

# Deciphering signaling pathways in hematopoietic stem cells: the molecular complexity of Myelodysplastic Syndromes (MDS) and leukemic progression

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## ABSTRACT

Myelodysplastic Syndromes, a heterogeneous group of hematological disorders, are characterized by abnormalities in phosphoinositide-dependent signaling, epigenetic regulators, apoptosis, and cytokine interactions within the bone marrow microenvironment, contributing to disease pathogenesis and neoplastic growth. Comprehensive knowledge of these pathways is crucial for the development of innovative therapies that aim to restore normal apoptosis and improve patient outcomes.

## 1. Introduction

Myelodysplastic Syndromes (MDS) are a heterogeneous group of hematological neoplasms characterized by dysfunctional hematopoiesis, peripheral blood cytopenia, and a heightened risk of progression to acute myeloid leukemia (AML) (Bernard et al., 2022). This group of disorders primarily affects the elderly population. The pathogenesis of MDS is multifaceted and involves various genetic, epigenetic, and microenvironmental factors that contribute to the disease initiation and progression (Garcia-Manero et al., 2020). Understanding the intricate signaling pathways involved in the pathogenesis and progression of MDS is crucial for the development of targeted therapies and the improvement of patient outcomes. That is why this review explores the complex network of signaling mechanisms implicated in MDS.

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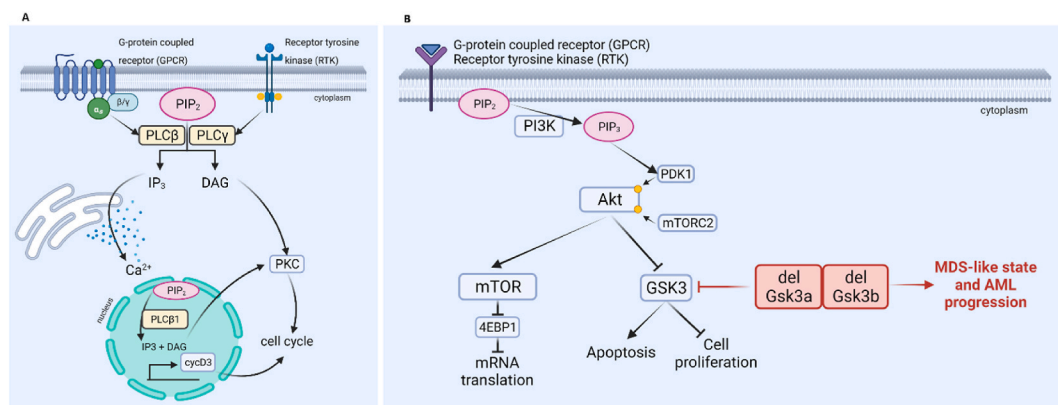
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## 2. Phosphoinositide signaling in MDS/AML

