CHARACTERIZATION OF MOLECULAR MECHANISM RESPONSIBLE FOR *FLT3* GENE OVEREXPRESSION IN ACUTE LEUKEMIAS

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INTRODUCTION

FLT3 overexpression is a recurrent event in many high-risk subtypes of acute leukemia. *FLT3* activating mutations (*FLT3*-AM) explain only a small fraction of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) cases with *FLT3* overexpression, supporting the idea that there are other regulatory mechanisms responsible for this aberrant profile (Figure 1).

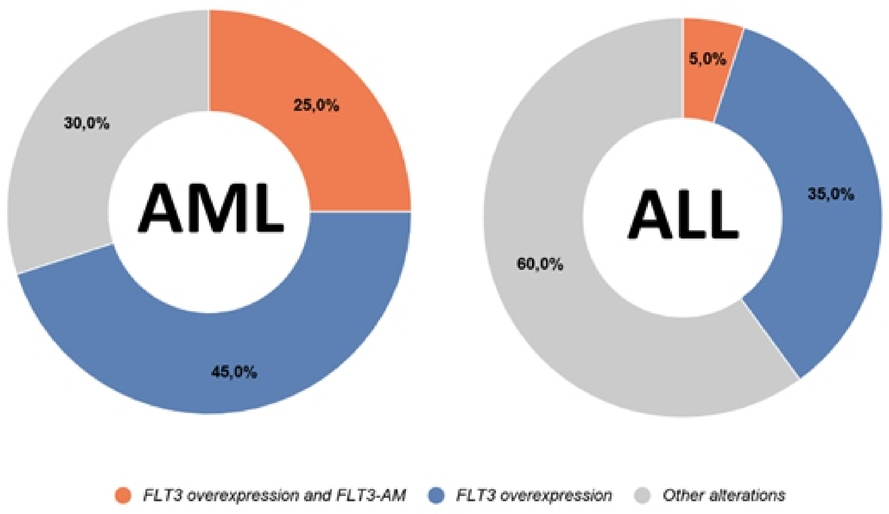

Figure 1. Frequency of *FLT3* overexpression in AML and ALL patients.
(Carow et al, 1996; Gilliland & Griffin, 2002; Armstrong et al, 2002; Libura et al, 2003; Garza-Veloz et al, 2015)

Recent studies have described that somatically acquired mutations in non-coding regulatory regions can create neomorphic enhancers leading to an aberrant expression of critical oncogenes. Therefore, we hypothesized that neomorphic enhancers could be a possible molecular mechanism responsible for *FLT3* overexpression both ALL and AML lacking *FLT3*-AM (Figure 2).

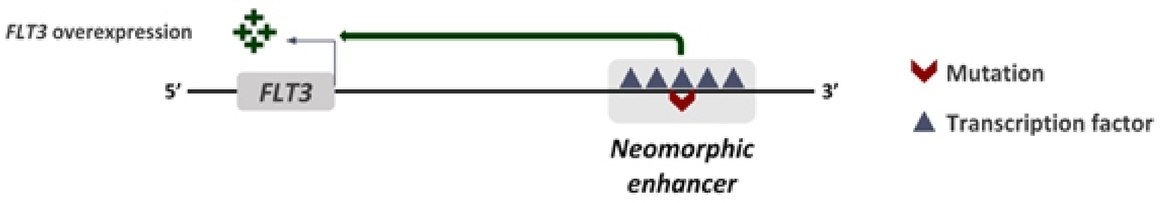

Figure 2. Schematization of the hypothesized mechanism responsible for *FLT3* overexpression.

