

A distinct biomolecular profile identifies monoclonal mast cell disorders in patients with idiopathic anaphylaxis

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Background: Clonal mast cell disorders are known to occur in a subset of patients with systemic reactions to Hymenoptera stings. This observation has prompted the question of whether clonal mast cell disorders also occur in patients with idiopathic anaphylaxis (IA).

Objective: We sought to determine the prevalence of clonal mast cell disorders among patients with IA, criteria to identify those patients who require a bone marrow biopsy, and whether the pathogenesis of IA involves a hyperresponsive mast cell compartment.

Methods: We prospectively enrolled patients with IA (≥ 3 episodes/y) who then underwent a medical evaluation that included a serum tryptase determination, allele-specific quantitative PCR (ASqPCR) for the *KIT* D816V mutation, and a bone marrow examination. Mast cells were cultured from peripheral blood CD34⁺ cells and examined for releasability after Fc ϵ RI aggregation.

Results: Clonal mast cell disease was diagnosed in 14% of patients referred with IA. ASqPCR for the *KIT* D816V mutation was a useful adjunct in helping identify those with systemic mastocytosis but not monoclonal mast cell activation syndrome. A modified overall clonal prediction model was developed by using clinical findings, a serum tryptase determination, and ASqPCR. There was no evidence of a hyperresponsive mast cell phenotype in patients with IA.

Conclusion: Patients with clonal mast cell disease can present as having IA. Distinct clinical and laboratory features can be used to select those patients more likely to have an underlying clonal mast cell disorder (monoclonal mast cell activation syndrome or systemic mastocytosis) and thus candidates for a bone marrow biopsy. (*J Allergy Clin Immunol* 2017;■■■:■■■-■■■.)

Key words: Anaphylaxis, mast cells, mastocytosis, monoclonal mast cell activation syndrome, mast cell activation, tryptase, *KIT*, allele-specific quantitative PCR

Idiopathic anaphylaxis (IA) is a diagnosis of exclusion and is based on the inability to identify a cause-and-effect relationship for a given anaphylactic event. Once the diagnosis is made, an associated question is whether there is an underlying clonal disorder, such as systemic mastocytosis (SM). Patients with a diagnosis of SM are reported to have an increased prevalence of anaphylaxis,¹ both unexplained and associated with Hymenoptera sensitivity.²⁻⁴ Although it is also known that some patients with IA have an underlying clonal mast cell disease,⁵ the frequency of the association between IA and clonal mast cell disease and how to identify patients with IA who should undergo a marrow examination to confirm this diagnosis has not been thoroughly explored.

Two studies have attempted to define characteristics that identify patients with severe mast cell mediator-related disease who are likely to have a clonal mast cell disorder.^{4,6} The first study examined patients presenting with severe mast cell mediator-related symptoms and compared the characteristics of this group with those of patients with a known diagnosis of indolent systemic mastocytosis (ISM) to identify clinical and biological characteristics that would predict those patients who would be more likely to have a clonal mast cell disorder and thus need a marrow examination.⁶ A second study retrospectively examined the records of patients with clinically suspected clonal mast cell disease based on 1 or more episodes of unexplained anaphylaxis who had a bone marrow examination. Based on their observations, they then suggested modifications to the previous scoring system.⁴

These reports led us to design a prospective study to determine the prevalence of clonal mast cell disorders in patients experiencing frequent episodes of IA without clinical evidence of mastocytosis, such as maculopapular cutaneous mastocytosis. Patients underwent a clinical evaluation, which included analysis for the *KIT* D816V mutation and a bone marrow biopsy. As an additional objective, we examined the bone marrow mast cell compartment *in vivo* and mast cells cultured from peripheral blood to determine whether there was evidence of a hyperresponsive mast cell phenotype in patients with IA. As will be shown, approximately 1 in 7 patients with IA had a clonal mast cell disorder. We found no evidence of a hyperresponsive mast cell phenotype. We also present a modified scoring system with increased specificity and sensitivity for identification of patients with recurrent IA who are candidates for bone marrow biopsy.

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Abbreviations used

ASqPCR: Allele-specific quantitative PCR
 bST: Baseline serum tryptase
 gDNA: Genomic DNA
 HV: Healthy volunteer
 IA: Idiopathic anaphylaxis
 ISM: Indolent systemic mastocytosis
 MMAS: Monoclonal mast cell activation syndrome
 NIH: National Institutes of Health
 qPCR: Quantitative PCR
 REMA: Spanish Network on Mastocytosis
 SM: Systemic mastocytosis

METHODS**Subjects**

Fifty-six subjects (aged 13–69 years) with a diagnosis of IA defined by current guidelines^{7,8} and meeting protocol entry requirements (see Table E1 in this article's Online Repository at www.jacionline.org) were enrolled from 22 states and 1 Canadian province (see Fig E1 in this article's Online Repository at www.jacionline.org) over a study period of 6 years on an institutional review board–approved National Institutes of Health (NIH) protocol (NCT00719719) for further evaluation. Anaphylaxis was diagnosed by the referral physician using criteria from the summary report on anaphylaxis.⁹ Patients had to experience 3 or more episodes of unexplained anaphylaxis within 12 months of study entry, at least 1 episode in the past 4 months, and at least 1 event evaluated in a medical facility in proximity to the episode where the diagnosis of anaphylaxis was confirmed by documenting hypotension and other physical findings.⁹ The median age of patients enrolled was 43 years. Thirty-seven (66.1%) were female, and 19 (33.9%) were male. The majority of patients were white (93%). On enrollment to the NIH protocol, all patients underwent a complete physical examination, with serum IgE and baseline serum tryptase (bST) levels measured and a bone marrow aspiration and biopsy performed. Healthy volunteers (HVs) for mast cell comparison studies were enrolled in an institutional review board–approved protocol (NCT00806364). All subjects provided informed consent before enrollment.

Laboratory studies**Serum tryptase, IgE, and venom-specific IgE levels.**

bST levels were determined by using a fluoroenzyme Immunoassay (ImmunoCAP; Phadia, Uppsala, Sweden) at Clinical Laboratory Improvement Amendments–approved laboratories. The normal reference range for this assay is 0.00 to 11.50 ng/mL. The serum IgE level was determined by using the Immulite XPI solid-phase chemiluminescence assay (Siemens Medical Solutions, Malvern, Pa). IgE levels to honeybee and yellow jacket venom were screened by using a fluoroenzyme immunoassay (Phadia ImmunoCAP) with a detection range of 0.35 to greater than 100 IU/mL in Dr Platts-Mills' laboratory, where values of greater than 0.35 IU/mL are considered positive.