Phosphoinositide (PI) signaling (Fig. 1) represents a multifaceted and pivotal regulatory pathway in MDS. PIs are phosphorylated lipid molecules that directly control vital cellular processes, such as cell proliferation, survival, adhesion, vesicle transport, and gene transcription (Ratti et al., 2021). PI metabolism involves a family of 13 PI-specific phospholipase C (PI-PLC) enzymes divided into 6 families, with PI-PLC $\beta$  being critical for nuclear inositol signaling and processes like cell cycle progression, proliferation, and differentiation (Fig. 1A) PI-PLC $\beta$ 1 plays a pivotal role in both the onset and progression from MDS to AML, impacting genetic and epigenetic processes (Cocco et al., 2015; Dexheimer et al., 2017; Follo et al., 2013). In genetic terms, a study involving 80 MDS cases found that 43.75% of patients showed a monoallelic deletion in the PI-PLC $\beta$ 1 gene, which was associated with poorer clinical outcomes and a higher risk of AML progression (Cocco et al., 2015; Follo et al., 2013). On the epigenetic front, CpG islands in the PI-PLC $\beta$ 1 gene promoter are frequently hypermethylated in high-risk MDS. Demethylating drugs, like Azacytidine, can restore the wild-type condition, leading to increased expression of cell cycle control genes, such as p15/INK4B, p21WAF/Cip1, and p27. In addition, higher PI-PLC $\beta$ 1 expression was associated with a favorable response to Azacytidine (Cocco et al., 2015), as well Cyclin D3, one of the molecular targets of PI-PLC $\beta$ 1 which plays a fundamental role in the G1/S cell cycle phase, hematopoietic differentiation, and MDS. Indeed, elevated Cyclin D3 levels resulted from increased PI-PLC $\beta$ 1 levels via activation of the Wnt/ $\beta$ -catenin pathway (Ramazzotti et al., 2017). Besides PI-PLC $\beta$ 1, another critical primary PI-PLC, known as PI-PLC $\gamma$  and existing in two isoforms (PI-PLC $\gamma$ 1 and PI-PLC $\gamma$ 2), was found in hematopoietic cells and was associated with erythroid and myeloid differentiation (Jackson et al., 2021; Wilde and Watson, 2001). The activity of PI-PLC $\gamma$ 1 is regulated by the enzyme PI3K and participates in different physiological functions, such as proliferation, migration, cell survival, and apoptosis. It is also implicated in cell migration and invasion, contributing to carcinogenesis (Piccolo et al., 2002). Recent analysis of PLCG1 expression in bone marrow (BM) mononuclear cells (MNCs) from 116 MDS patients, both with and without del(20q), revealed a significant reduction in PLCG1 expression in MDS patients, compared to a control group. This reduction was observed not only in MDS patients with del(20q), but also in the other cases. In addition, lower PLCG1 expression was associated with a lower overall survival (OS). Therefore, reduced PLCG1 expression may be an independent prognostic factor for OS and the level of PLCG1 expression at the time of diagnosis may serve as a valuable prognostic biomarker for MDS (Shiseki et al., 2020).

PI-PLC $\gamma$ 2 is another PI-PLC expressed in hematopoietic cells and is linked to the regulation of myeloid differentiation (Barbosa et al., 2014; Jackson et al., 2021). Recently, PLCG2 gene has been correlated to the lack of response to epigenetic therapy. Indeed, a molecular study, performed on few inositide-related genes, identified a small cluster of 6 frequently mutated genes, 3 of which showed the same point mutations: D133E in PIK3CD gene, D280G in AKT3 gene and Q548R in PLCG2 gene. Interestingly, when all present, there was a shorter OS, leukemia-free survival and duration of response, suggesting that patients with this mutational profile could be refractory to therapy (Follo et al., 2019).

This is particularly important, as the PI3K/Akt pathway plays a fundamental role in leukemogenesis, being involved in the regulation of cell metabolism, proliferation, survival, cell growth, angiogenesis, cell migration, and invasion (Engelman et al., 2006) (Fig. 1B). PI3K, a class of lipid kinases, plays a pivotal role in producing the lipid messenger phosphatidylinositol (3,4,5)-trisphosphate (PIP3), which activates downstream effectors, like Akt, and consequently mTOR and 4EBP1 (Liu et al., 2009). Deleting class 1 PI3K in mice resulted in a phenotype resembling MDS, characterized by HSC expansion, impaired differentiation, dysplasia, genomic instability, and impaired autophagy (Ames et al., 2023). Conversely, the hyperactivation of PI3K/Akt negatively affects the normal hematopoietic stem cell (HSC) function, leading to stem cell pool depletion (Magee et al., 2012). Previous studies demonstrated that lower-risk MDS patients showed only a weak positivity for p-Akt, compared to higher-risk cases, while healthy donors tested negative



**Fig. 1. Schematic representation of PLCs and PI3K/Akt pathways** (Created by BioRender.com). (A) PI-PLCs play a role in cell cycle regulation by cleaving phosphatidylinositol (4,5)-bisphosphate (PIP2) and producing two second messengers. PI-PLC $\beta$ 1 in the nucleus operates independently from the PI-PLCs counterpart in the plasma membrane. (B) The phosphoinositide pathway is also regulated by PI3K, which activates Akt and various downstream pathways (e.g., mTOR, GSK3). The image also shows that GSK3 deletion could contribute to a MDS-like state, linked to a major risk of AML progression.

for this marker (Follo et al., 2007). This could be explained by a decreased PTEN expression in MDS cells (50% of low-risk MDS patients exhibited higher PTEN levels compared to high-risk MDS patients), potentially contributing to the elevated levels of p-Akt observed in high-risk MDS (Nyåkern et al., 2006).

