

The Therapy of Myelofibrosis: Targeting Pathogenesis

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Abstract

Myelofibrosis with myeloid metaplasia (MMM) encompasses the diagnoses of agnogenic myeloid metaplasia (idiopathic myelofibrosis), as well as the advanced phases of polycythemia vera and essential thrombocythemia (post polycythemic and post thrombocythemia myeloid metaplasia, respectively). MMM is a clonal, hematopoietic stem cell disorder in which neither the pathogenesis, nor a broadly applicable effective therapy have been described. Clinically, these patients experience progressive marrow replacement by fibrotic tissue, ineffective hematopoiesis, problematic cytopenias, significant hepato-splenomegaly, extramedullary hematopoiesis, profound constitutional symptoms, and a risk of blastic transformation. Historically, therapies have been targeted at palliating symptoms (i.e. splenectomy, transfusions, hydroxyurea, erythropoietin, androgens, localized radiotherapy). Stem cell transplantation appears promising, but is often toxic and not broadly applicable due to co-morbidities and age of MMM patients. Non-myeloablative approaches to conditioning may broaden the applicability of stem cell transplantation in MMM, yet results to date are preliminary. Although a definitive molecular abnormality responsible for the pathogenesis of MMM has not been described, much has been learned about the aberrant expression of pro-fibrotic cytokines and the presence of increased angiogenesis in MMM. These pathogenetic insights have led to a series of pilot clinical trials with therapeutic agents targeting aberrantly expressed cytokines (and possibly angiogenesis) including Thalidomide (alone or in combination), Etanercept, and STI-571. Amongst these later agents Thalidomide has demonstrated the most promise (palliating disease associated cytopenias), whereas the TNF- α inhibitor Etanercept has aided with MMM associated constitutional symptoms. Although these later trials have been helpful in a subset of patients, no agent to date has led to solid complete responses in MMM across the spectrum of disease manifestations. Further insights into the pathogenetic mechanisms responsible for myeloproliferation (aberrant cell signaling pathways, apoptotic resistance, other) are necessary to guide selection and testing of the expanding number of novel anti-neoplastic agents in chronic myeloid disorders and MMM.

1. Introduction

Myelofibrosis with myeloid metaplasia (MMM) is a debilitating and progressive clonal [1] hematopoietic stem cell disorder. Although myelofibrosis can be seen with a variety of primary and secondary marrow insults, MMM encompasses the specific myeloproliferative disorders of agnogenic myeloid metaplasia, as well as the advanced phases of both polycythemia vera (PV) and essential thrombocythemia (ET) (post polycythemic (PPMM) and post thrombocythemic myeloid metaplasia (PTMM) respectively) [2]. MMM is an uncommon disorder (incidence 1.1/100,000 population/year) [3] with a wide array of disease manifestations and variable

prognosis [4]. The most aggressive cases of MMM experience early mortality that rivals the severity of many cancers, while others experience a more indolent disease [5].

Symptomatically these patients experience four main and variable disease manifestations [2]. First myeloproliferation, this manifests not only as variable degrees of leukocytosis but the universal feature of splenomegaly, the occasional development of hepatomegaly, and the more rare incidence of symptomatic extra-medullary hematopoiesis in other anatomic locations (i.e lung, spine, etc.). The second major disease manifestation in MMM is variable amounts of cytopenias (anemia, thrombocytopenia, or less often leukopenia). Anemia can range

from minimal anemia, or even temporarily normal hemoglobins (usually in patients with PPM), to complete dependence on erythrocyte transfusions for survival. Thrombocytopenia is the next most common cytopenia encountered, although again there is wide variability. Many MMM patients will have normal platelet counts, or even increased counts requiring pharmacologic control. Finally patients with MMM can have leukopenia, although leukocytosis is more common. Indeed, neutropenia is uncommon and neutropenic complications such as immunocompromised infections are quite uncommon. The third manifestation of MMM is variable degrees of constitutional symptoms. Fatigue can range from mild to debilitating, and is the most prevalent constitutional symptom. Variable amounts of night sweats, fevers and other hypercatabolic symptomatology are common. In addition bone pain, perhaps from the intramedullary changes in MMM, can occur and may require narcotic analgesia. The final disease manifestation of MMM is a predisposition to blastic transformation. Rates of leukemic transformation have been quoted from 10-15% [5] and have not traditionally responded to standard induction therapy.

