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Anti-tumor activity of CCI-779 in relapsed mantle cell lymphoma

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Mantle cell lymphoma (MCL) represents approximately 8% of cases of non-Hodgkin's lymphoma (NHL) and is an incurable, aggressive B-cell malignancy. The disease usually presents in an advanced stage and commonly involves extranodal sites such as the gut, bone marrow, and peripheral blood. Most patients are older adults and there is a male predominance. The characteristic tumor cell immunophenotype is a population of $CD20^+$, $CD10^-$, $CD5^+$, $CD23^-$ B-cells with monoclonal light chain expression on the cell surface.

MCL is also unique in that the tumor cells have a t(11;14)(q13;q32) chromosomal translocation that juxtaposes the cyclin D1 gene on chromosome 11 to the immunoglobulin heavy chain enhancer region on chromosome 14.[1-3] The transcription enhancers on 14q32, now linked to the cyclin D1 gene, result in the characteristic overexpression of cyclin D1 in the MCL tumor cells. There is currently no standard therapy for newly diagnosed or relapsed MCL. Many regimens have shown significant activity in this disease,[4-17] but relapse typically occurs, and patients usually die of progressive disease, with a median survival of 3 to 4 years. It is therefore clear that new treatments are needed for MCL.

Targeting molecular pathways

Even though cyclin D1 mRNA is constitutively expressed in MCL, it is subject to translational regulation by a pathway involving the mammalian target of rapamycin (mTOR).[18,19] Activated receptor tyrosine kinases and activated ras proteins enhance the catalytic activity of the lipid kinase phosphatidylinositol-3 kinase (PI3K), which converts phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphoate (PIP3). PIP3 activates the protein kinase phosphoinositide-dependent kinase 1 (PDK1), which, along with a second kinase such as integrin-linked kinase (ILK), contributes two phosphorylations required for maximal Akt activity. Akt then phosphorylates a number of substrates, including tuberous sclerosis (TSC) protein 2 (TSC2), which in its unphosphorylated state is complexed with TSC protein 1 (TSC1) and acts as a GTPase activating protein that diminishes activation of the small guanine nucleotide binding protein Rheb. When the TSC1/TSC2 complex is inactivated by Akt, Rheb remains in a GTPbound state that activates mTOR, a protein kinase that regulates mRNA translation by phosphorylating two critical substrates, eukaryotic initiation factor (eIF) 4E (eIF4E) binding protein (4E-BP1) and p70S6 kinase.[20,21]

Previous studies have shown that eIF4E is a component of a helicase complex that binds to the cap structure at the 5# end of mRNAs and enhances the ability of ribosome-eIF complexes to scan the mRNA in search of a translation initiation site.[22] The ability of eIF4E to bind to and participate in this helicase complex is inhibited when 4E-BP1 is bound. This inhibitory interaction is possible only when 4E-BP1 is unphosphorylated and is abrogated when 4E-BP1 is sequentially phosphorylated by mTOR and other kinases.[22,23] At the same time, mTOR mediated phosphorylation activates p70S6K, enabling its phosphorylation of ribosomal protein S6 and possibly other substrates, thereby enhancing the translation of messages with 5# terminal oligopyrimidine tracts.[18,22] Collectively, these events markedly enhance translation of a small but important group of messages, including those encoding c-myc, ornthithine decarboxylase, and cyclin D1, as well as ribosomal proteins themselves.[18,22,24,25] mTOR activity is modulated by mitogenic signals, which are transmitted through a signal transduction pathway involving PI3K, Akt, and TSC1 and TSC2.[18,19,26,27] In addition, mTOR-mediated signaling is also subject to modulation by the macrocyclic lactone rapamycin and its derivatives.[19,26,27] Once these agents bind to the 12 kDa cytosolic FK506-binding protein FKBP12, the resulting rapamycin-FKBP12 complexes bind to a specific site near the catalytic domain of mTOR and inhibit phosphorylation of mTOR substrates by a mech-

anism that remains somewhat poorly understood.[27]

As a consequence, translation of messages that require mTOR signaling is inhibited. This mechanism is thought to be responsible for the immunosuppressive effects of rapamycin as well as its putative antineoplastic activity.

