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A Knockout Combo: Eradicating AML Stem Cells with TKI plus SIRT1 Inhibition

Bernd B. Zeisig[1] and Chi Wai Eric So[1,*]
[1]Leukaemia and Stem Cell Biology Group, Department of Haematological Medicine, Division of Cancer Studies, King's College London, London WC2R 2LS, UK
*Correspondence: eric.so@kcl.ac.uk
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SIRT1 inhibition facilitates elimination of CML stem cells by Imatinib, in part via p53 activation. In this issue of *Cell Stem Cell*, Li et al. (2014) demonstrate a similar role for SIRT1 inhibition in eradicating FLT3-ITD AML stem cells, potentially through a positive feedback loop with c-MYC, highlighting SIRT1 as a potential target in combination cancer therapy.

Histone deacetylases (HDACs) are evolutionarily conserved transcriptional corepressors that are frequently deregulated in various human cancers (West and Johnstone, 2014). In addition to their well-recognized histone modification function involved in epigenetic regulation, HDACs also remove acetyl groups from a variety of nonhistone protein substrates critical for normal and disease development. Unlike class I/II/IV Zn^{2+}-dependent HDACs, class III HDACs comprising seven mammalian members of the Sirtuin protein family require NAD+ as a cofactor that links transcription with energy metabolism (Barneda-Zahonero and Parra, 2012). While the role of Sirtuins in oncogenesis varies based on tissue type (Brooks and Gu, 2009), recent evidence revealed a critical function for SIRT1 in regulating p53 activities in chronic myeloid leukemia (CML) (Li et al., 2012). In contrast to pan-HDAC inhibitors, genetic inactivation of SIRT1 or administration of the Sirtuin inhibitor Tenovin-6 (TV6) appears to be more tolerable to normal stem cells, and applying it as a treatment in combination with the BCR-ABL tyrosine kinase inhibitor (TKI) Imatinib can help to eradicate CML stem cells in mouse models, highlighting its potential as a therapeutic target for cancer stem cells. To gain further insights into the role of SIRT1 in other hematological malignancies, Li et al. (2014) now extend their findings to acute myeloid leukemia (AML) and propose a mechanism involving a positive feedback loop between SIRT1 and c-MYC in regulating AML stem cells (Figure 1).

In their current study published in this issue of *Cell Stem Cell*, Li et al. (2014) report that CD34+ cells from AML patients with intermediate to poor prognosis, and in particular those carrying the FLT3-ITD mutation, expressed high levels of SIRT1 protein and were particularly sensitive to the SIRT1 inhibitor TV6. FLT3-ITD stabilized SIRT1 protein levels, leading to reduced p53 target gene expression. Conversely, shRNA-mediated knockdown or pharmacological inhibition of SIRT1 in FLT3-ITD AML cells resulted in an increased level of acetylated p53 and its target gene expression, leading to an effective killing of FLT3-ITD-expressing cells, but not FLT3-WT or normal CD34+ cells, in vitro. More interestingly, inhibition of SIRT1 also enhanced the effects of FLT3 tyrosine kinase inhibitor Quizartinib (AC220) in suppressing FLT3-ITD AML. In spite of its effective inhibition of FLT3 kinase activity in FLT3-ITD AML cells, AC220 only partially reduced SIRT1 and p53 levels without significantly affecting p53 acetylation or its target gene expression. In contrast to the modest inhibition of primary FLT3-ITD CD34+ cells by AC220 in vitro, a combination treatment using TV6 and AC220 significantly reduced oncogenic potential of FLT3-ITD leukemic cells in vitro, in vivo, and in secondary xenograft transplant, suggesting an enhanced targeting of primitive AML stem cells by SIRT1 inhibition.