2. Therapy of MMM: Current Status

The management of MMM in 2002 is overall largely disappointing and usually palliative at best. Stem cell transplantation has shown promise in subsets of MMM patients and will be described first. Next, I will describe in the subsequent section the current general therapeutic maneuvers found to be of benefit in MMM. Keep in mind that there is currently no therapeutic agent with an FDA indication for the therapy of MMM, therefore the medical therapies described below have largely arisen from small pilot studies or anecdotal reports.

2.1. Stem Cell Transplantation

The concept of using stem cell transplantation for the therapy of MMM is attractive. MMM has been shown to be a clonal hematopoietic stem cell disorder through N-RAS [6], and X chromosome inactivation studies [7]. Therefore, if one were to ablate the diseased marrow and replace with healthy donor marrow in theory one would be cured of the disease. Indeed, the initial reports with allogeneic transplantation in MMM have shown that this therapy does have curative potential in these patients [8]. The most comprehensive report details the results of 55 MMM patients [9] (median age 42) who received an allogeneic stem cell transplant (1979-1997). This latter cohort with a median follow-up of 36 months experienced a 5 year survival was approximately 50% (depending on the subgroup analysis), with a 27% 1 year transplant related mortality. Graft versus host disease was significant with 33% experiencing grade 3-4 acute GVH. A full discussion as to the role of stem cell transplantation in MMM would require a complete manuscript but overall, the difficulty with applying this therapy broadly in MMM is several fold. First, many

MMM patients are not good candidates for the procedure either because advancing age increases risk of graft versus host disease and transplant related mortality (median age at diagnosis of MMM is 62 years [3]). In addition, the manifestations of MMM can either effect organ function or performance status to a degree that make stem cell transplant unadvisable. The second reason to be cautious in transplanting patients with MMM is that many patients may have a relatively good prognosis (5-10 year survival), and therefore subjecting these individuals to the upfront mortality associated with allogeneic stem cell transplant may be inappropriate. In those patients who are young, are good candidates, and have an adequate donor allogeneic stem cell transplant is probably the only therapy used to date that has curative potential in MMM.

Autologous stem cell transplantation has also been studied in MMM based on the hypothesis that autologous transplantation can be used in 1) older patients, 2) those patients without an HLA match, 3) those patients who would not tolerate an allogeneic transplant. A pilot, multi-center, study of 21 MMM patients who received an autologous stem cell transplant [10] was recently published with several interesting findings. First MMM patients were best mobilized with granulocyte stimulating factor (as opposed to merely harvesting peripheral blood CD34+ cells without stimulation), second successful engraftment was achievable in the patients with reasonable time to engraftment (5/21 needed infusion of additional CD34+ cells to achieve engraftment). The procedure was overall surprisingly well tolerated (transplant related mortality 3/21; 1 <day 100), with improvements seen in both cytopenias and myeloproliferative symptoms. The durability of benefits seen in MMM autologous stem cell transplant will need additional follow-up and subsequent trials to elucidate.

An autologous stem cell graft in MMM is going to contain many of the aberrant clonal cells, so why should autologous stem cell transplantation provide any benefit? Unlike non-Hodgkin's lymphoma or even multiple myeloma the "malignant cell" is intrinsic to myeloid function and current technology does not allow for purging of these aberrant cells from the graft. Nor is MMM a particularly chemotherapy sensitive malignancy in which one would use stem cell transplant as a mechanism to provide dose intensity to a therapy. Many speculations exist concerning the palliative benefit of autologous stem cell transplant in MMM. Does the high dose therapy somehow "set the clock back" on the intramedullary connective tissue deposition, or quiet the cytokine storm integral to marrow dysfunction and ineffective hematopoiesis. None of these questions currently have answers. Overall autologous transplant may have a palliative role for MMM, yet at a cost of morbidity and mortality that currently limit broad acceptance or applicability.