CCI-779 (also known as temsirolimus), a dihydroester of rapamycin that is suitable for intravenous use, is currently undergoing testing in solid tumor patients as a potential antineoplastic agent.[28-31] In view of the role of cyclin D1 in MCL, we conducted an initial phase II trial of single-agent temsirolimus at a dose level of 250 mg IV weekly for patients with relapsed MCL.[32] This study with weekly CCI-779 at a dose of 250mg demonstrated encouraging antitumor activity but thrombocytopenia was frequently observed and was dose limiting. Based on results from clinical trials in other malignancies, a subsequent cohort of patients was enrolled on the study and received a low-dose (25 mg) of CCI-779 to determine if a 10-fold decrease in the dose could produce a similar overall response rate.[33]

Clinical results

In the initial patient cohort in the study32, patients with relapsed or refractory MCL were eligible to receive temsirolimus 250 mg intravenously every week as a single agent. In the subsequent cohort of patients enrolled in the study33, patients received CCI-779 25mg IV weekly as a single agent. Eligible patients had biopsy proven cyclin D1 positive MCL and had relapsed or were refractory to therapy. Patients were required to have measurable disease, an adequate performance status, and adequate organ and bone marrow reserve. Patients were restaged after 1 cycle (4 doses), after 3 cycles, and every 3 cycles thereafter. Patients with a tumor response after six cycles were eligible to continue drug for a total of 12 cycles or two cycles after complete remission (CR), and were then observed without maintenance treatment.

In the initial study cohort, using temsirolimus at the 250mg dose level, 35 patients were enrolled and were assessable for toxicity. One patient had MCL by histology but was cyclin D1 negative and was ineligible for efficacy. The median age was 70 years (range, 38 to 89 years), 91% were stage 4, and 69% had two or more extranodal sites. Patients had received a median of three prior therapies (range, one to 11), and 54% were refractory to the last treatment. The overall response rate was 38% (13 of 34 patients; 90% CI, 24% to 54%) with one complete response (3%) and 12 partial responses (35%). The median time-to-progression in all patients was 6.5 months (95% CI, 2.9 to 8.3 months), and the duration of response for the 13 responders was 6.9 months (95% CI, 5.2 to 12.4 months). Hematologic toxicities were the most common, with 71% (25 of 35 patients) having grade 3 and 11% (four of 35 patients) having grade 4 toxicities. Thrombocytopenia was the most frequent cause of dose reductions but was of short duration, typically resolving within 1 week. However, dose reductions were necessary in the majority of patients.

Due to the finding that thrombocytopenia was the dose limiting factor, and the fact that dose intensity of the drug did not appear to correlate with clinical response, we decided to explore the use of a lower dose. Responses had been seen in other malignancies using 25mg IV weekly, and a second cohort of patients were enrolled and received a substantially lower dose of 25mg weekly IV. In this subsequent study cohort, using the 25mg dose level, 26 of the planned 27 patients have been enrolled. The design-mandated interim analysis on the first 13 evaluable patients confirmed 7 responders (54%; CI 16%-84%) and stable disease in the remaining 6 patients. One patient (8%) had a complete response and 6 (46%) had a partial response. Accrual to this study is almost complete and a final analysis will then be done. Significantly less thrombocytopenia was seen with the lower dose, but the frequency of anemia and neutropenia was similar in both studies.

In both studies, the most common adverse events of all grades were thrombocytopenia, hyperglycemia, anemia, neutropenia, increased triglycerides, mucositis, fatigue, infection without concomitant neutropenia, rash, nausea, weight loss, AST elevations, abnormal sense of taste, loss of appetite, hypercholesterolemia, and sensory neuropathy. No grade 5 events due to treatment were reported.

Pharmacodynamics

To develop an assay for mTOR inhibition that could be applied in clinical MCL samples, we initially treated MO258 cells with varying concentrations of rapamycin *in vitro* and probed whole cell lysates for phosphorylation of substrates downstream of mTOR using commercially available antiphosphoepitope antibodies. Blotting for phospho–4E-BP1 in this cell line and others proved difficult. In contrast, we observed that phospho-S6 and, with somewhat more difficulty, phospho-p70S6 kinase could be demonstrated. Moreover, inhibition of S6 phosphorylation was readily detectable at 0.1 nmol/L rapamycin and essentially complete at 1 nmol/L.

When this assay was applied to MCL samples from the blood of patients receiving temsirolimus 250mg weekly in the initial cohort, phosphorylation of S6 was more readily detectable than phosphorylation of p70S6K. Examination of serial samples revealed two distinct patterns. First, S6 phosphorylation was inhibited after temsirolimus treatment in three patients. Of these three patients, one responded to therapy, one was

stable, and one progressed without ever responding. In contrast, there was no evidence that S6 phosphorylation was inhibited in circulating MCL cells from two other patients. One of these patients had a PR; the other progressed on therapy.

Conclusions

Single-agent temsirolimus has substantial antitumor activity in relapsed MCL. The response rates appear similar at 250mg weekly and 25mg weekly.

This study demonstrates that agents that selectively target cellular pathways dysregulated in MCL cells can produce therapeutic benefit and this effect may not be dose dependant. Further studies of this agent in MCL and other lymphoid malignancies are therefore warranted.

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