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REVIEW

# HEDGEHOG SIGNALING PATHWAYS IN MULTIPLE MYELOMA

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## ABSTRACT

Multiple myeloma (MM) is a hematological disease characterized by the uncontrolled proliferation of bone marrow malignant plasma cells. Localization and survival of malignant cells relies on bone marrow niche, in turn determined by the interaction between MM cells and mesenchymal stromal cells (MSCs). Several reports suggest that Hedgehog (Hh) pathway plays an outstanding role in tumor microenvironment maintenance. Hh signaling orchestrates the transformation of the myeloma bone marrow microenvironment supporting the proliferation of malignant plasma cells by affecting NF-kB signaling. To date, different clinical approaches are currently undergoing to evaluate the role of Hh modulators as efficient MM therapy. In this review article, we discuss the recent advances in the understanding of Hh signaling pathway in MM microenvironment.

## KEY WORDS

*Hedgehog; myeloma multiple; tumor microenvironment.*

## IMPACT STATEMENT

Several reports investigated the role of Hedgehog signaling in multiple myeloma progression. In this review we discuss the recent advancements in this field, also considering the new drugs currently in clinical trial.

## INTRODUCTION

Multiple myeloma (MM) is a hematological disease characterized by bone marrow malignant plasma cells enhanced proliferation (1), usually resulting into hypercalcemia, renal impairment, anemia and bone pain (2). Myeloma bone disease is a devastating complication of MM observed in more than 80% of patients (3). The pathophysiology characterizing this outcome include a series of complex biochemical and cellular processes involving osteoclasts and osteoblasts activity, orchestrated by osteocytes. These cells act as mechano-sensors mediating the bone remodeling process by secreting cytokines such as Osteoprotegerin (OPG) and receptor activator of nuclear factor-kappa B ligand (RANKL) (4). In the MM context, several studies reported an increased RANKL/OPG ratio resulting into osteoclasts activation and disruption of bone marrow homeostasis (5-9). In physiological conditions, osteocytes inhibit osteoblasts differentiation by blockage of the canonical Wingless-type (Wnt) signaling, mediated by sclerostin and Dickkopf-1 (Dkk-1) secretion (10). Elevated amounts of DKK1 in MM patients correlated with the presence of focal bone lesions (11)**.** Moreover, the bone resorption is enhanced by malignant plasma cells, acting by i) releasing macrophage inflammatory protein-1a and b (MIP-1α-β), ii) inducing mature osteoblasts apoptosis and iii) inhibiting the differentiation of their precursors (12-14). As a result, bone matrix degradation releases the growth factors and cytokines boosting MM cells survival (15). For this reason, targeting the osteocytes-osteoblasts axis may represent a promising strategy counteracting MM progression.

The Hedgehog (Hh) signaling pathway holds a critical role for intercellular communication during the development of many organs, while its aberrant activation has been reported in several cancers (16). Hh signaling mostly relies on primary cilium, a microtubule-based organelle in the surface of vertebrate cells serving as mechano-sensory structure towards microenvironment stimuli (17). Consistently, primary cilium may act as a communication hub during organ and embryonic development, immune response, and tissue homeostasis, eventually triggering different cascade, including Wnt signaling (18).

Hh mammalian proteins have been grouped into three classes: Sonic Hedgehog (Shh), Desert Hedgehog (Dhh) and Indian Hedgehog (Ihh), in turn explicating different duties within the cellular context. The latter has been reported to play a major role in endochondral ossification during skeletal development, while Dhh expression has been described in pre-Sertoli cells leading male sexual differentiation, and Shh is secreted to mediate epithelial invagination, limbs patterning and nervous system commitment (19-21). Interestingly, activation of the Hh pathway has been reported to rely on two distinct mechanisms, namely canonical- and non-canonical- Hh activation (16). In the canonical pathway (**figure 1*A***), one of the Hh proteins binds to the hedgehog protein receptor Patched (Ptch), which is eventually internalized and degraded. Repression of the Ptch, occurring upon Hh binding, triggers 7-transmembrane protein Smoothened (Smo) activity, in turn promoting, downstream, GLI family zinc finger (Gli) nuclear traslocation. As a result, Gli modulates a plethora of genes widely identified as Hh targets (17), involved in cell cycle regulation, apoptosis, proliferation, angiogenesis, self-renewal, and epithelial-to-mesenchymal transition (22).

