system. For instance, the chemokine receptors CXCR4 and CCR5 interact with gp120, acting as HIV coreceptors and the chemokine CXCL16 interacts with oxidized LDL. Chemokine receptors can also form heterodimers with other chemokine receptors (Thelen et al., 2010) or with other membrane receptors modulating their function. For instance, CXCR2 and CXCR4 interacting with CD74 become functional receptors for the noncanonical chemokine macrophage migration inhibitory factor (MIF), and one could entertain the possibility that PITPNM3 forms a dimer with a conventional signaling GPCR.

Previous studies (Soria and Ben-Baruch, 2008) have shown that levels of the monocyte-attracting chemokines CCL2 and CCL5 are associated with macrophage infiltration and prognosis. Therefore, a comprehensive "chemokinome" system biology perspective may well be required to explore the clinical significance of chemokine levels in cancer.

The results reported here shed new light on the role of chemokines in cancer and raise important questions. There is no mouse counterpart of CCL18 on which to rely for rigorous genetic approaches to investigate its role in carcinogenesis. CCL18 was found in other human tumors, ovarian in particular. The present findings call for an appraisal of its prognostic significance in these pathologies. The actual structure of the CCL18-recognizing receptor complex will also need to be further investigated. Finally, and no less important, chemokine anticancer therapeutic strategies have entered clinical evaluation. In spite of stumbling blocks (e.g., lack of mouse counterpart, drugability of the receptor), CCL18 may be added to the list of potential therapeutic targets.

REFERENCES

Biswas, S.K., and Mantovani, A. (2010). Nat. Immunol. *11*, 889–896.

Chen, J., Yao, Y., Gong, C., Yu, F., Su, S., Chen, J., Liu, B., Deng, H., Wang, F., Lin, L., et al. (2011). Cancer Cell *19*, this issue, 541–555.

DeNardo, D.G., Barreto, J.B., Andreu, P., Vasquez, L., Tawfik, D., Kolhatkar, N., and Coussens, L.M. (2009). Cancer Cell *16*, 91–102.

Kulbe, H., Thompson, R., Wilson, J.L., Robinson, S., Hagemann, T., Fatah, R., Gould, D., Ayhan, A., and Balkwill, F. (2007). Cancer Res. *67*, 585–592.

Mantovani, A., Savino, B., Locati, M., Zammataro, L., Allavena, P., and Bonecchi, R. (2010). Cytokine Growth Factor Rev. *21*, 27–39.

Pollard, J.W. (2004). Nat. Rev. Cancer *4*, 71–78.

Soria, G., and Ben-Baruch, A. (2008). Cancer Lett. *267*, 271–285.

Steidl, C., Lee, T., Shah, S.P., Farinha, P., Han, G., Nayar, T., Delaney, A., Jones, S.J., Iqbal, J., Weisenburger, D.D., et al. (2010). N. Engl. J. Med. *362*, 875–885.

Tan, W., Zhang, W., Strasner, A., Grivennikov, S., Cheng, J.Q., Hoffman, R.M., and Karin, M. (2011). Nature *470*, 548–553.

Thelen, M., Munoz, L.M., Rodriguez-Frade, J.M., and Mellado, M. (2010). Curr. Opin. Pharmacol. *10*, 38–43.

Unswitch-ABL Drugs Overcome Resistance in Chronic Myeloid Leukemia

Leisl Packer[1] and Richard Marais[1,*]
[1]Division of Tumour Biology, The Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, UK
*Correspondence: Richard.Marais@icr.ac.uk
DOI 10.1016/j.ccr.2011.03.019

ABL inhibitors have revolutionized the clinical management of chronic myeloid leukemia, but the BCR-ABL^{T315I} mutation confers resistance to currently approved drugs. Chan et al. show, in this issue of *Cancer Cell*, that "switch-control" inhibitors block BCR-ABL^{T315I} activity by preventing ABL from switching from the inactive to active conformation.

