REVIEW

Novel strategies for the treatment of myelofibrosis driven by recent advances in understanding the role of the microenvironment in its etiology [version 1; peer review: 3 approved]

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Abstract

Myelofibrosis is the advanced stage of the Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs), characterized by systemic inflammation, hematopoietic failure in the bone marrow, and development of extramedullary hematopoiesis, mainly in the spleen. The only potentially curative therapy for this disease is hematopoietic stem cell transplantation, an option that may be offered only to those patients with a compatible donor and with an age and functional status that may face its toxicity. By contrast, with the Philadelphia-positive MPNs that can be dramatically modified by inhibitors of the novel BCR-ABL fusion-protein generated by its genetic lesion, the identification of the molecular lesions that lead to the development of myelofibrosis has not yet translated into a treatment that can modify the natural history of the disease. Therefore, the cure of myelofibrosis remains an unmet clinical need. However, the excitement raised by the discovery of the genetic lesions has inspired additional studies aimed at elucidating the mechanisms driving these neoplasms towards their final stage. These studies have generated the feeling that the cure of myelofibrosis will require targeting both the malignant stem cell clone and its supportive microenvironment. We will summarize here some of the biochemical alterations recently identified in MPNs and the novel therapeutic approaches currently under investigation inspired by these discoveries.

Keywords

Myelofibrosis, p53, transforming growth factor beta, combination therapy, MDM2 inhibitors, transforming growth factor beta inhibitors, animal models, pre-clinical studies

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Introduction

Overt myelofibrosis (MF) is the final stage of several disease entities collectively referred to as the Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) that include polycythemia vera (PV), essential thrombocythemia (ET), and prefibrotic or early stage primary myelofibrosis (pre-MF) and can also arise *de novo* as overt fibrotic-stage primary myelofibrosis (PMF)[1,2] These diseases share common clinical features including constitutional and microvascular symptoms, splenomegaly, a high risk of thromboembolic and hemorrhagic complications, and a propensity to progress to a form of acute myeloid leukemia (AML) termed MPN-blast phase (MPN-BP). Early studies have identified that MPNs arise within the hematopoietic stem/progenitor cell (HSPC) compartment, and recent advances have largely elucidated its molecular pathophysiology[3–5]. Constitutive activation of the JAK–STAT signaling pathway driven by one of several canonical somatic mutations results in myeloproliferation and contributes to genomic instability. Acquisition of additional genetic aberrations eventually leads to disease progression[5]. While PV, ET, and pre-MF are usually indolent hematological malignancies with a median survival spanning decades or several years, overt MF, which include PMF, carries worse prognosis and severely affects the patient's quality of life. Usually, MPN-BP has a prognosis of only several months[6]. The disease progression of MF exhibits a great range of patient-to-patient variability. The detailed genetic information currently available on large numbers of patients is providing evidence-based criteria for their risk stratification, which, in the future, may provide the basis for personalized therapy.

In contrast to the significant progress made in understanding the disease's pathogenesis, treatment for MF remains largely palliative. Although we can effectively reduce symptoms and prevent thromboembolic complications, a treatment that can modify the course of the disease and prevent progression to MPN-BP is lacking. The only therapeutic option that offers potential cure is allogeneic hematopoietic stem cell (HSC) transplantation (HSCT), but this approach is limited by the lack of donors to all patients and by associated morbidity and mortality. Improving the survival of patients with MF is a major unmet need in malignant hematology. Better understanding of the pathological pathways involved in MF disease progression has ushered the development of novel treatment strategies aimed at slowing or even reversing disease progression and prolonging patient survival. An excellent review on the genetic basis of MPNs has been recently published by Vainchenker *et al*. in *F1000Research*[5]. Here, we will summarize scientific information that is driving the search for a cure in MPNs, focusing our discussion on the most recent strategies targeting the microenvironment that are currently under investigation.

Mutational landscape in MPNs

In 2005, four groups reported the identification of a point mutation in exon 14 of the *JAK2* gene, *JAK2*V617F, in over 95% of patients with PV and 50–60% of patients with ET and PMF[7–10]. JAK2 is the tyrosine kinase that represents the first signal transduction element of the receptors for erythropoietin (EPO), thrombopoietin (TPO), and granulocyte-colony stimulating factor (GSCF)[5]. As such, JAK2 is necessary for normal hematopoietic cell growth and differentiation. *JAK2*V617 affects the inhibitory domain of the protein, rendering it constitutively active, independent of extracellular activation by the physiologic ligands. *JAK2* exon 12 mutations have later been found to drive most cases of *JAK2*V617-negative PV[11]. Inactivating mutations in the gene encoding the TPO receptor (*MPL*) have been identified in 3–5% of ET and PMF cases[12]. In 2013, frameshift mutations in exon 9 of the calreticulin (*CALR*) gene, encoding for an endoplasmic reticulum chaperone protein that interacts with MPL, were found in the majority of *JAK2*V617F and *MPL* mutation-negative ET and PMF patients, thereby completing the "missing piece in the puzzle" of MPN driver mutations[13,14]. In up to 10% of patients with ET and 15% of patients with PMF, a driver mutation cannot be identified. These "triple-negative" MPNs may be driven by non-canonical mutations in *JAK2* or *MPL* or by genetic lesions in other mediators of the JAK–STAT pathway such as *LNK* or *PPM1D*[5,15].

Advances in genotyping, such as the application of next-generation sequencing and high-resolution chromosomal microarrays, have led to the discovery of additional somatic mutations that usually arise following acquisition of the driver mutations but can also precede them and that can contribute to disease progression and transformation to MPN-BP. These mutations affect genes involved in epigenetic regulation and splicing, such as *ASXL1*, *DNMT3A*, *TET2*, *SRSF2, U2AF1*, and *SF3B1*, as well as signaling and apoptosis, and were found across all myeloid malignancies[5]. Mutations in *ASXL1*, *SRSF2*, *EZH2*, and *IDH1/2* have been associated with shortened survival and higher risk of progression to MPN-BP[16]. Mutations in *U2AF1* have been associated with anemia and additional poor prognostic features[17]. Mutations or other genetic lesions affecting the tumor suppressor p53 have been shown to play a central role in progression to MPN-BP and are highly predictive of leukemic transformation and poor outcomes[18,19]. The growing importance of genomic analysis in MPN patient assessment is reflected by the advent of updated risk stratification models integrating molecular and cytogenetic profiles with the more traditional clinical and morphological parameters to guide management decisions such as referral to HSCT[20–22]. For example, a Genetics-based International Prognostic Scoring System (GIPSS) has been proposed that is based exclusively on mutational and cytogenetic markers[20]. Recently, comprehensive genomic characterization of 2,035 MPN patients identified distinct genetic subgroups that correlate well with clinical course and prognosis and may arguably provide more accurate classification than current disease entities[15]. We hope that this influx of advanced molecular diagnostics will ultimately contribute to more personalized tailoring of treatment strategies and translate to improved survival.

Optimization of current treatment approaches for MF

Great efforts are dedicated to improving the treatment armamentarium currently available for MF. We will focus on what we perceive are the main trajectories within these efforts:

development of second-generation JAK inhibitors, advances in transplantation medicine and its utilization for MF, and evaluation of novel agents. Although a complete summary of current treatment approaches is beyond the scope of this review, it is important to note the emerging role for pegylated interferon formulations in the treatment of early stage (proliferative) MF[23–25] and of hypomethylating agents in accelerated and MPN-BP[26–28].

JAK inhibitors

Following the discovery of *JAK2*V617, a non-selective JAK1/2 inhibitor, ruxolitinib, was developed and has been shown in two pivotal phase III studies to induce significant spleen volume reduction and improvement in constitutional symptoms, leading to its approval by the USA and European regulatory agencies for intermediate-2 and high-risk MF[29,30]. The widespread use of ruxolitinib has changed the therapeutic landscape and significantly impacted the quality of life of many MF patients. Moreover, long-term follow up of the COMFORT studies has suggested a survival advantage with the use of ruxolitinib[31]. Since the biological effects of this drug on mutation allele burden or bone marrow histological findings have been inconsistent and overall modest[32–34], other analyses have attributed the beneficial effect of ruxolitinib on survival to the attenuation of systemic inflammation and reversal of cachexia owing to spleen volume reduction. Furthermore, the use of ruxolitinib is limited in patients with anemia and thrombocytopenia. These considerations inspired the development of second-generation JAK inhibitors that lack myelosuppressive effects and may allow effective treatment for patients with cytopenias. Three second-generation JAK inhibitors have been evaluated in phase III clinical trials, and their efficacy results are summarized in Table 1.

Pacritinib is an oral multi-kinase inhibitor with specificity for JAK2, FLT3, IRAK1, and CSF1R. The PERSIST-1 study

Table 1. Summary of the efficacy outcomes of the six larger scale (phase II and III) second-generation JAK inhibitor studies. BAT, best available therapy; DIPSS, Dynamic International Prognostic Scoring System; FED, fedratinib; IPSS, International Prognostic Scoring System; JAK, Janus kinase; MF, myelofibrosis; MOM, momelotinib; PAC, pacritinib; PLT, platelet; pts, patients; RBC, red blood cell; RUX, ruxolitinib; SVR, spleen volume reduction; TSS, total symptom score.

