available at http://www.tzuchimedjnl.com/

Tzu Chi Medical Journal

ELSEVIER

Original Article

Lestaurtinib is Cytotoxic to Oxaliplatin-resistant Transitional Cell Carcinoma Cell Line T24 *In Vitro*

Pei-Chun Lai[1,2], Te-Chao Fang[3,4], Chuan-Chu Cheng[5], Ted H. Chiu[1,6], Yen-Ta Huang[1,4,7]**

[1]*Institute of Pharmacology and Toxicology, Tzu Chi University, Hualien, Taiwan*
[2]*Department of Pediatrics, Buddhist Tzu Chi General Hospital, Hualien, Taiwan*
[3]*Department of Medicine, Tzu Chi University, Hualien, Taiwan*
[4]*Department of Nephrology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan*
[5]*Department of Research, Buddhist Tzu Chi General Hospital, Hualien, Taiwan*
[6]*Department of Pharmacology, Tzu Chi University, Hualien, Taiwan*
[7]*Department of Surgical Critical Care Unit, Buddhist Tzu Chi General Hospital, Hualien, Taiwan*

Article info

Article history:
Received: August 18, 2010
Revised: September 1, 2010
Accepted: September 6, 2010

Keywords:
Lestaurtinib
Oxaliplatin
Securin
Survivin
Transitional cell carcinoma

Abstract

Objective: Patients with bladder cancer have responded poorly to oxaliplatin therapy in clinical trials. Blockade of receptor tyrosine kinases is considered a good strategy in cancer therapy. Our previous studies have demonstrated the crucial roles of brain-derived neurotrophic factor and its receptor tropomyosin receptor kinase B (TrkB) in transitional cell carcinoma (TCC). The aim of this study was to examine the cytotoxic effects of lestaurtinib, a new pan-Trk inhibitor, and oxaliplatin on bladder cancer cell lines.

Materials and Methods: BFTC905 and T24 TCC cell lines were used for investigation *in vitro*. The effects of oxaliplatin and/or lestaurtinib on cell viability, apoptosis, and expression of survivin and securin were assessed. MTT assay was used for cytotoxic evaluation. DNA fragments were detected in both the culture medium and cytoplasm to differentiate the types of cell death (apoptosis *vs.* necrosis). Western blots were used to analyze the expression of survivin and securin after oxaliplatin and/or lestaurtinib treatment.

Results: Oxaliplatin at 3 μg/mL elicited cytotoxicity on BFTC905 but not T24 cells 48 hours after treatment. The addition of 1 or 3 μM lestaurtinib to oxaliplatin did not exert additive cytotoxic effects on BFTC905 cells. Although oxaliplatin at 3 μg/mL had no effect on T24 cells, the addition of 1 or 3 μM lestaurtinib demonstrated concentration-dependent inhibitory effects. Apoptosis of T24 cells was observed after treatment with lestaurtinib alone and lestaurtinib plus oxaliplatin. Furthermore, in T24 cells, the expression of survivin was inhibited by a combination of lestaurtinib and oxaliplatin, while securin expression was inhibited by lestaurtinib alone and lestaurtinib with oxaliplatin.

Conclusion: Lestaurtinib may be a potential new drug for the targeted therapy of oxaliplatin-resistant TCC. Further *in vivo* studies are needed. (*Tzu Chi Med J* 2010;22(3):125–130)

*Corresponding authors. Department of Nephrology, Buddhist Tzu Chi General hospital, 707, Section 3, Chung-Yang Road, Hualien, Taiwan.
E-mail addresses: thchiu@mail.tcu.edu.tw and butterdada@pchome.com.tw

1. Introduction

The distribution of urinary bladder cancer varies significantly between geographical regions and countries. The incidence is much higher in developed countries and some areas such as North America and Western Europe [1]. In Taiwan, the Bureau of Health Promotion, Department of Health reported that it was the eighth most common malignancy in 2007 with 1,457 new cases. Pathological classification indicates that more than 90% of bladder cancers are transitional cell carcinoma (TCC) [2]. Once distant metastasis occurs, the median survival is approximately 1 year even after aggressive treatment [3]. Therefore, we need to investigate new strategies and more effective treatments for metastatic bladder cancer.