Glycogen Synthase Kinase 3 (GSK3), a Serine/Threonine protein kinase associated with Akt, plays a critical role in the regulation of a broad range of biological activities (e.g., glycogen metabolism, cell signaling, cellular transport) (Fig. 1B). Akt phosphorylates and inhibits GSK3, along with other substrates (TSC2, FOXO proteins, and TBC1D4). GSK3, in turn, directly phosphorylates key components in the signaling network, including RICTOR, PTEN, and even Akt itself, potentially affecting feedback effects on signaling (Hermida et al., 2017). Previous research has highlighted the significant involvement of GSK3 in MDS and AML (Martelli et al., 2021). In mice, deleting *Gsk3b* alleles progressively reduced GSK3 signaling and gave rise to an MDS-like state, transforming HSCs into a pre-neoplastic condition resembling human MDS and disrupting the Wnt/Akt/mTOR signaling pathway. When combined with *Gsk3a* deletion, this genetic modification triggered the development of AML due to metabolic changes in HSCs. Furthermore, the molecular signature of GSK-3B-KO HSCs could predict clinical outcomes for MDS patients (Guezguez et al., 2016).

### 3. Disrupted epigenetic signaling

The pathophysiological process leading to the development of MDS involves genetic mutations that provide a selective advantage to the mutated clone, resulting in increase of its proliferation and expansion. Next-generation sequencing (NGS) uncovered a multitude of mutated genes in MDS, significantly advancing our understanding of the genetic alterations that contribute to this condition, particularly the changes in signaling pathways (Pellagatti and Boultonwood, 2015), and explaining the extensive disruption of epigenetic regulation (Ogawa, 2019). Thanks to increasingly advanced NGS technologies, a broad range of MDS driver gene mutations have been identified, including mutations in genes involved in DNA methylation and chromatin modifications (e.g., DNMT3A, TET2, IDH1/2, ASXL1, EZH2), RNA splicing (e.g., SF3B1, SRSF2, U2AF1/2), transcription (RUNX1, CEBPA), tyrosine kinase receptor (TKR)-mediated signaling (e.g., FLT3, KIT, NRAS, KRAS), and cell cycle regulation (e.g., TP53) (Ogawa, 2019). Notably, DNA methylation-related genes, as well as chromatin modification-associated genes, emerge as the most frequently mutated genes in MDS (Table 1) (Fig. 2).

TET2, a key epigenetic regulator, plays a role in DNA demethylation. TET2 mutations are present in approximately 22–35% of MDS cases, with a higher occurrence in lower-risk patients. Typically, these mutations are loss-of-function, leading to DNA hypermethylation (Boy et al., 2023) and increased stem cell self-renewal, thus expanding the HSC compartment and enhancing repopulation (Gurnari et al., 2022). Another commonly mutated gene in MDS, involved in the regulation of DNA methylation, is DNMT3A, encoding for an enzyme responsible for the methyl group transfer to specific DNA CpG sites. DNMT3A mutations are frequently observed in AML, occurring in over 20% of cases, whereas in MDS their presence is rarer, affecting no more than 10% of patients (Woods and Levine, 2015). Recent findings suggest that mutant DNMT3A leads to reduced methylation in specific regions and limits hypermethylation in AML cells (Boy et al., 2023). Therefore, these somatic mutations in DNMT3A disrupt the normal processes of differentiation and self-renewal in HSCs, providing a proliferative advantage and correlating with poorer OS in MDS patients. This increases the risk of malignant transformation in these individuals (Jawad et al., 2022).

