiles to paclitaxel and navelbine.

**Figure 2.** IRC-083927 inhibits tubulin polymerization and the binding of labeled colchicine on purified tubulin. **A,** ability of IRC-083927 (5 μmol/L) to inhibit polymerization of purified tubulin was assessed by turbidity change monitored spectrophotometrically at 340 μmol/L. DMSO was used as a vehicle. Colchicine (5 μmol/L) was used as positive control for tubulin polymerization inhibition. **B,** competitive-colchicine-binding site assay is based on scintillation proximity assay. IRC-083927 was tested at 20 μmol/L, whereas the reference compounds paclitaxel, colchicine, and vinblastine were tested at the concentration of 50 μmol/L. **C,** tubulin cytoskeleton (red) in DU-145 cells was assessed by IN Cell Analyzer 1000 showing that increasing concentrations of IRC-083927 or colchicine disorganized the cell structure. The nucleus is colored in blue with 4,6-diamidino-2-phenylindole.
IRC-083927 inhibited the proliferation of the three cell lines whatever their efflux pump status showing that its efficacy is not altered by the presence of efflux pumps in contrast to the approved drugs tested.

**IRC-083927 Is Potent on Tubulin Mutated Resistant Tumor Cell Lines**

A second mechanism of resistance to the tubulin-binding agents has been linked to the appearance of mutations of the tubulin genes. IRC-083927 was evaluated against cell lines A549.EpoB40 expressing one mutated β-tubulin and A549.EpoB480 expressing one mutated α-tubulin and one mutated β-tubulin. This effect has been compared with that observed in the parental cell line A549 (Table 1). As expected, epothilone B and paclitaxel were very active against the parental cell line A549 cells but much less active against A549.EpoB40 and poorly active against A549.EpoB480. In contrast, IRC-083927 was active against the three cell lines with similar IC50 values (10, 8, and 22 μmol/L). ABT-751 was much less potent against the three cell lines but remained active against the variants with IC50 values (1,271, 3,711, and 9,089 μmol/L).

**IRC-083927 Blocks Tubulin Mutated Resistant Tumor Cells in the G2-M Phase**

A characteristic effect of tubulin inhibitors on the cell cycle progression is that the treated cells accumulate in the G2-M phase. Fluorescence-activated cell sorting analysis presented in Table 2 shows that the percentage of A549 cells in G2-M phase increased in an IRC-083927 concentration-dependent manner from 25% (0 μmol/L) to 61% (60 μmol/L). At the same time, the percentage of G1 cells decreased from 53 to 16%. These results expected for a tubulin-binding agent in cells with a normal tubulin have been also observed on A549.EpoB40 expressing mutated tubulin gene. G2-M phase also increased in an IRC-083927 concentration-dependent manner in this variant from 46% (0 μmol/L) to 88% (60 μmol/L) concomitantly with a decrease of the percentage of cells in G1 from 38% to 2%.

**IRC-083927 Inhibits Angiogenesis In vitro**

Tubulin-binding agents reduce tumor perfusion and inhibit mitosis of tumor cells with markedly different effective dose ranges from agent to agent, but it is not always clear which effects contribute to the suppression of solid tumor growth. It was of interest to study the effects of IRC-083927 on angiogenesis looking at the endothelial cell proliferation (Fig. 3, top), migration, and vessel formation using an in vitro coculture model of angiogenesis (Fig. 3, middle and bottom). IRC-083927 completely inhibited the vascular endothelial growth factor–stimulated formation of vessel-like structures as assessed by CD31 staining. At 10 μmol/L, only a few small islands of endothelial cells were detected and virtually no CD31-positive cells at the 20 μmol/L concentration (Fig. 3). The lack of staining indicates that this compound may possess antiangiogenic activities as it blocks the proliferation of endothelial cells.

**IRC-083827 Is Bioavailable by the Oral Route in Mice**

To maintain a continuous blockade of tubulin polymerization, the drug exposure in vivo should be as long as possible. Hence, we assessed the bioavailability of IRC-083927 by the oral route in the mouse. Under our experimental conditions, in fed mice after an oral administration of IRC-083927 (10 mg/kg), the time to reach the maximum

<table>
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<tr>
<th>Cell line</th>
<th>IRC-083927 (μmol/L)</th>
<th>G2/M1 (%)</th>
<th>G2/M (%)</th>
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<tr>
<td>A549</td>
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<td>A549.EpoB40</td>
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Table 2. Percentage of cells in G2-M phase of cell cycle treated with IRC-083927.
plasma concentration ($T_{\text{max}}$) is 30 min with a concentration at this time point ($C_{\text{max}}$) of 10.3 μmol/L. Twenty-four hours after the bolus, the concentration is 0.9 μmol/L. Such a plasma concentration is higher than the IC50 observed in vitro to induce cell growth arrest; hence, a protracted administration of IRC-083827 seemed suitable to assess its potential against human tumors xenografted in athymic mice.

