Targeting metastatic leiomyosarcoma by rapamycin plus gemcitabine: An intriguing clinical observation

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Abstract. The emerging anti-cancer approach is based on combining a 'traditional' cytotoxic drug with a 'signaling' blocking agent. Such combination, if designed and applied properly, may increase selectivity towards tumor cells. The use of such combinations requires smart planning and choice of the drugs to be combined, their proper dosing as well as correct sequence and schedule of application. The combination of the anti-metabolite gemcitabine and the mTOR blocker, rapamycin, has achieved an impressive response in a patient with metastatic leiomyosarcoma.

Introduction

A major obstacle in treating sarcoma patients is achieving the right balance between the effectiveness of the drug and its cytotoxic side effects. Obviously the ultimate goal of any chemotherapy would be to selectively kill tumor, but not normal cells. However, the 'traditional' drugs used in chemotherapy are aimed either at damaging the cell DNA (e.g. cisplatin or adriamycin), blocking DNA synthesis (e.g. methotrexate), or interfering with cell mitosis (e.g. vincristine). As such these drugs lack selectiveness and affect all dividing cells, including blood stem cells and other tissues of rapid turnover. A new class of drugs has emerged in cancer treatment not targetting cell division itself, but rather blocking specific signaling events that eventually promote cell growth. Examples include inhibitors of tyrosine kinases, such as Imatinib (Gleevec), which was shown to block the oncogenic form of the kit receptor thereby demonstrating remarkable activity in gastrointestinal stromal tumors (GIST). While these drugs may be effective in 'one protein triggered cancer' such as GIST, which is driven by a single abnormal protein (oncogenic kit), most cancer cases involve multiple mutations associated with changes in the expression of tumor suppressor genes, proto-oncogenes and anti-apoptotic genes. Therefore, a single signaling-directed drug is unlikely to provide the ultimate solution in most cancers. The emerging alternative appears to constitute a combination of both a 'traditional' cytotoxic drug with a 'signaling' drug (1). Such a combination, if designed and applied properly, may increase selectivity towards tumor cells, rendering sensitivity to tumor cells, which have acquired chemo-resistance, allowing diminution of the cytotoxic drug doses, thereby reducing side effects and finally to be more effective than each agent separately. However, the use of such combinations requires smart planning and choice of drugs to be combined, their proper dosing as well as correct sequence and schedule of application. This is of crucial importance to enable synergism rather than antagonism between the combined drugs. This approach therefore requires full understanding of the molecular details and impact of the desired drug combination. The following clinical observation supports this notion.

Clinical observation

During the course of kidney transplantation in a diabetic, 49-year-old male, who reached end-stage renal failure, the surgeon noticed a tumor on the mesentery. Two months later a laparotomy, partial sigmoidectomy and complete resection of the tumor were performed. It was classified as an 8x7x5 cm intermediate grade T2N0M0 leiomyosarcoma, staining positive for smooth muscle markers and negative for the kit receptor (CD117). For the next 12 months, the patient was treated with immunosuppressant combinations that included cyclosporine (Novartis, Switzerland), CellCept (mycofenolate mofetil, Roche, Switzerland) and steroids, followed by Prograph (Fujisawa, Japan), CellCept and steroids, and finally Rappamune (Wyeth, USA) (4 mg daily), Cellcept (500 mg bid) and prednisone (5 mg daily). During this period no evidence of relapsing sarcoma was documented by repeated routine ultrasound studies of the abdomen and pelvis. The last combination was taken before and during the event of acute rejection accompanied by a strong inflammatory reaction, perirenal abscess and septic fever. Hemodialysis was introduced, and performed 3 times per week, and the rejected kidney was ablated by embolization. At this point immunosuppressants were withdrawn. Ultrasound study of the abdomen and pelvis

