Increased group 2 innate lymphoid cells in peripheral blood of adults with mastocytosis

Esmee K. van der Ploeg, MSc,a,b* Maud A. W. Hermans, MD, MSc,c,d* Vincent H. J. van der Velden, PhD,e Willem A. Dik, PhD,e Paul L. A. van Daele, MD, PhD,c,d,e and Ralph Stadhouders, PhDa,b

Rotterdam, The Netherlands

Background: Systemic mastocytosis is a hematological disease in which aberrant mast cells accumulate because of gain-of-function mutations in the KIT receptor. Group 2 innate lymphoid cells (ILC2s) are effector cells of type 2 immune responses that also express KIT and colocalize with mast cells at barrier tissue sites. In mouse models, mast cell-ILC2 crosstalk can drive local inflammation. However, a possible role for ILC2s in the pathophysiology of mastocytosis remains unexplored.

Objective: We sought to characterize circulating ILC2s in a clinically diverse cohort of patients with mastocytosis.

Methods: We included 21 adults with systemic mastocytosis and 18 healthy controls. Peripheral blood ILC2 abundance and phenotype were analyzed by flow cytometry and correlated to clinical characteristics, including the presence of the D816V KIT mutation.
Results: ILC2 levels were significantly higher in D816V+ patients with mastocytosis compared with D816V− patients or healthy controls. We observed increased proportions of KIT+ ILC2s among patients with mastocytosis, regardless of D816V status. Patients with skin involvement and itch showed the highest levels of ILC2s, which was independent from atopy or serum tryptase levels. Allele-specific quantitative PCR showed that the vast majority of ILC2s did not carry the D816V mutation.

Conclusions: Our findings suggest a role for ILC2s and pathogenic ILC2-mast cell crosstalk in mastocytosis. We hypothesize that a high cutaneous D816V+ mast cell burden alters the skin microenvironment to induce chronic local ILC2 activation and their dissemination into the circulation. Activated ILC2s could contribute to skin symptoms by producing inflammatory mediators and by further augmenting mast cell mediator release. (J Allergy Clin Immunol 2020; nnn- nnn.)

Key words: Mastocytosis, mast cell, innate lymphoid cell, ILC2, KIT, itch, maculopapular cutaneous mastocytosis

INTRODUCTION

Mastocytosis is a rare myeloproliferative disease in which aberrant mast cells accumulate. Most adult patients have systemic mastocytosis, which is defined by the involvement of at least 1 extracutaneous organ, most often the bone marrow.1 In 80% to 90% of patients with systemic mastocytosis, a somatic D816V mutation is detected in the KIT receptor tyrosine kinase. Under normal conditions, KIT requires binding of its ligand, stem cell factor (SCF), to induce mast cell proliferation and survival.2 The D816V mutation leads to constitutive ligand-independent activation of KIT signaling, resulting in uncontrolled mast cell proliferation.2 Patients with systemic mastocytosis can suffer from a wide variety of symptoms, including anaphylaxis, osteoporosis, itching, flushing, dyspepsia, diarrhea, fatigue, and psychological symptoms, which can greatly reduce quality of life.3 Many of these symptoms are presumably caused by increased levels of mediators released by mast cells such as histamine, tryptase, eicosanoids, and proinflammatory cytokines. However, it remains unclear why the type and severity of symptoms can vary so greatly between individual patients. In patients with mastocytosis, baseline serum tryptase and urine histamine levels do not correlate with individual symptoms,4 suggesting that other cell types might contribute to the pathophysiology of mastocytosis, possibly through their activation by mast cell signals.

