currently challenging because of difficulties in accessing donor tissue, the estimated low yield of residual LSEC, and non-optimized culture systems.[18] However, the growing field of inducible pluripotent cells may provide useful alternatives. In addition, LSEC are also attractive for their ability to induce antigen-specific immune tolerance.

Lastly, the fact that hepatocyte transplantation does not correct the hemophilia A phenotype in mice may have implications for translational studies on liver gene therapy for the disease. To date, the most successful trials for hemophilia B are using hepatocyte-specific promoters for the expression of factor IX. The fact that factor VIII is not normally secreted from human hepatocytes does not prevent the use of gene delivery to the hepatocyte, as promising data from preclinical studies on factor VIII expression in large animals support the concept that targeting hepatocytes has potential for translational studies.[19,20] However, this strategy may be limited by the intrinsic inability of the human hepatocyte to fully synthesize and secrete factor VIII, despite the presence of mRNA.[6] This may explain, at least in part, the higher dose of vectors required for expression of factor VIII compared to factor IX. On the other hand, despite the limiting effect on the efficacy of factor VIII secretion, the use of hepatocyte-specific expression is a favorable strategy in terms of immune tolerance induction to factor VIII, which is the most serious and common complication (~20%) of hemophilia A treatment.[19]

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## Dangerous liaisons: cooperation between Pbx3, Meis1 and Hoxa9 in leukemia


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Homeobox (*HOX*) genes have a longstanding association with human acute leukemias. In particular, high expression of the *HOXA9* gene is a highly significant marker of poor prognosis in acute myeloid leukemia,[1] and dysregulation of *HOXA9* appears to play a central role in several distinct leukemias. These include acute myeloid leukemia and acute lymphoblastic leukemias caused by translocations of the *Mixed Lineage Leukemia* (*MLL*) gene,[2-4] fusions of the *HOXA9* gene that produce a novel HOXA9-NUP98 fusion protein in acute myeloid leukemia,[5] and T-cell acute lymphoblastic leukemias that have translocations between the *TCRβ* and *HOXA9/A10* loci.[6] Interestingly however, despite this seemingly central role in a subset of acute leukemias, *Hoxa9* expression alone is only weakly oncogenic in mouse leukemia models and usually requires a second "hit" via overexpression of *Meis1*,[7,8] or in some cases *Pbx3*.[9] Much work has been done trying to understand the molecular function of the HOXA9 protein. MEIS1 and PBX3 are both members of the TALE (three amino acid loop extension) homeodomain-contain-

ing family of proteins and are able to modulate HOXA9 binding to DNA.[10] In a rigorous ChIP-seq experiment in mice, Hoxa9 was shown to co-bind with Meis1 at a large number of enhancer regions that control the activity of several key oncogenes,[11] but it was unknown if Pbx3 could contribute to this gene regulatory activity. In this issue of Haematologica, Garcia-Cuellar *et al.* extend our knowledge of this very important protein complex by showing that the TALE protein PBX3 is able to stabilize the TALE protein MEIS1 and contribute to HOXA9-mediated activation of gene targets and subsequent leukemogenesis.[12] Understanding the details of how this trimeric protein complex forms and interacts has the potential to aid the development of novel therapeutic inhibitors.[13]

### *Normal function of* Hox *genes*

The most famous *HOX* genes are the clustered *Hox* genes originally discovered in *Drosophila melanogaster*, in which they function as developmental regulators of body segment identity specification, along the anterio-posterior axis.[14] They similarly control body patterning in mammals, and also have a key role in controlling cell identity and differentiation of hematopoietic stem cells and progenitors.[15] Murine developmental studies have shown that *Hoxa* gene cluster expression is generally high in primitive hematopoietic populations and is subsequently down-regulated in more differentiated bone marrow cells.[16]