Drug	Trial	Patient population	Number of patients	Comparator	Spleen response (SVR≥35%) at 24 weeks	Symptom response (≥50% reduction in TSS) at 24 weeks	Cytopenia	Ref.
PAC	PERSIST-1 (phase III)	JAK-inhibitor-naïve pts with DIPSS intermediate or high-risk MF, with no exclusion for baseline anemia or thrombocytopenia	327	BAT (2:1) excluding JAK inhibitors	19% in PAC group versus 5% in BAT group (*P* <0.0003)	19% in PAC versus 10% in BAT	25% who were transfusion dependent achieved transfusion independence. Responses for pts with low PLT counts	35
PAC	PERSIST-2 (phase III)	MF pts with PLT counts <100 x 10^9/L	311	BAT (2:1) including RUX (48%)	18% in PAC group versus 3% in BAT group	25% in PAC group versus 14% in BAT group	Reduced transfusion burden in PAC group	36
MOM	SIMPLIFY-1 (phase III)	JAK-inhibitor-naïve adult pts with IPSS intermediate-2 or high-risk MF, or symptomatic intermediate-1 MF	432	Ruxolitinib (1:1) (the only head-to-head study)	26.5% in MOM group versus 29% in RUX group (non-inferior)	28.4% in MOM group versus 42.2% in RUX group (inferior)	66.5% of MOM pts transfusion independent at 24 weeks versus 49.3% with RUX	37
MOM	SIMPLIFY-2 (phase III)	Adult MF patients who had suboptimal responses or hematological side effects with RUX	156	BAT (RUX in 89%) (2:1)	7% in MOM group versus 6% in RUX group (not superior)	26% in MOM group versus 6% in BAT group (*P* = 0.0006)	RBC transfusion independence at 24 weeks achieved by 43% of MOM group versus 21% of BAT group	38
FED (two doses)	JAKARTA-1 (phase III)	JAK-inhibitor-naïve pts with intermediate-1 to high-risk MF	289	Placebo (2:1)	36% and 40% in FED 400 mg and 500 mg groups, respectively, versus 1% with placebo	36% and 34% for the two doses versus 7% with placebo	Not reported (drug myelosuppressive)	39
FED (400 mg)	JAKARTA-2 (phase II)	RUX resistant or intolerant patients with intermediate or high-risk MF	97	None (single-arm study)	55% (46 of 83 evaluable pts)	26% (23 of 90 evaluable pts)	Not reported (drug myelosuppressive)	40

randomized ruxolitinib-naïve patients irrespective of platelet count to either pacritinib at a dose of 400 mg once daily or best available therapy (BAT) excluding ruxolitinib. Spleen and symptom responses were significantly superior in the pacritinib arm. Notably, patients with low platelet counts achieved comparable benefits. Toxicities were overall manageable[35]. PERSIST-2 recruited only patients with thrombocytopenia and randomized them to pacritinib at one of two doses or BAT including ruxolitinib. Prior use of ruxolitinib was allowed. Pacritinib at both doses was superior to BAT and led to significant spleen and symptom responses, with tolerable myelosuppression[36]. The development of pacritinib was halted by a clinical hold placed by the FDA due to suspicion of excess cardiovascular deaths in patients treated with the drug. This hold was subsequently removed, and the results of a new study that may hopefully lead to pacritinib's approval are awaited.

Momelotinib is a JAK1/2 inhibitor with activity against activin receptors believed to contribute to a remarkable anemia response, rendering this drug attractive, especially in the setting of transfusion-dependent anemia. The two phase III SIMPLIFY studies that evaluated momelotinib, despite showing a compelling anemia response, each failed to show superiority or non-inferiority in a primary or key-secondary endpoint[37,38]. A more pragmatic study design that would take into account the anemia response and superior tolerability of this drug along with spleen and symptom responses could allow for a better evaluation of this potentially beneficial agent[41]. Recently, the development of momelotinib was renewed, and a new phase III study is recruiting patients.

Fedratinib is a selective JAK2 inhibitor. The JAKARTA-1 study evaluated two doses of fedratinib versus placebo and showed significantly superior spleen and symptom responses in both treatment arms[39]. JAKARTA-2 was a single-arm phase II study evaluating fedratinib in patients who were intolerant of or resistant to ruxolitinib[40]. This was the only clinical trial directly evaluating a second-generation JAK inhibitor as second-line treatment, and responses, especially with regards to spleen volume reduction, were compelling for this patient population. The development of fedratinib was also stalled by a clinical hold placed by the FDA due to a suspected association with Wernicke's encephalopathy, which was eventually lifted following re-evaluation. The phase III FREEDOM study (NCT03755518) is currently recruiting patients and will hopefully contribute to further development of this drug with apparent potential clinical benefit. Based on the results of the JAKARTA-1 trial, on 16 August 2019, the FDA approved fedratinib for the treatment of adult patients with intermediate-2 or high-risk MF.

Bone marrow transplantation

The discovery by Fialkow in 1976 that MPNs are a clonal disorder of HSCs[3], more recently confirmed at the molecular level by the Weissman laboratory[4], led to the hypothesis that MPNs may be cured by HSCT, a procedure which replaces the malignant HSCs of the recipient with healthy ones provided by the donor. However, since the disease also impairs the supportive bone marrow microenvironment, which remains patient derived after transplantation[42], this approach was initially received with skepticism. Reports of long-term survival of transplant recipients coupled by histological evidence of resolution of bone marrow fibrosis eventually provided compelling evidence for the curative potential of this approach[43–46]. However, HSCT remained limited to a relatively small proportion of patients owing to the older age and frequent comorbidities of most patients and the high non-relapse mortality reported in early studies.

Recently, advances in transplantation medicine and molecular diagnosis are being translated to a consistent improvement in HSCT outcomes in MF[47–49]. Several factors contribute to the increased utilization and improved outcomes of HSCT for MF treatment: 1) the use of reduced intensity conditioning (RIC) regimens in older patients, which have demonstrated decreased transplant-related mortality with comparable relapse rates and overall survival[50–52]; 2) increased accessibility to HSCT for patients lacking sibling or matched-unrelated donors owing to advances in the use of alternative donors such as haploidentical donors and cord blood[53–55]; 3) accumulating data regarding mutational profiles and their prognostic significance allowing earlier and more informed patient selection[56]; and 4) accumulating experience with the use of ruxolitinib before or after transplant.

HSCT is presently considered the standard of care in eligible patients with intermediate-2 and high-risk disease[34]. There is lack of consensus regarding its use in earlier stages of the disease predicted to have more prolonged survival. A subset of patients classified as having low/intermediate-1 risk disease may eventually experience rapid progression to advanced MF and shortened survival. The recent advances in linking genetic profiles with risk of progression may improve the prediction of disease course and allow us to identify early stage patients who should be considered for HSCT.

Evaluation of novel agents

Numerous investigational agents are being evaluated for the treatment of MF alone or in combination with ruxolitinib. These agents were designed with the aim to a) improve anemia, b) deplete the malignant HSCs by targeting molecular alterations downstream of the genetic lesions, c) reduce microenvironmental abnormalities that may synergize with the driver mutations in sustaining proliferation of the malignant HSCs, or d) boost patients' immune reactions. Comprehensive reviews focusing on investigational agents for the treatment of MF have been published recently[57,58]. We will highlight several agents representing the above aims that have shown a strong scientific and preclinical rationale and/or encouraging signs of activity in early phase clinical studies.

Agents that improve anemia

Activins are members of the transforming growth factor (TGF)-β superfamily that inhibit the differentiation of late-stage erythrocyte precursors in a mechanism independent of erythropoietin and are overexpressed in myelodysplastic

syndromes (MDS) and in MF. Two agents that antagonize the activity of activins, sotatercept and luspatercept, have demonstrated efficacy in the treatment of anemia in patients with MDS[59–63]. These agents are currently being evaluated in patients with MF and anemia with promising results in early phase studies[64].

Drugs targeting epigenomic alterations found in malignant HSCs

Members of the Bromodomain and Extra-Terminal (BET) family of proteins function as "readers" of histone modification marks, by interacting with acetylated lysine residues on histone tails, and regulate genes that are involved in inflammation and cancer such as *MYC, BCL-2*, and *NF-kB*[65,66]. The NF-kB pathway downstream to BET has been shown to be activated in MF via JAK–STAT and other inflammatory cytokine signaling[67]. In a preclinical study using two MF mouse models, treatment with a BET inhibitor (BETi) resulted in reduction of cytokine production, spleen volume, and bone marrow fibrosis, and these effects were enhanced in combination with ruxolitinib[68]. These data indicate that drugs that reduce NFκB function and expression of NF-kB target genes might be effective in treating MF[69]. CPI-610, a BET inhibitor, is currently being evaluated for the treatment of MF patients, alone or in combination with ruxolitinib (NCT02158858).

While most of the above investigational agents as well as JAK inhibitors target signaling pathways involved in myeloproliferation and systemic inflammation, agents directly targeting MF-HSCs are lacking. The development of such agents has been a major challenge in the effort to improve the therapeutic armamentarium for myeloid malignancies. Imetelstat is an inhibitor of telomerase enzymatic activity[70]. The rationale for its use in MF stems from the findings of enhanced telomerase activity in MPN granulocytes[71]. A pilot study evaluating the use of single-agent telomerase in 33 patients with intermediate-2 and high-risk MF demonstrated a modest overall response rate (ORR) of 21%; however, in all four patients who achieved a complete response, bone marrow fibrosis was reversed, and a molecular response occurred in three of these patients, suggesting that imetelstat targets the malignant HSCs[72]. Preclinical studies confirmed these findings by demonstrating direct targeting of MF-CD34$^+$ cells by imetelstat, reflected by decreased formation of megakaryocytic colonies and by reduced human chimerism in an immune-deficient mouse xenotransplantation model. Both of these effects were selective to cells from MF patients, but not normal individuals, and sustained over time[73]. Recently, a multicenter phase II study evaluating two doses of imetelstat in a cohort of MF patients that progressed after or were refractory to ruxolitinib treatment has demonstrated an estimated twofold prolongation of survival as compared to retrospective reports of comparable patient populations, warranting further clinical testing[74].

Targeting the inflammatory microenvironment

Several clinical studies have shown that MPN patients express increased levels of inflammatory mediators[75], including the key inflammatory cytokine interleukin-8 (IL-8), the plasma levels of which predict adverse clinical outcomes[76]. The pathogenetic role of these cytokines is also supported by the observation that JAK inhibition can attenuate features of MPNs *in vivo* through the inhibition of cytokine production in mutant and non-mutant cells[77]. However, there is limited insight on the pathobiological consequence of aberrant cytokine production in MPNs. It is conceivable that specific targeting of some of the cytokines altered in MPNs may lead to better clinical outcomes than those obtained with JAK inhibitors[77]. An altered cytokine pathway that holds potential clinical interest in MF is the lipocalin-2 (LCN2)/interleukin-8 (IL-8) axis and its down-stream signaling via NF-kB. There is evidence that the sequence of events leading to abnormalities in the microenvironment in MF spleens and marrow includes, at least in part, LCN2[78]. LCN2 increased the proliferation of splenic endothelial cells, and LCN2 treatment of splenic stromal cells led to increased elaboration of IL-8, which contributes to the creation of an endothelial cell niche supporting the proliferation of MF-HSCs[79,80]. This endothelial cell niche can be disrupted *in vitro* by reparixin, an antagonist of the receptors for IL-8 CXCR1/2[81,82] that are highly expressed on MF spleen CD34$^+$ cells[79]. These data indicate that reparixin may represent a novel therapy which can antagonize the effects of IL-8 and thereby disrupt the HSC niche function of splenic endothelial cells. A clinical trial to test the effects of reparixin in MF is planned to be opened by the Myeloproliferative Neoplasm Research Consortium.

