Finding the next Gleevec: FLT3 targeted kinase inhibitor therapy for acute myeloid leukemia

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Activating mutations in the FLT3 receptor tyrosine kinase occur in 30% of patients with acute myeloid leukemia. Small molecule FLT3 kinase inhibitors show selective antitumor activity in preclinical models. Clinical studies are underway.

The remarkable success of the small molecule tyrosine kinase inhibitor STI571 (also called Gleevec or imatinib) has galvanized the cancer research community to hasten the pursuit of new molecularly targeted therapy. Voices of caution warn that STI571 may be a one-time example because of the unique molecular features of chronic myeloid leukemia (CML) that make it the perfect candidate for single agent targeted therapy. CML results from the action of the BCR-ABL tyrosine kinase, created by the Philadelphia chromosome translocation. BCR-ABL is sufficient to cause leukemia in mouse models, but additional genetic hits occur over time as the disease progresses to the terminal phase called blast crisis (Sawyers, 1999). STI571 blocks BCR-ABL kinase activity and induces remissions in nearly all patients in the early stages of the disease, providing the best validation to date of the concept of molecularly targeted cancer therapy (Druker et al., 2001b; Kantarjian et al., 2002). In late stage CML, STI571 remains effective in a large fraction of patients (Druker et al., 2001a; Sawyers et al., 2002; Talpaz et al., 2002), but remissions are short-lived due to drug resistance that develops as a consequence of BCR-ABL kinase domain mutation or gene amplification (Branford et al., 2002; Gorre et al., 2001; von Bubnoff et al., 2002). The fact that STI571 is less effective in blast crisis CML, together with the lower response rates observed with other kinase inhibitors in solid tumors, has led to skepticism about whether this approach can be successfully extended to other cancers.

Soon we will have data from four new kinase inhibitors that speak to this question in acute myeloid leukemia (AML). Two papers in the current issue of *Cancer Cell* and a recent paper in *Blood* report promising preclinical results with three different inhibitors of the tyrosine kinase receptor FLT3, a therapeutic target in AML (Kelly et al., 2002c; Weisberg et al., 2002; Levis et al., 2002). Comparable results have been reported in abstract form for a fourth FLT3 inhibitor (O'Farrell et al., 2001). All four compounds are currently in clinical testing, with preliminary results expected in 6–12 months. Since AML is a hematologic malignancy characterized by multiple oncogenic hits (unlike CML), successful results with any of these new compounds would make a compelling case for the power of targeted molecular therapy in genetically complex cancers.

Rationale for targeting FLT3 in AML

Approximately one-third of patients with AML have mutations in FLT3 that lead to constitutive activation of the kinase as well as downstream signaling pathways (Nakao et al., 1996; Hayakawa et al., 2000; Kiyoi et al., 1999). Unlike BCR-ABL, which is activated by gene fusion due to the Philadelphia chromosome translocation, FLT3 activation in AML typically occurs due to internal tandem duplications in the kinase insert domain. An alternative mechanism is point mutation in the activation loop of the kinase (Yamamoto et al., 2001). The mutant FLT3 receptor causes cellular transformation and produces a myeloproliferative syndrome in mice (Kelly et al., 2002b), much like BCR-ABL (Daley et al., 1990). The fact that mutant FLT3 is not sufficient to cause full-blown AML supports a two-hit model for the genesis of acute leukemia. Experimental evidence suggests that an activated tyrosine kinase (which induces myeloid proliferation) must be paired with a second mutation affecting hematopoietic differentiation (such as a transcription factor translocation) to give the full disease phenotype (Kelly et al., 2002a).