Some receptor tyrosine kinases (RTKs) that are overexpressed in cancer tissues have been characterized as oncogenes. Thus, RTK blockade is currently considered a good strategy for clinical cancer therapy, especially for inoperable conditions [4]. Overexpression of some growth factors and their specific RTKs have been found in bladder cancer, including epidermal growth factor receptor, platelet-derived growth factor receptor β, and fibroblast growth factor 3, and have been correlated with poor clinical outcomes [5]. However, there have been only a few clinical trials of targeted therapy for bladder cancer because some preclinical trials of targeted therapies on certain RTKs showed disappointing results [5]. Thus, new RTKs should be investigated for targeted therapy of bladder cancer.

The tropomyosin receptor kinase (Trk) family of tyrosine kinase receptors consists of TrkA, TrkB and TrkC. These Trk receptors and their ligands play important roles in neuronal survival and differentiation [6]. TrkB and/or its ligand, brain-derived neurotrophic factor (BDNF), have also been found in solid malignancies such as pancreatic ductal adenocarcinoma [7], prostate cancer [8], and lung cancer [9]. In our previous reports, BDNF and TrkB, but not TrkA or TrkC, were found in three TCC cell lines, BFTC905, TSGH8301, and T24 [10]. Increased immunostaining for BDNF and TrkB was observed in human TCC specimens compared to normal tissues [11]. The proliferation and invasiveness of BFTC905 cells were enhanced by BDNF. TrkB antibody suppressed proliferation, elicited cytotoxicity, and inhibited migration [12]. These results indicate that both BDNF and TrkB play important roles in the progression of TCC. Drugs aimed at BDNF or TrkB receptors may provide a new potential approach for treating TCC.

Lestaurtinib (CEP-701) has been approved by the U.S. Food and Drug Administration for acute myeloid leukemia because of its potent inhibition of FMS-like tyrosine kinase 3 (also a member of the type III TRK superfamily) with an IC_{50} of 3 nM [13,14]. Lestaurtinib also inhibits the phosphorylation of TrkA and TrkB [15] as well as the tumor growth of pancreatic ductal adenocarcinoma xenografts through Trk blockade [16,17]. We hypothesize that lestaurtinib could elicit cytotoxicity and induce apoptosis in TCC cells *in vitro* and be a potential new drug for the targeted therapy of bladder cancer.

2. Materials and methods

2.1. Cell culture

Human TCC cell lines, BFTC905 and T24, were used for the study. The cells were maintained as described previously [10]. Gradings of BFTC905 [18] (originating from Taiwanese patients with TCC) and T24 (from ATCC, American Type Culture Collection, Manassas, VA, USA) were 3 and 2/3, respectively.

2.2. MTT cytotoxicity assay

For the cytotoxicity assay, 1×10^4 TCC cells per well were seeded in 96-well plates. After incubation overnight, the cells adhered on the plate. Lestaurtinib (LC Laboratories, Woburn, MA, USA), 1 or 3 μM, dissolved in 45% γ-cyclodextrin (Sigma, St. Louis, MO, USA) and oxaliplatin (Sanofi-Aventis, Paris, France) 3 μg/mL dissolved in phosphate-buffered saline (PBS) were administrated for 48 hours. The same volume of γ-cyclodextrin or PBS treatment served as a negative control. Conventional MTT (methylthiazolyldiphenyltetrazolium bromide; Sigma) assay was followed using the standard protocol [10].

2.3. DNA fragment assay

The apoptotic or necrotic condition of TCC cells after drug treatments was assessed by cell death detection ELISA^{plus} (Roche, Mannheim, Germany). Forty-eight hours after treatment with oxaliplatin and/or lestaurtinib, the supernatants of both the culture medium and the cytoplasmic fraction of 2×10^4 TCC cells in 24-well dishes were used. The O.D. value at 405 nm, representing the extent of DNA fragmentation, was measured by an ELISA reader.