Boosting the patient's immune system

An important emerging approach in cancer therapy is activation of endogenous anti-tumor activity by treatment with agents that suppress the immune evasion mechanisms activated by cancer cells[83]. Checkpoint inhibitors, which block the interaction between programmed cell death (PD)1 and its ligand PD-L1 that is exploited by cancer cells to prevent attack by cytotoxic T-lymphocytes, have shown impressive anti-tumor responses in several solid cancers and B-lymphoid malignancies[83]. There is a strong pre-clinical rationale for inhibition of PD1/PD-L1 interaction in MF[84–86], and several clinical trials are ongoing (NCT03065400, NCT02421354, and NCT02871323). In addition, recent studies suggest that mutant calreticulin and JAK2 can induce specific anti-tumor T-cell responses, which can be harnessed for the development of mutation-specific peptide vaccines[87,88]. These and other immune therapy approaches, such as bispecific antibodies, are still in early stages of development.

Harnessing the altered p53–TGF-β circuitry to treat MF

Although MF originates at the level of HSCs, the predominance of the malignant HSCs over the reservoir of normal HSCs is sustained by tumor-induced micro-environments of bone marrow and spleen[89,90]. Studies in animal models indicate that these microenvironmental abnormalities may induce MF independently from the presence of driver mutations[91]. It is believed that the interaction between MF and abnormal microenvironments determine, at least in part, the clinical sequelae of MF as well as the rate of progression and evolution to

MPN-BP[75]. In addition to abnormalities described in a previous section, "Targeting the inflammatory microenvironment", possibly inter-related abnormalities for which promising investigational agents are available are represented by reduced expression of p53 in malignant HSCs and increased TGF-β bioavailability in their supporting microenvironment.

Targeting the reduced expression of p53 in MF-HSCs

p53 is a tumor suppressor that regulates cell cycle, apoptosis, DNA repair, and senescence of many cell types[92,93]. Hypomorphic/loss-of-function mutations of *p53* are associated with tumor progression in most human cancers[18], and inactivating mutations are observed in 20% of MPN-BP while deletion of *p53* leads to the development of AML in *JAK2*V617F-harboring mice[94]. Although in patients with chronic-phase MPN *p53* is usually wild-type, as discussed in "Targeting the inflammatory microenvironment", malignant HSCs are p53 hypomorphic, as they express low levels of the protein[95,96].

Studies to determine the levels of p53 in MF patients with no obvious *p53* mutations were inspired by Takaoka *et al*., who reported that interferon suppresses tumor progression and activates the immune response by increasing the expression of p53 in mouse models of fibroblast transformation[97]. Since interferon is an effective therapy in patients with MPNs[98], it was hypothesized that interferon is at least partially effective in these patients by increasing the otherwise low levels of p53 expressed by the malignant HSCs[99]. As in other cancers[95,100], the mechanisms that reduce p53 in MPNs may be represented by overexpression of its major regulators MDM2 (HDM2 in humans) and MDM4 (HDM4/HDMX), which are expressed at levels higher than normal in MF CD34$^+$ cells[99]. Overexpression of HDM4 and HDM2 in MF may be genetically determined. The two genes are localized on chromosomes 1 and 12, respectively, and gain of 1q and 12q rearrangements are frequently associated with disease progression in MPNs[101]. Alternatively, the observation that high TGF-β1 induces the expression of HDM2 in breast cancer driving its late metastatic stage[102] suggests that, even in the absence of chromosomal duplication, the great levels of TGF-β expressed in the microenvironment (see following section) may drive high levels of HDM2 in MF.

The existence of a p53/HDM2 cycle in MF is important because binding of p53 to HDM2 resulting in p53 ubiquitination and degradation is inhibited by a class of compounds called Nutlins. Nutlins (Nutlin-3, RG7112, RG7388, HDM201, and KRT232) bind to the MDM2 p53-binding site, interfering with its interaction with p53, leading to p53 accumulation and activation[95,100,103–106]. Proof-of-principle for the therapeutic use of Nutlins in MF was provided by the observation that the orally available Nutlin RG7388 (idasanutlin) depletes MPN-HSCs in culture[99]. In addition, low doses of the Nutlin RG7112 induce apoptosis of MF CD34$^+$ cells and reduce donor cell chimerism and *JAK2*V617F allele burden in NSG mice transplanted with MF CD34$^+$ cells[107]. The promising results of an open label phase I study of idasanutlin as a single agent in patients with PV and ET presented by Dr. Mascarenhas *et al*. in 2017 at ASH[108] and the observation that in AML the patients with the highest overexpression of HDM2 are most responsive to therapy with idasanutlin[109] suggest that MF patients, who express higher levels of HDM2 than those observed in PV, will respond well to idasanutlin. A clinical trial with idasanutlin in MF will be conducted soon by the Myeloproliferative Neoplasm Research Consortium.

Targeting the TGF-β circuitry in MF

Megakaryocytic hyperplasia was the first histopathological hallmark identified in MF[110]. These early studies also identified that this abnormality is associated with increased TGF-β content and release due to pathological emperipolesis (the interaction between megakaryocytes and neutrophils)[111]. However, in spite of the overwhelming evidence linking TGF-β with fibrosis in other systems[112,113], the link between megakaryocyte and TGF-β abnormalities and disease progression in MF has been obscured for some time by the fact that they are not direct targets of the driver mutations.

The link between driver mutations, alterations of megakaryocyte maturation, and disease progression in MF has been recently clarified by the observations that mice expressing *JAK2*V617F only in megakaryocytes develop MF[114]; the driver mutations induce a ribosomopathy that reduces the content of the transcription factor GATA1 (which is essential for terminal maturation) in megakaryocytes, halting their maturation[115,116]; mice lacking the regulatory sequences which specifically drive GATA1 expression in megakaryocytes (*Gata1*^{low} mice) develop the same megakaryocyte abnormalities observed in MF and MF with age[117,118]; and, finally, treatment with an inhibitor of Aurora kinase A, a protein overexpressed in MF megakaryocytes, rescues GATA1 expression in these cells, curing MF in animal models[119], suggesting GATA1 as a druggable target in MF[120]. This clinical hypothesis was tested by demonstrating that an inhibitor of Aurora Kinase A has some efficacy in MF patients[121]. Owing to these exciting results, additional megakaryocyte abnormalities are currently being considered as potential therapeutic targets for MF.

The Vainchenker laboratory hypothesized for the first time that TGF-β represents a target to cure MF by demonstrating that malignant HSCs lacking the TGF-β gene fail to induce MF upon transplantation in healthy recipients[122]. Furthermore, malignant HSCs did not induce MF when transplanted into recipients lacking the TGF-β receptor 1 gene[123]. These results suggest that TGF-β produced by the progeny of the malignant HSCs exerts its pathobiological effects by activating its receptor on a cell population present in the microenvironment. However, attempts to explore the clinical potential of TGF-β in MF were hampered for some time by the fact that the gene ablation strategy used by Vainchenker is not practical in clinical settings.

TGF-β is known to play a major role in the development of fibrosis in multiple organs[124] and in the induction of tumor-supporting microenvironments in other cancers[125,126]. The experiments by Vainchenker do not clarify whether TGF-β

promotes MF by inducing fibrosis and therefore by hampering the ability of the bone marrow microenvironment to support normal HSCs and/or by inducing a microenvironment supporting the malignant HSCs in extramedullary sites. Using the *Gata1*^{low} mouse model, our laboratory has identified at least some of the events linking megakaryocyte abnormalities, increased TGF-β, and development of MF. We determined that, although plasma and washes from bone marrow and spleen of *Gata1*^{low} mice, as well as those from MF patients, contain levels of total and bioactive TGF-β modestly (twofold) greater compared to normal controls[127,128], the megakaryocytes and their surrounding microenvironment contain levels of TGF-β 1000–2000-fold greater than normal[128,129], suggesting that it is not increased TGF-β content per se but its increased bioavailability that plays a major role in inducing disease progression in MF. By careful electron-microscopy analyses, we also determined that the increased TGF-β bioavailability is established by a sequence of abnormal cellular interactions involving megakaryocytes, neutrophils, and activated fibrocytes that are mediated by adhesion receptors that are druggable (Figure 1). Ablation of one of them, P-selectin, rescues MF in *Gata1*^{low} mice, providing clinical rationale for the use of P-selectin inhibitors, alone or in combination with ruxolitinib, in MF.

To identify additional druggable TGF-β targets, we characterized the TGF-β expression profiling of bone marrow and spleen from MF patients and *Gata1*^{low} mice[128,130]. These experiments identified that the tissues from the patients and the mouse model express the same distinctive abnormalities which include reduced expression of the canonical SMAD 1, 2, and 4 signaling and increased expression of *JUNB*, *EVI1*, and *STAT1*, three genes downstream of the non-canonical MAPK signaling. These abnormalities predict the activation of non-canonical p38/ERK-dependent rather than canonical SMAD-dependent signaling. The knowledge that activation of SMAD signaling induces normal HSCs into quiescence[131,132] while in systemic sclerosis activation of the p38/ERK pathway promotes the development of fibrosis[133,134] suggests a model for progression from the indolent phase of