Why do we suddenly have four different inhibitors against the same kinase target? A review of the chemical structures and specificity profiles of these inhibitors provides some insight (Table 1). CEP-701 is an indolocarbazole derivative, originally reported to inhibit the TrkA receptor tyrosine kinase (George et al., 1999), and now shown to inhibit FLT3 (Levis et al., 2002). PKC412 is staurosporine derivative with activity against FLT3, as well as protein kinase C, KDR (also called VEGFR2 or FLT1), PDGFR, c-Kit, and FMS (Fabbro et al., 1999; Weisberg et al., 2002). SU11248, whose precise chemical structure has not been published, is an indolinone-based inhibitor of KDR, c-kit, and FLT3, similar to the less potent parent compound SU5416 (Fong et al., 1999; O'Farrell et al., 2001). Finally, CT53518 is a novel compound of the piperazinyl quinazoline class with activity against PDGFR, c-kit, and FLT3—a specificity profile similar to SU11248 despite quite distinct chemical structures (Kelly et al., 2002c).

All four compounds are postulated to function as ATP binding site inhibitors. How, then, can they all block ATP binding to FLT3, yet retain such distinct chemical structures and specificity profiles? Knowing the answer requires solving crystal structures of each inhibitor bound to the FLT3 kinase domain, but we can make educated guesses based on other kinase/inhibitor cocrystals. Kinase domains are dynamic structures which move from active to inactive conformations and undergo dramatic shape changes during these transitions (Huse and Kuriyan, 2002). Structurally different inhibitors can bind to the same kinase domain by taking advantage of these shape changes. For example, STI571 binds to Abl when the kinase domain is in the "off" configuration and locks it in an inactive state (Schindler et al., 2000), whereas, a second Abl inhibitor, PD 173955, binds Abl in the "on" configuration (J. Kuriyan, personal communica-

Table 1. Inhibitory activity (IC_{50}) of FLT3 kinase inhibitors against a panel of tyrosine kinase targets (all values are nM)

	Drug				
Kinase	CEP-701	CT 53518	PKC412	SU11248	SU5416[a]
FLT3	3 (iv)	220 (iv)	528 (iv); <10 (c)	10 (c)	250 (c)
PKC	218 (iv)	>30,000 (c)	22 (iv)	—	—
KDR	65 (iv)	>30,000 (c)	86 (iv)	10 (c)	1230 (iv)
PDGFR	773 (iv)	200 (iv)	80 (c)	10 (c)	20,000 (c)
KIT	—	170 (iv)	300 (c)	—	—
FMS	—	3430 (iv)	*	—	—
TRKA	3.7 (iv)	—	—	—	—
EGFR	>1,000 (iv)	>30,000 (c)	>100,000 (iv)	—	>100,000 (iv)
ABL	—	>30,000 (iv)	800 (iv)	—	—
SRC	—	>30,000 (iv)	>100,000 (iv)	—	—

IC_{50} = concentration of drug required to achieve 50% inhibition; iv = IC_{50} as measured using an in vitro kinase assay with purified kinase domain; c = IC_{50} as measured in cells expressing the kinase. — indicates data not available. Values in this table were obtained from the following references: George et al., 1999; Fabbro et al., 1999; Levis et al., 2002; Kelly et al., 2002c; O'Farrell et al., 2001; Fong et al., 1999; Weisberg et al., 2002.
[a]SU5416 is the parent compound for SU11248.
*Reported to be sensitive; precise IC_{50} not reported.

tion). This distinction provides a compelling explanation for the fact that PD 173955 is a potent inhibitor of both Abl and Src, whereas STI571 only inhibits Abl. The assumption, then, is that the active conformations of Src and Abl are structurally similar (allowing equivalent binding of PD 173955), whereas the inactive conformations are not (providing specificity for STI571). Similar logic applied to the four FLT3 inhibitors predicts that each might bind FLT3 in slightly different transition states between the active and inactive conformations of the kinase domain. The relative similarity of these transition states to those of other kinases in Table 1, such as KDR, PDGFR, and c-kit might explain the differences in crossreactivity.