Innate lymphoid cells (ILCs) orchestrate immune responses at mucosal surfaces in an antigen-independent manner. ILCs can be grouped into various subtypes depending on the inflammatory cytokines they produce, including group 2 ILCs (ILC2s), which play a central role in type 2 immunity and in allergic inflammatory disease.5 ILC2s and mast cells both reside at barrier sites of the human body, such as the skin, gut, and airways. Although ILC2s are largely tissue-resident, they can also be detected in the circulation. Interestingly, like mast cells, a subpopulation of ILC2s expresses KIT, and SCF can augment ILC2 activation.6 In mice, mast cell-ILC2 crosstalk is important not only to clear helminth infections but also to drive local inflammation.7 However, whether such crosstalk is also relevant in the context of mastocytosis remains to be addressed.

RESULTS AND DISCUSSION

Twenty-one patients with indolent systemic mastocytosis8 and 18 healthy controls were included in our study. Sixteen had a detectable D816V KIT mutation in bone marrow samples. Patients’ characteristics are summarized in Table I. The percentage of ILC2s was significantly increased in peripheral

| Characteristic                        | Healthy controls (N = 18) | Patients with mastocytosis (N = 21) | D816V+ Patients (N = 16) | D816V− Patients (N = 5) | P value (D816V+ vs D816V−) |
|--------------------------------------|---------------------------|-------------------------------------|--------------------------|--------------------------|---------------------------|
| Sex (male/female)                    | 7/11                      | 10/11                               | 7/9                      | 3/2                      | NS                        |
| Age (y)                              | 37.6 ± 10.4§               | 53.0 ± 15.4                         | 55.2 ± 13.8              | 46 ± 5.3                 | NS                        |
| Serum tryptase (μg/L)                | NA                        | 51.3 ± 72.2                         | 62.9 ± 78.7              | 11.8 ± 34.6              | .004                      |
| Neoplastic MC burden BM, n (%)†       | NA                        | 0.02 (0.13)                         | 0.14 (0.10)              | 0.04 (0.03)              | NS                        |
| Total IgE (U/mL)                     | NA                        | 26 ± 31                             | 29 ± 33                  | 13 ± 1.5                 | NS                        |
| Maculopapular cutaneous mastocytosis | NA                        | 81.0                                 | 93.8                     | 40                       | .008                      |
| Itch, %                              | NA                        | 66.6                                 | 68.8                     | 60                       | NS                        |
| Atopy, %§                            | 33.3                      | 19.0                                 | 31.25                    | 0                        | NS                        |
| Anaphylaxis, %                       | NA                        | 23.8                                 | 25                       | 20                       | NS                        |
| Flushing, %                          | NA                        | 42.9                                 | 43.8                     | 40                       | NS                        |
| Osteoporosis, %                      | NA                        | 42.9                                 | 50                       | 20                       | NS                        |
| Diarrhea, %                          | NA                        | 38.1                                 | 43.8                     | 20                       | NS                        |

Abbreviations used
- ILC2: Group 2 innate lymphoid cell
- MPCM: Maculopapular cutaneous mastocytosis
- SCF: Stem cell factor

BM, Bone marrow; MC, mast cell; NA, not applicable; NS, not significant.
§Continuous variables shown as mean ± SD.
†Controls were significantly younger than patients with mastocytosis (P = .001).
‡Neoplastic mast cell burden in the bone marrow was defined by aberrant expression of CD2 and/or CD25 as measured by flow cytometry.
§Atopy in patients with mastocytosis was defined by clinical symptoms of asthma, rhinoconjunctivitis, and/or proven sensitization to pollen or house-dust mite by skin prick test or specific IgE measurement. For healthy controls, atopy was defined as a history of allergic rhinoconjunctivitis and/or food allergy.
blood of patients with mastocytosis compared with healthy controls, but only in patients carrying the D816V mutation (Fig 1, A and B). Although the abundance of circulating KIT+ ILC precursors was similar across all groups (Fig 1, C), a larger proportion of the circulating ILC2 compartment was KIT+ in patients with mastocytosis, irrespective of D816V status (Fig 1, D). Chemokine (CCR4, CCR6) and costimulatory (inducible costimulator) receptor expression on ILC2s was similar between groups (Fig 1, E). D816V+ patients showed significantly higher serum tryptase levels ($P = .004$) and a trend toward higher neoplastic bone marrow mast cell burden ($P = .136$) compared with D816V− patients (Table I). However, we did not detect a significant correlation between blood ILC2 levels and bone marrow neoplastic mast cell burden (Spearman $r = -0.21$; $P = .435$) or serum tryptase levels (Spearman $r = -0.073$; $P = .752$).