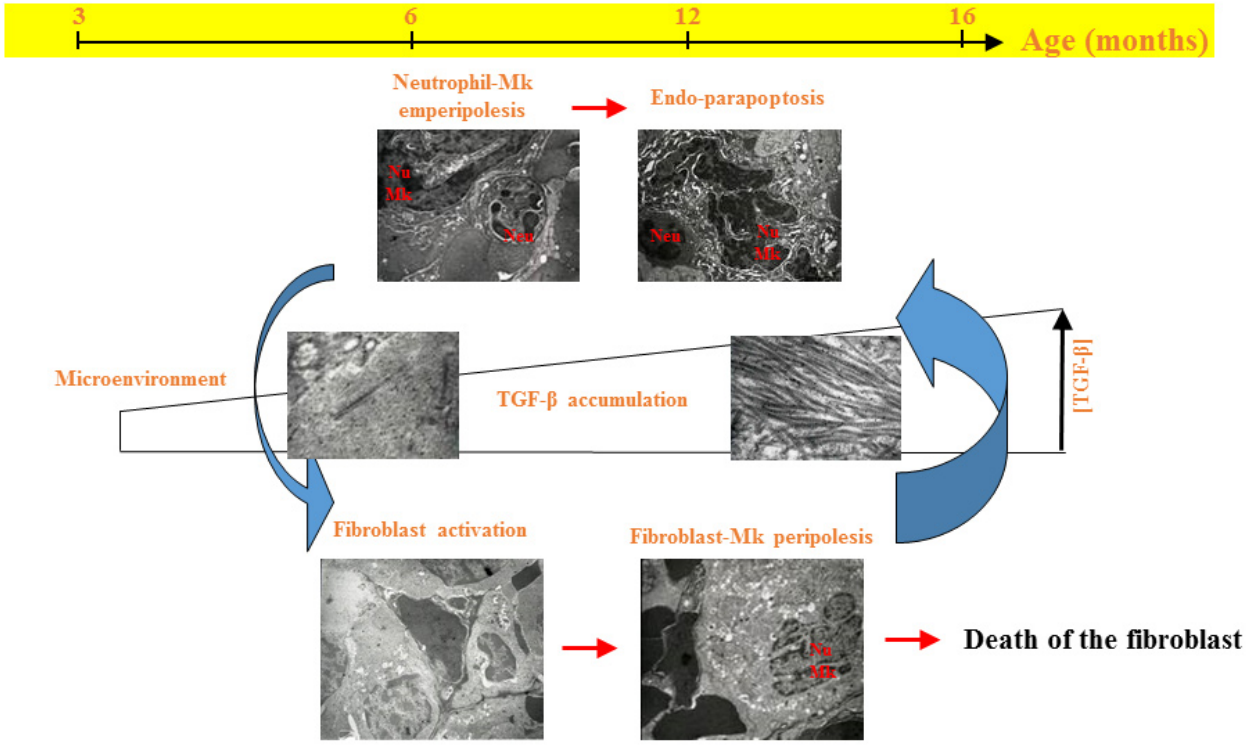

Figure 1. A cellular model for the establishment of increased transforming growth factor (TGF)-β bioavailability, which leads to fibrosis and disease progression in myelofibrosis (MF). This model was elaborated thanks to the fact that, in contrast with other animal models that develop a MPN phenotype that rapidly progress into its fatal MF phase (discussed in 114), *Gata1*^{low} mice slowly develop MF with age[117,118]. From 1–8 months, *Gata1*^{low} mice express pre-MF traits such as splenomegaly, increased rates of thrombosis, and osteosclerosis. From 8–12 months, they display MF traits including fibrosis and neovascularization, and from 12 months until their natural death they express a late-MF phenotype which includes increased stem/progenitor cell trafficking and extramedullary hematopoiesis in liver. The various phases are characterized by a sequence of abnormal cellular interactions that finally result in increased TGF-β bioavailability in the microenvironment. First, a pathological neutrophil-megakaryocyte (Mk) emperipolesis leads to death of the megakaryocytes by para-apoptosis, which releases TGF-β into the microenvironment. Second, TGF-β activates fibrocytes to produce collagen and to establish contacts with megakaryocytes, leading to the death of additional megakaryocytes and the release of activated lysyl-oxidase 2 (LOX2) into the microenvironment[135]. LOX2 polymerizes the collagen produced by the activated fibrocytes into collagen fibers, resulting in fibrosis. The collagen fibers are heavy binders of TGF-β, inducing the formation of areas of increased TGF-β bioavailability in the microenvironment (see also 128).

the disease (pre-MF) to MF depicted in Figure 2. In this model, increased levels of TGF-β promote disease progression by reducing the number of normal HSCs (inducing them into quiescence and reducing their supportive microenvironment in the bone marrow) while increasing the number of malignant HSCs (which are insensitive to TGF-β and are sustained by a specific microenvironment induced by TGF-β in the spleen).

Circumstantial evidence in support of the model for disease progression depicted in Figure 2 exists. First, we observed that the spleen, but not the bone marrow, from MF patients and *Gata1*^{low} mice contains greater numbers of activated fibrocytes that establish physical contacts with megakaryocytes, forming "nests", which lodge hematopoietic cells with the morphology of HSCs[128,136]. In the mouse model, the presence of these nests is induced by TGF-β. Second, we demonstrated that in contrast to normal HSCs, MF-HSCs are not induced into cycle by treatment with TGF-β receptor 1 kinase inhibitors[137].

Based on this overwhelming evidence, a proof-of-principle experiment was conducted that provided findings that one-month treatment with an inhibitor of LK5, the first element of the TGF-β receptor I signaling, completely rescues MF in *Gata1*^{low} mice[129,136]. Furthermore, a limited clinical trial with a human neutralizing antibody against TGF-β (fresolimumab, Sanofi Aventis) provided evidence for sustained improvements in hemoglobin level[137,138] and of reduced bone marrow fibrosis that lasted for at least one year after the drug was discontinued[137] in at least two of the three patients treated. A drawback of drugs targeting TGF-β is their lack of specificity that makes them prone to off-target effects. The availability of ligand-traps specific for TGF-β1, such as AVID200 developed by Formation Biologics[139], makes conducting clinical trials to test the efficacy of TGF-β inhibition in MF and eventually to halt the progression from pre-MF to MF in patients without fear of cardiovascular or osteological complications finally possible. AVID200 will be tested in a clinical trial to be opened soon by the Myeloproliferative Neoplasm consortium.

The existence of a circuitry between p53 and TGF-β in development and cancer progression has been extensively discussed[140–142]. In addition to the possibility that high levels of TGF-β may be responsible for reducing p53 in cancer cells by inducing HMD2 discussed in a previous section, "Targeting the reduced expression of p53 in MF-HSCs"[102], other feedbacks between the two pathways have also been described. In some cell models, low levels of p53 are responsible for rendering malignant cells insensitive to the inhibitory effects of TGF-β on proliferation[143]. It is therefore possible that, also in MF, low levels of p53 make the malignant HSCs TGF-β unresponsive. This consideration suggests that combination therapies

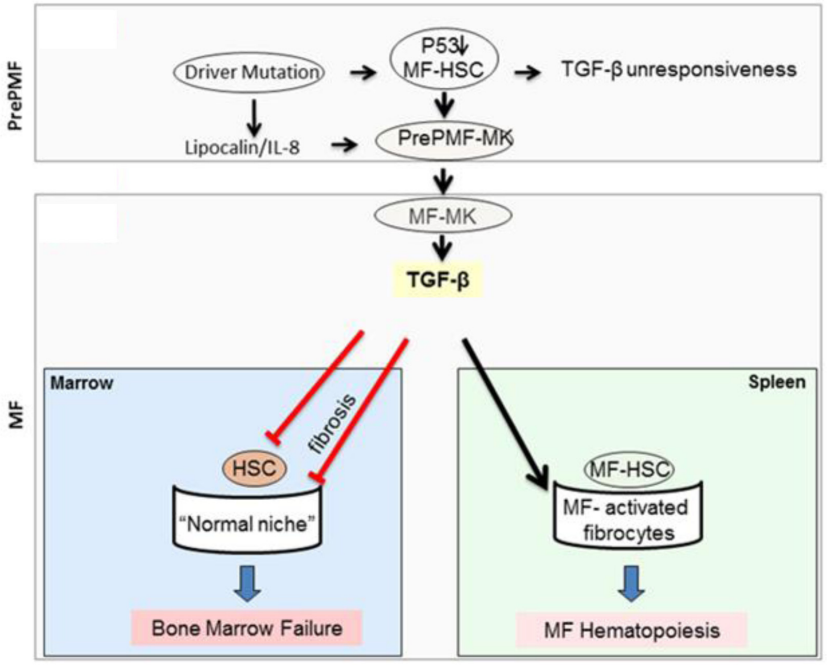

Figure 2. Circuitry between p53 abnormalities in the malignant hematopoietic stem cells (HSCs) and transforming growth factor (TGF)-β in the supporting microenvironment leading to disease progression in myelofibrosis (MF). It is suggested that in MF disease progression is driven by a p53/TGF-β circuitry. In the pre-MF stage, the driver mutations, possibly by inducing an inflammatory milieu (lipocalin-2 [LNC2]/interleukin-8 [IL-8]), reduce p53 in MF-HSCs, making these cells unresponsive to TGF-β and retarding megakaryocyte maturation. Retarded megakaryocyte maturation, associated with high IL-8 expression, induces, in an autocrine fashion, megakaryocytes to increase TGF-β bioavailability, which in turn is responsible for suppressing hematopoiesis from normal HSCs (inducing bone marrow failure) and for promoting an MF-HSC-supporting microenvironment in the spleen, facilitating the transition of pre-MF to MF (modified from 137). MK, megakaryocyte; PMF, primary myelofibrosis.

targeting p53 (Nutlins) and TGF-β (AVID200), by disrupting the cross-talks between the two pathways, have a greater potential than single therapies to cure MF. This hypothesis will be tested by ongoing preclinical studies.

Lessons learned from studying MF are broadly applicable to cancer pathogenesis and therapy

As previously discussed, the dissemination of malignant hematopoiesis beyond the marrow occurring during MF progression is associated with the establishment of malignant HSC-supporting microenvironments in the spleen. These events are strikingly similar to those occurring in many metastatic solid tumors[125,126], suggesting that lessons learned from studying MF may be applied to studying the progression of numerous other tumor types.

Induction of tumor-specific microenvironments as well as cell trafficking are complex processes that involve hundreds of genes, many of which are implicated in multiple biological processes. A comparison of gene dysregulation in *Gata1^{low}* mice and in the metastatic phase of other solid cancers provides support for the hypothesis that the two pathologies are determined by common mechanisms. Pathway analyses of expression profiling of bone marrow and spleen from *Gata1^{low}* mice identified two altered expression signatures involving 20–35 genes of the TGF-β1 signaling pathway with important hematopoietic functions[129]. In bone marrow, there was significant down-regulation of *Bmp2*, *Bmp5*, *Acvrl1*, and *Igf1*, whereas in the spleen, there was significant overexpression of *Cdkn1a* and *Ltbp1* and underexpression of *Gdf2* and *Nodal*. Interestingly, several of these events are reported also in other metastatic solid tumors. For example, TGF-β is dysregulated in colorectal cancer with similarity to those noted in the bone marrow, including downregulation of *Bmp2* and *Bmp5*[144]; ACVRL1 overexpression is also observed in colorectal cancer, where it has been suggested as a prognostic biomarker for the metastatic phase[145]; and TGF-β and its signaling cross-talk play a crucial role during the endothelial mesenchymal transition, which promotes the metastatic phase of various solid tumors, particularly in breast and pancreatic carcinoma[146]. These similarities strongly suggest that lessons learned from studying MF can be applied to studying the pathogenesis of other tumor types.

