Investigating the role of TANK-binding kinase 1 (TBK1) in *MLL-AF9+* acute myeloid leukemia.

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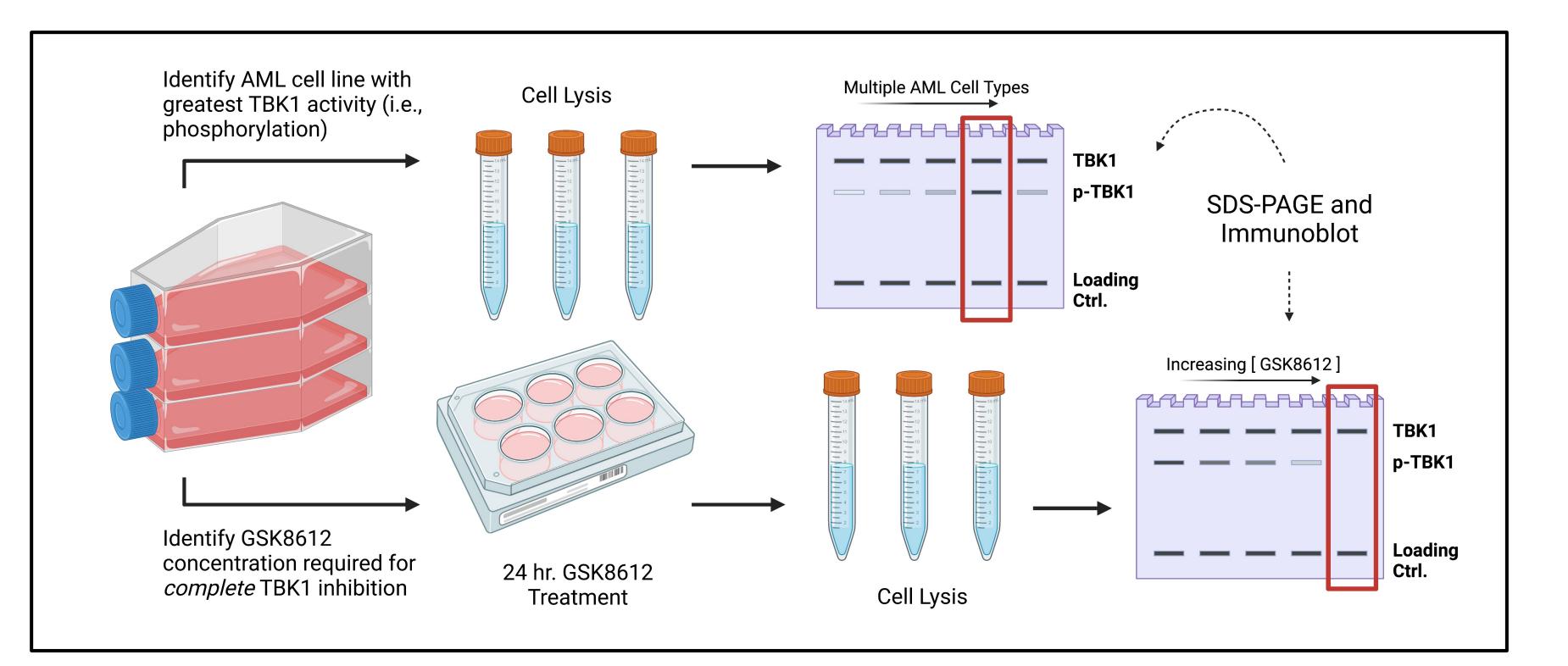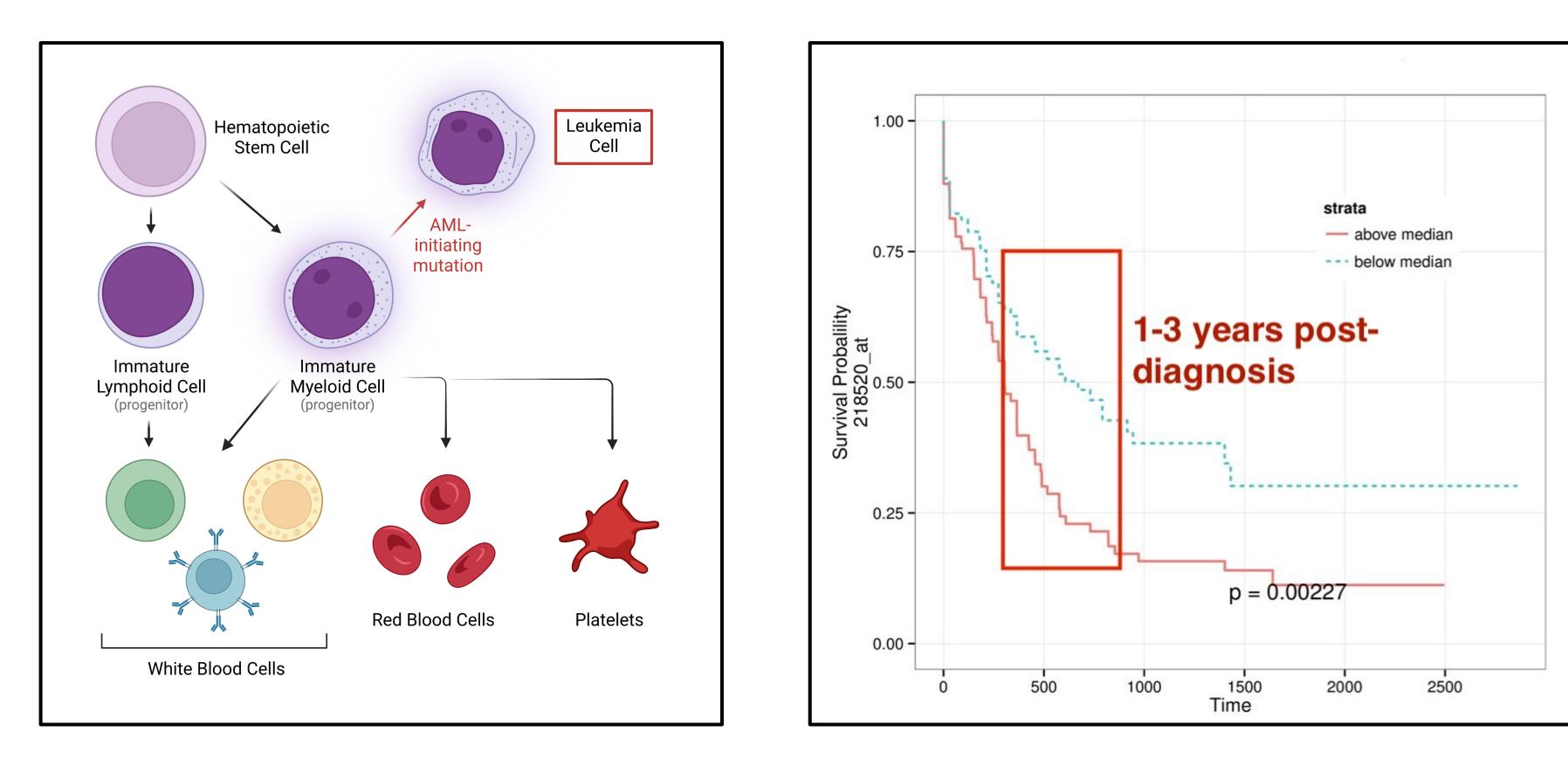
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Introduction