The BCR-ABL fusion protein is the primary driver oncogene in the majority of chronic myeloid leukemias (CML) and also in about 25% of adult acute lymphoblastic leukemias (ALL) (Druker et al., 2001). This protein is expressed from a fusion gene resulting from a reciprocal translocation between chromosome 9 and 22 (t(9;22)(q34;q11)), the so-called Philadelphia chromosome (Ph+). ABL is a tyrosine kinase and its fusion to BCR causes constitutive activation, driving hematopoietic cell transformation through activation of multiple signaling pathways. The treatment of CML was revolutionized by the development of imatinib, a small molecular-weight drug that inhibits ABL and mediates durable hematologic and cytologic responses in CML patients. The importance of imatinib cannot be underestimated, because as the first tyrosine kinase inhibitor (TKI) to achieve responses in cancer patients, it provided a paradigm shift in cancer treatment.

Although imatinib changed the clinical management of CML, some patients eventually fail on therapy due to acquired resistance. Resistance can be mediated by secondary mutations in BCR-ABL that block imatinib binding through steric hindrance or by switching ABL into the active conformation. Like imatinib, the

second generation BCR-ABL inhibitors nilotinib (Weisberg et al., 2007) and dasatinib (Shah et al., 2004) are also ATP-competitive inhibitors but are more potent and so retain binding to the majority of imatinib-resistant BCR-ABL mutants. Further, whereas imatinib only binds to the inactive conformation of ABL (type II inhibition), dasatinib binds both the active (type I inhibition) and inactive conformations, overcoming mutations that stabilize the active conformation.

Nilotinib and dasatinib provide vital second-line treatments for the majority of CML patients who relapse because of point mutations that block imatinib binding to BCR-ABL. However, a relatively common mutant, the T315I or "gatekeeper" mutation, is resistant to all three drugs (Gorre et al., 2001; Shah et al., 2004; Weisberg et al., 2007) because it not only stabilizes the active conformation of ABL to block imatinib and nilotinib binding, but also introduces a steric clash with dasatinib in the ATP pocket. Furthermore, by stabilizing the active conformation of ABL, T315I also mediates resistance to allosteric inhibitors (type III inhibitors) such as GNF2 or GNF5, because although they bind to BCR-ABL^{T315I} they do not block its activity. Thus, T315I represents a persistent clinical problem that accounts for 15%–20% of imatinib-resistant mutants.

To develop novel inhibitors of BCR-ABL^{T315I}, Chan et al. (2011) used structure-based drug design to create compounds that bind to the so-called "switch control" amino acids of ABL. In many kinases, activation is initiated by events such as phosphorylation, but their ability to switch between the inactive and active conformations is controlled by the switch control amino acids, which manage the thermodynamic, electrostatic, and pH changes that are needed to allow amino acid movements within the confines of the kinase domain. Critically, switch control amino acids adopt distinct orientations in the active and inactive conformation of the kinase and "switch-control inhibitors" are designed to stabilize their inactive orientation and lock the kinase in its inactive conformation.

By comparing the active and inactive conformations of ABL and LCK, Chan et al. (2011) identified R386 of ABL as a critical switch control amino acid. With this knowledge, they designed diarylureas to target this region and finally produced DCC-2036. Using structural approaches, Chan et al. show that DCC-2036 locks BCR-ABL in the inactive conformation even when it is phosphorylated on the activation site Y393. More importantly, they show that DCC-2036 also locks BCR-ABL^{T315I} into the inactive conformation. Thus, by targeting the switch pocket, DCC-2036 can overcome resistance that is mediated by mutations that lock ABL in the active conformation and also by those that introduce steric clashes in the ATP-binding pocket.

DCC-2036 is a potent ABL inhibitor (IC_{50} ~0.75 nM), but it does display rather broad activity and inhibits many other kinases, including TIE2, FLT3, and several SRC family kinases. Highly selective inhibitors are extremely valuable for proof-of-principle studies and they satisfy our purist ideas about the importance of inhibiting single targets with individual drugs. However, broad specificity drugs can achieve impressive responses in patients, not least because targeting several kinases simultaneously may mediate additive responses. In this context, the inhibition of SRC family kinases by DCC-2036 may provide unforeseen clinical benefits in CML patients. Furthermore, multikinase inhibitors can show efficacy is several indications, so the broad specificity of DCC-2036 may not be a disadvantage, but its advantages will need to be balanced against the possibility of increased mechanism-based and off-target toxicities.