Allele-specific quantitative PCR on peripheral blood.

Genomic DNA (gDNA) was prepared from 200 μ L of blood collected in EDTA from 37 patients and extracted with a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) in a volume of 100 μ L of elution buffer. Genomic DNA from HMC1.2 cells¹⁰ was used as the *KIT* D816V mutation positive control. gDNA from peripheral blood of an HV was used as a negative control. The concentration of each DNA sample was determined by using the NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Mass).

Both mutation-specific and control real-time quantitative PCR (qPCR) assays for *KIT* D816V mutations were performed for each sample in the same plate by using the TaqMan Universal PCR Master Mix with AmpErase UNG on the 7500 Real Time PCR System (Applied Biosystems, Foster City, Calif), as previously described.¹¹ Each real-time qPCR reaction was performed in triplicate with 50 ng of gDNA in a total volume of 25 μ L. Results were analyzed with SDS software (version 1.3.1; Applied Biosystems, Grand Island, NY). Samples with 2 of 3 or 3 of 3 analyses generating a cycle threshold

value of less than 41 were defined as mutation positive. Mutation-negative samples had negative test results, with 0 of 3 reactions producing a cycle threshold value of less than 44.¹² The percentage of cells carrying the *KIT* D816V allele among total nucleated blood cells was then calculated, as previously described.¹¹

Bone marrow examination

Bone marrow trephine biopsy specimens were fixed in B-5 fixative, embedded in paraffin, and processed for morphology by using standard procedures.¹³ Immunohistochemical studies with anti-tryptase, anti-CD117 (Cell Marque, Hot Springs, Ark), and anti-CD25 (Vision BioSystems, Norwell, Mass) antibodies were performed by using immunoperoxidase staining and an automated immunostainer (Ventana Medical System, Tucson, Ariz) per the manufacturer's instructions. Images were obtained with an Olympus DP72 digital camera (Canto II; BD Biosciences, San Jose, Calif). Bone marrow biopsy specimens were inspected and scored in a blind fashion by a single pathologist (I.M.).

Multiparameter flow cytometry was performed after bone marrow aspirates were processed and stained with antibodies for CD2, CD25, CD45, and CD117, as previously described.¹³ Activation markers were identified with additional antibodies for CD11c, CD35, CD59, CD63, CD69, and CD203c. The DIVA and FCS Express (De Novo software, Glendale, Calif) programs were used for data analysis.¹³

Bone marrow mononuclear cells were obtained from the Ficoll gradient in PBS. CD25⁺ cells were magnetically labeled with CD25 Microbeads II (Miltenyi Biotec, San Diego, Calif), and then the cell suspension was loaded onto a MACS column and placed in the magnetic field of the MACS separator. The labeled CD25⁺ cells were retained on the column and eluted as the positively selected cell fraction.

Detection of the *KIT* D816V mutation was performed by using RT-PCR/restriction fragment length polymorphism. The targeted gene region was amplified, and the PCR products were subjected to restriction enzymes to obtain fragments for analysis, as previously described.¹³

Mast cell culture and activation

Peripheral blood–derived CD34⁺ mast cells were cultured from 100 mL of blood obtained from patients and HVs, as previously described.¹⁴ Briefly, mononuclear cells were separated by means of density gradient centrifugation, and a human progenitor cell enrichment kit was used to obtain CD34⁺ cells by using negative selection per the manufacturer's guidelines (STEMCELL Technologies, Vancouver, British Columbia, Canada). Cells were then cultured in StemPro-34 Media (Life Technologies, Grand Island, NY) with 100 ng/mL recombinant stem cell factor and 100 ng/mL recombinant IL-6 (PeproTech, Rocky Hill, NJ) for 7 weeks, with 30 ng/mL recombinant IL-3 added during the first week only.¹⁴ The number of cells in culture was determined at 6 weeks and normalized to the percentage of CD34⁺ cells measured at the start of culture. At 6 to 7 weeks, peripheral blood–derived CD34⁺ mast cells were sensitized overnight in media containing biotinylated anti-IgE (100 ng/mL). After sensitization, the cells were activated by cross-linking with streptavidin (Sigma-Aldrich, St Louis, Mo), and percentage release of β -hexosaminidase was calculated.¹⁵

Statistics

bST values were compared by using the Student *t* test. A Wilcoxon rank sum test was used to compare absolute bone marrow mast cell values between different disease categories and control subjects. Mann-Whitney nonparametric analysis was used to compare surface antigens between the IA, monoclonal mast cell activation syndrome (MMAS), ISM, and HV groups. Scatterplots illustrate relationships between disease categories, tryptase levels, bone marrow mast cells, and IgE levels. Corresponding Spearman rank correlation coefficients were computed. Statistical graphics and analysis were performed with Prism software (6th ed; GraphPad Software, La Jolla, Calif).

TABLE I. Final diagnosis among patients presenting with IA

Diagnosis	MC aggregates, >15 MCs	Spindle-shaped, >25% MCs	CD25 ⁺ MCs	bST, >20 ng/mL	BM <i>KIT</i> D816V	Blood <i>KIT</i> D816V†
IA (n = 48)	0/48	0/48	0/48	1/48	0/48	0/29
MMAS (n = 4)*	0/4	2/4	1/4	2/4	3/4	0/4
ISM (n = 4)	3/4	4/4	4/4	2/4	4/4	3/4

BM, Bone marrow; MC, mast cell.

*There were 2 positive results for the bone marrow D816V mutation in CD25-enriched cells only.

†Blood *KIT* D816V allele-specific PCR was performed on 37 patients.

RESULTS

Diagnosis of clonal mast cell disease

All 56 patients underwent an evaluation, including a bone marrow examination with flow cytometry and *KIT* mutational analysis, to determine the presence or absence of an underlying clonal mast cell disorder. Four patients were given a diagnosis of ISM and 4 were given a diagnosis of MMAS based on existing criteria (Table I),^{16,17} leaving 48 patients with IA who had no evidence of a clonal disorder.

