MLN518 – an overview

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Chemistry

MLN518 (previously CT53518) is a 4-piperaziynylquinazoline derivative. Compounds derived from this pharmacophore were initially developed as inhibitors of the platelet derived growth factor receptors (PDGFR) α and β, tyrosine kinase receptors involved in a number of pathological processes including atherosclerosis, glomerulonephritis and liver cirrhosis. Both receptors additionally play a role in carcinogenesis as a result of constitutive activation by 5' fusion of domains that lead to dimerization in the absence of ligand or by point mutations.[1-3] PDGFR α and β belong to the type III family of receptor tyrosine kinases that is characterized by an extracellular domain containing 5 immunoglobulin-like repeats and a split kinase domain. Other members of this family are Kit, Flt-3 and the colony stimulating factor 1 (CSF-1) receptor. All of these kinases have been implicated in malignancy.

Activating mutations of Flt3 are the most common genetic abnormality in patients with acute myeloid leukemia (AML). Internal tandem duplications (ITD) in the juxtamembrane domain are found in approximately 30% of patients and in most studies are associated with hyperleukocytosis and a poor prognosis.[4-6] Mutations in the kinase activation loop occur in approximately 5% of patients.[7] Since imatinib, a highly active inhibitor of PDGFR and Kit is available for clinical use, MLN518 was primarily developed as a Flt3 inhibitor.

Preclinical evaluation

In vitro studies. In cellular phosphorylation assays (COS cells transduced with receptor constructs) the IC_{50} values for PDGFR?, Kit, Flt3 and CSF-1 were 0.2, 0.17 0.22 and 3.43 microM, respectively. The IC_{50} for inhibition of a panel of other tyrosine kinases, including KDR, EGFR, FGFR, InsR, Src, Abl and several serine/threonine kinases was ≥30 μM (Table 1).[8]

MLN518 was tested against a number of cell lines expressing ITDs of Flt3 (Flt3-ITD), including Baf3 cells engineered to express the respective constructs and human lines derived from patients with AML. The reported IC_{50} values for growth inhibition differ considerably between the two studies. Whereas Kelly et al. reported IC_{50} values between 10 and 30 nM[8], whereas more than 10-fold higher values were reported in a subsequent study of BaF3 cells.[9] The reasons for this discrepancy are not clear. In contrast to some other Flt3 kinase inhibitors such as PKC412,[10] MLN518 is generally less active against activation loop mutants.[9] In particular, the relatively frequent D835V mutant is highly drug resistant.

In addition to its well-established activity against wild type Kit, MLN518 was found to significantly inhibit the proliferation of cells expressing the D816V activation loop mutant, although with approximately 10-fold higher IC_{50} values (250–600 nM vs. 40 nM.[11] Since activation loop mutants are resistant to imatinib, this is potentially significant for the treatment of patients with systemic mastocytosis, where this mutant is common.[12]

Animal models

MLN518 was evaluated in two murine models. The survival of nude mice injected intravenously with BaF3 cells expressing Flt3-ITD was prolonged by oral MLN518 administered twice daily at total doses between 40 and 120 mg/kg/day. However all animals eventually died from leukemia, despite continued therapy. Results were more impressive in a murine model of FLT3-ITD-positive myeloproliferative disease. In this model, syngeneic recipients of bone marrow infected with a Flt3-ITD retroviral construct developed a myelopoliferative syndrome characterized by leukocytosis and splenomegaly. MLN518, when started between 25 and 32 days after injection of retrovirally transduced marrow, prevented the manifestation of disease in the majority of animals as assessed by normal and differential white cell counts. However, the disease was not eradicated as myeloprolif-

Table 1. Specificity and potency of CT53518 kinase inhibitor.

Receptor Tyrosine Kinases	CT53518	Staurosporin
βPDGFR	0.2μM	0.08μM
FLT3	0.22	0.10
c-Kit	0.17	0.01
CSF-1R	3.43	0.12
KDR	>30	0.02
EGFR	>30	ND
FGFR	>30	0.42
InsR	>30	<0.3
Nonreceptor tyrosine kinases		
Src	30	0.07
Abl	>30	0.10
Ser/Thr Kinases		
PKC, PKA	>30	0.08
MAP kinases and MAPK kinases		
Mek1, Mkk4, Mkk6, Erk2, p38	>30	<1

