# In silico Modeling of RNA-Based Spiegelmer Targeting Siglec-6 Receptors in Acute Myeloid Leukemia (AML)

RISHIMA ROUT

*Rishima Rout, Montgomery High School, Skillman, NJ*

*Published, December 2025*

**Acute Myeloid Leukemia (AML) is a form of blood cancer in which myeloid progenitor cells mutate into abnormal myeloid blasts that multiply quickly, take up space within the bone marrow, and prevent the production of normal blood cells. Siglec-6 is a receptor strongly expressed on the surface of these cancer stem cells. Spiegelmers are synthetic mirror-image RNA aptamers of L-RNA that bind specifi- cally to target molecules and exhibit high stability against nucleases. These spiegelmers could bind to the Siglec-6 receptor and, due to their L-form, may potentially evade nuclease degradation, thereby providing a foundation for novel targeted therapeutics. The 3D structure of Siglec-6 was modeled using AlphaFold 3 based on its amino acid sequence. The potential binding pockets on the surface of the protein were computed using P2Rank and ScanNet. Spiegelmers (S1=S5) were designed and structurally modeled using a multi-step process that included UNAfold for secondary structure prediction and ROSIE for tertiary structure modeling. Molecular docking simulations between Siglec-6 and spiegelmers were conducted to assess binding affinities and structural compatibility. Among the candidates, Spiegelmer S1 exhibited the most favorable interaction with Siglec-6, suggesting its potential as a lead molecule for targeted therapy. This computational pipeline provides a foundational framework for developing spiegelmer-guided CAR T-cell or aptamer-based therapies for AML, advancing precision medicine in hematologic oncology.**

## 1. INTRODUCTION

Leukemia is a form of cancer that affects blood cells (usually WBC) and the production of blood cells within the bone marrow. [1] The most common manifestation of leukemia are cases where an excessive amount of defective white blood cells are created, usually from a mutation that tells the malformed WBC to continue growing and dividing, and take up space inside the bone marrow and prevent the correct blood cells from being produced. Acute myeloid leukemia is a fast-growing leukemia that begins within the bone marrow and often spreads to the blood soon after. [5] AML typically affects myeloid progenitor cells that would turn into white blood cells. Symptoms often include general symptoms like fatigue, weight loss, fevers, and loss of appetite. [6] However, there are several more specific signs usually caused by the low blood cell count, such as anemic symptoms like weakness, feeling cold or lightheaded, headaches, shortness of breath, and/or paler skin. Symptoms caused by low WBC count include frequent or longer-lasting infections, and symptoms caused by low platelet count can include excessive bleeding/bruising. Leukemia cells known as blasts can clog blood vessels and block blood from travelling through the body, causing leukostasis, a rare condition with stroke-like symptoms like weakness in one side of the body, slurred speech, confusion, or sleepiness. Blasts can build up in other areas that the cancer could spread to, causing swelling and excessive bleeding in those areas. Current treatments include chemotherapy, targeted therapy, non-chemotherapy drugs, stem cell transplantation, or minor radiation therapy/surgery, all of which aim to put AML in complete remission where healthy blood cell counts are back to normal. Treatment usually occurs in stages of remission induction, consolidation, and maintenance. Issues such as myelosuppression (regular numbers of blood cells) can occur due to treatment. Many cancer treatments are taxing on the body, possibly creating more issues like further health fluctuation such as in chemotherapy treatment. Additionally, stem cell transplants require there to be a donor, and not every person that has AML can be a candidate. All treatments face the risk of not helping fix the illness, and even when they help make symptoms go into remission, cancer can still recur within the body. Siglec-6 is a receptor within the plasma membrane of hematopoietic stem and progenitor cells. Siglec-6 is expressed strongly on the surface of cancerous HSPCs.

Spiegelmers, otherwise known as L-RNA aptamers, are named as such because they are mirror-image L-nucleotides that act similarly to antibodies by binding to receptors on cells. These help identify harmful cells that aren't technically foreign to the body, like myeloid blasts. Spiegelmers, as mirror-image L-aptamers, display greater plasma stability compared to regular

D-aptamers, and the body doesn't break them down so quickly. This allows a greater positive impact because they last longer.

Nunes et al. have found that Siglec-6 gets overexpressed on the surface of the cancer cell and could be used as a potential target for AML cell inhibition. In this research we have designed spiegelmers that can not only bind to the Siglec-6 but can also prevent cancer cell activation. In addition, these spiegelmers are non reactive to nuclease enzyme thus are more stable than conventional aptamers.