Gene expression analysis, which was performed to provide insight into the underlying mechanism on FLT3-ITD AML patient samples, revealed a significant enrichment of c-MYC gene sets, which might be activated via STAT5-induced PIM kinase that phosphorylates and stabilizes c-MYC. c-MYC knockdown led to SIRT1 loss, whereas overexpression of c-MYC resulted in SIRT1 deubiquitination, which could be suppressed by USP22 deubiquitinase knockdown. Consistently, USP22 expression could be induced by c-MYC or FLT3-ITD. Intriguingly, SIRT1 knockdown or TV6 treatment reciprocally increased c-MYC acetylation and reduced its stability and activity in FLT3-ITD AML cells, suggesting the presence of a positive feedback loop between c-MYC and SIRT1. The effective clearance of c-MYC upon AC220 and TV6 combination treatment may also explain the enhanced efficacy as compared with single agents in targeting FLT3-ITD AML stem cells. Therefore, this report has not only extended the potential therapeutic application of SIRT1 inhibition in combination therapy to target cancer stem cells in a different hematological malignancy, it also reveals an underlying mechanism involving a positive feedback loop that regulates c-MYC/SIRT1 functions. This model is also compatible with the recent findings by Sasca et al. (2014) showing a potentially parallel FLT3/SIRT1 regulatory pathway mediated by ATM and DBC1 that both converge into the p53 pathway, which, when taken together, provide multiple interception points for therapeutic intervention in FLT3-mutated AML (Figure 1A).

Although FLT3 is one of the most common mutations found in AML, the heterogeneity of AML in which different subgroups are characterized by the presence of distinct genetic and cytogenetic aberrations/mutations that directly impact patient's prognosis and response to treatment (Zeisig et al., 2012) raises questions about the role of FLT3 mutation in disease development. In the case of common translocation leukemia involving MLL, CBF, or RARA fusions, FLT3

