A new kid on the block for acute myeloid leukemia treatment? Homoharringtonine interferes with key pathways in acute myeloid leukemia cells

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Treatment for acute myeloid leukemia (AML) has not changed substantially in the past 40 years. We still largely rely on the substances (antimetabolites and topoisomerase inhibitors) that were introduced for AML treatment in the 1970s. Although treatment outcomes for AML, other than acute promyelocytic leukemia (APM), have improved over the years, these improvements have been slow and incremental and been largely achieved by optimizing dosing schedules and better supportive care.[1] However, substantial improvements in treatment, with high cure rates, have been achieved in genetically well defined subgroups of leukemia like acute promyelocytic leukemia (driven by the PML/RARA fusion)[2] and chronic myeloid leukemia (driven by the BCR/ABL fusion).[3] AML, however, is a different beast. It is not a single disease but a genetically highly heterogeneous group of malignant diseases of the hematopoietic stem cell that have similar phenotypes. The great genetic heterogeneity of AML, which was recognizable even in the early cytogenetic studies, has become very apparent with the advent of next-generation sequencing which has allowed the analysis of AML genomes at base pair resolution.[4,5]

Considering that AML is a highly heterogeneous group of malignant diseases, it is not surprising that a 'one size fits all' standard treatment approach can not achieve satisfactory cure rates. One critical question is: how heterogenous is AML? How many different diseases are we dealing with? If we simply looked at the number of genes that have been shown to harbor somatic mutation in AML and the number of somatic mutations in each AML sample, the number of potential combinations, and thus AML subgroups, would be astronomical. However, this is most likely an overestimate of the heterogeneity and complexity of AML, and there is likely only a much smaller number of crucial pathways that are disrupted in AML pathogenesis. Identifying and targeting these crucial pathways should greatly improve AML therapy.

In their report in this issue of the Journal, Chen *et al.* present convincing evidence that homoharringtonine (HHT) (omacetaxine mepesuccinate), an alkaloid found in the Hainan plum yew,[6] is targeting such a critical pathway in AML pathogenesis.[7] HHT has been used in China for several years for the treatment of AML[8,9] and was approved in 2012 by the US Food and Drug Administration (FDA) for the treatment of chronic myeloid leukemia (CML) with resistance to tyrosine kinase inhibitors.[10] The mode of action of HHT in CML was reported to be through the inhibition of protein synthesis by binding to the A site of the ribosome.[11] Building on the success of HHT in the treatment of AML patients in China, where a remission rate of 83%

was achieved when HHT was used in combination with cytarabine and aclarubicin to treat *de novo* AML patients,[9] Chen *et al.* explored the mechanism of HHT efficacy in murine and human AML cell lines as well as in murine and xenotransplant AML models. HHT treatment led to apoptosis and differentiation in cell lines and resulted in increased survival in murine AML transplantation models and in human AML xenograft models. Then the group set out to elucidate the molecular mechanism responsible for the observed effects of HHT treatment.

In a series of elegant experiments, Chen *et al.* showed that HHT binds to the transcription factor SP1, thereby inhibiting the binding of SP1 to its cognate DNA sequences. However, the route to this discovery was not straightforward. Initially, the group examined global 5-hydroxymethylcytosine (5hmC) levels in cells treated with HHT and noticed a remarkable reduction in global 5hmC levels. 5hmC is generated by the oxidation of methylcytosine groups in DNA through the action of the three dioxygenase enzymes TET1, 2 and 3.[12] As only the expression of the *TET1* gene, and not of *TET2* or *TET3*, was significantly down-regulated after HHT treatment, the authors concluded that the reduction in *TET1* expression, and hence a reduced TET1 enzymatic activity, was probably the cause of the reduced 5hmC levels. Examining the promoter region of TET1, they found multiple binding motifs for the transcription factor SP1 and reasoned that SP1 might be important for the HHT-induced downregulation of TET1 expression. And indeed, they could show that HHT treatment reduced binding of SP1 to its binding motifs in the TET1 promoter region. Furthermore, a drug affinity responsive target stability assay and a cellular thermal shift assay provided strong evidence that HHT is indeed binding to the SP1 protein and preventing SP1 from binding to its recognition sites in the *TET1* promoter region.

What are the consequences of the HHT-induced reduction in *TET1* transcription and, consequently, the global reduction in 5hmC levels? The 5hmC mark is associated with active transcription. Thus, the reduction in 5hmC levels was accompanied by a genome-wide reduction in gene transcription. More than 1,600 genes showed reduced expression. Very interestingly, one of the most strongly repressed genes was *FLT3*, which codes for the tyrosine kinase receptor FLT3. The authors confirmed that the *FLT3* gene was one of the direct targets of TET1 action with chromatin immunoprecipiation-quantitative polymerase chain reaction (ChIP-qPCR). FLT3 signaling is critical in the maintenance of the leukemic phenotype and activates a number of genes, including *HOXA9* and *MEIS1*, which are highly expressed in hematopoietic stem and progenitor cells. There is a positive feed-back

loop between FLT3 and HOXA9 / MEIS1 expression. Enforced *FLT3* expression leads to upregulation of *HOXA9* and *MEIS1*, and enforced expression of *HOXA9* and *MEIS1* in turn results in upregulation of *FLT3*. In addition, looking at all of the genes that had reduced expression after HHT administration, the authors found a significant enrichment of MYC target genes. They were then able to confirm that MYC expression itself was reduced after HHT treatment. Myc is one of the master regulators of cellular growth, increasing ribosome biogenesis and protein synthesis.[13]

SP1 is a transcription factor which has many cognate binding sites in the promoter region of many genes. So what is the evidence that the HHT-mediated inhibition of SP1 binding to the *TET1* promoter is critical for the phenotype observed in AML cells after HHT treatment and not the repression of a number of other genes? Very elegantly, the authors showed that repression of *TET1* phenocopies the effect of HHT treatment.

