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Strategies targeting the PI3k/AKT pathway: rapamycin and its derivatives as lead compounds for downstream inhibition of the PI3kinase pathway

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mTOR (mammalian Target of rapamycin) appears to be a key target protein acting downstream to the activation of PI3K and Akt (see Figure 1). Cumulative evidences support the hypothesis that mTOR acts as a master switch of cellular catabolism and anabolism. In addition, mTOR has been recently found to have profound effects on the control of apoptosis. mTOR has a pleiotropic function in the regulation of cell death mainly dictated by the cellular context and downstream targets including p53, Bad, Bcl-2, protein kinase Cε,α,δ, Rb protein, STAT3, and c-Myc.

Rapamycin is a macrolide antibiotic produced by *Streptomyces hygroscopicus* (sirolimus) that interacts with FKBP12 (FK506-binding protein). The rapamycin-FKBP12 complex interacts with mTOR, to potently and selectively inhibit mTOR signaling to downstream targets. Rapamycin alone can induce apoptosis in a cell type-specific fashion and sensitize cancer cells to apoptosis induction by cisplatin, gemcitabine and taxanes. Interestingly, rapamycin antagonizes tumor growth induced by loss of the PI3K antagonist PTEN. PTEN +/– mice spontaneously develop neoplasia, associated with loss of the normal PTEN allele and an increased activation of Akt (PKB) and p70S6K. *In vivo* treatment of such mice with CCI-779, a rapamycin analog, normalizes p70S6K activity and reduces neoplastic proliferation. Similarly, PTEN-deficient human tumors are more sensitive to CCI-779-mediated growth inhibition than PTEN-expressing cells. This growth inhibition involves both a decrease in proliferation and an increase in apoptosis. Rapamycin analogues selected for clinical development are CCI-779 (i.v. formulation currently in phase II) and RAD001 (oral formulation currently in phase I). In clinical setting using intermittent administration of CCI-779, no evidence of immunosupressive effects was observed. Dose-limiting toxicity consisted of skin reaction and mucositis with minimal myelosupression. Evidence of antitumor activity were reported in patients with renal clear cell carcinoma and breast cancer and final results of phase II studies are pending. Selection of patients based on the detection of activated p70S6K/Akt and/or loss of PTEN expression to predict the sensitivity of tumor cells to rapamycin analogues will be discussed. Pharmacodynamic monitoring of the rapamycin activity in clinical trial using molecular endpoints such as the phosphorylation of Akt and p70S6K might help to determine biologically relevant dose(s) and plasma concentration(s) in human. Providing a better understanding of the tumor biology in individual patients, rapamycin, as well as its analogs, may become useful for the treatment of some classes of cancer including breast cancer.

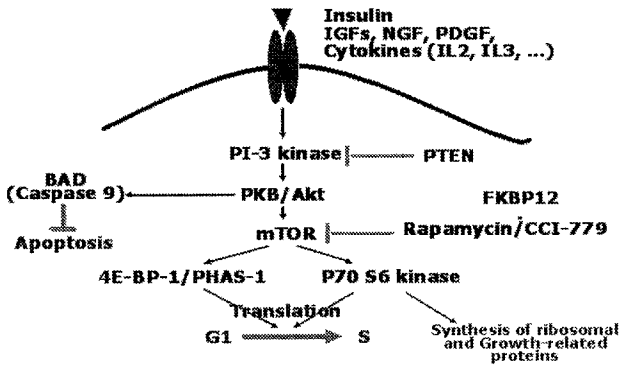

Fig. 1. PI3/mTOR/PI3k Molecular Pathway.

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Targeting the ubiquitin-proteasome pathway in breast cancer

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The 26S proteasome is an ATP-dependant multicatalytic protease that is responsible for most of the non-lysosomal intracellular protein degradation. It is composed of two functional entities: the 20S core catalytic complex and the 19S regulatory subunits. To be selected for proteasomal degradation, proteins must be previously tagged with a polyubiquitin chain, which is then recognized by a receptor in the 19S subunit; the ubiquitin chain is removed by isopeptidases and the protein is unfolded under hydrolysis of ATP; denatured proteins enter the inner catalytic chamber of the 20S complex to be hydrolysed to small polypeptides. In addition to removing damaged/unnecessary proteins, the proteasome is also an important mechanism of regulation of some key regulatory proteins and their inhibitors. This regulation is crucial for the control of many cellular processes, including activation of transcription factors, cell cycle progression and apoptosis. The critical role of the ubiquitin-proteasome (Ubi-Prot) pathway, in tumour cells, has led to the investigation of proteasome inhibition as a potential anticancer therapy.

