PEG IFN-alpha2a (Pegasys®) therapy in patients with chronic myeloproliferative diseases (excluding Philadelphia chromosome positive chronic myeloid leukemia).DM03-0109

1.0 Hypothesis

PEG IFN-a2a (Pegasys®) is an effective and tolerable treatment for patients with essential thrombocythemia (ET) and polycythemia vera (PV).

2.0 Objectives

2.1 The primary objective of this study is to determine the efficacy of PEG IFN-α2a in patients with chronic myeloproliferative diseases: ET and PV.

2.2 The secondary objective is to evaluate the toxicities of PEG IFN-α2a in patients with these disorders.

2.3 The tertiary objective is to evaluate pertinent morphologic and molecular characteristics of these diseases before and during the therapy with PEG IFN-α2a.

3.0 Background and Rationale

3.1 The Diseases and Current Treatment

ET, and PV are chronic myeloproliferative diseases, which are clinically defined entities within the broad category of chronic myeloid disorders. Because the clonal process originates at the stem-cell level, with retention of cellular differentiating capacity, chronic myeloproliferative diseases are characterized by bone marrow panhyperplasia and accumulation of mature myeloid cell lines. Currently, specific disease classification is based on the predominant pattern of myeloid-cell proliferation and the presence or absence of reactive bone-marrow fibrosis, with or without extramedullary hematopoiesis (Tefferi et al.). Thrombocytosis and absolute erythrocytosis are prerequisites for the diagnosis of ET and PV, respectively. Life expectancy in ET approaches that of an age- and sex-matched control population, and median

survival exceeds 15 years in young patients with PV. Nevertheless, these disorders are considered incurable and treatment is directed at reducing morbidity and preventing life-threatening complications. Vasomotor disturbances (headaches, dizziness, acral dysesthesia, erythromelalgia, visual symptoms), thrombohemorrhagic events, and the risk of transformation into acute myeloid leukemia or AMM/MF characterize the clinical course of both ET and PV (Tefferi et al.).

Vasomotor symptoms may occur in up to one-third of the patients and are usually controlled with aspirin. Bleeding complications, in the absence of platelet anti-aggregant therapy, are infrequent (< 1% pt/yr) and usually inconsequential. In contrast, thrombotic complications are more frequent and may even be fatal, especially in PV. In both ET and PV significant proportion of patients experience a thrombotic episode either before (10%-15%) or at diagnosis (10%-20%). During follow-up, the risk of thrombosis in both ET and PV depends on the patient's age and previous history of thrombosis (Rozman et al.). In comparison to a control population adequately matched for age, sex, and cardiovascular risk factors, patients with ET were found to be at a significantly increased risk for thrombosis (1% v 7% pt/yr). The risk was highest for patients older than 60 years (15% pt/yr) and for those with a previous history of thrombosis (31% pt/yr). The thrombogenic effects of advanced age and history of previous thrombosis were confirmed by other large studies. Therefore, patients with ET who are older than 60 years and/or have a history of previous thrombosis are considered ‘high-risk for thrombosis’. Additional risk factors may include the presence of cardiovascular risk factors, especially smoking. The degree of thrombocytosis or platelet-function abnormalities has not been correlated with thrombotic risk.

In one of the largest retrospective studies ever conducted in patients with PV, thrombosis was observed in 19% of 1213 patients followed up for a median of 5.3 years. Thrombotic risk was correlated with advanced age (> 4% pt/yr for patients older than 60 years versus 1.8% pt/yr for those younger than 40 years) and a history of thrombosis. These and other risk factors for thrombosis (treatment with phlebotomy alone, increased phlebotomy

requirement) were previously identified during the original Polycythemia Vera Study Group studies. Similar to the observation in ET, the absolute platelet count was not predictive of a thrombotic episode (Silverstein et al.).

3.1.1 ET

Transformation into acute myeloid leukemia or AMM/MF is rare in ET (< 2% in most large series). Therefore, treatment efforts in ET should strive to reduce thrombotic events in high-risk-for thrombosis patients without increasing the intrinsically low risk of leukemic transformation. To that effect, a recent randomized study of high-risk-for-thrombosis patients with ET demonstrated that treatment with hydroxyurea, compared with no myelosuppressive therapy, resulted in a significant decrease in thrombotic events (3.6% v 24% during a median follow-up of 27 months). Follow-up was too short to assess risk of leukemic transformation (Tefferi, Silverstein and Hoagland).

Acute myeloid leukemia has been reported in ET in the presence or absence of antecedent chemotherapy. In nonrandomized studies of patients with ET, acute myeloid leukemia was reported to develop after treatment with both alkylating and nonalkylating agents. Some case reports have underscored the significant risk of acute myeloid leukemia associated with long-term use of hydroxyurea in ET. Regardless, the leukemogenic potential of hydroxyurea is generally believed to be lower than that of alkylating agents. As such, the risk-benefit ratio favors the use of hydroxyurea therapy in high-risk-for-thrombosis patients with ET older than 60 years.

The choice of a platelet-lowering agent in younger patients who are at risk for thrombosis is currently a matter of controversy (Fenaux et al., Nand et al.). Anagrelide is an oral imidazoquinazolin derivative that is used to treat ET. Because anagrelide has an anticyclic AMP phosphodiesterase activity (and accordingly inhibits platelet aggregation in humans), the drug initially was tested for its potential use as a platelet antagonist. However, initial human studies at relatively low doses revealed a potent but reversible thrombocytopenic activity. To date, this thrombocytopenic effect has been observed only in humans and occurs at plasma

concentrations that are much lower than those capable of inhibiting platelet aggregation. Functional platelet abnormalities have not been observed with doses currently used to treat thrombocytosis.

Most of the side-effects of anagrelide observed in humans and animals are cardiovascular and stem from the direct peripheral vasodilatory effect and positive inotropic activity of the drug. The vas odilatory effect is believed to be the underlying cause of headache, which is the most frequent side-effect (occurring in more than one third of patients receiving treatment). Other side-effects related to the vasodilatory effect include fluid-retention or edema (24%), dizziness (15%), and postural hypotension, occurring at high single-dose levels. The cardiac effects include palpitations or forceful heart beats (27%), tachycardia and other arrhythmias (<10%), and congestive heart failure (2%). Less common side-effects include diarrhea, abdominal pain, nausea and transient rash. Rarely observed events with treatment have included hyperpigmentation of the lower extremity, pulmonary fibrosis, and abnormal liver function tests. In one small study of 20 patients with ET treated with anagrelide, eight patients experienced side-effects and treatment with the drug was discontinued in six patients. Therefore, anagrelide therapy should be avoided in patients with heart disease, and the drug should be given carefully to elderly patients. In addition, although anagrelide has no mutagenic activity, it is currently not advised for use during pregnancy (Anagrelide study group).