Finally, fibrosis is the end stage of all organ failures, including liver, kidney, heart, and lung, which, when associated with MF, have poor prognosis. It is conceivable that the identification of treatments which prevent or delay the progression of MF to PMF may inspire treatments for other organ failures as well.

Conclusion

Although MPNs are uniformly associated with the activation of the JAK/STAT signaling pathways, therapy with currently available JAK2 inhibitors is unable to deplete or eliminate MPN-HSCs. New developments in our knowledge on the biology of MPNs have identified an interplay among HSCs and microenvironmental abnormalities that may sustain disease progression. This cycle is druggable by BETi, CXCR1/2 inhibitors, p53 activators, and TGF-β inhibitors. These drugs are currently the subject of careful investigation in preclinical models and clinical studies in MF patients as single agents. We foresee that these careful approaches will soon identify the best agents that, alone or in combination, may take us closer to the cure of MF in the near future.

Author contributions

All of the authors have equally contributed to the literature search, the writing of the conceptualization of ideas, and the discussion of their perspectives, which led to the assembling of this manuscript.

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References

F1000 recommended

1. Arber DA, Orazi A, Hasserjian R, *et al.*: **The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia.** *Blood.* 2016; **127**(20): 2391–405.
PubMed Abstract | Publisher Full Text

2. Barosi G, Mesa RA, Thiele J, *et al.*: **Proposed criteria for the diagnosis of post-polycythemia vera and post-essential thrombocythemia myelofibrosis: a consensus statement from the International Working Group for Myelofibrosis Research and Treatment.** *Leukemia.* 2008; **22**(2): 437–8.
PubMed Abstract | Publisher Full Text

3. Adamson JW, Fialkow PJ, Murphy S, *et al.*: **Polycythemia vera: stem-cell and probable clonal origin of the disease.** *N Engl J Med.* 1976; **295**(17): 913–6.
PubMed Abstract | Publisher Full Text

4. Jamieson CH, Gotlib J, Durocher JA, *et al.*: **The *JAK2* V617F mutation occurs in hematopoietic stem cells in polycythemia vera and predisposes toward erythroid differentiation.** *Proc Natl Acad Sci U S A.* 2006; **103**(16): 6224–9.
PubMed Abstract | Publisher Full Text | Free Full Text

5. F Vainchenker W, Constantinescu SN, Plo I: **Recent advances in understanding myelofibrosis and essential thrombocythemia [version 1; peer review: 2 approved].** *F1000Res.* 2016; **5**: pii: F1000 Faculty Rev-700.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

6. Tefferi A: **Primary myelofibrosis: 2017 update on diagnosis, risk-stratification, and management.** *Am J Hematol.* 2016; **91**(12): 1262–71.
PubMed Abstract | Publisher Full Text

7. F James C, Ugo V, Le Couédic JP, *et al.*: **A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera.** *Nature.* 2005;

434(7037): 1144–8.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

8. Kralovics R, Passamonti F, Buser AS, *et al.*: **A gain-of-function mutation of JAK2 in myeloproliferative disorders.** *N Engl J Med.* 2005; **352**(17): 1779–90.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

9. Levine RL, Wadleigh M, Cools J, *et al.*: **Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis.** *Cancer Cell.* 2005; **7**(4): 387–97.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

10. Baxter EJ, Scott LM, Campbell PJ, *et al.*: **Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders.** *Lancet.* 2005; **365**(9464): 1054–61.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

11. Scott LM, Tong W, Levine RL, *et al.*: **JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis.** *N Engl J Med.* 2007; **356**(5): 459–68.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

12. Pikman Y, Lee BH, Mercher T, *et al.*: ***MPLW515L* is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia.** *PLoS Med.* 2006; **3**(7): e270.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

13. Klampfl T, Gisslinger H, Harutyunyan AS, *et al.*: **Somatic mutations of calreticulin in myeloproliferative neoplasms.** *N Engl J Med.* 2013; **369**(25): 2379–90.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

14. Nangalia J, Massie CE, Baxter EJ, *et al.*: **Somatic *CALR* mutations in myeloproliferative neoplasms with nonmutated *JAK2*.** *N Engl J Med.* 2013; **369**(25): 2391–405.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

15. Grinfeld J, Nangalia J, Baxter EJ, *et al.*: **Classification and Personalized Prognosis in Myeloproliferative Neoplasms.** *N Engl J Med.* 2018; **379**(15): 1416–30.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

16. Vannucchi AM, Lasho TL, Guglielmelli P, *et al.*: **Mutations and prognosis in primary myelofibrosis.** *Leukemia.* 2013; **27**(9): 1861–9.
PubMed Abstract | Publisher Full Text

17. Barraco D, Elala YC, Lasho TL, *et al.*: **Molecular correlates of anemia in primary myelofibrosis: a significant and independent association with U2AF1 mutations.** *Blood Cancer J.* 2016; **6**(4): e415.
PubMed Abstract | Publisher Full Text | Free Full Text

18. Harutyunyan A, Klampfl T, Cazzola M, *et al.*: **p53 Lesions in Leukemic Transformation.** *N Engl J Med.* 2011; **364**(5): 488–90.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

19. Rampal R, Ahn J, Abdel-Wahab O, *et al.*: **Genomic and functional analysis of leukemic transformation of myeloproliferative neoplasms.** *Proc Natl Acad Sci U S A.* 2014; **111**(50): E5401–10.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

20. Tefferi A, Guglielmelli P, Nicolosi M, *et al.*: **GIPSS: Genetically inspired prognostic scoring system for primary myelofibrosis.** *Leukemia.* 2018; **32**(7): 1631–42.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

21. Guglielmelli P, Lasho TL, Rotunno G, *et al.*: **MIPSS70: Mutation-Enhanced International Prognostic Score System for Transplantation-Age Patients With Primary Myelofibrosis.** *J Clin Oncol.* 2018; **36**(4): 310–8.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

22. Gagelmann N, Ditschkowski M, Bogdanov R, *et al.*: **Comprehensive clinical-molecular transplant scoring system for myelofibrosis undergoing stem cell transplantation.** *Blood.* 2019; **133**(20): 2233–42.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

23. Foucar CE, Stein BL: **Contemporary Use of Interferon Therapy in the Myeloproliferative Neoplasms.** *Curr Hematol Malig Rep.* 2017; **12**(5): 406–14.
PubMed Abstract | Publisher Full Text

24. Ianotto JC, Boyer-Perrard F, Gyan E, *et al.*: **Efficacy and safety of pegylated-interferon α-2a in myelofibrosis: A study by the FIM and GEM French cooperative groups.** *Br J Haematol.* 2013; **162**(6): 783–91.
PubMed Abstract | Publisher Full Text

25. Silver RT, Barel AC, Lascu E, *et al.*: **The effect of initial molecular profile on response to recombinant interferon-α (rIFNα) treatment in early myelofibrosis.** *Cancer.* 2017; **123**(14): 2680–7.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

26. Quintás-Cardama A, Tong W, Kantarjian H, *et al.*: **A phase II study of 5-azacitidine for patients with primary and post-essential thrombocythemia/polycythemia vera myelofibrosis.** *Leukemia.* 2008; **22**(5): 965–70.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

27. Thepot S, Itzykson R, Seegers V, *et al.*: **Treatment of progression of Philadelphia-negative myeloproliferative neoplasms to myelodysplastic syndrome or acute myeloid leukemia by azacitidine: a report on 54 cases on the behalf of the Groupe Francophone des Myelodysplasies (GFM).** *Blood.* 2010; **116**(19): 3735–42.
PubMed Abstract | Publisher Full Text

28. Daver N, Cortes JE, Pemmaraju N, *et al.*: **Ruxolitinib (RUX) in Combination with 5-Azacytidine (AZA) As Therapy for Patients (pts) with Myelofibrosis (MF).** *Blood.* 2016; **128**(22): 1127, Accessed July 31, 2019.
Reference Source

29. Verstovsek S, Mesa RA, Gotlib J, *et al.*: **A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis.** *N Engl J Med.* 2012; **366**(9): 799–807.
PubMed Abstract | Publisher Full Text | Free Full Text

30. Harrison C, Kiladjian JJ, Al-Ali HK, *et al.*: **JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis.** *N Engl J Med.* 2012; **366**(9): 787–98.
PubMed Abstract | Publisher Full Text

31. Verstovsek S, Gotlib J, Mesa RA, *et al.*: **Long-term survival in patients treated with ruxolitinib for myelofibrosis: COMFORT-I and -II pooled analyses.** *J Hematol Oncol.* 2017; **10**(1): 156.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

32. Martí-Carvajal AJ, Anand V, Solà I: **Janus kinase-1 and Janus kinase-2 inhibitors for treating myelofibrosis.** *Cochrane Database Syst Rev.* 2015; (4): CD010298.
PubMed Abstract | Publisher Full Text

33. Cervantes F, Pereira A: **Does ruxolitinib prolong the survival of patients with myelofibrosis?** *Blood.* 2017; **129**(7): 832–7.
PubMed Abstract | Publisher Full Text

34. Barbui T, Tefferi A, Vannucchi AM, *et al.*: **Philadelphia chromosome-negative classical myeloproliferative neoplasms: revised management recommendations from European LeukemiaNet.** *Leukemia.* 2018; **32**(5): 1057–69.
PubMed Abstract | Publisher Full Text | Free Full Text

35. Mesa RA, Vannucchi AM, Mead A, *et al.*: **Pacritinib versus best available therapy for the treatment of myelofibrosis irrespective of baseline cytopenias (PERSIST-1): An international, randomised, phase 3 trial.** *Lancet Haematol.* 2017; **4**(5): e225–e236.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

36. Mascarenhas J, Hoffman R, Talpaz M, *et al.*: **Pacritinib vs Best Available Therapy, Including Ruxolitinib, in Patients With Myelofibrosis: A Randomized Clinical Trial.** *JAMA Oncol.* 2018; **4**(5): 652–9.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

