

GATA-1–regulated genes has not been described. Thus, the findings of Elagib et al could provide new insight into the pathogenesis and, conceivably, the treatment of this human myeloproliferative disorder.

*Conflict-of-interest disclosure: The author declares no competing financial interests.* ■

● ● ● NEOPLASIA

Comment on Hallaert et al, page 5141

## Dis-Abl-ing CD40 buys toxic assets

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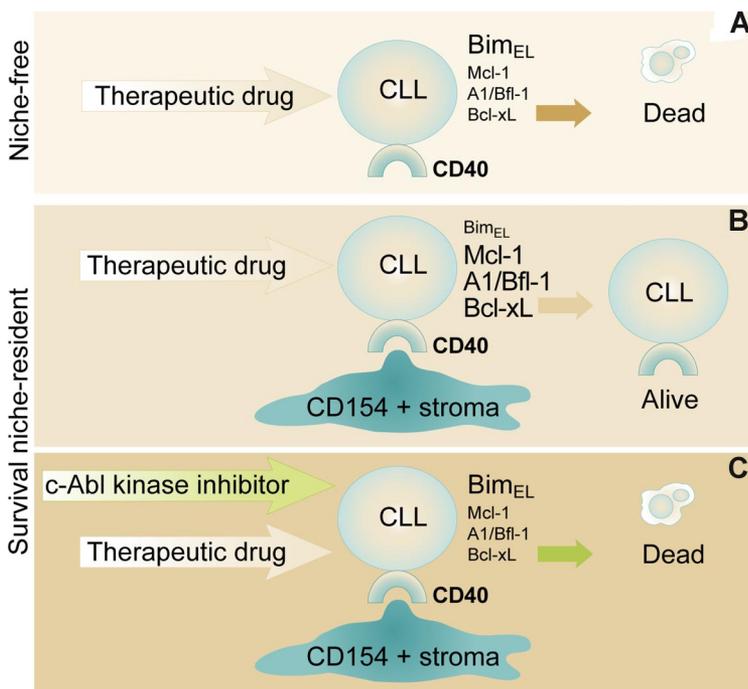
In this issue of *Blood*, Hallaert et al show that clinically approved *c-Abl* inhibitors reverse the protection against conventional chemotherapeutic drugs afforded to CLL cells from prolonged CD40 signaling.

Chronic lymphocytic leukemia (CLL), though well understood molecularly and functionally, essentially remains incurable. In vivo survival niches probably contribute to a surprisingly poor performance from drugs that seem very promising in vitro. Bone marrow stroma, blood-borne nerselike cells, and lymph node follicular dendritic cells are all culprits in the conspiracy.<sup>1</sup> At the heart of

### REFERENCES

1. Price DH. P-TEFb, a cyclin-dependent kinase controlling elongation by RNA polymerase II. *Mol Cell Biol.* 2000; 20:2629-2634.
2. Peterlin BM, Price DH. Controlling the elongation phase of transcription with P-TEFb. *Mol Cell.* 2006; 23:297-305.
3. Price DH. Poised polymerases: on your mark... get set...go! *Mol Cell.* 2008;30:7-10.

their operations lies the induction of antiapoptotic proteins, including the “usual suspects” of Mcl-1, Bcl-2, and Bcl-xL. One avenue of attack is to target the survival proteins directly as recently described using BH3-neutralizing AT-101.<sup>2</sup> In this issue of *Blood*, Hallaert et al have masterminded an alternative strategy that interrupts the signaling processes that promote CLL protection. To guide them toward



(A) CD40<sup>+</sup> CLL cells free of a survival niche (eg, in the circulation) constitutively expressing high levels of proapoptotic Bim-EL and low antiapoptotic Mcl-1, A1/BFI-1, and Bcl-xL successfully killed by therapeutic drugs. (B) Same cells receiving survival signals (here via CD154/stroma) down-regulate Bim-EL, up-regulate Mcl-1, A1/BFI-1, and Bcl-xL and are rendered resistant to therapeutic drugs. (C) Niche-protected CLL cells exposed to *c-Abl* kinase inhibitors reboot to niche-free levels of apoptosis regulators while restoring sensitivity to therapeutic drugs. Professional illustration by Paulette Dennis.

the guilty pathway, a neat piece of detective work was employed.