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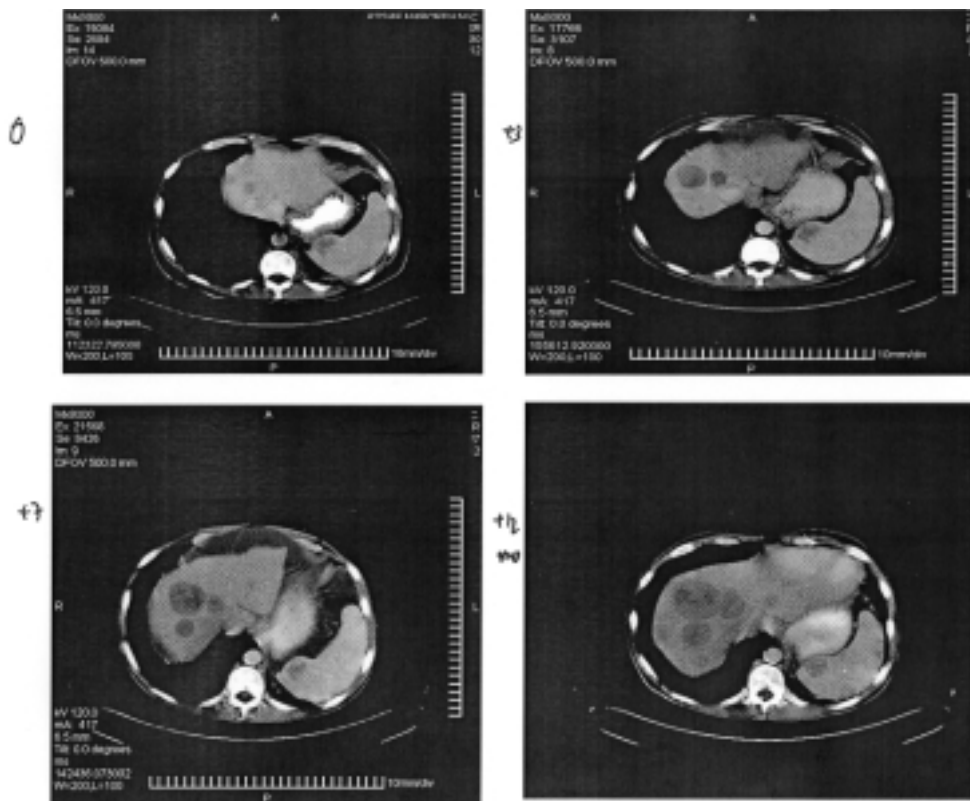E-mail: merimsky@zahav.net.il

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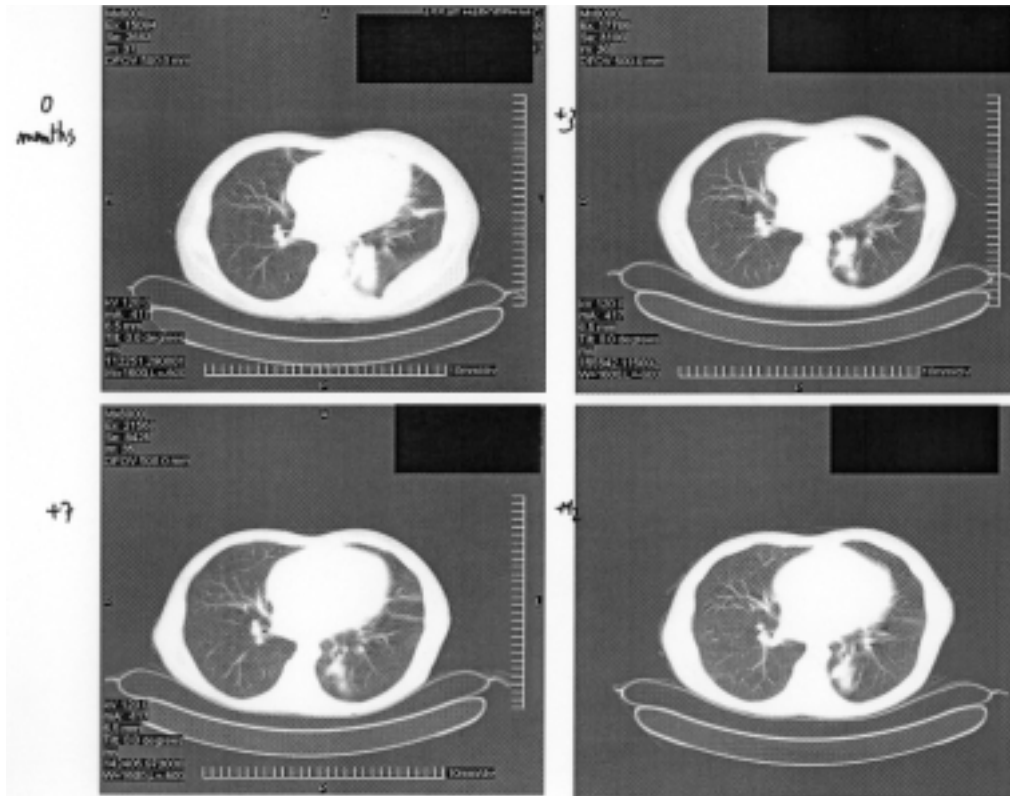
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Figure 1. Metastatic leiomyosarcoma treated by rapamycin plus gemcitabine. CT studies at baseline, 3, 7 and 12 months of therapy. (A) Lung; (B) spleen and liver.

