

Clinical and Scientific Advances in the Philadelphia-Chromosome Negative Chronic Myeloproliferative Disorders

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Abstract

The chronic myeloproliferative disorders are clonal hematopoietic stem cell disorders and include chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and agnogenic myeloid metaplasia (AMM). These diseases are characterized by clonal expansion of the myeloid compartment, increased marrow angiogenesis, and varying risks for blastic transformation. A clear molecular abnormality exists (t(9;22) leading to the fusion of BCR-Abl) only for CML, which led to effective targeted therapy (STI-571). Since no similar pathogenetic mechanism has been discovered for the t(9;22) negative chronic myeloproliferative disorders, their respective diagnosis is currently based on a variety of rather cumbersome diagnostic criteria. Polycythemia vera is distinguished from reactive erythrocytosis through erythropoietin independent growth of erythroid progenitors *in vitro*, suppressed levels of endogenous erythropoietin, possible overexpression of PRV-1 (polycythemia rubra vera-1), decreased c-Mpl expression on megakaryocytes, as well as overexpression of bcl-xL, and potentially aberrant activity of the Jak-Stat pathway. ET is defined by thrombocytosis and is distinguished from reactive states by decreased megakaryocyte c-Mpl expression, and a propensity for thrombosis. AMM has been associated with a variety of observations including increased concentrations of pro-fibrotic cytokines, increased angiogenesis, and myeloid expansion. AMM is often indistinguishable clinically and prognostically from the advanced phases of other CMPD (specifically post-polycythemic and post-thrombocythemia myeloid metaplasia), all of which are subentities of a diagnosis of myelofibrosis with myeloid metaplasia (MMM). The management of CMPD patients is quite varied given the broad range of disease severity and survival observed. The role of stem cell transplantation is limited by the age and comorbidities encountered in CMPD patients. Since no broadly applicable therapy effects the mortality of the CMPD, management currently focuses on the prevention/palliation of disease morbidity (i.e. vascular complications, pruritus, organomegaly, constitutional symptoms). Palliative strategies which currently focus on non-specific myelosuppression, will hopefully be soon replaced by targeted therapies as insight into pathogenetic mechanisms of these diseases evolves.

1. Introduction

The chronic myeloproliferative disorders (CMPD) are a group of clonal hematopoietic stem cell processes that lead to an overlapping group of diseases. Traditionally included in the classification of CMPD are patients with polycythemia vera (PV), essential thrombocythemia (ET), agnogenic myeloid metaplasia (AMM), and chronic myeloid leukemia (CML) [1]. Clinically these disorders share a variable spectrum of symptomatology arising

from myeloproliferation including peripheral blood manifestations of myeloproliferation (erythrocytosis, leukocytosis, or thrombocytosis) as well as target organ damage from the intramedullary proliferative state (organomegaly [2], vascular complications [3], skin manifestations [4], liver dysfunction [5], pulmonary hypertension [6], etc.). Chronic myeloid leukemia is the only CMPD with an identified pathogenetic mechanism, the Philadelphia chromosome or its molecular equivalent [7] (t(9;22)). The elucidation of the molecular defect in

CML has been a watershed event for that disease leading to a clear diagnostic criteria (the presence of t(9;22) on conventional cytogenetic analysis or the discovery of the BCR/Abl transcript on FISH (fluorescent in-situ hybridization)) and the development of targeted therapies (inhibition of the BCR/Abl product by Imatinib Mesylate) [8] that appear very promising for this disorder. The Philadelphia chromosome negative CMPD are currently defined by their respective phenotypic manifestations. Specifically, PV and ET are mainly defined by idiopathic erythrocytosis and thrombocytosis, respectively [7]. AMM is also a phenotypic diagnosis of idiopathic intramedullary fibrosis, myeloproliferation, splenomegaly and is virtually indistinguishable from the advanced phases of PV or ET (post polycythemic myeloid metaplasia and essential thrombocythemia respectively) [9]. Although the Philadelphia Chromosome negative CMPD still do not have clear pathogenetic etiologies or definitive therapies, recent scientific inroads have been made into the diagnosis and management of the CMPD. Scientific insight into any one of the CMPD may or may not have relevance to the related disorders, I will attempt to synthesize recent information as it pertains to the diagnosis, pathogenesis, and management of the Philadelphia chromosome negative CMPD.