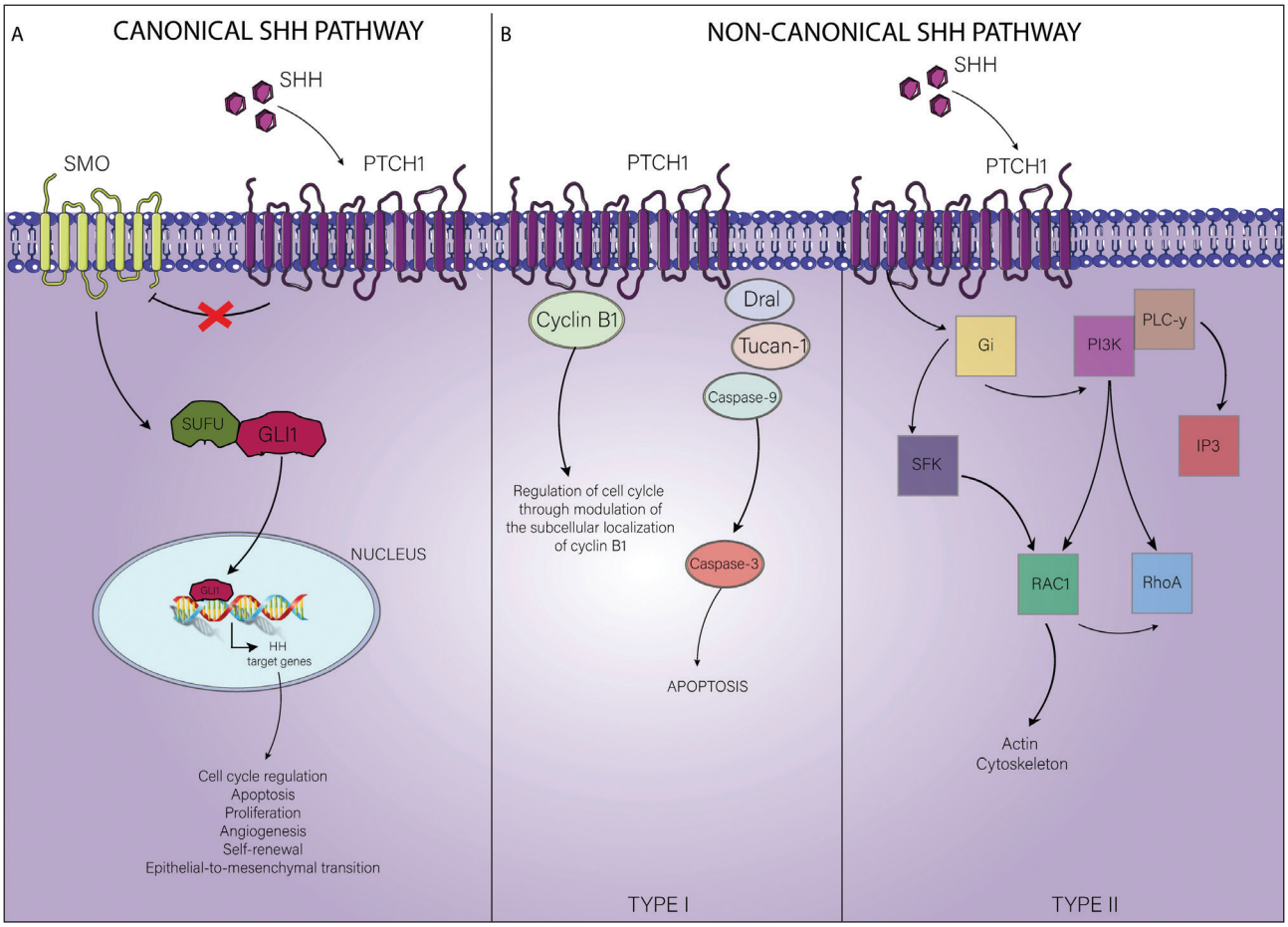
Besides, Gli are also regulated by a family of tumor suppressor proteins, namely Suppressor of Fused (SUFU) (23). When Ptch ligands are missing, Gli proteins are recruited by Sufu, which are in charge of inhibiting their nuclear translocation (24). For this reason, the full-length Gli proteins are converted to a C-terminal shorten repressed form (Gli-R). This structure is phosphorylated by glycogen synthase kinase 3 beta (GSK3β), casein kinase I (CK1), and

protein kinase A (PKA) (25). Gli proteins retained at the cytoplasm by Sufu are then degraded or processed, overall triggering Hh inhibition. However, how these last steps are operated in mammals are elusive still (23).

Non-canonical Hh activation (**figure 1 *B***), on the other hand, has been characterized to be orchestrated by two separate pathways. Type I non-canonical Hh activation relies on Ptch1-activity when Ptch ligands are missing. This noncanonical signaling activity regulates the cell cycle through modulation of the subcellular localization of cyclin B1 (26). Type II non-canonical Hh activation is Smo-dependent Gli-independent. Small GTPases RhoA and Rac1 are the main players in charge for triggering this pathway, in a cellular-context manner (27-29). In carcinogenic processes these mechanisms have been reported to be profoundly affected as a result of Hh signaling misregulation (30). Given the role of Hh pathways in cell development, its aberrant activation might thus contribute to hematological malignancies progression, overall representing a promising strategy to target for developing novel drug-based approaches (31).

## HEDGEHOG SIGNALING IN MULTIPLE MYELOMA

MM cells-mesenchymal stromal cells (MSCs) interactions have been described to play an outstanding role in MM pathogenesis, eventually contributing to MM cell survival, proliferation and chemoresistance (32). Shh produced by the stromal cells supports proliferation of hematopoietic stem cells, prompts germinal-center B cells survival and antibody production (33-35). In tumor context, MSC-induced Shh signaling is important in protecting my-




**Figures 1. *A.*** *Canonical activation of Shh pathway. Canonical pathway is triggered by interaction between Shh and Ptch1. In response to this binding, Ptch1 no longer inhibits Smo, which in turn promotes downstream Gli nuclear translocation and target genes activation;* ***B.*** *Non-canonical Shh pathway. Non-canonical activation can be orchestrated by two separate pathways. Type I Smo-independent activation relies on Ptch interaction with cyclin B1, leading to cell cycle regulation. Type II is Smo-dependent Gli-independent. When Shh binds Ptch1, Smo activates Gi protein and small GTPases RhoA and Rac1, as well as calcium release stimulation from endoplasmic reticulum and PLC-y- catalyzed the opening of IP3-depedent channels by the generation of IP3.*

elodysplastic syndrome (MDS) cells from apoptosis (36) Despite accumulation of a plethora of genetic lesions, myeloma PCs lose their dependency on BM microenvironment only in the latest stages of disease and therefore long-term culture of primary MM cells without stromal support is rarely possible in vitro (37). Among the main MSC-released soluble factors contributing to myeloma cells survival, Shh allow survival and growth of MM cells. Indeed, its proliferative effect is inhibited by cyclopamine, an alkaloid which binds to SMO stabilizing its inactive conformation (37).