3.1.2 PV

All patients with PV require phlebotomy, with the goal of keeping the hematocrit less than 45% in men and 42% in women. This maneuver prolongs survival by decreasing, but not abolishing, the risk of thrombosis. The residual risk of early thrombosis (33%) is reduced further by supplementing phlebotomy with myelosuppressive therapy (10%). In contrast, the addition of chemotherapy has not been shown to decrease the risk of transformation into acute myeloid leukemia (< 2%) or myelofibrosis with myeloid metaplasia (9%) observed in patients receiving treatment with phlebotomy alone. Instead, some of the currently available

myelosuppressive agents have been shown to increase the risk of leukemic transformation to 11% (chlorambucil), 8% (radiophosphorus), 7% (pipobroman), or 6% (hydroxyurea). On the basis of these observations, hydroxyurea is currently recommended as a supplement to phlebotomy for patients with PV who are at risk for thrombosis.

The goal of therapy in PV is not only to prevent thrombosis but also to reduce the risk of transformation into acute myeloid leukemia or AMM/MF. As such, new therapeutic agents need to be explored in both 'low-risk' and 'high-risk' patients with PV. 'Low-risk' patients with PV remain at an increased risk for thrombosis and transformation into acute myeloid leukemia or AMM/MF. Regardless, it currently is assumed that the risk-benefit ration favors treatment with phlebotomy alone in this group of patients. Such an assumption may not hold true when the specific agents are considered that are less leukemogenic than alkylating agents or radiophosphorus (Silverstein et al.).

In 'high-risk' patients with PV the decision to institute a myelosuppresive agent should take into account not only the potential benefit in preventing thrombosis but also the risk of inducing therapy-related acute myeloid leukemia and the possible negative impact on pregnancy. The leukemogenic potential of the currently preferred agent, hydroxyurea, appears to be lower than that of alkylating agents or radiophosphorus. However, several studies have suggested that in comparison with phlebotomy alone, the long-term use of hydroxyurea may be associated with an increased risk of the development of myelodysplastic syndrome or acute myeloid leukemia. Therefore, in 'high-risk' patients younger than 60 years, interferon-α may be considered a reasonable alternative to hydroxyurea as a myelosuppresive agent. Specifically, interferon-α may be considered for 'high-risk' young women who may be pregnant or are of childbearing age and for control of refractory pruritus.

Anagrelide is a nonmyelosuppressive, selective platelet-lowering agent. The decrease in thrombotic risk associated with PV has been shown only with the use of myelosuppressive agents. However, the degree of thrombocytosis has not been correlated with thrombotic

risk in PV. Therefore, no theoretical rationale currently exists to encourage the use of anagrelide in PV (Berk et al.).

3.2 Interferon as a Therapeutic Agent in Myeloproliferative Diseases

The precise mechanism of action of interferon-α in the chronic myeloproliferative disease has not been established clearly, but it largely appears to be the result of an antiproliferative effect on hematopoietic progenitor cells from which the abnormal clone arises. Dose-dependent suppression of progenitor cells has been demonstrated in vivo and in vitro and it affects both normal and clonal progenitors. However, there is evidence for a selective action on clonal progenitor cells. In patients with ET treated with interferon-α, the decrease in platelet count has been associated with a significant decrease in the number and size of bone-marrow megakaryocyte progenitors (CFU-M). Similarly, a significant decrease in erythroid (BFU-E) and granulocyte-macrophage (CFU-GM) progenitors was associated with the elimination of the need for phlebotomy in patients with PV treated with interferon-α. This synchronous decrease in both circulating mature hematopoietic cells and progenitor cells is consistent with an inhibitory effect on the progenitors as the mechanism of controlling thrombocytosis and erythocytosis (Partaneu et al.).

Earlier work by our group at MDACC revealed that interferon-α could suppress excessive thrombocytosis in Ph-positive CML (Talpaz et al. 1983). Subsequent reports confirmed the platelet-lowering activity of interferon-α in chronic myeloproliferative diseases. Numerous investigators have since reported on the therapeutic effects of interferon-α in ET. These include platelet-reduction (up to 80% - 100% response rate), resolution of splenomegaly and control of disease-associated symptoms. Also, several authors have reported sustained, unmaintained remissions following discontinuation of long-term interferon-α therapy. Remission induction doses ranged from 3 to 5 million units per day given subcutaneously, with an average response time of 12 weeks. Maintenance doses were lower, consistent with a decrease in bone-marrow

megakaryocyte mass seen with continuous interferon-α therapy. Interferon-α is not know to be leukemogenic or teratogenic, and it does not cross the placenta. Consequently, treatment with interferon-α may be considered during pregnancy.

Preliminary reports on interferon-α as a new therapy for PV appeared in the late 1980s. Subsequent studies confirmed the benefits of interferon-α in PV, which include induction of complete hematologic remission (up to 70% - 80% response rate) and resolution of disease-associated symptoms and/or splenomegaly. It is now thought that interferon-α antagonizes the action of platelet-derived growth factor (PDGF) in addition to its direct effect on erythroid progenitors (Silver RT). Inhibition of PDGF may be effective in MF as well. Doses used to obtain these responses varied between 4 and 27 million units subcutaneously per week, with an average maintenance does of 3 million units three times a week. One of the major benefits of interferon-α therapy in PV has been the ability to control refractory pruritus (75% response rate) and to improve quality of life. Recent reports suggest that interferon-α may have the potential to suppress the abnormal clone in PV, analogous to its ability to induce cytogenetic remission in CML. Eradication or suppression of the malignant clone has major implications for altering the natural history of PV, with its inherent risk of transformation into leukemia or myelofibrosis (Lewis et al.).

Toxicity and cost are major obstacles to the use of interferon-α therapy. The adverse dropout rate has been approximately 17% and 20% among treated patients with ET or PV, respectively. Upon initiation of therapy, most patients experience a transient influenza-like syndrome associated with fever and chills, myalgias, headache, and arthralgias. Premedication with acetaminophen is usually helpful in ameliorating these acute side-effects. Chronic side-effects include fatigue, nausea, anorexia, weight-loss, diarrhea, increased liver transaminase, altered mental status, and depression. Also, the exacerbation or development of autoimmune disease, including autoimmune thyroiditis, immune-mediated hemolytic anemia or thrombocytopenia, and symmetric polyarthropathy in patients receiving long-term interferon-α therapy has been described (Silver RT).