Marrow findings in Fig 1 show the spectra of marrow findings in patients referred with the diagnosis of IA. Fig 1, A, shows results of a representative marrow biopsy and flow cytometric analysis for CD25 from a patient with IA who did not meet the criteria for a clonal mast cell disorder. All marrow mast cells are CD25[−], and results of marrow *KIT* D816V mutation analysis were negative. The patient had increased bST levels (29.4 ng/mL). In Fig 1, B, findings are shown for a patient given a diagnosis of MMAS (bST, 16.7 ng/mL). This patient's marrow exhibited both CD25⁺ and CD25[−] mast cell populations and was positive for the D816V mutation in the enriched CD25⁺ mononuclear cell population. The patient whose marrow results are shown in Fig 1, C, had a bST of 88.7 ng/mL and met both the major and minor criteria for the diagnosis of ISM.

Of interest, results of peripheral blood analysis for the presence of the *KIT* D816V mutation were consistently negative in patients given a diagnosis of IA alone (100%) and positive in patients with ISM (allelic burden range, 0.037% to 1.19%), who showed mast cell aggregates of more than 15 mast cells on the bone marrow biopsy (100%). However, results of peripheral blood allele-specific quantitative PCR (ASqPCR) were negative in all patients with MMAS and in 1 patient with ISM who had no marrow aggregates, the major criterion for the diagnosis of SM. Thus, based on this test alone, the negative and positive predictive values for ASqPCR to diagnose ISM are 90.74% and 100%, respectively, in the population of patients with IA.

As shown in Table II, patients with IA (no evidence of a clonal mast cell disorder) were more likely to be female, but age did not differ among the 3 groups. IgE levels were lowest in patients with ISM, but median values were not significantly different between groups. bST levels were significantly higher in patients with MMAS and ISM compared with those in patients with IA ($P < .0001$). On average, patients with IA had more frequent episodes of anaphylaxis per year and a longer duration of disease compared with patients with clonal disorders. Only 1 patient with IA and none with clonal disease reported clinical symptoms to Hymenoptera. Thus venom sensitization was not a distinguishing feature of clonal mast cell disease in this population.

Examination of the mast cell compartment *in vivo* and *in vitro*

We next examined the characteristics of the bone marrow mast cell compartment in patients with IA, MMAS, and ISM versus findings in HVs. The absolute mast cell count was similar between patients with IA and HVs, whereas the mast cell count was greater in patients with MMAS and ISM (Table III). In addition, CD25, CD2, CD11c, CD59, CD69, and CD35 mean fluorescence intensity ratios, as expected, were increased in patients with ISM¹⁸ but not in patients with IA or MMAS. As expected, we found overexpression of CD63 on mast cells in the marrow of patients with MMAS and ISM and CD203C on mast cells in patients with ISM but not in patients with IA and HVs (Fig 2, A and B). Thus, an assessment using immunophenotypic markers of mast cells in marrow provided no evidence that the mast cell compartment in patients with IA differed from what was observed in HVs. We were unable to obtain sufficient mast cells from marrow aspirates of patients with IA or from HVs to examine their activation through the IgE and C5a receptors, as has been reported in a study on bone marrow mast cells from patients with clonal disease.¹⁹

Mast cells were cultured from 48 patients with IA, 4 patients with MMAS, and 4 patients with ISM to further examine the mast cell compartment. Each culture was followed for mast cell growth, and at 6 to 7 weeks, IgE-mediated degranulation was determined in comparison with mast cell culture from an HV performed in parallel. Mast cell growth from patients with MMAS and HVs was similar, whereas it was statistically greater in patients with IA and ISM (Fig 2, C). IgE-mediated mast cell degranulation, as assessed by β -hexosaminidase release, was similar among all groups (Fig 2, D). Hence we found no evidence that mast cells cultured from patients with IA were intrinsically more reactive.

Tryptase determinations in association with anaphylaxis and over time

We were able to obtain serum tryptase levels in 13 patients at baseline and within 4 hours after an episode of anaphylaxis. All patients had a significant increase in serum tryptase levels¹⁷ associated with an anaphylactic episode (Fig 3, A and B). Patients with a clonal disorder had a greater increase in serum tryptase levels after the event when compared with patients with IA alone (average, 502 vs 25 ng/mL). We obtained sequential bST levels in 35 patients. Tryptase levels in patients with IA tended to remain stable, whereas patients with clonal mast cell disorders trended upward (Fig 3, C and D).

Predictors of clonal mast cell disorders

Two groups have proposed scoring systems termed either the Spanish Network on Mastocytosis (REMA) score or the modified