2. Diagnostic Insights

2.1. PV

Polycythemia vera, the most common of the CMPD with an incidence of 2.3/100,000/year [10], has had the most diagnostic scrutiny of these disorders. A variety of clinical diagnostic criteria have been published in the absence of an absolute marker. These have ranged from the original PV study group [11] which focused on an increased red cell mass in the absence of secondary causes of erythrocytosis, to more modern adjusted criteria that attempt to take into account biological features associated with PV (i.e. inappropriately low serum erythropoietin levels [12]).

2.2. ET

Simply put, the diagnosis of ET in the current day is the presence of persistent thrombocytosis, in the absence of any secondary cause. There are a variety of published diagnostic criteria for ET, but most of the criteria are aimed at the exclusion of confounding diseases [13-15]. When a patient presents with thrombocytosis one needs to exclude the possibility that the patient may have either a reactive thrombocytosis (RT) or another primary thrombocytotic state (other CMPD). Distinguishing ET from RT relies upon several factors including the duration of thrombocytosis, and occasionally platelet morphology [16]. Intramedullary manifestations of megakaryocyte proliferation as demonstrated by clumping [17] have also been of diagnostic benefit. Serum markers of occult iron deficiency (serum ferritin) or inflammatory/ connective tissue diseases (c-reactive

protein, interleukin- 6, or other acute phase reactant) [18] have also been of diagnostic discriminatory value. Various other histologic features or *in vitro* progenitor growth characteristics have recently been studied to augment the distinction between ET and RT in difficult cases.

2.3. AMM

AMM, the least common of the CMPD with an incidence of 1.1/100,000/year [19], is indistinguishable from the advanced phases of PV and ET (post-polycythemic myeloid metaplasia and post-thrombocythemic myeloid metaplasia respectively) and is a sub-diagnosis of myelofibrosis with myeloid metaplasia (MMM). The diagnosis of MMM is clinically, as opposed to pathogenetically, based upon the presence of myeloproliferation, the development of intramedullary fibrosis, and splenomegaly all in the absence of a secondary cause [9]. It is interesting that a diagnosis of CML can be almost clinically indistinguishable (leukocytosis, splenomegaly) from cellular phase AMM or even ET (except for the presence of the Philadelphia chromosome). Attempts to isolate a unifying and defining karyotypic abnormality for AMM, or MMM, have yielded various karyotypic abnormalities, however none of these abnormalities were either defining or universal [20].

2.3.1. Growth Factor/Cytokine Hypersensitivity

Endogenous erythroid colony formation (EEC) of in the absence of the usually prerequisite cytokines (i.e erythropoietin) has been well described in PV, and even MMM [21,22]. Amongst the EEC assays available there is data to suggest that serum free assays which utilize collagen (as opposed to a methycellulose base) may be more sensitive, and easier to interpret [23]. EEC formation is clearly a feature of PV (and helpful for distinguishing PV from secondary erythrocytosis), but may be present in a subset of patients with ET (some of which end up developing PV) [24]. The presence of EEC in CMPD led to the evaluation of spontaneous megakaryocyte colony formation in ET [25]. The spontaneous growth of megakaryocyte colonies, in the absence of the prerequisite cytokine TPO, has been described as a specific method for distinguishing ET from RT [26,27].

Spontaneous myeloid colony growth/ cytokine hypersensitivity in the CMPD are of potential interest from a pathogenetic mechanistic standpoint. However, the variability in potential interpretation of these colony assays, the potential effect of concurrent platelet reducing therapy on spontaneous growth, and the lack of laboratory infrastructure limit these *in vitro* methods to centers which specialize in myeloid disorders. Never-the-less spontaneous myeloid colony formation can help in the diagnosis of difficult cases but is probably of greater importance in potentially yielding pathogenetic insight as discussed in the subsequent section.