First, they constructed a survival niche: CD154-transfected fibroblasts furnishing both CD40 stimulation and a range of stromal factors. CD40 ligation affords, in all manner of B cells, substantial protection to both default

and deliberately provoked apoptosis, and CLL is no exception. In addition to confirming previously described CD40-driven antiapoptotic changes in CLL populations, the authors identified for the first time a reduction in the proapoptotic protein Bim-EL. It was known from other systems that ERK-signaling could alter levels of Bim-EL protein, and they went on to show that not only did CD154 stimulate ERK phosphorylation in CLL cells, but also that inhibiting ERK activity negated the ensuing Bim-EL decline. However, ERK proved an innocent bystander when considering resistance to therapeutic drugs. Thus, even with ERK incapacitated, fludarabine, bortezomib, and others were all powerless to outmaneuver the defenses bolstered by CD40 and stroma.

The authors next trained the spotlight on the increased Mcl-1 protein expression they had noted. This is when *c-Abl* began to audition for the leading role. Already a star of the Philadelphia chromosome, survival signaling via dysregulated *Abl* underpins pathobiology in chronic myeloid leukemia (CML). Not only Mcl-1 but also other antiapoptotic signatures from niche-protected CLL cells conformed to those emanating constitutively from BCR-*Abl*. With all the pieces now so elegantly in place, it does not require a Lieutenant Columbo to pronounce the denouement: *c-Abl* inhibitors used so successfully for treating CML likewise overcome conferred resistance to underperforming therapeutic drugs in CLL. Both widely used imatinib (gleevec) and even more so second-generation dasatinib (sprycel) rebooted CLL cells to drug sensitivity. This desired outcome was accompanied by a requisite reversal of the antiapoptotic profile imprinted by the survival niche. Neither IgV<sub>H</sub> mutational status nor p53 dysfunction tempered the success. As an added bonus, the authors were able to recapitulate findings on blood CLL cells with samples from lymph node biopsies.

This is an exciting advance. The data are persuasive and internally consistent. Questions do remain but not necessarily of a quality to warrant concern with respect to therapeutic options. The authors themselves confess to their model being “supraphysiological.” While B cells are constitutively programmed for CD154 receptivity, the opportunity for ligand

delivery is, by necessity, fleeting. It would be interesting to learn the impact of short-term, low-level CD154 exposure on the outcomes monitored.<sup>3</sup> Moreover, evidence for CD154 majorly contributing to in situ CLL survival is not convincing. Once more, reappraisal of the system, here replacing the transfected mouse fibroblasts with more natural accessory support, would be informative. The authors are equally open with respect to the targets of the “c-Abl inhibitors” within the context of CLL. Rationally designed to target the tyrosine kinase activity constitutive to BCR–Abl, imatinib and dasatinib are by no means Abl-specific. Though leaving open a mechanism-of-action in this new setting, the efficacy of these compounds (at least in vitro) remains.

Defeating the complicity between hijacked extrinsic and autonomous intrinsic forces that currently limit therapeutic benefit in CLL may now be a step nearer.

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#### REFERENCES

1. Caligaris-Cappio F, Ghia P. Novel insights in chronic lymphocytic leukemia: are we getting closer to understanding the pathogenesis of the disease? *J Clin Oncol*. 2008;26:4497–4503.
2. Balakrishnan K, Burger JA, Wierda WG, Gandhi V. AT-101 induces apoptosis in CLL B-cells and overcomes stromal cell-mediated Mcl-1 induction and drug resistance. *Blood*. Prepublished on October 3, 2008, as DOI 10.1182/blood-2008-02-138560.
3. Stewart R, Wei W, Challa A, et al. CD154 tone sets the signalling pathways and transcriptome generated in model CD40-pluricompetent L3055 Burkitt's lymphoma cells. *J Immunol*. 2007;179:2705–2712.

#### ● ● ● PHAGOCYTES

Comment on Anderson et al, page 5202

## PI3K and NADPH oxidase: a class act

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In this issue of *Blood*, Anderson and colleagues show that class III PI3K is essential for CD18-dependent activation of the NADPH oxidase, and production of ROS mediated by this phagocytosis pathway does not require antibody or FcγR for the subsequent synthesis of PtdIns(3)P at phagosome membranes.