2.2. Current Palliative Strategies in MMM

The only potential curative therapy for MMM is allogeneic transplantation, in the very small subset of

MMM patients in whom such a therapy is both appropriate and desired. No other therapy has been shown to effect the survival of patients afflicted with the disease, therefore current utilized management strategies are really palliative in orientation and goal. It is important in choosing any of these palliative modalities to truly weigh the status of the patient. One can never make an asymptomatic patient feel better, and often even our palliative therapies carry toxicities that can easily negate their benefit. When palliative therapy is appropriate it is necessary to identify which disease manifestations truly affect quality of life. Currently there has been modest success with palliating myeloproliferative and cytopenia associated symptoms, with no real success with either MMM associated constitutional symptoms or blastic transformation.

2.3. Palliation of Myeloproliferation

The marked myeloproliferation characteristic of MMM is accompanied by a marked increase in the circulation of immature myeloid progenitors in various stages of maturation [11]. Indeed, marked increases in circulating CD34+ cells are hallmarks of the diagnosis of MMM [12]. Aggregations of myeloid progenitors outside of the medullary cavity are identified as extramedullary hematopoiesis (EMH). Circulating myeloid progenitors have a propensity for accumulation foremost in the reticulo-endothelial system of the spleen and liver [13]. The resulting hepato-splenomegaly may lead to pain, early satiety, sequestration of erythrocytes and platelets, and portal hypertension [14]. EMH has been shown to accumulate and cause symptoms in various other critical areas including the lungs (leading to pulmonary hypertension), abdomen, spine, pericardium, etc [15].

The palliation of symptomatic EMH in MMM is currently accomplished through either non-specific myelosuppression in an attempt to decrease the contributory circulating myeloid progenitor pool, or targeted cyto-reduction through either radiotherapy or surgery.

2.3.1. Hydroxyurea

Hydroxyurea is a useful, oral, well tolerated, non-specific myelosuppressive agent which can reduce the leukocytosis and occasional thrombocytosis associated with MMM [16]. The reduction in thrombocytosis can be helpful in the setting of high thrombotic risk or extreme thrombocytosis [17]. The reduction of leukocytosis in MMM is clinically useful only if the leukocytosis is extreme and symptomatic, or if the reduction in leukocytosis leads to a significant reduction in splenomegaly (seen in around 25% of patients). Occasionally one will need to utilize a substantial dose of hydroxyurea (2-3 grams/day) to achieve a meaningful reduction in splenomegaly. Hydroxyurea has the unwanted effect of potentially exacerbating anemia or thrombocytopenia (if present). When high dose hydroxyurea is utilized and effective supplemental exogenous erythropoietin may be used to ameliorate the associated

anemia. Long term complications of hydroxyurea include lower extremity ulceration [18] and a small (yet still unproven) potential risk of increasing the risk of blastic transformation [19].

2.3.2. Interferon Alpha

Interferon alpha (INF- α) is a myelosuppressive, non specific immunosuppressive, and potentially anti-angiogenic cytokine [20]. INF α has had palliative benefit in polycythemia vera [21] and based upon this benefit in a related chronic myeloproliferative disorder a Phase II trial was undertaken in MMM [22]. 11 patients with MMM were treated with 3×10^6 IU three times/week for the first three months, then increased to 5×10^6 IU three times/week for the remainder of the study. Toxicity at these doses was universal, and lead to premature withdrawal in 7/11 patients. Constitutional symptoms observed with INF α (i.e. fatigue) were additive to those associated with MMM. The four patients that completed the trial (1 year of therapy) had no objective benefit in anemia, organomegaly, or intramedullary markers of MMM (angiogenesis, reticulin fibrosis). In addition, *in vitro* assays [23] have shown that INF α does not induce critical cellular effects (tyrosine phosphorylation of the Vav proto-oncogene [24]) associated with the agent's myelo-suppressive effects. There have however been reports showing a beneficial impact of INF α [25] on MMM patients particularly in decreasing leukocytosis and thrombocytosis, but at doses that are difficult to tolerate ($2.5-5 \times 10^6$ IU/day). In addition there are anecdotal, un-published, accounts of improvements of splenomegaly and myeloproliferation with doses of INF α as low as 1×10^6 IU three times/week. However, there is currently no objective reason to utilize INF α in MMM, outside of a study setting, given the lack of objective efficacy and the significant constitutional toxicities associated with this therapy.

