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EFFICACY OF VENETOCLAX AND AZACITIDINE FOR TARGETING LEUKEMIC STEM CELL IN ACUTE MYELOID LEUKEMIA

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ABSTRACT

Venetoclax in combination with Azacitidine has demonstrated the ability to regulate cell survival, with tolerable safety and favourable overall responses in older adult patients with AML. This novel combination regimen has shown efficacy even in high risk groups, such as those aged 75 or older, patients with poor cytogenetics, and those with secondary AML. The addition of Venetoclax enhanced the effect of Azacitidine on in vivo survival, and this combination has been effective in disrupting the metabolic machinery that drives energy metabolism, leading to the eradication of leukemia stem cells. In this study, the growth of hematopoietic and leukemic stem cells were analyzed using a mathematical model that considers the bone marrow as a system of "well-mixed" tanks of cells at different stages of differentiation. Mathematical analysis focused on parameters such as self-renewal, proliferation, and apoptosis, assessing the impact of Venetoclax in combination with Azacitidine. The B-cell lymphoma 2 (BCL-2) protein is critical for the survival and persistence of AML blasts, and Venetoclax targets this protein to regulate cell survival. In AML, overexpression of BCL-2 contributes to the survival of leukemic cells, including AML blasts. Azacitidine, on the other hand, works by inhibiting DNA methylation and promoting cell death.

Overall, the combination of Venetoclax and Azacitidine offers a promising therapeutic approach for AML, particularly in older adults and high risk patient groups. By targeting key pathways involved in cell survival and metabolism, this combination regimen holds potential for improving outcomes in AML patients.

Keywords: Venetoclax; Azacitidine; Acute Myeloid Leukaemia (AML); Hematopoietic Stem Cells (HSC); Leukemic Stem Cells (LSC)

INTRODUCTION:

Acute myeloid leukemia (AML) is a challenging disease, particularly in older adults, who commonly experience poorer responses to standard induction chemotherapy due to adverse genomic features and increased treatment resistance. Additionally, comorbidities and compromised organ function often render older patients ineligible for aggressive cytotoxic treatments. Lower-intensity regimens, such as hypomethylating agents (HMAs) like azacitidine or decitabine, or low-dose cytarabine, are typically utilized in elderly AML patients unfit for intensive chemotherapy **[14, 17, 28]**. However, these treatments yield modest response rates, take several months to achieve the best response, and are not curative, with a median overall survival of less than one year. Given these limitations, there's a pressing need for targeted therapies that can rapidly induce high rates of clinical response, with improved tolerability and durable outcomes for elderly AML patients. The B-cell lymphoma 2 (BCL-2) protein is integral to the survival and persistence of AML blasts, regulating the mitochondrial apoptotic pathway **[3, 9]**. BCL-2 maintains myeloblast survival by inhibiting the pro-apoptotic protein BAX, thus preventing mitochondrial outer membrane permeabilization and subsequent cell death. Venetoclax, an oral inhibitor of BCL-2, has shown promising clinical activity and a tolerable safety profile as a single agent in patients with relapsed or refractory AML. The development of targeted therapies like venetoclax represents a significant step forward in addressing the unmet needs of elderly AML patients. Further research and clinical trials exploring the efficacy of venetoclax, alone or in combination with other agents, hold promise for improving outcomes in this challenging patient population **[11, 16, 20, 24]**.

Acute myeloid leukemia (AML) poses significant challenges in elderly patients, often presenting with adverse genomic features and resistance to standard induction chemotherapy. Additionally, older patients may have comorbidities and compromised organ function, making them unsuitable for aggressive treatment options. In such cases, lower-intensity regimens become the standard of care. Hypomethylating agents

(HMAs) like azacitidine or decitabine, along with low-dose cytarabine, are commonly used in these situations. However, the response rates with these therapies are modest, and achieving the best response may take several months. Furthermore, these treatments are not curative, and the median overall survival remains less than one year. Given these challenges, there is a critical need for the development of targeted therapies tailored for elderly AML patients. These therapies should aim to induce rapid and durable clinical responses with better tolerability. Research efforts are underway to address this unmet need and improve outcomes for this vulnerable patient population. The B-cell lymphoma 2 (BCL-2) protein plays a crucial role in the survival and persistence of acute myeloid leukemia (AML) blasts by regulating the mitochondrial apoptotic pathway. BCL-2 maintains the survival of myeloblasts by binding to and sequestering pro-apoptotic BAX, thereby preventing mitochondrial outer membrane permeabilization and subsequent cell death. When BCL-2 is inhibited or antagonized, BAX is released, leading to mitochondrial outer membrane permeabilization and ultimately cell death.

Venetoclax is an oral inhibitor of BCL-2 that has shown promise in the treatment of relapsed or refractory AML. It works by selectively targeting and inhibiting BCL-2, thereby promoting apoptosis in AML blasts. Clinical studies have demonstrated that venetoclax as a single agent has significant clinical activity and a tolerable safety profile in patients with relapsed or refractory AML. This suggests that targeting BCL-2 with venetoclax may be an effective therapeutic strategy for AML patients, particularly those who have relapsed or are refractory to standard treatments. Further research is ongoing to explore the potential of venetoclax in combination with other agents or in different patient populations to optimize its efficacy in the treatment of AML.