In summary, by binding to the transcription factor SP1, HHT affects one of the core pathways that are responsible for growth (MYC and targets) and impaired differentiation (HOXA9 and MEIS1) of AML cells. The central players in this pathway are the dioxygenase TET1 and the receptor tyrosine kinase FLT3. FLT3-activating mutations are found in about 40% of AML cases and it is overexpressed in an even larger proportion of AML. Thus, HHT promises to be effective in the majority of AML cases. HHT might thus be efficacious in a larger number of AML patients than several of the more recently introduced targeted AML drugs like FLT3 inhibitors (e.g. midostaurin, gilteritinib), IDH1/2 inhibitors (enasidenib, evosidenib), or hedgehog pathway inhibitors (glasdegib).[14] At the same time, one would hope that it is more specific for AML than conventional chemotherapeutic agents such as nucleoside analogs or anthracyclines, which affect very basic cellular functions like DNA metabolism and are thus highly toxic to normal cells.
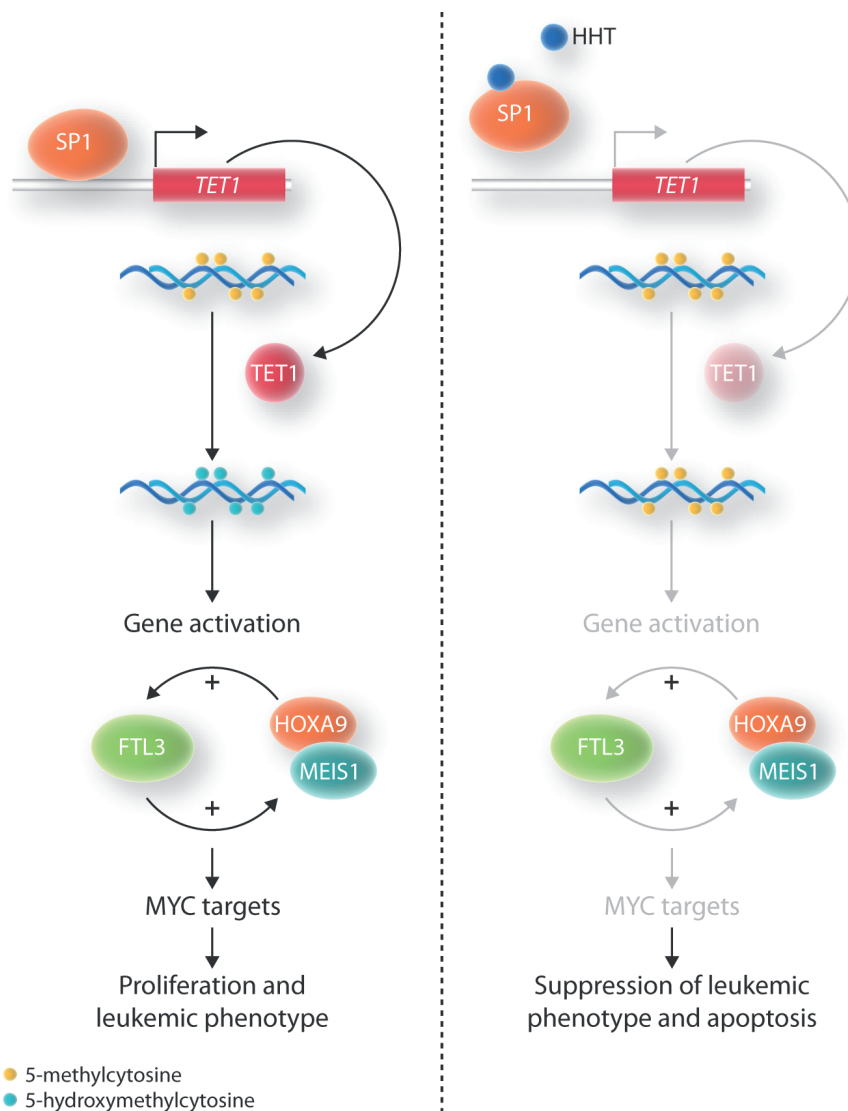
Figure 1. Diagram of the pathways affected by homoharringtonine (HHT). (A) Status of the pathways in the leukemic cells in the absence of HHT. (B) When HHT is present, it binds to SP1 which leads to a reduction of the activity of downstream pathways.

However, as HHT has been shown to bind to SP1, and SP1 is a widely expressed transcription factor whose expression is not restricted to AML cells, it is not surprising that HHT treatment can have considerable side effects, including myelosuppression. Another unanswered question is whether HHT only binds to SP1 or whether it also binds to other cellular proteins which, again, could cause side effects and adverse reactions.

Although it is unlikely that HHT will provide a cure for AML, it could become an important component of ever more diversified and targeted treatment strategies in this disease. The effectiveness of HHT and its therapeutic window need to be assessed rigorously in controlled clinical trials. These should be accompanied by a detailed genetic work up to identify those AML subgroups that are especially responsive to HHT therapy.

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