2.3.3. Splenectomy

The surgical removal of the spleen is one of the oldest acknowledged therapies for MMM [26]. Originally physicians speculated that the spleen was integral to the pathogenesis of the disorder, since splenomegaly was the key clinical feature [27]. We now understand that the spleen is probably an innocent bystander to the pathogenesis of MMM, and that the pathogenetic mechanism of splenomegaly in MMM is accumulation of immature myeloid precursors in the splenic sinusoids [13]. Hence it has been well shown that splenectomy is neither a cure nor even prolongs survival, therefore splenectomy is merely a palliative procedure for MMM [14]. In addition, splenectomy in MMM has significant risks of both peri-operative morbidity and mortality. In the peri-operative period there is risk of hemorrhage, thrombosis and infection with a 30 day surgical mortality of 8-10%. Long term morbidity is also a consideration with risks of significant post-splenectomy thrombocytosis (often leading to vascular complications), as well occa-

sional hepatomegaly (from accumulation of myeloid precursors in the hepatic reticuloendothelial system) [28].

If splenectomy is only palliative and risky in MMM, should any MMM patient be splenectomized? In a recent retrospective review from our institutional experience with 223 splenectomized MMM patients we found certain surgical indications were more likely to experience clinical benefit [14]. Specifically we found durable symptomatic relief in patients whose primary indication for splenectomy was painful splenomegaly, and occasional improvement in refractory anemia. However we found only marginal benefit in patients splenectomized to ameliorate portal hypertension, and adverse outcomes in patients splenectomized for thrombocytopenia. The latter group also experienced increased morbidity and mortality with the procedure. Additionally, post-operative risks of significant thrombocytosis were associated with increased pre-operative platelet counts. Therefore, our current recommendations are to offer palliative splenectomy to individuals with refractory, severely symptomatic, splenomegaly (unresponsive to hydroxyurea) with aggressive post-operative control of thrombocytosis (with platelet lowering agents +/- platelet apheresis).

2.3.4. External Beam Radiotherapy

Myeloid progenitors are sensitive to the cytotoxic effects of external beam radiotherapy (XRT), therefore it is reasonable to hypothesize that judicious use of radiotherapy may reduce symptomatic aggregations of extramedullary hematopoiesis. Indeed, we have described that a useful role for targeted XRT for palliation of MMM associated splenomegaly [29] and hepatomegaly [30]. In addition, XRT has aided in palliating EMH associated pulmonary hypertension, as well as non-hepatosplenic accumulations of EMH [15].

Spleen: 2 separate reports have described the palliative benefit to external beam radiation in improving symptomatic splenomegaly in MMM [29,31]. Our reported experience [29] described a group of 23 MMM patients who received a median radiation course of 277 cGy in a median of 8 fractions. An objective decrease in spleen size was noted in 94% of patients, however 44% of patients experienced post-treatment cytopenias (26% were severe; 13% fatal). In addition, splenic radiation seemed to increase morbidity and mortality of subsequent splenectomy when undertaken. Splenic radiation is effective for palliating MMM associated splenomegaly but should be limited to patients with adequate platelet counts (normal); and in who are not likely to ever be splenectomized.

Liver: The liver, like the spleen, grows in MMM from the sequestration of circulating myeloid precursors. There are few ways of reducing of symptomatic hepatomegaly in MMM patients. We reported on the use of localized, low-dose, radiotherapy in MMM [30]. Specifically, we described the outcomes of 14 patients who received XRT for symptomatic hepatomegaly (median dose 150 cGy). Although most patients experienced symptomatic relief (86%), objective decrease in

hepatomegaly was less common (35%) and the duration of benefit was brief (3 months). Hepatic radiation has a very limited palliative role in MMM.

Lungs: The sequestration of myeloid precursors into the pulmonary parenchyma can lead to symptomatic pulmonary hypertension in MMM [32]. We have recently reported our experience with single fraction, low-dose (100 cGy), external beam radiation to the lungs for palliating MMM associated pulmonary hypertension [33]. Currently a total of five patients at our institution have received a palliative benefit from pulmonary XRT demonstrated by improved performance status, decreased pulmonary artery pressures on echocardiography, and less EMH on lung technetium⁹⁹-sulfur colloid scans. MMM patients with symptoms consistent with pulmonary hypertension should be evaluated for pulmonary EMH (exclude post-thrombotic or other causes of pulmonary hypertension) and if present may benefit from a single 100 cGy fraction of XRT to the lungs by an experienced radiation oncologist.