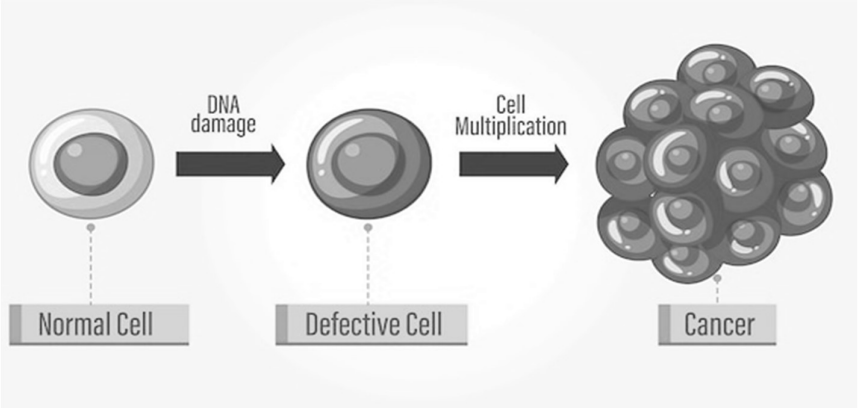

Figure 1: Process of cancer cell development

The combination of Venetoclax with Azacitidine has shown a synergistic effect in preclinical models of AML cells. Additionally, Azacitidine may reduce levels of MCL-1, an anti-apoptotic protein crucial in AML pathogenesis and a potential source of resistance to Venetoclax. Researchers have investigated the safety and efficacy of this combination in elderly patients with treatment-naive AML who are ineligible for intensive chemotherapy. Preliminary data from dose-escalation studies have been published, providing insights into the safety and potential effectiveness of this combination therapy. These preliminary findings suggest that combining Venetoclax with Azacitidine may offer a promising treatment option for elderly AML patients who cannot tolerate intensive chemotherapy. Further clinical trials are needed to validate these findings, determine optimal dosing regimens, and assess long-term outcomes. Nonetheless, the initial results indicate a potential new approach to improve outcomes for this challenging patient population **[4, 7, 22, 29]**. Venetoclax is a medication that targets the B-cell lymphoma 2 (BCL-2) protein, which plays a crucial role in regulating cell survival. In Acute Myeloid Leukemia (AML), the overexpression of BCL-2 contributes to the survival and persistence of leukemic cells, including AML blasts. Azacitidine is a chemotherapy drug commonly used in the treatment of AML, which works by inhibiting DNA methylation and promoting cell death. When Venetoclax is combined with Azacitidine, it enhances the effectiveness of treatment by targeting different pathways involved in AML cell survival **[2, 15, 19, 23, 26]**. Venetoclax inhibits the anti-apoptotic function of BCL-2, leading to programmed cell death (apoptosis) of leukemic cells. Azacitidine, on the other hand, disrupts DNA methylation and induces cell death in AML blasts **[11, 16, 20, 24]**.

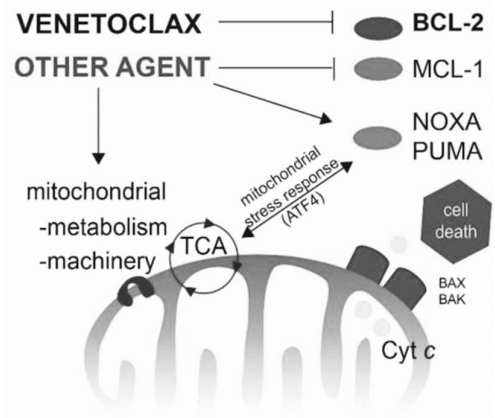

Figure 2: Pathway of targeting Venetoclax resistance

The combination of Venetoclax and Azacitidine has shown promising results in clinical trials, particularly in older adults with previously untreated AML or those ineligible for intensive chemotherapy **[1, 10]**. This combination therapy has been associated with higher response rates, including higher rates of complete remission, and improved overall survival compared to Azacitidine alone. Thus, the relationship between Venetoclax, Azacitidine, and the BCL-2 protein underscores the potential for targeted therapy to disrupt key survival pathways in AML and improve treatment outcomes **[6, 21]**.

The combination of Venetoclax with Azacitidine represents an example of inhibiting an antiapoptotic agent, as it releases the antiapoptotic brake, leading to re-sensitization of cells to apoptosis. Preclinical data strongly suggest synergistic effects when using Venetoclax and Azacitidine concurrently. Another strategy involves increasing the levels of proapoptotic proteins, which has shown promising preclinical activity when combined with Venetoclax. This approach aims to enhance the apoptotic response of cancer cells by promoting the activity of proteins that induce cell death. Furthermore, targeting mitochondrial metabolism to synergize with Venetoclax is another promising avenue. Mitochondria play a crucial role in both cell death and metabolism, and dysregulation of mitochondrial metabolism is often linked to changes in protein expression. Combining Venetoclax with agents that target mitochondrial metabolism may enhance its efficacy by disrupting the metabolic processes that support cancer cell survival. These approaches highlight the diverse strategies being explored to enhance the effectiveness of Venetoclax in the treatment of cancer, particularly in AML. By targeting multiple aspects of apoptotic regulation and cellular metabolism, combination therapies hold promise for improving outcomes in patients with hematologic malignancies **[12, 18, 25, 27]**.

The combination of Azacitidine and Venetoclax has been effectively used to treat patients with AML with disruption of the metabolic machinery driving energy metabolism leading to eradication of leukemia stem cells. For this study growth of hematopoietic and leukemic stem cells was studied as a system of "well-mixed" tanks of cells present in the bone marrow, considering the different stages of differentiation as compartments. Mathematical analysis for three parameters used for the analysis are self-renewal, proliferation, and apoptosis and the impact of Venetoclax in combination with Azacitidine have studied. In cancer patient B-cell lymphoma 2 (BCL-2) protein plays

an important role in the survival and persistence of AML blasts **[5, 8, 13]**. Venetoclax targets the B-cell lymphoma 2 (BCL-2) protein, which plays a crucial role in regulating cell survival. In Acute Myeloid Leukemia (AML), the overexpression of BCL-2 contributes to the survival and persistence of leukemic cells, including AML blasts. Azacitidine works by inhibiting DNA methylation and promoting cell death.

