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TAK1 and *TBK1* are Differentially Required by GMP- and LMPP-like Leukemia Stem Cells

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ABSTRACT

Acute myeloid leukemia (AML) encompasses a diverse group of cancers that originate in the blood-forming tissues of the bone marrow. Aside from the M3 subtype (*PML-RARA*$^+$), AML carries a 5-year survival rate of 28% for patients 20+ years of age. AML is the most common cancer of the hematopoietic system and is slightly more common in biological males; the average age at diagnosis is 68 years.

Standard frontline treatment for AML is a 2-phase regimen of intensive chemotherapy (CTx) employing daunorubicin and cytarabine. Despite 60-70% of patients achieving complete remission (CR), at least half of CR-achieving patients experience relapse within 3 years from their diagnosis. Additionally, 30-40% of patients present with refractory AML, experiencing little to no benefit from frontline treatment.

AML relapses when a pool of undetectable, CTx-resistant leukemia stem cells (LSCs) survives & proliferates after frontline CTx.[1] Notably, the poor performance status of many AML patients precludes use of the standard CTx regimen; while reduced-intensity CTx still offers therapeutic benefit, it is less effective at killing LSCs and, as a result, relapse is more likely.

Goardon, *et al*. determined that **AML patients harbor two types of LSCs**: granulocyte-macrophage progenitor **(GMP)-like LSCs** and FLT3$^+$ lymphoid-primed multipotential progenitor **(LMPP)-like LSCs.**[2] Eradication of both types of LSCs is necessary to maintain CR in AML.

Our group and others have established that **~40% of AML patients express upregulated Toll-like receptor (TLR) signaling (TLR$^+$)**. TLR$^+$ disease is associated with specific genetic abnormalities, such as *MLL* rearrangements (*MLL*-r$^+$), and is inversely associated with prognosis (**Figure 1**).[3,4] TLR$^+$ AML represents a challenging, treatment-sparse subset of an already difficult-to-treat disease. To study TLR$^+$ AML, we utilize an *MLL*-r$^+$ model using the *MLL-AF9* oncogene.

We have also demonstrated that both **GMP- and LMPP-like LSCs require TLR-associated Ser/Thr protein kinases for their survival**.[5-7] Specifically, GMP-like LSCs require TAK1 and LMPP-like LSCs require TBK1. The loss of either *Tak1* or *Tbk1* ablates the corresponding LSC pool and enriches for the opposite LSC pool *in vitro* and *in vivo*. Recently, our group determined that the genetic loss of *Tak1* sensitizes mouse AML cells to TBK1 blockade *in vitro*.

Strikingly, **the loss of *Tbk1* also seems to extend overall survival (OS) despite causing extramedullary AML.** While mice given *Tbk1*^{NULL} AML cells develop a subcutaneous tumor of AML cells (chloroma) near the pelvis, they survive longer than mice given control AML cells. The clinical significance is unknown, but these data support our impression that the loss of *Tbk1* forces AML cells to differentiate; this *should* be therapeutically favorable, as inducing the differentiation of AML cells is an effective treatment strategy. Theoretically, chloromas may form in *Tbk1*^{NULL} AML due to the enrichment of GMP-like LSCs, which express higher levels of chemokine receptors.