Choosing the optimal clinical development strategy

With four FLT3 inhibitors racing into the clinic, is it possible to predict the winner? One concern frequently voiced during the clinical planning stages of kinase inhibitors is toxicity, particularly if the compound has broad activity. Intuition would tell us that clinical success is more likely if an inhibitor is highly specific. The compounds in Table 1 show clear differences here that may impact safety and tolerability. But we should learn from the STI571 experience. The STI571 dose used in CML (for BCR-ABL inhibition) also blocks wild-type Abl, c-kit, and PDGFR. Yet the drug is extremely well tolerated in animals and patients—despite the fact that knockout mice lacking either Abl, Kit, or PDGFR all have severe phenotypes. Traditional toxicology studies are still the best guide for preclinical safety evaluation, and we should not rule out a possible drug solely on the basis of the kinase inhibition profile.

What is the best clinical trial design? The preclinical data for all four compounds argues that AML patients whose leukemia cells have FLT3 mutations should respond. All compounds showed selective activity against cell lines and murine leukemia models engineered to express mutant FLT3 (Kelly et al., 2002c; Levis et al., 2002; Weisberg et al., 2002). One group also demonstrated a correlation between presence of FLT3 mutation and antileukemia activity by studying AML patient material cultured in vitro (Levis et al., 2002; Levis et al., 2001). A potential problem with the logic of testing these drugs only in AML patients with FLT3 mutation is that FLT3 may not be the true target of these inhibitors. While there is very little evidence to support this contention, it is curious that the inhibitor with the broadest activity (PKC412) shows a discrepancy between inhibition of purified FLT3 kinase domain in vitro (IC_{50} = 528 nM) and inhibition of endogenous FLT3 in cells (IC_{50} < 10 nM) (Weisberg et al., 2002). Although differences between in vitro and cellular IC_{50}s are common with kinase inhibitors, the trend is typically in the opposite direction and is usually explained by issues involving transport across the cell membrane. The PKC412 discrepancy might be technical, since the in vitro assay uses the wild-type FLT3 kinase domain rather than mutant FLT3. However, it remains formally possible that the target for this compound is another kinase closely associated with FLT3. The authors addressed this issue by deriving PKC412-resistant cell lines, and observed amplification of the FLT3 transgene (Weisberg et al., 2002). This result provides strong evidence that FLT3 is the true target, but the most convincing proof would come from isolating a PKC412-resistant FLT3 mutant, analogous to BCR-ABL mutants that confer STI571 resistance (Gorre et al., 2001; von Bubnoff et al., 2002). Assuming that FLT3 is the relevant drug target in these models, we must still decide if enrollment to clinical trials should be restricted to AML patients with FLT3 mutations. My personal bias would be to restrict enrollment in the initial trials so that the experimental hypothesis is tested cleanly and efficiently. I believe this approach affords the best chance of a clinical result that will generate excitement in the cancer community, which will lead to rapid patient accrual to the large registration trials required for FDA approval. But a word of caution is in order, since clinical responses have already been observed in AML patients in the phase I studies of SU5416, the precursor molecule to SU11248 (Yee et al., 2001). It is not known if these patients had FLT3 abnormalities; therefore, it remains possible that inhibition of other SU5416 targets, such as KDR or c-Kit, could explain the clinical responses.

With all four compounds entering the clinic simultaneously, it is interesting to speculate on the potential outcomes. Safety and tolerability are essential benchmarks that are impossible to predict in advance, and proper dose selection for achieving blood levels that give sustained FLT3 inhibition in tumor cells requires careful pharmacokinetic and pharmacodynamic

assessment. In this regard, PKC412 may have a head start since phase I clinical testing has been completed in patients with solid tumors (targeting PKC), and favorable safety and pharmacokinetic data were obtained that predict for successful FLT3 inhibition (Propper et al., 2001). Assuming that sustained FLT3 inhibition can be achieved in tumor cells, what type of clinical results can we expect? Because of the phenotypic similarities between AML and blast crisis CML, we might consider the STI571 experience in myeloid blast crisis as a guide. Response rates in phase I–II trials were 50–60 percent, with complete remissions in 10–20 percent of cases, but relapses were common (Druker et al., 2001a; Sawyers et al., 2002). If all four compounds meet the milestone of FLT3 inhibition in tumor cells with acceptable toxicity, it is unlikely that there will be any losers in the group. Extrapolating from the STI571 experience, we can anticipate that resistance to single agent therapy will occur, presumably through kinase domain mutations or gene amplification. How wonderful it would be to have four structurally distinct FLT3 inhibitors, so that combination therapy against the same target could be tested as a strategy to block the emergence of resistance, much like current HIV treatment regimens.