Other: Symptomatic accumulations of myeloid precursors can arise in a variety of anatomical locations and cause problematic EMH in MMM patients, including pleural effusions, pericardial effusions, and even spinal cord compression. We have found judicious use of external beam radiotherapy can alleviate spinal cord compression arising from EMH in MMM [15]. Localized, palliative XRT, may be worth considering in areas of symptomatic EMH in MMM patients which could tolerate XRT.

2.3.5. 2-CDA

Post splenectomy thrombocytosis and leukocytosis is a difficult to manage morbidity in MMM patients [14]. In addition, progressive hepatomegaly after removal of the spleen often negates the palliative benefits of splenectomy in MMM patients [34]. 2-chlorodeoxyadenosine (2-CDA) is a purine nucleoside analog with myelosuppressive effects that has been found to help palliate the post-splenectomy myeloproliferation in MMM [35]. Specifically, Tefferi et. al. reported on 9 MMM patients with post-splenectomy with progressive hepatomegaly or symptomatic thrombocytosis who were treated prospectively with 2-CDA (0.1mg/kg/day for 7 days for up to 5 cycles). After a median of four cycles, an objective decrease in hepatomegaly was seen in 7/9 (78%) of patients, with 4 of these responding group experiencing a durable benefit (4-28 months). Excess myelosuppression (neutropenia or thrombocytopenia) was observed in some patients, yet did not lead to any major complications. Subsequent reports have similarly described the activity of this regimen in this setting [36] for MMM patients. There is no data on using 2-CDA in non-splenectomized MMM patients, yet may be worthy of study in patients resistant to the effects of hydroxyurea on myeloproliferative symptoms.

2.4. Palliation of MMM Associated Cytopenias

MMM associated cytopenias are multifactorial in

origin. First there is potentially ineffective hematopoiesis stemming from the effects of the aberrant myeloid clone on myelopoiesis. Second, it there may be direct suppression of hematopoiesis by the various increased cytokines (such as TNF- α) on the marrow. Next, the splenomegaly intrinsic to MMM can lead to sequestration of significant numbers of mature as well as immature myeloid elements. Functionally, anemia is the most prevalent and problematic of the MMM associated cytopenias. The anemia can range from mild, to partial or even complete erythrocyte transfusion dependence. Anemia contributes to the multifactorial fatigue associated with the disease, and is indeed one of the most significant adverse prognostic factors in MMM. Leukopenia, when present, is an adverse prognostic factor yet is not very common in a disease in which leukocytosis is more common. In addition, neutropenic complications (in the absence of myelosuppressive therapy) are not common features of the disease. Thrombocytopenia can lead to morbidity in MMM from hemorrhagic complications, and complicates elective splenectomy. Palliation of cytopenias is accomplished through either direct replacement (transfusions) or medical therapy aimed at augmenting residual "normal" myelopoiesis.

2.4.1. Transfusions

Direct replacement of insufficient erythrocytes through transfusion is the cornerstone of palliating anemia related symptoms in MMM. Clearly transfusions, although beneficial, carry the problems of potential transfusion reactions, the exposure to infectious conditions, and the potential development of allo-immunization leading to future transfusion resistance. In addition, the expense of transfusions and the effect on quality of life of the transfusion process make decreasing the need for transfusions to be important in the management of MMM. Erythrocyte transfusions should be minimized to palliate symptoms of decreased oxygen carrying capacity (i.e. dyspnea or angina) or for very severe anemia (hemoglobin <7.5 g/dL).

Transfusion of platelets in MMM is less frequently required in MMM but more quickly leads to alloimmunization than erythrocyte transfusion, therefore platelet transfusions in MMM should be limited to hemorrhagic episodes or thrombocytopenia severe enough that risks of spontaneous bleeding are unacceptable (platelet counts $<10 \times 10^9/L$ or higher in clinical scenarios such as fever etc.).