Early studies with the long acting pegylated interferon-α suggest that this agent induces significantly less side effects while it is probably more active. We conducted a pilot study of PEG-interferon α-2b (PEG Intron) in patients with ET. Patients with a history of persistent (greater than 2 months) platelet counts > 600 x 10^9/L, with hyperplasia of bone marrow megakaryocytes in the absence of an alternate identifiable cause of thrombocytosis were eligible. Patients were required to have either thrombo-hemorrhagic signs and/or symptoms in a previously untreated patient; persistence of thrombo-hemorrhagic signs and/or symptoms in a patient receiving anagrelide, interferon-α, or hydroxyurea; or intolerance to anagrelide, interferon-α, or hydroxyurea. The initial PEG-interferon α-2b dose was from 1.5 to 4.5 μg/kg/week subcutaneously with subsequent dose adjustments as indicated by response and adverse events. Eleven patients (9 female, median age 54 years (range 26-69) were treated. PEG-interferon α-2b rapidly controlled platelet counts and resolved symptoms in all patients. The median duration of PEG-interferon α-2b therapy on study was 9 months (4-17). No patient had signs or symptoms of thrombosis or hemorrhage while on study. Ten (91%) patients were in CR after 2 months of therapy, 11 (100%) after 4 months. One patient discontinued therapy at 4 months because of persistent Grade 3 fatigue and a second at 5 months because of anxiety and depression. Thus, we believe that PEG-interferon α-2b has significant activity in patients with ET (Alvarado et al.)

3.3 Angiogenesis in Myeloproliferative Diseases

The putative role of angiogenesis in the neoplastic phenotype was first described thirty years ago (Folkman). Over the last decade, advances in molecular, cytogenetic, and cellular techniques have highlighted the powerful prognostic and therapeutic implications of the angiogenic process as it relates to human malignancy. Most of the early work focused on identification, quantification, and implication of vascularity on the biology and clinical behavior of solid tumors (Scappaticci). More recently, malignant hematologic disorders have been studied and the importance of bone marrow vascularity (angiogenesis) on the natural history

and possible therapy of these diseases is beginning to emerge (Thomas, Kerbel and Folkman).

One of the earliest reports describing the role of angiogenesis in a hematologic malignancy examined bone marrow from children with ALL (Perez-Ataye et al.). Median microvessel density was significantly higher in the bone marrow of patients with ALL compared to normal controls. Studies of microvessel density have been duplicated in patients with AMM/MF, CML, ET, acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and chronic lymphocytic leukemia (CLL): bone marrow from patients with these diseases all demonstrated statistically higher levels of vascular density compared with normal bone marrow (Thomas, Di Raimondo et al.).

The molecular and cellular mechanisms responsible for the angiogenic process are becoming better understood. As a substitute for microvessel density, other surrogate markers are being explored, particularly serum or plasma levels of known angiogenic factors. Vascular endothelial growth factor (VEGF) remains one of the most potent positive regulators of endothelial cell proliferation that has been described. New data suggest some overlap between endothelial and hematopoietic cells, and the possibility that angiogenic factors may also play a role in the growth and proliferation of hematopoietic cells (Kerbel and Folkman, Albitar). To understand the role of VEGF in the neoplastic process, studies have correlated serum levels in patients with a variety of solid human malignancies and clinical outcome (Scappaticci). Similar studies are emerging in patients with hematologic malignancies (Thomas, Albitar, Di Raimondo et al.). For example, VEGF was first identified as a possible paracrine growth factor in patients with AML (Fiedler et al.), and was shown to be an independent predictor of outcome in patients with AML (Aguayo et al.). Our recent study found increased expression of VEGF in marrow cells from patients with CML and the high VEGF levels correlated with poor outcome of patient with chronic phase CML (Verstovsek et al.). In addition, we found high levels of VEGF receptor 1 and 2 in CML patients. While levels of receptor expression did not correlate with any particular disease phase, there was a statistically significant correlation between higher levels of VEGF-R2

expression and prognosis. These findings support VEGF-R2 over-expression as an independent prognostic indicator for shortened survival in patients with chronic phase CML. Similarly, a number of studies found not only increased bone marrow microvessel density but also increased circulating angiogenic factors in other myeloproliferative diseases (ET, PV, MF) (Di Raimondo et al., Musolino et al.). Finally, the levels of circulating angiogenic factors are being used in the assessment of efficacy of anti-angiogenic therapy: for example, circulating levels of VEGF have been correlated with thalidomide's efficacy in hematological diseases.

Interferon-α has been shown to have antiangiogenic effects (reviewed in Kerbel and Folkman). The first evidence that interferon-α had anti-endothelial activity was reported in 1980 when it was found to inhibit the motility of vascular endothelial cells *in vitro* in a dose-dependent and reversible manner, and subsequently found to inhibit angiogenesis *in vivo*. Experimental studies in mice showed that the anti-angiogenic efficacy of interferon-α is optimal at low doses and declines at higher doses. The first use of anti-angiogenic therapy in humans was in 1988 when pulmonary hemangiomatosis in 12-year old boy was successfully treated with daily low dose interferon-α. New blood-vessel growth in proliferating haemangiomas has been associated with increased expression of angiogenic factor called fibroblast growth factor (FGF) and interferon-α has been shown to downregulate FGF expression in human cancer cells. There is now evidence that interferon-α therapy in CML may be successful at least in part due to its antiangiogenic properties as angiogenic factors are suppressed in treated patients (Di Raimondo et al.).

4.0 Background Drug Information

4.1 Interferons

The interferons are a family of naturally occurring, small proteins with molecular weights of approximately 15,000 to 21,000 daltons. Although first described by Isaacs and Lindermann (Isaacs et al.) as substances produced by virally infected cells that could induce resistance in other cells to lytic virus infections, they are produced and secreted by virtually all eukaryotic cells in response to

viral infections or to various biologic and synthetic inducers. Three major classes of interferons have been identified: alfa, beta, and gamma. These classes are not homogenous, and each may contain several different molecular species of interferon. For example, at least 14 genetically distinct human alfa interferons have been identified thus far.

The genes for a number of these proteins have been cloned, and recombinant forms of interferon α, including interferon α-2a and interferon α-2b, have undergone extensive clinical investigation. To date, interferon has been approved in numerous countries for the treatment of chronic hepatitis B and C (CHC), hairy-cell leukemia, AIDS-related Kaposi's sarcoma, condylomata acuminata, renal cell carcinoma, malignant melanoma, chronic myelogenous leukemia, basal cell carcinoma, non-Hodgkin's lymphoma, multiple myeloma, cutaneous T-cell lymphoma (mycosis fungoides), chronic delta hepatitis and laryngeal papillomatosis. Interferon α is also being used in combination with a multiplicity of chemotherapeutic agents in the investigational treatment of a variety of malignancies and in combination with ribavirin, amantadine, mycophenolate mofetil, and nonsteroidal anti-inflammatory drugs in the investigational treatment of patients with CHC.

Current treatment regimens require frequent administration of interferon α. Treatment intervals may range from three times per week to as often as once daily for periods of several months to a year or longer. The frequency of interferon α administration is determined by therapeutic requirements of the disease (daily for certain oncology indications and three times weekly for virology indications) and the pharmacokinetic properties of the protein. In various studies, the reported terminal elimination half-life for interferon α ranges from 4 to 10 hours, with peak serum concerntrations occurring at 3 to 8 hours following intramuscular or subcutaneous administration, there is little or no detectable interferon α remaining in the serum. Thus, frequent administration of interferon α has been considered necessary for sustained efficacy. Furthermore, treatment with interferon α results in several dose-dependent side effects that cause difficulties associated with frequent administration. The typical acute toxicity profile that tends to occur after every

injection includes flu-like symptoms of fever, chills, headache, myalgia, and dizziness.

