months of treatment due to intolerance. Among the 31 patients who received PEG-IFN for >3 months, the median dose per cycle was 4.0 μg/kg (range: 1.5-5.9) and the median maximum dose was 5.0 μg/kg (range: 1.7-6.0), with only 15 patients reaching the 6.0 μg/kg dose. Nineteen patients did not receive Ara-C (early withdrawal, n=4; PEG-IFN hematologic toxicity, n=15). In the remainder, the median number of Ara-C cycles was 2 (range: 1-10).

Of the 22 patients not in complete hematologic response (CHR) at the start of treatment, 16 (73%) achieved a CHR by a median of 3 weeks (range: 2-12). In the 22 evaluable patients, the best observed response was a MCR in 32%, including 14% who had a CCR. Extra-hematologic toxicity is shown in Table 2. One patient developed fever, chills and muscle and bone pain at the time of the first PEG-IFN dose (6.0 μg/kg). This patient was managed with hydroxyurea for three months, and then PEG-IFN (2.0 μg/kg) was reintroduced. Immediately after, he developed fever, acute respiratory distress and lung infiltrates. Microbiological studies were negative and, despite standard measures, the patient died. Grade II-IV neutropenia was recorded in 20% of cases but only one patient required hospital admission because of infection.

With a median follow-up of 17.2 months (range: 3.7-24.8), two patients died (PEG-IFN toxicity and transplant complications, one case each), one remains on PEG-IFN and maintains a CCR at 19 months, two switched to regular IFN and attained CCR, four received a transplantation and one switched to hydroxyurea. The remaining 26 were converted to imatinib; 19 of 22 assessed for response achieved a CCR. Talpaz *et al.* reported the results of PEG-IFN treatment in 27 patients with chronic or accelerated phase CML resistant/intolerant to IFN.[1] The starting dose was 0.75 μg/kg/week and the recommended weekly dose 6.0 μg/kg, since higher doses involved substantial hematologic and extra-hematologic toxicity. Treatment was effective, especially in patients already in CHR. Garcia-Manero *et al.*[6] administered PEG-IFN plus Ara-C to 76 patients with early chronic phase CML. The starting dose was initially 6.0 μg/kg but, due to toxicity, the starting dose was then reduced to 4.5 μg/kg. CHR was obtained in 73% of cases and MCR in 56%, including 21% CCR. It was recommeded that the PEG-IFN dose be decreased to 3.0 μg/kg or less. Michallet *et al.*,[7] in a randomized study of 344 newly-diagnosed patients, compared PEG-IFN α-2b and standard IFN, reporting 23% and 21% MCR, respectively, at one year, with similar toxicity. The results of our study are comparable, although we found more toxicity. The preliminary results of two studies of imatinib plus PEG-IFN in newly-diagnosed CML indicate the efficacy of this combination but also its substantial toxicity.[4,5]

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Chronic Myeloproliferative Disorders

FIP1L1-PDGFRA and *c-kit* D816V mutation-based clonality studies in systemic mast cell disease associated with eosinophilia

Laboratory methods to detect both *FIP1L1-PDGFRA* and *c-kit* D816V mutations were combined with immunomagnetic cell separation to study the extent of clonal involvement by both myeloid and lymphoid cells in 3 patients with systemic mastocytosis associated with eosinophilia. The results suggested an early stem cell origin for the *FIP1L1-PDGFRA* mutation.