2.4. Western blot

Standard procedures were followed as previously described [10]. Primary antibodies used were anti-survivin (#2808; Cell Signaling Technology Inc., Danvers, MA, USA) and anti-securin (ab3305; Abcam Inc., Cambridge, MA, USA). Expression of α-tubulin (sc-8305; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was used as the internal standard. The intensity of immunoreactive

proteins was calculated using National Institutes of Health software ImageJ version 1.40. The intensity ratio was the intensity of survivin or securin divided by that of α-tubulin, and was used to compare the drug effects.

2.5. Statistical analysis

Data are expressed as mean±standard error of the mean, and were evaluated by Student's *t* test. In all cases, $p<0.05$ was considered statistically significant.

3. Results

3.1. Cytotoxicity of oxaliplatin alone or in combination with lestaurtinib on BFTC905 and T24 cells

As shown in Fig. 1, oxaliplatin at 3 μg/mL inhibited 33% of BFTC905 cell viability (66.7±9.6% of viability compared to PBS control; $n=3$) 48 hours after treatment. However, 3 μg/mL of oxaliplatin did not exert significant cytotoxicity on T24 cells (96.4±5.8% of viability compared to PBS control; $p=0.33$, $n=3$).

A combination of oxaliplatin and lestaurtinib showed no additive cytotoxic effects on BFTC905 cells (62.8±3.1% and 62.8±6.3% of viability, after treatment with 1 and 3 μM lestaurtinib plus 3 μg/mL oxaliplatin, respectively; $n=3$). In contrast, 1 and 3 μM lestaurtinib when combined with 3 μg/mL oxaliplatin concentration-dependently inhibited the viability of T24 cells (57.3±2.7% and 34.2±1.2% of viability after treatment with 1 and 3 μM lestaurtinib plus 3 μg/mL oxaliplatin, respectively; $n=3$).

3.2. Lestaurtinib induced apoptosis in T24 cells

To determine the characteristics of cytotoxicity mediated by lestaurtinib on T24 cells, DNA fragment ELISA assay was carried out (Fig. 2). Low basal level DNA fragments bound by anti-histone and anti-DNA antibodies in the ELISA assay kit were detected in the culture medium of T24 cells after lestaurtinib and/or oxaliplatin treatment. However, a large amount of cytoplasmic DNA fragments was observed after treating T24 cells with 1 μM lestaurtinib alone or in combination with 3 μg/mL oxaliplatin when compared with treatment with 3 μg/mL oxaliplatin alone ($p=0.000001$ and 0.00002, respectively; $n=3$). A small amount of cytoplasmic DNA fragments was detected in the control and oxaliplatin-treated cytoplasmic samples ($p=0.33$, $n=3$). These results indicated that lestaurtinib but not oxaliplatin induced apoptosis of T24 cells.

3.3. Expression of survivin and securin in T24 cells after lestaurtinib treatment

Decreased expression of survivin in bladder cancer specimens has been reported to be a good predictor

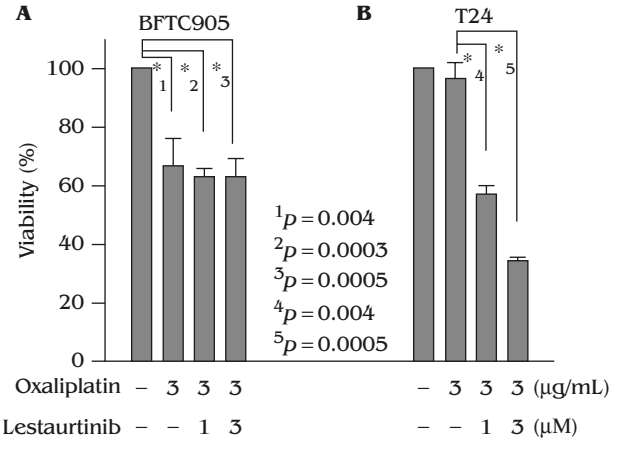

Fig. 1 — Cytotoxic effects of oxaliplatin alone and in combination with lestaurtinib on BFTC905 and T24 cells. The viability of (A) BFTC905 and (B) T24 cells was measured by MTT assay 48 hours after treatment with 3 μg/mL oxaliplatin or 3 μg/mL oxaliplatin plus 1 or 3 μM lestaurtinib. The viability ratio was the ratio of the O.D. value from the drug-treated sample divided by that of the control sample. *$p<0.05$.