Adapted from Kelly et al.[8]

erative disease developed with a latency of 45 days after discontinuation of therapy, even in the mice that had responded initially.[8] MLN518 was also effective in a murine model of acute promyelocytic leukemia (APL) associated with Flt3-ITD in combination with all trans retinoic acid (ATRA). In this model, bone marrow from PML-RARα-transgenic mice is transduced with a FLT3-ITD retrovirus. Compared to the transgenic mice the penetrance of APL is 100% in the Flt3-ITD-positive animals. Both MLN518 and ATRA were effective as single agents but there was synergism with the combination.[13]

In contrast to patients with chronic phase chronic myeloid leukemia (CML) there is a significant rate of primary refractory disease and a high rate of relapse in patients with blast crisis CML and Philadelphia chromosome-positive AML treated with imatinib.[14] This suggests that responses to kinase inhibitor therapy in AML may be incomplete and transient rather than stable, which implies that Flt3 inhibitor may eventually be combined with conventional chemotherapy and/or stem cell transplant approaches. Given the critical role of Flt3 in hematopoiesis and the fact that MLN518 inhibits Kit, another tyrosine kinase receptor with a central role in hematopoietic cell development, it was relevant to test whether MLN518 would significantly increase the toxicity of chemotherapy or delay engraftment after marrow ablative radiation and stem cell transplantation. Reassuringly, recovery to *safe* neutrophil counts was not delayed in mice receiving MLN518 prior to, during and after chemotherapy with cyclophosphamide and engraftment after stem cell transplantation was not delayed and stable.[15] These data provide the basis of combining MLN518 with conventional therapeutic approaches to AML.

Clinical trials

Phase 1 study in AML. MLN518 was first tested in a phase I study of 40 patients with AML or high-risk myelodysplasia. Flt3-ITD mutations were not required for study entry. Non dose-limiting toxicities were nausea, vomiting, diarrhea and edema. At 525 mg orally BID generalized weakness occurred in 1/6 patients, at 700 mg BID in 2/3, defining this as the dose limiting toxicity. Steady state trough levels at this dose were > 2000 nM. At 525 mg BID, the dose chosen for the phase II study, mean plasma trough levels were 453 nM. MLN518 had an apparent half-life of 33 hours. Activity was moderate: 2 patients with wild type Flt3 experienced stabilization of peripheral blood counts for >5 months. Only one patient with FLT3-ITD was examinable for response and experienced a reduction of peripheral blood and bone marrow blasts for > 28 days.[16]

Phase II study in AML. In the phase II study 20 AML patients with FLT3-ITD were treated with 525 mg MLN518 BID. All patients achieved steady state trough levels > 150 ng/mL, and partial or complete dephosphorylation of FLT3 in peripheral blood blasts, this was demonstrated in 4/4 patients evaluated in this way. Of 15 examinable patients, 7 had progressive disease. Two patients with stable disease for > 50 days subsequently underwent allogeneic stem cell transplantation. Six patients had evidence of some antileukemic effect characterized by a reduction of peripheral blood by a mean of 92% (range 85-100%) and bone marrow blasts by a mean of 62% (range 44-94%). These effects lasted for 1-3 months. No partial or complete responses were seen.[17]

Perspective

Overall, the results with FLT3 inhibitors in AML and with MLN518 in particular have not quite fulfilled hopes. Much of this disappointment is due to the expectation that a FLT3 inhibitor may produce similarly good results as imatinib does in CML. However, AML is clearly more similar to CML in blast crisis than to CML in chronic phase, so it is not surprising that results are far less spectacular. Even more important, whereas BCR-ABL is most likely the initiating event in CML, this may not be the case for FLT3 mutations in AML. Thus, eliminating the FLT3 mutant clone is not equal to eliminating the leukemia, which explains why responses if they occur tend to be incomplete. On the other hand, side effects at pharmacologically active doses are manageable and patients that do respond may gain time that may allow them to proceed to an allogeneic transplant, without added toxicity. The value of MLN518 and other FLT3 inhibitors may be best exploited in combination with conventional chemotherapy regimens. A phase I study that will test MLN518 in combination with standard *3+7* combination therapy in newly diagnosed patients with AML, irrespective of FLT3-ITD status is about to open. If successful this study will soon be followed by a phase III trial that will require FLT3-ITD as an entry criteria (Michael Heinrich and Michael Cooper, personal communication, August 2005). Another study in untreated patients who decline or are not eligible for chemotherapy is planned to open in 2006. Compared to some other FLT3 kinase inhibitors MLN518 has the disadvantage of being inactive against most activation loop mutants. On the other hand, activity was observed in patients with unmutated FLT3, which would extend the spectrum of patients who may benefit. Whether the *in vitro* activity against activation loop mutants of Kit is clinically relevant is currently unknown, and more potent agents may soon become available.

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