the primary tumor, which suggests that CLV dilation may have a far more central role in the metastatic process than hitherto appreciated. Interestingly, Etodolac also diminished metastatic burden in the lung. These results suggest that a level of control over the lymphatic and systemic dissemination could potentially be achieved by administration of relatively safe anti-inflammatory agents.

This provocative study adds an important dimension to the process that might be viewed as vascular system "conditioning" for cancer metastasis. While the focus of the present study is on CLV dilation, others observed lymphangiogenesis within lymph nodes prior to their metastatic colonization (Tobler and Detmar, 2006), a process that may be attributed to remote influences of growth factors or exosomes (Hood et al., 2011). Analogous pre-metastatic niches were also described at sites of blood borne metastases (Kaplan et al., 2005).

The enlargement of macroscopic vessels located outside of a growing tumor is not restricted to CLVs. Similar increases in diameter are often observed in the case of blood vessels that supply tumor microcirculation (feeding arteries and collecting veins), which is also apparent from some of the images included in the study by Karnezis et al., (2012). Although this is a commonly observed phenomenon, the underlying biological process has thus far attracted minimal attention (Yu and Rak, 2003). In contrast to angiogenesis, which occurs at the level of microscopic capillaries (Carmeliet and Jain, 2011), formation of larger tumor-feeding blood vessels may involve such mechanisms as dilation, similar to that occurring in CLVs, or circumferential growth ("tumor arteriogenesis") (Yu and Rak, 2003). Whether such macroscopic changes control tumor microenvironment, growth, or hematogenous metastasis (by analogy to CLVs) remains to be studied.

The novel and fascinating link between CLV dilation and lymphatic metastasis described by these authors raises several important questions. For example, how does CLV dilation promote metastasis? Is this merely a wider conduit ("plumbing") effect, or does it involve more subtle regulatory mechanisms (e.g., tumor-LEC interactions)? Since the VEGF-D-induced increase in prostaglandin levels is detected in peripheral blood, could such a change be indicative of impending lymphatic metastasis in the clinic? How early in progression of human cancers would increase in prostaglandins occur, and how discrete, how detectable, would this event be? What systemic consequences may be associated with VEGF-D-induced increase in prostaglandins in blood, e.g., for the vascular system? What turns on lymphangiogenic growth factors in metastatic cancers, and is there a link between oncogenic pathways and CLV dilation?

It is fascinating to think that a pharmacological blockade of the pathological CLV dilation and metastasis could be achieved with already available agents (VEGF/VEGFR3/2 inhibitors and NSAIDs). However, one wonders whether such treatment could interfere with the lymph outflow from the primary tumor mass leading to a build up of interstitial fluid pressure (IFP)? Increase in IFP has been linked to impaired drug delivery and could result in vascular compression, hypoxia, and perhaps in hematogenous metastasis. It is unclear if any of these effects might accompany therapeutic interference with CLV dilation. Indeed, the work of Karnezis et al., (2012) opens up several new lines of inquiry and a new domain in the field of lymphangiogenesis and cancer progression.

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aSIRTing Control over Cancer Stem Cells

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Cancer stem cells lie at the root of chronic myelogenous leukemia (CML) and mediate its continued growth. Their resistance to current therapies results in an inability to eradicate the disease. In this issue of *Cancer Cell*, Li et al. identify SIRT1 as a new target for eliminating CML cancer stem cells.

Chronic myelogenous leukemia (CML) is a cancer that begins in hematopoietic stem cells. Triggered by the BCR-ABL translocation (Melo and Barnes, 2007), additional mutations can induce its progression from a slow-growing chronic