## 2. METHOD

UniProt is an online resource that catalogues protein sequences along with the information about those protein chains. [7] We used it to copy the amino acid sequence below to model protein chains by searching for Siglec-6 in humans, which has a UniProt ID of 043699, then going to 'Sequence and Isoforms' and down- loading the amino acid sequence of isoform 043699. AlphaFold 3 is an AI tool that predicts the structure of a biomolecule chain like the one above and generates a model. [3] We used it to see a 3-D model of Siglec-6 by inputting the amino acid sequence. PROTTER is a resource that has 2-D diagrams of proteins that show how they interact with the cell membrane. We use it to access a labelled diagram of siglec-6 to see which parts of this receptor are outside of, within, or inside a virus's membrane. P2Rank is a program that predicts the ligand binding sites of proteins. We used it to simulate the structure of the molecule, and found no pockets in the structure. We first pasted the APT- MER sequence into the RNA Folding Form in UNAFold Web Server. [4] Leaving all settings as is, we then clicked 'Fold RNA' and then downloaded the CT file. Next, we opened RNA Struc- ture and selected the CT file that was downloaded. Designating the email address before submitting the query, we then copied the dot and bracket form. Then, going onto ROSIE, we pasted both the APTMER sequence and the dot and bracket form, leav- ing all other settings as is, then submitted the job. Lastly, we opened HDOCK and uploaded both the receptor PDB file and the ligand PDB file to their respective sections, before entering an email and job name then submitting the job. [2] The molecular docking simulation used (HDOCK) is a computing simulator that predicts the orientation of ligands when they bind to proteins. We used it to visualise the most likely binding patterns of the ligand with the receptor. Five patterns stood out most, which were then inputted into the Protein-Ligand Interaction Profiler site, and subsequently used the Binding Energy site IEM to gauge the strength of interaction between the receptor siglec-6 and the Spiegelmers. S5 proved to be the best match with the most negative binding energy. NUPACK was used to estimate the free energy of each variation's structure.

## 3. RESULTS

This research shows the compatibility of different binding pat- terns of spiegelmers with the intended siglec-6 receptor. These results discuss the process of finding a way to identify myeloid blasts by binding an aptamer to a receptor that is overexpressed on these cancerous cells (siglec-6). Figure 3 shows the location (in relation to the membrane of a cell) of the receptor siglec-6, which was found using PROTTER.

Figure 4 shows the charges of the external section of siglec-6. Figure 4a shows the most likely locations to bind; the predicted binding site is the most positive area on siglec-6, Figure 4b. Figure 5 shows the secondary structures of spiegelmers to help give context for the shape of each of the sets S1-S5. Figure 6 shows the molecular docking structures of siglec-6 and spiegelmers, simulated using the online HDOCK model. We used this to provide a physical representation of the positions of each receptor and aptamer orientation in each pairing. Figure 7 displays the number of hydrogen bonds and salt bridges between the spigelmer and siglec-6 at the five binding sites selected. We used PLIP webserver to compare the number of interactions between each set to find they were all fairly similar. Figure 8 displays the binding energy of each of the five orientations compared to each other. The software used was PRODIGY, which we used to graph each trial's binding energy, while the data was collected from the IEM Binding Energy site.

## 4. DISCUSSION

Acute Myeloid Leukemia (AML) remains one of the most clin- ically challenging hematologic malignancies due to its hetero- geneity, high relapse rates, and limited availability of durable targeted therapies. Despite advancements in genomic profiling and the introduction of FLT3, IDH1/2, and BCL2 inhibitors, a considerable subset of patients fail to achieve complete remission or develop treatment resistance. Therefore, developing novel molecular strategies that selectively recognize and eliminate leukemic stem cells (LSCs) is essential for improving therapeutic outcomes. In this study, we investigated the potential of RNA- based spiegelmers—chemically mirrored L-RNA aptamers—as targeted ligands for the SIGLEC-6 receptor, which is strongly overexpressed on AML-associated hematopoietic stem and pro- genitor cells (HSPCs). Because SIGLEC-6 displays restricted expression on normal blood and immune cells but is signifi- cantly elevated in leukemic populations, it represents an appeal- ing therapeutic target. Our computational modeling pipeline, combining AlphaFold3-based structural prediction, P2Rank and ScanNet binding pocket identification, ViennaRNA/UNAfold secondary structure modeling, and HDOCK docking simula- tions, enabled us to systematically evaluate the binding potential of five spiegelmer candidates (S1–S5).