taken 3 months later documented a normal sized liver with two suspicious nodules. CT inspection documented several nodules in the lower lobe of the left lung, fifteen liver nodules, a 2x2.5x3.5 splenic nodule and a 4.5x4.5x15.4 cm left renal solid mass. Ultrasound FNA from the liver failed to reveal any tumor cells. A month later, a second ultrasound study documented a tumor on the left native kidney. A biopsy was taken from the renal mass, but yielded no malignant cells. The nodules were therefore suspected as abscess linked to the former rejection episode.
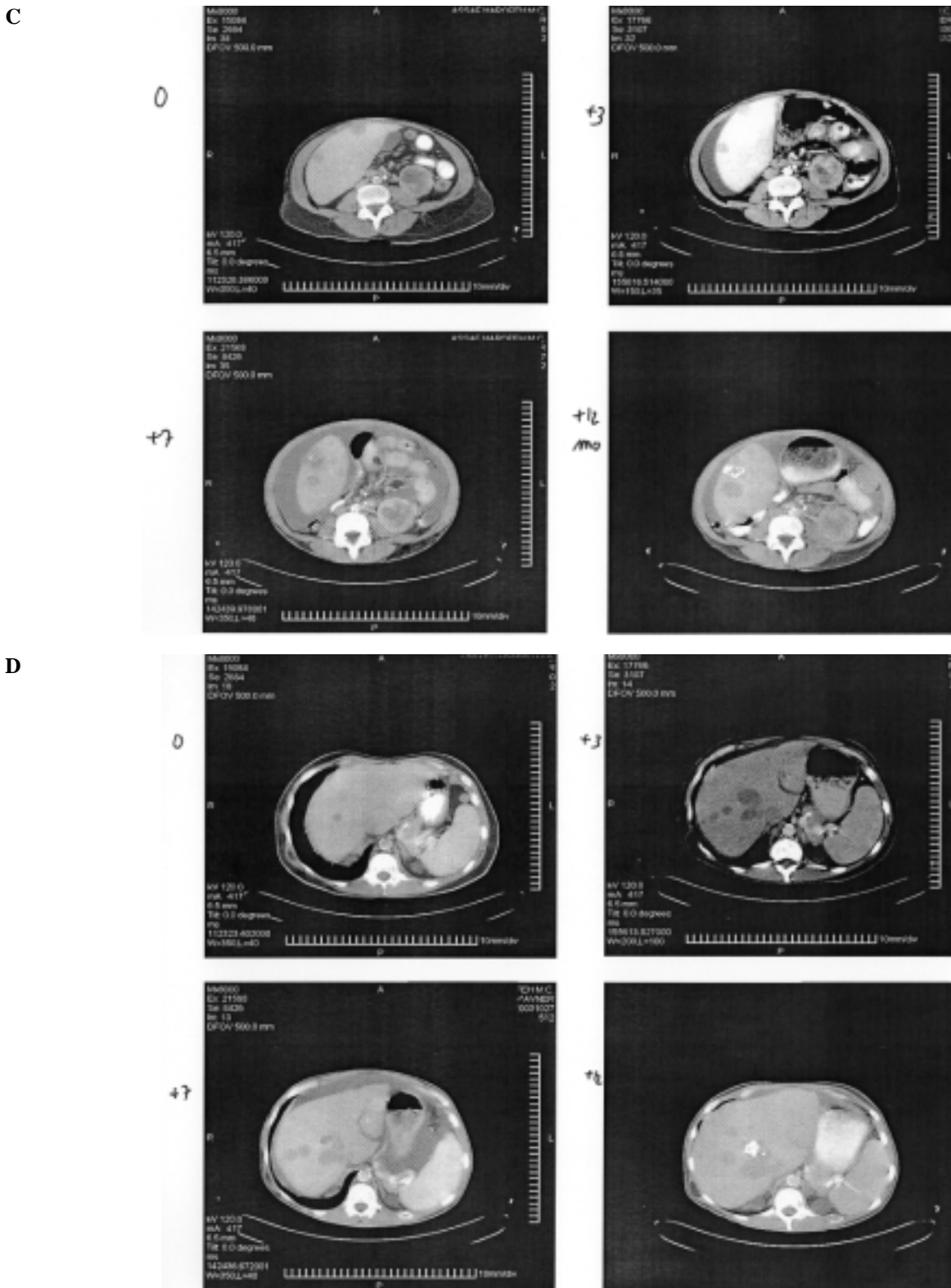
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Figure 1. (C) liver and kidney, the progression of liver nodules by size, but the development of nodule calcifications; and (D) liver; note calcifications in nodules.