Although total CD4+ T-cell levels did not differ, proportions of CRTH2+ T_{H}2 cells, the functional counterparts of ILC2s belonging to the adaptive immune system, were also significantly increased in peripheral blood of patients with mastocytosis compared with healthy controls (Fig 2). However, we found no significant correlation between the levels of CRTH2+ T_{H}2 cells and ILC2s (Spearman $P = .13$; $P = .58$).

**FIG 1.** ILC2s are increased in peripheral blood of D816V+ patients with MC. A, Flow cytometry gating strategy for ILC precursors, KIT+ ILC2s, and KIT− ILC2s. B, Proportions of total ILC2s (left) and of KIT+ ILC2s (right) in peripheral blood of healthy controls (HCs) and D816V negative (D816V−), and D816V positive (D816V+) patients with MC. C, Proportions of ILCPs in peripheral blood of HCs, D816V−, and D816V+ patients. D, KIT+ ILC2 proportions of total ILC2s in peripheral blood of HCs and patients with MC. D816V− patients are indicated by yellow symbols; D816V+ patients by red symbols. E, Proportions of CCR4+, CCR6+, and ICOS+ ILC2s in peripheral blood of HCs and patients with MC. D816V− patients are indicated by yellow symbols; D816V+ patients by red symbols.

ICOS, Inducible costimulator; ILCP, innate lymphoid cell precursor; MC, mastocytosis. Data are shown as symbols for individual patients/controls, together with bar graphs for mean values ± SEM. Comparisons between groups were evaluated using a Mann-Whitney U test; $P < .05$ was considered statistically significant.
We next explored associations between circulating ILC2 populations and clinical symptoms in patients with mastocytosis. Patients with maculopapular cutaneous mastocytosis (MPCM) showed elevated levels of ILC2s as compared with healthy controls and patients without MPCM (Fig 3, A). This is in line with the high D816V prevalence (88.2%) in the MPCM group and only a single D816V patient without MPCM. Additional analysis using clinical data from our complete cohort of 263 adults with mastocytosis confirmed increased skin involvement in D816V patients as compared with D816V patients: 73% versus 42%, respectively (P = .002, chi-square test). Compared with healthy controls, D816V patients who reported itch had higher levels of ILC2s (Fig 3, B), irrespective of their KIT expression status (Fig 3, C). Of note, D816V patients with or without atopy showed similar levels of ILC2s, and we found no significant correlation between serum IgE levels and ILC2 abundance (Spearman ρ = .007; P = .98) (Fig 4, A and B). Furthermore, ILC2 abundance and KIT expression were not associated with anaphylaxis, diarrhea, osteoporosis, or flushing, and we found no significant correlations with D816V allelic burden in peripheral blood (P > .05). CRTH2+ CD4+ T cells were not specifically increased in patients with the D816V mutation, MPCM, or itch (P > .05), emphasizing the specificity of the association between ILC2s and D816V mastocytosis with skin involvement.

To assess whether the altered ILC2 compartment in patients with mastocytosis is linked to the occurrence of the D816V KIT mutation in the ILC lineage, we performed allele-specific quantitative PCR on isolated genomic DNA from ILC2s and innate lymphoid cell precursors. Despite an approximately 1% to 2% mutant allele detection sensitivity (see this article’s Online Repository at www.jacionline.org), we could not detect D816V allelic DNA in ILC2s or innate lymphoid cell precursors (see Fig E1, A and B, in this article’s Online Repository at www.jacionline.org), indicating that the D816V mutation is absent or very rare in circulating ILCs.