Acute myeloid leukemia (AML) is the most common cancer of the blood-forming (hematopoietic) tissue.[1] It occurs when an immature myeloid cell acquires an AML-initiating mutation that causes it to transform into a malignant leukemia cell.[2] Among other novel characteristics, this new cell type exhibits increased proliferation and a reduction in both differentiation and cell death. These changes enable the leukemic population to expand, consuming resources in the bone marrow and interrupting normal hematopoiesis. This disturbance in normal hematopoiesis is what leads to the bruising and fatigue that are characteristic of AML.

Currently-available chemotherapies are very effective, inducing remission in approximately 60-70% of AML patients; however, among those who achieve remission, at least 50% will relapse.[3] This is due to the survival of a rare, stem-like population of leukemia cells (LSCs) that are resistant to the initial treatment.[4] Unlike their more abundant blast/ "bulk" cell counterparts (which comprise a majority of the liquid tumor), LSCs are responsible for disease initiation and AML relapse, giving rise to a treatment-resistant disease that is more difficult to treat.[4] Despite this paradigm, there are currently no LSC-specific agents available for AML treatment. **Thus, there is a dire need for LSC-specific therapies that can help prevent relapse**.

TANK-binding kinase 1 (TBK1), a serine/ threonine kinase, has shown promise as a therapeutic target. In addition to promoting MYC activity and FLT3 expression in AML cells, it plays a critical role in cellular health, acting as a positive regulator of mitophagy – a process by which reactive oxygen species (ROS) are removed from the cell.[5] Although important for all cell types, mitophagy is especially critical for stem cells (normal *and* malignant – e.g., LSCs), as elevated ROS levels can ablate their self-renewal ability.[6] High *TBK1* mRNA expression in AML patients has also been associated with reduced odds of overall survival at about the same time that remission tends to occur (i.e., 1-3 years post diagnosis). [7]

We hypothesize that TBK1 inhibition could antagonize the stem-like characteristics of LSCs, promoting their loss-of-function and death, thereby increasing the odds of achieving a cure.

Objectives

☐ **Characterize GSK8612**, a selective inhibitor of TBK1, by determining which concentration is sufficient to achieve *complete* inhibition of TBK1. To this end, immunoblot will be used to quantify phosphorylated TBK1 (p-TBK1) and total TBK1 expression in response to varying concentrations of GSK8612 across human AML cells lines; TBK1 substrates (e.g., IRF3) will be examined as well.

☐ **Characterize the effect of GSK8612 on the functional characteristics of human AML cell lines**. To this end, *in vivo* (e.g., xenograft ability) and *in vitro* (e.g., colony-forming ability, expression of myeloid surface markers [CD11b & CD115], and expression of stem-cell markers [FLT3 & cKIT]) assays will be used.