Phagocytosis of microorganisms by neutrophils and other phagocytic leukocytes is mediated in part by receptors for opsonins, such as the complement receptor, CD11b/CD18, and/or those specific for antibody Fc (eg, FcγR receptors). Ingestion of microbes triggers generation of reactive oxygen species (ROS), which are important for the antibacterial and antifungal activity of phagocytes. ROS are produced by the NADPH oxidase, a multi-component enzyme that assembles at the plasma or phagosome membrane. In resting phagocytes, the redox component of NADPH oxidase, flavocytochrome *b*<sub>558</sub>, resides in the plasma membrane and membranes of granules, whereas other proteins of the enzyme complex, the GTPase–Rac, p47<sup>phox</sup>, p67<sup>phox</sup>, and p40<sup>phox</sup>, are present in the cytosol.<sup>1</sup> During phagocytosis, the cytosolic components translocate to the membrane where they assemble with flavocytochrome *b*<sub>558</sub> (composed of gp91<sup>phox</sup> and p22<sup>phox</sup>) to form the active NADPH oxidase.<sup>1</sup> Translocation of cytosolic components is highly regulated and involves

multiple SH3-domain interactions and interaction of *phox* homology domains (PX) with phosphoinositides.<sup>1–3</sup> Defects in gp91<sup>phox</sup>, p22<sup>phox</sup>, p47<sup>phox</sup>, or p67<sup>phox</sup> cause chronic granulomatous disease, a rare hereditary disorder characterized by recurrent bacterial and fungal infections due to the inability of leukocytes to produce ROS.<sup>1</sup> A critical role for p40<sup>phox</sup> in NADPH oxidase function has remained elusive, but recent work indicates that p40<sup>phox</sup> is essential for optimal killing of bacteria by neutrophils.<sup>1,4</sup>

Phosphatidylinositol 3-kinases (PI3Ks) are important for signal transduction events, leading to the production of ROS following FcγR phagocytosis.<sup>2,3,5–8</sup> class I and class III PI3Ks have unique functional and temporal activities within phagocytic leukocytes and their individual roles in phagocytosis and NADPH oxidase assembly/activation are currently an intense area of investigation. class I PI3Ks, which generate PtdIns(3,4,5)P<sub>3</sub> and PtdIns(3,4)P<sub>2</sub>, are necessary for formation of phagosomes.<sup>7,8</sup> Recent studies indicate that

the PtdIns(3)P generated by class III PI3K contributes to phagosome maturation, and importantly, the binding of PtdIns(3)P to p40<sup>phox</sup> regulates NADPH oxidase activation during phagocytosis.<sup>2,5</sup> Although PI3Ks are known to play a key role in NADPH oxidase activation during FcγR-mediated phagocytosis, there is little or no information bearing on the role of complement- or nonantibody receptors in this process.

A new study by Anderson et al reveals that class III PI3K and PtdIns(3)P are essential for activation of the NADPH oxidase during CD18-mediated phagocytosis. The authors use mice lacking CD18 or antibody to show that ROS production following phagocytosis of serum-opsonized *Staphylococcus aureus* or *Escherichia coli* is dependent on serum complement and CD18 rather than antibody and FcγRs. Human or mouse neutrophils treated with wortmannin, a general PI3K inhibitor, had significantly reduced capacity to produce ROS following phagocytosis of opsonized *S aureus* or *E coli*. By comparison, inhibitors specific for class I PI3Ks failed to block ROS production under these conditions, and the findings were confirmed by assays with neutrophils from mice genetically deficient in class I or class II PI3Ks in which ROS generation was normal. Collectively, these results indicate that class I or class II PI3Ks do not contribute to production of ROS following complement receptor-mediated phagocytosis. Unlike class I or class II PI3Ks, there is no mouse knockout model for the single eukaryotic class III PI3K (known as Vps34). To circumvent this problem, the authors use an RNA interference approach to demonstrate that class III PI3K regulates accumulation of PtdIns(3)P on bacterial phagosomes and CD18-dependent ROS production in macrophage-like cell lines. These results led to a final series of experiments from which the authors conclude that there are probably additional p40<sup>phox</sup> PX-domain-independent roles for PtdIns(3)P in the regulation of phagosomal ROS generation.

The study is the first to provide a direct link between PI3Ks and ROS production following complement receptor-mediated phagocytosis. It is interesting that the phagocytic particle, eg, IgG-opsonized particles versus serum-opsonized bacteria, appears to dictate whether class I or class III PI3K is involved in phagocytosis and subsequent



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