Mechanistic Interpretation ofSpiegelmer–SIGLEC-6 Binding. The electrostatic analyses revealed a consistently positive binding interface on SIGLEC-6, particularly around the extracel- lular immunoglobulin-like domains. Spiegelmers, being polyan- ionic RNA molecules, are naturally predisposed to interact with positively charged pockets. The docking results aligned with this expectation: all modeled spiegelmers made interactions within the predicted positively charged surface region.

Our computational docking results showed that all five spiegelmers formed comparable numbers of hydrogen bonds and salt bridges with SIGLEC-6, indicating general compatibility and stable interaction potential. However, spiegelmer S5 demonstrated the most favorable binding energy profile, suggesting a tighter and potentially more specific interaction. This increased affinity may be attributed to its more stable secondary structure, enhanced base stacking interactions, or more optimal positioning of functional groups in its tertiary RNA fold.

This finding is notable because spiegelmers, due to their L-RNA configuration, are not recognized by endogenous nucleases. Therefore, a spiegelmer such as S5, which binds tightly to SIGLEC-6 while maintaining robust structural stability, is

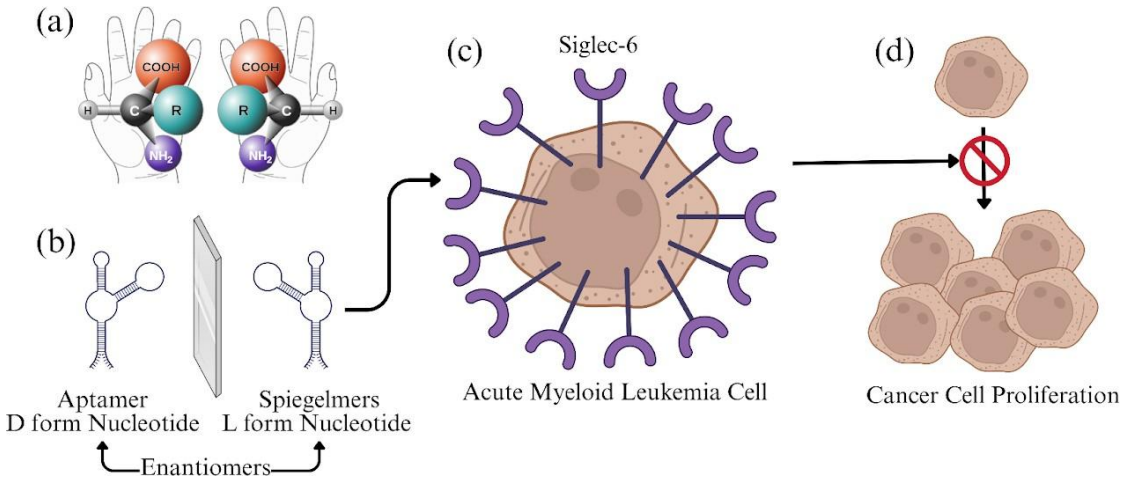


**Fig. 1.** The process of targeting cancer cells. A/B: The molecular structure of an aptamer and a spiegelmer, both of which are reflections of each other (enantiomers). C: A myeloid blast, with an abundance of siglec-6 receptors on it. D: By binding to these receptors, spiegelmers prevent continued cancer growth.