Six months later, CT scan revealed an enlarged liver with multiple lesions, a tumor on the native left kidney and another tumor in the left lower lobe of the lung. A core biopsy was taken from the liver and yielded a c-kit-negative leiomyosarcoma, similar to the primary tumor. The patient was already symptomatic, and complained of pain in the left shoulder and left flank, left pleuritic pain and fever,

weakness and loss of appetite. On the basis of time-events relations it seemed likely that the sarcoma recurred at the time of rejection and rapidly progressed on cessation of the immunosuppressant therapy. Proceeding on this observation, the patient resumed taking rapamycin 4 mg/day, which is known to exhibit remarkable anti-cancer activity in several tumor models, and according to our own experience and

literature data, chemotherapy with gemcitabine was suggested. Our patient was not a candidate for standard chemotherapy for soft tissue sarcoma due to his deteriorated medical condition. Gemcitabine-based chemotherapy was the only feasible treatment. Rapamycin was chosen to be added to gemcitabine. After receiving an ethics committee approval for using gemcitabine and rapamycin in this case, the patient signed an informed consent. Rapamycin was taken for 4 weeks prior to gemcitabine introduction, and interrupted for 2 days before the administration of the first dose of gemcitabine (1000 mg/m^2). Hemodialysis was performed on the next day, 18-20 h after gemcitabine infusion. Complete blood count and biochemical serum analysis were performed on a regular basis before each session of hemodialysis. Rapamycin was resumed one day after each gemcitabine infusion, and re-interrupted 2 days before the subsequent dose of gemcitabine. Treatment was to be given on days 1 and 8 q3w. Four cycles were given on days 1 and 8 but the intervals were hardly kept due to several adverse events, which included thrombocytopenia, neutropenia, hypo-albuminemia, anemia (Fig. 1A-D) and upper gastrointestinal bleeding enforcing blood transfusion after each chemo-therapy treatment. Ascites were noted after 3 cycles and was possibly related to hypoalbuminemia or to gemcitabine toxicity rather than disease progression (2). Therefore, the schedule of gemcitabine was changed into day 1 q2w from the fifth cycle and its dose gradually reduced, while the dose and schedule of rapamycin remained unchanged. Indeed, the combination of rapamycin (4 mg/day) and gemcitabine (350 mg/m^2) at a 1 q2w was well tolerated (Fig. 1).

The response to rapamycin plus gemcitabine combination was impressive. Pain alleviation and improvement of appetite and well-being were reported by the patient. These were accompanied by an increase in blood hemoglobulin levels and serum albumin. Evaluation of CT scans suggested significant tumor regression in the lung (good partial response) and disappearance of the pleural fluid on the left side. The renal mass and the splenic nodule stayed within the range of stabilization, but the disease in the liver became larger (Fig. 1A-D). Notably, calcifications appeared in the liver nodules after a 5 to 6-month treatment period. An acute myocardial infarction and congestive heart failure and shock occurred after 10 months of treatment. However, this event is most likely an outcome of the patient's diabetic disease and his dependence on hemodialysis. Overall, the patient has been doing well for more than 14 months considering his widespread multi-organ metastatic disease in conjunction with basal diseases of diabetes and renal failure.