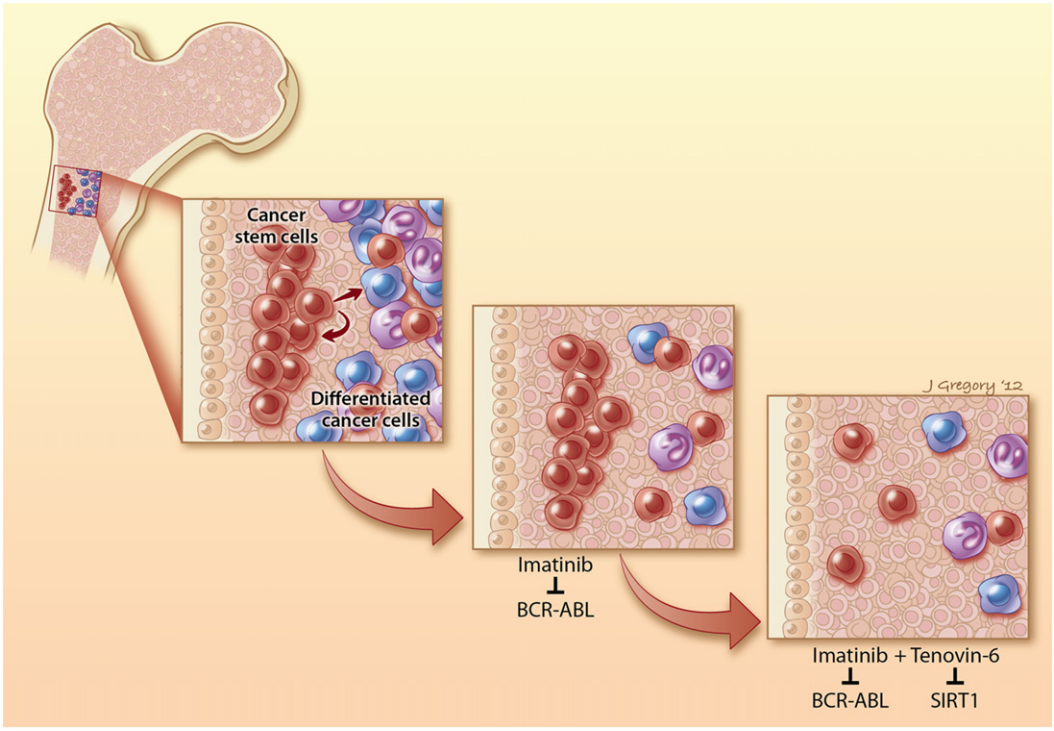

Figure 1. SIRT1 Inhibition Effectively Targets CML Cancer Stem Cells
Chronic myelogenous leukemia (CML) is composed of differentiated cells (blue and purple) as well as a more primitive pool of cancer stem cells (red) that have the capacity to propagate the disease (left). The kinase inhibitor Imatinib can eliminate differentiated CML cells but cannot effectively target cancer stem cells (middle). Though insensitive to Imatinib, cancer stem cells remain dependent on SIRT1. Thus, the combined use of the SIRT1 inhibitor Tenovin 6 and Imatinib effectively removes residual cancer stem cells and may block CML at its root (right).

phase to a more aggressive and undifferentiated blast crisis phase. The discovery of the kinase inhibitor imatinib mesylate revolutionized the treatment of CML. Over the years, however, it has become clear that while kinase inhibitors can hold CML at bay, they are unable to eradicate the disease, leading to a life-long dependence on the drug and an increased risk of relapse and progression. In addition, kinase inhibitors are ineffective against drug-resistant and advanced stage disease. Although such patients may not form a large group in developed countries, the global face of CML is very different, and many patients are not diagnosed until the disease is at an advanced stage. Insight into the limitations of targeted kinase therapy came from an understanding that CML is composed of differentiated cells as well as a more undifferentiated pool of cancer stem cells that have the capacity to propagate the disease (Wang et al., 1998). Emerging evidence suggests that differentiated CML cells are addicted to ABL and can be eliminated by kinase inhibitors, while cancer stem cells can become ABL independent and thus persist despite therapy (Graham et al., 2002; Corbin et al., 2011). Thus, identifying regulators that are required for CML cancer stem cell growth and renewal is critical for effectively targeting the disease. In this issue of *Cancer Cell*, Li et al. (2012) identify SIRT1, the founding member of the Sirtuin family of proteins, as an exciting new target for eradicating CML cancer stem cells and thereby stopping CML growth.

Sirtuins, mammalian homologs of the yeast protein silent information regulator 2, represent a unique subclass of histone deacetylases; their substrates can include both histones and non histone proteins, and unlike other HDACs, they act in an NAD-dependent manner (Haigis and Sinclair, 2010). Sirtuins exert a powerful influence on a wide array of cellular processes including DNA repair, cell survival, metabolism, and aging in diverse organisms (Haigis and Sinclair, 2010).