4.2 Rationale for Use of PEG IFN-α2a

Subject compliance with daily interferon α dosing during the prolonged treatment course of therapy is an important factor for achieving clinical benefit. In general, despite the absence of direct comparisons, anti-tumor efficacy appears inferior in those studies where the median interferon α dose delivered is less than 3.6 MIU/m2/day. Similarly, trials performed in Ph-positive CML suggest that an increased AUC, with associated prolonged tumor exposure to the interferon α effect, may be more critical than peak interferon α levels in mediating an anti-leukemic effect. Early discontinuation of interferon α due to toxicity could adversely affect a patient's ability to achieve a response.

If the same clinical benefit can be achieved with a long-acting interferon α, the requirement for multiple injections will be reduced and result in great subject convenience. Given the pharmacokinetics and pharmacodynamics of PEG IFN-α2a and preliminary assessments of tolerability, PEG IFN-α2a may widen the therapeutic index for conventional interferon- and offer an improved risk benefit ratio.

4.3 PEG IFN-α2a

PEGs are amphiphilic polymers of ethylene glycol of varying average molecular weights that can be covalently attached to proteins. Modification of proteins with PEG has resulted in increased serum half-life and reduced immunogenicity for a number of proteins. The properties of pegylated proteins vary with the number of PEG molecules attached per protein molecule and the structure (e.g., linear or branched) and average molecular weight of the PEG polymer. A number of proteins used for patient therapy have now been modified with PEG and evaluated clinically. All of these pegylated proteins have increased half-lives relative to the unmodified proteins. Pegylated proteins are also less immunogenic or nonimmunogenic.

Hoffmann-La Roche has developed a modified form of interferon α-2a, pegylated interferon α-2a (PEG IFN-α2a, Pegasys), which is chemically modified by the covalent attachment of a 40K branched methoxy polyethylene glycol moiety. Compared with interferon α-2a, PEG-IFN α-2a exhibits sustained absorption, decreased systemic clearance, and an approximately tenfold increase in serum half-life. The biological activity of PEG IFN-α2a, as measured by 2,5-OAS activity, is similarly prolonged, resulting in a significantly improved pharmacodynamic response compared with interferon α-2a. Pharmacokinetic and pharmacodynamic data obtained from animals (rat and monkey), phase I studies in healthy volunteers, and phase II and III studies in patients with chronic hepatitis C (CHC) indicate that PEG IFN-α2a injected once a week has the potential for superior efficacy as compared with interferon α-2a injected three times a week. The adverse effect profile to date is similar to that observed with interferon α-2a. A phase I ascending dose study in patients with renal cell carcinoma has been completed, and the dose being used in the phase II study is 450 µg. Enrollment in a phase I study in patients with Ph-positive CML has been completed. Although a true MTD was not reached, the dose used in phase III CML study was also 450 µg.

4.4 Pharmacokinetics of PEG IFN-α2a

The structure of the PEG moiety directly affects the clinical pharmacology of PEG-IFN α-2a. Specifically, the size and branching of the 40 kDa PEG moiety define the absorption, distribution, and elimination of PEG-IFN α-2a. The pharmacokinetics of PEG-IFN α-2a were studied in healthy subjects and hepatitis C infected patients. Absorption of PEG-IFN α-2a was sustained, peak serum concentrations were reached 72 to 96 hours after dose administration, and measurable concentrations were seen within 3 to 6 hours of a single dose. Dose proportional increases in AUC and Cmax were seen in patients who received once weekly doses of PEG-IFN α-2a. The absolute bioavailability was 84% and was similar to that seen with IFN α-2a.

The systemic clearance was about 100 mL/h, which was 100-fold lower than that of interferon α-2a. After an

intravenous dose, the terminal half-life was about 60 hours, compared to 3 to 4 hours for standard interferon. The terminal half-life after subcutaneous dosing was longer (about 80 hours, range of 50 to 140 hours). After intravenous dosing, the steady-state volume of distribution was 6 to 14 liters. Based on studies in rats, the drug is distributed to the liver, kidney, and bone marrow as well as being highly concentrated in the blood.

Metabolism is the main clearance mechanism for PEG-IFN α-2a. The kidneys eliminated less than 10% of a dose as the intact PEG-IFN α-2a. The metabolic profile of PEG-IFN α-2a has not been fully characterized. Studies in rats showed that the PEG moiety remained attached as the protein portion was metabolized. The metabolic products of PEG-IFN α-2a (including the PEG moiety attached to the metabolized interferon) were excreted in the urine and bile. In rats, the half-life of the radiolabeled PEG moiety was about 10 days and, thus, within about 40 days the PEG moiety was eliminated from the body.

In patients with CHC, steady-state serum concentrations increased twofold to threefold compared with single-dose values and reached steady state within 5 to 8 weeks of once weekly dose administration. Once steady state was achieved, there was no further accumulation of PEG-IFN α-2a. The peak to trough ratio after 48 weeks of treatment was about 1.5 to 2.0. PEG-IFN α-2a serum concentrations were sustained throughout the 1-week dose interval (168 hours).

4.5 Preclinical Studies with PEG IFN-α2a

The pharmacologic and immunomodulatory properties of PEG IFN-α2a relative to those of interferon α-2a were evaluated in both *in vitro* and *in vivo* studies. Since the biological activity of interferon α-2a is species restricted, these studies were performed using cells (bovine, human) or animals (cynomolgus monkeys) in which interferon α-2a is active. The following were the main observations:

- PEG IFN-α2a retains both the antiviral and antiproliferative activities of unmodified

interferon α-2a, although usually with lower specific activity.

- PEG IFN-α2a causes less of an immunogenic response in mice than interferon α-2a.
- PEG IFN-α2a is immunogenic in monkeys, as was anticipated based on the immunogenicity of interferon α-2a in monkeys, but this observation is not considered predictive of the immunogenic potential of PEG IFN-α2a in humans.