2.3.2. *c-Mpl/Thrombopoietin (TPO)*

The binding of TPO to its ligand c-Mpl on megakaryocytes is responsible for megakaryocyte growth and development in normal hematopoiesis [28], and therefore the TPO-c-Mpl axis has been a subject of scrutiny in thrombocytosis. Indeed, the abnormal proliferation of platelets, which characterizes essential thrombocythemia (ET), has been associated with dysregulation of the thrombopoietin (TPO) c-Mpl axis. Specifically, recent studies have demonstrated inappropriately elevated levels of thrombopoietin with corresponding decreased surface expression of the corresponding receptor (c-Mpl) on both platelets and megakaryocytes in the chronic myeloproliferative disorders. Moliterno and colleagues were the first to describe the impaired expression of c-Mpl in PV [29], and subsequently showed this impaired expression arose from a post-translational processing defect of the receptor [30]. In addition, the decrease in c-Mpl expression on the surface of megakaryocytes may aid in distinguishing PV from secondary causes of erythrocytosis [31]. Recent data from Dr. Moliterno and colleagues suggests that despite reduced c-Mpl expression in PV the CD34+ cells from these patients still respond to TPO stimulation [32]. This latter intriguing observation suggests that decreased c-Mpl expression in PV patients may arise from more than just downregulation, and that this abnormality may have relevance on the cytokine hypersensitivity observed in the myeloid progenitors from these patients.

Similar to the aberrancy in the c-Mpl/TPO axis seen in PV a defect has been described in ET [33]. In a recent report of 183 patients with thrombocytosis 164 with essential thrombocythemia (ET) and 19 with reactive thrombocytosis (RT) megakaryocyte c-Mpl staining was decreased in ET patients compared with both the normal comparison group ($P < 0.0001$) and those individuals with RT ($P < 0.0001$) [34]. However, neither decreases nor heterogeneity of megakaryocyte c-Mpl staining correlated with either clinical presentation, laboratory studies, vascular events, or survival. The corresponding mechanism by which c-Mpl is decreased in ET has not been shown [35]. The decrease surface expression of c-Mpl in ET may be responsible for elevated TPO levels through lack of ligand binding, in fact an inverse correlation between TPO and c-Mpl has been previously reported in ET [36]. However, TPO levels have been shown to be increased in secondary thrombocytosis, perhaps as an acute phase reactant [37,38]. We observed normal c-Mpl expression in patients with RT, therefore the elevated TPO levels observed in these patients may be the cause of their thrombocytosis. The lack of prognostic significance to the degree of c-Mpl expression in ET suggests this assay may be helpful in distinguishing ET from RT, but does not necessarily give any pathogenetic insight.

c-Mpl expression does appear to be decreased in megakaryocytes across the spectrum of CMPD including not only ET and PV but also AMM [39]. The immuno-

histochemical analysis of bone marrow trephines for c-Mpl may aid in the distinction of primary from secondary myeloproliferative states, yet the lack of specificity or clear correlation with any disease parameters suggest that the changes seen may not be pathogenic. However, correlations between the TPO axis and the pathogenesis of the CMPD is still preliminary and is worthy of further investigation.

2.3.3. *Angiogenesis*

Neoangiogenesis has been shown to be important for the growth and proliferation of many solid malignancies [40]. Similarly, pathologic increases in bone marrow stromal microvessels have been demonstrated across a spectrum of primary marrow disorders including lymphoproliferative disorders (multiple myeloma [41], chronic lymphocytic leukemia [42], acute lymphoblastic leukemia [43]), and myeloid disorders (acute myeloid leukemia [44], myelodysplasia [45], and chronic myeloid leukemia [45]). The observed increases in angiogenesis have been found to be both of prognostic significance and potentially a useful therapeutic target in myeloma [46,47]. Indeed, the proliferation of microvessels to support proliferative marrow activity is of at least diagnostic relevance across the CMPD.

We recently reported on the marked increase in angiogenesis observed in myelofibrosis with myeloid metaplasia [48]. In a cohort of 114 patients with MMM and a control group of 44 normals, 15 PV, and 17 ET patients we demonstrated a significant increase in bone marrow angiogenesis in 70% of the patients with MMM, 33% with polycythemia vera, 12% with essential thrombocythemia, and 0% of normal controls. A total of 112 of the 114 patients with MMM (98%) had some degree of increase in angiogenesis. In a multivariate analysis, increased angiogenesis in MMM correlated significantly with increased spleen size and was found to be a significant and independent risk factor for overall survival. Increases in marrow angiogenesis correlated with hypercellularity and megakaryocyte clumping. In contrast, these latter two features were inversely proportional to reticulin fibrosis, whereas increases in marrow angiogenesis were independent of reticulin fibrosis. The marked prominence of angiogenesis in MMM has been further corroborated [49] and has led to clinical trials with angiogenesis inhibitors such as thalidomide [50] and SU5416 [51] (an inhibitor of VEGF; the vascular endothelial growth factor). In addition, the cause for the vascular proliferation is still not certain as it appears the degree of angiogenesis observed may be independent of the cytokine VEGF (vascular endothelial growth factor) [52].