CD138+ cells from MM patients exhibit overexpression of Hh signaling components, such as *PTCH*, *GLI1* and *GLI2* through the activation of non-canonical Smo-independent pathway (16). Moreover, a significative down-regulation of Hh repressor gene *GLI3* has been described in malignant plasma cells compared to the healthy counterpart (16). MM is characterized by two distinct populations: CD138-CD19+ stem cells, resembling memory B cells, and malignant CD138+CD19- terminally differentiated plasma cells (38). Peacock et. al (32) demonstrated a marked down-regulation of *PTCH1* in CD138-CD19+ stem cell compartment, together with an increase of *SMO* and *GLI1* expression. On the other hand, CD138+CD19- differentiated plasma cells showed increased *PTCH1* levels. Therefore, CD138-CD19+ stem cell populations are more sensitive to Hh ligand than malignant CD138+CD19-terminally differentiated plasma cells (32).

In addition to the stromally induced Hh signaling, MM cells are able to produce and secrete themselves the Hh ligands. Autocrine Shh signaling enhances tumor proliferation and protects CD138+ cells from spontaneous and stress-induced apoptosis increasing BCL-2 expression levels (39). This evidence correlates with an independent study reporting that an Hh-gene signature is able to cluster MM patients in two subgroups characterized by the opposite Hh pathway expression in mature PCs and their precursors. In particular, patients with Hh hyperactivation in MM cells, but not in their B cells, show higher genomic instability associated to shorter progression-free survival and overall survival (40).

Hh signaling is also associated with the nuclear transcription factor-kB (NF-kB) pathway in several tumors such as liver cancer, breast cancer, prostate cancer, pancreatic cancer, diffuse large B-cell lymphoma (DLBCL) (41-44). NF-kB is a heterodimeric complex consisting of a p50 (NF-kB1) and p65 (RelA) subunits, which form an inactive cytoplasmic ternary complex with the inhibitory protein IKBa. In response to an extracellular stimulus, IKBa may be degraded and NF-kB can translocate into the nucleus to activate the expression of genes involved in the immune and inflammatory responses, such as interleukin 2 receptor alpha chain gene, interleukin 6, granulocyte colony-stimulating factor, interferon-beta (IFN-b) (45). Interestingly, it has been reported that canonical pathway activation of Sonic Hedgehog is responsible for the enhancement of NF-KB activity in MM cells, preventing their apoptosis (46).

Tumor-derived Hh signaling can favor the production of receptor activator of nuclear factor-kB ligand (RANKL) in osteoblasts, stimulating osteoblastogenesis and increasing bone resorption (47). Hh signaling has also been found to stimulate MSCs differentiation on osteoblasts regulating expression of Runt-related transcription factor (RUNX2) and Osterix (OSX) expression (48). Activation of osteoblastogenesis is directly modulated by SMO and GLIs-induced signaling (48). Indeed, inhibition of Shh signaling by using cyclopamine strongly reduces osteoblastogenesis (49). Myeloma PCs acts as GLI1 suppressor on MSCs, thus, reducing the potential of MSCs to differentiate in osteoblasts (50).

## TARGETING HH PATHWAY

Given the crucial role played by Hh pathway in MM progression, recent reports focused on developing new therapeutic strategies aiming to its inhibition. One of them targets the Smo receptor using cyclopamine, eventually resulting in the inhibition of Hh signaling (51). Since Hh signaling regulates NF-κB through both its classical pathway (SHh/PTCH1/SMO/GLI1) and non-classical pathway by SMO recruitment of TRAF6 to ubiquitination, the SMO inhibitor cyclopamine in combination with bortezomib enhance the proteasome inhibitor-induced cytotoxic effects (46) These results enforce the hypothesis describing a proteasome-Hh axis which may be targeted in the feature studies. Despite the promising results, cyclopamine showed teratogenic potential, toxicity and poor bioavailability, overall discouraging further application aiming to clinical outcomes (52). However, cyclopamine opened the path towards further drug development aiming to target Hh pathway for MM treatment.

