The Biology Behind

Is KIT an Important Therapeutic Target in Small Cell Lung Cancer?

Commentary re: B. E. Johnson *et al.*, Phase II Study of Imatinib in Patients with Small Cell Lung Cancer. Clin. Cancer Res., *9:* 5880–5887, 2003.

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Introduction

SCLC[1] represents a significant minority of lung cancer (15–25%) and typically presents as extensive-stage disease. Although SCLC is highly responsive to cisplatin-based combination chemotherapy, most patients have disease recurrence and few patients are cured. A plateau in the development of effective medical therapy for this difficult cancer has led to a search for new active agents (1). In this issue, Johnson *et al.* (2) report on their efforts to clinically target the KIT RTK in SCLC using imatinib (formerly STI571; Gleevec in the United States and Glivec in Europe; Novartis Pharma, Basel, Switzerland) . The goal of this commentary will be to review the rationale for this novel therapeutic approach.

KIT is a M_r 145,000 transmembrane glycoprotein that serves as the receptor for KITLG (also known as stem cell factor, mast cell growth factor, Steel factor) and has intrinsic tyrosine kinase activity. A member of the large family of RTKs, KIT is closely related to the receptors for PDGF, colony stimulating factor 1, and FMS-related tyrosine kinase 3-ligand. Binding of KITLG to KIT results in receptor homodimerization and the activation of tyrosine kinase activity, leading to phosphorylation of a variety of signaling intermediates. In many cases, these substrates are themselves kinases and serve to initiate and/or amplify intracellular signal transduction.

KIT is critical to the development of the ICC, hematopoietic progenitor cells, mast cells, melanocytes, and germ cells. Not surprisingly, many of the KIT-expressing cells in adults are the same cells that have a developmental requirement for normal functional activity of KIT. Specifically, KIT is normally expressed by germ cells, mast cells, ICC, melanocytes, and certain hematopoietic progenitors. KIT is also expressed by a variety of neoplasms, including some neoplasms that arise from cell types that normally express KIT (*e.g.*, ICC→GISTs). In addition, KIT is expressed by a variety of neoplasms, including SCLC, whose putative cells of origin are not developmentally dependent on KIT (3). PDGF(RA), platelet derived growth factor (receptor A).

Evaluating Candidate Diseases for Treatment with KIT Kinase Inhibitors

In general, RTKs can be activated by one of four different mechanisms (Fig. 1). In the case of KIT, the normal mode of activation binding is binding of KITLG. The source of KITLG can be distant (classic hormonal mechanism) or local (paracrine or autocrine). A second mechanism of KIT activation is through intragenic mutations. Such mutations, which are common in GISTs and mast cell tumors, may foster spontaneous ligand-independent receptor homodimerization or directly activate the kinase domain. In theory, KIT could also be activated by genomic amplification resulting in protein overexpression, as is seen with HER2 in breast cancer. This mechanism of KIT activation has not been reported in any human cancer. Finally, KIT could be activated through a chromosomal translocation or other gene rearrangement. Although this has yet to be observed in human cancer, a variety of other RTKs are activated through such mechanisms, which lead to the formation of fusion proteins with high levels of kinase activity (*e.g.*, Ets variant gene 6 (TEL oncogene), FIP1-Like 1-PDGFRA; Refs. 4, 5).

In considering the treatment of a given tumor with a KIT TKI, three key questions must be addressed. First, is there evidence that KIT is activated in the tumor cells? Second, does KIT activation in these cells play a significant role in supporting proliferation and/or survival? Third, can the activated KIT in a tumor be blocked by a given TKI? In the case of GISTs, preclinical studies provided strong affirmative answers to all of these questions. Using IHC techniques, almost all GISTs strongly express KIT. Furthermore, immunoblotting analysis of GIST lysates revealed abundant phosphorylation of KIT, a direct sign of kinase activation. In addition, a high percentage of GISTs harbor intragenic mutations of KIT, and these mutations were shown to be kinase-activating when studied *in vitro*. Finally, KIT TKIs such as imatinib were shown not only to be potent inhibitors of GIST cell growth in culture but also resulted in a dose-dependent induction of apoptosis. These preclinical studies provided a strong foundation for the clinical trials that subsequently demonstrated excellent activity of imatinib against these tumors (6–10).