Increases in angiogenesis, as manifested by increased microvessel density, were recently reported in a large cohort of ET patients as compared to both normal marrows ($P < 0.0001$) and RT ($P = 0.006$) [34]. Although the degree of increase in microvessel density was variable amongst the ET patients (See Table 1), no patient with documented RT had a significant increase

Table 1.

Results of Recent Pilot Studies in Myelofibrosis with Myeloid Metaplasia.

Agent	Ref #	Therapeutic goal	n	Outcome
Thalidomide (Single Agent) 4 Trials	109 110 111 112	Inhibit Angiogenesis	21 10 6 15	Anemia Responses (20–43%) Platelet response (25–66%) Spleen Response (20–31%) Excess toxicity including myeloproliferation seen, less at lower doses (<100mg/day)
Pirfenidone	113 Fibrosis	Inhibit	28	No clinical/marrow benefit
Etanercept	114	Inhibit TNF- α	22	60% Improvement in constitutional symptoms 20% Improvement in Cytopenia/ Splenomegaly Well Tolerated but expensive
Imatinib Mesylate (STI-571)	115	Inhibit Tyrosine Kinases (?PDGF)	23	Poorly tolerated Possible splenic rupture risk No durable clinical response
Melphalan	116	Non-specific Myelosuppressive	104	66% response rate after 7 months of therapy Potentially accelerates leukemic transformation

in microvessel density. Increases in microvessel density correlated with the presence of: palpable splenomegaly ($P=0.004$), reticulin fibrosis ($P=0.005$) (in absence of known transformation to PTMM), and marked bone marrow megakaryocyte proliferation ($P=0.002$). However, no correlation between increased microvessel density and clinical presentation, platelet count or other laboratory studies, vascular events (thrombosis or hemorrhage), or survival was observed in the ET cohort. Although, angiogenesis was clearly increased in ET these increases were less than patients with either ET associated myelofibrosis with myeloid metaplasia (post-thrombocytopenic myeloid metaplasia (PTMM) $n=19$) ($P=0.0002$) or *de novo* MMM ($n=96$) ($P<0.0001$).

Angiogenesis is clearly increased in the CMPD, and increases may correlate with disease progression and severity. In addition, increased angiogenesis is helpful for distinguishing primary versus secondary myeloproliferative states, but may not be of benefit in distinguishing amongst the various CMPD. However, what is not clear is whether angiogenesis is central to the pathogenesis of these diseases or merely epiphenomenon. Increases in microvasculature appear to be a feature in most intramedullary hematologic malignancies. This latter lack of specificity is concerning as to whether angiogenesis inhibition will be of any therapeutic value.

2.3.4. Clonality

Another proposed diagnostic method for ET (or other CMPD) is to evaluate patients with for evidence of clonal versus polyclonal hematopoiesis using X-chromosome inactivation. A prospective study evaluating pat-

terns of clonality in patients with thrombocytosis found that patients with a clinical diagnosis of ET were heterogenous for clonality testing [53]. There are many technical limitations to clonality analysis in ET including restriction to female patients (X chromosome inactivation patterns), possible existence of clonal and polyclonal hematopoiesis (thereby needing a very sensitive method to detect the clone), or restriction of clonality to a certain lineage (such as megakaryocytes). These multiple limitations limit any solid conclusions which can be drawn from clonality analysis in ET from either a diagnostic or prognostic vantage.