2.4.2. Erythropoietin

The administration of exogenous erythropoietin has been occasionally helpful in patients with MMM. Rodriguez et al. [37] described their experience and a meta-analysis of patients with MMM treated with erythropoietin described in the literature. An overall response rate of 33% was seen with doses of up to 600 units/kg/week being required. Patients with serum erythropoietin levels of <125 mU/ml had the highest

likelihood of response. Responses to exogenous erythropoietin most frequently occur within 2-3 months with responses ranging from mild decreases in transfusion dependence to complete transfusion independence. There is no data available on the efficacy of longer acting erythropoietin analogs (i.e. darbopoyetin) in MMM associated anemia.

2.4.3. Androgens

Androgens have been beneficial for the palliation of MMM associated anemia. Various androgen formulations have been utilized for MMM patients including nandrolone [38], oxymethalone, and danazol [39,40]. Doses of danazol in the range of 600-800 mg/day lead to responses in 4/7 patients treated from 3-6 months with MMM anemia in the report by Cervantes et al. [39]. In this latter study responders had either previously been splenectomized, or had only modest splenomegaly. In addition, it has been suggested that MMM patients with karyotypic abnormalities are less likely to respond to palliative androgen therapy [38]. Currently a trial of danazol is reasonable in palliating patients with MMM associated anemia (after being satisfied there is no occult prostatic cancer). There is no data comparing the palliative benefits of erythropoietin to androgen therapy or using those agents in combination in MMM patients with anemia.

2.4.4. Splenectomy

The overall role and limitations of palliative splenectomy in MMM patients was detained previously in this manuscript. I will make special mention that splenectomy can produce a durable palliative benefit with regards to anemia in 23% of MMM patients [14]. The potential benefit from splenectomy for anemia in MMM is in many ways negated by the significant risks associated with that procedure and should be felt to be inferior to less invasive medical therapies.

3. Therapy of MMM: Results of Recent Clinical Trials

Upon objectively reviewing the status of therapeutic options for MMM one realizes that there is only one potentially curative, yet very toxic and not widely appropriate, therapy (allogeneic stem cell transplantation and ? non-myeloablative stem cell transplant), and that there is no FDA approved therapy for MMM. Available therapies are agents used for off label indications with only palliative effects in universally the minority ($<50\%$) of MMM patients for which they are utilized. Several pilot clinical trials have been undertaken recently utilizing a variety of agents aimed at the inhibition of the various pathogenetic mechanisms in MMM (pro-fibrogenic cytokines, angiogenesis, etc.).

3.1. Thalidomide

Exciting data has recently emerged, demonstrating that thalidomide has potent anti-angiogenic properties. The drug has been shown to be a potent inhibitor of bFGF-induced angiogenesis when tested using the rat cornea micropocket angiogenesis assay [41]. These anti-angiogenic properties of thalidomide have been explored in the realm of cancer therapy. In a mouse model of metastatic malignancy, thalidomide was shown to reduce the incidence of lung metastases from primary Lewis Lung tumors [42]. Thalidomide has been found to be a valuable adjunct in the therapy of multiple myeloma [43], a hematologic malignancy characterized by intramedullary angiogenesis. MMM has also been shown to have marked intra-medullary angiogenesis and this was the basis for the piloting of thalidomide in the therapy of this disease. By virtue of the fact that the anti-angiogenic activity of thalidomide is largely secondary to the inhibition of bFGF, which is a mediator of many of the pathogenic processes in MMM, points to a unique and specific, directed therapeutic approach. In addition to the inhibition of angiogenesis, specific inhibition of fibroblast proliferation and possibly the inhibition dysregulated, hyperproliferative hematopoiesis is postulated.