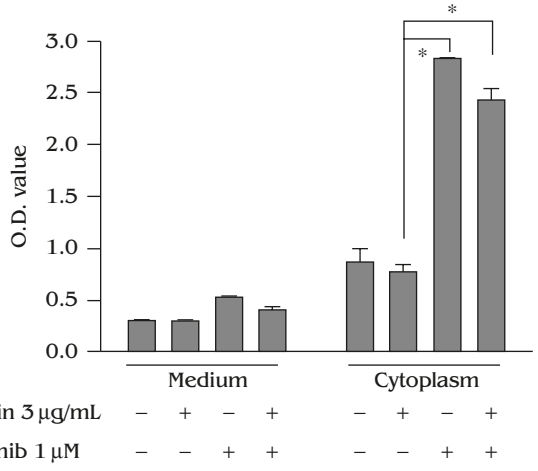

Fig. 2 — DNA fragments in the culture medium or cytoplasm of T24 cells detected by ELISA. Forty-eight hours after treatment with oxaliplatin and/or lestaurtinib, the supernatants of both the culture medium and the cytoplasmic fraction of 2×10^4 transitional cell carcinoma cells were collected. Increased expression of DNA fragments in the medium indicates necrosis due to membrane rupture, while increased expression in the cytoplasm alone indicates apoptosis. *$p<0.05$.

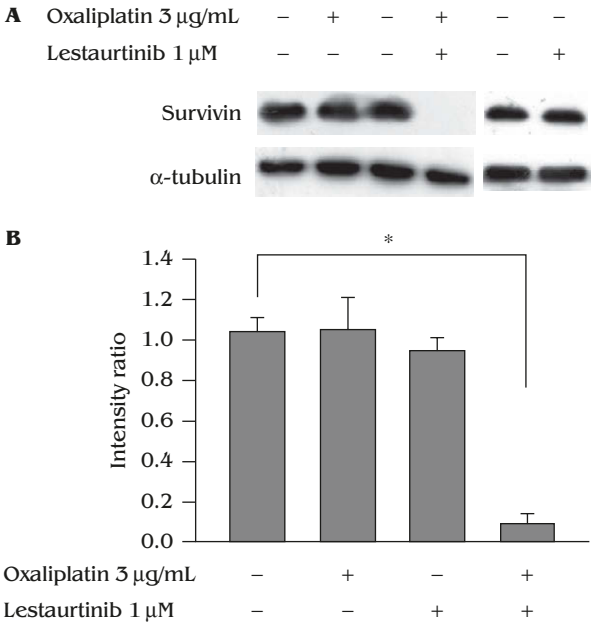

Fig. 3 — Expression of survivin after oxaliplatin and/or lestaurtinib treatment. (A) A representative Western blot of the expression of survivin examined 48 hours after oxaliplatin and/or lestaurtinib treatment. Expression of α-tubulin was used as an internal standard. (B) Quantitative comparison of the survivin expression in each group (n=3). *p<0.05.