Mathematical Model:

In this study, the time evolution of cellular growth of the hematopoietic and leukemic cell lines was studied by considering a mathematical model. In the bone marrow, we considered a system of "well-mixed" pools of cells that are differentiating in different substages as compartments. The characteristics used for the analysis were self-renewal rate, proliferation rate and death rate. The classical model proposed by some researchers **[4, 9]** was also converted into a fractional order derivative based model, and results were obtained that were also consistent with the classical model. The understanding how fast the cells are growing and renewing themselves is a more important for finding ways to treat the disease. The following mathematical model developed by **[17, 23]** describes the dynamics of hematopoietic and leukemic cells in acute myeloid leukaemia based on three primary parameters, self-renewal rate (a_i^c, a_i^l), proliferation rate (p_i^c, p_i^l), and death rate (d_i^c, d_i^l). The mathematical model (Eq. 1-6) is based on understanding of the haematopoiesis process such that stages of cell differentiation are assumed to be compartments (ordered sequence of differentiation). The time-dependent ordinary differential equations were developed to describe the cell densities (or populations) of hematopoietic and leukemic cells Hematopoietic cell line:

$$\frac{dc_1}{dt} = \left(2a_{1,max}^c s(t) - 1\right)p_1^c c_1(t) - d_1^c c_1(t) \quad (1)$$

$$\frac{dc_i}{dt} = 2\left(1 - a_{i-1,max}^c s(t)\right)p_{i-1}^c c_{i-1}(t) + \left(2a_{i,max}^c s(t) - 1\right)p_i^c c_i(t) - d_i^c c_i(t) \quad (2)$$

$$\frac{dc_n}{dt} = 2\left(1 - a_{n-1,max}^c s(t)\right)p_{n-1}^c c_{n-1}(t) - d_n^c c_n(t) \quad (3)$$

Leukemic cell line:

$$\frac{dl_1}{dt} = \left(2a_{1,max}^l s(t) - 1\right)p_1^l l_1(t) - d_1^l l_1(t) \quad (4)$$

$$\frac{dl_i}{dt} = 2\left(1 - a_{i-1,max}^l s(t)\right)p_{i-1}^l l_{i-1}(t) + \left(2a_{i,max}^l s(t) - 1\right)p_i^l l_i(t) - d_i^l l_i(t) \quad (5)$$

$$\frac{dl_m}{dt} = 2\left(1 - a_{m-1,max}^l s(t)\right)p_{m-1}^l l_{m-1}(t) - d_m^l l_m(t) \quad (6)$$

The number of compartments is denoted by n. In the hematopoietic cell line, the first compartment denotes the hematopoietic stem cell population, while the n^{th} compartment denotes the post mitotic mature population.

The number of cell compartments in between 1 and n is denoted by i, where $i \in [2, n-1]$. Similarly, the first compartment in the leukemic cell line denotes the leukemic stem cell population, and the post mitotic mature blasts are denoted by m^{th} compartment. The cell densities of the hematopoietic cell population in the compartment j at time t are denoted by $c_j(t)(j = 1, 2, \ldots . n)$, while $l_j(t)(j = 1, 2, \ldots . n)$ denotes the cell densities for the leukemic cell population.

The classical time-based differential equations are based on the treatment of the cell cycle as a well-mixed population, from which cells may either proliferate at the rate $p(t)$ or die at the death rate d. For simplicity, the death rate can be considered zero for every compartment except the post mitotic cell compartment, i.e., n^{th} compartment.

For the i^{th}compartment where $i < n$, the flux to mitosis, in which the mother cell divides to produce two daughter cells, is given by $p_i(t)c_i(t)$, while the outflux to mitosis equals to $2p_i(t)c_i(t)$. In the following process, the fraction of cells that stay within compartment i, referred to as self-renewal, is given by $2a_i(t)p_i(t)c_i(t)$.

It is also assumed that $[1 - a_i(t)]$ is the probability of each daughter cell moving to the next compartment, while $a_i(t)$ fraction ensures that the cell population stays in the same compartment from where they have formed. Further, the fraction of cells that differentiate and move to compartment $i + 1$ is given by $2(1 - a_i(t))p_i(t)c_i(t)$. Cells in the n^{th} compartment have a zero-proliferation rate, but a non-zero death rate. Therefore, the cell population in the mature compartment depends on the flux of differentiated cells from $(n-1)^{th}$ compartment (1^{st}term) and the death of the mature cells (2^{nd}term) **[6, 24]**.

The negative feedback signal of cytokines regulates the formation of blood cells. Cytokines are crucial external signalling molecules in stem cells that regulate the dynamics of cell differentiation and proliferation, but their precise nature is still unknown. When released, cytokines such as erythropoietin (EPO) in erythropoiesis and granulocyte colony stimulating factor (G-CSF) for granulopoiesis in hematopoietic stem cells and NF-κB and phosphatidyl-inositide-3 kinase (PI3K) in leukemic stem cells regulate the growth of cells in the body **[4, 9]**.

The increase in the concentration of cytokines indicates that there is a need for

more blood cells of a certain type, which stimulates the formation of mature cells. It is also assumed that their densities depend majorly on postmitotic cell densities, and leukemic and hematopoietic cells respond to the same cytokines and complete for them. In the following model, cytokine is denoted by s(t) and given by:

$$s(t) = \frac{1}{1 + k_c c_n(t) + k_l l_n(t)} \in (0, 1] \qquad (7)$$

where k_c and k_l are positive constants.

