Farnesyltransferase Inhibitors and Their Role in the Treatment of Multiple Myeloma

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Background: Ras mutations are among the most common oncogene mutations found in multiple myeloma (MM). Patients with mutated Ras are less likely to respond to chemotherapy and have a shortened median survival. Therefore, targeting Ras farnesylation may be a valuable approach to treatment of MM. R115777 (tipifarnib) is a potent farnesyltransferase inhibitor (FTI) presently undergoing phase II/III clinical trials.

Methods: We reviewed the preclinical and clinical experience of FTIs as antineoplastic agents and describe their potential role in the treatment of MM.

Results: FTIs are a novel group of agents that selectively inhibit farnesyltransferase, an enzyme responsible for the posttranslational modification of several proteins including Ras. Since Ras is among the most commonly mutated oncogenes associated with cancer, this class of drugs has been evaluated in clinical trials in a diversity of tumors. R115777 has been evaluated in a phase II clinical trial in patients with advanced myeloma and found to be well tolerated. It induced disease stabilization in more than 60% of patients with advanced myeloma.

Conclusions: The drug selectively targets farnesyltransferase, but this effect did not correlate with disease stabilization, suggesting that these drugs may be targeting a survival pathway independent of Ras processing. Further studies will evaluate the use of FTI in maintenance therapy as well as in combination with other agents in advanced myeloma.

Introduction

In recent years, it has become evident that the interactions between the myeloma cells and the stromal cells in the tumor microenvironment play an important role in tumor growth, drug resistance, and bone disease. This understanding has led to the development of new treatments that can not only induce myeloma cell cytotoxicity but also interfere with...
myeloma-stromal cell interactions either directly or by interrupting signal transduction pathways involved in disease activity and progression. An important mediator of myeloma-stromal cell interaction is interleukin-6 (IL-6). The adhesion of myeloma cells to stromal cells induces IL-6 secretion by the stromal cells, which in turn mediates myeloma cell growth through the activation of signal transduction pathways involved in cell growth and transformation, such as the STAT, AKT and Ras/ERK1-2 pathways. These signal transduction pathways downstream from IL-6, represent potential treatment targets in multiple myeloma (MM). Among these, the Ras oncogene is of particular interest as a potential therapeutic target, as Ras mutations represent the most common gene mutation in MM.

Farnesyltransferase Inhibitors (FTIs) are a group of drugs that selectively inhibit the enzyme farnesyltransferase (FTase) that is responsible for the transfer of a farnesyl group to Ras and other proteins involved in signaling concerning cell transformation and survival. FTIs comprise a novel class of antineoplastic agents recently developed to inhibit FTase. While these inhibitors were designed to target Ras, it is evident in many instances that Ras may not be the only target of FTIs. In many tumor cell lines including myeloma, the antitumor activity of FTI does not correlate with mutated Ras status. The finding that K-ras and N-ras can be prenylated by geranylgeranyl transferase also argues against Ras as the dominant target, as preclinical models bearing these mutations are sensitive to FTI treatments. To date, many proteins have been suggested as potential FTI targets.

Ras Oncogene in Myeloma

The Ras oncoprotein is a monomeric membrane-localized G protein signal transducer of 21-kD molecular weight that requires prenyl lipid modification and membrane association for signal transduction activity. This modification involves the covalent addition of either farnesyl (15-carbon) or geranylgeranyl (20-carbon) groups to conserved carboxy terminal cysteine residues of certain proteins. The enzymes that catalyze this modification are FTase and geranylgeranyl transferase, respectively. Mutations in Ras result in constitutive activity that can lead to uncontrolled proliferation and inhibition of apoptosis.

In 1996, Liu et al found Ras mutations in 39% of newly diagnosed myeloma patients as well as a correlation between Ras mutation and shorter survival. Patients with Ras mutations had a median survival of 2.1 years compared with 4.0 years for patients with wild-type Ras. Bezlieau et al reported a similar incidence of Ras mutations at diagnosis that increased to 81% at the time of disease relapse. Furthermore, Kalakonda and colleagues reported that N-ras 61 mutation-positive cells could be detected in subpopulations of tumor cells in all cases of newly diagnosed myeloma patients. These findings provide further evidence that Ras mutations are the most prevalent oncogenic mutations in MM.

R115777

R115777 (tipifarnib) is an imidazole-containing heterocyclic compound that is a potent nonpeptidomimetic inhibitor of FTase. The growth of several human tumor cell lines, including those with either
wild-type or mutant Ras, is inhibited with 50% inhibitory concentrations (IC$_{50}$s) ranging from 1.7 to 50 nmol/L. In vivo bid dosing shows a dose-dependent inhibition of human colon and pancreatic cancer xenografts, with antitumor effects including apoptosis, decrease of proliferation, and antiangiogenesis. In phase I trials, R115777 has been administered at doses up to 1300 mg p.o. b.i.d. for 5 days every 2 weeks without significant toxicities. Two additional phase I studies have investigated dosing for 14 or 21 days followed by 7 days of rest. The dose-limiting toxicity was reversible myelosuppression. The drug has demonstrated clinical activity in phase I and II studies of patients with metastatic breast cancer, myelodysplastic syndrome, and acute myelogenous leukemia.