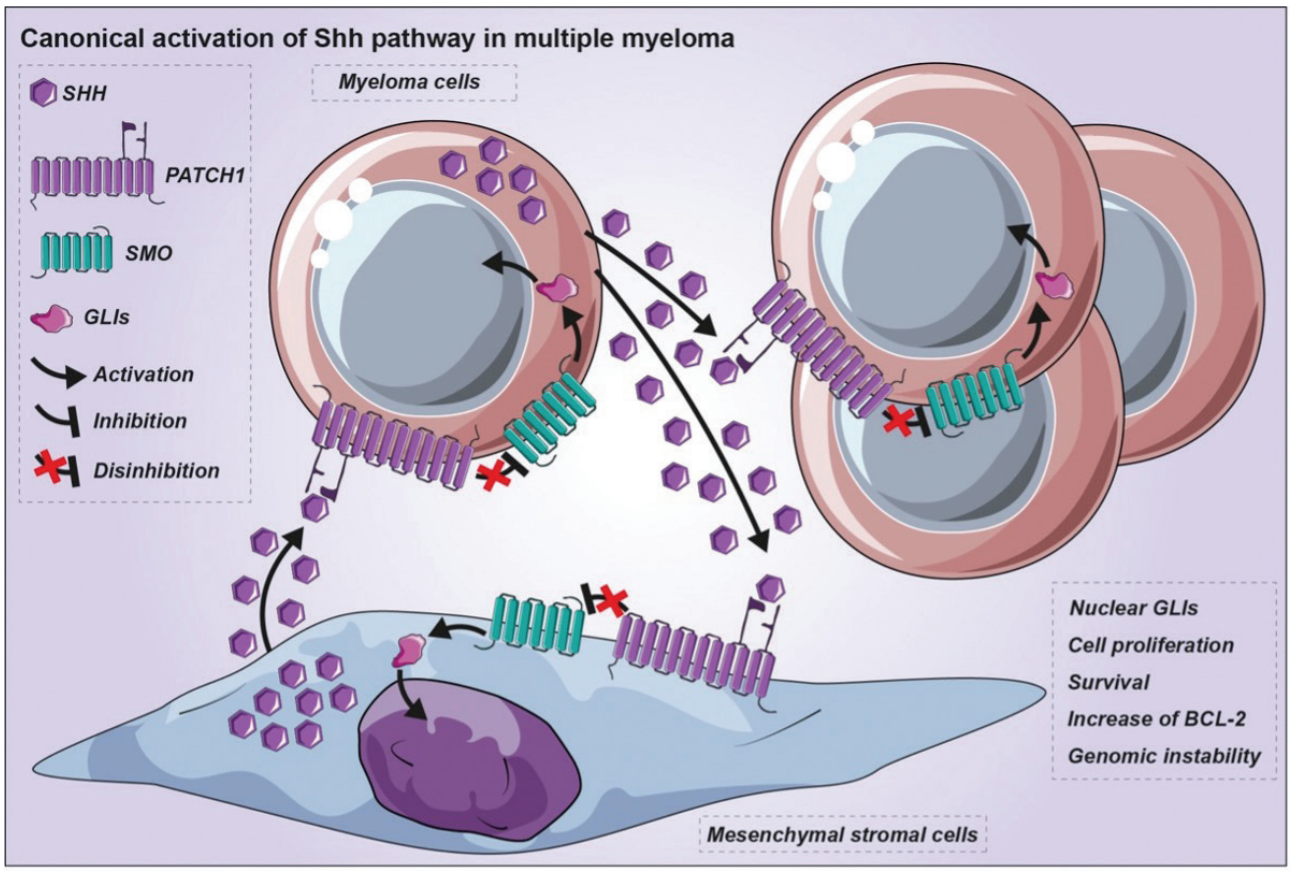



**Figure 2.** *Crosstalk of mesenchymal stroma cells and myeloma cells: the role of Sonic Hedgehog pathway. Stromal compartment is the most important source of Shh in bone marrow, mediating proliferation of hematopoietic stem cells, prompting germinal-center B cells survival and antibody production.*

Currently, a newer drug namely Vismodegib acting through Hh pathway inhibition has been approved by US Food and Drug Administration's (US FDA) priority review program on January 30th, 2012 for the treatment of advanced basal-cell carcinoma (BCC) (53). Since Vismodegib was found to have an acceptable safety profile and antitumor activity in patients with BCC and medulloblastoma (54, 55), new clinical trials are being planned in other malignances, including MM (**table I**). However, patients undergoing Vismodegib treatment against BCC showed bone toxicities, with premature fusion of the epiphyses reported in pediatric patients (56). Moreover, cramps or dysgeusia over the course of the therapy appeared in several patients, requiring interruption of the standard therapy, eventually shifting to an intermittent Vismodegib schedule (57).

In parallel, Sonidegib (Odomzo™), a SMO receptor antagonist, has being developed by Novartis for the treatment of BCC (58). This drug was reported to hamper cell viability, neurosphere formation, and Gli transcriptional activity, triggering the apoptotic cascade by activation of caspase-3 and cleavage of poly (ADP-ribose) polymerase *in vitro* (59). In a transgenic mouse model of islet cell neoplasms Sonidegib significantly reduced tumour volume by 95% compared with untreated littermates by inhibition of Hh signaling (59). Given the efficacy and tolerability of a topical formulation of Sonidegib in BCC patients, phase I/II investigation are underway on other malignancies including medulloblastoma, small cell lung cancer, breast cancer, myelofibrosis, chronic myeloid leukaemia, and MM (table I) (60-64). As for Vismodegib, clinical trials displayed a set of typical side effects associated with Sonidegib administration. Muscle spasms, alopecia, dysgeusia, nausea, increased Creatin Kinase, fatigue, decreased weight, diarrhea, decreased appetite, myalgia, and vomiting were frequent in patients, eventually undergoing dose interruptions, reductions, or treatment discontinuation (65). For this reason, further studies are needed to evaluate the usage and dosage of both of these Hh inhibitor for clinical approaches.

| DRUG | TRIAL REGISTRATION NUMBER | LOCATION | YEARS |
|---|---|---|---|
| ATO (Arsenic Trioxide) | NCT00469209 | U.T.M.D Anderson Cancer Center Huston, Texas, US | 2006-2008 |
| | NCT00258245 | Barbara Ann Karmanos Cancer Institute Detroit, Michigan, US | 2005-2008 |
| | NCT00661544 | U.T.M.D Anderson Cancer Center Huston, Texas, US | 2004-2007 |
| | NCT00201695 | Ohio State University Columbus, Ohio, US | 2004-2008 |
| | NCT00006021 | Mount Sinai Comprehensive Cancer Center at Mount Sinai Medical Center Miami Beach, Florida, US | 2000-2007 |
| | NCT00017069 | Arizona Clinical Research Center Tucson, Arizona, US | 2001-2005 |
| | NCT000193544 | CTI BioPharma Seattle, Washington, US | 2002-2009 |
| | NCT000193544 | City of Hope Duarte, California, US | 2005-2009 |
| | NCT00003395 | Memorial Sloan Kettering Cancer Center New York, New York, US | 1998-2000 |
| Vismodegib (GDC-0449) | NCT02465060 | University of Alabama at Birmingham Cancer Center Birmingham, Alabama, US | Recruiting |
| | NCT03297606 | Cross Cancer Institute Edmonton, Alberta, CA, US | Recruiting |
| | NCT03878524 | OHSU Knight Cancer Institute Portland, Oregon, US | Recruiting |
| Sonidegib (LDE-225) | NCT02254551 | Colorado Blood Cancer Institute Denver, Colorado, US | 2015 |
| | NCT02086552 | Mayo Clinic Rochester, Minnesota, US | 2014-2021 |