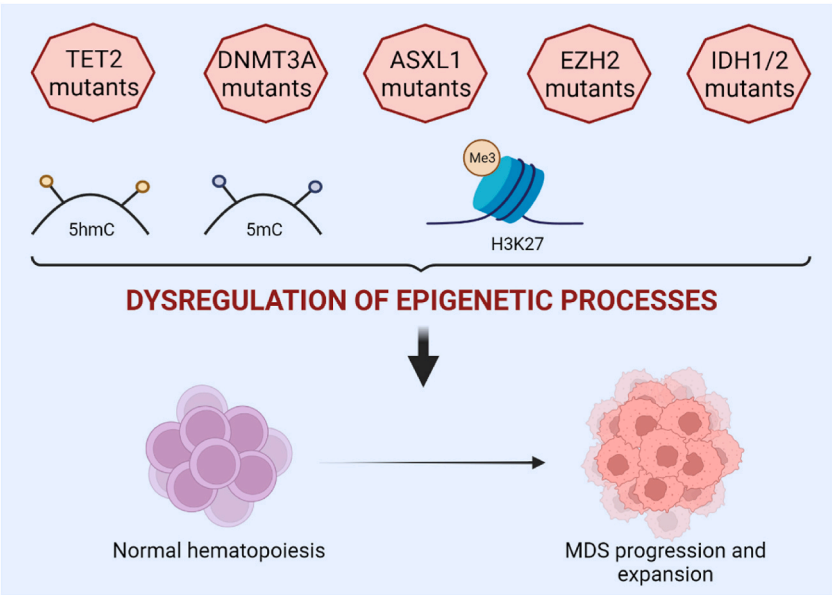
IDH1 and IDH2 mutations, although relatively uncommon in MDS (occurring in only 2–5% of cases), impact almost 10% of AML patients (Komrokji et al., 2022). IDH1 mutations in hematopoietic cells lead to the expansion of progenitor cells, resulting in splenomegaly and anemia and inducing leukemia with other collaborating oncogenes (DiNardo et al., 2016). ASXL1 and EZH2 mutations are recognized as loss-of-function mutations in MDS (Sashida et al., 2014; Wang et al., 2014). ASXL1 mutations, occurring in approximately 15–21% of all higher-risk MDS cases (Thol et al., 2011; Yang and Agosto-Peña, 2023), are associated with high aggressiveness and poor prognosis in both high-risk MDS and secondary AML (sAML) (Yang and Agosto-Peña, 2023). Meanwhile, EZH2 mutations are less common, affecting 5–6% of MDS cases and being rare in AML (Sakhdari et al., 2022), contributing to myeloid disease, myelofibrosis, collaborate with mutant RUNX1 to accelerate MDS, and induce chemoresistance in AML patients (Göllner et al., 2017; Muto et al., 2013). Furthermore, both ASXL1 and EZH2 mutations potentially influence histone H3 lysine27 trimethylation (H3K27Me3) (Issa, 2013).

### 4. Dysfunctional apoptotic signaling

In MDS, dysfunctional apoptotic signaling plays a critical role in the pathogenesis of the disease. Apoptosis, or programmed cell death, is a tightly regulated process that maintains the balance between cell proliferation and cell death. When this balance is disrupted, it can lead to the accumulation of abnormal and malignant cells, which is a hallmark of MDS.

**Table 1**  
Mutated epigenetic regulators in MDS.

Mutated gene	Frequency in MDS	Function
TET2	22–35% (Boy et al., 2023; Gurnari et al., 2022)	Methylcytosine dioxygenase
DNMT3A	<10% (Woods and Levine, 2015)	<i>De novo</i> DNA methylation
IDH1	2% (Komrokji et al., 2022)	No direct epigenetic activity
IDH2	4% (Komrokji et al., 2022)	No direct epigenetic activity
ASXL1	15–20% (Thol et al., 2011; Yang and Agosto-Peña, 2023)	Accessory protein of PRC2
EZH2	6% (Göllner et al., 2017; Muto et al., 2013)	Histone methyltransferase



**Fig. 2. Schematic representation of mutated epigenetic regulators in MDS** (Created by [BioRender.com](#)). MDS often exhibit mutations in key epigenetic regulators, such as TET2, DNMT3A, ASXL1, EZH2 and IDH1/2. At a molecular level, TET2 mutations could alter 5-hydroxymethylcytosine (5hmC), while 5-methylcytosine (5mC) is possibly altered by DNMT3a mutations, and H3K27me3 is potentially influenced by ASXL1 and EZH2 mutations ([Issa, 2013](#)). These mutated epigenetic regulators play a critical role in shaping the epigenome, leading to aberrant gene expression patterns, disrupting the normal processes of hematopoiesis and contributing to the development and progression of MDS.