Here, we describe, to our knowledge, the first explorative investigation into the abundance and phenotype of ILC2s in...
patients with mastocytosis. We found that patients with the activating D816V KIT mutation in mast cells showed elevated frequencies of circulating ILC2s compared with healthy controls or D816V patients. The increased circulating ILC2 levels in D816V patients were linked to the presence of MPCM and itch. Together, our observations reveal positive associations between ILC2 frequencies, the D816V mutation, and specifically skin symptoms.

Because we could not detect the D816V mutation in peripheral blood ILCs, the increased ILC2 abundance in the circulation of patients with mastocytosis with cutaneous symptoms is likely a consequence of excessive mast cell activity in the bone marrow or skin rather than a cell-intrinsic ILC2 defect. However, increased bone marrow output of ILC2s appears an unlikely scenario, because circulating ILC2 levels did not correlate with bone marrow mast cell burden or serum tryptase levels. Hence, we postulate that constitutive mediator release by D816V skin mast cells, including inflammatory molecules such as IL-1β, TGFβ, and IL-33–activating proteases (eg, chymase and tryptase), could activate skin-resident ILC2s and create a favorable microenvironment for recruiting circulating ILC2s (Fig 4, C). Moreover, mast cell–derived prostaglandin D2 has previously been shown to stimulate ILC2 cytokine production and migration via CRTH2. Furthermore, soluble SCF levels are higher in lesional skin of patients with mastocytosis, potentially increasing the local SCF availability for KIT+ ILC2s. Chronic ILC2 activation could in turn contribute to a cutaneous cytokine milieu that promotes inflammation, mast cell mediator release, and skin symptoms—similar to mechanisms suggested for atopic dermatitis. Prolonged stimulation of tissue ILC2s can trigger their systemic dissemination, which can explain the increased ILC2 presence in the circulation in D816V mastocytosis.

Interestingly, KIT+ ILC2s express the skin-homing chemokine receptor CCR10, implying that this ILC2 subset is capable of efficiently migrating toward the skin (Fig 4, C). In line with enhanced skin migration of KIT+ ILC2s in mastocytosis, we observed that patients with frequent itch showed a reduced relative proportion of KIT+ cells within their circulating ILC2 compartment compared with patients without itch.

In summary, we conclude that circulating ILC2s are elevated in D816V mastocytosis and are associated with the presence of MPCM and itch, providing a strong rationale for performing in-depth studies into the role of ILC2s in the pathophysiology of mastocytosis.

For detailed methods, please see the Methods section in this article’s Online Repository at www.jacionline.org.
FIG 4. ILC2 levels do not correlate with atopy status and the proposed model for pathological ILC2-mast cell interplay in the skin of D816V+ patients with MC. A, Total ILC2 proportions in D816V+ patients with MC and healthy control (HC) subjects with or without atopy. B, Scatter plot showing an absence of correlation between total IgE levels (U/mL) and ILC2 abundance (Spearman $r = .007; P = .98$). Data are shown as symbols for individual patients/controls, with bar graphs for mean values ± SEM. Comparisons between groups were evaluated using a Mann-Whitney U test; $P < .05$ was considered statistically significant. C, Constitutive mediator release by D816V+ skin mast cells, including inflammatory molecules such as IL-1β, TGFβ, PGD2, and IL-33–activating proteases (eg, chymase and tryptase) activate skin-resident ILC2s and create a favorable microenvironment for recruiting circulating ILC2s (ie, via CCR10). Elevated soluble SCF levels further augment KIT+ ILC2 activation. Chronic ILC2 activation in turn contributes to an inflammatory cutaneous cytokine milieu (eg, via production of IL-9) that further promotes mast cell activity and skin symptoms but also triggers ILC2 dissemination into the circulation. Solid arrows denote activation via indicated signaling molecules; dashed arrow indicate cleavage of IL-33 into mature active IL-33; striped arrows depict cellular migration. MC, Mastocytosis; PGD2, prostaglandin D2.
Key messages

- Peripheral blood ILC2 abundance is increased in patients with indolent systemic mastocytosis who carry the D816V KIT mutation in mast cells.
- Although circulating KIT\(^{+}\) ILC2s are increased in mastocytosis, ILC2s do not appear to harbor the D816V mutation.
- Elevated ILC2 levels correlate with skin symptoms such as itch, but are independent from atopy status and serum tryptase levels.