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Certain mutations in either the stem cell (*c-kit*) or platelet-derived growth factor receptor (*PDGFRA*) genes have been pathogenetically linked to systemic mast cell disease (SMCD) (e.g. *c-kit* D816V, *FIP1L1-PDGFRA*).[1,2] While *FIP1L1-PDGFRA*+ SMCD is invariably associated with prominent blood eosinophilia,[2] the D816V *c-kit* mutation has been demonstrated in both the presence[2] and absence[3] of associated eosinophilia. The availability of highly reliable laboratory assays to detect these specific mutations has offered the opportunity for lineage-specific clonal studies in SMCD. Accordingly, *c-kit* D816V mutation-based clonality studies have previously demonstrated clonal involvement of both myeloid (monocytes, neutrophils, eosinophils, erythrocytes) and lymphoid cells (B or T lymphocytes) in both indolent and aggressive SMCD.[4-8] In the current study, we used immunomagnetic bead cell separation techniques in combination with either fluorescence *in situ* hybridization (FISH) or polymerase chain reaction (PCR)-based DNA sequencing to study the extent of clonal involvement in various cell types of myeloid as well as lymphoid lineage in both *FIP1L1-PDGFRA*+ and *c-kit*

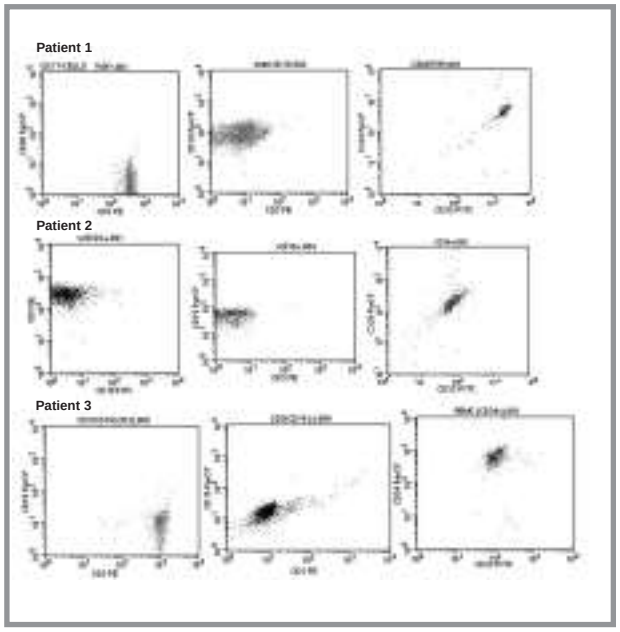

Figure 1. Immunophenotypic distribution of purified T (CD3⁺), B (CD19⁺), and myeloid progenitor (CD34⁺) cell fractions in 3 patients with systemic mast cell disease associated with eosinophilia.

D816V⁺ SMCD with eosinophilia (SMCD-eos). Double density gradient centrifugation (Histopaque-1077™ layered over Histopaque-1119™, Sigma Diagnostics, St. Louis, MO, USA) was used to separate out the granulocyte and mononuclear cell layers from each sample. The mononuclear cell layer was further fractionated by magnetic cell sorting (MACS; Miltenyi Biotech, Auburn, CA, USA) using antibodies that are specific to myeloid progenitor cells (CD34⁺) as well as both T (CD3⁺) and B (CD19⁺) lymphocytes. The granulocyte cell layer was also fractionated further using MACS Automated Cell Separation by the incubation of an antibody/magnetic bead complex specific for neutrophils (CD16). Suspensions were processed according to the manufacturer's protocol and both the positive and negative fractions were kept; CD16⁺ for purified neutrophils and CD16⁻ for eosinophils. The cell purity of the various fractions was analyzed using a FACScalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and, for the eosinophil and neutrophil fractions, further confirmed by morphologic evaluation of a Wright-Giemsa stained smear. The presence of *FIP1L1-PDGFRA* was indirectly assessed by using probes covering the *CHIC2* locus at chromosome 4q12, as previously described.[2] It is to be recalled that the *FIP1L1-PDGFRA* fusion results from an interstitial deletion on chromosome 4q12 and the *CHIC2* gene is located within the deleted region.[9] Previously published methods were also used to detect *c-kit* D816V mutation from genomic DNA.[3]

