A novel combination therapy approach for the treatment of acute myeloid leukemia: the multi-kinase inhibitor sorafenib and the HDM2 inhibitor nutlin-3

Ellen Weisberg and Martin Sattler

Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

E-mail: ellenweis@yahoo.com *doi:10.3324/haematol.2012.078451*

Like other aggressive malignancies, acute myeloid leukemia (AML) is commonly associated with genomic instability, resulting in drug resistance and disease progression. The current goal is to identify therapies that inhibit cancer growth as well as providing a long-lasting clinical response. Growth factor receptors and growth signaling pathways are frequently the target of activating mutations in AML and have led to the identification of small molecule kinase inhibitors, although these have had mixed clinical success. For example, activating mutations of the receptor tyrosine kinase FLT3 (fms-related tyrosine kinase-3) occur in approximately 30% of AML and confer a poor prognosis.[1] First generation FLT3 inhibitors, including midostaurin, and newer, more potent second generation FLT3 inhibitors such as quizartinib or sorafenib, can lead to improved disease outcome but do not provide a cure.[2,3]

Resistance to these multi-kinase inhibitors in AML patients often arises due to the development of secondary mutations within mutant FLT3 as well as other factors.[4] In a study of 13 patients with relapsed or chemo-refractory mutant FLT3-expressing AML treated with sorafenib, following an initial clinical response, the majority of the patients lost their response over time, despite continued inhibition of FLT3.[2] Drug resistance in this study was believed to be in part due to sorafenib selection of resistant leukemic cells bearing the D835 mutation in FLT3.

As limited clinical efficacy and drug resistance are common features of tyrosine kinase inhibitors, such as sorafenib, which target mutant FLT3-positive leukemia, current preclinical studies of FLT3 inhibitors often include combination treatment strategies that pair FLT3 inhibitors with other agents. One novel combination treatment approach consists of a small molecule inhibitor able to inhibit the association between HDM2 (human double minute 2) and p53. HDM2 is a protein that regulates the activity of the p53 tumor suppressor protein, a key regulator of apoptosis, which functions upstream in the apoptotic cascade by both indirectly and directly regulating Bcl-2 family proteins. In cells expressing wild-type (wt) p53, the HDM2 protein binds to p53 and blocks its activity. Thus, inhibition of the association between HDM2 and p53 activates p53 and causes apoptosis of cells expressing wt p53. Disease targets for which use of an HDM2 inhibitor makes intuitive sense include hematopoietic malignancies and renal cell carcinoma, as the majority of hematologic malignancies are characterized by wt p53,[5] and the incidence of p53 mutations in renal cell carcinoma has been reported to be only around 20%.[6]

In this issue of the journal, Zauli *et al.* report the results of their investigation into the mechanism of synergy between sorafenib and nutlin-3, an inhibitor of the HDM2/p53 interaction,[7] against primary AML cells and cell lines characterized by wt p53, mutant p53, or deleted p53. It should be pointed out that oncogenic FLT3 was unlikely to have been a primary target of sorafenib in these experiments due to its efficacy in wt FLT3-expressing cells. Vatsyayan *et al.* have recently established that sorafenib and nutlin-3 also have a synergistic effect against renal cell carcinoma, further hinting at a yet to be identified target of sorafenib.[8] Additional targets of the multi-kinase inhibitor, sorafenib, include VEGFR-2, PDGFR, Raf, and c-Kit,[9] which may play an important role in this drug's efficacy in combination with nutlin-3. The use of sorafenib as a single agent has limitations in these diseases but the recent reports effectively demonstrate a novel approach for the development of a promising therapeutic strategy that relies on the combined use of multi-kinase inhibitors and inhibitors of HDM2.

The data from Vatsyayan *et al.* demonstrated synergy between sorafenib and nutlin-3 against renal cell carcinoma in terms of inhibition of cell viability, pro-apoptotic effects, reduction of renal cell carcinoma migration, and induction of p53 levels and activity. In renal cell carcinoma, sorafenib as a single agent activates p53, and the combination of nutlin-3 and sorafenib leads to an increase in the half-life of p53. In the study by Zauli *et al.*, sorafenib and nutlin-3 were observed to have a synergistic effect in terms of induction of apoptosis and autophagy against each of a panel of AML cell lines expressing different combinations of either wt or mutant FLT3 or wt or mutant p53, as well as primary AML cells. Importantly, the cells that were most sensitive to the combination of sorafenib and nutlin-3 were those harboring mutant FLT3. This finding is of potential clinical importance in that while FLT3-ITD has been proposed to be an oncogenic driver as well as a valid therapeutic target in AML,[10] tyrosine kinase inhibitors, such as midostaurin,[11] as single agents generally induce transient decreases in peripheral blood blasts and partial responses in patients harboring mutant FLT3.[3] Due to their limited therapeutic efficacy, it is common for FLT3 inhibitors in clinical development to be combined with standard chemotherapy, which is associated with unwanted adverse side effects/toxicity. The coupling of FLT3 inhibitors that have limited efficacy as single agents with targeted inhibitors of signaling molecules having an influence on the apoptotic machinery is an alternative approach to effective killing of mutant FLT3-positive cells, while also potentially circumventing the toxicity resulting from commonly used AML therapeutics such as doxorubicin and cytarabine.