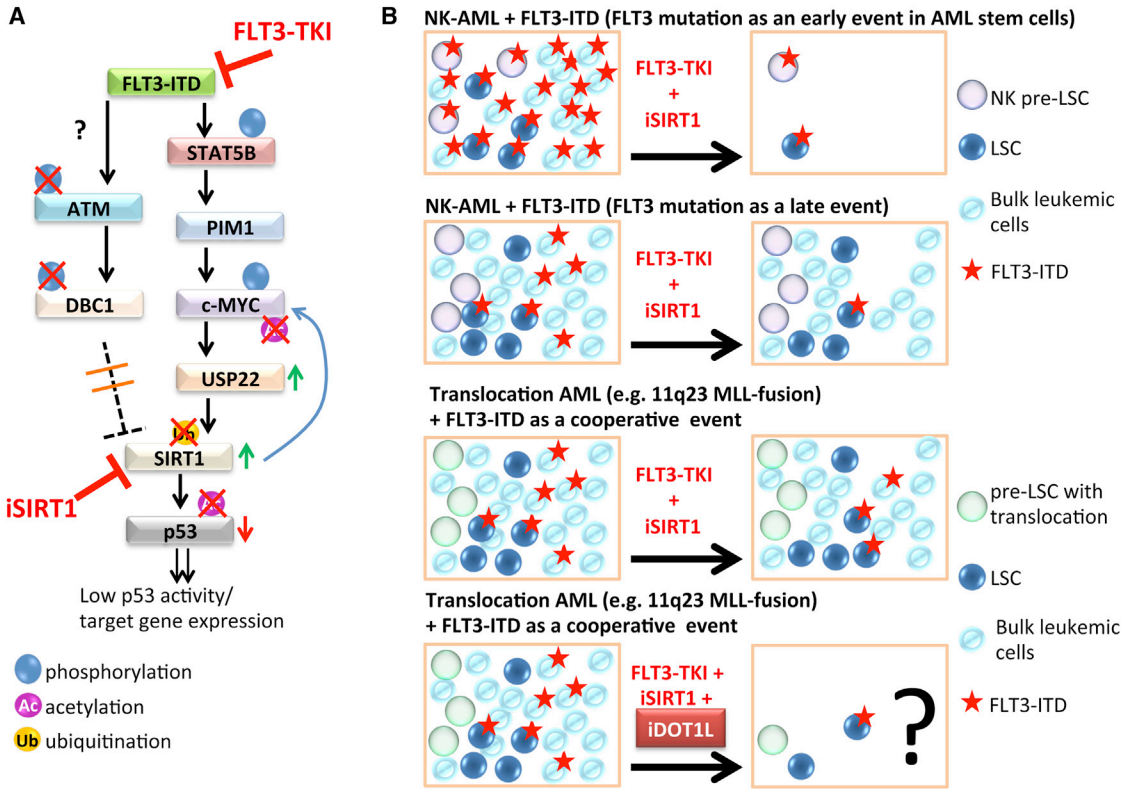

Figure 1. Molecular Regulation and Targeting of SIRT1 in AML

(A) Proposed pathways and potential therapeutic intervention involving SIRT1 and its downstream effectors (e.g. p53 and c-MYC) in FLT3-ITD AML. The right arm of the diagram based on the study by Li et al. (2014) shows that FLT3-ITD activates c-MYC via STAT5 and PIM1 kinase. c-MYC expression in turn enhances expression of the deubiquitinase USP22 that stabilizes SIRT1 protein by protecting SIRT1 from proteasomal degradation. While a high level of SIRT1 expression leads to a reduced activity of p53 by deacetylation of p53, it also stabilizes c-MYC. On the left arm of the diagram, based on the study by Sasca et al. (2014), both ATM and DBC1 are dephosphorylated in the presence of FLT3-ITD. DBC1, inhibiting SIRT1 by binding to its catalytic domain, has much reduced binding affinity to SIRT1 when it is dephosphorylated, leading to enhanced SIRT1 activity. Both studies show that FLT3-ITD AML subgroups can be targeted with FLT3-TKI and iSIRT1 (SIRT1 inhibitor).

(B) Genetic and cellular heterogeneity influence the efficacy of SIRT1 inhibition and its therapeutic strategy in AML treatment. The current study from Li et al. demonstrates that targeting SIRT1 in combination with FLT3-TKI can be an effective treatment avenue in NK-AML with FLT3-ITD (first panel). Combination therapy with iSIRT1 and FLT3-TKI may be much less effective in NK-AML where the FLT3-ITD mutation does not occur at the pre-LSC level but only later during the clonal evolution resulting in only a subset of leukemic cells harboring FLT3-ITD (second panel). In translocation AML (e.g., 11q23 translocations carrying MLL fusions), where chimeric fusion proteins represent strong initiating events at the pre-LSC level, FLT3-ITD may only be acquired later during clonal evolution. Targeting these subsets of AML with iSIRT and FLT3-TKI may not be effective (third panel). It is hypothesized that these subgroups of AML may be more efficiently targeted by combination treatment of iSIRT1/FLT3-TKI and other agents that target the initiating events (e.g., iDOT1L that suppresses a critical component of the MLL fusion transcriptional complex in 11q23 leukemia) (fourth panel).

mutation might represent one of the important cooperating events, but not the dominant initiating driver (Figure 1B). Similarly, even in normal karyotype (NK) AML, where the FLT3 mutation can also exist just in subclones (Ding et al., 2012; Welch et al., 2012), FLT3/SIRT1 inhibition may only be effective in targeting a subset of AML stem cells in the patients (Figure 1B). This may explain the failure to eradicate FLT3-ITD+ AML stem cells in both primary and secondary transplantation models using the AC220 and TV6 combination treatment by the authors. Although the study by Li et al. has not investigated common translocation leukemia, the report by Sasca et al. using well-established primary murine models of MLL-AF9 or AML1-ETO with FLT3-ITD demonstrated rather modest in vitro effects from SIRT1 inhibition. Therefore it is very likely that the effect of SIRT1 inhibition in FLT3-ITD+ AML will largely depend on the dominant driver mutations and the architecture of the leukemic clones. It is tempting to speculate that SIRT1 inhibition in combination with other agents targeting the initiating event may be an attractive therapeutic avenue in certain AML subsets (Figure 1B). To further realize the therapeutic potentials, future studies are needed to determine the efficacy of combination treatments involving SIRT1 inhibition on various AML subtypes before one can potentially translate these findings into patients' benefits. In addition, the putative tumor suppressor functions of SIRT1 (Wang et al., 2008) also need to be carefully considered for any clinical treatments. Nevertheless, the discovery of the critical role of SIRT1 in mediating oncogenic potentials of FLT3-ITD opens up a new opportunity to improve the efficacy of targeting FLT3-mutated AML.