METHODS AND RESULTS

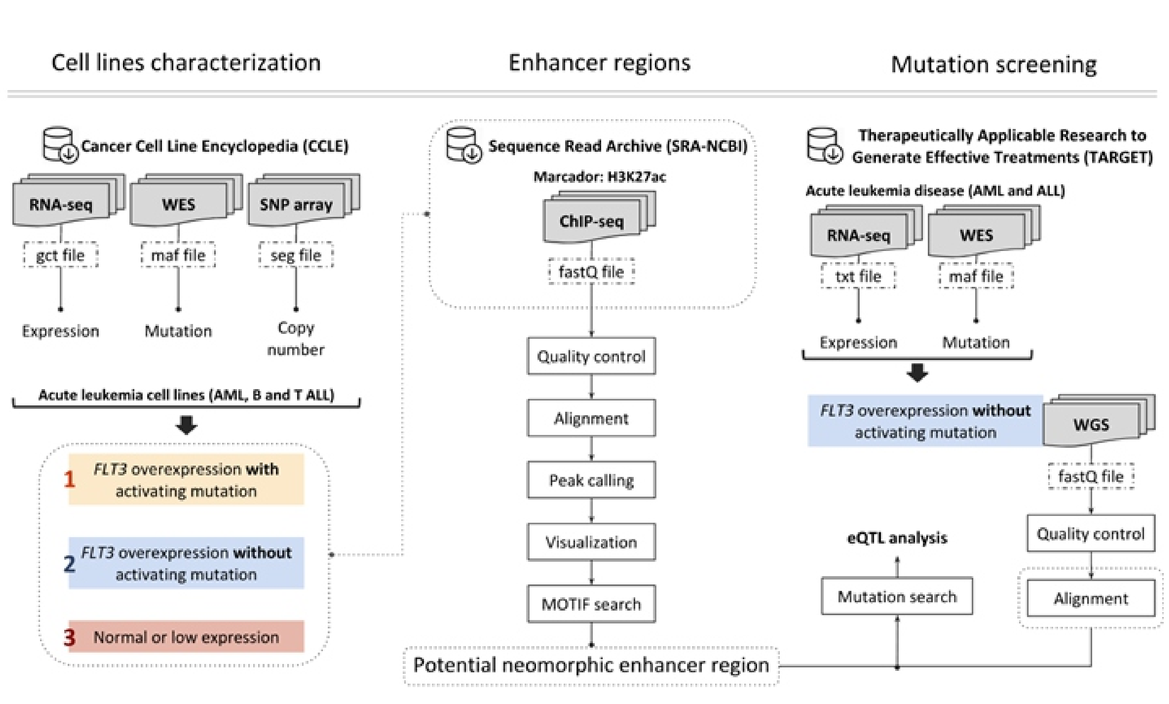

Figure 3. Design of the study.

