Pegylated Interferon for the Treatment of Early Myelofibrosis: Correlation of Serial Laboratory Studies with Response to Therapy

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Online Resource 1.

Case 1

Patient 1 is a 61-year-old Asian female with JAK2-negative, CALR-positive ET diagnosed 24 years prior to starting Peg-IFN. She did not require therapy for almost 20 years. Then due to development of other cardiovascular risk factors and a steadily rising platelet count, hydroxyurea was recommended, but she was unable to tolerate it due to profound fatigue. Therefore, anagrelide was initiated with effective platelet control for 5 years. During the year prior to starting Peg-IFN, she developed anemia with a drop in hemoglobin from 12.4 g/dL to a nadir of 9.7 g/dL. A workup for bleeding and nutritional deficiencies was unrevealing and her renal function was normal. A bone marrow biopsy performed three months prior to starting Peg-IFN was a markedly hypercellular marrow (80%) with hyperplasia, dysplasia and clustering of megakaryocytes, mild reticulin fibrosis, and normal cytogenetics (Table 3). Anagrelide was discontinued and 45 mcg of weekly Peg-IFN was initiated.

Platelet count normalized within the first month of therapy and the hemoglobin had normalized by the fifth month of therapy without any other intervention to correct the anemia. LDH levels were normal prior to Peg-IFN but increased to a peak of 290 units/L at 4 months of treatment and normalized by the 7th month of therapy (Figure 1). Band forms and nucleated red cells were noted in the peripheral blood after starting pegylated interferon, and these disappeared by 12 months of therapy (Figure 2). Toxicities included grade 2 fatigue, grade 1 rhinitis, and grade 2 night sweats, which resolved when the dosing interval was reduced to every 3 weeks. Repeat bone marrow biopsy was performed following one year of treatment with Peg-IFN (Table 3). It demonstrated normocellularity (60%) and stable fibrosis compared to the previous biopsy. Bone marrow biopsy performed at 3 years showed a moderately hypercellular marrow (70%) with megakaryocytic hyperplasia, dysplasia, and clustering, stable fibrosis and normal cytogenetics (Table 3). She is now in her fifth year of therapy and is being treated with 45mcg of monthly Peg-IFN with excellent platelet control and normal hemoglobin.

Case 2

Patient 2 is an 83-year-old woman with JAK2-negative ET diagnosed five years prior to starting Peg-IFN. A spleen tip was palpated on physical exam. A bone marrow biopsy performed at the time of the initial diagnosis revealed a normocellular marrow with increased megakaryocytes, some forming clusters, and mild reticulin fibrosis, consistent with ET. She was promptly initiated on treatment with hydroxyurea, which resulted in reduction of her platelet count to below 600 x10^9/L as well as a slight reduction in her hemoglobin. She experienced a gradual rise in her platelet count and decline in her hemoglobin with a nadir of 7.9 g/dL after 4 years of treatment with hydroxyurea. Although her creatinine clearance remained within normal range, her anemia was responsive to treatment with an erythropoietin stimulating agent (ESA). Her platelets remained difficult to control ranging between 600-900 x10^9/L. A repeat bone marrow biopsy demonstrated a normocellular marrow (50%) with dysplastic and clustered megakaryocytes and
mild reticulin fibrosis. She refused anagrelide therapy but agreed to Peg-IFN, which was initiated at a starting dose of 45 mcg weekly.

Platelet control was achieved within 2 months of therapy with Peg-IFN, and the hemoglobin gradually rose without further ESA administration to a peak of 11.3 g/dL after 10 months of Peg-IFN therapy. After 3 months of Peg-IFN, her spleen size had increased with a maximum excursion of 6 cm below the left costal margin. Moreover, following initiation of Peg-IFN, immature cell forms appeared in the peripheral blood (Figure 2). While the splenomegaly completely resolved by month 7 of treatment, the presence of immature cells did not diminish with ongoing therapy. Peg-IFN was discontinued after 18 months of treatment due to grade 2 fatigue and grade 2 night sweats. She was subsequently initiated on the JAK2 inhibitor ruxolitinib, which she has now been taking for 2 years with good disease control but significant anemia again responsive to treatment with an ESA.