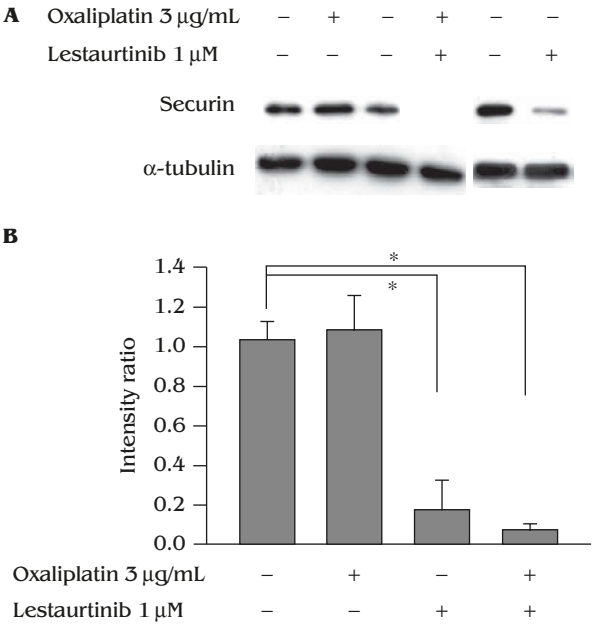

Fig. 4 — Expression of securin after oxaliplatin and/or lestaurtinib treatment. (A) A representative Western blot of the expression of securin examined 48 hours after oxaliplatin and/or lestaurtinib treatment. Expression of α-tubulin was used as an internal standard. (B) Quantitative comparison of the securin expression in each group (n=3). *p<0.05.

of response to therapy [19,20]. Securin, also called pituitary tumor-transforming gene, plays important roles in tumorigenesis and invasiveness in various neoplasms [21]. As shown in Fig. 3, survivin expression in T24 cells was not altered 48 hours after treatment with lestaurtinib (1 μM) or oxaliplatin (3 μg/mL), but was almost completely inhibited when treated with a combination of lestaurtinib and oxaliplatin. Oxaliplatin at 3 μg/mL alone had no effect on securin expression. Lestaurtinib at 1 μM markedly inhibited the expression of securin, but there was no further inhibition when combined with oxaliplatin (Fig. 4).

4. Discussion

Oxaliplatin is a third-generation platinum derivative with a 1,2-diamino-cyclohexane carrier ligand. It has less renal toxicity than cisplatin, but is at least equally as cytotoxic as cisplatin on cancer cells. Many cisplatin-resistant cancer cell lines are sensitive to oxaliplatin because it demonstrates multiple mechanisms of action [22]. The present study suggests that oxaliplatin could be an alternative agent for bladder cancer treatment. However, a disappointing response rate was observed in patients with previously treated, unresectable or metastatic TCC [23]. In two recent phase II trials, a combination of oxaliplatin and gemcitabine showed a satisfactory overall response rate (47–48%) in patients with locally advanced or metastatic urothelial cancer [24,25]. However, the above data reflect that this drug combination was ineffective in half of the patients with TCC. In addition, the median time to disease progression was delayed by just 5 months under this combination treatment [24]. T24 cells have been reported to be an oxaliplatin-resistant TCC cell line [26]. Thus, we examined if enhanced cytotoxicity on TCC cells could be achieved by a combination of oxaliplatin plus other agents.

Oxaliplatin elicited cytotoxicity on BFTC905 but not T24 cells in this study. The characteristics of oxaliplatin resistance in T24 cells are consistent with a previous report [26]. Several mechanisms of oxaliplatin resistance, including reduced drug accumulation similar to that of cisplatin [27], and increased cellular glutathione [28], have been reported. Uptake of oxaliplatin in T24 cells is apparently less than that in oxaliplatin-sensitive testicular 833k cells [26]. Increased heat shock protein 60 mRNA may also be a factor of resistance to platinum analogs in human ovarian and bladder carcinoma cell lines [29]. The mechanisms of differential sensitivity between T24 and BFTC905 cells toward oxaliplatin require further investigation.

Recent strategies for cancer therapy have employed a combination of cytotoxic and targeted agents, but variable results have been reported in pre-clinical