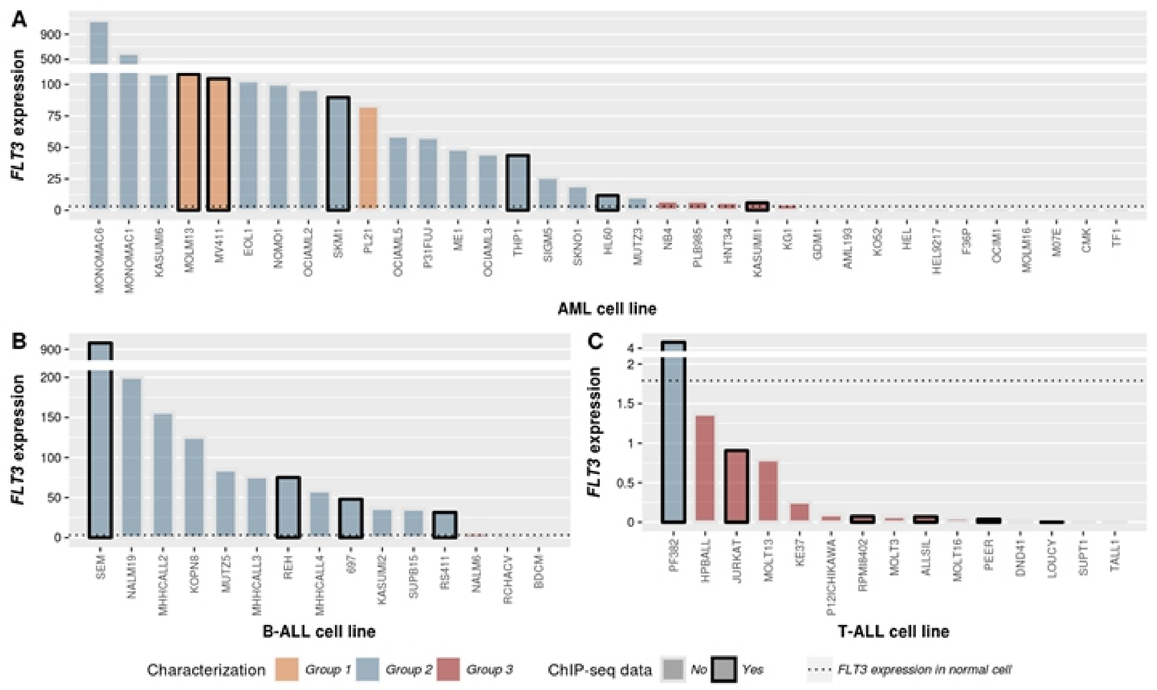

Figure 4. Characterization of leukemia cell lines. Bar graphs demonstrating the *FLT3* expression in AML **(A)**, B-ALL **(B)** and T-ALL **(C)** cell lines. Group 1: cell lines with *FLT3* overexpression and activating mutation (orange); Group 2: cell lines with *FLT3* overexpression but not activating mutation (blue); Group 3: cell lines with *FLT3* normal or low expression (red). Healthy bone marrow (for AML and B-ALL) or thymus (for T-ALL) samples were used as a calibrator for determination of *FLT3* overexpression. Due to data availability, six AML, four B-ALL and six T-ALL cell lines were evaluated for H3K27ac Chip-Seq (black border).

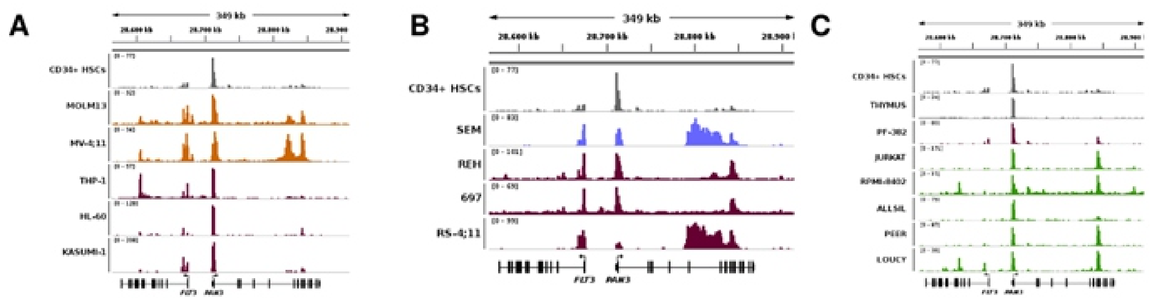

Figure 5. ChIP-Seq Analysis. ChIP-seq tracks for H3K27ac at the *FLT3* and adjacent *PAN3* gene locus in AML **(A)**, B-ALL **(B)** and T-ALL **(C)** cell lines that over-expresses *FLT3* with activating mutations (orange), with amplification (light purple) or without alterations (dark purple). Normal or low expression were representing by green peaks. We used human normal hematopoietic stem cell (CD34+ HSCs) and thymus cells as control.

CONCLUSION

Our initial data have shown the presence of a potential neomorphic enhancer region in cell lines with *FLT3* overexpression, however, further analyses are required to confirm these evidences. For this, ChIP-seq data sets of DNase hypersensitivity and other histone-modifications will be analysed. Then, we will search for alterations with potential neomorphic enhancer formation in this non-coding regulatory region. The results will be validated by *in vitro* assays in order to demonstrate the association between the neomorphic enhancer with *FLT3* overexpression.

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