Buried amongst the optimism that STI571 has generated for targeted cancer therapy are the economic forces at large pharmaceutical companies that make it difficult to develop drugs for diseases with small market potential like CML. While AML is more common than CML, marketing departments must be aware that FLT3 inhibitors may only be effective in AML patients with FLT3 mutations (30 percent of all cases). Has there been a change in the traditional "big pharma" business model to allow pursuit of such niche markets? We can only guess. Early decisions behind the development of PKC412 and SU11248 were presumably based on large market potential, since their original targets (PKC and KDR, respectively) are widely implicated in cancer. FLT3 offers the advantage of a much better validated target, presumably at low additional cost for a potentially high payoff. Whatever the reasons underlying the decisions to go forward, the impact on future business models for cancer drug development could be substantial if any of these drugs are financial successes. We all envision the day when oncologists will select from a wide array of nontoxic, molecularly targeted therapies based on the molecular phenotype of the tumor. The ongoing FLT3 clinical trials provide a powerful series of experiments that will teach us a great deal about the science (and the business) of developing kinase inhibitors for cancer. We should have some answers soon.

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Selected reading

Branford, S., Rudzki, Z., Walsh, S., Grigg, A., Arthur, C., Taylor, K., Herrmann, R., Lynch, K.P., and Hughes, T.P. (2002). Blood *99*, 3472–3475.

Daley, G.Q., Van Etten, R.A., and Baltimore, D. (1990). Science *247*, 824–830.

Druker, B.J., Sawyers, C.L., Kantarjian, H., Resta, D.J., Reese, S.F., Ford, J.M., Capdeville, R., and Talpaz, M. (2001a). N. Engl. J. Med. *344*, 1038–1042.

Druker, B.J., Talpaz, M., Resta, D.J., Peng, B., Buchdunger, E., Ford, J.M., Lydon, N.B., Kantarjian, H., Capdeville, R., Ohno-Jones, S., and Sawyers, C.L. (2001b). N. Engl. J. Med. *344*, 1031–1037.

Fabbro, D., Buchdunger, E., Wood, J., Mestan, J., Hofmann, F., Ferrari, S., Mett, H., O'Reilly, T., and Meyer, T. (1999). Pharmacol. Ther. *82*, 293–301.

Fong, T.A., Shawver, L.K., Sun, L., Tang, C., App, H., Powell, T.J., Kim, Y.H., Schreck, R., Wang, X., Risau, W., et al. (1999). Cancer Res. *59*, 99–106.

George, D.J., Dionne, C.A., Jani, J., Angeles, T., Murakata, C., Lamb, J., and Isaacs, J.T. (1999). Cancer Res. *59*, 2395–2401.

Gorre, M.E., Mohammed, M., Ellwood, K., Hsu, N., Paquette, R., Rao, P.N., and Sawyers, C.L. (2001). Science *293*, 876–880.

Hayakawa, F., Towatari, M., Kiyoi, H., Tanimoto, M., Kitamura, T., Saito, H., and Naoe, T. (2000). Oncogene *19*, 624–631.

Huse, M., and Kuriyan, J. (2002). Cell *109*, 275–282.

Kantarjian, H., Sawyers, C., Hochhaus, A., Guilhot, F., Schiffer, C., Gambacorti-Passerini, C., Niederwieser, D., Resta, D., Capdeville, R., Zoellner, U., et al. (2002). N. Engl. J. Med. *346*, 645–652.