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Reinventing the Neural Crest: Direct Reprogramming Makes iNCCs

Sandra Varum[1] and Lukas Sommer[1,*]
[1]Institute of Anatomy, University of Zurich, 8057 Zurich, Switzerland
*Correspondence: lukas.sommer@anatom.uzh.ch
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Aberrant neural crest (NC) development is at the origin of many congenital diseases. Given the limitations in human NC cell isolation and expansion, the development of new strategies for NC generation is crucial. In this issue of *Cell Stem Cell*, Kim et al. (2014) report the direct reprogramming of postnatal fibroblasts into multipotent NC cells.

Neural crest (NC) cells occupy a unique position among stem cell populations found in the embryo. First, the NC innovation was a crucial event in vertebrate evolution by making possible the development of paired sense organs and, with the development of NC-derived jaws, the transition to a more predatory life style. Second, the NC has one of the broadest differentiation potentials in vivo, surpassed only by the inner cell mass of the blastocyst (Bronner and LeDouarin, 2012). As a consequence, anomalous NC development is at the root of many congenital diseases including craniofacial, cardiovascular, and bowel syndromes (Takahashi et al., 2013). Aberrant NC biology has also been associated with cancer originating from NC-derived tissues, such as melanoma, the most aggressive skin cancer (Shakhova et al., 2012). Understanding how NC cells develop and generate their distinct progeny is thus highly relevant. In this issue of *Cell Stem Cell*, Kim and colleagues report the direct reprogramming of postnatal fibroblasts into induced neural crest cells (iNCCs). This approach constitutes an excellent platform to investigate NC biology in development and disease (Kim et al., 2014).

The NC is a transient stem cell population that emerges from the dorsal margin of the neural plate in vertebrate embryos. During neural tube closure, NC cells undergo an epithelial to mesenchymal transition (EMT) and migrate extensively to populate different parts of the embryo. In their target structures, they give rise to a plethora of cell types and tissues, such as the ectomesenchyme of the craniofacial elements, glia and neurons of the peripheral nervous system (PNS), melanocytes in the skin, and smooth muscle cells of the outflow track of the heart.

The existence of cells with NC potential is not restricted to early embryonic development because NC-like cells have been identified in NC-derived tissues during the fetal period and adulthood of various vertebrate species, including human. Moreover, NC-like cells have been isolated from many sources, such as skin, sciatic nerve, gut, and bone marrow. Although they display cell-intrinsic differences depending on the source and timing of isolation, postmigratory NC-like cells are capable of differentiating into several neural and nonneural cell types. Therefore, given their broad developmental potential and their accessibility in adult organs, it has been proposed that NC-like cells might have therapeutic value. However, a major limitation for the use of postmigratory NC-like cells is their restricted availability and expandability (Dupin and Sommer, 2012).

Given the broad range of diseases with NC etiology and the restrictions in the isolation of human NC, the development of new model systems for the generation of this cell population is imperative. Such models should provide good tools not only for the study of mechanisms underlying NC self-renewal and cell fate specification but also for human disease modeling. In the last decade, a major effort has focused on the derivation of NC cells from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). Several studies have reported the differentiation of NC derivatives from hESCs or iPSCs, including melanocytes, nociceptive neurons, and