IRC-083827 Inhibits the Growth of Human Tumors Xenografted in Athymic Mice

IRC-083927 was evaluated with a curative approach in athymic mice xenografted with two different models: the human cervical tumor C33-A (Fig. 4A and B) and the human hormone-independent breast tumor cell MDA-MB-231 (Fig. 4C and D). The treatment was initiated only when the tumor volume reached 100 mm³. In both models, the regimen (5 mg/kg qd5, 2 days off during 4 weeks, per os) leads to a significant tumor growth inhibition (Fig. 4A and C) without toxicity in terms of weight loss or animal death (Fig. 4B and D). The efficacy of IRC-083927 was comparable with that of ABT-751 (100 mg/kg qd5, 2 days off during 4 weeks, per os) in the MDA-MB-231 model.

Discussion

Drug resistance is one of the main problems in the management of neoplastic diseases, and the effectiveness...
of many clinically useful drugs is limited by the intrinsic or the adaptive appearance of drug resistance mechanisms (37, 38). Drugs that affect the tubulin-microtubule equilibrium (Taxol and Vinca alkaloids) are effective anticancer drugs but subject to drug resistance mechanisms. Therefore, there is a need to design new agents to interact with tubulin at sites different from those of Vinca alkaloids and taxanes but which must conserve good antitumor activity along with an acceptable toxicity profile (39). The design of compounds that interact with tubulin at sites different from those of Vinca alkaloids, taxanes, or epothilones entails the optimization of original chemical scaffolds that should display different pharmacologic properties (linked to chemical stability, biodistribution, biological effects, etc.). Such new chemical entities represent a particular medical interest for the treatment of human tumors resistant to currently used therapeutic agents.

Here, we report a novel chemical entity IRC-083927, which inhibits polymerization of purified tubulin and disorganizes microtubules in human tumor cells. IRC-083927 binds to the tubulin colchicine-binding site, arrests the cell cycle progression in G2-M phase, and displays high antiproliferative activity against drug-resistant tumor cell lines. Moreover, IRC-083927 shows significant activity in animal models bearing human tumors treated by the oral route without apparent macroscopic toxicity. In addition, in view of the tubulin-binding ability of IRC-083927, it was important to examine if it also exerts an inhibitory effect on angiogenesis like other tubulin-binding agents (40, 41). As expected, IRC-083927 inhibited human umbilical vein endothelial cell proliferation and vessel formation in these systems (42). Using this coculture system, IRC-083927 was found to be active from 10 μmol/L in inhibiting vessel formation and is consequently a potent antiangiogenic agent.

**Figure 4.** Responses of human tumor xenografts to IRC-083927. **A,** significant growth inhibition of human cervical tumor C33-A in nude mice (n = 8 per group) treated with IRC-083927 by oral route. Both regimens gave statistical benefits for the reduction of tumor volumes at the end of the treatment (2.5 mg/kg: P = 0.0016 and 5 mg/kg: P = 0.0016) and at the end of the follow-up (2.5 mg/kg: P = 0.0008 and 5 mg/kg: P = 0.0087). B, no change of the body weight of the C33A xenografted nude mice treated with IRC-083927 using the schedules described above. C, significant growth inhibition of human breast tumor in nude mice (n = 6 per group) treated with both IRC-083927 (end of treatment: P = 0.0039 and end of follow-up: P = 0.0039) or ABT-751 (end of treatment: P = 0.0039 and end of follow-up: P = 0.0039). D, no change of the body weight of the MDA-MB-231 xenografted nude mice treated with IRC-083927 or ABT-571 using the schedules described above. (--), duration of treatment.
Two mechanisms of drug resistance have been challenged with IRC-083927: (a) the presence of drug efflux pumps and (b) the presence of mutations on the tubulin-encoding genes (α and β; refs. 14–17) even if the clinical effect of such mutations is still under debate (43, 44). The presence of drug efflux pumps in the NCI-H69 variants (PgP+ or MRP+) strongly altered the drug sensitivity of the three compounds already approved regarding the resistance factors of paclitaxel (resistance factor > 26) and navelbine (resistance factor > 55) on the PgP+ variant and of Adriamycin on both PgP+ and MRP+ variants (resistance factor > 35 in both cases). This was not the case with IRC-083927, which remains a highly potent inhibitor of cell proliferation (8 μmol/L parental cell line NCI-H69 versus 13 μmol/L MRP+ variant and 42 μmol/L PgP+ variant; Table 1).