One of the targets of this pathway is p53, which acts as a negative regulator of cell growth and also plays an important function in apoptosis. Cyclins and CDK inhibitors (p21 and p27) are regulated by the Ubi-Prot pathway, and its inhibition can sensitise cells to apoptosis. This pathway is also required for transcriptional regulation, particularly of the nuclear factor-kB (NF-kB), which has been implicated in many tumours including breast cancer (BC); after degradation of IkB by the proteasome, NF-kB translocates to the nucleus and regulates crucial genes involved in tumour metastasis, angiogenesis and apoptosis: tumour necrosis factor (TNF), interleukins, pro-inflammatory enzymes (NOS, COX-2), cell adhesion molecules (E-selectin, ICAM-1, VCAM-1), members of the Bcl-2 and the inhibitor of apoptosis (IAP) families. The latter two play an important role in resistance to both chemotherapy (CT) and radiotherapy (RT). The Ubi-Prot pathway is also implicated in the turnover of ER, growth factor receptors such as HER-2 and EGFR, and proteins of oncogenes (c-fos/c-jun, c-myc, N-myc).

Proteasome inhibitors: The dipeptide boronic acid analogue Bortezomib (Velcade™), formerly known as PS-341 (PS), was the first proteasome inhibitor used in the clinical setting and is now in advanced stages of development, due to its potent, highly selective and reversible inhibition of proteasome activity. It can be administrated orally, intravenous, intraperitoneal or intratumoral. PS specifically and selectively inhibits the proteasome by binding tightly to the enzyme's active site and leads to exclusive blockage of the proteasome chymotrypsin activity.

Preclinical data: *In vitro* and *in vivo* studies have shown that PS is active against a variety of malignancies, including haematological and solid tumours (i.e. breast, prostate, lung, pancreas, colon, ovarian, head & neck). PS has activity as single agent and in combination with several cytotoxic agents, such as 5-FU, irinotecan, gemcitabine, doxorubicin and docetaxel, and with radiation, enhancing both CT- and RT-induced apoptosis.

Malignant cells are more sensitive to proteasome inhibition than their normal counterparts. Actively dividing cells are considerably more sensitive to proteasome-induced apoptosis than non-proliferating cells; however this difference in sensitivity cannot be totally explained by the high replication rate of malignant cells and other, still poorly understood, factors must be involved.

Accurate PS dosing was performed using a rapid and reliable proteasome activity bioassay from whole blood or white blood cells (maximum point of proteasome inhibition: 1 hour post infusion). Most organs receive a similar amount of drug, with the exception of the central nervous system, eyes and testis where PS was not detected. Side effects of PS are dose-related and generally well tolerated; the most common are gastrointestinal (G.I.) such as anorexia, vomiting, and diarrhoea. Toxicity became more pronounced and severe when proteasome inhibition exceeded 80%, and persistent maximal inhibition led to significant toxicity and death. Since PS is a reversible proteasome inhibitor, pharmacological/toxicity studies in animals indicated that the baseline proteasome activity was restored between 48 and 72 hours after cessation of administration. Based on such preclinical studies, a target level of 80% of proteasome inhibition for a transient duration, and an interval of at least 72h between 2 administrations was recommended for clinical studies.

Mechanisms of action: Table 1 summarizes the most important mechanisms of action of PS. Of note, the actual molecular targets at which PS elicits its anti-tumour activity may vary among different tumour types, and the extent to which each target is critical to the inhibition of tumour growth can also differ.

Table 1: Bortezomib mechanisms of action

1	Stabilization of cell-cycle regulatory proteins, i.e. p21, p27, wild-type p53
2	Inhibition of NF-κ B activation (potential imp target in ER-negative breast cancers)
3	Induction of apoptosis, through increased levels of key proteins (p21, bax) and induction of cell cycle arrest at G1 or G2-M phase
4	Override of Bcl-2 resistance
5	Inhibition of cell growth signalling pathways
6	Anti-angiogenesis
7	Inhibition of cellular adhesion molecule expression – ICAM-1, VCAM-1, E-selectin

Clinical experience: Almost 1000 patients have been enrolled in phase I and II clinical trials of PS. Phase I clinical trials demonstrated activity in