HOX proteins all contain a homeodomain, a protein domain with known DNA binding activity. Because of this, HOX proteins are widely regarded to be transcription factors. Direct evidence for their role in transcriptional regulation comes from the fact that both Hoxa9 and Meis1 have been shown to bind directly to enhancer regions, and inactivation of these proteins can cause both up-regulation and down-regulation of their target genes.[11] However, it is also worth noting that evidence exists for HOX function that does not require DNA binding. They have been shown to interfere with CBP-mediated transcriptional activation (via interactions with the HOX homeodomain) by blocking histone acetyltransferase activity.[17] Additionally, Hoxa9 can regulate hematopoietic stem cell and progenitor activity through direct down-regulation of the cell-cycle regulator Geminin, via association with a ubiquitin ligase complex.[18] Although these alternate mechanisms of HOX molecular function are potentially very interesting, they have not been fully elucidated and most research has focused on the role of HOX proteins in transcriptional regulation.

If HOX proteins function primarily as transcription factors, this raises a potential problem. Individual HOX proteins all have highly conserved homeodomains with very similar DNA binding activities, and yet they often display significantly different phenotypes. This raises an interesting question: if HOX proteins do function primarily as transcription factors, what controls their phenotypic specificity?

### *HOXA9 DNA binding and TALE family proteins*

Early *in vitro* DNA binding experiments with HOX proteins identified the cooperation of a PBX family cofactor (extradenticle in fly) that contributes to both the specificity and selectivity of these DNA interactions. Further

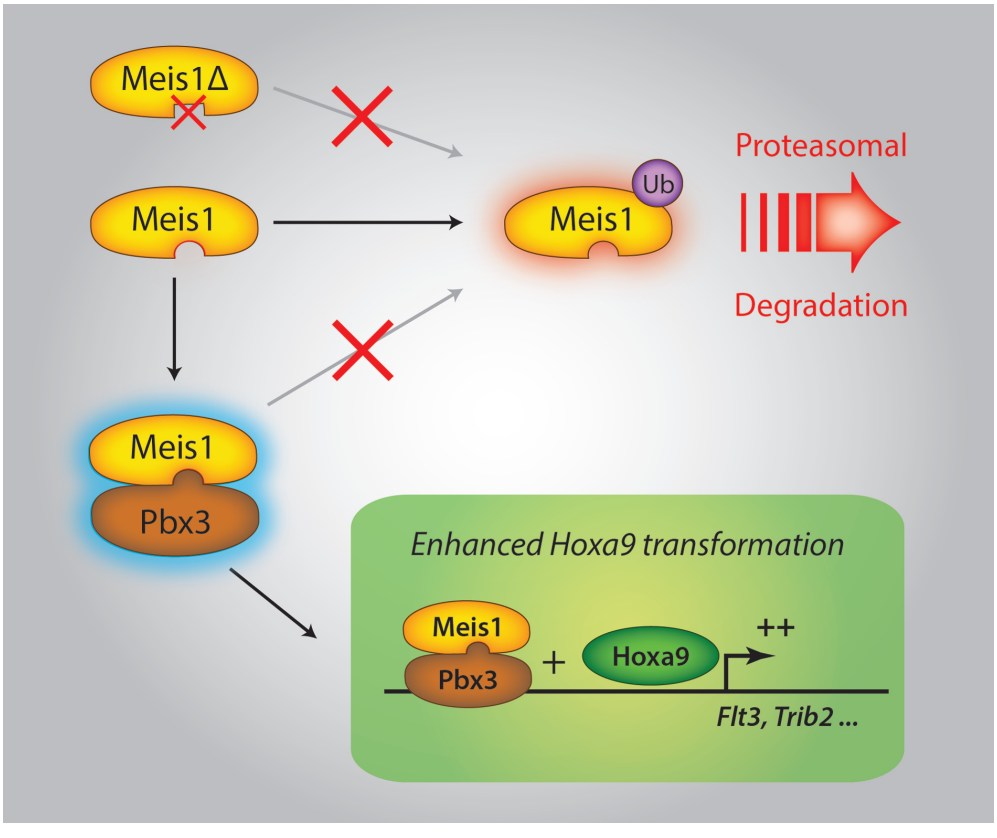


**Figure 1.** Schematic representation of TALE protein cooperation. Meis1-Pbx3 dimerzation (highlighted in blue) stabilizes Meis1 protein levels and enhances Hox-mediated transformation by increasing transcription of Meis1 target genes such as *Flt3* and *Trib2*. Unbound Meis1 is ubiquitinated and subsequently degraded by the proteasome. Mutating the Pbx interaction domain of Meis1 (Meis1Δ) also prevents Meis1 ubiquitination. Ub: ubiquitin.

