role of sexual transmission and microcephaly. Ultimately, one of the most useful applications of this model would be to screen antiviral therapies and vaccines with the goal of stopping or lessening maternal-fetal ZIKV transmission. Pregnancy produces a unique challenge for some of the aforementioned reasons, and drugs that work effectively in nonpregnant adults might be contraindicated for a pregnant female. Therefore, the generation of models specifically tailored to look at fetal outcomes is an absolutely critical step in the development of therapies to protect against the most vulnerable populations at risk.

It is likely that the murine model will not be suitable for studying every aspect of disease, and so additional models will be needed. Nonhuman primate models will likely produce the most relevant data since the anatomy more closely mimics that of a human's. Both adult and pregnancy primate models are currently under way. Several groups, including those at UC Davis and the University of Wisconsin, have begun studies to address these concerns. Real-time data reporting can be found online (zika.labkey.com) and more traditional manuscripts will follow. These multipronged approaches are essential to understanding the natural history and pathogenesis of ZIKV infection and the evaluation of candidate vaccines and antivirals in a timely manner.

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Eliminating Cancer Stem Cells in CML with Combination Transcriptional Therapy

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Leukemia stem cells (LSCs) are resistant to current therapies used to treat chronic myeloid leukemia (CML). Abraham et al. (2016) have identified a molecular network critical for CML LSC survival and propose that simultaneously targeting two of their major transcriptional regulators, p53 and c-Myc, may facilitate their eradication.

Chronic myeloid leukemia (CML) is a lethal hematopoietic malignancy characterized by the fusion of the ABL proto-oncogene 1 (ABL1) and the breakpoint cluster region (BCR) genes, which results in the expression of a constitutively active protein tyrosine kinase—the BCR-ABL1 oncoprotein. In the past 15 years, tyrosine kinase inhibitors (TKIs) such as Imatinib (Gleevec), Nilotinib, and Dasatinib have been introduced and used with great success as the standard of care for patients with CML (Jabbour and Kantarjian, 2014). However, discontinuation of TKI therapy results in disease recurrence in more than 50% of patients (Jabbour and Kantarjian, 2014; Mahon et al., 2010). Therefore, in order to achieve durable remissions, CML patients require continuous treatment with TKIs, increasing their risk of developing resistance, harmful side effects due to drug toxicity, and the associated expenses of long-term treatment. In recent years, it has become increasingly clear that TKIs are not effective at killing the leukemic stem cells (LSCs) in CML, which are the malignant cells responsible for maintaining the disease (Chu et al., 2011; Corbin et al., 2011). Therefore, a true "curative" treatment for CML remains elusive, and a better understanding of the molecular mechanisms regulating LSC survival is imperative for

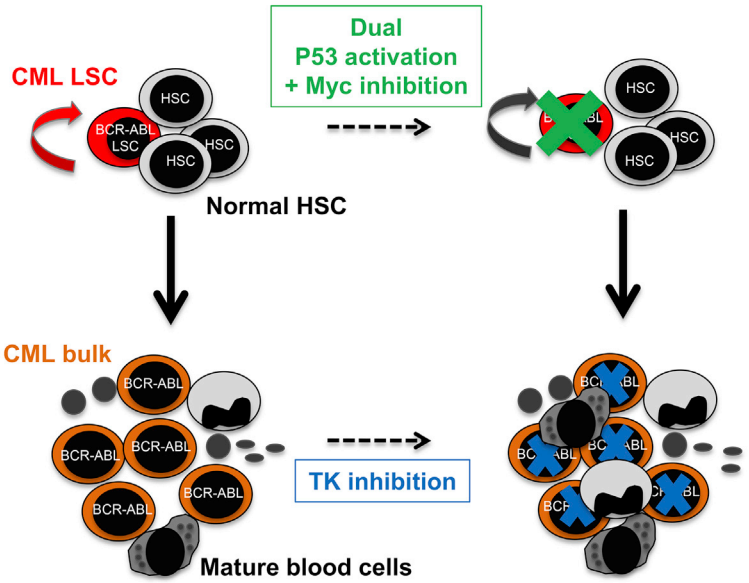

Figure 1. Schematics of Combinatorial Targeting of p53 and c-Myc as a Therapeutic Approach to Eliminate Cancer Stem Cells in Chronic Myeloid Leukemia

Tyrosine kinase inhibitors (TKIs) (such as Imatinib [Gleevec], Dasatinib, and Nilotinib) have been used with great success as the standard of care for patients with Chronic Myeloid Leukemia (CML). However, a number of reports suggest that while TKIs are very effective in targeting the more mature bulk cell population in CML, they are not effective at eradicating the cancer/leukemia stem cells (LSCs) responsible for initiation, maintenance, and relapse of the disease. Abraham et al. (2016) propose a therapeutic approach to eliminate CML LSCs by simultaneously targeting two major perturbed transcriptional networks. Activation of p53 (e.g., by HDM2 inhibitors) and simultaneous inhibition of c-Myc (e.g., by BET inhibitors) led to inhibition of CML LSCs but had no measurable effects on normal HSCs. Such a therapeutic approach at the stem cell level (upper panels) could complement TKI-mediated inhibition and disease control of the CML bulk (lower panels).

developing more effective targeted strategies that will eliminate TKI-resistant LSCs in CML.