The antiproliferative effects of PEG IFN-α2a and interferon α-2a were compared in an *in vitro* assay against a series of tumor cell lines, including a CML line (K562), four renal cell carcinoma cell lines (ACHN, A-498, G-402 and Caki-1), and four malignant melanoma cell lines (HS-294-T, A-375, SK-MEL-28 and SK-MEL-3). The data demonstrate that PEG IFN-α2a maintains antiproliferative activity against this range of human tumor cells. As *in vitro* models cannot account for the pharmacokinetic advantage of PEG IFN-α2a, the most appropriate assessment would be *in vivo* models, in which this potential benefit of the molecule should be observed. The *in vivo* antitumor effects of PEG IFN-α2a were evaluated in nude mice bearing human tumor xenografts. Nude mice were injected with tumor cells subcutaneously and tumors were allowed to grow until well-established tumors were present. Treatment with either PEG IFN-α2a once per week or unmodified interferon α-2a three times per week was administered sc in the opposite flank from the tumor for 4 to 6 weeks. The total weekly doses for PEG IFN-α2a and interferon α-2a were equivalent. In A-498–bearing nude mice, treatment with 100 g of interferon α-2a three times per week significantly inhibited growth of this tumor compared with untreated controls. In animals treated with 300 µg (measured as micrograms of interferon α-2a) of PEG IFN-α2a once per week, PEG IFN-α2a was more active than interferon α-2a in inhibiting tumor growth and also resulted in tumor regression. In similar experiments using two other renal cell carcinoma cell lines (ACHN and Caki-1), a clear trend toward increased activity of PEG IFN-α2a over interferon α-2a was found but without the statistical significance seen with A-498. In conclusion, PEG IFN-α2a shows marked antitumor activity when

administered to nude mice bearing human renal cell carcinoma tumor xenografts.

4.6 Clinical Studies in Healthy Volunteers

PEG IFN-α2a was administered by subcutaneous injection to 92 healthy volunteers in a phase I double-blind study. Volunteers received single ascending doses of 45, 135 or 270 µg of PEG IFN-α2a or 3 MIU (15 µg) or 18 MIU (90 µg) of unmodified interferonα -2a. Results showed that:

- PEG IFN-α2a had a more favorable pharmacokinetic profile than interferon α-2a, with decreased clearance and a serum t½ approximately 10-fold higher.
- PEG IFN-α2a had an improved pharmacodynamic profile as compared with interferon α-2a, as measured by a prolonged elevation of 2'5'-oligoadenylate synthetase following single-dose administration.
- The safety and tolerability profiles of PEG IFN-α2a are similar to those of interferon α-2a. Neutropenia was more frequent and more marked in PEG IFN-α2a treated patients, but neutrophil counts recovered in all cases in less than two weeks following drug administration.

4.7 Clinical Studies in Chronic Hepatitis C (CHC)

In a phase II dose-finding study in patients with non-cirrhotic CHC, doses of 45, 90, 180 and 270 µg of PEG IFN-α2a once weekly vs 3 MIU interferon α-2a tiw were evaluated in an ascending dose design. The side effect profile was similar to the adverse events observed with interferon α-2a, and a dose of 180 µg has been found acceptable in patients with CHC. At the 270 µg dose level, 18 of 40 patients required dose modification; however, for patients with CHC a neutrophil count of less than 1.0×10^9 /L was considered dose limiting. There were no cases of neutropenic sepsis. A second phase II study has been conducted in 268 cirrhotic patients, and two phase III studies in 1170 patients with CHC have been completed; all studies used Roferon-A as a comparator. In total, data have been generated in 995 hepatitis patients treated with doses of 45 µg (20 pts), 90

µg (116 pts), 135 µg (215 pts), 180 µg (604 pts), or 270 µg (40 pts) PEG IFN-α2a. Results showed that:

Results from a Phase II PEG IFN-α2a ascending dose study confirm the optimal dose of PEG IFN-α2a for the treatment of CHC to be 180 µg once weekly. Sustained virologic response was 36% in CHC patients without cirrhosis treated with this dose. These results also confirm significant improvements in liver histology for patients treated with either PEG IFN-α2a or standard interferon α2a therapy. Pharmacokinetic data from a Phase III multinational study further support the once weekly 180 µg dose of PEG IFN-α2a for the treatment of CHC.

Results from a Phase II PEG IFN-α2a pharmacokinetics study confirm the feasibility of similar once-weekly PEG IFN-α2a dosing in CHC patients with cirrhosis as in healthy volunteers and noncirrhotic hepatitis C patients.

One Phase III PEG IFN-αa study reports virologic sustained responses of 39% in CHC patients without cirrhosis (28% in CHC patients with Genotype 1 infection) when treated with 180 g PEG IFN-α2a once weekly for 48 weeks. A second Phase III study reports virologic sustained responses of 38% in previously untreated CHC patients with or without cirrhosis treated with either 135 g or 180 g of PEG IFN-2a once weekly for 48 weeks. Sustained virologic response among CHC patients with Genotype 1 infection were 22% and 19% following 180 g and 135 g of PEG IFN-α2a once weekly for 48 weeks, respectively. Additionally, histologic improvement (defined as 2-point improvement from baseline on post-liver biopsy) occurred in 58% and 48% of patients treated once-weekly with 180 g and 135 g of PEG IFN-α2a, respectively.

A Phase II/III PEG IFN-α2a study reports virologic sustained responses of 30% in difficult-to-treat CHC patients with cirrhosis when treated with 180 µg PEG IFN-α2a once weekly for 48 weeks. Study results also confirm statistically significant improvements in both liver histology and quality of life for patients treated with 180 µg PEG IFN-α2a when compared with standard interferon α2a therapy.

The overall frequency and types of adverse events, laboratory abnormalities and drug discontinuations reported during a 48-week course of PEG IFN-α2a therapy administered once weekly, appear similar to that of a 48-week course of interferon α2a therapy administered three times a week. Adverse events included fatigue, headache, myalgia, arthralgia, flu-like symptoms, nausea, vomiting, injection site reactions, fever, chills, diarrhea, alopecia, abdominal pain, depression, irritability, insomnia, rigors, dizziness and anorexia. Laboratory abnormalities included neutropenia, thrombocytopenia, abnormal ALT values, hypothyroidism and hyperthyroidism. The incidence of premature withdrawals for adverse events and laboratory abnormalitites were similar between the PEG IFN-α2a dose groups and the interferon α-2a group (approximately 10%).

4.8 Clinical Studies in Renal Cell Carcinoma (RCC)

A phase I/II dose-finding study in advanced or metastatic RCC is fully recruited. Twenty-seven patients had been entered into the phase I portion of this study and dosed in cohorts of six patients with once weekly PEG IFN-α2a 180 µg, 270 µg, 360 µg, 450 µg or 540 µg. Dose limiting toxicities occuring at 540 µg PEG IFN-α2a included elevated liver transaminases (1 patient with grade 3 ALT), and fatigue (1 patient with grade 3 fatigue). For this reason, the MTD was determined to be 540 µg, and the dose chosen to be taken forward into further development is 450 µg, which has been well tolerated for 3 months or more. Forty additional patients have now been entered into the Phase II portion of the study at the dose of 450 µg. Three patients have stopped treatment for safety reasons: thrombocytopenia (180 µg), increased alkaline phosphatase (450 µg) and GI bleeding/sepsis/seizure, unrelated to treatment (450 µg). Most patients in the RCC study are tolerating PEG IFN-α2a well. The types of adverse events have been similar in nature to that of unmodified α-interferons. Adverse event reports are available for 66 patients, and the most frequently occurring (>5%) adverse events include: fatigue, fever, chills, myalgia, nausea, headache, decreased appetite, back pain, dizziness and diarrhea. Changes in laboratory values have generally been mild to