In myeloma, the prenylation inhibitors FTI-277 and geranylgeranyl transferase I inhibitor (GGTI-2166) were shown to induce apoptosis in myeloma cell lines selected for resistance to classic cytotoxics, including doxorubicin and melphalan. Similarly, we and others have shown that R115777 induces a dose- and time-dependent growth inhibition and apoptosis in myeloma cell lines.

**Phase II Trial of R115777 in Advanced Multiple Myeloma**

We conducted a phase II trial to evaluate the activity and tolerability of R115777 and also to correlate response to inhibition of protein farnesylation and oncogenic/tumor survival pathways in patients with advanced MM. Eligibility criteria included patients that meet criteria for relapsed or refractory myeloma, ECOG performance status <3, normal renal function, and measurable disease. FTI 300 mg given orally b.i.d. was administered for 3 weeks and was repeated every 4 weeks. The dose was to be escalated after 1 cycle to 400 mg b.i.d. in the absence of grade 3 toxicity. Patients were evaluated after 2 cycles and treatment was continued if they had a response, improvement, or stabilization of disease according to modified SWOG criteria for disease response. Forty-three patients entered the study. The median age was 62 years (range = 33 to 82). The patients were all heavily pretreated, with a median of 3.7 chemotherapy regimens prior to entering the study. Fifty-four percent of the patients had prior thalidomide or high-dose chemotherapy and stem cell/bone marrow transplant. On entering the study, half of the patient group was refractory to their most recent treatment. The most common toxicity was fatigue, which occurred in 66% of patients. Other toxicities included diarrhea, nausea, neuropathy, anemia, and thrombocytopenia. Sixty-two percent of the patients had a reduction of the monoclonal protein of less than 50% consistent with disease stabilization. Treatment with R115777 suppressed FTAse but not GGTase I activity in bone marrow and peripheral blood mononuclear cells of patients with MM. Similarly, R115777 inhibited the prenylation of the farnesylated protein HDJ-2 in all patients, and it decreased the levels of phosphorylated Akt and STAT3 but not Erk1/2 in bone marrow from patients in whom these oncogenic tumor survival pathways were constitutively activated. Inhibition of farnesylation did not correlate with clinical activity. We conclude that R115777 is tolerable and can induce disease stabilization in patients with MM, and that 300 mg b.i.d. is sufficient to inhibit FTAse activity, protein farnesylation, and the oncogenic/tumor survival pathway.

**Current and Future Studies**

We learned from this clinical trial that while protein farnesylation was inhibited in all patients, this event did not correlate with clinical activity. Clinical results indicated that R115777 reduced the levels of phosphorylated Akt and STAT3 in bone marrow from patients in whom these tumor survival pathways were constitutively active, and the former correlated with disease stabilization in the limited number of patients examined. The PI 3-kinase/AKT2 pathway has been shown to be a critical target for FTI-induced apoptosis in ovarian cancer cell lines. Therefore, we examined the mechanisms of cytotoxicity of R115777 on myeloma cell lines and its correlation with AKT activity. R115777 inhibited proliferation in all cell lines except MM1 at concentrations <5 µm, with RPMI 8226 showing the most sensitivity (IC$_{50}$ 2 × 10$^{-8}$ M) and U266 and H929 showing a more moderate sensitivity of 2 × 10$^{-7}$ M and 4 × 10$^{-7}$ M, respectively. Propidium iodide cell cycle analysis indicated that 8226 and H929 cells accumulate in G$_2$-M phase and G$_2$-G$_0$ phase, respectively, in a dose-dependent manner. Annexin V-PI analysis indicated a dose-dependent increase in the number of apoptotic cells in all cell lines except MM1s. A dose of 1 µm R115777 induced procaspase 3 cleavage in 8226 cells, but not in MM1 cells, between 12 and 24 hours after treatment. R115777 induced a dose-dependent pro-caspase 3 cleavage in 8226, U266, and H929 cells within 72 hours. MM1 cells under the same conditions failed to exhibit a similar response after 72 hours of treatment. FTI inhibited AKT phosphorylation in a dose-dependent manner in all MM cell lines examined. The levels of phosphorylated AKT detected correlated with resistance to FTI, with the more resistant cell lines showing higher levels of phosphorylated AKT and incomplete inhibition when treated with FTI. Our data suggest that the AKT tumor survival pathway plays an important role in R115777-induced apoptosis in myeloma.
Conclusions

FTIs are a new class of agents with significant antmyeloma activity in vitro. In patients, R115777 induced stabilization of disease in 62% of patients with advanced MM. Further clinical studies will examine the clinical activity of this agent in combination with other cytotoxics or as maintenance therapy in patients with MM.

References