Initial pilot studies with thalidomide in MMM were dose escalating in nature beginning at doses of 100 mg of thalidomide/day [44-46]. Increasing doses of thalidomide were associated with activity (Table 1), yet tox-

icity in terms of undesired myeloproliferation, sedation, and neuropathy. The published trials of thalidomide as a single agent therapy in MMM demonstrated several interesting findings. First, the activity observed in these trials was mainly manifest in the improvement of cytopenia's (anemia and thrombocytopenia) with less frequent improvement in splenomegaly. The next feature that was universally observed was that patients with MMM did poorly with dose escalation, and in fact did not tolerate the agent very well even as doses of 200 mg/day of thalidomide. Lastly, there is a subset of MMM patients that can experience unwanted myeloproliferation with Thalidomide therapy [47]. Interestingly, myeloproliferation has even been observed in patients without prior identified myeloproliferative disorders [48]. Dose reductions of thalidomide to only 50 mg (or 1 tablet/day) may maintain the beneficial effects seen with this agent in MMM, and is currently being studied at that dose (alone or in combination). Overall, current data would suggest single agent thalidomide, probably at 50 mg/day, is most likely to benefit MMM patients with anemia or thrombocytopenia but close observation is advised for potential toxicities as well as strict adherence to the contraceptive precautions mandated by the manufacturer (Celgene, Co. New Jersey, USA). There is currently no data concerning combination therapy of thalidomide and other active agents in MMM, but the possibilities are intriguing and the subject of current clinical trials.

3.2. Etanercept

TNF- α is a cytokine implicated both in the pathogenesis of MMM, as well as being pro-fibrogenic [49] and potentially a direct inhibitor of hematopoiesis [50] and cause of MMM associated constitutional symptoms (fatigue and cachexia) [51]. Etanercept (*Enbrel*; Immunex, Seattle, WA, USA) is an inhibitor of TNF- α (dimeric soluble recombinant form of the extracellular domain of human p75 TNF receptor fused to Fc fragment of human immunoglobulin) that is helpful for abrogating the TNF- α associated morbidities of rheumatoid arthritis and based on its activity and safety was piloted in MMM [52]. 22 patients with MMM were prospectively treated with twice weekly subcutaneous injections of Etanercept (25 mg per injection) for up to 24 weeks. The drug was well tolerated and was successful in improving MMM associated constitutional symptoms in 60% of those enrolled. However, only modest benefit was observed in either improving peripheral cytopenias or reducing splenomegaly (20%). In addition no objective changes in intramedullary manifestations of MMM were observed. Etanercept appears to help palliate severe constitutional symptoms in MMM (i.e. fever and fatigue), but due to the lack efficacy on key disease features (i.e. myeloproliferation or cytopenia's) probably will not (at least as single agent) significantly affect the natural history of MMM.

Table 1.

Published Trials of Single Agent Thalidomide in Myelofibrosis with Myeloid Metaplasia.

Trial	Ref #	Patient numbers	Thalidomide dose	Outcome
Barosi et al.	44	21	100-400/day	Anemia response (3/7; 43%) Platelet response (2/3; 66%) Spleen response (31%) Excess toxicity 91%
Canepa et al.	45	10	200-800/day	3/4 with AMM improved 0/6 with sMMM improved Poorly tolerated >400 mg
Pozzato et al.	71	6	100-?	Responses seen in cellular phase MMM
Elliott et al.	46	15	200-400	Anemia response (20%) Spleen response (23%) Unexpected myeloproliferation seen

3.3. STI-571

Imatinib Mesylate (aka STI-571, or Gleevec) is an orally bioavailable tyrosine kinase inhibitor that has demonstrated profound *in vitro* and *in vivo* activity against the bcr/abl kinase in chronic myeloid leukemia [53] (CML), and this activity has led to significant clinical benefit. STI-571 inhibits various other kinases, including both c-kit [54] and the PDGF (platelet derived growth factor receptor) [55]. The latter two kinases have been implicated in the pathogenesis of MMM, and therefore our group undertook a Phase II trial of this agent in MMM [56]. Twenty-three patients with MMM were treated at the approved dose for CML (400 mg orally per day). Overall little or no clinical activity was observed with no improvement seen in either anemia, or meaningful decreases in myeloproliferation. In addition the agent proved to be poorly tolerated and displayed considerable toxicity from thrombocytosis, neutropenia, muscular pain, and even splenic rupture [57]. There is currently no role for the use of STI-571 in MMM in any non-protocol setting.