moderate, and are consistent with those expected with the use of alpha interferons. The most important laboratory abnormalities have been elevations in transaminase levels, ranging from minor increases to grade 3 ALT elevations experienced by three patients in the highest dose group tested. These elevations have returned to near baseline either on treatment or after holding a dose. Other laboratory abnormalities include mild to moderate decreases in white cells, neutrophils, lymphocytes, platelets and hemoglobin over the first few weeks of treatment. The only notable instance of hematological toxicity is one patient who experienced neutropenia and developed pneumonia beginning on the third day after the first 180 µg PEG IFN-α2a injection. Twenty-seven patients have discontinued treatment due to progressive disease. Efficacy assessments are available from the first 27 patients entered in the study. Five of the 27 phase I patients have demonstrated confirmed partial response (18.5%); 12 patients completed the 6-month study and 10 continued treatment in a maintenance phase.

4.9 Prior Clinical Experience with PEG IFN-α2a in Hematological Diseases

4.9.1 Safety of PEG-IFN -α2a in the Treatment of Ph-positive CML: Study NO15764

Study NO15764 is a phase I, open-label, nonrandomized, dose-escalation trial of the safety and efficacy of PEG IFN-α2a in Ph-positive CML. A total of 43 patients received either PEG-IFN α-2a monotherapy (three at 270 µg and six each at 360, 450, 540 or 630 µg) or PEG-IFN α-2a plus cytosine arabinoside (ara-C) combination (six at 450 µg of PEG-IFN α-2a plus 10 mg of ara-C, six at 540 µg of PEG-IFN α-2a plus 10 mg or ara-C, and four at 540 µg of PEG-IFN α-2a plus 20 mg of ara-C). The PEG-IFN α-2a doses were given once weekly. The ara-C doses were given daily. However, for patients in the 540-µg PEG-IFN α-2a plus 20-mg ara-C group, ara-C was given only on 10 days a month.

All patients from all treatment groups had one or more adverse events. The types of adverse events were similar to those seen with α-interferons. The most frequently observed adverse events included fatigue,

headache, rigors, myalgia, decreased appetite, diarrhea, nausea, night sweats, pyrexia, and injection site pain or irritation.

As of November 20, 2001, four patients had died. One patient treated with 450 µg of PEG-IFN -α2a died of lymphoma. The reasons for the other three deaths (one from the PEG-IFN -α2a 270-µg group and two from the PEG-IFN -α2a 630-µg group were not specified. These three deaths occurred 5, 14, and 16 months after treatment ended, and were assumed to had been related to the underlying disease.

A total of 19 serious adverse events were reported in 13 patients. All treatment groups had at least one patient who experienced a serious adverse event. The serious adverse events that were reported included thrombocytopenia, transfusion reaction, gastroenteritis, herpes simplex, viral infection, unevaluable reaction (pregnancy in spouse), pyrexia, device failure, hernia repair, lymphoma, dehydration, diarrhea, liver fatty, blood bilirubin increased, weakness, pleuritic pain, and atherosclerosis. About two-thirds of the serious adverse events were possible, probably, or remotely related to the study drug and usually needed dose reduction or, in a few cases, premature treatment discontinuation. However, most of these serious adverse events resolved without sequelae.

A total of 12 patients withdrew from the study for safety-related reasons. Four of the six patients with 540 µg of PEG-IFN α-2a plus 10 mg of ara-C withdrew, while none of the patients treated with 360 µg of PEG-IFN or 540 µg of PEG-IFN α-2a plus 20 mg of ara-C withdrew for safety related reasons. One or two patients from each of the remaining treatment groups withdrew for safety-related reasons. Of the 12 patients whose treatment was discontinued prematurely for safety reasons, eight patients were withdrawn for adverse events including irritation of the buccal mucosa, lymphoma, mucositis and pruritic rash, fatigue, leg pain, neuropathy, and weakness of the lower extremities; and four patients were withdrawn for laboratory abnormalities including thrombocytopenia, elevated liver enzymes, abnormal liver function tests, and elevated bilirubin.

4.9.2 Safety of PEG-IFN α-2a in the Treatment of Ph-positive CML: Study NO16006

Study NO16006 is a phase III, randomized, open-label, parallel-arm, multicenter trial comparing the efficacy and safety of PEG-IFN α-2a and interferon α-2a in patients with recently diagnosed Ph-positive CML never treated with interferons. Patients have been randomly assigned to receive subcutaneous injection of either 450 µg of PEG-IFN α-2a once weekly or 9 MIU of interferon α-2a daily (titrated upward from a starting dose of 3 MIU daily) for up to 12 months. Responders to treatment may continue treatment for an additional year. As of November 19, 2001, 71 and 74 patients who received PEG-IFN α-2a and interferon α-2a, respectively, had safety data available.

Eight-five percent and 77% of patients treated with PEG-IFN α-2a and interferon α-2a, respectively, had one or more adverse events. The most common adverse events from both treatment groups were similar to those seen with interferon treatment, such as pyrexia, myalgia, fatigue, rigors, diarrhea, nausea, arthralgia, headache, appetite decreased, and anorexia. Most of these events occurred more frequently in the PEG-IFN α-2a-treated patients than in the interferon α-2a-treated patients.

Two patients from each treatment group have died. CML, aggravated general condition, and thrombotic thrombocytopenic purpura each led to the death of one patient. The reason for the fourth death was not specified.

Fourteen percent of patients treated with PEG-IFN α-2a and 15% of patients treated with interferon α-2a had experienced serous adverse events. About one-third of the reported serious adverse events were possibly, probably, or remotely related to the study drug and required dose reduction or premature treatment discontinuation. However, most of these serious adverse events resolved without sequelae. For the PEG-IFN α-2a-treated patients, serious adverse events were reported more often as general disorders and disorders of blood and the lymphatic system; for the interferon α-2a-treated patients, serious adverse events were reported more often as general disorders,

musculoskeletal, connective tissue, and bone disorders, and cardiac disorders.

Six PEG-IFN α-2a-treated patients (8%) and eight interferon α-2a-treated patients (11%) withdrew because of adverse events or laboratory abnormalities. For the PEG-IFN α-2a treatment group, the safety reasons for patient withdrawal included acute hip pain, thrombocytopenia, very high WBC counts, and low neutrophil and platelet counts and low hemoglobin concentration. For the interferon α-2a treatment group, the safety reasons for patient withdrawal included myalgia, depression, anaphylaxis, femoral head necrosis, and positive chest x-ray and flu-like syndrome.