Chan et al. (2011) show that DCC-2036 inhibits BCR-ABL and blocks phosphorylation of its substrates STAT5 and CrkI in CML, and Ph+ ALL cell lines. In mice, DCC-2036 has excellent oral bioavailability, easily reaching blood plasma levels above its predicted active concentration. It is well tolerated and does not appear to affect normal hematopoietic cells. Critically, it prolongs mouse survival in BCR-ABL^{T315I} CML and ALL models. DCC-2036 is currently undergoing Phase I clinical trials, and preliminary data show that it mediates a striking reduction in phospho-STAT5 in the cells from patients treated with 300 mg. It also mediates a minor reduction in phospho-CrkI in BCR-ABL^{T315I} CML cells in patients treated with 100mg DCC-2036. While it is unclear how these effects will translate to tumor response, its ability to inhibit both "wild-type" and mutant BCR-ABL in patients is extremely promising.

DCC-2036 is not the first drug to show activity against leukemias harboring BCR-ABL^{T315I}. Following promising Phase I results, the potent BCR-ABL and BCR-ABL^{T315I} inhibitor ponatinib (AP24534; O'Hare et al., 2009) is currently undergoing Phase II clinical trials in CML and Ph+ ALL patients who are resistant or intolerant to nilotinib and dasatinib. HG-7-85-01 combines features of imatinib and dasatinib and has anti-BCR-ABL^{T315I} activity, particularly when combined with nilotinib (Weisberg et al., 2010), and although the allosteric inhibitors GNF2 and GNF5 lack intrinsic anti-BCR-ABL^{T315I} activity, at high concentrations they are active when combined with nilotinib (Zhang et al., 2010).

These continue to be exciting times in CML research, and it seems probable that one or more of these third-generation drugs will show clinical efficacy against BCR-ABL^{T315I}. An optimist may wonder if the final chapter of the CML story is now being written, but this seems unlikely. The last decade has shown that CML is extraordinarily adaptable and appears to escape new treatments with relative ease.

It may therefore be pertinent to consider how the cells will escape drugs such as DCC-2036. Clues to the answer may already be available. Although rare, compound BCR-ABL mutants harboring more than one point mutation have been described in patients sequentially treated with ABL inhibitors (Shah et al., 2007). Where these include the T315I mutation, the compound mutants exhibit greater resistance to TKIs than the T315I single mutation. It would be interesting to know how sensitive these compound mutants are to DCC-2036. It should also be remembered that only 60% of CML resistance is mediated by BCR-ABL-dependent mechanisms, so resistance to switch control drugs may generally be driven by BCR-ABL-independent mechanisms. Again, it would be interesting to test this broad specificity drug against cells whose resistance is BCR-ABL independent. Presumably, if resistance is mediated by DCC-2036 targets such as the SRC family kinases (Donato et al., 2003), sensitivity will be retained. However, other mechanisms may provide resistance, such as mutations in the switch control amino acids. Finally, it remains unclear if any of these

compounds target the stem cell population effectively. This is important because these cells are thought to be the source of the residual disease that remains even after long-term drug treatment.

If it lives up to its promise, DCC-2036 will play a role in the CML and AML stories. Regardless of its final contribution to the clinical management of these diseases, switch control drugs are certainly a very elegant solution to the BCR-ABL^{T315I} problem.

REFERENCES

Chan, W.W., Wise, S.C., Kaufman, M.D., Ahn, Y.M., Ensinger, C.L., Haack, T., Hood, M.M., Jones, J., Lord, J.W., Lu, W.P., et al. (2011). Cancer Cell *19*, this issue, 556–568.

Donato, N.J., Wu, J.Y., Stapley, J., Gallick, G., Lin, H., Arlinghaus, R., and Talpaz, M. (2003). Blood *101*, 690–698.