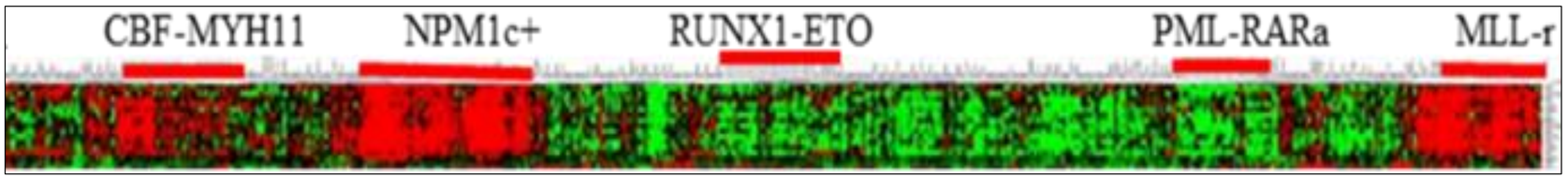
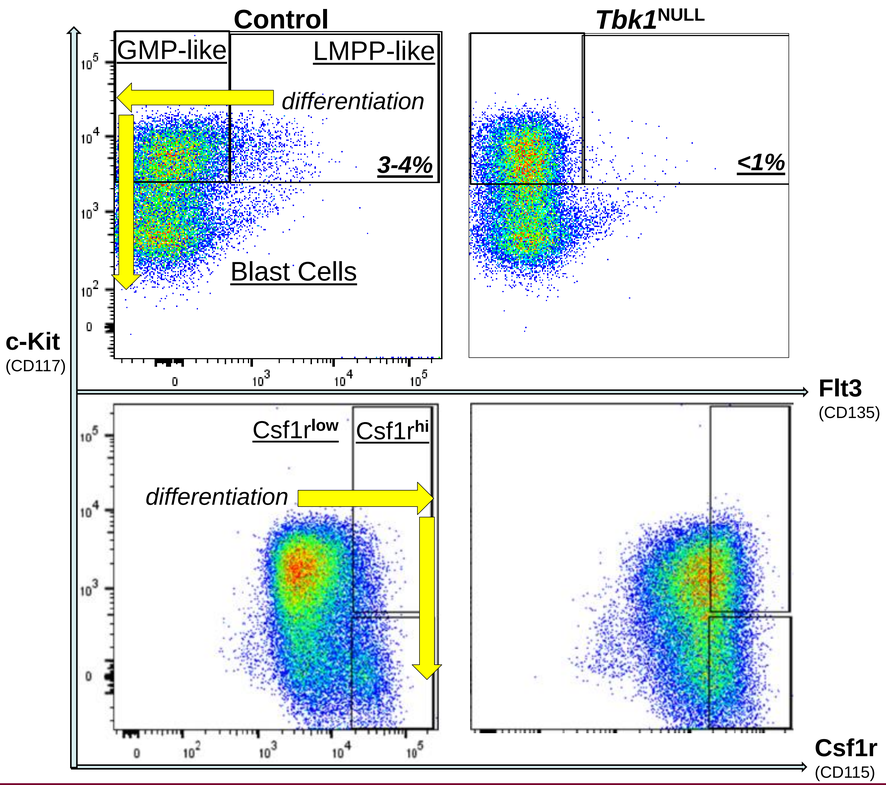


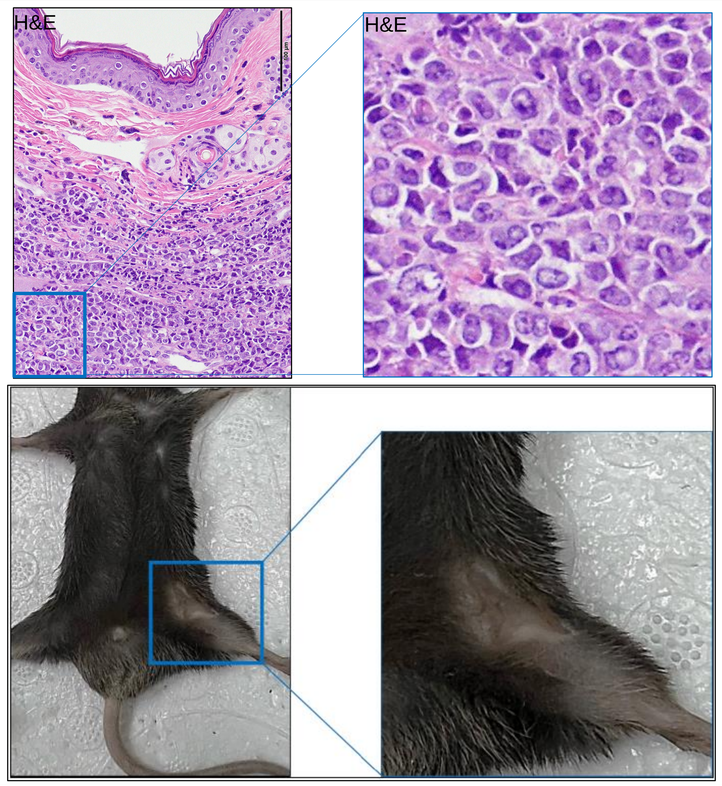
Figure 1: **The expression of TLR-associated genes is associated with AML genetic subtype and inversely correlates with prognosis (*e.g.*, *MLL*-r$^+$ VS *RUNX1-ETO*$^+$).** *Heatmap depicting results from microarray analysis of peripheral-blood AML cells isolated from human patients, grouped by genetic subtype. Increased/decreased levels of TLR-associated mRNA are shown in red/green, respectively.*