4.10 Rationale for Dosage Selection

The PEG-IFN dose for this phase II study has been selected based on safety results, the maximum tolerated dose (MTD) determined in the phase I study in patients with Ph-positive CML, in combination with ara-C (Talpaz et al. 2000), and a minimum of 3 months safety data from the patients taking the projected phase III dose. In the phase I RCC study, the MTD was 540 µg, therefore the dose used in the phase II portion of that study is 450 µg. Patients in the phase I CML study have shown neutropenia and elevated transaminases starting at 540 µg, although a formal MTD has not been declared, even at the dose of 630 µg. When given once weekly, 450 µg of PEG-IFN α-2a is the dose that is considered to deliver the best balance between immunostimulatory/antiproliferative effect and long-term tolerability. To increase safety of the medication, the starting dose, will be 90µg and may be increased to doses of 180µg, 270µg, 360µg, and 450µg provided patients do not experience significant side effects.

4.11 Update of study results as of 01/15/2009 and plans

At present the study has accrued 40 patients with PV and 36 patients with ET. Most patients had extensive prior therapies with hydroxurea and anagrelide and all 40 patients with PV had phlebotomies. With a median folllowup of 24 months on Pegasys therapy, the overall hematologic repsonse rate was 81% with PV (complete

in 78%) and 92% with ET (complete in 86%). These results are the best ever achieved with any form of therapy in PV and ET. In addition, measuring JAK2 V617F mutations, we observed complete molecular responses in 12% of patients and any molecular response in 53% of patients. Side effects were accetable. At the Pegasys dose of 90 mcg weekly Grade 3 neutropenia was observed in only 8% of patients and other side effects (fatigue, dizziness, depression) in only 4% of patients each. These findings are a true therapeutic breakthrough in the managment of PV and ET and have been also reproduced by investigators in France (Kiladjian et.al Blood 112;3065,2008) who reported with Pegasys therapy in 40 patients with PV a CHR in 78%, a complete molecular response in 24%, and a major molecular response in 69%.

Hoffman-LaRoche, the Company, declined to expand the study and provide free drug. Our results suggest this is the best available treatment for these patients. We would like to expand the study and continue therapy with Pegasys commercial preparation. We propose to include up to 100 patients with PV and 100 patients with ET. This will allow a more precise estimate of hematologic and molecular response with long term follow up, a better estimate of long term survival and toxicity profiles, and a treatment of patients on protocol which affords better care by our leukemia experts and allows future analyses of data in a large cohort including an evaluation of prognostic factors that determin molecular response and long term outcome. Our data was presented at ASH 2008 (Quintas-Cardama, Blood 112:653, 2008).

Update September, 2019

Sixteen patients remain on study for a median of 12 years (range 11 - 13). All patients are on a stable dose of pegylated interferon with normal blood counts. None of the patients have experience drug-related adverse reactions in 9 years. Changes in the protocol reflect standard of care monitoring of patients in long term complete hematologic remission. This study represents the longest follow up of patients with ET or PV treated with pegylated interferon.

5.0 Patient Eligibility Criteria

5.1 Inclusion Criteria

- Following diagnoses:

  --ET: Patients with PLT > 600 x10^9/l documented in the past 12 months; hyperplasia of marrow megakaryocytes in the absence of identifiable cause of thrombocytosis and in the absence of Ph chromosome. Patients with ET and lower PLT will be eligible if attributable to prior ET therapy.

  --PV: Patients should have Hb >/= 15g/dl (except if patient is having phlebotomies done or is taking hydroxyurea) and documented past diagnosis

- Performance status $\leq$ 2 (ECOG scale; see Appendix A).
- Age greater than 18 years since disease is extremely rare in younger age group.
- Adequate liver function: total bilirubin of $\leq$ 2.0 mg/dl (except for patients with Gilbert's Syndrome) and AST (SGOT) or ALT (SGPT) < 3 X ULN (or < 5 X ULN if considered due to tumor), and renal function (serum creatinine $\leq$ 2.0 mg/dl)
- Signed informed consent indicating that patients are aware of the investigational nature of this study in keeping with the policies of the M.D. Anderson Cancer Center. The only acceptable consent form is the one approved by the M.D. Anderson Cancer Center IRB.
- Willingness and ability to comply with the requirements of the protocol for the duration of the study.
- Patients must have been off chemotherapy for 1 week prior to beginning Pegasys and have recovered from the toxic effects of that therapy. Patients may have received hydroxyurea or anagrelide immediately before study entry, and may continue into therapy if treating physician determines that this is in the best interest of the patient.

5.2 Exclusion Criteria

- Pregnant or lactating women.

- Patients with prior history of another malignancy or concurrent malignancy, except for the following: basal cell carcinoma of the skin, carcinoma in situ of the cervix, or other malignancies if the patient is disease free >3 years.
- Patients with history of ischemic retinopathy.
- Patients with history of severe cardiac disease: NYHA Functional Class III or IV, myocardial infarction within 6 months, uncontrolled ventricular tachyarrythmias or unstable angina.
- Patients with history of medically significant psychiatric disease if not controlled, especially endogenous depression (does not include reactive depression post-cancer diagnosis), psychosis and bipolar disease.
- Patients with seizure disorders requiring anticonvulsant therapy.
- Patients with known infection with HBV, HIV, or other active systemic infection.
- Patients with known autoimmune disease except for rheumatoid arthritis.
- Patients with renal disease on hemodialysis.
- Patients taking continuous or chronic high-dose systemic steroids; if discontinued, there must be a minimum washout period of one month before study drug is begun.
- Patients with known hypersensitivity to PEG-IFN α-2a or its components.

6.0 Treatment Plan

6.1 Start of Therapy

This is a Phase II (activity), non-randomized, open-label study. All patients will be registered in the Clinical Oncology Research Database (CORE) at MD Anderson Cancer Center after the informed consent is signed. Medication will be dispensed to patients from MD Anderson Pharmacy.

- PEG-IFN α-2a will be given subcutaneously once weekly and may be self-administered.
- Starting dose of PEG-IFN α-2a is 90 micrograms subcutaneously weekly.
- Treatment will be given as long as it benefits the patient (see section 9.1)

The patient may be given the first injection in the clinic or may be taught self-injection techniques for self-administration at home, if the patient and investigator feel comfortable with this arrangement. Patients not able to return to MD Anderson Cancer Center for injection teaching may receive teaching from their local physician. Prior to receiving multi-dose vials, patients local physician will be provided with a letter explaining the teaching needs of the patients along with a teaching documentation form (see appendix I).The teaching documentation form must be completed and returned to MD Anderson Cancer Center prior to the patient receiving study drug in multi-dose vials. PEG-IFN α-2a will be provided as either ready-to-use solution in single use glass vials or as multi-dose vials. Contents require no mixing before use.