REFERENCES

1. Pardanani A. Systemic mastocytosis in adults: 2019 update on diagnosis, risk stratification and management. Am J Hematol 2019;94:363-77.
2. Chatterjee A, Ghosh J, Kapur R. Mastocytosis: a mutated KIT receptor induced myeloproliferative disorder. Oncotarget 2015;6:18250-64.
3. Vermeiren MR, Kranenburg LW, van Daele PLA, Gerth van Wijk R, Hermans MAW. Psychological functioning and quality of life in patients with mastocytosis: a cross-sectional study. Ann Allergy Asthma Immunol 2020;124:373-8.e2.
4. van Doormaal JJ, van der Veer E, van Voorst Vader PC, Kluin PM, Mulder AB, van der Heide S, et al. Tryptase and histamine metabolites as diagnostic indicators of indolent systemic mastocytosis without skin lesions. Allergy 2012;67:683-90.
5. Doherty TA. At the bench: understanding group 2 innate lymphoid cells in disease. J Leukoc Biol 2015;97:455-67.
6. Hochdorfer T, Winkler C, Pardali K, Mjosberg J. Expression of c-Kit discriminates between two functionally distinct subsets of human type 2 innate lymphoid cells. Eur J Immunol 2019;49:884-93.
7. Shimokawa C, Kanaya T, Hachisuka M, Ishiwata K, Hisaeda H, Kurashima Y, et al. Mast cells are crucial for induction of group 2 innate lymphoid cells and clearance of helminth infections. Immunity 2017;46:863-74.e4.
8. Valenta P, Akin C, Metcalfe DD. Mastocytosis: 2016 updated WHO classification and novel emerging treatment concepts. Blood 2017;129:1420-7.
9. Kristensen T, Vestergaard H, Møller MB. Improved detection of the KIT D816V mutation in patients with systemic mastocytosis using a quantitative and highly sensitive real-time qPCR assay. J Mol Diagn 2011;13:180-8.
10. Mukai K, Tsai M, Saito H, Galli SJ. Mast cells as sources of cytokines, chemokines, and growth factors. Immunol Rev 2018;282:121-50.
11. Xue L, Salimi M, Panse I, Mjosberg JM, McKenzie AN, Spits H, et al. Prostaglandin D2 activates group 2 innate lymphoid cells through chemokreceptor-homologous molecule expressed on TH2 cells. J Allergy Clin Immunol 2014;133:1184-94.
12. Hamann K, Haas N, Grabbe J, Czarnetzki BM. Expression of stem cell factor in cutaneous mastocytosis. Br J Dermatol 1995;133:203-8.
13. Roediger B, Kyle R, Le Gros G, Weninger W. Dermal group 2 innate lymphoid cells in atopic dermatitis and allergy. Curr Opin Immunol 2014;31:108-14.
14. Ricardo-Gonzalez RR, Schneider C, Liao C, Lee J, Liang H, Locksley RM. Tissue-specific pathways extrude activated ILC2s to disseminate type 2 immunity. J Exp Med 2020;217:e20191172.
15. Bernink JH, Ohne Y, Teunissen MBM, Wang J, Wu J, Krabbendam L, et al. c-Kit-positive ILC2s exhibit an ILC3-like signature that may contribute to IL-17-mediated pathologies. Nat Immunol 2019;20:992-1003.
METHODS

Subjects

Adult patients who fulfilled the World Health Organization criteria\(^1\) for indolent systemic mastocytosis were recruited from the Erasmus MC Mastocytosis Center outpatient clinic. Healthy controls were recruited by the Franciscus Gasthuis & Vlietland hospital in Rotterdam. This study was performed according to the latest Helsinki guidelines. All subjects provided a written informed consent, and all experimental procedures were approved by the Medical Ethical Committee of the Erasmus MC in Rotterdam, the Netherlands.

