



[haematologica]  
2004;89:303-308

## The diagnostic value of CD123 in B-cell disorders with *hairy* or *villous* lymphocytes

ILARIA DEL GIUDICE  
ESTELLA MATUTES  
RICARDO MORILLA  
ALISON MORILLA  
KWASI OWUSU-ANKOMAH  
FURHEEN RAFIQ  
ROGER A'HERN  
JULIO DELGADO  
MOHAMMED BADIE BAZERBASHI  
DANIEL CATOVSKY

A B S T R A C T

**Background and Objectives.** CD123 is an antibody that identifies the  $\alpha$  chain of the human interleukin-3 receptor and is expressed in a variety of normal hematopoietic cells, acute leukemia and hairy cell leukemia (HCL). The aim of the study was to investigate the diagnostic value of CD123 expression in B-cell disorders with circulating *hairy* and *villous* lymphocytes.

**Design and Methods.** We investigated the diagnostic value of CD123 expression in neoplastic cells from 59 patients with B-cell disorders with circulating *hairy* or *villous* lymphocytes: HCL (n=24), the variant form of HCL (n=11) and splenic lymphoma with villous lymphocytes (SLVL) (n=24). Cells from 12 patients with chronic lymphocytic leukemia were used as controls. Immunophenotypic analysis was performed by flow cytometry on 77 samples from peripheral blood (n=48), bone marrow (n=25) and spleen cell suspensions (n=4).

**Results.** Our findings show that cells from 95% of typical HCL express CD123 with strong to moderate intensity while this molecule is absent in circulating cells from most cases of HCL-variant (91%) and SLVL (97%).

**Interpretation and Conclusions.** We conclude that CD123 is a useful new marker for distinguishing B-cell disorders with circulating villous lymphocytes as its expression is characteristic of typical HCL with high sensitivity and specificity. However CD123 does not allow the distinction between HCL-variant and SLVL, as both are CD123 negative.

**Key words:** hairy cell leukemia, chronic lymphocytic leukemia, splenic lymphoma with villous lymphocytes, CD123, immunophenotype.

From the Academic Department of Hematology and Cytogenetics (IDG, EM, RM, AM, KO-A, FR, JD, MBB, DC); Department of Information, The Royal Marsden Hospital and Institute of Cancer Research, London, UK (RA'H).

Correspondence: Estella Matutes, MD, PhD, FRCPath Academic Department of Haematology and Cytogenetics, Royal Marsden Hospital, 203 Fulham Road, SW3 6JJ, London (UK).  
E-mail: estella.matutes@icr.ac.uk

©2004, Ferrara Storti Foundation

CD123 is a monoclonal antibody (McAb) that identifies a 378 amino acid glycoprotein (70 kDa), the  $\alpha$  chain of the human interleukin-3 receptor (IL-3R), a heterodimer comprising an  $\alpha$  and a  $\beta$  chain.<sup>1</sup> The  $\alpha$  subunit binds IL-3 with high specificity but low affinity. The  $\beta$  chain is involved in signal transduction and has been found to be part of the IL-5 and granulocyte-monocyte colony-stimulating factor (GM-CSF) receptors. The interaction of these two subunits leads to the formation of a high affinity receptor complex.<sup>2-4</sup>

Flow cytometry studies using monoclonal antibodies against the  $\alpha$  chain of IL-3R — 9F5;<sup>5</sup> N3A;<sup>6</sup> 7G3<sup>7</sup> — have shown IL-3R expression on human normal monocytes,<sup>8</sup> eosinophils,<sup>9</sup> basophils,<sup>10</sup> vascular endothelial cells<sup>5</sup> and a subpopulation of B-lymphocytes but not on neutrophils<sup>11</sup> and T-lymphocytes. In addition, the IL-3R  $\alpha$  chain

shows a variable degree of expression in cells committed to the myeloid-lineage and CD34<sup>+</sup> hematopoietic progenitors according to their differentiation stages<sup>12-13</sup> and a strong expression in a subpopulation of dendritic cells and their precursors in adult peripheral blood, fetal bone marrow and cord blood.<sup>14-15</sup> There is little information on the expression of CD123 in cells from lymphoproliferative disorders of mature B-cells. A study of 122 cases showed that cells from 74 out of 77 chronic lymphocytic leukemia (CLL) and 15/17 mantle and follicular lymphomas were CD123 negative, while CD123 was strongly expressed in hairy cell leukemia (HCL).<sup>16</sup>

However the diagnostic value of CD123 in distinguishing B-cell disorders with circulating *hairy* or *villous* lymphocytes has not been specifically evaluated. All these three disorders are recognized as distinct

entities in the WHO classification of Tumours of Haematopoietic and Lymphoid tissues, including HCL-variant as a rare condition.<sup>17</sup> Although a set of markers compounded in a scoring system allows typical HCL to be distinguished from its variant form (HCL-variant) and from splenic lymphoma with villous lymphocytes (SLVL), there is an overlap of marker expression in the latter two disorders.<sup>18-20</sup> Indeed, the differential diagnosis between HCL-variant and SLVL is based on the morphology of peripheral blood lymphocytes and chiefly on bone marrow and spleen histology but so far not on immunophenotype.

The aim of this study was to investigate further the diagnostic value of CD123 expression in B-cell disorders with circulating hairy and villous lymphocytes.