The challenge in tackling other cancers, particularly high-grade carcinomas such as SCLC, with KIT TKIs lies in developing assays or model systems that can provide insight into the activity (and importance) of KIT in such malignancies. As will be discussed, several approaches are currently being used. The most common, and perhaps least satisfactory, is IHC. In the case of KIT, expression is evident in 95% of GISTs and the majority of these tumors respond to imatinib. But KIT expression is also

Received 10/15/03; accepted 1/30/03.
Grant support: In part, Merit Review Grant from the Department of Veterans Affairs (to M. C. H.).
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[1] The abbreviations used are: SCLC, small cell lung cancer; RTK, receptor tyrosine kinase; KITLG, KIT ligand; ICC, interstitial cell(s) of Cajal; GIST, gastrointestinal stromal tumor; TKI, tyrosine kinase inhibitor; IHC, immunohistochemical; PDGF(RA), platelet derived growth factor (receptor A).

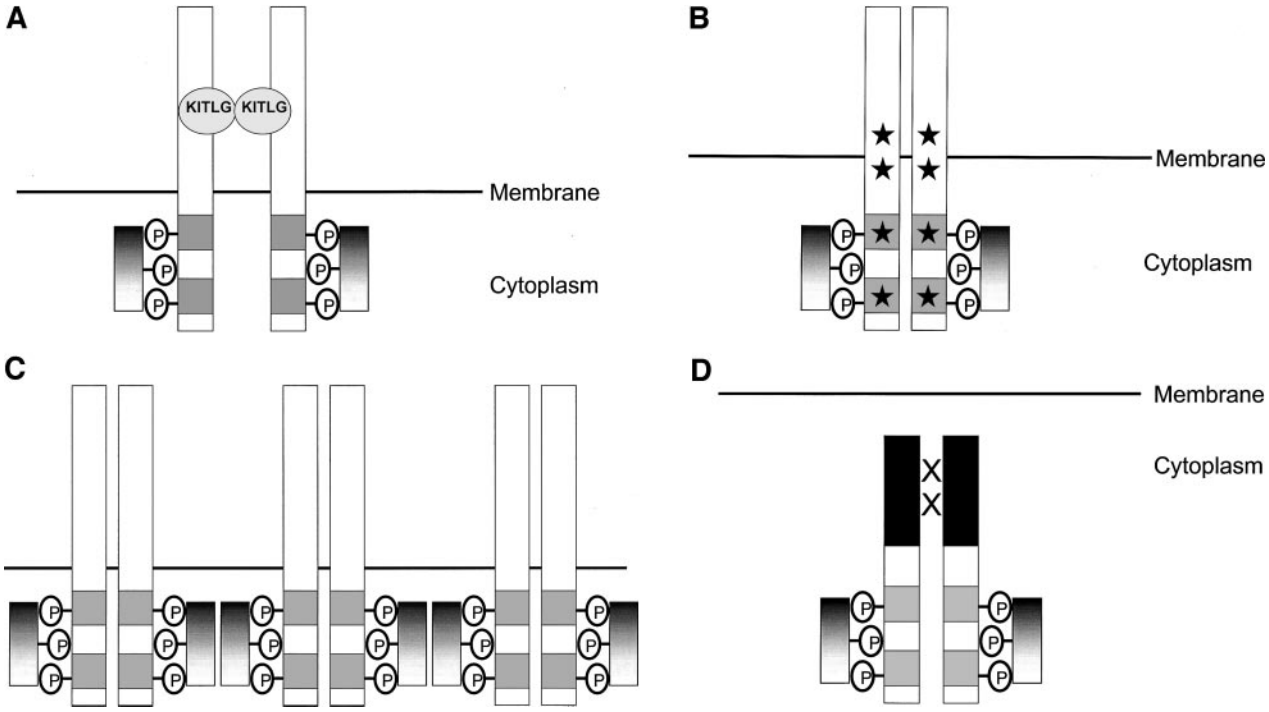

Fig. 1 Molecular mechanisms of KIT activation. *A,* normal ligand-dependent activation. Binding of homodimeric KITLG induces receptor homodimerization with resultant activation of tyrosine kinase activity. KITLG may be produced by distant or local sources. An autocrine source of KITLG has been postulated in some cases of SCLC. *B,* intragenic activating mutations. Activating mutations (point mutations, insertions, or deletions) are common in GISTs and mast cell neoplasms; *stars,* location of these mutations. No intragenic activating mutations of KIT have been found in SCLC. *C,* overexpression. Overexpression due to genomic amplification and/or other mechanisms can result in ligand-independent dimerization because of increased receptor density. This mechanism of activation has not been proven in any human malignancy. *D,* chromosomal translocations resulting in expression of a fusion oncoprotein. The NH_2-terminal domain of the fusion oncoprotein is encoded by a highly expressed gene and contains one or more dimerization/oligomerization domains. The COOH-terminal domain contains the entire tyrosine kinase domain. This mechanism of activation of KIT has not been reported in any human malignancy.