and clinical trials [30–33]. Consequently, we assessed the effects of a novel combination involving lestaurtinib and oxaliplatin *in vitro*, especially in oxaliplatin-resistant TCC cell lines. Lestaurtinib plus oxaliplatin elicited far more potent cytotoxicity on T24 cells than oxaliplatin alone, and a higher concentration of lestaurtinib exhibited greater cytotoxicity when combined with oxaliplatin. However, lestaurtinib and lestaurtinib plus oxaliplatin exerted similar cytotoxic effects on T24 cells (results not shown). In contrast, the cytotoxic effects on BFTC905 cells seemed to result from oxaliplatin alone. Our preliminary results also showed that lestaurtinib has a higher IC_{50} ($\fallingdotseq 5\,\mu M$) on BFTC905 cells than T24 cells ($\fallingdotseq 1\,\mu M$). This observation may partially explain why 3 μM lestaurtinib only inhibited the cell viability by 40%. The apoptosis and inhibition of securin expression on T24 cells after lestaurtinib and/or oxaliplatin treatment are consistent with results from the cytotoxic MTT assay. The concentration used in our study was within the trough level, between 1.1 and 13.3 μM, found on day 28 in a study of patients with acute myeloid leukemia who received 60 mg lestaurtinib twice daily [34]. These results demonstrated the potential of lestaurtinib for bladder cancer therapy. Combinations of lestaurtinib and other chemotoxic agents that exert greater cytotoxicity should be explored.

Survivin, a member of the inhibitor of apoptosis protein family, has been shown to be a promising biomarker for the diagnosis and prognosis of bladder cancer [20,35,36]. Our results showed that neither oxaliplatin nor lestaurtinib altered survivin expression in T24 cells. However, lestaurtinib together with oxaliplatin markedly reduced the expression of survivin. In previous reports, downregulation of survivin by oxaliplatin diminished radioresistance and enhanced the effects of paclitaxel on head and neck squamous carcinoma cells *in vitro* [37,38]. This may explain why oxaliplatin administration did not alter survivin expression in oxaliplatin-resistant T24 cells. However, the observation that lestaurtinib did not affect survivin expression in T24 cells was not consistent with its cytotoxic effect and inhibition of securin expression. Our preliminary experiments also revealed that lestaurtinib did not change the survivin expression in BFTC905 cells. Recently, we have shown that blockade of TrkB by its specific TrkB antibody inhibited survivin expression on these TCC cells [12]. There might be compensatory mechanisms to restore the expression of survivin, which is inhibited by lestaurtinib beyond its TrkB blocking effect.

Securin expressed in nuclei was originally demonstrated as an inhibitor of premature sister chromatid separation as well as a potential activator of transcription, but recently it has also been proved to mediate tumor invasiveness and recurrence [21]. One example is that overexpressed securin in glioma specimens is associated with an unfavorable outcome [39], yet no study addressing the relationship between securin and bladder cancer has been reported. Our previous study has demonstrated the expression of securin in TCC cells and the inhibition of securin expression by TrkB antibody [12]. Therefore, it was not surprising to find decreased expression of securin after treatment with the pan-Trk inhibitor lestaurtinib. Because of the consistent results among apoptosis, MTT assay, and securin inhibition, we believe that inhibition of securin expression is also a good indicator for TCC therapy.

In the present study, we demonstrated the potential of lestaurtinib for TCC therapy mediated by the inhibition of survivin and securin, even on the oxaliplatin-resistant TCC T24 cell line, resulting in apoptosis.

Acknowledgments

This study was partially supported by a grant-in-aid from Tzu Chi General Hospital, Hualien, Taiwan, and a grant from the National Science Council (NSC-97-2314-B-303-016, YTH), Taiwan.