37. Mesa RA, Kiladjian JJ, Catalano JV, *et al.*: **SIMPLIFY-1: A Phase III Randomized Trial of Momelotinib Versus Ruxolitinib in Janus Kinase Inhibitor-Naïve Patients With Myelofibrosis.** *J Clin Oncol.* 2017; **35**(34): 3844–50.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

38. Harrison CN, Vannucchi AM, Platzbecker U, *et al.*: **Momelotinib versus best available therapy in patients with myelofibrosis previously treated with ruxolitinib (SIMPLIFY 2): a randomised, open-label, phase 3 trial.** *Lancet Haematol.* 2018; **5**(2): e73–e81.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

39. Pardanani A, Harrison C, Cortes JE, *et al.*: **Safety and Efficacy of Fedratinib in Patients With Primary or Secondary Myelofibrosis: A Randomized Clinical Trial.** *JAMA Oncol.* 2015; **1**(5): 643–51.
PubMed Abstract | Publisher Full Text

40. Harrison CN, Schaap N, Vannucchi AM, *et al.*: **Janus kinase-2 inhibitor fedratinib in patients with myelofibrosis previously treated with ruxolitinib (JAKARTA-2): a single-arm, open-label, non-randomised, phase 2, multicentre study.** *Lancet Haematol.* 2017; **4**(7): e317–e324.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

41. Mascarenhas J, Hoffman R: **Don't judge a JAK2 inhibitor by spleen response alone.** *Lancet Haematol.* 2018; **5**(2): e56–e57.
PubMed Abstract | Publisher Full Text

42. Simmons PJ, Przepiorka D, Thomas ED, *et al.*: **Host origin of marrow stromal cells following allogeneic bone marrow transplantation.** *Nature.* 1987; **328**(6129): 429–32.
PubMed Abstract | Publisher Full Text

43. Guardiola P, Anderson JE, Bandini G, *et al.*: **Allogeneic stem cell transplantation for agnogenic myeloid metaplasia: a European Group for Blood and Marrow Transplantation, Société Française de Greffe de Moelle, Gruppo Italiano per il Trapianto del Midollo Osseo, and Fred Hutchinson Cancer Research Center Collaborative Study.** *Blood.* 1999; **93**(9): 2831–8.
PubMed Abstract

44. Deeg HJ, Gooley TA, Flowers ME, *et al.*: **Allogeneic hematopoietic stem cell transplantation for myelofibrosis.** *Blood.* 2003; **102**(12): 3912–8.
PubMed Abstract | Publisher Full Text

45. Migliaccio AR, Martelli F, Verrucci M, *et al.*: **Altered SDF-1/CXCR4 axis in patients with primary myelofibrosis and in the Gata1^{low} mouse model of the disease.** *Exp Hematol.* 2008; **36**(2): 158–71.
PubMed Abstract | Publisher Full Text | Free Full Text

46. Kröger N, Zabelina T, Alchalby H, *et al.*: **Dynamic of bone marrow fibrosis regression predicts survival after allogeneic stem cell transplantation for myelofibrosis.** *Biol Blood Marrow Transplant.* 2014; **20**(6): 812–5.
PubMed Abstract | Publisher Full Text

47. Kröger N, Giorgino T, Scott BL, *et al.*: **Impact of allogeneic stem cell transplantation on survival of patients less than 65 years of age with primary myelofibrosis.** *Blood.* 2015; **125**(21): 3347–50; quiz 3364.
PubMed Abstract | Publisher Full Text | Free Full Text

48. Keyzner A, Han S, Shapiro S, *et al.*: **Outcome of Allogeneic Hematopoietic Stem Cell Transplantation for Patients with Chronic and Advanced Phase Myelofibrosis.** *Biol Blood Marrow Transplant.* 2016; **22**(12): 2180–6.
PubMed Abstract | Publisher Full Text

49. Ali H, Aldoss I, Yang D, *et al.*: **Long-Term Survival in Myelofibrosis after Allogeneic Hematopoietic Cell Transplantation Using Fludarabine/Melphalan Conditioning Regimen.** *Blood.* 2017; **130**(Suppl 1): 199. Accessed July 10, 2019.
Reference Source

50. Gupta H, Klisovic R, Galvin J, *et al.*: **Exploring the Potential of JAK1/2 Inhibitor Ruxolitinib with Reduced Intensity Hematopoietic Cell Transplantation (HCT) for Myelofibrosis: Stage I Results of a Prospective Trial Conducted through the Myeloproliferative Disorders - Research Consortium (MPD-RC).** *Blood.* 2016; **128**(22): 1126.
Reference Source

51. Kröger N, Holler E, Kobbe G, *et al.*: **Allogeneic stem cell transplantation after reduced-intensity conditioning in patients with myelofibrosis: a prospective, multicenter study of the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation.** *Blood.* 2009; **114**(26): 5264–70.
PubMed Abstract | Publisher Full Text

52. Rondelli D, Goldberg JD, Isola L, *et al.*: **MPD-RC 101 prospective study of reduced-intensity allogeneic hematopoietic stem cell transplantation in patients with myelofibrosis.** *Blood.* 2014; **124**(7): 1183–91.
PubMed Abstract | Publisher Full Text | Free Full Text

53. Bregante S, Dominietto A, Ghiso A, *et al.*: **Improved Outcome of Alternative Donor Transplantations in Patients with Myelofibrosis: From Unrelated to Haploidentical Family Donors.** *Biol Blood Marrow Transplant.* 2016; **22**(2): 324–9.
PubMed Abstract | Publisher Full Text

54. Takagi S, Ota Y, Uchida N, *et al.*: **Successful engraftment after reduced-intensity umbilical cord blood transplantation for myelofibrosis.** *Blood.* 2010; **116**(4): 649–52.
PubMed Abstract | Publisher Full Text

55. Robin M, Giannotti F, Deconinck E, *et al.*: **Unrelated cord blood transplantation for patients with primary or secondary myelofibrosis.** *Biol Blood Marrow Transplant.* 2014; **20**(11): 1841–6.
PubMed Abstract | Publisher Full Text

56. F Kröger N, Panagiota V, Badbaran A, *et al.*: **Impact of Molecular Genetics on Outcome in Myelofibrosis Patients after Allogeneic Stem Cell Transplantation.** *Biol Blood Marrow Transplant.* 2017; **23**(7): 1095–101.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

57. Vannucchi AM, Harrison CN: **Emerging treatments for classical myeloproliferative neoplasms.** *Blood.* 2017; **129**(6): 693–703.
PubMed Abstract | Publisher Full Text

58. F Shreenivas A, Mascarenhas J: **Emerging drugs for the treatment of Myelofibrosis.** *Expert Opin Emerg Drugs.* 2018; **23**(1): 37–49.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

59. Carrancio S, Markovics J, Wong P, *et al.*: **An activin receptor IIA ligand trap promotes erythropoiesis resulting in a rapid induction of red blood cells and haemoglobin.** *Br J Haematol.* 2014; **165**(6): 870–82.
PubMed Abstract | Publisher Full Text | Free Full Text

60. Suragani RN, Cadena SM, Cawley SM, *et al.*: **Transforming growth factor-β superfamily ligand trap ACE-536 corrects anemia by promoting late-stage erythropoiesis.** *Nat Med.* 2014; **20**(4): 408–14.
PubMed Abstract | Publisher Full Text

61. Iancu-Rubin C, Mosoyan G, Wang J, *et al.*: **Stromal cell-mediated inhibition of erythropoiesis can be attenuated by Sotatercept (ACE-011), an activin receptor type II ligand trap.** *Exp Hematol.* 2013; **41**(2): 155–166.e17.
PubMed Abstract | Publisher Full Text

62. F Komrokji R, Garcia-Manero G, Ades L, *et al.*: **Sotatercept with long-term extension for the treatment of anaemia in patients with lower-risk myelodysplastic syndromes: a phase 2, dose-ranging trial.** *Lancet Haematol.* 2018; **5**(2): e63–e72.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

63. F Platzbecker U, Germing U, Götze KS, *et al.*: **Luspatercept for the treatment of anaemia in patients with lower-risk myelodysplastic syndromes (PACE-MDS): a multicentre, open-label phase 2 dose-finding study with long-term extension study.** *Lancet Oncol.* 2017; **18**(10): 1338–47.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

64. Bose P, Daver N, Pemmaraju N, *et al.*: **Sotatercept (ACE-011) Alone and in Combination with Ruxolitinib in Patients (pts) with Myeloproliferative Neoplasm (MPN)-Associated Myelofibrosis (MF) and Anemia.** *Blood.* 2017; **130**(Suppl 1): 255. Accessed July 11, 2019.
Reference Source

65. F Filippakopoulos P, Qi J, Picaud S, *et al.*: **Selective inhibition of BET bromodomains.** *Nature.* 2010; **468**(7327): 1067–73.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

66. F Pérez-Salvia M, Esteller M: **Bromodomain inhibitors and cancer therapy: From structures to applications.** *Epigenetics.* 2017; **12**(5): 323–39.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

67. Hayden MS, Ghosh S: **Shared principles in NF-kappaB signaling.** *Cell.* 2008; **132**(3): 344–62.
PubMed Abstract | Publisher Full Text

68. F Kleppe M, Koche R, Zou L, *et al.*: **Dual Targeting of Oncogenic Activation and Inflammatory Signaling Increases Therapeutic Efficacy in Myeloproliferative Neoplasms.** *Cancer Cell.* 2018; **33**(1): 29–43.e7.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

69. F Saenz DT, Fiskus W, Manshouri T, *et al.*: **BET protein bromodomain inhibitor-based combinations are highly active against post-myeloproliferative neoplasm secondary AML cells.** *Leukemia.* 2017; **31**(3): 678–87.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

70. Mocellin S, Pooley KA, Nitti D: **Telomerase and the search for the end of cancer.** *Trends Mol Med.* 2013; **19**(2): 125–33.
PubMed Abstract | Publisher Full Text

71. F Vasko T, Kaifie A, Stope MB, *et al.*: **Telomeres and Telomerase in Hematopoietic Dysfunction: Prognostic Implications and Pharmacological Interventions.** *Int J Mol Sci.* 2017; **18**(11): pii: E2267.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