The TP53 gene, a tumor suppressor gene encoding the p53 transcription factor, is frequently mutated in cancers and associated to unfavorable outcomes ([Sabapathy and Lane, 2018](#)). TP53 mutations, occurring in 10% of de novo MDS cases and in 40% of therapy-related MDS ([Jädersten et al., 2011](#)), suggest high-risk MDS, rapid progression to AML, resistance to standard therapies, and a poor prognosis. ([Klimovich et al., 2022](#); [Patel et al., 2023](#)). TP53 plays a fundamental role in initiating both intrinsic and extrinsic apoptotic pathways, regulating the expression of pro-apoptotic molecules, including Bax, Bak, Bid, Noxa, and Puma, and leading to the removal or breakdown of anti-apoptotic Bcl-2 family members ([Hernández Borrero and El-Deiry, 2021](#)), which regulate the mitochondrial pathway of apoptosis. In MDS, the balance between pro-survival and pro-apoptotic proteins of the Bcl-2 family is substantially disturbed during disease progression, as it inhibits apoptosis and promotes cell survival ([Reidel et al., 2018](#)). In higher-risk MDS, flow cytometry analyses revealed a higher expression of Bcl-2 relative to Bcl-XL, along with lower levels of pro-apoptotic proteins like Bax and Bad ([Invernizzi et al., 2001](#)). Even immunocytochemistry on BM smears from high-risk MDS patients demonstrated a significantly greater number of Bcl-2-positive cells compared to lower-risk MDS patients ([Invernizzi et al., 2001](#)), who showed an increase in apoptosis in the BM. Therefore, high-risk MDS patients are potential good candidates for treatment with Venetoclax, a Bcl-2 specific inhibitor ([Reidel et al., 2018](#)), but also lower-risk MDS patients, in case of disease progression, may acquire resistance to apoptosis and start showing an abnormal expression of Bcl-2 proteins ([Invernizzi et al., 2001](#)).

Bcl-2 family proteins also have crucial roles in regulating the mitochondrial membrane potential. Both MDS and AML cells are characterized by low levels of reactive oxygen species (ROS), which apparently result from a combination of low mitochondrial activity and high activity of ROS-removing pathways, such as autophagy. These mitochondrial dysfunctions can disrupt the intrinsic

**Table 2**  
Cytokines supporting or inhibiting MDS/AML cells.

Cytokine	Physiological function	Function in MDS/AML
TNFα	Pro-inflammatory cytokine	Increase of cell death and disruption of microenvironmental equilibrium ( <a href="#">Shi et al., 2019</a> ; <a href="#">Yang et al., 2015</a> )
IFNγ	Pro-inflammatory cytokine	Inhibition of hematopoiesis ( <a href="#">Yang et al., 2015</a> ) and generation of immunosuppressive BM microenvironment enriched of Tregs ( <a href="#">Corradi et al., 2022</a> )
TGFβ	Anti-inflammatory cytokine	Promotion of fibrosis within the BM and alteration of normal hematopoiesis ( <a href="#">Bataller et al., 2019</a> )
IL-6	Pro-inflammatory cytokine	Promotion of hematopoietic cells proliferation and alteration of apoptosis ( <a href="#">Habbel et al., 2020</a> )
IL-8	Chemoattractant cytokine	Migration and expansion of LSCs and MSCs( <a href="#">Kuett et al., 2015</a> ; <a href="#">Vijay et al., 2019</a> )
IL-1β	Pro-inflammatory cytokine	Inhibition of normal hematopoietic cells growth and promotion of LSCs survival within the BM( <a href="#">Arranz et al., 2017</a> )

apoptotic pathway (Mattes et al., 2019), thus potentially play a role in MDS.

As for other players of the Bcl-2 signaling, consistently elevated MCL-1 (Myeloid cell Leukemia sequence 1) levels are detected in nearly all BM cell samples from AML patients. Overexpression of MCL-1 is linked to treatment resistance, particularly to Venetoclax, and a poor prognosis. Therefore, MCL-1 inhibitors represent a promising therapeutic strategy to treat malignancies that rely on MCL-1, potentially enhancing the effectiveness of anti-cancer drugs when used in combination (Wei et al., 2020).