3.4. Pirfenidone

Pirfenidone (5-methyl-1-phenyl-2-[1H]-pyridone) is a novel anti-fibrosing agent with *in vitro* inhibitory activity against several key cytokines implicated in MMM (PDGF, TNF- α , TGF- β) [58-60]. This agent had displayed encouraging activity against a variety of non-malignant fibrosing diseases (i.e. idiopathic pulmonary fibrosis [61]) and hence was piloted in MMM. Unfortunately in a phase II trial, using doses of Pirfenidone felt to be adequate, of 28 patients with MMM treated for 1 year no patient had an objective response [62]. Although Pirfenidone displayed no activity in MMM, perhaps newer generations of anti-fibrosing agents may be useful in the disease and will certainly merit scientific scrutiny.

3.5. Melphalan

Melphalan is an orally bioavailable alkylating agent with myelosuppressive properties. Alkylators have activity in MMM by causing a direct, non-specific, myelosuppression and therefore may potentially palliate symptoms associated with myeloproliferation [63]. The difficulty with the use of alkylators in MMM is that these agents are genotoxic [64] and may increase the baseline risk (10-15%) of leukemic transformation in the disease [65]. A clinical trial was undertaken in MMM utilizing low dose melphalan [66] on the presumption that this agent is less leukemogenic than busulfan, the alkylator used for myeloproliferative disorders in the past (however there is no data in the literature comparing the various alkylators for leukemic acceleration). Over a seven year period 104 patients with MMM were treated with 2.5mg of oral melphalan three times/week. The agent was active with 66% of patients achieving a

response after a median of 7 months of therapy, with the greatest impact being upon myeloproliferative manifestations. However, the major reason for study discontinuation was blastic transformation 26%, and 48% in pretreated patients. There are many potential contributors to leukemic transformation in MMM that are inherent to the disease and independent of the alkylator therapy these patients received. Never-the-less the long term use of melphalan in MMM may increase the risk of leukemic transformation and should therefore be used in appropriate circumstances (i.e. elderly patient who needs myelosuppression and is not a candidate/or has not responded to hydroxyurea).

3.6. Non-Myeloablative SCT

Non-myeloablative allogeneic stem cell transplant approaches are potentially of interest in MMM because of the limited applicability and toxicity of standard myeloablative allogeneic transplants in these patients [9]. Earlier this year an intriguing report of allogeneic transplant with a reduced intensity condition regimen in MMM patients was published [67]. Four MMM patients (ages 48-58) received a conditioning regimen of fludarabine (30 mg/m² IV daily for 5 days) and melphalan (70 mg/m² IV for 2 days). These patients all had significant regression of their intramedullary manifestations of MMM, and all four were alive at last follow-up with stable chimerism. In addition, the graft versus host disease described (grade 1 in 1 patient) were minimal. These preliminary results are encouraging for the role of non-myeloablative allogeneic stem cell transplant in MMM but will need further follow-up and study to answer the key questions. First how durable are the responses seen in non-myeloablative stem cell transplant in MMM, second how broadly applicable (?age limits) will this therapy be for MMM patients, and how reproducible are these preliminary results?

4. Therapy of MMM: Future Directions

The therapy of MMM is currently still very disappointing in 2002. Stem cell transplantation is still very toxic and rarely an option in most MMM patients, non-myeloablative approaches are intriguing but there are still many unanswered questions. Although there are various medical therapies that may palliate aspects of the disease, no medical therapy that has had any demonstrable effect on survival in MMM. In addition no medical therapy to date has really been shown to have a decisive effect on intramedullary manifestations of the disease. Effective control of MMM may require targeted therapy against the intrinsic malignant clone. Targeting the pathogenetic mechanisms of the disease may well require additional insights into the molecular biology of MMM. Perhaps genomic analysis through micro-array analysis or proteomics will lead to the discovery of a unifying pathogenetic mechanism that can be exploited for therapy. In parallel to the basic science investigation of the disease empiric testing of novel therapeutic agents

with activity against related myeloid diseases should continue. One such example of empiric testing of potentially active agents is the farnesyl transferase inhibitor R115777. This latter compound has been beneficial in refractory acute leukemia [68], and subsequently has been shown to have intriguing *in vitro* activity in MMM myeloid progenitors [69] and preliminary activity in MMM patients [70]. Continued active collaborative investigation between clinicians, basic scientists, and industry is necessary to improve the understanding and therapy of MMM.

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