Discussion

This interesting and unusual case points to the activity and safety of combining rapamycin and gemcitabine in a patient with metastatic leiomyosarcoma. Gemcitabine is a pyrimidine antimetabolite that is activated intracellularly by nucleoside kinases to generate active di- and triphosphate nucleosides. Gemcitabine diphosphate inhibits ribonucleotide reductase leading to reduction in deoxynucleotides concentrations, while gemcitabine triphosphate competes with dCTP for incorporation into DNA. These combined activities result in

chain termination. Therefore, gemcitabine is a cell phase-specific drug primarily killing cells undergoing DNA synthesis at their S phase. While gemcitabine alone or in combination with docetaxel has already been given to patients with soft tissue sarcoma, and especially in cases of leiomyosarcoma (3-5), this is the first report of an *in vivo* administration of gemcitabine and rapamycin combination chemotherapy. Rapamycin, a natural product produced by Streptomyces hygroscopicus, was originally identified 20 years ago during antibiotic screening and was subsequently found to possess highly potent immunosuppression properties (6). It is currently the drug of choice in renal transplantation. However, recognition of the growth inhibitory effects of rapamycin alongside the identification of its cellular target have marked rapamycin as a potential anti-cancer therapeutic. Through its highly selective binding to its intracellular receptor protein FKBP12, rapamycin forms a complex that inhibits the function of the signaling kinase 'mTOR' (mammalian target of rapamycin) (7). The latter belongs to the family of phosphatidylinositol-3-kinase (PI3K)-related kinases (PIKK), that are involved in the control of essential cell functions, including cell cycle progression, cell cycle check points, DNA repair, and DNA recombination (8). Specifically, mTOR, is a downstream component in the PI3K/Akt (protein kinase B) pathway, which participates in the transduction of signaling events ultimately linked to the activation of cyclin-dependent kinases (CDKs), increase in the cellular levels of cyclins such as cyclin D1, and phosphorylation of the retinoblastoma (Rb) protein. As such, mTOR plays a central role in the control of cell proliferation, cell survival and adhesion-independent survival and migration (9,10). Through the inhibition of mTOR, rapamycin causes cell cycle arrest in the G1 phase, prevents CDK activation, inhibits Rb protein phosphorylation, and accelerates the turnover of cyclin D1, leading to a deficiency of active CDK4/cyclin D1 complexes. These events then contribute to the prominent inhibitory effects of rapamycin at the G1/S boundary of the cell cycle (11). Notably, rapamycin also displays anti-angiogenic activities linked to a decrease in production of vascular endothelial growth factor (VEGF) thereby markedly inhibiting response of vascular endothelial cells to stimulation by VEGF (12).

The anti-proliferative activity of rapamycin was first evaluated in variety of murine tumor cell lines and tumor model systems (reviewed in ref. 13). In those experiments rapamycin was found to exhibit impressive anti-tumor activity and to render radiation sensitivity to otherwise resistant tumors. Moreover, rapamycin effectively synergizes with and markedly enhances the efficacy of gemcitabine in inhibiting the growth of human pancreatic xenografts in a mouse model (14). However, to the best of my knowledge, this is the first demonstration of the therapeutic value of this combination in a human.

The idea of treating sarcoma patients with a combination of rapamycin and gemcitabine is particularly intriguing. Gemcitabine appears to be an agent with activity, particularly in patients with leiomyosarcomas, while mTOR is a central effector of mitogenic stimuli promoting smooth muscle proliferation. Indeed, consistent with this notion, rapamycin displays profound anti-proliferative activity on smooth

muscle (15) and also strongly attenuates growth of rhabdo-myosarcoma cell lines (16) and Ewing's sarcoma (17). The present observation adds some important information on the possible therapeutic role of rapamycin in combination with gemcitabine in leiomyosarcoma. This study clearly shows that such combination is safe, well tolerated and of significant efficacy. The response in the lung was impressive, while the progression in the liver was finally arrested, with the development of nodule calcifications. Furthermore, although the chronic renal failure and dialysis complicated the course of the disease and the administration of chemotherapy, this study indicates that gemcitabine and gemcitabine/rapamycin can be safely administered by a short infusion in patients with CRF while on hemodialysis. Hemodialysis should be performed 18-20 h following gemcitabine infusion in order to allow the drug to exert its activity and to clear it from the circulation. The profile of toxicity in our patient included myelosuppression and fluid retention in the form of ascites while no rash or other side effects were noted. Ascites on the other hand could be related to renal failure and dialysis-related fluid retention. We have already launched a phase II study to examine the efficacy of this combination in patients with metastatic soft tissue sarcomas.