**Table I.** *Clinical trials. The table reports registered clinical trial on https://clinicaltrials.gov focused on Hh inhibitors for MM treatment.*

Because the GLI proteins are the final effectors of Shh pathway, the development of a GLI-targeted approach might be useful to inhibit tumor growth and therapy resistance. Among GLI antagonists, there are GANT58 and GANT61 (GLI-ANTagonist) (66) GANT61 is more specific toward GLI proteins and effectively reduces GLI1 and GLI2 DNA-binding ability. Arsenic Trioxide (ATO) (a Food and Drug Administration (FDA)-approved drug with sub-micromolar potency against GLI1/267 (67) was shown to inhibit GLI1 directly inhibiting its transcriptional activity (68). MM cells treated with ATO also show inhibition of NF-kB, hampered adhesion to MSCs with consequent disruption of tumor growth and survival (69). A first phase II study of ATO in a MM cohort was designed to assess the response to therapy of patients with relapsed or resistant MM, previously treated with autologous stem cell (70). Eligible patients (n = 10) received a 2-hour daily infusion of ATO 0.15 mg/kg for 60 days. The treatment was supplemented for 30 days more in patients showing a response, defined as a reduction in myeloma paraprotein at days 30 and 60. Three out of ten patients who completed more than 30 days of ATO infusion were characterized by >50% reduction in serum paraprotein levels (n = 2), a more stable disease (n = 1). Furthermore, one out of ten progressed. Surprisingly, five patients belonging to the initial cohort, displayed stable disease (n = 2) and progressed (n = 3), already upon < 30 days treatment. Table 1 lists completed clinical trials, providing a strong basis for the use of ATO in MM patients. Interestingly, ATO found an important clinical path in counteracting relapsed or refractory acute promyelocytic leukemia. However, ATO usage has been discouraged as a consequence of its side effects on healthy tissues, eventually resulting in cardiotoxicity (71). Notably, QT prolongation, torsades de pointes and sudden cardiac death have been reported upon ATO administration. The main reason behind ATO-related cardiac toxicity is related to the large amount of ROS produced following ATO treatment, which in cardiac cells, as a consequence of the low amount of antioxidants, it is enhanced (71, 72).

Interestingly, two parallel phase II trials aim to assess the safety and efficacy of Sonidegib in combination with bortezomib and lenalidomide, in patient with relapsed/refractory MM (NCT02254551) or as maintenance therapy following autologous stem cell transplantation of refractory multiple myeloma (NCT02086552), respectively.

Further approaches to enhance Hh inhibitors efficiency include synergic strategies with molecules targeting the Hh signaling cascade at multiple levels. With this regard such ATO has been recently tested together with Itraconazole, Vismodegib or Sonidegib (73).

For this reason, it should not be surprising if multiple combinations of Hh-targeting agents will be disclosed soon. For this purpose, we listed the literature currently available investigating the crosstalk between Hh and multiple myeloma in **table II**.

## CONCLUSION AND FUTURE PERSPECTIVES

MM is a hematological disease characterized by an aberrant activation of several molecular mechanisms, eventually reshaping the bone microenvironment, and resulting in MM progression. In this landscape, researchers are aiming to identify novel therapeutic targets to improve patients' prognosis. With this regards, Hh activation has been reported to cover an underestimated role in bone marrow development, thus prompting different groups to target this cascade in different hematological diseases (74). In the MM context, Hh aberrant signaling, in turn mediated by Shh release, affects bone marrow microenvironment transformation, supporting the proliferation of malignant plasma cells by enhancing NF-kB signaling, also resulting in chemotherapy resistance (75). In this context, targeting the Hh pathway may represent a valuable strategy. To date, the main strategies are represented by three drugs (ATO, Vismodegib, and Sonidegib) which are currently being tested in different clinical trials (**table I**). However, the usage of drugs targeting Hh cascade may not be useful enough to hamper MM progression. For this reason, a further effort could be done to design more powerful Hh modulators. In alternative, the ones currently available may be tested together with molecules targeting Hh cascade on different levels to enhance Hh modulators' effect. Ultimately, an outstanding strategy may also be represented by supplementation of currently clinical-available drugs in combination with Hh modulators, aiming to obtain a more beneficial treatment. In this regard administration of Ixazomib reshapes the MM microenvironment also stimulating Hh cascade. However, further studies are needed to fully understand the regulatory mechanisms underlying Hh signaling pathway and how PIs affect them, towards the development of new treatment to efficiently hamper MM progression.

| REFERENCE | TITLE | JOURNAL |
|---|---|---|
| 3 | A novel Bruton's Tyrosine Kinase inhibitor CC-292 in combination with the proteasome inhibitor carfizomib impacts the bone microenvironment in a multiple myeloma model with the resultant antimyeloma activity. | Leukemia, **2014** |
| 16 | Canonical and noncanonical Hedgehog pathway in the pathogenesis of multiple myeloma. | Blood, **2012** |
| 31 | Aberrant activation of the Hedgehog signaling pathway in malignant hematological neoplasm. | The American Journal of Pathology, **2012** |
| 32 | Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. | Proceedings of the National Academy of Sciences, **2007** |
| 34 | Sonic Hedgehog is produced by follicular dendritic and protects germinal center B cells from apoptosis. | Journal of Immunology, **2005** |
| 37 | Essential role of stromally induced Hedgehog signaling in human CD138+ myeloma cell survival and drug resistance. | Nature Medicine, **2007** |
| 39 | A critical role of stromally induced Hedgehog signaling in B-cell malignancies. | Blood, **2014** |
| 40 | Opposite activation of the Hedgehog pathway in CD138+ plasma cells and CD138- 19+ B cells identifies two subgroups of patients with multiple myeloma and different prognosis. | Leukemia, **2016** |
| 46 | Targeting the cross-talk between the Hedgehog and NF-kappaB signaling pathways in multiple myeloma. | Leukemia & Lymphoma, **2019** |
| 47 | The role of Hedgehog signaling in tumor induced bone disease. | Cancers (Basel), **2015** |
| 50 | Ixazomib improves bone remodeling and counteracts Sonic Hedgehog signaling inhibition mediated by myeloma cells. | Cancers (Basel), **2020** |
| 70 | Ascorbic acid enhances arsenic trioxide-induced cytotoxicity in multiple myeloma. | Blood, **2001** |
| 75 | Effect of Hedgehog pathway abnormality on chemotherapeutic resistance of multiple myeloma. | Zhongguo Shi Yan Xue Ye Xue Za Zhi, **2017** |