observed in other malignancies, including adenoid cystic carcinoma and melanoma, that are not clinically sensitive to imatinib. Thus, the widely held concept that a positive KIT stain implies imatinib sensitivity is too simplistic. Expression of KIT may reflect the differentiation state of the tumor cell, but this does not necessarily equate with a significant biological role of KIT in tumor homeostasis (3, 11).

Demonstration of KIT activation in tumor cells is a more direct approach. Unfortunately, although studies of KIT expression in different neoplasms abound, evidence for KIT activation in such tumors is largely nonexistent. Currently, studies of KIT activation require the use of fresh or fresh-frozen tumor cells to prepare protein lysates for immunoblotting and/or immunoprecipitation experiments. In the future, the generation of specific reagents for detection of phosphorylated forms of KIT may allow such studies to be performed using formalin-fixed, paraffin-embedded tissue specimen. Regardless of which approach is used, documentation of activated KIT in the tumors of individual patients may be critical in the interpretation of clinical trial outcomes, because heterogeneity may underlie pathological subtypes that are thought to be morphologically and immunophenotypically uniform, including SCLC (3, 10, 12).

The next most stringent standard in assessing KIT as a treatment target is evidence that its activation is pathogenetic and/or critical for tumor maintenance. Genetic, *in vitro*, and animal studies demonstrating the importance of KIT in tumor initiation and/or progression are crucial for selecting the best candidate diseases for treatment with KIT TKIs. *In vitro* and/or *in vivo* studies are particularly important in cases in which the target is not activated by a genomic mechanism and overexpression and/or a ligand-dependent mechanism of activation is the proposed mechanism of activation (see above). For example, KIT activation (autophosphorylation) is found in the vast majority of GISTs, but response rates are dramatically lower for tumors lacking a KIT mutation compared with tumors expressing a mutant KIT kinase (10, 12).

In tumors for which KIT kinase activity is the major determinant of cellular proliferation *and* survival, tumor regressions resulting in objective responses would be expected with KIT TKI treatment. However, if a KIT TKI influenced tumor proliferation but not survival, then the best that could be expected is a cytostatic effect. Furthermore, if KIT kinase activity in a tumor is supportive but not mandatory for tumor cellular proliferation, the observed clinical effect with a TKI would be a delayed time to tumor progression (due to a decrease in tumor growth rate). Therefore, predictions of the likely treatment effect with a given agent should be used in designing clinical studies. It should be noted that the failure of a KIT TKI as

monotherapy does not mean that a given agent is without clinically exploitable biological activity. "Negative" studies must be interpreted carefully to avoid discarding agents or disease indications that can successfully be approached using combination therapy as discussed below.

A final consideration is whether the activated KIT target in a tumor is "druggable." For many targets, suitable compounds for clinical development have yet to be identified. In the case of KIT, not all activating mutations can be inhibited by any given compound. Imatinib, for example, is a potent inhibitor of GIST-associated KIT exon 11 mutations but is inactive against mastocytosis-associated KIT exon 17 mutations (13). In considering candidate diseases for treatment with a KIT TKI, the greatest likelihood of success exists in diseases in which all of the above criteria have been met.

KIT Biology in SCLC. Early studies of KIT expression in SCLC demonstrated a high frequency of coexpression of KIT and KITLG, suggesting the possibility of an autocrine growth loop. Most of these studies used immortalized cell lines and assayed for the presence of *KIT* transcripts rather than actual protein expression (14–16). More recent IHC studies of primary tumors suggest that the frequency of KIT-positive SCLC tumors is much lower, in the range of 25–50% (2, 15, 17–20). In addition, 50% of SCLC cases that were KIT-positive at the time of initial diagnosis were subsequently found to be KIT negative using a postchemotherapy relapse specimen (21). Because of the lack of suitable IHC reagents for KITLG, the actual percentage of SCLC tumors that coexpress KIT and KITLG is unknown.