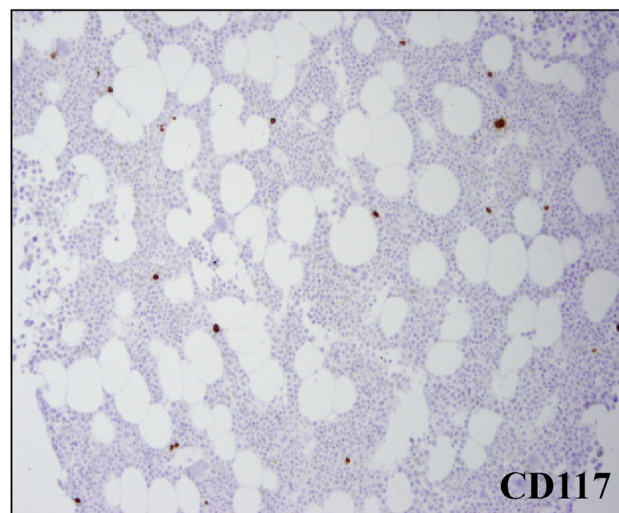
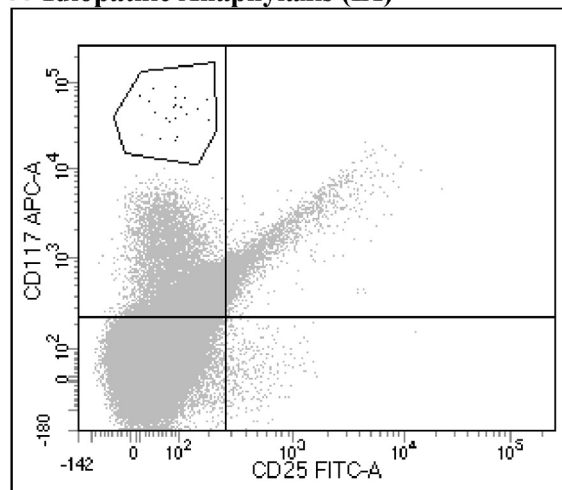
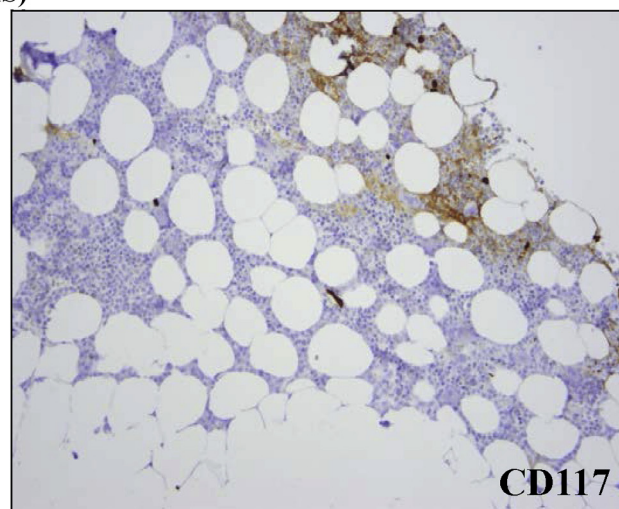
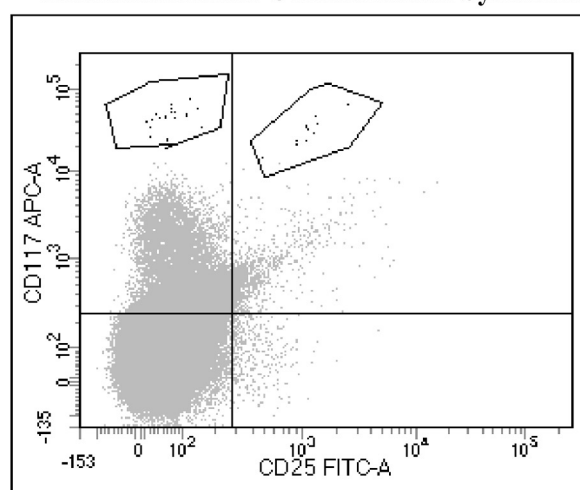
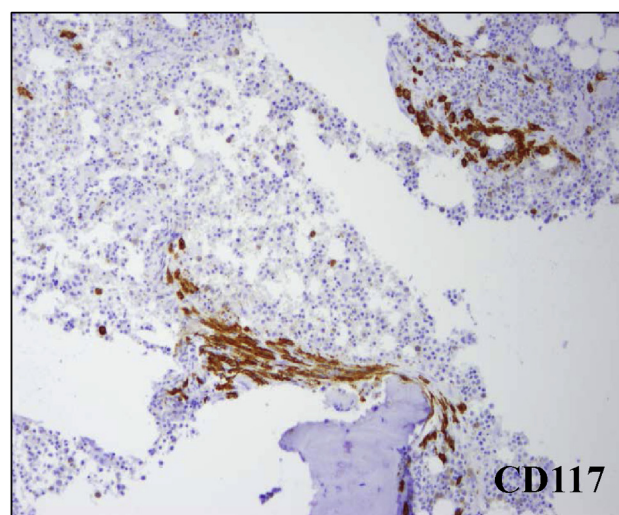
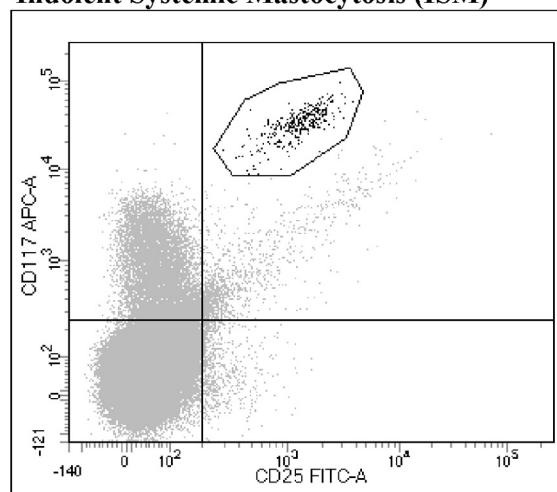
A Idiopathic Anaphylaxis (IA)**B Monoclonal Mast Cell Activation Syndrome (MMAS)****C Indolent Systemic Mastocytosis (ISM)**

FIG 1. Bone marrow mast cell flow cytometry and immunohistochemistry in patients with IA (A), patients with MMAS (B), and patients with ISM (C). Flow cytometric results show that CD117⁺ mast cells gain CD25 surface expression in patients with clonal disease, as shown by no abnormal CD25⁺ mast cells in patients with IA (Fig 1, A), 2 populations of mast cells in patients with MMAS (CD25⁺ and CD25⁻ cells; Fig 1, B), and abnormal CD25⁺ mast cells in patients with ISM (Fig 1, C). Bone marrow biopsy specimens showed no mast cell clusters in patients with IA and those with MMAS, with multiple clusters in patients with ISM. APC, Allophycocyanin; FITC, fluorescein isothiocyanate.

TABLE II. Patients' characteristics

Final diagnosis	Male/female	Age (y), median (range)	Serum IgE (IU/mL), median (range)	bST (ng/mL), median (range)	Anaphylaxis frequency number/y, average (range)	Anaphylaxis duration (y), average (range)	Venom sensitivity by clinical history*
IA	14/34	43.0 (13-69)	97.5 (5.1-2457)	4.1 (1.2-29.6)	9 (3-24)	7 (1-34)	1/48
MMAS	3/1	48.5 (40-53)	87.6 (31.8-128)	18.4 (3.1-32.4)	6 (5-8)	4 (1-7)	0/4
ISM	2/2	35.5 (17-58)	40.8 (21.2-805)	18.6 (3.7-88.7)	5 (3-9)	3 (1-8)	0/4

P values for serum bST levels for patients with MMAS and those with ISM compared with patients with IA were .0001 for both.

*All patients underwent ImmunoCAP for IgE to honeybee and yellow jacket venoms. Seven of 48 patients with IA, 1 of 4 patients with MMAS, and 1 of 4 patients with MMAS had positive results.