**Table II.** *Currently available literature investigating Hh-MM crosstalk. The table reports the available works investigating the role played by Hh in MM progression as they are cited along the main body of the text.*

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## ETHICS

### Conflicts of interests

The authors have declared no conflict of interests.

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### Authors' contribution

Conceptualization: ID, DT, CG, AR, SG, NV, FDR, GAP; validation: AR, GS, AB, LL, ID; writing-original draft preparation: ID, DT, CG, SG, GAP, ELS, NV, TZ, FDR, GLV, RP, RP; supervision: DT, GAP, DLF, FDR, NV, DC, RP, GLV, RA, ID, GLV. All authors have read and agreed to the published version of the manuscript.

### Availability of data and materials

No new data were generated or analysed in this research.

### Ethical approval

N/A.

## REFERENCES

1. Brigle K. Rogers B. Pathobiology and Diagnosis of Multiple Myeloma. Semin Oncol Nurs 2017;33:225-36.
2. Palumbo A. Multiple myeloma. Curr Opin Oncol 2012;24(2):S1-2.
3. Eda H, Santo L, Cirstea DD, et al. A novel Bruton's tyrosine kinase inhibitor CC-292 in combination with the proteasome inhibitor carfilzomib impacts the bone microenvironment in a multiple myeloma model with resultant antimyeloma activity. Leukemia 2014;28:1892-901
4. Bonewald LF. The amazing osteocyte. J Bone Miner Res 2011;26:229-38.
5. Giuliani N, Bataille R, Mancini C, Lazzaretti M, Barille S. Myeloma cells induce imbalance in the osteoprotegerin/osteoprotegerin ligand system in the human bone marrow environment. Blood 2001;98:3527-33.
6. Pearse RN, Sordillo EM, Yaccoby S, et al. Multiple myeloma disrupts the TRANCE/ osteoprotegerin cytokine axis to trigger bone destruction and promote tumor progression. Proc Natl Acad Sci USA 2001;98:11581-86.
7. Croucher PI, Shipman CM, Lippitt J, et al. Osteoprotegerin inhibits the development of osteolytic bone disease in multiple myeloma. Blood 2001;98:3534-40.
8. Terpos E, Szydlo R, Apperley JF, et al. Soluble receptor activator of nuclear factor kappaB ligand-osteoprotegerin ratio predicts survival in multiple myeloma: proposal for a novel prognostic index. Blood 2003;102:1064-9.
9. Giallongo C, Parrinello NL, La Cava P, et al. Monocytic myeloid-derived suppressor cells as prognostic factor in chronic myeloid leukaemia patients treated with dasatinib. J Cell Mol Med 2018;22:1070-80.
10. Baron R, Kneissel M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. Nat Med 2013;19:179-92.
11. Tian E, Zhan F, Walker R, et al. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. N Engl J Med 2003;349:2483-94.
12. Choi SJ, Cruz JC, Craig F, et al. Macrophage inflammatory protein 1-alpha is a potential osteoclast stimulatory factor in multiple myeloma. Blood 2000:96:671-75.
13. Silvestris F, Cafforio P, Tucci M, Grinello D, Dammacco F. Upregulation of osteoblast apoptosis by malignant plasma cells: a role in myeloma bone disease. Br J Haematol 2003; 122:39-52.
14. Camiolo G, Tibullo D, Giallongo C, et al. alpha-Lipoic Acid Reduces Iron-induced Toxicity and Oxidative Stress in a Model of Iron Overload. Int J Mol Sci 2019, 20.
15. Abe M, Hiura K, Wilde J, et al. Osteoclasts enhance myeloma cell growth and survival via cell-cell contact: a vicious cycle between bone destruction and myeloma expansion. Blood 2004;104(8):2484-91.