Case 3

Patient 3 is a 79-year-old woman with JAK2-negative, CALR-positive ET diagnosed nine years prior to starting Peg-IFN. Her initial therapy consisted of combination of hydroxyurea and anagrelide, and her platelets ranged from 247-820 x10^9/L on this regimen. As she was experiencing intolerable side effects from these agents including mild anemia with a hemoglobin nadir of 11.1 g/dL, Peg-IFN was initiated at a starting dose of 90 mcg weekly. The hydroxyurea and anagrelide were titrated off over the next 1 (hydroxyurea) and 9 (anagrelide) months. A bone marrow biopsy performed shortly after starting Peg-IFN treatment revealed a normocellular marrow (50%), megakaryocyte hyperplasia and clustering, moderate reticulin fibrosis, and normal cytogenetics (Table 3).

Platelet control was achieved following 3 months of treatment with Peg-IFN. Upon initiation of Peg-IFN, her anemia worsened with a drop in hemoglobin to 10.0 g/dL and she was started on an ESA. After 2 years of Peg-IFN treatment, her hemoglobin stabilized at 10-11 g/dL and she no longer required ESA support. Her hemoglobin continued to rise to a peak of 12.1 g/dL after four years on Peg-IFN. LDH levels had been elevated and stable prior to Peg-IFN. Following treatment start, LDH steadily increased to a peak of 727 units/L at 3 months of treatment (Figure 1). The LDH subsequently decreased to below her baseline levels. She had the appearance of immature cells in the peripheral blood within one month of therapy, which resolved after two years on Peg-IFN (Figure 2). She experienced grade 2 fatigue and grade 2 arthritis with the Peg-IFN but has opted to continue with therapy. Repeat bone marrow biopsy at 4 years showed a hypocellular marrow (30%) with trilineage hematopoiesis, megakaryocytic hyperplasia with clustering, and improved reticulin fibrosis (Table 3). She is currently in her fifth year of therapy with Peg-IFN, now at 60 mcg bimonthly with excellent platelet control (less than 600 x10^9/L) and a stable hemoglobin (11.2-12.3 g/dL).
**Case 4**

Patient 4 is a 56 year-old Caucasian female with JAK2-negative, CALR-positive ET diagnosed nine years prior to starting Peg-IFN when she presented with a cerebrovascular event. She was treated with anagrelide for several years and had been unable to tolerate hydroxyurea. She had progressive anemia during the year prior to initiating Peg-IFN with a hemoglobin nadir of 9.3 g/dL as well as poor platelet control on anagrelide (474-919 x10^9/L). The spleen was palpable at 5 cm below the costal margin. A bone marrow biopsy performed three months prior to starting Peg-IFN revealed a markedly hypercellular marrow (80%) with trilineage hyperplasia, megakaryocytic hyperplasia with clustering and moderate dysplasia, moderate reticulin fibrosis, and normal cytogenetics (Table 3). She was started on 45 mcg of Peg-IFN and titrated off anagrelide after four months on Peg-IFN.

Platelet control was achieved after 2 months of therapy. Her hemoglobin gradually improved to 11.6 g/dL after almost two years on Peg-IFN with no requirement for EPO-stimulating agents. Prior to starting Peg-IFN, bands and metamyelocytes were noted in the peripheral blood. There was progressive left shift after starting Peg-IFN, including 1% blasts at 6 months of treatment, which resolved after 12 months of therapy (Figure 2). LDH was elevated to 478 units/L but had been stable for three months before starting Peg-IFN. Following Peg-IFN, LDH rose to a peak of 637 units/L after five months of treatment then normalized by 18 months of treatment (Figure 1). She had a decrease in spleen size to 2.5 cm below the costal margin. A repeat bone marrow biopsy performed after 1 year of therapy demonstrated stable reticulin fibrosis (Table 3). The frequency of Peg-IFN was reduced after 18 months of treatment to 90 mcg every three weeks with no drug-related toxicity, excellent platelet control and stable hemoglobin. A third bone marrow biopsy performed after 2 years of Peg-IFN revealed improvement in reticulin fibrosis (Table 3).