Fractional ordered differential equation, in the recent times, has gained attention due to its ability to provide a better precision between the actual and simulated data as compared to the classical models. The fractional order derivative is advantageous due to its memory effect property, which indicates that the future state of the system depends on the current state as well as the past state **[23, 29]**. Fractional Derivative Equations (FDE) is not a new concept; it was introduced back in 1695 by Gottfried Leibniz in a letter written to Guillaume de L'Hôpital. Over the years, mathematicians, namely Riemann-Liouville, Caputo, Jumarie, Hadamard, and Weyl, have introduced their own definitions of fractional order derivatives with some advantages and disadvantages, but the best known is the Riemann-Liouville definition (Abu-Shady & Kaabar, 2021). The derivate of order is given by:

$$D_{0+}^{\alpha} f(t) = \frac{1}{\Gamma(1-\alpha)} \left(\frac{d}{dt}\right)^n \int_0^t \frac{f(s)}{(t-s)^{\alpha-n+1}} ds, \quad n = [\alpha] + 1, (8)$$

where $\alpha \in R, [n-1, n)$ and $0 < \alpha < 1$ for $n \in Q$, Γ is the gamma function, and $[\alpha]$ is the greatest integer value of α. Riemann–Liouville satisfies the linear property of fractional derivates, but failed to solve the differentiation of a constant value when replaced by Riemann–Liouville differential operator of order α.

$$D^{\alpha} c = \frac{c}{\Gamma(1-\alpha)} t^{-\alpha} \neq 0, \qquad \text{c = constant} \quad (9)$$

While the Caputo definition for FDE is as follows.

$$D_{0+}^{\alpha} f(t) = \frac{1}{\Gamma(1-\alpha)} \int_0^t \frac{f^n(s)}{(t-s)^{\alpha-n+1}} ds, \quad n = [\alpha] + 1, (10)$$

Following the Caputo type fractional derivative of order α, the modified model for stem cell growth of hematopoietic and leukemic cell lines is:

Caputo-fractional based hematopoietic cell line:

$$\frac{d^{\alpha} c_1}{dt^{\alpha}} = \left(2\left(a_{1,max}^c\right)^{\alpha} s(t) - 1\right)(p_1^c)^{\alpha} c_1(t) - (d_1^c)^{\alpha} c_1(t) \qquad (11)$$

$$\frac{d^{\alpha} c_i}{dt^{\alpha}} = 2\left(1 - (a_{i-1,max}^c)^{\alpha} s(t)\right)(p_{i-1}^c)^{\alpha} c_{i-1}(t) + \left(2(a_{i,max}^c)^{\alpha} s(t) - 1\right)(p_i^c)^{\alpha} c_i(t) - (d_i^c)^{\alpha} c_i(t) \qquad (12)$$

$$\frac{d^{\alpha} c_n}{dt^{\alpha}} = 2\left(1 - (a_{n-1,max}^c)^{\alpha} s(t)\right)(p_{n-1}^c)^{\alpha} c_{n-1}(t) - (d_n^c)^{\alpha} c_n(t) \qquad (13)$$

Caputo-fractional based leukemic cell line:

$$\frac{d^{\alpha}l_1}{dt^{\alpha}} = \left(2\left(a^{l}_{1,max}\right)^{\alpha}s(t) - 1\right)(p^{l}_{1})^{\alpha}l_1(t) - \left(d^{l}_{1}\right)^{\alpha}l_1(t) \quad (14)$$

$$\frac{d^{\alpha}l_i}{dt^{\alpha}} = 2\left(1 - \left(a^{l}_{i-1,max}\right)^{\alpha}s(t)\right)(p^{l}_{i-1})^{\alpha}l_{i-1}(t) + \left(2(a^{l}_{i,max})^{\alpha}s(t) - 1\right)(p^{l}_{i})^{\alpha}l_i(t) - \left(d^{l}_{i}\right)^{\alpha}l_i(t) \quad (15)$$

$$\frac{d^{\alpha}l_m}{dt^{\alpha}} = 2\left(1 - \left(a^{c}_{m-1,max}\right)^{\alpha}s(t)\right)(p^{l}_{m-1})^{\alpha}l_{m-1}(t) - (d^{l}_{m})^{\alpha}l_m(t) \quad (16)$$

The above model is based on the simple dimensional analysis that both, left-hand and right-hand sides have the same dimension of $(\text{time})^{-\alpha}$. To maintain the dimensionality, we introduced the order α on the constants, viz., self-renewal rate, proliferation rate, and death rate, on the right-hand side, and changed the order of differentiation to α on the left-hand side.

RESULTS AND DISCUSSION:

In **Figure 3**, the hematopoietic and leukemic cell lines are assumed to be a 2-compartment system. In a hematopoietic cell line, hematopoietic stem cells are immature cell types in the first compartment, and the second compartment comprises post-mitotic mature cells. Similarly, leukemic stem cells and mature blasts are the first and second compartment cells, respectively, in a leukemic cell line. The cell lines are regulated by negative feedback signalling from cytokines. In this case, we re-analysed the necessary properties for the establishment of a leukemic stem cell population.

The following example highlights the establishment of a leukemic cell line where leukemic stem cells have an enhanced self-renewal rate and a decreased proliferation rate compared to healthy stem cells.

The initial conditions were set to values of healthy equilibrium, while the leukemic compartments have 10 and 0 cells, respectively. The results have shown a significant decrease in the cell number for hematopoietic cells, while the leukemic cell line shows an increase in the cell count over the course of 3500 days. The cytokine density also decreased, which indicates the decline of healthy cell lines and the establishment of leukemic cell lines. For the same problem, a fractional-ordered model has been developed and numerically solved using predictor-corrector algorithms **[3, 16]**. The fractional-ordered based solution for the following scenario has shown consistency in results with the classical model. Here, the values of α were 0.9, 0.85, and 0.8, and with the decreasing α, the stability curves shift with increasing time with no significant change in the count of cells **(Figure 3)**.

