FLT3/ITD AML and the law of unintended consequences

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Acute myeloid leukemia with a FLT3 internal tandem duplication (FLT3/ITD) mutation is an aggressive hematologic malignancy with a generally poor prognosis. It can be successfully treated into remission with intensive chemotherapy, but it routinely relapses. At relapse, the blasts tend to have higher mutant allelic ratios and, in vitro, are more addicted to the aberrant signaling from the FLT3/ITD oncoprotein. They remain highly responsive to FLT3 ligand, the levels of which rise several-fold during the course of chemotherapy. The question now arises as to whether these high levels of FLT3 ligand are actually promoting relapse, and, if so, how we can use this information to adjust our therapeutic approach and improve the cure rate for acute myeloid leukemia with FLT3/ITD. (*Blood*. 2011;117(26): 6987-6990)

Introduction

The law of unintended consequences teaches us that, when we intervene in a complex system, we invariably create unanticipated and sometimes undesirable outcomes. Our attempts to treat the disease known as acute myeloid leukemia with a FLT3 internal tandem duplication (FLT3/ITD AML) mutation certainly could be characterized as an intervention in a complex system, and the question can now be raised as to whether we are creating an unintended consequence with our therapeutic approach.

FLT3 is a cytokine receptor that is expressed on the leukemic blasts in most cases of acute leukemia.[1-5] On binding FLT3 ligand (FL), FLT3 dimerizes and undergoes a conformational change, causing its activation loop to assume an open conformation and to allow ATP access to the ATP-binding pocket. Ligand-activated FLT3 undergoes autophosphorylation and, through a series of kinase cascades, transduces signals promoting cell growth and inhibiting apoptosis through proteins such as Ras-GTPase activating protein, phospholipase C β, STAT5, and ERK1/2.[6-12] The ligand, FL, is expressed in virtually all cell types thus far examined, including leukemia cells.[13-15] In contrast, the receptor, FLT3, has a fairly narrow range of cell expression, being localized primarily to hematopoietic and neural tissues, which presumably confines its functions to these cell types.[16] FL acts in synergy with other cytokines to promote hematopoietic precursor expansion, and targeted disruption of either FLT3 or FL in mice, although not embryonically lethal, leads to a reduction in hematopoietic precursors.[17-24]

FLT3/ITDs were first described in patients with AML in 1996 by Nakao et al.[25] These mutations, which disrupt the autoinhibitory function of the receptor's juxtamembrane domain, result in constitutive autophosphorylation of FLT3 within the blasts that harbor them.[26,27] Fifteen years after this initial discovery, FLT3/ITD AML now stands as a distinct clinical entity, an often lethal subtype of AML that has been a considerable challenge to those of us who treat it.[28] Some recent clinical and laboratory findings about this disease may provide insight into why these patients relapse so quickly, and how we might improve their outcomes.

FLT3/ITD mutations are present in roughly a quarter of adult AML cases.[29] In a minority of cases they represent a presumably late mutation in AML, evolving out of an antecedent myelodysplastic syndrome.[30] However, the more characteristic presentation is that of de novo disease, presenting with a high leukocyte count and normal cytogenetics. Numerous retrospective analyses of clinical trial results have established that patients with FLT3/ITD AML achieve complete remission at or near the rate for patients with AML lacking these mutations.[31-34] However, equally well established is the fact that patients with FLT3/ITD are far more likely to relapse and do so more rapidly than their FLT3 wild-type counterparts. The median survival of FLT3 mutant AML after first relapse has been reported to be < 5 months.[35,36]

Coincident with the recognition of FLT3/ITD AML as a disease entity was the emergence of an important new anticancer therapy: tyrosine kinase inhibitors (TKIs). The remarkable clinical activity of imatinib mesylate for blast crisis chronic myelogenous leukemia and Philadelphia-positive acute lymphocytic leukemia (2 diseases with a number of similarities to FLT3/ITD AML) spurred the development of FLT3 TKIs.[37,38] Currently, older, multitargeted FLT3 inhibitors are in advanced clinical trials, whereas newer, more FLT3-selective inhibitors are entering development.[39]

There are important differences between the FLT3/ITD and BCR-ABL oncoproteins however similar the diseases they cause might seem to be. Unlike BCR-ABL, FLT3 is a transmembrane protein primarily localized to the plasma membrane, where it binds its cognate ligand, the cytokine FL. Why would the ligand have any effect on a receptor tyrosine kinase that is supposed to be constitutively activated? Recent evidence suggests that the ITD-mutated receptor is, in fact, heavily influenced by FL. FL is coexpressed with FLT3 in leukemia cells and is up-regulated in response to FLT3 inhibition. When the FLT3/ITD receptor is expressed in cells that completely lack FL (derived from an $FL^{-/-}$ mouse), it shows only weak autophosphorylation.[40] This finding casts the FLT3/ITD oncoprotein in a wholly different light: not as an autonomously activated receptor but rather as one that is simply hyperresponsive to its ligand. To complicate matters further, FL markedly interferes with the ability of TKIs to inhibit FLT3 signaling.[41] Indeed, the surge of FL after chemotherapy may have been responsible for the generally poor level of in vivo FLT3