TABLE III. Immunophenotypic characteristics of bone marrow mast cells

Diagnosis	Mast cell absolute count*	CD25 MFI ratio (range)	CD2 MFI ratio (range)	CD11c MFI ratio (range)	CD35 MFI ratio (range)	CD59 MFI ratio (range)	CD63 MFI ratio (range)	CD69 MFI ratio (range)	CD203c MFI ratio (range)
Patients with IA	10.50 (4-65) n = 44	1.02 (0.05-2.2) n = 44	0.79 (0.08-3.7) n = 43	5.09 (0.35-25.3) n = 39	1.14 (0.02-14) n = 42	29.09 (1.71-70.8) n = 42	7.54 (0.21-24.9) n = 41	2.63 (0.0-10.7) n = 40	11.13 (0.40-49.2) n = 40
Patients with MMAS†	25.50 (16-142) n = 4	1.26 (0.53-7.8) n = 4	1.16 (0.61-137.8) n = 4	5.45 (2.46-11.3) n = 3	0.98 (0.57-2.5) n = 3	35.78 (32.47-128.3) n = 3	24.90 (18.40-54.7) n = 3	2.75 (1.5-3.6) n = 3	28.41 (15.98-40.8) n = 2
Patients with ISM	216.0 (69-327) n = 4	4.79 (2.68-6.9) n = 4	14.87 (6.47-39.3) n = 4	12.35 (5.38-31.3) n = 4	15.47 (4.75-35.4) n = 4	85.86 (64.62-134.6) n = 4	106.60 (17.30-165) n = 4	5.99 (5.13-8.9) n = 4	30.20 (15.84-66.4) n = 4
HVs	10.0 (7-36) n = 4	0.94 (0.8-1.3) n = 4	0.67 (0.5-1.2) n = 4	4.40 (4.2-13.2) n = 4	1.08 (0.5-1.4) n = 4	33.50 (24.9-56) n = 4	5.10 (3.4-16.5) n = 4	2.40 (1.6-4.2) n = 4	7.89 (5.15-10.5) n = 4

Data are expressed as medians (ranges).

MFI, Mean fluorescence intensity.

*Number of mast cells per 300,000 cells counted based on flow on a marrow aspirate.

†One patient with a split mast cell population in the MMAS category was excluded from analysis for the following surface antigen marker ratios: CD11c, CD35, CD59, CD63, CD69, and CD203c. There was insufficient sample to complete labeling for all markers.

REMA score to predict the likelihood of a clonal mast cell disorder based on clinical findings exclusive to a bone marrow biopsy.^{4,6} When either of these approaches were applied to our patient population, the probable diagnosis of a clonal disorder then verified by means of a bone marrow examination had a sensitivity and specificity of 62.5% and 72.9%, respectively, with either system. We created a modified version of the clinical variables; changed the serum tryptase limits from 25 and 20 ng/mL, respectively, to 11.4 ng/mL or less for IA and greater than 11.4 ng/mL for clonal disease; and added the results of ASqPCR (Table IV). When applied to the 37 patients from our cohort who had ASqPCR performed with a score of 2 or greater, the diagnosis of a clonal mast cell disorder was obtained with a sensitivity and specificity of 75% and 100%, respectively (Table V). Thus by using this further modified scoring system, termed the NIH Idiopathic Clonal Anaphylaxis Score, no patients with IA would have undergone an unnecessary bone marrow procedure, and all patients with ISM would have received a correct diagnosis, as would have 75% of patients with MMAS.

DISCUSSION

Here we report the first prospective study to investigate the prevalence of a clonal mast cell disorder in patients with frequent episodes of IA. Patients with IA entered into this study were referred with this diagnosis by physicians who found no precipitating factors related to anaphylaxis and no reason to suspect a clonal mast cell disorder, including the presence of cutaneous mastocytosis. On admission to the NIH Clinical Center, all 56 patients had an evaluation that included bone

marrow biopsy and aspiration. Based on the results of a marrow biopsy and aspiration, 4 patients were given a diagnosis of ISM (7%) based on World Health Organization guidelines,¹⁶ and 4 patients were given a diagnosis of MMAS (7%).²⁰⁻²² Note that because not all patients given a diagnosis of IA were able to have tryptase levels drawn in association with their episodes of anaphylaxis and the concept of MCAS has evolved, now requiring demonstration of an increase in mast cell mediators, some would now use the term monoclonal mast cells of uncertain significance in referring to these 4 patients.²³ The extent of bone marrow pathology in patients who fulfilled the criteria for ISM was consistent with the relatively low bST values. The bone marrow pathology in patients with MMAS fulfilled only 1 to 2 minor criteria for ISM, which is consistent with the consensus diagnosis of MMAS (Table E2).²² As an example, 1 patient with MMAS had 2 populations of mast cells (CD25⁺ and CD25⁻ cells) on flow cytometry, indicating the presence of both normal and clonal mast cells (Fig 1, A and B). This highlights the importance of performing a bone marrow analysis, which examines all parameters listed in the World Health Organization criteria for the diagnosis of mastocytosis (see Table E2 in this article's Online Repository at www.jacionline.org).

Flow cytometric analysis showed that immunophenotypic characteristics of bone marrow mast cells were abnormal in patients with ISM or MMAS (Fig 2, A and B, and Table III) with expression of surface makers, such as CD25, and upregulation of CD63 and CD203c. The bone marrow mast cell compartment in patients with IA was no different from that of HVs, including the degree of *in vivo* activation, as shown by expression of CD63 and CD203c. Thus there was no evidence