experiments suggested an interesting model whereby HOX protein-mediated activation/repression of target gene transcription could be switched, by co-binding or absence (respectively) of extradenticle.[19] Human HOXA9, MEIS1 and PBX2 were shown to form a trimeric DNA-binding complex, and further studies confirmed an important role of *HOXA9/MEIS1* in leukemia.[20,21] However, exactly which factors control HOXA9 activity in mammalian systems and how much this activity affects normal hematopoiesis *versus* leukemogenesis is not completely understood.

MEIS1 first became implicated in leukemia as a result of experiments using the BXH2 murine myeloid leukemia model to identify disease genes by proviral tagging. *Hoxa9*, *Hoxa7* and *Meis1* were almost always targeted for activation in these leukemias,[22] and synergy between *Hoxa9* and *Meis1* expression is required to produce aggressive leukemia in mice.[7] *Pbx1* fails to copy this requirement, despite it having a role in maintaining definitive hematopoiesis.[23]

The discovery that PBX3 (not PBX1 or PBX2) is an important player in HOX-dysregulated leukemias reconciled the apparent primacy of Hoxa9 and Meis1-mediated transformation, with earlier studies that demonstrated Pbx involvement in the Hoxa9 trimeric complex.[9,24] While these studies showed that PBX3 was needed for HOXA9-mediated induction of leukemia, the molecular mechanism for this contribution was not completely understood.

### *Pbx3 contributes to Hoxa9 leukemogenesis through stabilization of the Meis1 protein*

In this issue of Haematologica, Garcia-Cuellar and colleagues make an important step towards elucidating the regulation of Hox function through Hoxa9-Meis1-Pbx3 interactions. They were able to show that a direct interaction with Pbx3 protects Meis1 from ubiquitination and subsequent proteasome-mediated degradation, thus crucially extending the half-life of the Meis1 protein.[12] Interestingly, making mutations in the Meis1 protein that disrupt this Pbx3-Meis1 interaction domain (i.e. Meis1Δ for short) abrogated Meis1 ubiquitination and degradation, suggesting that Pbx3 may compete directly with a ubiquitin ligase for binding to the same region of Meis1. However, they went on to show that Meis1 stabilization alone is not sufficient for increased Meis1 activity, as Meis1Δ mutants were unable to cooperate with Hoxa9 in colony-forming assays or *in vivo* leukemia assays. They further showed that Pbx3-Meis1 binding is required for an efficient Meis1-Hoxa9 interaction, indicating that the overall effect is that Pbx3 stabilizes Meis1 protein levels, as well as enhancing Meis1-Hoxa9 interactions and subsequent gene regulatory activity[12] (see Figure 1). Additionally, when Hoxa9 and Pbx3 are co-expressed, they also cause increased expression of the *Meis1* gene, suggesting that there is another layer of cooperation between these factors.

What does all this mean for the function of Hoxa9 and the promotion of leukemogenesis? The authors were able to show that while Hoxa9/Pbx3 or Hoxa9 alone are unable to produce leukemia in mice even after 180 days, Hoxa9 expression along with both Pbx3 and Meis1 shows some degree of cooperation. This contrasts with the results of Li *et al.* who were able to see a cooperative effect between Hoxa9 and Pbx3 without Meis1,[9] but this could potentially be explained by differences in expression levels between the two different model systems used. Whatever the final explanation might be, the role of Pbx3 in enhancing Hoxa9/Meis1-mediated leukemogenesis provides novel insights into how these two TALE cofactors mediate the transcriptional function and leukemic role of the Hoxa9 protein.

