4SC-202 induces differentiation of AML cells by upregulating promoter histone acetylation

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4SC-202 and acute myeloid leukemia (AML)

4SC-202 is an orally available, clinical stage epigenetic small molecule which inhibits histone deacetylases HDAC 1-3 and the lysine-specific demethylase LSD1. Acute myeloid leukemia (AML) is characterized by neoplastic proliferation of immature myeloid cells which accumulate in the bone marrow and interfere with normal hematopoiesis. Standard therapy consists of induction and consolidation therapy to achieve hematological remission and complete eradication of tumor cells, respectively. One therapeutic strategy is differentiation induction of immature AML cells which may result in decreased proliferation rates, apoptosis and/or enhanced chemotherapeutic sensitivity. In addition, appropriately differentiated AML cells may be able to regain APC functions such as presentation of tumor antigens to the immune system and enhancement of an anti-tumoral immune response.

To explore a potential rationale for 4SC-202 as AML therapy, we investigated the impact of 4SC-202 on the differentiation of AML cell lines THP-1, HL-60 and MOLM-13.

4SC-202 increases histone acetylation at promoters of differentiation genes. THP-1 cells were treated with increasing doses of 4SC-202 for 24h. Promoter histone acetylation was analyzed by chromatin immunoprecipitation (ChIP) detecting acetylated lysine 9 and 27 on histone H3 (H3K27ac, H3K9ac). Quantification is shown as mean % input of 2 technical ChIP replicates with corresponding standard deviation compared to IgG control (dotted line). qPCR amplicon location is shown in respective genomic loci of CD86 and ITGAM (CD11b).

4SC-202 induces differentiation genes

4SC-202 increases CD86 and CD11b protein levels in AML cells. AML cell lines THP-1 and HL-60 were treated with 4SC-202 or vehicle (DMSO 0.1%) for 24h and protein expression levels were analyzed by flow cytometry. Bars represent mean fluorescence intensity (MFI) with standard deviation of 3 biological replicates. Isotype control was used to analyze unspecific background signal.

4SC-202 is more efficacious in differentiation than reference compounds. AML cell lines THP-1 and HL-60 were treated with 4SC-202 [1 µM], ATRA [20 µM], Bexarotene [10 µM], GM-CSF [10 ng/mL], TNF-α [10 ng/mL], IFN-γ [10 ng/mL] or vehicle (DMSO 0.1%) for 24h. Protein expression levels were analyzed by flow cytometry. Bars represent mean fluorescence intensity (MFI) with standard deviation of 3 biological replicates.

4SC-202 favors a mature APC phenotype

• Mature antigen-presenting cells (APC):
  • MHC molecules for antigen presentation
  • co-stimulatory signal
e.g. HLA-DR
e.g. CD86

4SC-202 in combination with IFN-γ induces a CD86highHLA-DRhigh phenotype. AML cell lines THP-1, MOLM-13 and HL-60 were treated with 4SC-202 [3 µM] and/or interferon-γ (IFN-γ) [10ng/mL] for 24h. Protein expression levels were analyzed by flow cytometry.

A: Representative density plots for CD86 and HLA-DR fluorescence signal intensity.
B: Quantification of CD86highHLA-DRhigh. Bars represent mean fluorescence intensity (MFI) with standard deviation of 3 biological replicates.

Conclusions

4SC-202 in AML cells:
• Increases promoter histone acetylation of differentiation genes
• Induces expression of differentiation genes superior to reference compounds
• Promotes mature antigen-presenting cell (APC) phenotype in combination with IFN-γ

preliminary rationale for consolidation therapy of AML patients:

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