The second mechanism of resistance is more directly related to the tubulin-binding agents. The mutation of the amino acid residue at position 292 located on β-tubulin has been observed in human patients (45). The use of these particular cell lines enabled the discrimination of tubulin-binding agents on cell lines A549.EpoB40 (mutation on β-tubulin Gln 292→Glu) and A549.EpoB480 (mutation on β-tubulin (Gln292→Glu; Val60→Phe and on α-tubulin Leu195→Met). Epothilone, paclitaxel, and navelbine are poorly active in these models. IRC-083927 is ~1,000 times more active on A549.EpoB480 mutated cell line than paclitaxel and epothilone B. Only ABT-751 displays a similar profile to IRC-083927 but with a considerably lower potency.

In the studies reported here, IRC-083927 was evaluated in animal models bearing human cervical tumor C33-A and human breast tumor MDA-MB-231. Our results indicate that IRC-083927 exhibits strong activity in both models leading to a stabilization of the tumor size during the treatment period. In addition, we have compared its efficacy with that of the colchicine-binding site binding agent ABT-751, which is the most advanced compound in clinical trials. Both compounds displayed a high and comparable efficacy, with a potency advantage observed for IRC-083927 (5 versus 100 mg/kg). It is noteworthy that this potency advantage was also observed in in vitro cell proliferation assays.

It remains to determine the drug exposure achieved in mice where a robust antitumor activity against tumor xenografts has been observed and then ensure that this exposure can be achieved in patients. In addition, the oral administration of IRC-083927 compared with an injectable formulation represents an advantage for both patients and physicians (46). The toxicity profile needs to be fully documented before the relevance of IRC-083927 can be definitely established. The full assessment of the overall adverse effects sides will be done in the next steps of the preclinical development after the scaling up of the synthesis. Based on the observations of compounds binding to the colchicine-binding site already in clinical trials such as ABT-751, the effect on the digestive tract should be assessed in the light of the abdominal pain, constipation, and fatigue (47). Observed constipation would appear unexpected in view of the laxative effects observed with the colchicines (48). However, these observed toxicities differ from those observed with epothilones [diarrhea associated with patupilone or neurotoxicity and neutropenia encountered with other epothilones (ixabepilone, BMS-310705, and KOS-1584) and with taxanes and Vinca alkaloids (neurotoxicity and hematologic toxicity)].

In conclusion, the data show that the novel imidazole derivative IRC-083927 has properties distinct from Adriamycin, navelbine, epothilone B, and paclitaxel and that it is efficacious in suppressing cell growth in a variety of tumor models despite PgP, MRP, or mutated tubulin status. Furthermore, it exhibits significant antiangiogenic activity that could offer considerable potential for drug combinations. Access to novel tubulin inhibitors binding at different sites will allow their use in combination with other drugs (49) and particularly to tubulin-binding agents (taxanes or Vinca alkaloids) to obtain synergism (50). This work is currently in progress.

IRC-083927 is a promising preclinical compound with a potential for the management of patients with demonstrated resistance to anticancer drugs such as taxanes or Vinca alkaloids.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Iwasaki S, Shirai R. Natural organic compounds that affect to microtubule functions: syntheses and structure-activity relationships of combretastatins, curacin A and their analogs as the colchicine-site ligands on tubulin. Yakugaku Zasshi 2000;120:875–89.


Correction

Effects of IRC-083927 on human tumor growth

In the article on the effects of IRC-083927 on human tumor growth in the August 2008 issue (1), several values listed as micromoles per liter (μmol/L) should have been listed as nanomoles per liter (nmol/L). On page 2429, the change should appear on lines 5, 7, 17, 18, 19, and 20 of the first paragraph and on lines 10 and 11 of the second paragraph. On page 2430, the change should appear in Tables 1 and 2; on lines 19, 22, 30, 31, and 37 of the first paragraph; and on lines 13 and 15 of the second paragraph. On page 2431, the change should appear in the legend to Fig. 3. On page 2432, the change should appear on line 16 of the second paragraph. On page 2433, the change should appear on lines 14 and 15 of the first paragraph.

Reference

Molecular Cancer Therapeutics

IRC-083927 is a new tubulin binder that inhibits growth of human tumor cells resistant to standard tubulin-binding agents
Anne-Marie Liberatore, Hélène Coulomb, Dominique Pons, et al.

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