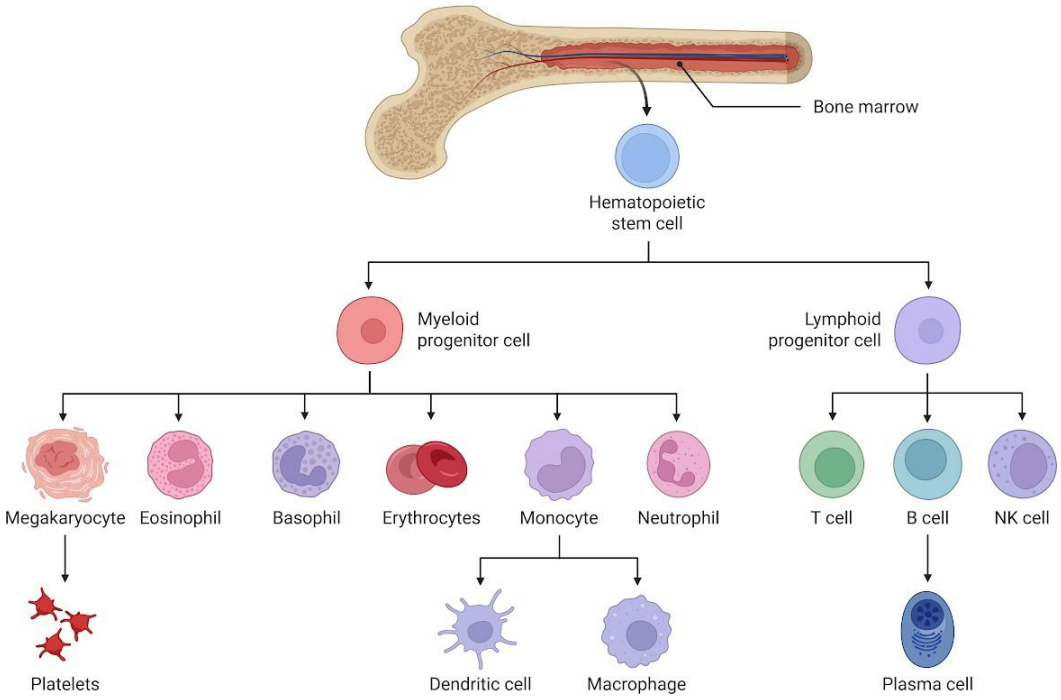


**Fig. 2.** Classification of hematopoietic stem cells in bone marrow. Acute Myeloid Leukemia is formed in Myeloid progenitor cells, and causes the receptor siglec-6 to be over-expressed, providing a prime target for detecting and treating AML—figure created with Biorender software.

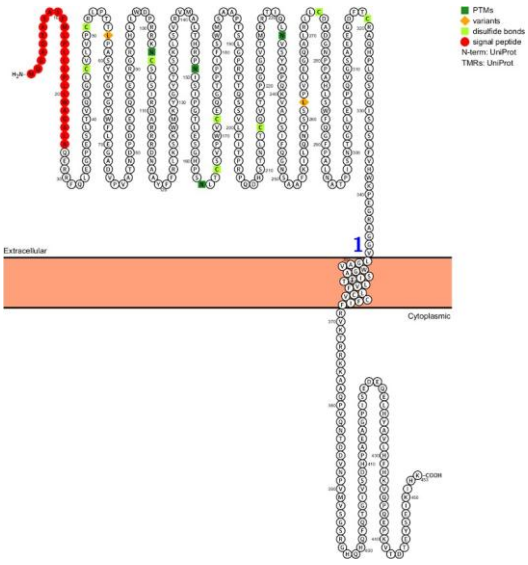


**Fig. 3.** The location of siglec-6 outside, within, and inside the membrane of a cell. Figure created with PROTTER software.

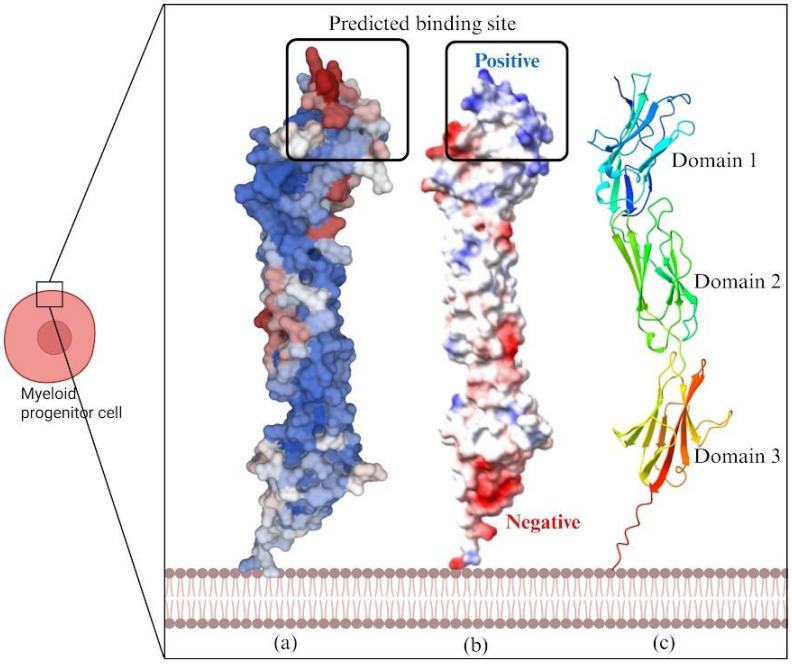


**Fig. 4.** (a) the predicted binding site of siglec-6 in the box, generated by Scannet; (b) electrostatic surface potential (the charge of the protein), with red as negative and blue as positive, generated by ChimeraX software; (c) The three domains of siglec-6, generated by ChimeraX.