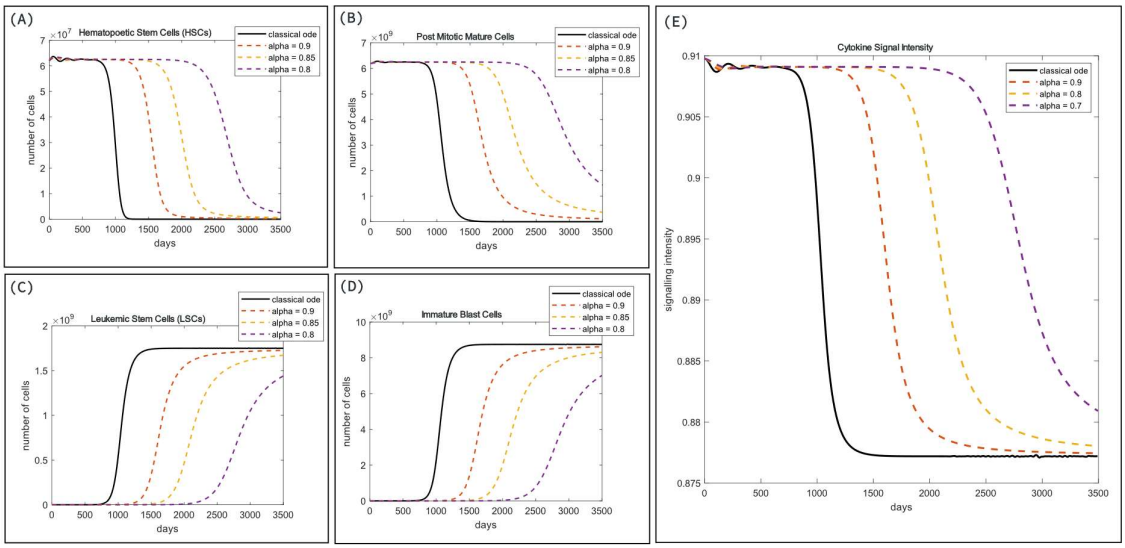

Figure 3: FDE solution for the steady state establishment for leukemic cell line and extinction of hematopoietic cell line for 2-compartment system

In **Figure 4**, the self-renewal fractions for both cell lines are assumed to be equal, i.e., they have equal ability of self-renewal. However, the proliferation rate for the hematopoietic cell line is double than that of the leukemic cell line **[14, 28]**. The following result depicts the coexistence of leukemic and hematopoietic cell lines with a negligible fluctuation in the cell lines as well as the cytokine signal intensity. But on a clinical scale, these changes are invisible. It can also be said that the hematopoietic cell line is co-existing with the leukemic cell line while the parameters are in favour of the former cell line.

The leukemic stem cells show reduced self-renewal and enhanced proliferation compared to the hematopoietic stem cells. However, the enhanced proliferation could not compensate for the reduced self-renewal potential. The numerical solution indicates a negligible change in the hematopoietic cell counts, which thus remained constant after minimal fluctuation. A similar pattern was also observed for the cytokine signal intensity. On the other hand, leukemic cell counts drastically decreased in a short time **[12, 24]**. The following result is an indication of the importance of self-renewal potential for the establishment of cell lines. The number of cells was observed over a period of 1000 days in both hematopoietic and leukemic cell lines to underline the effect of self-renewal and proliferation rates. In the following simulation, the self-renewal rate for leukemic stem cells (LSCs) was higher than that of hematopoietic stem cells (HSCs), while the reverse was the case with leukemic and hematopoietic progenitor cells (LPCs and HPCs) **[13, 28]**. The proliferation rate for hematopoietic cell lines was lower than that of leukemic cell lines. The resultant

simulation depicts the decline in the growth of hematopoietic cell lines and enhanced leukemic cell lines. And if cell counts of all compartments are compared for both cell lines, the growth of HPCs and LPCs in their respective cell lines is much greater than that of other compartments **[4, 16]**.

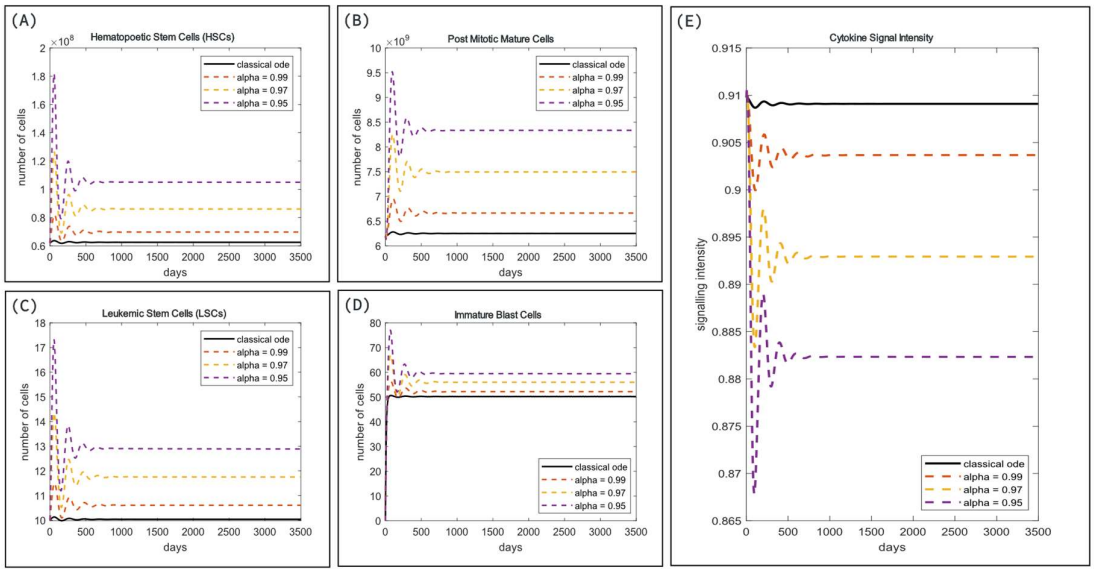

Figure 4: FDE solution for state of equilibrium when hematopoietic and leukemic cells are coexisting for 2-compartment system