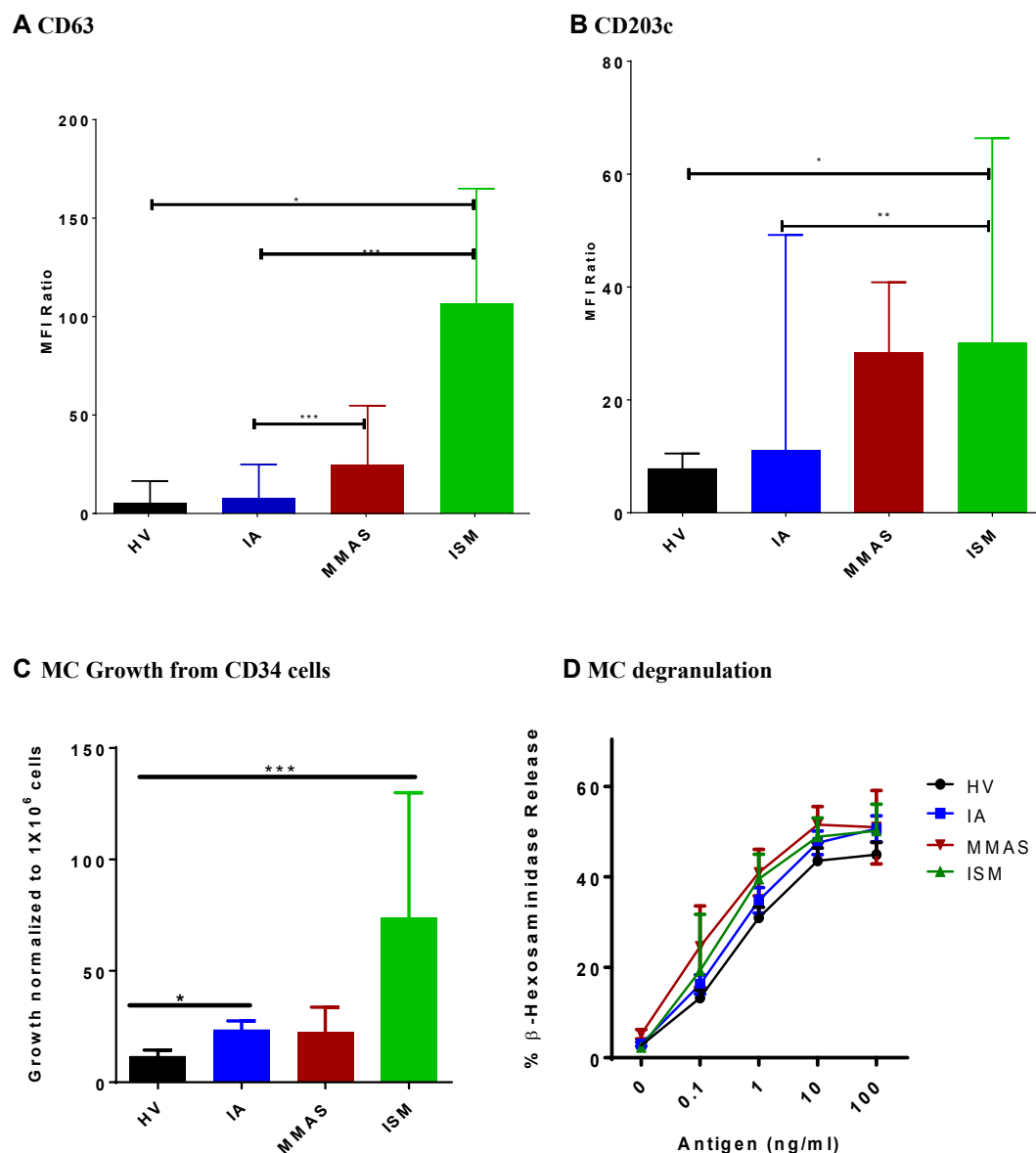


FIG 2. Immunophenotypic mast cell markers expressed by bone marrow mast cells, mast cell growth, and degranulation of cultured human mast cells. The mast cell markers CD63 and CD203c are compared in nonclonal (HVs and patients with IA) and clonal (patients with MMAS and those with ISM; **A** and **B**) patient populations. Patients with ISM demonstrated the highest expression of CD63 and CD203c, which were both significantly higher than in both nonclonal populations. Nonclonal populations were similar. Mast cell proliferation from CD34⁺ progenitor cells produced more mast cells from patients with IA or those with ISM (**C**). Degranulation of mast cells through the IgE receptor was similar among groups (**D**). Fig 2, A: * $P = .02$ and *** $P < .0001$; Fig 2, B: * $P = .04$ and ** $P = .002$; Fig 2, C: * $P = .01$ and *** $P = .0002$.

of *in vivo* activation of mast cells in the bone marrow of patients with IA.

When cultured, CD34⁺ cells from the peripheral blood of patients with ISM or IA produced more mast cells compared with those obtained from HVs. When all groups were compared, there was no difference in mast cell responsiveness, as examined based on IgE-dependent β -hexosaminidase release (Fig 2, C and D). Thus we found no evidence that mast cells from patients with IA were hyperresponsive to IgE-mediated activation when compared with mast cells from HVs, although their growth appeared greater for reasons that are unclear.

The symptoms reported in patients with IA were typical of those associated with mast cell mediator release. Most patients (approximately 91%) had cutaneous manifestations associated with episodes of anaphylaxis. Other signs and symptoms included hypotension, wheezing, shortness of breath, laryngospasm, vomiting, and diarrhea.⁶

Findings that supported the diagnosis of a clonal disorder included relatively higher bST levels and more severe reactions. Only 1 patient with IA had a history of a clinical reaction to venom with supporting laboratory test results. None of the patients with clonal disease reported an adverse clinical response to venom stings, although 2 had positive results for serum specific