72. Tefferi A, Lasho TL, Begna KH, *et al.*: **A Pilot Study of the Telomerase Inhibitor Imetelstat for Myelofibrosis.** *N Engl J Med.* 2015; **373**(10): 908–19.
PubMed Abstract | Publisher Full Text

73. F Mosoyan G, Kraus T, Ye F, *et al.*: **Imetelstat, a telomerase inhibitor, differentially affects normal and malignant megakaryopoiesis.** *Leukemia.* 2017; **31**(11): 2458–67.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

74. Mascarenhas J, Komrokji RS, Cavo M, *et al.*: **Imetelstat Is Effective Treatment for Patients with Intermediate-2 or High-Risk Myelofibrosis Who Have Relapsed on or Are Refractory to Janus Kinase Inhibitor Therapy: Results of a Phase 2 Randomized Study of Two Dose Levels.** *Blood.* 2018; **132**(Suppl 1): 685–685.
Reference Source

75. Hasselbalch HC: **Perspectives on chronic inflammation in essential thrombocythemia, polycythemia vera, and myelofibrosis: is chronic inflammation a trigger and driver of clonal evolution and development of accelerated atherosclerosis and second cancer?** *Blood.* 2012; **119**(14): 3219–25.
PubMed Abstract | Publisher Full Text

76. F Tefferi A, Vaidya R, Caramazza D, *et al.*: **Circulating interleukin (IL)-8, IL-2R, IL-12, and IL-15 levels are independently prognostic in primary myelofibrosis: a comprehensive cytokine profiling study.** *J Clin Oncol.* 2011; **29**(10): 1356–63.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

77. F Kleppe M, Kwak M, Koppikar P, *et al.*: **JAK-STAT pathway activation in malignant and nonmalignant cells contributes to MPN pathogenesis and therapeutic response.** *Cancer Discov.* 2015; **5**(3): 316–31.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

78. Lu M, Xia L, Liu YC, *et al.*: **Lipocalin produced by myelofibrosis cells affects the fate of both hematopoietic and marrow microenvironmental cells.** *Blood.* 2015; **126**(8): 972–82.
PubMed Abstract | Publisher Full Text | Free Full Text

79. Lu M, Xia L, Salama ME, *et al.*: **Splenic Micro Environmental Cells from Patients with Myelofibrosis Elaborate a Cascade of Cytokines and Serve As a Niche for Malignant Hematopoiesis.** *Blood.* 2016; **128**(22): 953.
Reference Source

80. Wang X, Prakash S, Lu M, *et al.*: **Spleens of myelofibrosis patients contain malignant hematopoietic stem cells.** *J Clin Invest.* 2012; **122**(11): 3888–99.
PubMed Abstract | Publisher Full Text | Free Full Text

81. Goldstein L, Perez RP, Yardley DA, *et al.*: **A single-arm, preoperative, pilot study to evaluate the safety and biological effects of orally administered reparixin in early breast cancer patients who are candidates for surgery.** *Cancer Res.* 2016; **76**(14): Suppl.
Publisher Full Text

82. Pawlick RL, Wink J, Pepper AR, *et al.*: **Reparixin, a CXCR1/2 inhibitor in islet allotransplantation.** *Islets.* 2016; **8**(5): 115–24.
PubMed Abstract | Publisher Full Text | Free Full Text

83. Mellman I, Coukos G, Dranoff G: **Cancer immunotherapy comes of age.** *Nature.* 2011; **480**(7378): 480–9.
PubMed Abstract | Publisher Full Text | Free Full Text

84. F Choi DC, Tremblay D, Iancu-Rubin C, *et al.*: **Programmed cell death-1 pathway inhibition in myeloid malignancies: Implications for myeloproliferative neoplasms.** *Ann Hematol.* 2017; **96**(6): 919–27.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

85. F Prestipino A, Emhardt AJ, Aumann K, *et al.*: **Oncogenic JAK2^{V617F} causes PD-L1 expression, mediating immune escape in myeloproliferative neoplasms.** *Sci Transl Med.* 2018; **10**(429): pii: eaam7729.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

86. Craig R, Tripp SR, Deininger MSM: **Programmed death ligand (PD-L1) expression is increased in spleens of myelofibrosis patients**. In: *United States and Canadian Academy (Annual Meeting)*. 2016; 1353.

87. F Holmström MO, Martinenaite E, Ahmad SM, *et al.*: **The calreticulin (CALR) exon 9 mutations are promising targets for cancer immune therapy.** *Leukemia.* 2018; **32**(2): 429–37.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

88. Cimen Bozkus C, Finnigan JP, Mascarenhas J, *et al.*: **Immune Checkpoint Blockade Enhances Mutated Calreticulin-Induced T Cell Immunity in Myeloproliferative Neoplasms.** *Blood.* 2017; **130**(Suppl 1): 384. Accessed July 16. 2019.
Reference Source

89. Desterke C, Martinaud C, Ruzehaji N, *et al.*: **Inflammation as a Keystone of Bone Marrow Stroma Alterations in Primary Myelofibrosis.** *Mediators Inflamm.* 2015; **2015**: 415024.
PubMed Abstract | Publisher Full Text | Free Full Text

90. F Martinaud C, Desterke C, Konopacki J, *et al.*: **Osteogenic Potential of Mesenchymal Stromal Cells Contributes to Primary Myelofibrosis.** *Cancer Res.* 2015; **75**(22): 4753–65.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

91. F Walkley CR, Olsen GH, Dworkin S, *et al.*: **A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor gamma deficiency.** *Cell.* 2007; **129**(6): 1097–110.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

92. Vousden KH, Lane DP: **p53 in health and disease.** *Nat Rev Mol Cell Biol.* 2007; **8**(4): 275–83.
PubMed Abstract | Publisher Full Text

93. Reinhardt HC, Schumacher B: **The p53 network: Cellular and systemic DNA damage responses in aging and cancer.** *Trends Genet.* 2012; **28**(3): 128–36.
PubMed Abstract | Publisher Full Text | Free Full Text

94. F Tsuruta-Kishino T, Koya J, Kataoka K, *et al.*: **Loss of p53 induces leukemic transformation in a murine model of Jak2 V617F-driven polycythemia vera.** *Oncogene.* 2017; **36**(23): 3300–11.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

95. Shangary S, Wang S: **Targeting the MDM2-p53 interaction for cancer therapy.** *Clin Cancer Res.* 2008; **14**(17): 5318–24.
PubMed Abstract | Publisher Full Text | Free Full Text

96. F Nakatake M, Monte-Mor B, Debili N, *et al.*: **JAK2^{V617F} negatively regulates p53 stabilization by enhancing MDM2 via La expression in myeloproliferative neoplasms.** *Oncogene.* 2012; **31**(10): 1323–33.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

97. F Takaoka A, Hayakawa S, Yanai H, *et al.*: **Integration of interferon-alpha/beta signalling to p53 responses in tumour suppression and antiviral defence.** *Nature.* 2003; **424**(6948): 516–23.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

98. Gowin K, Thapaliya P, Samuelson J, *et al.*: **Experience with pegylated interferon α-2a in advanced myeloproliferative neoplasms in an international cohort of 118 patients.** *Haematologica.* 2012; **97**(10): 1570–3.
PubMed Abstract | Publisher Full Text | Free Full Text

99. Lu M, Wang X, Li Y, *et al.*: **Combination treatment *in vitro* with Nutlin, a small-molecule antagonist of MDM2, and pegylated interferon-α 2a specifically targets JAK2V617F-positive polycythemia vera cells.** *Blood.* 2012; **120**(15): 3098–105.
PubMed Abstract | Publisher Full Text | Free Full Text

100. Wade M, Li YC, Wahl GM: **MDM2, MDMX and p53 in oncogenesis and cancer therapy.** *Nat Rev Cancer.* 2013; **13**(2): 83–96.
PubMed Abstract | Publisher Full Text | Free Full Text

101. F Marcellino BK, Hoffman R, Tripodi J, *et al.*: **Advanced forms of MPNs are accompanied by chromosomal abnormalities that lead to dysregulation of TP53.** *Blood Adv.* 2018; **2**(24): 3581–9.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

102. Araki S, Eitel JA, Batuello CN, *et al.*: **TGF-beta1-induced expression of human Mdm2 correlates with late-stage metastatic breast cancer.** *J Clin Invest.* 2010; **120**(1): 290–302.
PubMed Abstract | Publisher Full Text | Free Full Text

103. F Lu M, Breyssens H, Salter V, *et al.*: **Restoring p53 function in human melanoma cells by inhibiting MDM2 and cyclin B1/CDK1-phosphorylated nuclear iASPP.** *Cancer Cell.* 2013; **23**(5): 618–33.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

104. Vassilev LT: **p53 Activation by small molecules: application in oncology.** *J Med Chem.* 2005; **48**(14): 4491–9.
PubMed Abstract | Publisher Full Text

105. Ray-Coquard I, Blay JY, Italiano A, *et al.*: **Effect of the MDM2 antagonist RG7112 on the P53 pathway in patients with *MDM2*-amplified, well-differentiated or dedifferentiated liposarcoma: an exploratory proof-of-mechanism study.** *Lancet Oncol.* 2012; **13**(11): 1133–40.
PubMed Abstract | Publisher Full Text

106. Ding Q, Zhang Z, Liu JJ, *et al.*: **Discovery of RG7388, a potent and selective p53-MDM2 inhibitor in clinical development.** *J Med Chem.* 2013; **56**(14): 5979–83.
PubMed Abstract | Publisher Full Text

107. Lu M, Xia L, Li Y, *et al.*: **The orally bioavailable MDM2 antagonist RG7112 and pegylated interferon α 2a target *JAK2V617F*-positive progenitor and stem cells.** *Blood.* 2014; **124**(5): 771–9.
PubMed Abstract | Publisher Full Text | Free Full Text

108. Mascarenhas J, Lu M, Virtgaym E, *et al.*: **Open Label Phase I Study of Single Agent Oral RG7388 (idasanutlin) in Patients with Polycythemia Vera and Essential Thrombocythemia.** *Blood.* 2017; **130**(Suppl 1): 254.
Reference Source

109. Reis B, Jukofsky L, Chen G, *et al.*: **Acute myeloid leukemia patients' clinical response to idasanutlin (RG7388) is associated with pre-treatment MDM2 protein expression in leukemic blasts.** *Haematologica.* 2016; **101**(5): e185–8.
PubMed Abstract | Publisher Full Text | Free Full Text

110. Zucker-Franklin D: **Ultrastructural studies of hematopoietic elements in relation to the myelofibrosis-osteosclerosis syndrome, megakaryocytes and p latelets (MMM or MOS).** *AdvBiosci.* 1974; **16**: 127–143.