In the study by Zauli *et al.*, the sorafenib and nutlin-3 combination was effective in promoting apoptosis and autophagy in leukemic cells regardless of their wt or deleted/mutated p53 status. The pro-apoptotic Bcl2 family member Bax was observed to contribute to the induction of apoptosis by sorafenib combined with nutlin-3 in AML cells characterized by wt p53, whereas the pro-apoptotic

Bcl2 family member Bak contributed to drug combination-induced apoptosis of AML cells characterized by deleted p53. It should be noted that, as in the study by Vatsyayan *et al.* in which high concentrations of sorafenib (up to 50 μM) and nutlin-3 (up to 20 μM) were administered, Zauli *et al.* used concentrations of sorafenib and nutlin-3 ranging from 3-10 μM. It cannot, therefore, be entirely excluded that additional drug targets contributed in part to the observed effects.

As a multi-kinase inhibitor, sorafinib may have a broad spectrum of application. Sorafenib was approved for the treatment of advanced renal cancer in 2005, and showed sufficient efficacy in hepatocellular carcinoma to warrant its approval for this indication in 2007. The drug is also in late stage clinical trials for non-responsive thyroid cancer, and in early clinical trials for recurrent glioblastoma. In this respect, sorafenib might be unique for its broad spectrum of potential targets that gives it some efficacy in a variety of cancers. Mutations in p53 have been reported to be frequent in poorly differentiated human thyroid carcinomas[12] and sporadic in glioblastomas.[13] Malignancies such as AML and renal cancer, in which the incidence of wt p53 is high, would be expected to show the best clinical response to a combination therapy regimen that includes sorafenib and an HDM2 inhibitor. Studies such as those by Zauli *et al.* and Vatsyayan *et al.* highlight the possible benefit of using HDM2 inhibitors as part of a potentially efficacious combination therapy strategy that also includes sorafenib. Further investigations, utilizing pro-apoptotic agents as part of a combination treatment approach aimed at enhancing the clinical potency of sorafenib and other tyrosine kinase inhibitors with efficacy limited by factors contributing to drug resistance, are warranted.

Ellen Weisberg is Principal Associate in Medicine at the Dana Farber Cancer Institute, Harvard Medical School (Boston, MA, USA). Her main field of interest is drug development and drug resistance mechanisms. Martin Sattler is an Assistant Professor of Medicine at the Dana-Farber Cancer Institute and Harvard Medical School (Boston, MA, USA). His main field of interest is molecular mechanisms involved in the induction of genomic instability.

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References

1. Zhang W, Konopleva M, Shi YX, McQueen T, Harris D, Ling X, et al. Mutant FLT3: a direct target of sorafenib in acute myelogenous leukemia. J Natl Cancer Inst. 2008;100(3):184-98.
2. Man CH, Fung TK, Ho C, Han HH, Chow HC, Ma AC, et al. Sorafenib treatment of FLT3-ITD(+) acute myeloid leukemia: favorable initial outcome and mechanisms of subsequent nonresponsiveness associated with the emergence of a D835 mutation. Blood. 2012; 119(22):5133-43.
3. Stone RM, DeAngelo DJ, Klimek V, Galinsky I, Estey E, Nimer SD et al. Patients with acute myeloid leukemia and an activating mutation in FLT3 respond to a small-molecule FLT3 tyrosine kinase inhibitor, PKC412. Blood. 2005;105:54-60.
4. Williams AB, Nguyen B, Li L, Brown P, Levis M, Leahy D, et al. Mutations of FLT3/ITD confer resistance to multiple tyrosine kinase inhibitors. Leukemia. 2012 [Epub ahead of print]
5. Mitani N, Niwa Y, Okamoto Y. Surveyor nuclease-based detection of p53 gene mutations in haematological malignancy. Ann Clin Biochem. 2007;44(Pt6):557-9.
6. Girgin C, Tarhan H, Hekimgil M, Sezer A, Gurel G. P53 mutations and other prognostic factors of renal cell carcinoma. Urol Int. 2001;66(2):78-83.
7. Zauli G, Celeghini C, Melloni E, Voltan R, Ongari M, Tiribelli M, et al. The sorafenib plus nutlin-3 combination promotes synergistic cytotoxicity in acute myeloid leukemic cells irrespectively of FLT3 and p53 status. Haematologica. 2012;97(11):1722-30.
8. Vatsyayan R, Singhal J, Nagaprashantha LD, Awasthi S, Singhal SS. Nutlin-3 enhances sorafenib efficacy in renal cell carcinoma. Mol Carcinog. 2011 Oct 17. doi: 10.1002/mc.20875. [Epub ahead of print]
9. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, et al. Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med. 2007;356(2):125-34.
10. Smith CC, Wang Q, Chin CS, Salerno S, Damon LE, Levis MJ, et al. Validation of ITD mutations in FLT3 as a therapeutic target in human acute myeloid leukemia. Nature. 2012;485(7397):260-3.
11. Weisberg E, Boulton C, Kelly LM, Manley P, Fabbro D, Meyer T, et al. Inhibition of mutant FLT3 receptors in leukemia cells by the small molecule tyrosine kinase inhibitor PKC412. Cancer Cell. 2002;1(5): 433-43.
12. Fagin JA, Matsuo K, Karmakar A, Chen DL, Tang SH, Koeffler HP. High prevalence of mutations of the p53 gene in poorly differentiated human thyroid carcinomas. J Clin Invest. 1993;91(1):179-84.
13. Gomori E, Doczi T, Pajor L, Matolcsy A. Sporadic p53 mutations and absence of ras mutations in glioblastomas. Acta Neurochir (Wien). 1999;141(6):593-9.