6.2 Dose Escalation and Dose Reduction

Dose Level	Dose of Pegasys in micrograms SQ weekly
+4	450 mcg
+3	360 mcg
+2	270 mcg
+1	180 mcg
0(starting dose)	90 mcg
-1	45 mcg
-2	45 mcg every other week

Any patient who experiences grade 1 or 2 adverse event (see NCI-CTC guidelines, appendix C) should be maintained on the assigned dose if possible. Treatment should be put on hold if a patient experiences any of the following:

- grade 3-4 laboratory abnormalities
- grade 3-4 constitutional toxicity

Dose adjustments may be made at the discretion of the treating physician.

Questionable cases should be resolved by clinical judgment of the investigator.

7.0 Pretreatment evaluation

The pre-treatment evaluation will be aimed at confirming/establishing the diagnosis. The evaluation will consist of a through assessment of the following tests: and will be done within a month of enrollment in the study (unless noted otherwise):

- A complete primary evaluation with physical examination
- CBC, platelet counts, differential
- Serum creatinine, bilirubin, SGPT
- Pregnancy test (if of child bearing potential)
- Bone marrow aspiration and biopsy; cytogenetic studies; if not done in the last 3-6 months

8.0 Evaluation During Study

- Follow up visits at MD Anderson every 3-6 months with review of symptoms and physical exam for the first year, then every 6-12 months and thereafter approximately once yearly or at the discretion of the treating physician.

- CBC, platelets, differential, creatinine, bilirubin, and SGPT are recommended twice per year or at the discretion of the treating physician.

- Thyroid function tests are recommended once per year or at the discretion of the treating physician.
- Bone marrow aspiration and biopsy every 3-6 months during the first year, then if needed; cytogenetics once when in complete remission if abnormal prior to therapy.

8.1 Monitoring of Patients on Study

Patient will be given instructions and the M.D. Anderson contact name (principal investigator/MD Anderson Leukemia staff treating physician), phone, and fax numbers. The patient's local doctor will be identified, contacted, and the protocol details discussed.

All therapy will be given under the care of the patient's local doctor and MD Anderson Leukemia staff treating physician. The patient's local doctor should contact the MD Anderson Leukemia staff treating physician before any dose modifications are considered, and the modifications should be initiated only by the MD Anderson Leukemia staff treating physician or principal investigator, and complications will be dictated at each follow-up visit.

From past experience, it is recognized that treatment provided in collaboration with patient's local doctor outside MD Anderson may occasionally result in some difficulties such as 1) not performing tests as required, 2) not sending results of tests or updates of patient courses, 3) and not informing on toxicities and hospitalizations. This type of therapy is already broadly practiced in the community. Therefore: 1) we will discuss with the patient's local doctor about the protocol and doses, 2) we will make significant efforts (contact at least twice and document) to retrieve the information (tests, toxicities, hospitalizations), and 3) we will document all the information on follow-up visits. Since information may still be missing in one or more components in some patients (e.g. missing CBC or SMA-7, missing drug administration record), these types of difficulties will be collected and documented in the patient's medical record. This will reflect the oncology community practice of the program.

9.0 Criteria for Response and Toxicity

9.1 Criteria for Response

9.1.1 ET

CR = Reduction of PLT to $<440x10^9$/l and disappearance of thromboembolic events, without the use of anagrelide or hydroxyurea

PR = Reduction of PLT by 50% but still >440x10^9/l or reduction of thromboembolic events by 50%, without the use of anagrelide or hydroxyurea

9.1.2 PV

CR = Normalization of Hb (< 15.0g/dl), no phlebotomies needed, and disappearance of splenomegaly by palpation, without the use of anagrelide or hydroxyurea

PR = A 50% reduction in phlebotomies rate or splenomegaly reduced by 50% by palpation, without the use of anagrelide or hydroxyurea

9.2 Toxicity

10.0 Criteria for Removal from the Study

Subjects have the right to withdraw from the study at any time for any reason. An excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of subjects should be avoided. Should a subject decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible. Criteria for discontinuation of therapy include:

- Clearly documented progressive disease (increasing requirement for blood transfusion, increasing spleen size, increasing platelet or white blood cell count, increasing frequency of phlebotomies, or frequency of thromboembolic events) or no response (see section 9.1) within 6 months from the start of the therapy on a given dose (i.e. continuously on the same dose level of PEG-IFN α-2a). Caution: Patient without response after 3 months on a given dose should have the dose escalated if possible (see section 6.2); if no response when on the escalated dose for 6 months, then therapy should stop.
- Severe toxicities not responding to dose adjustments (see section 6.2)
- Non-compliance to protocol requirements (see section 5.0)

- Development of other conditions for which, in the Investigator's opinion, it is in the subject's best interest to be withdrawn from the study.

11.0 Statistical Considerations

11.1 This is a phase II trial of PEG IFN-a2a (Pegasys®) as a treatment for ET and PV. In each disease, a response rate of 35% or more would be accepted as evidence for a good efficacy to be further pursued in this disease subsets in larger randomized or combination studies. Therefore, in each disease category, the study will stop if response is 07, < 1/11, <2/15. Otherwise it will accrue 80 patients to better define the response and toxicity profiles. Also, in the total study group, a severe toxicity rate of <20% is allowed. Therefore, the study will also stop if severe toxicity is observed in >7/15, 11/30, 15/45.

11.2 Update of statistical considerations as of 1/15/2009

Experience in 76 patients has shown very high response rates of 78% to 86% in ET and PV. This expansion of the study is to provide patients with the best available treatment, Pegasys, at an optimal dose schedule, in the context of a study using the commercial drug. Up to 100 patients with ET and 100 patients with PV will be treated. This will allow for 1) a large scale capture of more precise response data (hematologic, molecular), 2) detailed analysis of side effects, 3) evaluation of longer term prognosis and survival, and 4) analysis of potential prognostic factors associated with molecular response. Such analyses will be conducted using standard analytical methods.

12.0 Reporting Requirements

12.1 Serious Adverse Events will be reported per institutional policy. Adverse events will be reported in the medical record.

See Appendix G

12.2 **The research protocol includes adequate plans to protect identifiers from improper use.** HIPAA Identifiers (name, medical record number)

will be collected but will be replaced by study numbers in the analytical file; complete confidentiality will be maintained during this retrospective evaluation, manuscript preparation, and submission.

(a) **The research protocol includes an adequate plan to destroy the identifiers at the earliest opportunity consistent with conduct of the research.** Identifiers (name, medical record number) will be collected but will be replaced by study numbers in the analytical file; Complete confidentiality will be maintained during this retrospective evaluation, manuscript preparation, and submission. Identifiers will be destroyed within 1 year of publication

(b) **The research protocol includes adequate written assurances that the PHI will not be reused or disclosed to any other person or entity, or for other research.**

Complete confidentiality will be kept during this retrospective evaluation, manuscript preparation, and submission. This data will not be used for any other purpose and will not be reused or disclosed to any other person or entity, or for other research.

Anonymized information will be retained by the investigator in locked files or password protected databases

2. **The research could not practicably be conducted without access to and use of the PHI.** Access and use of PHI is essential to conducting this study and meeting the study objectives.

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