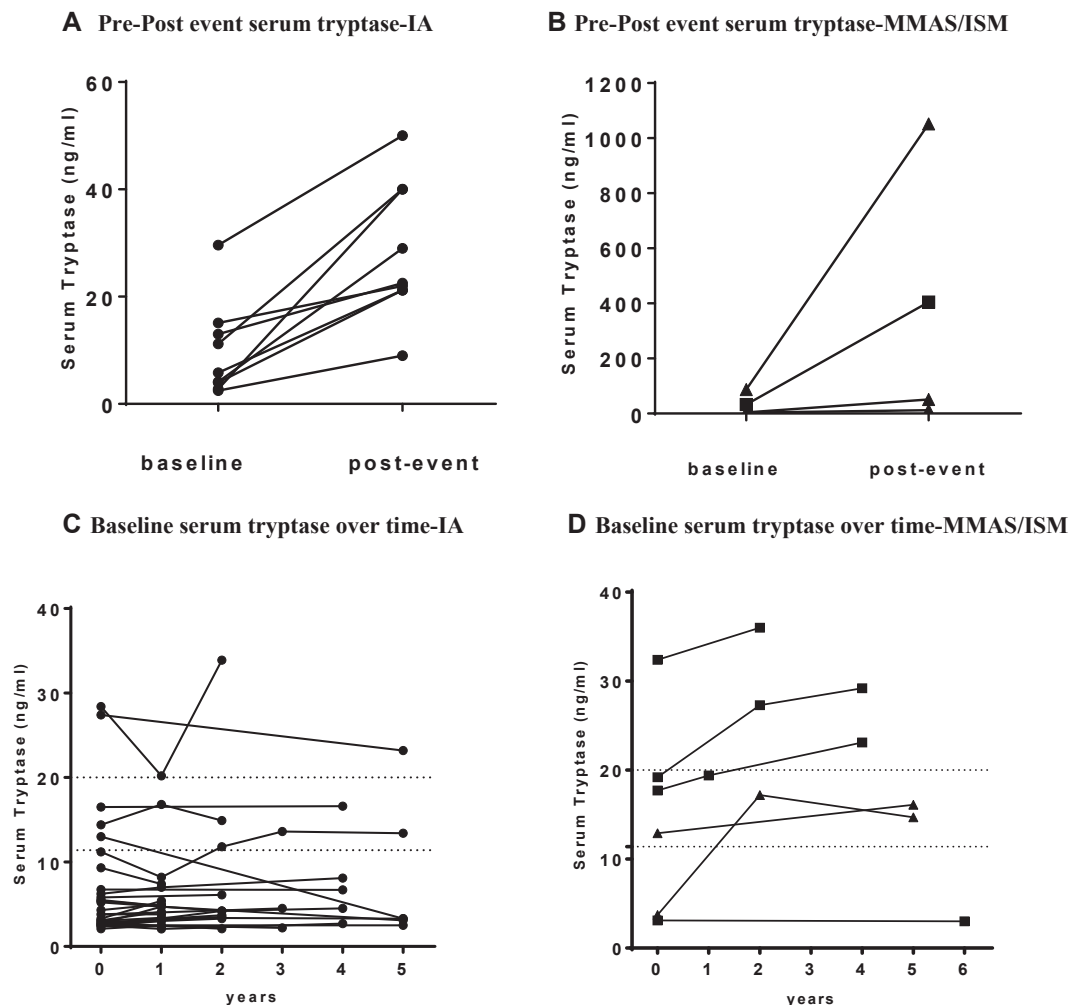


FIG 3. Pre-event/postevent serum tryptase (A and B) and bST (C and D) levels over time in patients with nonclonal and clonal mast cell disease. When compared with baseline values, both patients with IA ($P = .001$; Fig 3, A) and those with clonal disease (MMAS and ISM; $P = .002$; Fig 3, B) had a significant increase in serum tryptase levels with an anaphylactic event. However, the average increase was 502 versus 25 ng/mL for patients with clonal disease versus those with IA, respectively. In most patients with IA, the bST level remained stable over time (Fig 3, C), whereas the majority of patients with clonal disease experienced a gradual increase over time (Fig 3, D). Circles, Patients with IA; triangles, patients with ISM; squares, patients with MMAS.

TABLE IV. Clonal mast cell predictability tables: Mast cell activation symptom scores (NICAS)

Variable	Score
Sex	
Male	+1
Female	-1
Clinical symptoms	
Absence of angioedema	+1
Flushing	-1
Urticaria	+1
Syncope	+3
Tryptase	
<11.4 ng/mL	-1
>11.4 ng/mL	+1
Allele-specific PCR	
Negative	-1
Positive	+3

A total score of 2 or greater is predictive of clonal disease.
NICAS, NIH Idiopathic Clonal Anaphylaxis Score.

IgE (Table II). Hence venom-induced anaphylaxis was not predictive of clonal disease.

Our study, which focused on patients with severe recurrent IA, differs from a recent study that reported the presence of mastocytosis in patients presenting with any cause of anaphylaxis, including venom, and regardless of a previous diagnosis of ISM. They found an incidence of clonal disease of 4.3% compared with 14% in our study. In their study 75% had a clinical reaction to wasp venom, also differing from our cohort. However, their study also recognized the value of ASqPCR, which we similarly found useful in identifying clonal disorders.²⁴⁻²⁷ We would note that in our study patients with the diagnosis of IA who had a marrow test performed by their physician and had positive results for mastocytosis would not have been referred to our study, and therefore the prevalence of a clonal mast cell disorder in patients with IA is likely to be greater than 14%.

Another study, as previously mentioned, resulted in a scoring system (REMA score) designed to predict clonality among

TABLE V. Clonal mast cell predictability tables: Sensitivity and specificity for clonal prediction scores

Scoring system on NIH patient data	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
REMA score ⁶	62.5% (24.4% to 91.5%)	72.92% (58.1% to 83.4%)	27.78% (9.7% to 53.5%)	92.11% (78.6% to 98.3%)
Modified REMA score ⁷	62.5% (24.4% to 91.5%)	72.92% (58.1% to 83.4%)	27.78% (9.7% to 53.5%)	92.11% (78.6% to 98.3%)
ASqPCR	37.5%	100%	100%	90.57%
NICAS with ASqPCR	75% (34.9% to 96.8%)	100% (92.6% to 100%)	100% (54.1% to 100%)	96% (86.3% to 99.5%)

NICAS, NIH Idiopathic Clonal Anaphylaxis Score.

patients presenting with severe mast cell mediator-related symptoms.⁶ This scoring system was further refined in a subsequent study previously discussed that retrospectively examined the records of patients with clinically suspected clonal mast cell disease based on 1 or more episodes of unexplained anaphylaxis who had a bone marrow examination.⁴ When either of these algorithms was applied to our patient population, the positive and negative predictive values for clonality were 27.8% and 92.1%, respectively (Tables IV and V). We further modified these scoring systems for our population with IA by using a serum tryptase level cutoff of 11.4 ng/mL and ASqPCR to detect the presence or absence of the *KIT* D816V mutation in peripheral blood, which increased the positive and negative predictive values to 100% and 96%, respectively. Note, however, that our algorithm was specifically developed for the evaluation of patients presenting with severe recurrent IA and thus is not meant to replace previous scoring systems (Tables IV and V). These findings and recommendations are in line with previous guidelines, including the REMA score and the European Competence Network on Mastocytosis guidelines.^{6,17}

Serum tryptase measurements in association with an episode of anaphylaxis have been shown to be valuable in implicating the mast cell compartment in patients with anaphylaxis.²⁸ We found that the increase in serum tryptase levels with an anaphylactic event was greater in patients with a clonal disease compared with those with nonclonal disease (Fig 3, A and B). In addition, when followed over time, most patients (83.3%) with clonal disease demonstrated a gradual increase in bST levels compared with patients without clonal disease (7.1%; Fig 3, C and D). Because the management for ISM is generally symptomatic, these parameters could be used as additional measures to follow patients with mast cell mediator symptoms for a more tempered diagnostic approach.