2.3.5. PRV-1

A novel and intriguing observation in PV has been the isolation and characterization of the polycythemia rubra vera-1 gene (PRV-1) by Dr. Pahl and colleagues [54]. Subtractive hybridization was used to isolate the PRV-1 gene which was overexpressed in granulocytes from PV, and found to be a member of the uPAR receptor superfamily. Expression of PRV-1 occurred only in stimulated normal patients (stimulated with granulocyte colony stimulating factor) so it was felt to be specific for PV. Subsequent characterizations have found PRV-1 to be anchored on the cell membrane, expressed in all myeloid lineages, and not due to a rearrangement or structural alteration of the gene. Subsequent, and exhaustive, probing of other genes with potential homology have found that CD177 is highly homologous to PRV-1 and may well be the same gene (or at least different alleles of the same gene) [55]. CD177 is poorly expressed in normal neutrophils and is a target

for auto and allo antibodies. The pathogenetic role of PRV-1 in PV is unclear and may well be epiphenomenon. Never-the-less PRV-1 may well have diagnostic value in the diagnosis of cases of PV. In a prospective study using PCR to detect PRV-1 expression in patients with PV (n=48) and controls (4 with secondary erythrocytosis, 33 normals), PRV-1 was expressed only in PV and not in controls or in patients with CML or familial PV⁵⁶. In the latter report PRV-1 expression was also compared in PV to patients with ET (n=29) since the specificity of PRV-1 expression was not well established. PRV-1 expression was performed by PCR and compared to parallel assays measuring endogenous erythroid colony formation (EEC). PRV-1 and EEC assays were positive in all the PV patients, and ET patients who displayed PRV-1 also would form EEC. It has been suggested that ET patients who form EEC often end up developing the PV phenotype and may be diagnosed in a phase of their disease that precedes the erythrocytosis [24]. Overall, PRV-1 may not play a causal role in PV, it may really be CD177, but appears to be of potential discriminatory benefit between PV and secondary erythrocytosis. The value of PRV-1 for differentiating PV from ET is still unclear, nor is it clear that distinguishing between ET and PV is clinically important in patients who overexpress PRV-1.

Overall, the additional insights gained in the CMPD have yielded additional histologic, phenotypic, or even molecular features that aid in distinguishing primary from secondary causes of myeloproliferation. Never-the-less in the absence of a clear pathogenetic mechanism for the individual CMPD we will be left with various cases in which diagnostic uncertainty exists (albeit this is the vast minority of CMPD patients).

2.3.6. CD34+ Cell Measurement in Peripheral Blood

The development of MMM, either primary (i.e. AMM) or secondary (PPMM/PTMM) is associated with progressive leukoerythroblastosis [9]. Indeed it has been shown that accompanying the various immature myeloid elements in the peripheral blood of patients with MMM one finds increased numbers of CD34+ cells. Two reports have shown a clear diagnostic and discriminatory value to increased measurements of CD34+ cells in MMM [57,58]. In addition, the degree of increase in circulating CD34+ cells appeared to correlate to the degree of myeloproliferation (leukocyte count, circulating blast percentage, splenomegaly) and prognosis (as estimated by the Dupriez prognostic score for AMM [59]). The ability to use CD34+ measurements to discriminate between the various CMPD is probably greatest in those cases in which little uncertainty exists because marked increases in CD34+ cells is most often accompanied by evidence of advanced MMM.

2.3.7. Diagnostic Conclusions

The vast majority of cases of ET, PV, and AMM (MMM) will be able to be diagnosed by combining

clinical features, peripheral blood hematologic parameters, serum erythropoietin levels, CD34+ cell quantification, and standard bone marrow aspirates and biopsies. Indeed histologic analysis can frequently discriminate between primary and secondary causes of myeloproliferation [60,61]. The next tier of discriminatory testing to distinguish a primary and secondary process may include immunohistochemical staining of the marrow for angiogenesis (CD34) and c-Mpl, and perhaps PRV-1 when such assays become available (either PCR based or immunohistochemical). Karyotypic analysis is of prognostic value [62] and may well be helpful in diagnosing a primary myeloproliferative syndrome. *In vitro* colony forming assays remain too cumbersome for widespread applicability. Even after all of the above testing is performed there may still be overlap amongst the various CMPD, this overlap will be unavoidable until specific markers (molecular or otherwise) are found for the respective disease entities.

3. Pathogenetic Insights

The lack of unifying pathogenetic mechanism in the CMPD has been a major limiting factor in improving the diagnosis or therapy of these disorders. Although patients with CMPD's may display a variety of karyotypic abnormalities (deletions of chromosomes 13, 20; abnormalities of chromosome 1,9; or therapy associated changes such as deletions of chromosomes 5 or 7) attempts to identify a causative karyotypic abnormality (i.e. such as the t(9;22) in CML) have been unsuccessful for AMM [62], PV [63], or ET [64]. Never-the-less there has been a variety of insightful observations regarding various properties of the myeloid progenitors in patients with CMPD's that may assist in identifying pathogenetic mechanisms in these disorders.