16. Blotta S, Jakubikova J, Calimeri T, et al. Canonical and noncanonical Hedgehog pathway in the pathogenesis of multiple myeloma. Blood 2012;120(25):5002-13.
17. Carballo GB, Honorato JR, de Lopes GPF, Spohr TCLSE. A highlight on Sonic hedgehog pathway. Cell Commun Signal 2018;16(1):11.
18. Pala R, Alomari N, Nauli SM. Primary Cilium-Dependent Signaling Mechanisms. Int J Mol Sci 2017;18(11):2272.
19. Bechtold TE, Kurio N, Nah HD, Saunders C, Billings PC, Koyama E. The Roles of Indian Hedgehog Signaling in TMJ Formation. Int J Mol Sci 2019;20(24):6300.
20. Bitgood MJ, Shen L, McMahon AP. Sertoli cell signaling by Desert hedgehog regulates the male germline. Curr Biol 1996;6(3):298-304.
21. Hosoya A, Shalehin N, Takebe H, Shimo T, Irie K. Sonic Hedgehog Signaling and Tooth Development. Int J Mol Sci 2020;21(5):1587.
22. Litingtung Y, Dahn RD, Li Y, Fallon JF, Chiang C. Shh and Gli3 are dispensable for limb skeleton formation but regulate digit number and identity. Nature 2002;29;418(6901):979-83.
23. Huang D, Wang Y, Tang J, Luo S. Molecular mechanisms of suppressor of fused in regulating the hedgehog signalling pathway. Oncol Lett 2018;15(5):6077-86.
24. Gonnissen A, Isebaert S, Haustermans K. Targeting the Hedgehog signaling pathway in cancer: beyond Smoothened. Oncotarget 2015;6(16):13899-913.
25. Lee H, Ko HW. Ciliary smoothened-mediated noncanonical hedgehog signaling promotes tubulin acetylation. Biochem Biophys Res Commun 2016;480(4):574-79.
26. Barnes EA, Kong M, Ollendorff V, Donoghue DJ. Patched1 interacts with cyclin B1 to regulate cell cycle progression. EMBO J 2001;20(9):2214-23.
27. Jenkins D. Hedgehog signalling: emerging evidence for non-canonical pathways. Cell Signal 2009;21(7):1023-34.
28. Robbins DJ, Fei DL, Riobo NA. The Hedgehog signal transduction network. Sci Signal 2012;5(246):re6.
29. Tibullo D, Caporarello N, Giallongo C, et al. Antiproliferative and Antiangiogenic Effects of Punica granatum Juice (PGJ) in Multiple Myeloma (MM). Nutrients 2016;8(10):611.
30. Scales SJ, de Sauvage FJ. Mechanisms of Hedgehog pathway activation in cancer and implications for therapy. Trends Pharmacol Sci 2009;30(6):303-12.
31. Ok CY, Singh RR, Vega F. Aberrant activation of the hedgehog signaling pathway in malignant hematological neoplasms. Am J Pathol 2012;180(1):2-11.
32. Peacock CD, Wang Q, Gesell GS, et al. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. Proc Natl Acad Sci 2007;104:4048-53.
33. Gering M, Patient R. Hedgehog signaling is required for adult blood stem cell formation in zebrafish embryos. Dev Cell 2005;8:389-400.
34. Sacedón R, Díez B, Nuñez V, et al. Sonic hedgehog is produced by follicular dendritic cells and protects germinal center B cells from apoptosis. J Immunol 2005;174(3):1456-61.
35. Romano A, Parrinello NL, Simeon V, et al. High-density neutrophils in MGUS and multiple myeloma are dysfunctional and immune-suppressive due to increased STAT3 downstream signaling. Sci Rep 2020;10(1):1983.
36. Zou J, Hong Y, Tong Y, et al. Sonic hedgehog produced by bone marrow-derived mesenchymal stromal cells supports cell survival in myelodysplastic syndrome. Stem Cells Int 2015;2015:957502.
37. Dierks C, Grbic J, Zirlik K, et al. Warmuth M. Essential role of stromally induced hedgehog signaling in B-cell malignancies. Nat Med 2007;13(8):944-51.
38. Matsui W, Huff CA, Wang Q, et al. Characterization of clonogenic multiple myeloma cells. Blood 2004;103(6):2332-6.
39. Liu Z, Xu J, He J, et al. A critical role of autocrine sonic hedgehog signaling in human CD138+ myeloma cell survival and drug resistance. Blood 2014;124(13):2061-71.
40. Martello M, Remondini D, Borsi E, et al. Opposite activation of the Hedgehog pathway in CD138+ plasma cells and CD138-CD19+ B cells identifies two subgroups of patients with multiple myeloma and different prognosis. Leukemia 2016;30(9):1869-76.
41. Cao X, Geradts J, Dewhirst MW, Lo HW. Upregulation of VEGF-A and CD24 gene expression by the tGLI1 transcription factor contributes to the aggressive behavior of breast cancer cells. Oncogene;31(1):104-15.
42. Hyun J, Wang S, Kim J, et al. MicroRNA-378 limits activation of hepatic stellate cells and liver