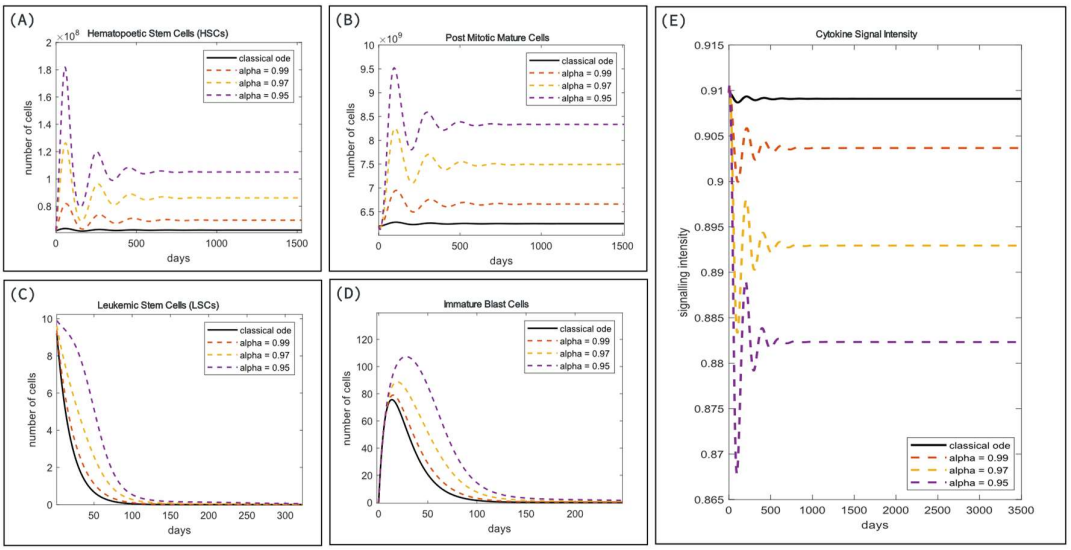

Figure 5: FDE solution for simulation depicting extinction of the leukemic cell population and re-establishment of hematopoietic equilibrium for 2-compartment system

This result has been consistent with the assumption in the literature that LPC proliferated faster than LSC, even though the self-renewal of LSC was higher than that of LPC. Based on this, it can be proposed that the cell count of the LPC compartment depends on its self-renewal rate. A low self-renewal rate leads to a lesser LPC

population, but the number of mature blasts per LPC would be higher. While a high self-renewal rate of LSC would lead to a higher LPC population but a lesser population of mature blasts per LPC, it would tend to keep more LPC population in the mother compartment **[14,19, 23]**.

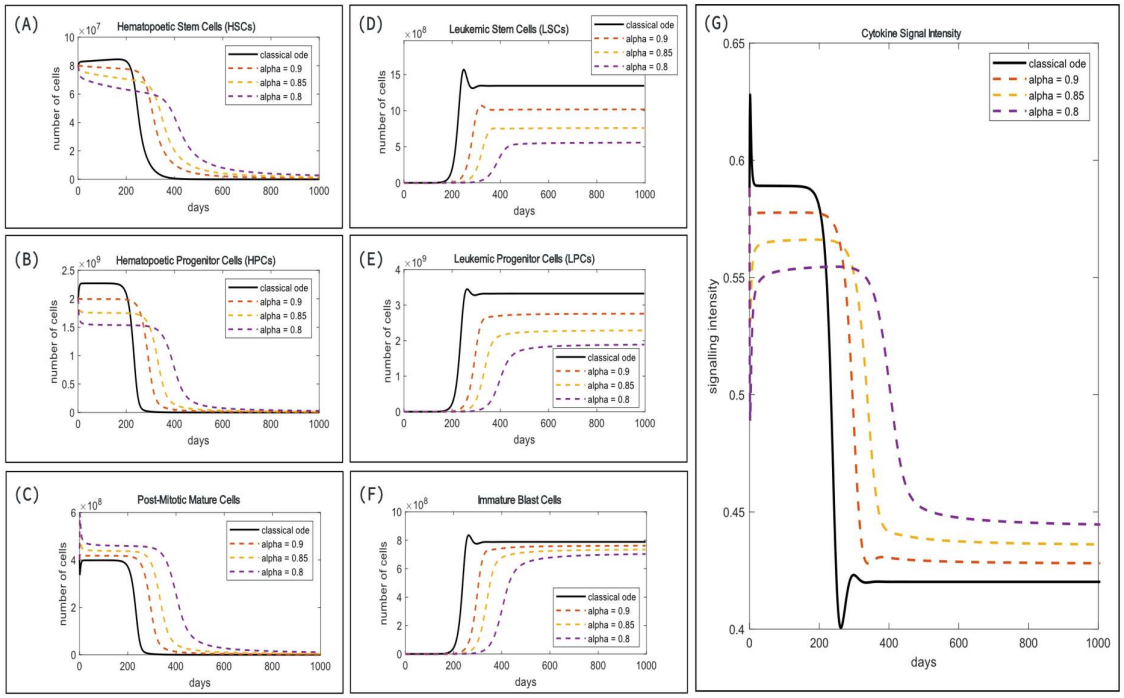

Figure 6: FDE solution for the generation of leukemic cells and extinction of hematopoietic cells for a 3-compartment system