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inhibition observed in the recent trial of lestaurtinib after chemotherapy.[36] The influence of FL on FLT3/ITD signaling will probably be quite problematic to efforts aimed at incorporating FLT3 inhibition into conventional AML chemotherapy regimens. Aplasia-inducing radiation or chemotherapy has been well established to induce significant increases in FL levels,[42-44] which in turn would block the effects of FLT3 inhibitors.

There is another important difference between the FLT3/ITD and BCR-ABL oncoproteins. Inhibition of the downstream signaling of BCR-ABL by an inhibitor such as imatinib mesylate results in rapid apoptosis of the bulk population of leukemia cells.[45] This addiction of the leukemia cells to the oncoprotein is a signature feature of the Philadelphia-positive diseases that can be exploited for clinical benefit. In contrast to this, addiction to FLT3/ITD signaling is not necessarily a consistent feature of FLT3/ITD AML, at least according to in vitro studies. Inhibition of FLT3 alone is insufficient to induce apoptosis in a significant fraction of FLT3/ITD AML primary samples collected at initial diagnosis.[46] However, samples collected at the inevitable relapse and tested in vitro are much more likely to undergo apoptosis in response to FLT3 inhibition in comparison to the diagnostic samples.[46] The best predictor for an AML sample to have a cytotoxic response to FLT3 inhibition is a high FLT3/ITD mutant allelic burden. Perhaps not coincidentally, the FLT3/ITD mutant allelic ratio tends to be increased at relapse compared with diagnosis (although in a minority of cases the mutation can be lost altogether).[47-49] Relapse, a higher mutant allelic ratio, and addiction to FLT3/ITD signaling appear inextricably linked in FLT3/ITD AML.

To summarize, then, FLT3/ITD AML is a disease that appears to evolve between diagnosis and relapse, with the leukemia cells becoming more addicted to FLT3 signaling after recurrence after chemotherapy. Treatment of a patient with chemotherapy leads to high levels of FL in the plasma throughout the period of recovery and during consolidation. FL is a cytokine that acts directly on the mutant FLT3/ITD receptor, maximizing its activity and promoting the survival of blasts. Although these findings have practical implications, in that these properties could be used to predict clinical response and to design treatment regimens, they uncover a potentially larger issue.

Are we promoting relapse of FLT3/ITD AML with successive rounds of chemotherapy? If induction regimens de-bulk the BM of blasts, leaving a residual leukemia stem cell population, do the recurrent waves of FL that follow select for the emergence of FLT3-addicted subclones? Patients with FLT3/ITD AML often relapse during consolidation. It is conceivable, given the above-mentioned findings, that we could be doing more harm than good by administering repeat cycles of high-dose cytarabine, or whichever consolidation regimen is being used. Indeed, in a recent randomized trial of induction chemotherapy using more intensive anthracycline use, patients with FLT3/ITD AML did not appear to benefit from intensifying therapy, in contrast to patients with FLT3 wild type.[50]

The clonal evolution of FLT3/ITD AML almost certainly occurs through >1 mechanism. The lower allelic burden often seen at diagnosis may represent simple heterozygosity of the mutation in an otherwise uniform population of blasts. At relapse, deletion or point mutations of the wild-type allele, gene conversion, or outright loss of one copy of chromosome 13 (the chromosome on which the FLT3 gene is localized) could lead to a hemizygous or homozygous state.[51,52] Increased expression of the mutant allele (or loss of the wild-type allele) presumably provides a selective advantage, resulting in expansion of these particular clones. Alternately, the blast population at diagnosis can be nonuniform, with some cells completely lacking the FLT3/ITD mutation and others harboring heterozygous or homozygous mutants.[53] The leukemia stem cells harboring the FLT3/ITD mutation could have a survival advantage over their wild-type counterparts and emerge at relapse as the dominant clone. In any of these scenarios, however, the FLT3/ITD clones are still highly responsive to FL, which could contribute significantly to their ability to survive successive rounds of chemotherapy.

The patients who present with low mutant allelic ratio at diagnosis present an interesting counter-argument to the concept that FL promotes or influences relapse. Those relatively few patients with FLT3/ITD AML who have low allelic ratios at presentation often (but not always) lose the mutation altogether at relapse.[48-49] FL clearly does not select for the expansion of these apparent subclones. However, patients with low allelic burden seem to have a prognosis that is similar to that of patients with AML with wild-type FLT3.[34] It is possible that in these cases the ITD mutation occurred relatively late in leukemogenesis, perhaps in a leukemia stem cell with lower long-term renewal potential.