3.1. Apoptosis Resistance/ Bcl-xL

PV has long been characterized by erythroid colony erythropoietin independent growth or EEC [65]. One potential mechanism that has been proposed is an intrinsic resistance to apoptosis in these cells, suggesting myeloproliferation may also include some degree of myelo-accumulation. Silva et. al. recently demonstrated that erythroid precursors from PV patients express high levels of the anti-apoptotic Bcl-2 family member Bcl-x_L [66], including mature erythroid cells that ordinarily do not express Bcl-x_L. It is known that hematopoietic growth factors may function through the inhibition of normal apoptosis [67] and that perhaps the endogenous myeloid colony formation (a clear feature of PV, ET, and even AMM) arises from an intrinsic apoptotic defect. Clearly, Bcl-x_L is a promising starting place for investigation, but whether overexpression of that protein is the mechanism (and not a secondary effect of some other apoptotic defect) is still unclear. Future therapies aimed at the stimulation of apoptosis, or correction of apoptotic defects may be worthy of clinical investigation in the CMPD.

3.2. Protein Phosphatases

Intracellular signaling from cytokine receptor pathways relies on a balance of interaction between protein kinases (usually an activator) and phosphatases (usually degrades or inhibits downstream signaling). Two manuscripts have been published to date looking at phosphatase activity in PV. In the first (Dai et. al. [68]) inhibition of tyrosine phosphatase increased erythroid colony formation in normal marrows but not in PV marrows, suggesting that in PV the wild type phosphatase is already defective and thus further inhibition would be irrelevant. In the second manuscript (Sui et. al. [69]) a potentially novel erythroid protein phosphatase was described which had more activity (3x) in PV than normals. Further research needs to be performed to corroborate either of these observations, and to determine whether phosphatases have any role in the pathogenesis of PV or the other CMPD (no data on AMM or ET).

3.3. Jak-Stat Pathway

The development of erythropoietin independent erythroid colonies in PV is very curious since the erythropoietin receptor is structurally and functionally normal in PV [70]. Investigators have focused on potential downstream effectors of erythropoietin to look for potential mechanisms of growth factor independence/hypersensitivity. One such potential downstream pathway of interest is the STAT (signal transducers and activators of transcription) pathway. Erythropoietin signals through JAK 2 and STAT 5, whereas STAT3 has been associated with G-CSF signaling. Dr. Pahl and colleagues have reported on the presence of constitutive STAT3 activation in the peripheral blood granulocytes from a cohort of PV patients [71], but no constitutive activation in STAT1 or STAT5. How the constitutive activation of STAT3 relates to the pathogenesis of PV is still not known, nor whether this observation has relevance to the other CMPD.

3.4. FLT3

FLT3 (FMS like receptor tyrosine kinase) is expressed on hematopoietic progenitors and 25% of patients with acute myeloid leukemia (AML) [72]. Indeed, FLT3 mutation detection has prognostic relevance in AML [73] and has been the subject of great scientific interest. Kelly et. al. recently transduced the myeloid progenitors of mice with FLT3 mutants isolated from human patients with AML and transplanted these cells into mice [74]. The mice developed a phenotype of intramedullary reticulin fibrosis, leukocytosis, and splenomegaly (i.e. phenotypically similar to the CMPD). The presence or relevance of FLT3 in the CMPD is still not known but potentially of interest given the above observations.

3.5. Genomic Insights

Current investigations into the origins and pathogenesis of the CMPD have been based on rationally designed experimentation into candidate pathways and genes based on various disease associated properties (i.e. growth factor independent growth). Although this latter approach has yielded new avenues to pursue scientifically it is an exhaustive process, and one where significant efforts could be spent pursuing cellular phenomena of the myeloproliferative phenotype. In an attempt to screen many pathways, and genes simultaneously the use of oligonucleotide microarray technology has been used in CMPD patients [75]. Specifically, Jones and colleagues used commercially available oligonucleotide microarrays for the analysis of purified CD34+ cells from 6 patients with MMM. A variety of marked overexpression/ underexpression of various intriguing genes was observed. Further corroboration with future specimens, clinical parameters, and further use of bioinformatics may aid in narrowing the list of potentially involved candidate genes. The overall technology of microarray analysis and proteomics is advancing rapidly and holds great promise for identifying new and potentially previously uninvestigated genes and pathways in the origin of the various CMPD.