Methods

Immunoblot was used to determine basal TBK1 and p-TBK1 expression following GSK8612 treatment. Our first goal was to determine which human AML cell line (e.g., MOLM-13, MM6, or THP-1) exhibited the greatest p-TBK1 expression and would thus serve as a reliable, clear reporter of TBK1 activity.
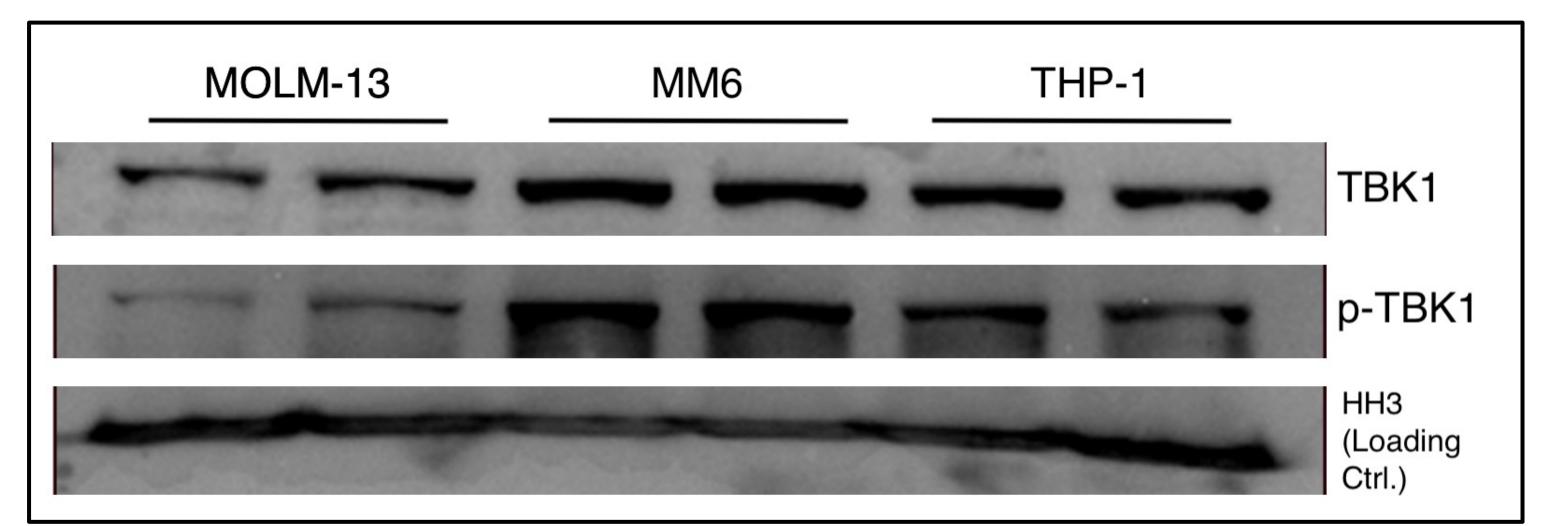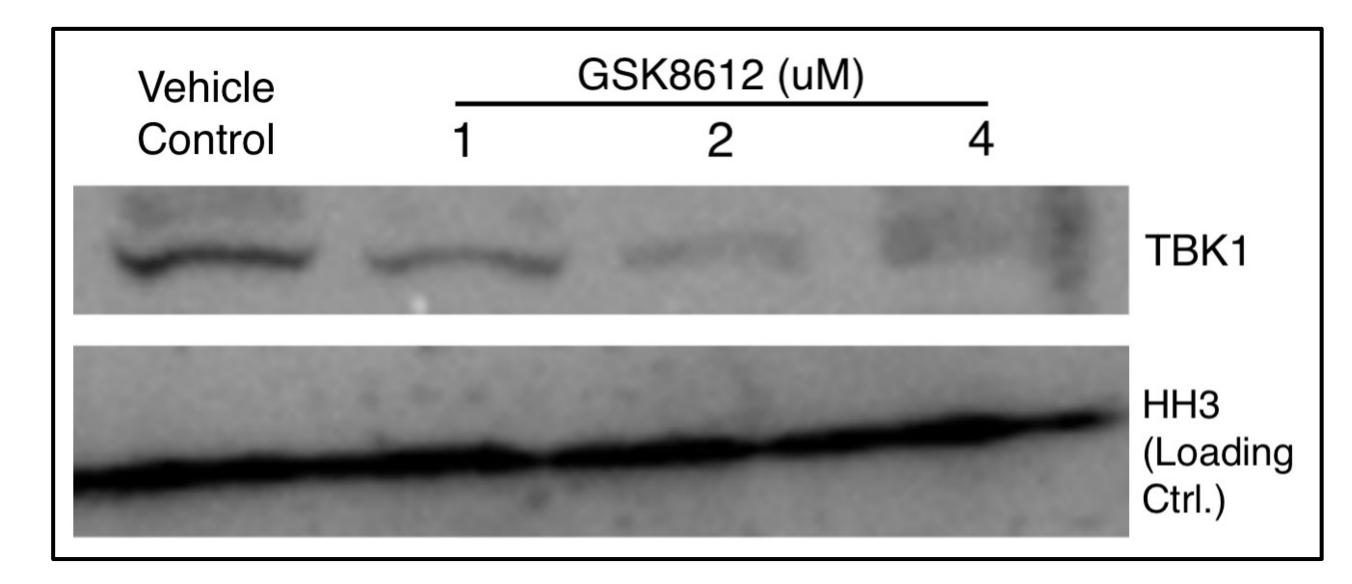
Once a cell line was identified, it was cultured in a 6-well plate. To each well, increasing concentrations of GSK8612 were added; following a literature review, 1-4 uM was determined to be an appropriate concentration range to begin with. After allowing the cells to incubate with the inhibitor for 24 hours, they were harvested and immunoblot was performed. We again probed for p-TBK1. However, this time, our goal was to determine the concentration at which the intensity of the associated band was significantly reduced.

Results

The MM6 human AML cell line exhibits a higher level of TBK1 activity (p-TBK1) compared to MOLM-13 and THP-1.

A dose-dependent reduction in total TBK1 follows GSK8612 treatment in MOLM-13 cells.

Conclusions

☐ The MM6 human AML cell line should primarily be used to interrogate the effect of GSK8612 on p-TBK1 expression.

☐ The reduction in total TBK1 following GSK8612 treatment was not anticipated. As a specific and potent inhibitor of TBK1, we expected that only a reduction in p-TBK1 would occur. Because of this surprising result, the experiment must be repeated. If our reported results are true, the TBK1 homolog IKKε should be examined, as a compensatory upregulation of IKKε could be expected.

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