In this prospective study of IA, 14% of patients were given a diagnosis of a clonal mast cell disorder. None of these patients presented with venom-induced anaphylaxis, and therefore this patient group differed from those described by other groups.^{2,29} Although no single parameter was diagnostic for a clonal disorder, peripheral blood ASqPCR to identify the presence of the *KIT* D816V mutation was beneficial in identifying those patients with IA and a clonal disorder with a 100% positive predictive value. However, the sensitivity of ASqPCR alone for clonal disease was only 37.5%. Our scoring system reliably screens out patients without clonal disease who would otherwise undergo an unnecessary bone marrow procedure. The NIH Idiopathic Clonal Anaphylaxis Score using ASqPCR increased the sensitivity and specificity of predicting a clonal disorder in patients with IA to 75% and 100%, respectively. We offer this study and its conclusions as a contribution to the identification of patients with IA who have an underlying clonal mast cell disorder.

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Key messages

- Fourteen percent of patients referred with the diagnosis of IA had an underlying clonal mast cell disorder.
- A distinct biomolecular profile identifies those patients with IA who are likely to have a clonal mast cell disorder and thus candidates for a bone marrow study to confirm the diagnosis.
- We did not find evidence of a hyperresponsive mast cell phenotype in patients with IA in the absence of a clonal mast cell disorder.

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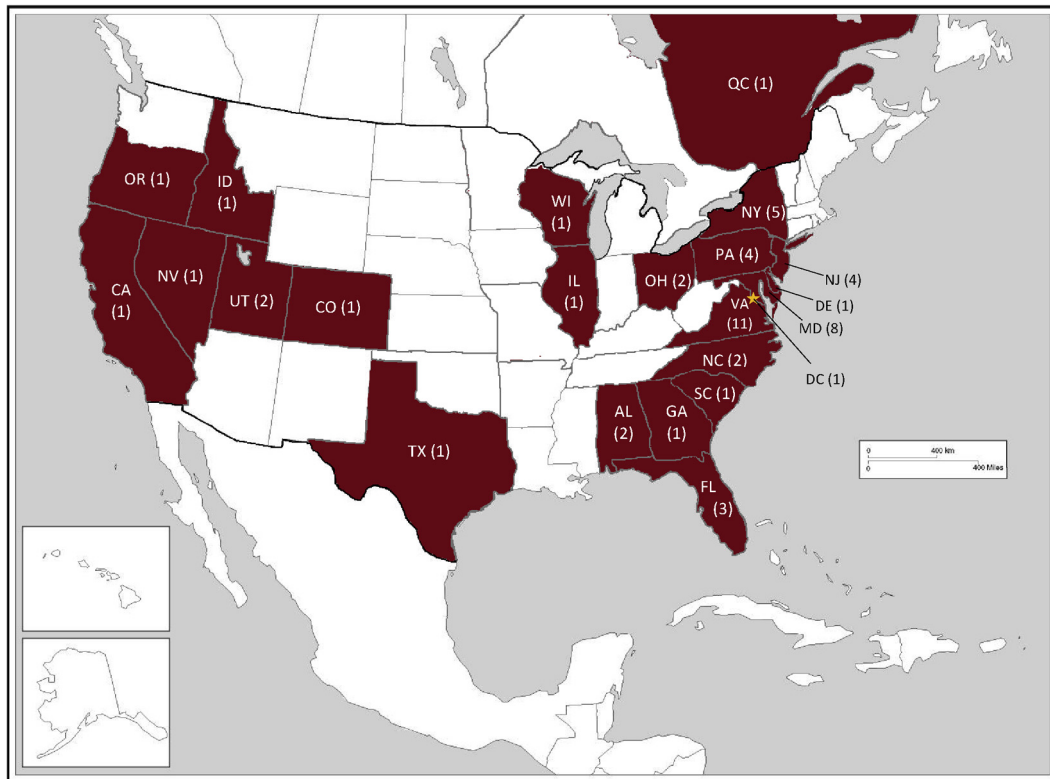


FIG E1. Residential demographics of patients. Patients were enrolled mainly from the East Coast but also included Western states and Canada. Fifty-six patients were enrolled from 22 states, the District of Columbia, and 1 Canadian province over a period of 6 years.

TABLE E1. Entry criteria for the protocol

Inclusion criteria	
IA	Involvement of skin or mucosal tissue with ≥ 1 of the following: ● Increased serum tryptase level ● Respiratory compromise ● Gastrointestinal symptoms ● Hypotension with or without end-organ dysfunction
Frequency	≥ 1 Episode(s) within 4 mo ≥ 3 Episodes within 12 mo of study entry
Documentation	≥ 1 Episode(s) observed and documented by physician
Age	13-70 y
Other	Willing/able to undergo bone marrow biopsy and aspirate
Referring physician/local care provider identified	
Exclusion criteria	
Risk assessment	Comorbid condition that might increase risk of participation
Diagnosis	Mastocytosis
Cognitive	Inability to provide informed consent

TABLE E2. Evidence for clonal mast cell disease: Evidence of clonality

Patients	MC aggregates, >15 MCs	Spindle-shaped, >25% MCs	CD2/CD25 ⁺	bST, >20 ng/mL	BM D816V ⁺	Blood D816V ⁺
MMAS						
1	No	No	No	No	Yes*	No
2	No	No	Yes	No	Yes*	No
3	No	Yes	No	Yes	No	No
4	No	Yes	No	Yes	No	No
ISM						
1	No	Yes	Yes	No	Yes	Yes†
2	No	Yes	Yes	No	Yes	No
3	Yes	Yes	Yes	Yes	Yes	Yes
4	Yes	Yes	Yes	Yes	Yes	Yes

*CD25⁺ enriched fraction of bone marrow mononuclear cells.

†At the time of blood D816V mutational analysis, the tryptase level was 14.7 ng/mL; the initial tryptase level was 3.7 ng/mL.