References

1. Peters GJ, van der Wilt CL, van Moorsel CJ, Kroep JR, Bergman AM and Ackland SP: Basis for effective combination cancer chemotherapy with antimetabolites. Pharmacol Ther 87: 227-253, 2000.
2. Merimsky O, Meller I, Kollender Y and Inbar M: Palliative effect of gemcitabine in osteosarcoma resistant to standard chemotherapy. Eur J Cancer 34: 1296-1297, 1998.
3. Merimsky O, Meller I , Flusser G, Kollender Y, Issakov J, Weil-Ben-Arush M, Fenig E, Neuman G, Sapir D, Ariad S and Inbar M: Gemcitabine in soft tissue or bone sarcoma resistant to standard chemotherapy: a phase II study. Cancer Chemother Pharmacol 45: 177-181, 2000.
4. Patel SR, Gandhi V, Jenkins J, Papadopolous N, Burgess MA, Plager C, Plunkett W and Benjamin RS: Phase II clinical investigation of gemcitabine in advanced soft tissue sarcomas and window evaluation of dose rate on gemcitabine triphosphate accumulation. J Clin Oncol 19: 3483-3489, 2001.
5. Hensley ML, Maki R, Venkatraman E, Geller G, Lovegren M, Aghajanian C, Sabbatini P, Tong W, Barakat R and Spriggs DR: Gemcitabine and docetaxel in patients with unresectable leiomyosarcoma: results of a phase II trial. J Clin Oncol 20: 2824-2831, 2002.
6. Dupont P and Warrens AN: The evolving role of sirolimus in renal transplantation. QJM 96: 401-409, 2003.
7. Chen J, Zheng XF, Brown EJ and Schreiber SL: Identification of an 11-kDa FKBP12-rapamycin-binding domain within the 289-kDa FKBP12-rapamycin-associated protein and characterization of a critical serine residue. Proc Natl Acad Sci USA 92: 4947-4951, 1995.
8. Keith CT and Schreiber SL: PIK-related kinases: DNA repair, recombination, and cell cycle checkpoints. Science 270: 50-51, 1995.
9. Huang S, Bjornsti MA and Houghton PJ: Rapamycins: mechanism of action and cellular resistance. Cancer Biol Ther 2: 222-232, 2003.
10. Chen J: Novel regulatory mechanisms of mTOR signaling. Curr Top Microbiol Immunol 279: 245-257, 2004.
11. Mita MM, Mita A and Rowinsky EK: Mammalian target of rapamycin: a new molecular target for breast cancer. Clin Breast Cancer 4: 126-137, 2003.
12. Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, Bruns CJ, Zuelke C, Farkas S, Anthuber M, Jauch KW and Geissler EK: Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat Med 8: 128-135, 2002.
13. Dancey JE: Clinical development of mammalian target of rapamycin inhibitors. Hematol Oncol Clin North Am 16: 1101-1114, 2002.
14. Bruns CJ, Koehl GE, Guba M, Yezhelyev M, Steinbauer M, Seeliger H, Schwend A, Hoehn A, Jauch KW and Geissler EK: Rapamycin-induced endothelial cell death and tumor vessel thrombosis potentiate cytotoxic therapy against pancreatic cancer. Clin Cancer Res 10: 2109-2119, 2004.
15. Zohlnhofer D, Nuhrenberg TG, Neumann FJ, Richter T, May AE, Schmidt R, Denker K, Clauss MA, Schomig A and Baeuerle PA: Rapamycin effects transcriptional programs in smooth muscle cells controlling proliferative and inflammatory properties. Mol Pharmacol 65: 880-889, 2004.
16. Hosoi H, Dilling MB, Shikata T, Liu LN, Shu L, Ashmun RA, Germain GS, Abraham RT and Houghton PJ: Rapamycin causes poorly reversible inhibition of mTOR and induces p53-independent apoptosis in human rhabdomyosarcoma cells. Cancer Res 59: 886-894, 1999.
17. Mateo-Lozano S, Tirado OM and Notario V: Rapamycin induces the fusion-type independent down-regulation of the EWS/FLI-1 proteins and inhibits Ewing's sarcoma cell proliferation. Oncogene 22: 9282-9287, 2003.