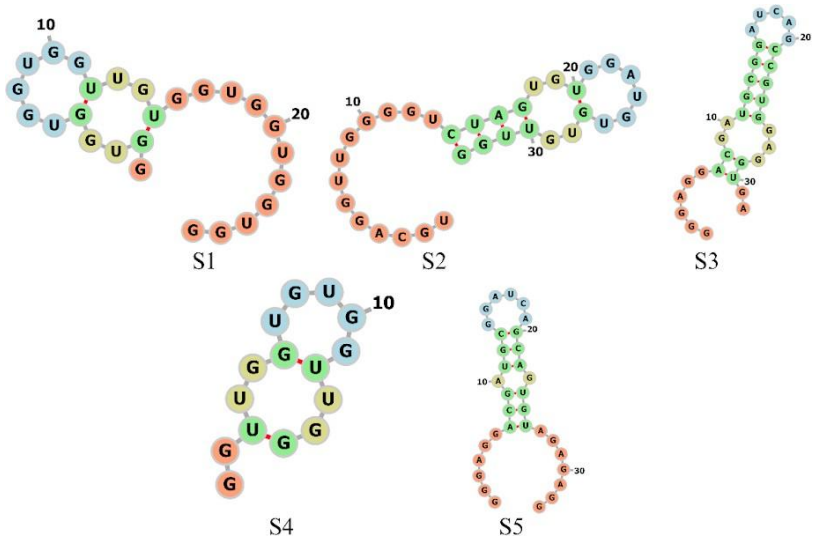


**Fig. 5.** The secondary structure ofspiegelmers S1-S5. Figure generated with ViennaRNA

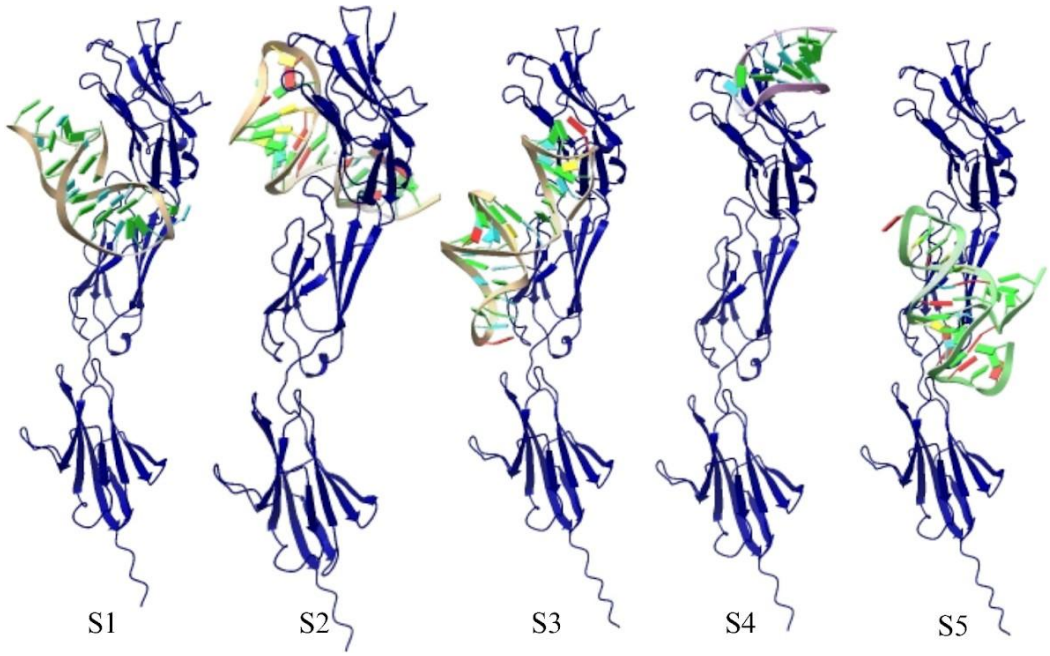


**Fig. 6.** The molecular docked structure of siglec-6 (in blue) andspiegelmers (in brown) used in this research. Figure generated by HDOCK.