Flow cytometry analysis and fluorescence assisted cell sorting

PBMCs were isolated from whole blood by density gradient centrifugation using Ficoll-PaqueTM PLUS (GE Healthcare Life Sciences, Eindhoven, The Netherlands). PBMCs were subjected to extracellular staining with antibodies for 60 minutes at 4°C, and for 15 minutes at 4°C with LIVE/DEAD Fixable Green Dead Cell Stain Kit (Thermofisher, Breda, The Netherlands). Stainings were performed with 5 to 10 million PBMCs to obtain a sufficient number of ILCs. The following antibodies to human proteins were used (including manufacturer, dilutions used, and clone numbers): From BioLegend (Amsterdam, The Netherlands): Peridinin chlorophyll protein–cyanine 5.5 (PerCP-Cy5.5)-conjugated anti-CCR4 (1:40, L291H4), Brilliant violet (BV) 421-conjugated anti-CD117 (1:20, 104D2), fluorescein isothiocyanate (FITC)-conjugated anti-CD94 (1:20, DX22), and Alexa Fluor 700-conjugated anti-NKp46 (1:20,9E2). From Thermofisher: Allophycocyanin (APC)-conjugated anti-CD127 (1:20, eBioRDR5), FITC-conjugated anti-CD3 (1:100, UCHT1), anti-CD14 (1:200, 61D3), and phycoerythrin (PE)-indotricarbocyanine (Cy7)-conjugated streptavidin (1:1000). From Milltenyi Biotec (Leiden, The Netherlands): Biotinylated anti-CRTH2 (1:50, BM16). From BD Biosciences (Vianen, The Netherlands): FITC-conjugated anti-CD19 (1:300, HIB19), anti-CD16 (1:400, 3G8), anti-TCRgd (1:20, B1), PE-conjugated anti-CCR6 (1:20, 11A9), BV650-conjugated anti-inducible costimulator (1:20, DX29), BV605-conjugated anti-CD56 (1:50, NCAM16.2), and BV785-conjugated anti-CD4 (1:30, SK3). For flow cytometry analysis, data were acquired on an LSR II or a FACSymphony flow cytometer using FACS Diva software (BD Biosciences) and analyzed using FlowJo 10 (BD Biosciences). We sorted approximately 2000 ILC2s (Lineage “CD127+ CRTH2+) or innate lymphoid cell precursor (Lineage “CD127+CD117- CRTH2+) in 1.5 mL eppendorf tubes rinsed with 10% FCS for real-time quantitative D816V mutation detection PCR using a FACSARia (BD Biosciences).

Real-time quantitative D816V mutation detection PCR

Genomic DNA was isolated from sorted ILC2 and innate lymphoid cell precursor fractions (with a FACSARia, see above) using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer’s instructions. The presence of KIT D816V mutant alleles was evaluated using a previously described\(^2\) routine diagnostics real-time quantitative PCR assay that uses wild-type and mutation-specific primer/probe sets. The D816V mutation was deemed undetectable if cycle threshold values were greater than 38. DNA from HMC1.2 cells was used as a control for the D816V mutant allele, and DNA from peripheral blood Buffy coat as control for the wild-type allele. The quantitative range of the assay was controlled for by including a standardized serial dilution of HMC1.2 or peripheral blood Buffy coat genomic DNA in the same run. Accurate detection of both alleles was observed down to the 0.01% dilution (as indicated by the positive control bars in Fig E1).