Kelly, L., Clark, J., and Gilliland, D.G. (2002a). Curr. Opin. Oncol. *14*, 10–18.

Kelly, L.M., Liu, Q., Kutok, J.L., Williams, I.R., Boulton, C.L., and Gilliland, D.G. (2002b). Blood *99*, 310–318.

Kelly, L.M., Yu, J.-C., Boulton, C.L., Apatira, M., Li, J., Sullivan, C.M., Williams, I., Amaral, S.M., Curley, D.P., Duclos, N., et al. (2002c). Cancer Cell, this issue, 421–432.

Kiyoi, H., Naoe, T., Nakano, Y., Yokota, S., Minami, S., Miyawaki, S., Asou, N., Kuriyama, K., Jinnai, I., Shimazaki, C., et al. (1999). Blood *93*, 3074–3080.

Levis, M., Allebach, J., Tse, K.F., Zheng, R., Baldwin, B.R., Smith, B.D., Jones-Bolin, S., Ruggeri, B., Dionne, C., and Small, D. (2002). Blood *99*, 3885–3891.

Levis, M., Tse, K.F., Smith, B.D., Garrett, E., and Small, D. (2001). Blood *98*, 885–887.

Nakao, M., Yokota, S., Iwai, T., Kaneko, H., Horiike, S., Kashima, K., Sonoda, Y., Fujimoto, T., and Misawa, S. (1996). Leukemia *10*, 1911–1918.

O'Farrell, A.M., Abrams, T., Yuen, H., Ngai, T., Louie, S., Wong, L., Heinrich, M., Yee, K., Smolich, B., Murray, L., et al. (2001). SUGEN Compounds SU5416 and SU11248 Inhibit Flt3 Activity: Therapeutic Application in AML. American Society of Hematology 43rd Annual Meeting, Orlando, Florida.

Propper, D.J., McDonald, A.C., Man, A., Thavasu, P., Balkwill, F., Braybrooke, J.P., Caponigro, F., Graf, P., Dutreix, C., Blackie, R., et al. (2001). J. Clin. Oncol. *19*, 1485–1492.

Sawyers, C.L. (1999). N. Engl. J. Med. *340*, 1330–1340.

Sawyers, C.L., Hochhaus, A., Feldman, E., Goldman, J.M., Miller, C.B., Ottmann, O.G., Schiffer, C.A., Talpaz, M., Guilhot, F., Deininger, M.W., et al. (2002). Blood *99*, 3530–3539.

Schindler, T., Bornmann, W., Pellicena, P., Miller, W.T., Clarkson, B., and Kuriyan, J. (2000). Science *289*, 1938–1942.

Talpaz, M., Silver, R.T., Druker, B.J., Goldman, J.M., Gambacorti-Passerini, C., Guilhot, F., Schiffer, C.A., Fischer, T., Deininger, M.W., Lennard, A.L., et al. (2002). Blood *99*, 1928–1937.

von Bubnoff, N., Schneller, F., Peschel, C., and Duyster, J. (2002). Lancet *359*, 487–491.

Weisberg, E., Boulton, C., Kelly, L.M., Manley, P., Fabbro, D., Meyer, T., Gilliland, D.G., and Griffin, J.D. (2002). Cancer Cell, this issue, 433–443.

Yamamoto, Y., Kiyoi, H., Nakano, Y., Suzuki, R., Kodera, Y., Miyawaki, S., Asou, N., Kuriyama, K., Yagasaki, F., Shimazaki, C., et al. (2001). Blood *97*, 2434–2439.

Yee, K.W.H., O'Farrell, A.M., Smolich, B.D., Cherrington, J.M., Wait, C.L., Griffith, D.J., McGreevey, L.S., and Heinrich, M.C. (2001). SU5416 and SU5614 Inhibit Wild-Type and Activated Mutant FLT3 Signaling in Leukemia Cells. American Society of Hematology 43rd Annual Meeting, Orlando, Florida.