fibrosis by suppressing Gli3 expression. Nat Commun 2016;7:10993.
43. Qu C, Liu Y, Kunkalla K, et al. Trimeric G protein-CARMA1 axis links smoothened, the hedgehog receptor transducer, to NF-κB activation in diffuse large B-cell lymphoma. Blood 2013;121(23):4718-28.
44. Singh AP, Arora S, Bhardwaj A, et al. S. CXCL12/CXCR4 protein signaling axis induces sonic hedgehog expression in pancreatic cancer cells via extracellular regulated kinase- and Akt kinase-mediated activation of nuclear factor κB: implications for bidirectional tumor-stromal interactions. J Biol Chem 2012;287(46):39115-24.
45. Baeuerle PA. The inducible transcription activator NF-kappa B: regulation by distinct protein subunits. Biochim Biophys Acta 1991;1072:63-80.
46. Cai K, Na W, Guo M, et al. argeting the crosstalk between the hedgehog and NF-κB signaling pathways in multiple myeloma. Leuk Lymphoma 2019;60(3):772-81.
47. Cannonier SA, Sterling JA. The Role of Hedgehog Signaling in Tumor Induced Bone Disease. Cancers 2015;7(3):1658-83.
48. Lv WT, Du DH, Gao RJ, et al. Regulation of Hedgehog signaling Offers A Novel Perspective for Bone Homeostasis Disorder Treatment. Int J Mol Sci 2019;20(16):3981.
49. Felber K, Croucher P, Roehl HH. Hedgehog signalling is required for perichondral osteoblast differentiation in zebrafish. Mech Dev 2011;128(1-2):141-52.
50. Tibullo D, Longo A, Vicario N, et al. Ixazomib Improves Bone Remodeling and Counteracts sonic Hedgehog signaling Inhibition Mediated by Myeloma Cells. Cancers 2020;12(2):323.
51. Gould A, Missailidis S. Targeting the hedgehog pathway: the development of cyclopamine and the development of anti-cancer drugs targeting the hedgehog pathway. Mini Rev Med Chem 2011;11(3):200-13.
52. Lipinski RJ, Hutson PR, Hannam PW, et al. Dose- and route-dependent teratogenicity, toxicity, and pharmacokinetic profiles of the hedgehog signaling antagonist cyclopamine in the mouse. Toxicol Sci 2008;104(1):189-97.
53. Sandhiya S, Melvin G, Kumar SS, Dkhar SA. The dawn of hedgehog inhibitors: Vismodegib. J Pharmacol Pharmacother 2013;4(1):4-7.
54. Sekulic A, Migden MR, Oro AE, et al. Efficacy and safety of vismodegib in advanced basal-cell carcinoma. N Engl J Med 2012;366(23):2171-9.
55. Lorusso PM, Jimeno A, Dy G, et al. Pharmacokinetic dose-scheduling study of hedgehog pathway inhibitor vismodegib (GDC-0449) in patients with locally advanced or metastatic solid tumors. Clin Cancer Res 2011;17(17):5774-82.
56. Ciciarelli V, Cortellini A, Ventura A, Gutiérrez García-Rodrigo C, Ficorella C, Fargnoli MC. Rare bone toxicity associated with vismodegib. JAAD Case Rep 2020;6(6):482-5.
57. Tronconi MC, Solferino A, Giordano L, et al. Tailored Toxicity-Driven Administration of Vismodegib in Patients With Multiple or Locally Advanced Basal Cell Carcinoma: A Pilot Analysis. Front Oncol 2020;10:563404.
58. Burness CB. Sonidegib: First Global Approval. Drugs 2015;75(13):1559-66.
59. Fu J, Rodova M, Nanta R, et al. NPV-LDE-225 (Erismodegib) inhibits epithelial mesenchymal transition and self-renewal of glioblastoma initiating cells by regulating miR-21, miR-128, and miR-200. Neuro Oncol 2013;15(6):691-706.
60. Kieran MW, Chisholm J, Casanova M, et al. Phase I study of oral sonidegib (LDE225) in pediatric brain and solid tumors and a phase II study in children and adults with relapsed medulloblastoma. Neuro Oncol 2017;19(11):1542-52.
61. Pietanza MC, Litvak AM, Varghese AM, et al. A phase I trial of the Hedgehog inhibitor, sonidegib (LDE225), in combination with etoposide and cisplatin for the initial treatment of extensive stage small cell lung cancer. Lung Cancer 2016;99:23-30.
62. Gupta V, Wolleschak D, Hasselbalch H, et al. Safety and efficacy of the combination of sonidegib and ruxolitinib in myelofibrosis: a phase 1b/2 dose-finding study. Blood Adv. 2020;4(13):3063-71.
63. Hartmann-Johnsen OJ, Kåresen R, Schlichting E, Nygård JF. Better survival after breast-conserving therapy compared to mastectomy when axillary node status is positive in early-stage breast cancer: a registry-based follow-up study of 6387 Norwegian women participating in screening, primarily operated between 1998 and 2009. World J Surg Oncol 2017;15(1):118.
64. Irvine DA, Zhang B, Kinstrie R, et al. Deregulated hedgehog pathway signaling is inhibited by the smoothened antagonist LDE225 (Sonidegib) in chronic phase chronic myeloid leukaemia. Sci Rep 2016;6:25476.
65. Jain S, Song R, Xie J. Sonidegib: mechanism of action, pharmacology, and clinical utility for

advanced basal cell carcinomas. Onco Targets Ther 2017;10:1645-53.
66. Bhateja P, Cherian M, Majumder S, Ramaswamy B. The Hedgehog Signaling Pathway: A Viable Target in Breast Cancer? Cancers 2019;11(8):1126.
67. Kim J, Lee JJ, Kim J, Gardner D, Beachy PA. Arsenic antagonizes the Hedgehog pathway by preventing ciliary accumulation and reducing stability of the Gli2 transcriptional effector. Proc Natl Acad Sci 2010;107(30):13432-7.
68. Beauchamp EM, Ringer L, Bulut G, et al. Arsenic trioxide inhibits human cancer cell growth and tumor development in mice by blocking Hedgehog/GLI pathway. J Clin Invest 2011;121(1):148-60.
69. Anderson KC, Boise LH, Louie R, Waxman S. Arsenic trioxide in multiple myeloma: rationale and future directions. Cancer J 2002;8(1):12-25.
70. Grad JM, Bahlis NJ, Reis I, Oshiro MM, Dalton WS, Boise LH. Ascorbic acid enhances arsenic trioxide-induced cytotoxicity in multiple myeloma cells. Blood 2001;98(3):805-13.
71. Vineetha VP, Raghu KG. An Overview on Arsenic Trioxide-Induced Cardiotoxicity. Cardiovasc Toxicol 2019;19(2):105-19.
72. Costa VM, Carvalho F, Duarte JA, Bastos Mde L, Remião F. The heart as a target for xenobiotic toxicity: the cardiac susceptibility to oxidative stress. Chem Res Toxicol 2013;26(9):1285-311.
73. Ghirga F, Mori M, Infante P. Current trends in Hedgehog signaling pathway inhibition by small molecules. Bioorg Med Chem Lett 2018;28(19):3131-40.
74. Irvine DA, Copland M. Targeting hedgehog in hematologic malignancy. Blood 2012;119(10):2196-204.
75. Hu JS, Huang X, Huang YD, Lu YY, Lu QY. [Effect of Hedgehog Signaling Pathway Abnormality on Chemothe-rapeutic Resistance of Multiple Myeloma]. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2017;25(2):465-70.