Tryptase and IgE measurements

Serum tryptase level was measured by fluoroenzymeimmunoassay technology using the Phadia250 system (Thermo Fisher Scientific, Uppsala, Sweden) according to the manufacturer’s instructions. Total serum IgE level (U/mL) was also measured by fluoroenzymeimmunoassay on the Phadia 250 system using the ImmunoCAP Total IgE test (Thermo Fisher Scientific), according to manufacturer’s instructions.

Immunophenotyping of neoplastic mast cells in bone marrow

Flow cytometry immunophenotyping of mast cells from bone marrow aspirate is performed routinely in our laboratory for diagnostic purposes, and precise methods have been previously described in detail.\(^3\) Briefly, bone marrow aspirate is collected in heparin tubes and processed within 24 hours. Erythrocytes are lysed using ammonium chloride after which leukocytes are washed with PBS/BSA and resuspended at 6 × 10⁵ cells/mL. Fifty microliter of this suspension is stained (10 minutes at room temperature) using the following antibodies: CD117-PE (104D2), CD45-PerCP (2D1), CD25-APC (2A3), CD117-PE-Cy7 (104D2; custom conjugated), CD34-APC-Cy7 (8G12; custom conjugated) (all from BD Biosciences); CD2-FITC (T11), CD2-PE (T11), CD33-PE (My9) (all from Beckman Coulter); and CD117-APC (104D2; Dako Cytomation, Glostrup, Denmark). Subsequently, cells are washed with PBS/BSA and resuspended in FACSFLOW Solution (BD Biosciences). Data are acquired using a FACSCompHist or FACS Canto B. Of note, 1 × 10⁶ cells are recorded per tube. Data are analyzed using FACS Diva software. Mast cells are gated on the basis of strong CD117 expression and subsequent gating is performed in the 6-color analysis using CD33 positivity and CD34 negativity. Neoplastic mast cells are CD2 and/or CD25 positive.

Statistical analysis

Depending on the type of variables, we used nonparametric Mann-Whitney U tests, chi-square tests, and Spearman correlation analyses to determine statistical significance. IBM SPSS Statistics (25) and Prism Graphpad software (8.0.2) were used for statistical analyses.

REFERENCES

E1. Valent P, Akin C, Metcalfe DD. Mastocytosis: 2016 updated WHO classification and novel emerging treatment concepts. Blood 2017;129:1420-7.
E2. Kristensen T, Vestergaard H, Moller MB. Improved detection of the KIT D816V mutation in patients with systemic mastocytosis using a quantitative and highly sensitive real-time qPCR assay. J Mol Diagn 2011;13:180-8.
E3. van Daele PL, Beukenkamp BS, Geertsma-Kleinekoort WM, Valk PJ, van Laar JA, van Hagen PM, et al. Immunophenotyping of mast cells: a sensitive and specific diagnostic tool for systemic mastocytosis. Neth J Med 2009;67: 142-6.
FIG E1. Failure to detect D816V allelic DNA in genomic DNA isolated from circulating ILC2s or ILCPs. Shown are cycle threshold (Ct) values from diagnostic quantitative PCR assays for the wild-type and D816V KIT alleles on genomic DNA isolated from approximately 2000 sorted ILCPs (A) and ILC2s (B) of 6 patients with mastocytosis with detectable D816V KIT alleles in bone marrow samples. Serially diluted DNA from HMC1.2 cells was used as a positive control for the D816V mutant allele; DNA from peripheral blood buffy coats was used as control for the wild-type allele. Internal controls ("pos. ctrl") for both PCR assays (10% and 0.01% dilutions) are indicated in the figure. The D816V mutation was deemed undetectable ("ND") if Ct values were greater than 38. Combining the number of input cells, the successful amplification of the 0.01% dilution control, and the approximately 8 Ct remaining detection range, this experimental set up translates to an approximately 1% to 2% mutant allele detection sensitivity. ILCP, Innate lymphoid cell precursor.