In this study, the authors use a combination of genetically engineered mouse models and primary leukemia xenografts to assess the role of SIRT1 in mouse and human CML growth. The authors first examined the expression of SIRT1 in normal and CML cells, focusing on the stem cell enriched $CD34^+$ population. SIRT1 was expressed at higher levels in human CML $CD34^+$ cells than in normal $CD34^+$ cells. Moreover, knockdown of SIRT1 in $CD34^+$ CML cells led to reduced proliferation, enhanced apoptosis, and impaired colony-forming ability. Importantly, SIRT1 knockdown had less of an effect on proliferation and apoptosis of normal $CD34^+$ cells, suggesting that CML and normal stem cells display a differential dependence on SIRT1. Further, the combined use of SIRT1 inhibition together with imatinib led to an increase in cell death, suggesting that suppression of SIRT1 could cooperate with imatinib to more effectively block CML stem cells (Figure 1).

To test if the dependence of CML on SIRT1 could be useful in a therapeutic context, the authors used the small molecule Tenovin 6 (TV-6), which blocks the activity of sirtuin family proteins (Lain

et al., 2008). In vitro treatment with TV-6, and to a greater extent with TV-6 and imatinib, reduced colony formation and in vivo engraftment more effectively than imatinib alone, highlighting the potential utility of SIRT inhibition in the context of combination therapy.

While the experiments involving ex vivo exposure suggested that pharmacologic blockade of SIRT1 was effective against CML, it was critical to assess whether the drug could affect disease in a physiological context. To test this, the group isolated leukemic cells from an inducible BCR-ABL transgenic mouse and transplanted them into irradiated recipients. These mice were subsequently treated with imatinib, TV-6, or the combination daily for 21 days. Although imatinib alone impaired leukemia growth, it failed to target CML stem cells. In contrast, TV-6 alone, and to a greater extent TV-6 and imatinib, led to a very significant loss of CML stem cells. Consistent with this, mice treated with the combination showed improved survival, with reduced numbers of residual leukemic cells in the bone marrow after discontinuation of treatment. Although the changes in survival were perhaps not as dramatic as the drop in cancer stem cell content may have predicted, it is important to note that the drug was discontinued after 3 weeks; thus, continued treatment, modified dosing or the use of alternate inhibitors might show further benefits in vivo. In a key experiment, the authors also tested the effect of TV-6 on mice xenografted with an imatinib-resistant blast crisis CML patient sample and found that it led to a significant reduction in engraftment at multiple sites of leukemia growth. This suggests that targeting SIRT1 may be effective against both chronic phase and in imatinib-resistant advanced stage disease. More broadly, this work identifies Sirtuins as an important control point for cancer stem cells and provides a strong rationale for considering SIRT1 inhibitors for treatment of myeloid leukemias and perhaps other malignancies that display activation of this pathway.

How does SIRT1 inhibition eliminate CML cancer stem cells? SIRT1 has previously been shown to deacetylate p53 and thereby regulate its transcriptional activity (Haigis and Sinclair, 2010). In support of this notion, SIRT1 inhibition elevated acetylated and total p53 levels in both chronic and blast crisis phase CML $CD34^+$ cells, triggering a rise in p53 target genes. Loss of function studies indicated that TV-6 depends on p53 to affect CML, consistent with the fact that p53 activation can effectively target CML. This suggests that consideration of SIRT1 as a target should take into account a patient's p53 status, since the 30% of blast crisis patients whose disease display p53 mutations are unlikely to respond to this strategy (Melo and Barnes, 2007).

In the last few years, basic and translational work has identified several pathways that are critical for CML stem cell function and renewal, including promyelocytic leukemia protein (PML), β-catenin, Alox5, and Smoothened (reviewed in Chen et al., 2010). These studies shed light on the molecular mechanisms that protect and sustain CML cancer stem cells, allowing them to evade imatinib. Some have been of immediate translational interest because they can be readily targeted; this is true in particular for PML and Smoothened, which can be inhibited by arsenic trioxide and by Hedgehog pathway antagonists (Dierks et al., 2008; Ito et al., 2008; Zhao et al., 2009). Both strategies are currently being tested in trials of myeloid leukemia, and it will be of great interest to see how effective and durable they turn out to be. But considering the fact that kinase inhibitors can hold CML at bay in many patients, the bar for a new therapeutic in this disease may be high. At this stage, it is not unreasonable to hope for eradication of residual cancer stem cells and an ability to discontinue therapy without relapse. Perhaps the blockade of SIRT1 will allow us to finally assert control over CML cancer stem cells and accelerate progress toward this goal.

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