The hypothesis that FL promotes relapse of FLT3/ITD AML is, in at least some ways, a testable one. In patients with newly diagnosed FLT3/ITD AML undergoing induction and consolidation there is significant interpatient variability in the degree to which FL rises from baseline.[41] We might predict that relapses will be more likely to occur, and will occur earlier, in patients with high FL levels compared with low FL levels. The hypothesis could be refuted by finding no effect of FL on relapse risk or by finding that high levels actually predict for better outcomes. In fact, high FL levels after chemotherapy could be a surrogate for the intensity of aplasia, which from a traditional perspective is thought to be beneficial. We are prospectively examining this issue by serial measurements of FL levels during induction and consolidation in patients with FLT3/ITD AML, both at our own institution and in collaboration with ongoing cooperative group trials.

Many advocate the use of allogeneic transplantation as the most effective consolidation for FLT3/ITD AML.[54,55] This is an issue that remains quite controversial.[56-60] Certainly, there are numerous variables that could have influenced the outcomes of trials involving allogeneic transplantation (any one of which could cloud the interpretation of the results), including time to transplantation, preparative regimens, transplantation-related mortality, and graft-versus-host prophylaxis. However, if FL levels do contribute to relapse, and if the "graft-versus-leukemia" effect is a real one, then the best approach for the patient would be to proceed as rapidly as possible to allogeneic transplantation once remission is achieved. Allogeneic transplantation, of course, also involves chemotherapy, usually more intensive than a single course of consolidation. However, in substitution for 4 cycles of high-dose cytarabine, it probably results in a less prolonged overall elevation of FL (although this should be confirmed prospectively), and it also introduces a different type of therapeutic effect, that of immunotherapy. At our institution, where we have aggressively pursued allogeneic transplantation for these patients in first remission, the survival for patients with FLT3/ITD AML is equivalent to non–FLT3-mutated AML.[55] Intriguingly, in a recent report from the AML Study Group in Ulm, Germany, where a similar strategy has been used since 2006, it was noted that patients with FLT3/ITD who received a transplant sooner rather than later after achieving remission had better outcomes.[61] Normally, in landmark analyses of AML transplantation studies, longer time to transplantation is associated with better outcomes, presumably because patients who

are well enough for transplantation after several months represent a generally favorable risk group. However, longer time to transplantation, which would necessitate extra courses of consolidation, was associated with worse overall survival in their analysis. This is exactly what would be predicted if those recurrent courses of consolidation were actually promoting relapse.

On the basis of these findings, it can be proposed that the optimal therapeutic approach for a patient with FLT3/ITD AML would be a single course of induction therapy followed as rapidly as possible by allogeneic transplantation, including the use of alternative donors (matched unrelated, haploidentical, or cord blood derived) if necessary. Novel therapeutic agents can be introduced into this paradigm. FLT3 inhibitors can be used early in therapy (before the rise in FL levels, before their effectiveness is limited) to improve remission rate and, if the FL levels return to baseline, to maintain the patient in remission until transplantation. FLT3 inhibition could also be used as maintenance therapy after allogeneic transplantation, as is commonly done with BCR-ABL inhibitors,[62] particularly in light of the mounting anecdotal evidence of activity in this setting.[63,64] Finally, consideration could be given to targeting FL with monoclonal antibodies.

Obviously, it would be far more preferable to avoid allogeneic transplantation, with its concomitant short- and long-term risks of morbidity and mortality,[65] by curing our patients with a combination of chemotherapy and FLT3 inhibition. Indeed, this approach continues to look promising, because preliminary results from large combination trials appear to show a survival improvement from this approach (compared with historic controls).[66,67] The final results of these trials, however, may be difficult to interpret in light of the reported high rates of allogeneic transplantations occurring in first remission in the enrolled patients.[66,67]

Great strides have been made in improving the survival of patients with AML over the past several decades.[68] It has been increasingly recognized that AML is not a single disease, and the improvements in survival have been brought about as a result of tailoring therapy according to the molecular features of the disease. In this case, we may need to tailor the therapy to try to account for the law of unintended consequences. Even recent medical history is full of examples of the best intentions gone awry: estrogen therapy that prevents osteoporosis but causes breast cancer,[69] erythropoietin therapy that decreases transfusion requirements but kills patients with cardiovascular and thromboembolic events,[70] and intensive blood glucose control that results in hypoglycemic deaths.[71] More is not always better, and this may well apply to chemotherapy and FLT3/ITD AML.

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