References

1. Ploeg M, Aben KK, Kiemeney LA. The present and future burden of urinary bladder cancer in the world. *World J Urol* 2009;27:289–93.
2. Tavora F, Epstein JI. Bladder cancer, pathological classification and staging. *BJU Int* 2008;10:1216–20.
3. Calabro F, Sternberg CN. High-risk metastatic urothelial cancer: chances for cure? *Curr Opin Urol* 2002;12:441–8.
4. Krause DS, Van Etten RA. Tyrosine kinases as targets for cancer therapy. *N Engl J Med* 2005;353:172–87.
5. Black PC, Agarwal PK, Dinney CP. Targeted therapies in bladder cancer—an update. *Urol Oncol* 2007;25:433–8.
6. Heumann R. Neurotrophin signalling. *Curr Opin Neurobiol* 1994;4:668–79.
7. Miknyoczki SJ, Lang D, Huang L, Klein-Szanto AJ, Dionne CA, Ruggeri BA. Neurotrophins and Trk receptors in human pancreatic ductal adenocarcinoma: expression patterns and effects on in vitro invasive behavior. *Int J Cancer* 1999;81:417–27.
8. Montano X, Djamgoz MB. Epidermal growth factor, neurotrophins and the metastatic cascade in prostate cancer. *FEBS Lett* 2004;571:1–8.
9. Ricci A, Greco S, Mariotta S, et al. Neurotrophins and neurotrophin receptors in human lung cancer. *Am J Respir Cell Mol Biol* 2001;25:439–46.
10. Huang YT, Lai PC, Wu CC, et al. BDNF mediated TrkB activation is a survival signal for transitional cell carcinoma cells. *Int J Oncol* 2010;36:1469–76.
11. Lai PC, Chiu TH, Huang YT. Overexpression of BDNF and TrkB in human bladder cancer specimens. *Oncol Rep* 2010;24:1265–70.
12. Huang YH, Lai PC, Wu CC, Cheng CC, Chiu TH. TrkB antibody elicits cytotoxicity and suppresses migration/invasion of transitional cell carcinoma cells. *Int J Oncol* 2010;37:943–9.