111. Schmitt A, Jouault H, Guichard J, *et al.*: **Pathologic interaction between megakaryocytes and polymorphonuclear leukocytes in myelofibrosis.** *Blood.* 2000; **96**(4): 1342–7.
PubMed Abstract

112. Massagué J: **TGFbeta in Cancer.** *Cell.* 2008; **134**(2): 215–30.
PubMed Abstract | Publisher Full Text | Free Full Text

113. Flavell RA, Sanjabi S, Wrzesinski SH, *et al.*: **The polarization of immune cells in the tumour environment by TGFbeta.** *Nat Rev Immunol.* 2010; **10**(8): 554–67.
PubMed Abstract | Publisher Full Text | Free Full Text

114. Zhan H, Ma Y, Lin CH, *et al.*: **JAK2^{V617F}-mutant megakaryocytes contribute to hematopoietic stem/progenitor cell expansion in a model of murine myeloproliferation.** *Leukemia.* 2016; **30**(12): 2332–41.
PubMed Abstract | Publisher Full Text | Free Full Text

115. Gilles L, Arslan AD, Marinaccio C, *et al.*: **Downregulation of GATA1 drives impaired hematopoiesis in primary myelofibrosis.** *J Clin Invest.* 2017; **127**(4): 1316–20.
PubMed Abstract | Publisher Full Text | Free Full Text

116. Zingariello M, Sancillo L, Martelli F, *et al.*: **The thrombopoietin/MPL axis is activated in the Gata1^{low} mouse model of myelofibrosis and is associated with a defective RPS14 signature.** *Blood Cancer J.* 2017; **7**(6): e572.
PubMed Abstract | Publisher Full Text | Free Full Text

117. F Vannucchi AM, Bianchi L, Cellai C, *et al.*: **Development of myelofibrosis in mice genetically impaired for GATA-1 expression (GATA-1^{low} mice).** *Blood.* 2002; **100**(4): 1123–32.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

118. Martelli F, Ghinassi B, Panetta B, *et al.*: **Variegation of the phenotype induced by the Gata1^{low} mutation in mice of different genetic backgrounds.** *Blood.* 2005; **106**(13): 4102–13.
PubMed Abstract | Publisher Full Text

119. F Wen QJ, Yang Q, Goldenson B, *et al.*: **Targeting megakaryocytic-induced fibrosis in myeloproliferative neoplasms by AURKA inhibition.** *Nat Med.* 2015; **21**(12): 1473–80.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

120. Ling T, Crispino JD, Zingariello M, *et al.*: **GATA1 insufficiencies in primary myelofibrosis and other hematopoietic disorders: consequences for therapy.** *Expert Rev Hematol.* 2018; **11**(3): 169–84.
PubMed Abstract | Publisher Full Text | Free Full Text

121. Gangat N, Stein BL, Marinaccio C, *et al.*: **Alisertib (MLN8237), an Oral Selective Inhibitor of Aurora Kinase a Has Clinical Activity and Restores GATA1 Expression in Patients with Myelofibrosis.** *Blood.* 2018; **132**(Suppl 1): 688.
Reference Source

122. Chagraoui H, Komura E, Tulliez M, *et al.*: **Prominent role of TGF-beta 1 in thrombopoietin-induced myelofibrosis in mice.** *Blood.* 2002; **100**(10): 3495–503.
PubMed Abstract | Publisher Full Text

123. Gastinne T, Vigant F, Lavenu-Bombled C, *et al.*: **Adenoviral-mediated TGF-beta1 inhibition in a mouse model of myelofibrosis inhibit bone marrow fibrosis development.** *Exp Hematol.* 2007; **35**(1): 64–74.
PubMed Abstract | Publisher Full Text

124. F Wernig G, Chen SY, Cui L, *et al.*: **Unifying mechanism for different fibrotic diseases.** *Proc Natl Acad Sci U S A.* 2017; **114**(18): 4757–62.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

125. Kalluri R, Weinberg RA: **The basics of epithelial-mesenchymal transition.** *J Clin Invest.* 2009; **119**(6): 1420–8.
PubMed Abstract | Publisher Full Text | Free Full Text

126. Scheel C, Weinberg RA: **Cancer stem cells and epithelial-mesenchymal transition: Concepts and molecular links.** *Semin Cancer Biol.* 2012; **22**(5–6): 396–403.
PubMed Abstract | Publisher Full Text | Free Full Text

127. Vannucchi AM, Bianchi L, Paoletti F, *et al.*: **A pathobiologic pathway linking thrombopoietin, GATA-1, and TGF-beta1 in the development of myelofibrosis.** *Blood.* 2005; **105**(9): 3493–501.
PubMed Abstract | Publisher Full Text

128. Zingariello M, Ruggeri A, Martelli F, *et al.*: **A novel interaction between megakaryocytes and activated fibrocytes increases TGF-β bioavailability in the *Gata1*^{low} mouse model of myelofibrosis.** *Am J Blood Res.* 2015; **5**(2): 34–61.
PubMed Abstract | Free Full Text

129. Zingariello M, Martelli F, Ciaffoni F, *et al.*: **Characterization of the TGF-β1 signaling abnormalities in the Gata1^{low} mouse model of myelofibrosis.** *Blood.* 2013; **121**(17): 3345–63.
PubMed Abstract | Publisher Full Text | Free Full Text

130. Ciaffoni F, Cassella E, Varricchio L, *et al.*: **Activation of non-canonical TGF-β1 signaling indicates an autoimmune mechanism for bone marrow fibrosis in primary myelofibrosis.** *Blood Cells Mol Dis.* 2015; **54**(3): 234–41.
PubMed Abstract | Publisher Full Text | Free Full Text

131. Bruno E, Horrigan SK, Van den Berg D, *et al.*: **The Smad5 gene is involved in the intracellular signaling pathways that mediate the inhibitory effects of transforming growth factor-beta on human hematopoiesis.** *Blood.* 1998; **91**(6): 1917–23.
PubMed Abstract

132. Scandura JM, Boccuni P, Massagué J, *et al.*: **Transforming growth factor beta-**

induced cell cycle arrest of human hematopoietic cells requires p57KIP2 up-regulation. *Proc Natl Acad Sci U S A.* 2004; **101**(142): 15231–6.
PubMed Abstract | Publisher Full Text | Free Full Text

133. F Gerber EE, Gallo EM, Fontana SC, *et al.*: **Integrin-modulating therapy prevents fibrosis and autoimmunity in mouse models of scleroderma.** *Nature.* 2013; **503**(7474): 126–30.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

134. F Akhmetshina A, Palumbo K, Dees C, *et al.*: **Activation of canonical Wnt signalling is required for TGF-β-mediated fibrosis.** *Nat Commun.* 2012; **3**: 735.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

135. Eliades A, Papadantonakis N, Bhupatiraju A, *et al.*: **Control of megakaryocyte expansion and bone marrow fibrosis by lysyl oxidase.** *J Biol Chem.* 2011; **286**(31): 27630–8.
PubMed Abstract | Publisher Full Text | Free Full Text

136. Spangrude GJ, Lewandowski D, Martelli F, *et al.*: **P-Selectin Sustains Extramedullary Hematopoiesis in the *Gata1*^{low} Model of Myelofibrosis.** *Stem Cells.* 2016; **34**(1): 67–82.
PubMed Abstract | Publisher Full Text

137. Ceglia I, Dueck AC, Masiello F, *et al.*: **Preclinical rationale for TGF-β inhibition as a therapeutic target for the treatment of myelofibrosis.** *Exp Hematol.* 2016; **44**(12): 1138–1155.e4.
PubMed Abstract | Publisher Full Text | Free Full Text

138. Mascarenhas J, Li T, Sandy L, *et al.*: **Anti-transforming growth factor-β therapy in patients with myelofibrosis.** *Leuk Lymphoma.* 2014; **55**(2): 450–2.
PubMed Abstract | Publisher Full Text

139. Varricchio L, Mascarenhas J, Migliaccio AR, *et al.*: **AVID200, a Potent Trap for TGF-β Ligands Inhibits TGF-β1 Signaling in Human Myelofibrosis.** *Blood.* 2018; **132**(Suppl 1): 1791.
Reference Source

140. Elston R, Inman GJ: **Crosstalk between p53 and TGF-β Signalling.** *J Signal Transduct.* 2012; **2012**: 294097.
PubMed Abstract | Publisher Full Text | Free Full Text

141. Dupont S, Zacchigna L, Adorno M, *et al.*: **Convergence of p53 and TGF-beta signaling networks.** *Cancer Lett.* 2004; **213**(2): 129–38.
PubMed Abstract | Publisher Full Text

142. F Cordenonsi M, Dupont S, Maretto S, *et al.*: **Links between tumor suppressors: p53 is required for TGF-beta gene responses by cooperating with Smads.** *Cell.* 2003; **113**(3): 301–14.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

143. Gerwin BI, Spillare E, Forrester K, *et al.*: **Mutant p53 can induce tumorigenic conversion of human bronchial epithelial cells and reduce their responsiveness to a negative growth factor, transforming growth factor beta 1.** *Proc Natl Acad Sci U S A.* 1992; **89**(7): 2759–63.
PubMed Abstract | Publisher Full Text | Free Full Text

144. F Pellatt AJ, Mullany LE, Herrick JS, *et al.*: **The TGFβ-signaling pathway and colorectal cancer: Associations between dysregulated genes and miRNAs.** *J Transl Med.* 2018; **16**(1): 191.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

145. F Hanna DL, Loupakis F, Yang D, *et al.*: **Prognostic Value of ACVRL1 Expression in Metastatic Colorectal Cancer Patients Receiving First-line Chemotherapy With Bevacizumab: Results From the Triplet Plus Bevacizumab (TRIBE) Study.** *Clin Colorectal Cancer.* 2018; **17**(3): e471–e488.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

146. F Melzer C, Hass R, von der Ohe J, *et al.*: **The role of TGF-β and its crosstalk with RAC1/RAC1b signaling in breast and pancreas carcinoma.** *Cell Commun Signal.* 2017; **15**(1): 19.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

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