## 5. Dysregulated cytokine signaling in the bone marrow microenvironment

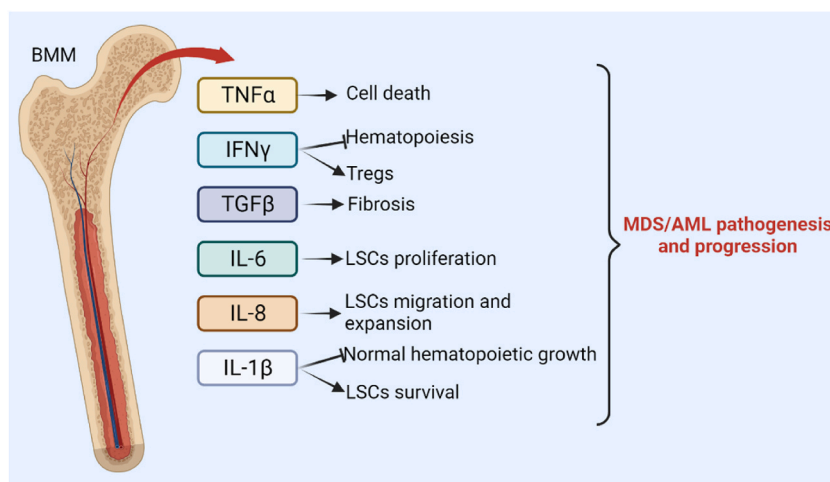
In the context of MDS, BM microenvironment (BMM) plays a crucial role in determining the destiny of HSCs. Dysregulated signaling and communication within this altered niche can stimulate the proliferation and survival of abnormal clonal populations, thereby anchoring the distinctive ineffective hematopoiesis observed in MDS (Table 2) (Fig. 3).

MDS patients exhibit abnormal expression of more than 30 cytokines in their peripheral and marrow blood. Notably, increased levels of specific cytokines, including tumor necrosis factor alpha (TNF- $\alpha$ ), interferon gamma (IFN- $\gamma$ ), transforming growth factor beta (TGF- $\beta$ ), interleukin (IL)-6, IL-8, and IL-1 $\beta$  have been identified (Banerjee et al., 2019).

Abnormalities in TNF- $\alpha$ , a pro-apoptotic cytokine, enhances accelerated cell death in lower-risk MDS, disrupting the microenvironmental equilibrium (Shi et al., 2019; Yang et al., 2015). Furthermore, IFN- $\gamma$ , another pro-apoptotic cytokine, has been shown to inhibit hematopoiesis and increase inducible nitric oxide synthase (iNOS) production, associated with the stimulation of tumorigenesis in different type of cancer (Yang et al., 2015). In AML, cells can indeed reshape the BMM by releasing high levels of IFN- $\gamma$ . This leads to an overexpression of indoleamine 2,3-dioxygenase-1 (IDO1) in mesenchymal stromal cells (MSCs), resulting in an immunosuppressive phenotype enriched with regulatory T cells (Tregs) (Corradi et al., 2022). Tregs play a role even in MDS, as the interaction between immune cells and elevated cytokines may contribute to several functional alterations, like the activation of apoptosis in lower-risk cases, the inhibition of immune response in higher-risk MDS, and the secretion of other cytokines, e.g., TGF- $\beta$  (Lynch and Calvi, 2022), associated with the promotion of fibrosis within the BM, further complicating the microenvironment and hindering normal hematopoiesis. Therefore, the increased levels of TGF- $\beta$  can impact the differentiation of hematopoietic cells, exacerbating the ineffective hematopoiesis observed in MDS (Bataller et al., 2019).