The three study patients were 2 males (patients 1 and 3; both with the *FIP1L1-PDGFRA* mutation) and a female (patient 2) with the *c-kit* D816V mutation; their respective ages were 49, 51, and 80 years. Lineage-specific clonal studies were performed in each cell fraction in which sample purity was demonstrated to be > 98%, as depicted in Figure 1 for T (CD3⁺), B (CD19⁺) and myeloid progenitor (CD34⁺) cells. The results of the FISH-based CHIC2 deletion (a surrogate for *FIP1L1-PDGFRA*) and PCR-based *c-kit* D816V studies are illustrated in Table 1. The findings clearly establish a major clonal presence in eosinophils among all 3 study patients, which confirms our earlier observations in this regard involving both *FIP1L1-PDGFRA*⁺ and *c-kit* D816V⁺ patients with SMCD-eos.[2,8] Although to a lesser degree, both neutrophils and CD34⁺ cells were also clonally involved in all three patients. In contrast, only one patient (*FIP1L1-PDGFRA*⁺ SMCD) showed evidence of clonal lymphoid involvement with the specific mutation being detected in T lymphocytes (Table 1).

Table 1. Detection of either *c-kit* D816V or *FIP1L1-PDGFRA* mutation in 3 informative patients with systemic mast cell disease associated with eosinophilia.

pt	*Mutation*	*Eosinophil*	*Neutrophil*	*CD34⁺*	*CD3⁺*	*CD19⁺*
1	CHIC2-	22.5%	6%	27%	8.5%	3.5%
2	D816V	+	+	+	–	–
3	CHIC2-	79%	16%	8%	1%	3%

Percentages represent the proportion of purified cell populations with deletion of the CHIC2 locus, a surrogate for FIP1L1-PDGFRA. CD34, myeloid progenitor cells; CD3, T lymphocyte; CD19, B lymphocyte. The median abnormal signal percentage in 25 controls was 0% (range 0-2%).

With regard to *c-kit* D816V⁺ SMCD, previous studies had demonstrated clonal involvement by monocytes, erythrocytes, B cells, T cells, and bone marrow mast cells.[4–7] Because the current study included only one patient with *c-kit* D816V⁺ SMCD, it would be inappropriate to consider the possibility of potentially conflicting results between this study and those previously published in regards to the extent of clonal involvement by either B or T lymphocytes.[4,5] On the other hand, the observations from the current study support the early stem cell origin of the *FIP1L1-PDGFRA* mutation and provide additional evidence regarding the clonal inclusion of T cells in various myeloproliferative disorders[10]

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Malignant Lymphomas

Detection of *bcl-2* rearrangement in mucosa-associated lymphoid tissue lymphomas from patients with hepatitis C virus infection

It has been shown that t(14;18)(q32;q21) involving fusion of *IGH* with *MALT1* occurs frequently in mucosa-associated lymphoid tissue (*MALT*) lymphomas. Results of the present study indicate that the classical form of t(14;18)(q32;q21) involving fusion of *IGH* with *bcl-2* can be detectable in a subset of MALT lymphomas in patients with hepatitis C virus (HCV) infection.

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Extranodal marginal zone B-cell lymphomas of mucosa-associated lymphoid tissue (MALT lymphomas) comprise approximately 8% of non-Hodgkin's lymphomas (NHL) and are among the 6 most common forms of NHL. MALT lymphomas usually result from chronic stimulation of B-cells by persistent infection or autoimmune processes.[1] Among the genetic abnormalities associated with MALT lymphomas, t(11;18)(q21;q21) is the most common. This translocation fuses apoptosis inhibitor-2 (*API2*) with MALT lymphoma-associated translocation gene-1 (*MALT1*).[1] However, Streubel *et al.* showed that t(14;18)(q32;q21) involving fusion of the immunoglobulin heavy chain gene (*IGH*) with *MALT1* also occurs in MALT lymphomas.[2] This finding was supported by recent reports from Sanchez-Izquierdo *et al.* and Murga Penas *et al.*[3,4] The translocation described by these 3 groups was detected most frequently in MALT lymphomas of the conjunctiva, liver, skin, parotid gland, and salivary gland. Interestingly, all of these anatomical sites are susceptible to hepatitis C virus (HCV) infection.[5]