13. Levis M, Allebach J, Tse KF, et al. A FLT3-targeted tyrosine kinase inhibitor is cytotoxic to leukemia cells in vitro and in vivo. *Blood* 2002;99:3885–91.
14. Knapper S, Burnett AK, Littlewood T, et al. A phase 2 trial of the FLT3 inhibitor lestaurtinib (CEP701) as first-line treatment for older patients with acute myeloid leukemia not considered fit for intensive chemotherapy. *Blood* 2006; 108:3262–70.
15. Festuccia C, Muzi P, Gravina GL, et al. Tyrosine kinase inhibitor CEP-701 blocks the NTRK1/NGF receptor and limits the invasive capability of prostate cancer cells in vitro. *Int J Oncol* 2007;30:193–200.
16. Miknyoczki SJ, Dionne CA, Klein-Szanto AJ, Ruggeri BA. The novel Trk receptor tyrosine kinase inhibitor CEP-701 (KT-5555) exhibits antitumor efficacy against human pancreatic carcinoma (Panc1) xenograft growth and in vivo invasiveness. *Ann N Y Acad Sci* 1999;880:252–62.
17. Miknyoczki SJ, Chang H, Klein-Szanto A, Dionne CA, Ruggeri BA. The Trk tyrosine kinase inhibitor CEP-701 (KT-5555) exhibits significant antitumor efficacy in preclinical xenograft models of human pancreatic ductal adenocarcinoma. *Clin Cancer Res* 1999;5:2205–12.
18. Tzeng CC, Liu HS, Li C, et al. Characterization of two urothelium cancer cell lines derived from a blackfoot disease endemic area in Taiwan. *Anticancer Res* 1996;16:1797–804.
19. Akhtar M, Gallagher L, Rohan S. Survivin: role in diagnosis, prognosis, and treatment of bladder cancer. *Adv Anat Pathol* 2006;13:122–6.
20. Margulis V, Lotan Y, Shariat SF. Survivin: a promising biomarker for detection and prognosis of bladder cancer. *World J Urol* 2008;26:59–65.
21. Salehi F, Kovacs K, Scheithauer BW, Lloyd RV, Cusimano M. Pituitary tumor-transforming gene in endocrine and other neoplasms: a review and update. *Endocr Relat Cancer* 2008;15:721–43.
22. Raymond E, Faivre S, Chaney S, Woynarowski J, Cvitkovic E. Cellular and molecular pharmacology of oxaliplatin. *Mol Cancer Ther* 2002;1:227–35.
23. Winquist E, Vokes E, Moore MJ, Schumm LP, Hoving K, Stadler WM. A phase II study of oxaliplatin in urothelial cancer. *Urol Oncol* 2005;23:150–4.
24. Carles J, Esteban E, Climent M, et al. Gemcitabine and oxaliplatin combination: a multicenter phase II trial in unfit patients with locally advanced or metastatic urothelial cancer. *Ann Oncol* 2007;18:1359–62.
25. Theodore C, Bidault F, Bouvet-Forteau N, et al. A phase II monocentric study of oxaliplatin in combination with gemcitabine (GEMOX) in patients with advanced/metastatic transitional cell carcinoma (TCC) of the urothelial tract. *Ann Oncol* 2006;17:990–4.
26. Hah SS, Sumbad RA, de Vere White RW, Turteltaub KW, Henderson PT. Characterization of oxaliplatin-DNA adduct formation in DNA and differentiation of cancer cell drug sensitivity at microdose concentrations. *Chem Res Toxicol* 2007;20:1745–51.
27. Hector S, Bolanowska-Higdon W, Zdanowicz J, Hitt S, Pendyala L. In vitro studies on the mechanisms of oxaliplatin resistance. *Cancer Chemother Pharmacol* 2001;48:398–406.
28. El-Akawi Z, Abu-Hadid M, Perez R, et al. Altered glutathione metabolism in oxaliplatin resistant ovarian carcinoma cells. *Cancer Lett* 1996;10:5–14.
29. Abu-Hadid M, Wilkes JD, Elakawi Z, Pendyala L, Perez RP. Relationship between heat shock protein 60 (HSP60) mRNA expression and resistance to platinum analogues in human ovarian and bladder carcinoma cell lines. *Cancer Lett* 1997;119:63–70.
30. Doi T, Boku N, Kato K, et al. Phase I/II study of capecitabine plus oxaliplatin (XELOX) plus bevacizumab as first-line therapy in Japanese patients with metastatic colorectal cancer. *Jpn J Clin Oncol* 2010;40:913–20.
31. Augustine CK, Toshimitsu H, Jung SH, et al. Sorafenib, a multikinase inhibitor, enhances the response of melanoma to regional chemotherapy. *Mol Cancer Ther* 2010;9: 2090–101.
32. Ulivi P, Arienti C, Zoli W, et al. In vitro and in vivo antitumor efficacy of docetaxel and sorafenib combination in human pancreatic cancer cells. *Curr Cancer Drug Targets* 2010 May 21. (Epub ahead of print)
33. Dal Lago L, D'Hondt V, Awada A. Selected combination therapy with sorafenib: a review of clinical data and perspectives in advanced solid tumors. *Oncologist* 2008; 13:845–58.
34. Smith BD, Levis M, Beran M, et al. Single-agent CEP-701, a novel FLT3 inhibitor, shows biologic and clinical activity in patients with relapsed or refractory acute myeloid leukemia. *Blood* 2004;103:3669–76.
35. Shariat SF, Karakiewicz PI, Godoy G, et al. Survivin as a prognostic marker for urothelial carcinoma of the bladder: a multicenter external validation study. *Clin Cancer Res* 2009;15:7012–9.
36. Nouraee N, Mowla SJ, Ozhand A, Parvin M, Ziaee SA, Hatefi N. Expression of survivin and its spliced variants in bladder tumors as a potential prognostic marker. *Urol J* 2009;6:101–8.
37. Khan Z, Khan N, Tiwari RP, Patro IK, Prasad GB, Bisen PS. Down-regulation of survivin by oxaliplatin diminishes radioresistance of head and neck squamous carcinoma cells. *Radiother Oncol* 2010;96:267–73.
38. Khan Z, Khan N, Varma AK, et al. Oxaliplatin-mediated inhibition of survivin increases sensitivity of head and neck squamous cell carcinoma cell lines to paclitaxel. *Curr Cancer Drug Targets* 2010 Jun 25. (Epub ahead of print)
39. Genkai N, Homma J, Sano M, Tanaka R, Yamanaka R. Increased expression of pituitary tumor-transforming gene (PTTG)-1 is correlated with poor prognosis in glioma patients. *Oncol Rep* 2006;15:1569–74.