On one hand, interleukin-6 (IL-6), frequently elevated in MDS, has been associated with the activation of the JAK-STAT signaling pathway, supporting the proliferation of hematopoietic cells and disrupting apoptosis (Habbal et al., 2020). On the other hand, in CD34<sup>+</sup> MDS cells, there is a remarkable increase in IL-8 expression, suggesting its involvement in the recruitment of undifferentiated hematopoietic progenitors into the peripheral blood. Through its CXCR2 receptor, IL-8 prevents the expression or activation of adhesion molecules within the BM, thereby promoting the transendothelial migration of these progenitors (van Eeden and Terashima, 2000). Additionally, AML cells have been observed to release IL-8 in vitro and this secretion has been shown to be implicated in the migration and expansion of both AML cells and MSCs that populate the dysfunctional BM (Kuett et al., 2015; Vijay et al., 2019). In the context of AML, it has been demonstrated that even IL-1 $\beta$  hampers the self-renewal capacity of hematopoietic cells, inhibiting their growth and serving as a critical survival factor for leukemic stem cells (LSCs) within the BM (Arranz et al., 2017), where an autocrine signaling loop mediated by IL-1 $\beta$  is established (Cozzolino et al., 1989).

This heightened cytokine milieu disrupts the balance between cell death and proliferation, favoring cell survival over programmed cell death. As hematopoietic cells develop resistance to apoptotic signals, this imbalance leads to the characteristic cytopenias and dysplastic changes observed in MDS (Lynch and Calvi, 2022). Consequently, this proinflammatory environment fosters the survival and expansion of abnormal myeloid clones, further contributing to the pathogenesis of the disease (Sallman and List, 2019).



**Fig. 3. Dysregulated cytokine signaling in the Bone Marrow Microenvironment (BMM)** (Created by BioRender.com). The cartoon depicts the main cytokines involved in signaling within the bone marrow microenvironment that leads to MDS/AML onset and progression.



## 6. Conclusions

As illustrated, the PI-dependent signaling pathway has a key role in MDS, involving PI-PLC $\beta$ 1 and PI-PLC $\gamma$  in the regulation of different cellular processes, impacting both genetic and epigenetic mechanisms, and correlating with unfavorable clinical outcomes and increased risk of AML progression. Even dysregulation of the PI3K/Akt/GSK3 pathway, another key regulator of PI-related pathways, leads to an MDS-like phenotype, emphasizing its importance. Therefore, focusing on nuclear PI-PLC $\beta$ 1 and PI-PLC $\gamma$  holds potential significance in the regulation of MDS cell proliferation and differentiation, offering an alternative treatment strategy to overcome resistance to PI3K/Akt inhibitors in MDS patients (Mongiorgi et al., 2016). Similarly, Bcl-2 signaling is often altered in higher-risk patients (Jilg et al., 2016). That is why Venetoclax, an oral Bcl-2 inhibitor, has proven to be particularly effective in treating MDS, especially in combination with hypomethylating agents (HMAs), thus representing a promising new therapeutic strategy (Tsao et al., 2012). However, BCL-2 gene mutations are often retrieved in MDS, and cases need to be re-evaluated. Indeed, as previously described, NGS analyses advanced the comprehension of genetic alterations in MDS, further complicating the landscape of MDS pathogenesis and progression. For instance, TET2 mutations cause hypermethylation and stem cell self-renewal, DNMT3A mutations are responsible of the disruption of HSC differentiation and are correlated with poorer OS, and ASXL1 and EZH2 mutations cause high cancer aggressiveness and poor prognosis. Therefore, new treatments are now based on gene mutations. This is the case of IDH inhibitors, like Enasidenib, that are FDA-approved for IDH2-mutated AML, and are currently investigated alone or in combination with HMAs (DiNardo et al., 2023). This intricate network of pathways is intensified by perturbed cytokine signaling within the bone marrow niche, creating a favorable microenvironment to MDS onset and progression. Targeting the overactive TGF $\beta$  pathway with Luspatercept in low-risk MDS has shown encouraging results and stands as the only approved therapy in a specific subset of patients, with ongoing developments in similar agents (Bazinet and Bravo, 2022). Additionally, the anti-IL-1 $\beta$  antibody Canakinumab is being assessed for its potential in treating lower-risk MDS, either alone or in combination with other drugs (Bazinet and Bravo, 2022).

All in all, the exploration of targeted therapies and the identification of specific gene mutations may pave the way for the development of personalized treatments tailored to the specific molecular aberrations present in each patient. As research advances, uncovering the interplay between these pathways offers new strategies for the management of MDS, possibly also enhancing the quality of life for MDS patients.

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## CRediT authorship contribution statement

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## Declaration of competing interest

The authors have not conflicts of interest to declare.

## Data availability

No data was used for the research described in the article.

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