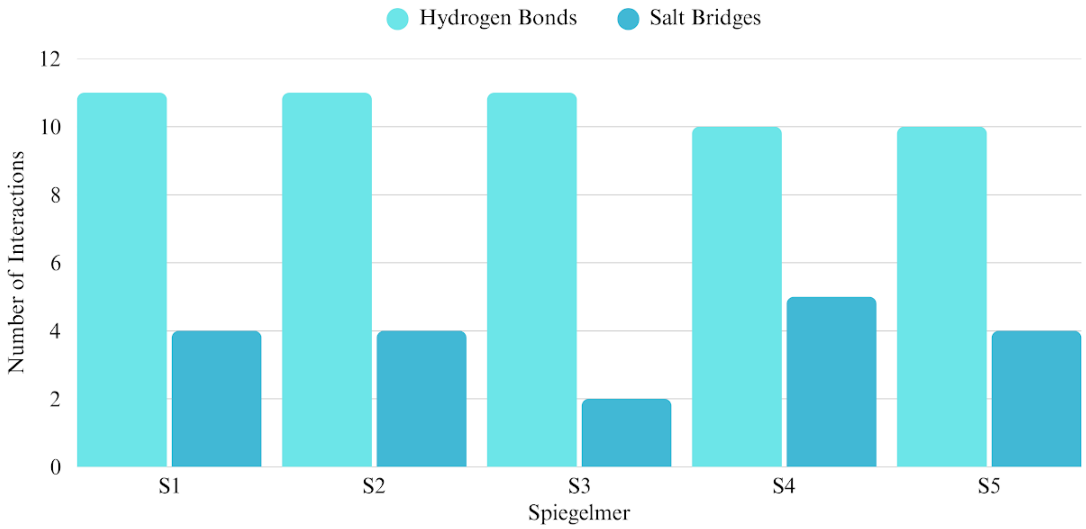


**Fig. 7.** The number of interactions between siglec-6 and 5spiegelmers (L-RNA aptamers). All spiegelmers formed a similar number of interactions—figure generated by PLIP webserver.


**Fig. 8.** The binding energy of spiegelmers S1 through S5. S5 is clearly proven to have the least binding energy, being the most appropriate candidate. Figure generated with PRODIGY.

particularly well suited for therapeutic development.

## 5. CONCLUSION

In this study, we have demonstrated the potential of RNA-based spiegelmers as a targeted therapeutic strategy for AML by computationally modeling their interactions with SIGLEC-6 receptors. The selected spiegelmers are promising candidates with the strongest binding affinity for the cancer receptor; hence, they will bind strongly to the cancer cell. In addition, it is also stable against the nuclease enzyme, thus has a longer half-life. This work provides a framework for the rational design of spiegelmer-guided therapies that may contribute to precision medicine approaches in blood cancer.

## REFERENCES

1. G. Juliusson and R. Hough, Leukemia. Prog Tumor Res **43**, 87–100 (2016).
2. Y. Yan, D. Zhang, P. Zhou, B. Li, and S. Y. Huang, "HDOCK: a web server for protein-protein and protein-DNA/RNA docking based on a hybrid strategy," Nucleic Acids Res **45**(2017).
3. J. Abramson, J. Adler, J. Dunger, R. Evans, T. Green, A. Pritzel, . . Jumper, and J. M, "Accurate structure prediction of biomolecular interactions with AlphaFold 3," Nature **630**, 493–500 (2024).
4. N. R. Markham and M. Zuker, "UNAFold," in "Bioinformatics: Structure, Function and Applications," J. M. Keith, ed. (Humana Press, 2008), pp. 3–31.
5. F. Bayón-Calderón, M. L. Toribio, and S. González-García, "Facts and Challenges in Immunotherapy for T-Cell Acute Lymphoblastic Leukemia," Int J Mol Sci **21** (2020).
6. C. H. Pui and W. E. Evans, "Treatment of Acute Lymphoblastic Leukemia," New England Journal of Medicine **354**, 166–178 (2006).
7. T. U. Consortium, "UniProt: a hub for protein information," Nucleic acids research **43** (2014).