RESULTS[7]

Tbk1 deletion ablates LMPP-like LSCs & forces differentiation of AML cells.



Tbk1 deletion extends OS but causes AML cells to form chloroma.

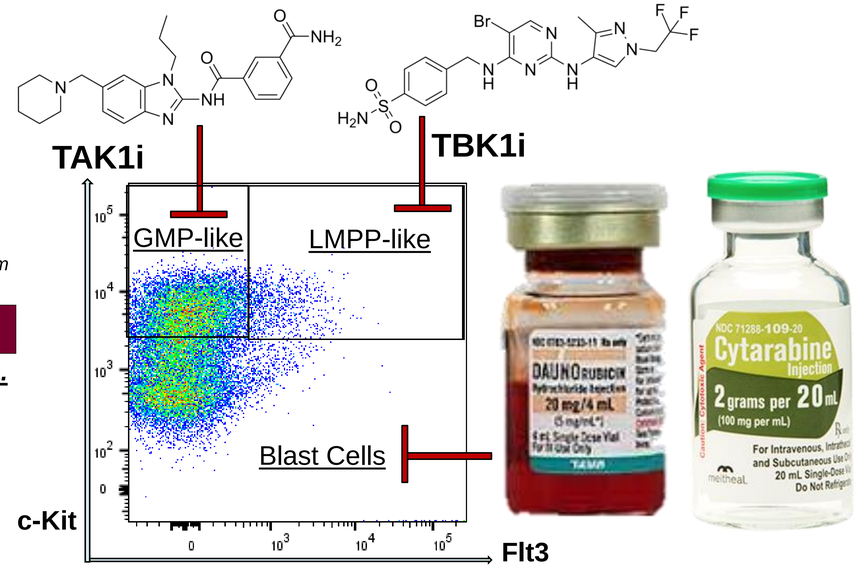


MATERIALS/METHODS

- **Syngeneic Mouse Model of AML**
  - ✓ *MLL-AF9*$^+$ *mouse bone marrow cells (Tbk1*^{NULL} *or control [Tbk1*^{WT}*]) delivered 48h after conditioning C57BL/6J recipients with sublethal, 20mg/kg IP busulfan*
- ***In Vitro* Analyses**
  - ✓ *Surface markers of MLL-AF9*$^+$ *mouse bone marrow cells (Tbk1*^{NULL} *or control [Tbk1*^{WT}*]) analyzed via FACS*
  - ✓ *MLL-AF9*$^+$ *mouse bone marrow cells treated for 24h with either HS276 (TAK1i) or GSK8612 (TBK1i), then analyzed via FACS*

Thank You to the LUMC/CBCC Comparative Medicine Facility, FACS Core Facility, Department of Pathology, and Department of Cancer Biology.

CONCLUSIONS



We hypothesize that the differentiation & eradication of LSCs can be induced by blocking TAK1/TBK1 in combination with standard CTx (and possibly targeted agents like *Mylotarg*®, *Venclexta*®, and/or *Xospata*®).

We propose TAK1/TBK1 parallel blockade as augmentation to standard CTx, ideally allowing for a dose-reduction of CTx & promoting improved patient outcomes.

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