3.6. Origins of Thrombosis

The development of the vascular complications of thrombosis or hemorrhage are a source of considerable morbidity and mortality in the CMPD [76]. Management, and prevention, of vascular events in CMPD patients has relied upon the use of anti-platelet therapy in patients with significant thrombocytosis and significant thrombotic risk factors [77]. Various investigators have attempted to better define the pathogenetic mechanisms of thrombosis in CMPD patients to better stratify thrombotic risk and treat the appropriate patients prior to an event, in addition to gain further insight into the optimal therapy/thrombotic prophylaxis in these patients. A recent large prospective study comparing the presence of various hemostatic gene polymorphisms and clinical history and found the presence of the P1^{A2} allele of GPIIIa to be associated with arterial thrombosis in PV patients [78]. Of note there was no association in the latter study with expression of the prothrombin 20210 (G20210A) or factor V G1691A (Leiden) mutations and thrombosis. Other investigators have found that increased expression of phosphatidyl serine on the surface of ET and PV platelets may lead to aberrant and excessive platelet activation [79]. Perhaps, there is endothelial dysfunction as a pathogenetic contributor to thrombosis in the CMPD [80]. Further research is needed to augment our current understanding and appropriate management/ prevention of vascular complications in the CMPD.

4. Management Insights

The CMPD carry a variable prognostic outlook. AMM/MMM carries the worst prognosis with a median survival rivaling many lethal solid malignancies (may be <2 years). Various prognostic features can help stratify the suspected outcomes in MMM patients [81] including anemia [59], karyotypic abnormalities [62], age, the presence of circulating blasts [59], and markedly increased angiogenesis in the marrow [48]. The prognosis for PV and ET is more favorable, however vascular complications, progression into MMM, and blastic transformation are all developments which significantly worsen prognosis. In addition, it has been suggested that early therapy of young patients with ET or PV with genotoxic agents for disease control may lead to the undesired development of long term toxicity (leukemic transformation) [82-84]. Therapy of MMM has currently focused on palliative myelosuppression or attempts to diminish severe disease associated cytopenias. Various recent clinical trials have been recently performed in MMM and are discussed below. The therapy of ET and PV have traditionally been aimed at controlling disease manifestations and attempting to prevent vascular complications (all while trying to avoid long term complications). There are several excellent, and complete, reviews discussing standard therapeutic options in the CMPD [9,85-88]. The subsequent section will deal with recent clinical trials of interest in the management of CMPD patients.

4.1. Is There a Cure for the CMPD?

Currently there is no broadly applicable therapy that is curative for PV, ET, or MMM. Specifically, no drug therapy that is appropriate for patients with the age and co-morbidities usually found in the overall elderly population with CMPD has been shown to significantly prolong survival. However, stem cell transplantation (allogeneic, or possibly non-myeloablative allogeneic) may in some appropriate candidates provide curative potential. The concept of using stem cell transplantation for the therapy of MMM is attractive given this CMPD disorder has the poorest survival. Initial reports with allogeneic transplantation in MMM have shown that this therapy does have curative potential in these patients [89]. The most comprehensive report details the results of 55 MMM patients [90] (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. The significant toxicity of the latter reports of full allogeneic transplant in MMM begs the question whether a non-myeloablative conditioning regimen would broaden the applicability of this therapy to older MMM patients. In a recent report [91] 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. Autologous stem cell transplantation has also been studied in MMM in a pilot, multi-center, study of 21 MMM patients who received an autologous stem cell transplant [92] 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.

There is currently no data on the use of stem cell transplantation in stable phase ET or PV. Indeed, the significant risks of any of the stem cell transplantation procedures is difficult to justify given the overall modest prognosis of these patients. There is however data on the use of stem cell transplantation in the spent phases of ET and PV, PTMM and PPMM respectively. Indeed, it appears that there may be a significant chance of salvage for these patients that otherwise would have a poor prognosis. In a report of 19 patients with PTMM/PPMM (some with transformation into acute leukemia), 10 experienced significant clinical benefit [93], while seven died of transplant related mortality. The latter study is of note for several reasons. First, significant clinical benefit was seen in a good percentage of patients who would traditionally not respond well to standard induction chemotherapy. Second, the median age of the patients reported was 43 years, well below disease medians [19] calling in to question the broad applicability of this therapy.