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

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

O'Hare, T., Shakespeare, W.C., Zhu, X., Eide, C.A., Rivera, V.M., Wang, F., Adrian, L.T., Zhou, T., Huang, W.S., Xu, Q., et al. (2009). Cancer Cell *16*, 401–412.

Shah, N.P., Skaggs, B.J., Branford, S., Hughes, T.P., Nicoll, J.M., Paquette, R.L., and Sawyers, C.L. (2007). J. Clin. Invest. *117*, 2562–2569.

Shah, N.P., Tran, C., Lee, F.Y., Chen, P., Norris, D., and Sawyers, C.L. (2004). Science *305*, 399–401.

Weisberg, E., Manley, P.W., Cowan-Jacob, S.W., Hochhaus, A., and Griffin, J.D. (2007). Nat. Rev. Cancer *7*, 345–356.

Weisberg, E., Choi, H.G., Ray, A., Barrett, R., Zhang, J., Sim, T., Zhou, W., Seeliger, M., Cameron, M., Azam, M., et al. (2010). Blood *115*, 4206–4216.

Zhang, J., Adrián, F.J., Jahnke, W., Cowan-Jacob, S.W., Li, A.G., Iacob, R.E., Sim, T., Powers, J., Dierks, C., Sun, F., et al. (2010). Nature *463*, 501–506.

Resisting Targeted Therapy: Fifty Ways to Leave Your EGFR

Paul Workman[1,*] and Paul A. Clarke[1]
[1]Signal Transduction and Molecular Pharmacology Team, Cancer Research UK Cancer Therapeutics Unit, Division of Cancer Therapeutics, The Institute of Cancer Research, Haddow Laboratories, 15 Cotswold Road, Sutton SM2 5NG, UK
*Correspondence: Paul.workman@icr.ac.uk
DOI 10.1016/j.ccr.2011.03.020

Despite the promise of the new generation of molecularly targeted drugs, intrinsic and acquired resistance is proving to be as problematic as with cytotoxic drugs. Two recent papers have identified novel ways by which non-small cell lung cancers can exhibit resistance to EGFR inhibitors and suggest new therapeutic workarounds.

Paul Simon famously sang about the fifty ways to leave your lover. Two recent studies by Sequist et al. (2011) and Bivona et al. (2011) have revealed several additional new ways by which non-small cell lung cancers (NSCLC) can escape the clutches of small molecule inhibitors of the epidermal growth factor receptor (EGFR) tyrosine kinase. Whether there will turn out to be fifty, or even more mechanisms through which resistance can be mediated—either de novo or acquired during treatment—remains to be seen. What is clear is that there are a growing number of ingenious molecular means by which cancers can circumvent inhibitors of EGFR and other oncogenic kinases. Although representing therapeutic challenges to the clinician, these mechanisms also suggest rational new therapeutic opportunities to improve clinical outcomes. Furthermore, by using kinase inhibitors as chemical probes (Workman and Collins, 2010) to interrogate human cancers, we are gaining considerable fundamental as well as translational insights into the diverse mechanisms of human oncogenesis.

The extraordinary success of imatinib in prolonging the lives of patients with chronic myeloid leukemia (CML) through the inhibition of the pathogenic tyrosine kinase activity of BCR-ABL has had a major scientific impact in validating the concept of single kinase addiction in the clinic (Druker et al., 2006). Furthermore, the principle of treating such oncogene addiction (Weinstein, 2002) with inhibitors of the respective major driver kinases has proved to be applicable to many other types of cancer. On the other hand, the tumor regression and prolonged survival obtained are commonly not as sustained as in CML (Sawyers, 2009).

NSCLC is a leading cause of death worldwide (www.who.int/cancer/en/, http://globocan.iarc.fr/factsheets/populations/factsheet.asp?uno=900), and cytotoxic chemotherapy has limited effectiveness. Approximately 10% of NSCLCs in western populations harbor somatic mutations in exons encoding the tyrosine kinase domain of EGFR, and these occur with an increased frequency in adenocarcinomas arising in nonsmokers, females, and individuals of Asian ethnicity. These mutations cause activated signaling