Chronic antigenic stimulation of B-cells resulting from HCV infection has been suggested to contribute to the development of lymphoproliferative disorders.[6] Previous studies have also suggested a role for the *bcl-2* rearrangement during development of lymphoproliferative disorders among HCV-infected individuals.[7] However, the incidence of the classical form of t(14;18)(q32;q21) involving fusion of *IGH* with *bcl-2* has not been investigated in MALT lymphoma patients. In the present study, MALT lymphoma tissue from 11 HCV-infected and 9 HCV-negative patients was analyzed for the presence of this translocation (Table 1). DNA isolated from MALT lymphoma biopsy specimens was analyzed for the presence of *bcl-2* rearrangement at the major (MBR) and minor breakpoint regions (mcr) by polymerase chain reaction (PCR) as previously described.[8] The MALT lymphoma biopsy specimens were collected from the primary site of disease.

Rearrangement of *bcl-2* was detected in 5 of 20 (25%) MALT lymphoma biopsy specimens after both the first and second rounds of amplification. Control experiments were performed to verify that the methods used to test for *bcl-2* rearrangement were reliable. Tumor biopsy specimens from 15 of 15 HCV-negative follicular lymphoma (FL) patients were positive for *bcl-2* rearrangement. Of these translocations, 13 occurred at the MBR and 2 occurred at the mcr. In contrast, *bcl-2* rearrangement was not detected in peripheral blood mononuclear cell from 50 healthy HCV-negative donors. These positive and negative control experiments demonstrate that the methods used to test for bcl-2 rearrangement reliably detected the genetic abnormality without yielding false positive results.

MALT lymphoma biopsy specimens were also analyzed for expression of Bcl-2, Bcl-6, and CD10 by immunohistochemistry. All MALT lymphoma biopsy specimens tested were negative for both Bcl-6 and CD10 expression, which excludes the possibility that these tumors were of follicular origin. Bcl-2 expression was analyzed in MALT lymphoma biopsy specimens from 3 of 5 patients positive for the *bcl-2 r*earrangement and from 12 of 15 patients negative for the *bcl-2* rearrangement. Bcl-2 expression was detected in 3 of 3 patients positive for the *bcl-2* rearrangement, a result consistent with those of previous studies correlating Bcl-2 expression with the *bcl-2* rearrangement.[9] Bcl-2 was expressed in 8 of 12 MALT lymphoma biopsy specimens neg-

Table 1. Clinical characteristics and *bcl-2* rearrangement status of the MALT lymphoma patients studied.

Patient	*Age/ Sex*	*Sites of Disease*	*HCV Status*	*MC*	*bcl-2 Rearr.*
1	65/F	Stomach, BM	+	+	+
2	66/F	Skin	+	+	–
3	59/M	Liver, BM	+	+	+
4	66/F	Stomach	+	+	–
5	55/F	Salivary gland, BM	+	+	–
6	69/F	Salivary gland, BM	+	–	+
7	51/F	Skin, BM	+	–	–
8	79/M	Salivary gland, stomach	+	–	–
9	72/F	Stomach	+	–	+
10	57/F	Salivary gland, BM	+	–	–
11	60/F	Lung, BM	+	–	–
12	38/M	Ocular adnexa, BM	–	–	–
13	70/F	Stomach, BM	–	–	+
14	69/F	Stomach, spleen	–	–	–
15	64/M	Fatty renal capsule, BM	–	–	–
16	61/F	Stomach, BM	–	–	–
17	65/F	Stomach, BM	–	–	–
18	60/F	Stomach, spleen, BM	–	–	–
19	71/M	Stomach, BM	–	–	–
20	67/M	Stomach, BM	–	–	–

Rearr.: rearrangement; BM: bone marrow; F: female; M: male; MC: type II mixed cryoglobulinemia syndrome.