The following result is a depiction of the maturation of the 6-compartment system of hematopoietic stem cells. Haematopoiesis has been considered a multistage process of successive cell proliferation and self-renewal to reach a maturation stage. The compartment 1 here depicts the long-term repopulating stem cells (LT-HSC), which gradually, through successive stages, viz., short-term repopulating stem cells (ST-HSC), multipotent progenitor cells (MPC), committed progenitor cells (CPC), and precursors, develop into mature cells. These stages are denoted by compartment 2, compartment 3, compartment 4, compartment 5, and compartment 6, respectively **[5, 9, 24]**.

For this simulation, we assumed the initial numbers of LT-HSC, ST-HSC, and MPC to be $10^5, 10^6$ and 10^7, respectively. While CPC, precursors, and post-mitotic mature cells are considered as null values. The values used to generate the following result and the analysis done by clearly indicate that stem cells in this system can be solely maintained by self-renewal rate, given that

the self-renewal rate of HSC has to be ≥ 50% and higher than that of all successive stages. And our analysis also revalidates the same **[6, 9, 14]**.

For the problem discussed above, the fractional-ordered model has been analysed. For the following analysis, the values of α are taken to be $0.9, 0.8,$ and 0.7. The FDE solution depicts minimal yet considerable changes in the cell-growth number; however, the results are consistent with the classical model.

In **Figure 8**, shows the effect of Venetoclax and the **Figure 9**. shows the combination of Venetoclax with Azacitidine and demonstrated good tolerability among adult patients with previously untreated AML who were ineligible for standard induction therapy. The safety profiles were similar across all arms of the dose escalation and expansion phases. Similar frequencies of adverse events (AEs) were observed in Azacitidine groups. The lowest incidences of gastrointestinal symptoms, such as nausea, diarrhea, and decreased appetite, were noted in the cohort receiving 400mg of Venetoclax in combination with Azacitidine. Common grade 3/4 AEs were primarily hematologic and consistent with those reported with single agent Azacitidine in previous studies. Neutropenia, a common side effect, was managed by delaying treatment cycles upon confirmation of morphologic clearance of leukemia. Recurrent grade 3/4 neutropenia in subsequent cycles was addressed through Venetoclax dose interruptions, reduction in treatment duration, delayed treatment cycles, and intermittent use of growth factors according to institutional standards. Despite the exclusion of CYP3A inhibitor azole antifungals, the frequency of fungal infections was low. This can be attributed to the prophylactic use of alternative antifungals such as echinocandins in 46% of patients and the relatively low rate of invasive fungal infections observed in the patient population. Overall, the combination of Venetoclax and Azacitidine demonstrated manageable toxicity and an acceptable safety profile in this cohort of previously untreated AML patients ineligible for standard induction therapy. These findings support the continued investigation of this combination regimen in larger clinical trials.

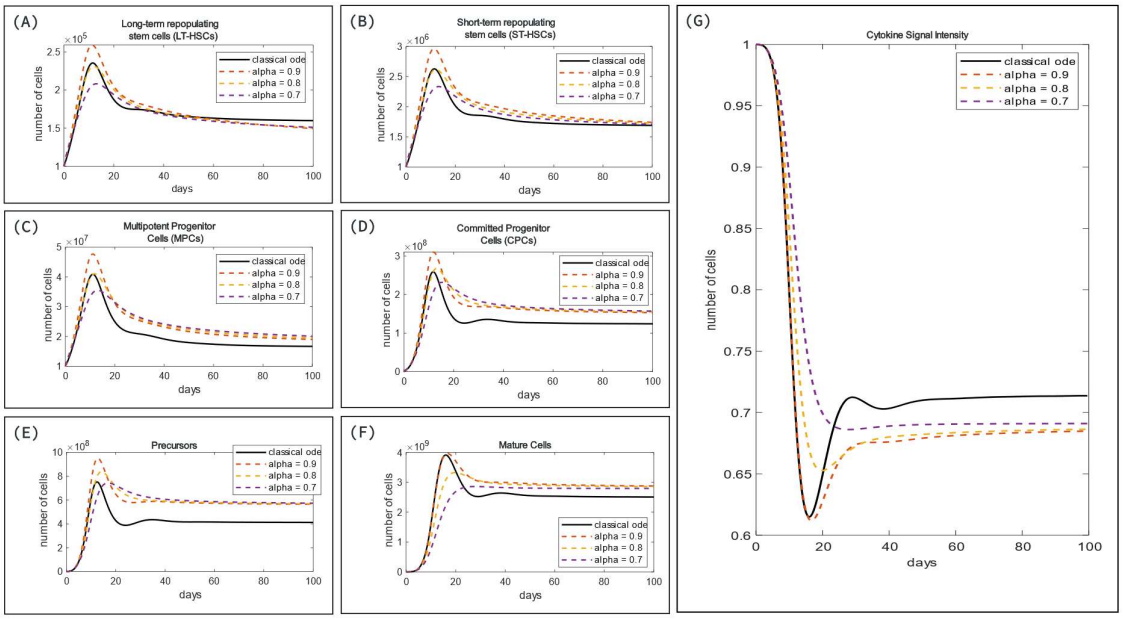

Figure 7: FDE solution for the time evolution of the cell population of hematopoietic stem cells for 6-compartment system