Abraham and colleagues sought out to identify potential therapeutic targets in CML by using an unbiased systems biology approach. They combined proteomics, transcriptomics, and network analyses to identify aberrantly expressed protein networks critically important for LSC survival in primary CML patient samples. This approach revealed a unique protein network regulated by the tumor suppressor p53 and the oncogene c-Myc in CML LSCs. While both p53 and c-Myc have well-characterized roles in CML and other human cancers (Li et al., 2012; Reavie et al., 2013), combinatorial targeting of these pathways has yet to be considered as a therapeutic approach. Therefore, the authors asked whether dual activation of the p53 pathway and inhibition of c-Myc could lead to a viable and selective therapeutic strategy to eliminate LSCs in CML (Figure 1). To ascertain the effects of p53 activation and c-Myc inhibition, CML CD34+ cells were transduced with shRNA lentiviral constructs targeting HDM2 (a p53 E3 ubiquitin ligase), c-Myc, or both. Dual modulation of p53 and c-Myc resulted in a synergistic decrease in viability, increased apoptosis, and decreased colony formation, suggesting that both pathways combined are critical for CML cellular survival. These findings were recapitulated using small molecules such as RITA, which stabilizes p53 by preventing its degradation, and CPI-203, a BET bromodomain inhibitor that targets c-Myc. Interestingly, treatment with RITA alone resulted in increased apoptosis and decreased cell viability; CPI-203 primarily induced differentiation of CML CD34+ cells, suggesting that dual modulation of these two pathways synergistically kills CML cells via two independent cell-biological mechanisms. Moreover, RNA-seq performed on individual or dual-treated CML CD34+ cells showed a high degree of synergism in genes enriched for p53/apoptosis or c-Myc/differentiation, confirming the phenotypic results. These findings argue that single and combined treatments result in the modulation of distinct molecular pathways.

LSCs are believed to share some characteristics with normal hematopoietic stem cells (HSCs) including quiescence, the ability to self-renew, and the ability to regenerate multi-lineage hematopoiesis (Passegué et al., 2003). However, CML network analyses predicted that CML cells, but not normal HSCs, are dependent on p53/c-Myc signaling, suggesting that the combination treatment could selectively target CML cells. To formally test this hypothesis, healthy CD34+ cells were treated with the single agents or in combination, and no significant effects on proliferation or apoptosis were observed. These findings showed that dual targeting of p53 and c-Myc results in selective inhibition of CML, but not normal HSCs. Similar effects were also observed in phenotypically defined LSCs (CD34+ CD38−), suggesting that the combination treatment selectively targets these rare cells, which are believed to be responsible for leukemia initiation and maintenance. To more precisely test whether the combination treatment would inhibit the ability of normal HSCs and LSCs to self-renew and to regenerate the hematopoietic system, the authors used a pre-treatment/xeno-transplantation mouse model. While pre-treatment of cells with Dasatinib had no effect on CML CD34+ cells' ability to regenerate multi-lineage hematopoiesis, the combination treatment with RITA and CPI-203 significantly inhibited the ability of CML CD34+ cells to engraft. Of note, the combination pre-treatment had no effect on the reconstitution properties of healthy CD34+ cells in vivo, which further strengthens the argument that this dual activation of p53 and inhibition of c-Myc selectively targets CML LSCs. Using a genetic mouse model of CML as well as human CML xenografts, the authors

provide further proof of principle demonstrating that available oral agents such as RG7388 (HDM2 inhibitor) and CPI-0610 (BET inhibitor), which are currently in clinical trials, can selectively inhibit CML cell growth in vivo. In future studies, it will be interesting to assess whether such dual inhibition has an effect on overall survival of treated mice and to test whether the combination treatment leads to reduction or elimination of leukemia-initiating cells in secondary transplantation assays.

Interestingly, the authors found that the p53/c-Myc network is also deregulated in TKI-non-responder (TKI-NR) patients and more advanced forms of CML. And indeed, combination treatment significantly induced apoptosis in CD34+ cells from TKI-NR patients.

In conclusion, Abraham et al. (2016) demonstrate the power of using an unbiased systems biology approach to identify novel regulatory pathways and therapeutic vulnerabilities in leukemic stem cells using primary patient samples. Their findings strongly support the further exploration and testing of dual p53 and c-Myc targeting, in addition to TKI therapy, for patients with CML. Furthermore, as transcriptional and epigenetic dysregulation is currently emerging as one of the hallmarks of the earliest origins of cellular transformation and cancer stem cells (Corces-Zimmerman et al., 2014; Will et al., 2015), the combinatorial targeting of key dysregulated transcription factors may be an approach with broader applicability including other types of cancer.

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Fate by Chance, not by Choice: Epidermal Stem Cells Go Live

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The skin epidermis is constantly renewed by epidermal stem cells. In a recent *Science* paper, Rompolas et al. utilize live imaging to track epidermal stem cells over their lifetimes. Their findings provide new insights into epidermal stem cell behaviors and unravel how newly generated cells are integrated into pre-existing tissues.

Homeostasis involves the replacement of old tissues with new tissues generated from somatic stem cells. Advances in lineage-tracing strategies have enabled the identification of stem cells and lineage relationships of their progeny. However, the vast majority of these studies rely on fixed samples taken at different time points, which do not inform how an individual stem cell behaves throughout the process. Using two-photon microscopy combined with live imaging, Rompolas et al. (2016) followed individual stem cells over their lifetimes, and in so doing, elucidated new principles of epidermal homeostasis.

The skin epidermis undergoes constant turnover. Proliferating cells are located at the basal layer, while differentiating cells move upward to first form the spinous layer, followed by the granular layer, and eventually the most outer stratum corneum, a dead cell layer that sheds. A classical hypothesis, inspired by the columnar stacks seen with the cornified layer, suggests that each stack of cornified cells is maintained by the basal cells underneath and each is an "epidermal proliferative unit" (the EPU hypothesis). Each EPU contains one slow-cycling stem cell that divides asymmetrically to renew itself and generate the fast-dividing transit-amplifying cells, which undergo limited rounds of divisions before differentiating upward (Potten, 1974) (Figure 1A).

Recent studies have suggested that the EPU hypothesis is not accurate in