**Case 5**

Patient 5 is a 68 year-old Caucasian female with JAK2-positive PV diagnosed 12 years prior to starting Peg-IFN. She was maintained on hydroxyurea and periodic phlebotomies with adequate control of her blood counts. She had a drop in hemoglobin from 12.6 g/dL to 9.9 g/dL during the three months prior to starting Peg-IFN and a spleen tip was palpable on physical exam. A bone marrow biopsy was then performed and revealed a markedly hypercellular (80%) bone marrow with no increased blasts and mild reticulin fibrosis. She was started on 45 mcg of pegylated interferon in the setting of increased fibrosis and inability to tolerate hydroxyurea due to anemia.

Her hemoglobin improved to 15.7 g/dL within 4 months of starting Peg-IFN and she began requiring monthly phlebotomy. A left-shift was noted in the peripheral blood after 6 months of treatment, which resolved after 12 months (Figure 2). LDH was increased at 330 units/L prior to Peg-IFN but had been stable for two months. Within 3 months of treatment, LDH had increased to a peak of 528 units/L with a subsequent decrease to normal levels after 12 months of Peg-IFN (Figure 1). She had progressive
splenomegaly after starting Peg-IFN to a maximum span of 5 cm below the costal margin at 6 months of treatment, which resolved by 15 months of treatment. Repeat bone marrow biopsy performed after 15 months of treatment with Peg-IFN showed a moderately hypercellular marrow (70%), stable reticulin fibrosis, and normal cytogenetics (Table 3). She is in her third year of therapy with 180 mcg of weekly Peg-IFN with no dose-limiting toxicities, normal white blood cell and platelet counts, and monthly phlebotomy.

Case 6

Patient 6 is a 73 year-old Caucasian male with JAK2-positive ET diagnosed 13 years prior to starting Peg-IFN. He was started on anagrelide with effective platelet control for several years. Nine years after diagnosis, there was concern for progression to myelofibrosis in the setting of anemia, progressive splenomegaly and poikilocytosis. Bone marrow biopsy revealed a markedly hypercellular marrow (90%) with increased reticulin fibrosis. He was switched to hydroxyurea with reduction of splenomegaly, stabilization of his hemoglobin, and good platelet control for four years. He again developed splenomegaly and anemia and a repeat bone marrow was performed, which was normocellular (60%) with trilineage hyperplasia and abnormal megakaryocyte clusters, 5% blasts, and mild reticulin fibrosis (Table 3). Cytogenetics revealed clonal abnormalities in three of the 27 cells analyzed, and these included deletion of the short arm of 17 and deletion of the long arm of chromosome 20. One cell had both deletions and two cells had one of either. Peg-IFN was initiated at a dose of 45 mcg.

His hemoglobin subsequently dropped from 13.2 g/dL to 10.8 g/dL. Immature granulocytes were noted on labs within one month of starting Peg-IFN with a concurrent increase in LDH from 468 units/L to a peak of 743 units/L (Figures 1 and 2). He experienced fatigue, weight loss, and mild depression and therefore Peg-IFN was stopped after two months. Splenomegaly improved from 7 cm to 4.5 cm while on interferon. His hemoglobin improved to above 13 g/dL after stopping Peg-IFN. Immature granulocytes disappeared from the peripheral blood and LDH returned to pre-interferon levels. A bone marrow biopsy performed almost one year after starting Peg-IFN was normocellular (50%) with trilineage hematopoiesis, clustered atypical megakaryocytes, blasts less than 5%, and stable reticulin fibrosis (Table 3). Cytogenetics revealed clonal abnormalities in 10 of 20 cells analyzed with detection of three unrelated clonal populations however the deletion of 17q previously identified had disappeared. He is currently being managed on hydroxyurea and his hemoglobin has remained above 13 g/dL.