Evidence for KIT activation in SCLC is largely absent. In SCLC cell lines that putatively coexpress KIT and KITLG, there is no compelling *in vitro* evidence of KIT activation in the absence of exogenous KITLG (22–26). Unexpectedly, a recent xenograft study using NCI-H526 cells found strong evidence of KIT activation *in vivo* even though there was minimal evidence of KIT activation using the same cells in tissue culture experiments (22). Because murine KITLG is 100-fold less active than human KITLG in stimulating KIT, this finding does suggest a tumor-dependent mechanism of activation.

Whether KIT activation is important in SCLC growth and survival remains an open question. There is no evidence for a genomic mechanism of KIT activation in SCLC (17). *In vitro* studies of KIT TKIs and SCLC cell lines have yielded mixed results. In experiments using low-serum conditions, growth dependence on exogenous KITLG can be demonstrated and such ligand-dependent KIT activation can be inhibited using KIT TKIs such as imatinib or SU11248. In contrast, in the presence of "normal" levels of fetal bovine serum (10%), the growth of the same cell lines is KITLG independent, and the dose of KIT TKIs required to inhibit cellular growth is 10–100-fold higher than the IC_{50} for inhibiting KIT activation. Significantly, KIT TKIs induce apoptosis of these same cell lines in low-serum but not normal-serum conditions (22, 24, 26).

In xenograft models, KIT TKIs had a cytostatic rather than a cytotoxic effect on SCLC tumor cells. As noted above, xenografts of NCI-H26 in athymic mice expressed activated KIT. Treatment of tumor-bearing mice with either imatinib or SU11248 partially or completely inhibited KIT activation, respectively. On the other hand, protein lysates from these tumors also had evidence of activated PDGFRB, possibly derived from the tumor stroma. The effect of imatinib or SU11248 on PDGFRB phosphorylation paralleled the effects on KIT activation. Treatment with either agent slowed tumor growth but did not induce tumor regression. Paradoxically, SU11248 had a more profound inhibitory effect on the tumor growth of a KIT-negative SCLC cell line (NCI-H82). Overall, SU11248 was more "active" than imatinib in these experiments, possibly because of effects of SU11248 on other non-imatinib kinase targets such as vascular endothelial growth factor receptors (22). Thus, available xenograft data do not provide proof that the observed slowing of tumor growth with imatinib or SU11248 is actually due to KIT inhibition.

Future Studies of KIT As a Target in SCLC

On the basis of the above criteria, it is unlikely that KIT TKIs will be effective as monotherapy for SCLC. However, targeting KIT might still be of clinical benefit in some cases. Preclinical studies of KIT activation in human tumor specimens as opposed to cell line models would be helpful in strengthening the rationale for such clinical studies. In future clinical studies, it will be crucial to prescreen patients based on KIT expression or, better still, KIT activation. On the basis of the recent report by Rossi *et al.* (21), it may be necessary to assess KIT expression/activation using a current specimen rather than materials obtained at the time of original diagnosis.

As noted above, KIT inhibitors can induce apoptosis in SCLC cells when signaling via other growth factor pathways is dampened by the use of low-serum conditions. Therapeutic targeting of these other pathways in SCLC in combination with a KIT TKI might, therefore, induce apoptosis in addition to inhibiting cellular proliferation. Additional studies are needed to identify other crucial pathways in SCLC for such combined approaches. The utility of this approach is suggested by xenograft experiments in which SU11248, a multitargeted kinase inhibitor, had efficacy that was superior to that of imatinib, which has a more restricted spectrum of activity (22).

Until other growth and survival pathways are identified and effectively targeted, an alternative approach would be to combine KIT TKIs with standard chemotherapy. In xenograft models, the combination of SU11248 and chemotherapy had greater activity than either modality alone (22). Such a combined approach is more likely to result in objective clinical responses than monotherapy with a KIT TKI. The most effective way to gauge the utility of this approach would be to use an increase in the time-to-treatment failure as the primary end point in a clinical study. Given the short progression times that are typically seen in SCLC, an efficacious combined approach would likely give a clear "signal" in an appropriately designed and powered Phase II study.

In summary, the existing evidence does not suggest that KIT TKIs will be effective as monotherapy for SCLC. However, future preclinical and clinical studies targeting KIT in combination with other "targeted" or conventional agents may still lead to improvements in the treatment of this common and all too lethal disease. Development of such therapies may also serve as a paradigm for future clinical use of KIT TKIs in other KIT-positive malignancies.

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