The findings are to underscore the promising therapeutic potential of venetoclax in combination with azacitidine for the treatment of acute myeloid leukemia (AML) in older adult patients. This novel combination regimen has demonstrated the ability to regulate cell survival effectively while maintaining tolerable safety profiles and eliciting favorable overall responses. Importantly, this regimen has shown efficacy even in traditionally high-risk patient groups, including those aged 75 or older, patients with poor cytogenetics, and those with secondary AML. The addition of Venetoclax to Azacitidine has not only enhanced the therapeutic effect but also played a crucial role in disrupting the metabolic machinery driving energy metabolism, thereby leading to the eradication of leukemia stem cells. The study utilized a mathematical model to analyze the growth dynamics of hematopoietic and leukemic stem cells within the bone marrow, considering various parameters such as self-renewal, proliferation, and apoptosis. This analysis provided insights into the impact of the Venetoclax and Azacitidine combination on cellular processes critical to AML pathogenesis.

Central to the mechanism of action, Venetoclax targets the B-cell lymphoma 2 (BCL-2) protein, a key regulator of cell survival in AML. By inhibiting BCL-2, Venetoclax disrupts the survival and persistence of leukemic cells, complementing the cytotoxic effects of Azacitidine, which works by inhibiting DNA methylation and promoting cell death. Overall, the combination of Venetoclax and

Azacitidine represents a promising therapeutic approach for AML, particularly in older adults and high-risk patient groups. By targeting essential pathways involved in cell survival and metabolism, this combination regimen holds significant potential for improving outcomes and addressing the unmet medical needs of AML patients. Continued research and clinical trials will further elucidate the efficacy and long-term benefits of this treatment approach.

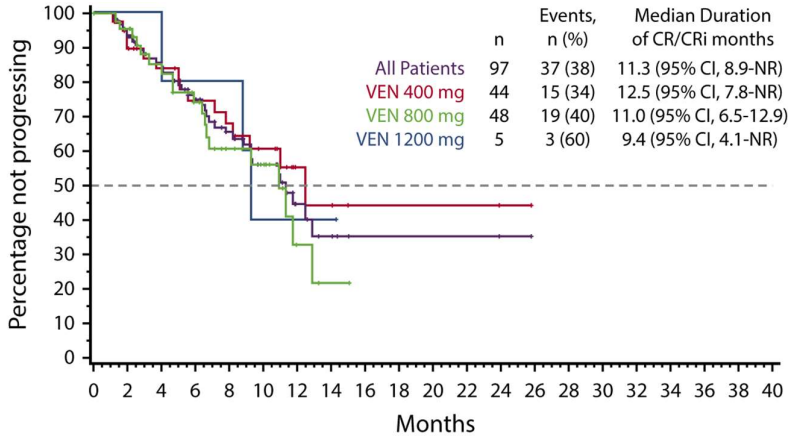

Figure 8: Overall Survival by Venetoclax

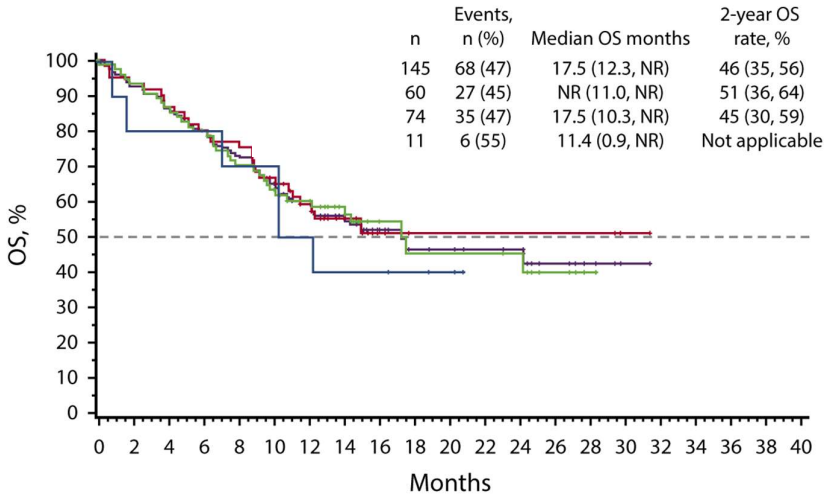

Figure 9: Overall Survival by Venetoclax with Azacitidine

CONCLUSION:

This study delved into the time evolution of cellular growth in hematopoietic and leukemic cell lines, particularly focusing on the bone marrow as a system of "well-mixed" pools of cells at various differentiation stages. Key characteristics such as self-renewal rate, proliferation rate, and death rate were analyzed, with the classical model transformed into a fractional order derivative-based model, yielding results consistent with the classical

approach. Understanding the pace of cellular growth and renewal is paramount for devising effective treatments for diseases such as acute myeloid leukemia (AML). In the context of AML, the B-cell lymphoma 2 (BCL-2) protein assumes a critical role in the survival and persistence of leukemic cells, including AML blasts. Venetoclax's targeting of BCL-2 represents a strategic approach to disrupt cell survival mechanisms implicated in AML progression. Azacitidine, through its mechanisms of inhibiting DNA methylation and promoting cell death, in combination with Venetoclax, emerges as a promising therapeutic avenue for elderly AML patients ineligible for intensive chemotherapy, offering both efficacy and tolerability. The combined effect of Venetoclax and Azacitidine demonstrates a significant impact on the growth dynamics of hematopoietic and leukemic cells. By understanding and targeting these fundamental pathways, advancements in treatment strategies for AML, particularly in elderly patients with limited therapeutic options, hold promise for improved clinical outcomes.

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