Overall, stem cell transplantation holds potential promise as potentially curative therapy in a subset of CMPD patients. However, there are several key unanswered questions. First, given the potential long range survival of patients with ET, PV, or even minimally symptomatic AMM, which of these individuals (if any) should be subjected to the significant upfront morbidity and mortality of stem cell transplantation. Second, up to what age should stem cell transplantation be considered? Third, what is the ideal conditioning regimen (myeloablative versus non-myeloablative)? Fourth, should unrelated transplantations be considered (and with which conditioning regimen). In the absence of complete data it may be most prudent to consider stem cell transplant in those CMPD patients with poor prognosis, and if possible as part of a clinical trial.

4.2. Interferon in CMPD Management

Interferon alfa (INF- α) has been of therapeutic benefit in the CMPD to various degrees. The greatest evidence for the use of INF- α is in the palliation of myeloproliferative symptomatology in patients with PV. Erythrocytosis, relief from pruritus, and decreased splenomegaly may be controlled in up to 76% of PV patients receiving significant doses of this agent (average 3 million units subcutaneously three times per week) [94,95]. In addition, the use of INF- α may be superior to phlebotomy alone. However, the side effects of INF- α can be significant [96], and there is no randomized trial available for review between INF- α and other myelosuppressive agents (such as hydroxyurea).

The use of INF- α in ET patients has been limited because of the agents toxicity and the ability of both hydroxyurea [77] and anagrelide [97] to control problematic thrombocytosis in these patients. Pegylated interferon alpha 2b (PEG-Intron) is a formulation that can be administered weekly and there is preliminary evidence for myelosuppressive activity and improved tolerability in ET patients [98,99]. Never-the-less there is no data suggesting the equivalence of INF- α (pegylated or not) to hydroxyurea for the prophylaxis of vascular complications in ET patients at risk [77].

The role of INF- α in the management of MMM is limited at best. Recently we reported on 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 [100]. 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). Indeed even the early reports of INF- α in MMM were disappointing in terms of activity and toxicity [101,102]. In addition, *in vitro* assays [103] have shown that INF α does not induce critical cellular effects (tyrosine phosphorylation of the Vav proto-oncogene [104]) associated with the agent's myelo-suppressive effects. There have however been reports showing a beneficial impact of INF α [105] 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).

4.3. Management of Pruritus

Pruritus is a significant symptom in up to 50% of patients with PV, and may be associated with iatrogenic iron deficiency (potentially from therapeutic phlebotomy) [106]. A recent intriguing, but anecdotal, observation [107] suggests selective serotonin reuptake inhibitors may be an effective palliative therapy in PV patients suffering the CMPD related morbidity. The mechanism

by which these latter agents would relieve pruritus is unknown, but suggests the related biologic amine pathways to be potentially worthy of study in PV.

4.4. Recent Clinical Trials in MMM

Recently there have been a series of clinical trials in MMM using a variety of agents aimed at the inhibition of various cytokines felt to play various roles in the pathogenesis or phenotype of MMM (pro-fibrotic, pro-angiogenic, inhibitors of erythropoiesis). Complete discussions regarding these trials are included in the accompanying manuscript focusing on the therapy of MMM. These trials are summarized in tabular format in Table 1. Although several of these probative pilot trials have displayed some objective palliative clinical benefit, no trial of a therapeutic agent has lead to a complete, and durable remission in MMM. Novel agents are currently under investigation [108], but overall further pathogenetic insight (and perhaps good luck) will be required to significantly impact the clinical course of MMM.

5. Conclusions

Many significant questions remain as to the critical pathogenetic pathways, and hence optimal therapy of the CMPD. In the absence of definitive, and broadly applicable, curative therapy the clinician is faced with several challenges in the care and management of CMPD patients. Accurate diagnosis, the palliation and prevention of CMPD associated morbidity, all while trying to prevent inadvertent iatrogenic therapy associated toxicity are challenges to overcome. A pathogenetically targeted, and insightful therapy (akin to Imatinib Mesylate in CML) is and continues to be the scientific and clinical goal of the "other" (Philadelphia chromosome negative) chronic leukemia's of myeloid origin.

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