### *Conclusions*

These findings provide a mechanistic analysis for the long-standing enigma of the trimeric Hoxa9-Meis1-Pbx3 complex in leukemia. They suggest a number of specific molecular mechanisms by which Pbx3 individually supports the function of Meis1, and the complex as a whole, and explore the mechanisms by which Pbx3 may be contributing to HOXA9 molecular function.

In doing so, the work by Garcia-Cuellar and colleagues provides a better understanding of the complex interactions that control HOXA9 protein activity, an entity that plays an important role in a number of severe hematologic malignancies. This knowledge could provide further possible avenues for targeting the HOX-mediated leukemic transcription program. As new therapeutic technologies mature, such as small peptide inhibitors, detailed knowledge of the molecular interaction between oncogenic transcription factors and their cofactors will be essential in the design of novel therapeutics.

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## Rituximab maintenance therapy in diffuse large B-cell lymphoma: is XY the most important variable?

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In B-cell non-Hodgkin lymphomas (NHL) rituximab has extended the disease-free intervals of hundreds of thousands of patients. At the inception of rituximab a considerable amount of academic vigor was invested in finding the appropriate dose and frequency during induction therapy. This was followed by consideration of rituximab maintenance or extended dosing strategies. However, if maintenance rituximab does not significantly improve treatment outcomes it only represents expensive plasma. An integral step in harnessing the excitement for maintenance rituximab is to look for patients' characteristics that can help to tailor or risk-adapt rituximab dose and/or duration of use with the goal of providing benefit to all. The primary endpoint of interest, improvement in overall survival, has only been seen in meta-analyses, leaving surrogate markers of benefit, such as event-free survival and progression-free survival in trials, to be debated at podiums and in patients' examination rooms without a clear consensus being reached.[1]

The original report that triggered the spark of enthusiasm for maintenance rituximab was published by Dr. Ghielmini and colleagues and concerned patients with follicular lymphoma (FL) in whom prolonged rituximab treatment extended the duration of remission.[2] The use of rituximab in FL subsequently expanded as results of randomized trials emerged showing remission prolongation with maintenance rituximab after single agent rituximab and combined rituximab-chemotherapy and then similar results in mantle cell lymphoma.[3-7] A theme began to develop: rituximab maintenance was most useful in B-cell NHL subtypes in which the majority of patients do not have durable remissions. However, in diffuse large B-cell lymphoma (DLBCL), the most common NHL, in which the majority of patients who achieve a complete remission after rituximab-chemotherapy are cured, maintenance rituximab therapy has not been felt to be efficacious.

Nevertheless, Huang and colleagues reported a randomized trial of maintenance rituximab in patients with an objective response after six cycles of R-CHOP-14 (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone). The maintenance rituximab was administered monthly during the first 12 months and once every 3 months during the second year.[8] Patients who received maintenance rituximab had a progression-free survival rate at 5 years of 45% compared to 34% in the patients who were observed ($P$=0.006). The overall survival rate at 5 years was 62% with maintenance rituximab and 49% with observation ($P$=0.03). Maintenance rituximab improved the progression-free and overall survival of patients in all International Prognostic Index groupings. The lower progression-free and overall survival rates might be expected and were probably related to the fact that all patients who had an objective response (i.e., not just complete remissions) were included in the analysis. In this study, the results were not reported by gender, so it is not possible to determine whether the observed benefit was greater in males than in females.

In a subsequent, larger randomized trial carried out by the Eastern Cooperative Oncology Group (EGOG; ECOG 4494) in the USA, patients over 60 years of age with DLBCL were randomly assigned to receive R-CHOP or CHOP; there was then a second randomization to maintenance rituximab or no maintenance rituximab.[9] Thus, this was a four-arm study including patients who received R-CHOP and no maintenance rituximab, R-CHOP with maintenance rituximab, CHOP with maintenance rituximab, and CHOP without maintenance rituximab. The results were comparable within the three groups who