## Design and Methods

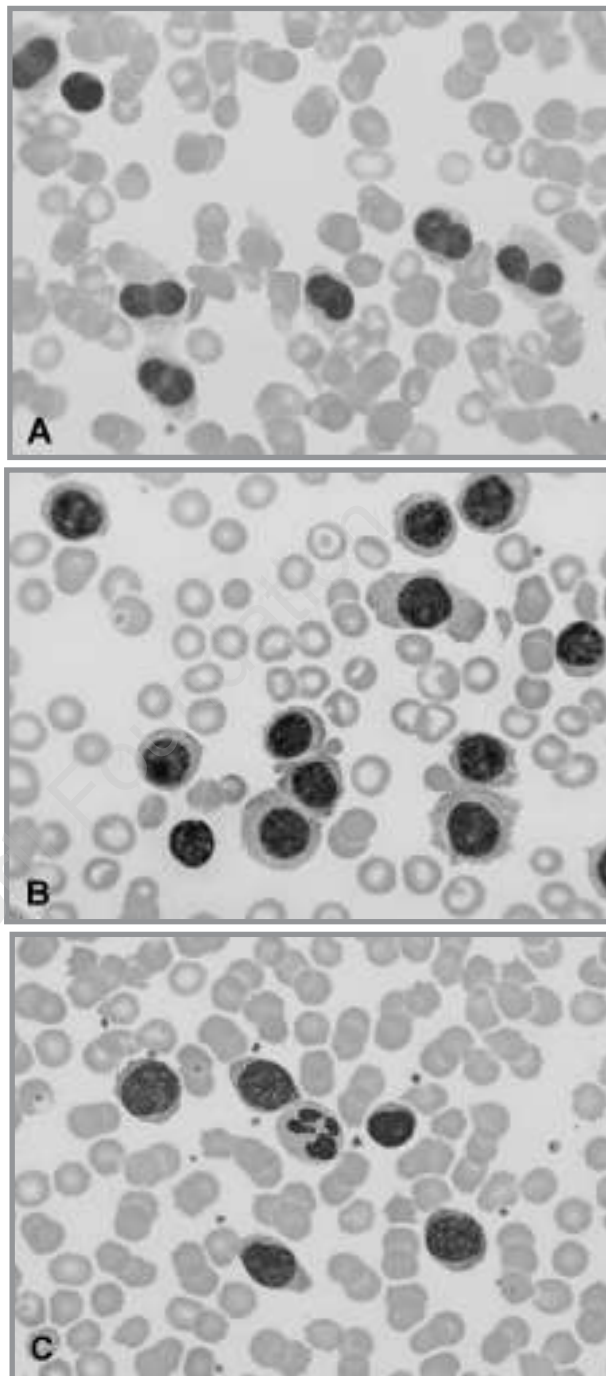
### Patients and samples

Immunophenotypic analysis was performed on 77 samples from peripheral blood (n=48), bone marrow (n=25) and spleen cell suspensions (n=4) from 71 patients with a B-cell lymphoproliferative disorder referred to our Department. These cases were: CLL (n=12), HCL (n=25), HCL-variant (n=11) and SLVL (n=29). The diagnoses were based on clinical features, lymphocyte morphology (Figures 1 A, B and C) and immunophenotype, and in 49 cases confirmed by bone marrow (n=32), spleen (n=14) and/or lymph node (n=3) histology.

Fresh samples were used in 68 cases and mononuclear cells cryopreserved in liquid nitrogen in 9 cases. Sixty-seven cases were investigated at diagnosis and 10 specimens were collected during follow-up. All samples had a clonal B-cell population (ranging from 10% to 98%), detected by membrane immunoglobulin light chain restriction (Smlg).

### Immunophenotyping

Immunophenotypic analysis was performed using two-color immunostaining with directly conjugated McAbs with a panel of B-cell markers and the HCL-associated markers CD25, CD11c, and CD103. An isotype-matched negative control (Caltag, San Francisco, USA) was used in all cases. The clone and source of the McAbs are as follow: CD123-PE (9F5) and CD25-PE (2A3) from Becton Dickinson, San Jose', CA, USA; CD2-PE (G11), CD5-FITC (5D7); CD11c-PE (3.9); CD19-FITC and-PE (SJ25-C1); CD20-FITC (HI47); CD22-FITC (RFB4), and CD23-FITC (TU1) all from Caltag, San Francisco, USA; CD79b-PE (CB3-1) from Immunotech, Marseille, France; CD103-FITC (B-ly7) from Immunology, Groningen, The Netherlands; FMC7-nc-FITC (FMC7-nc)<sup>21</sup> from Caltag, San Francisco, USA; anti-κ-



**Figure 1. Peripheral blood films illustrating the morphologic features in three cases representative of HCL (a), HCL-V (b) and SLVL (c).**

FITC (polyclonal) and anti-λ-PE (polyclonal) from DakoCytomation, Glostrup, Denmark.

For the immunostaining, cells were incubated with McAbs for 10 min at room temperature. Fresh samples were treated with a hypotonic lysing solution. κ and λ light chains were detected with directly conjugated

**Table 1. Results of immunophenotype in disorders with villous/hairy cells.**

Marker	HCL			HCL-variant			SLVL		
	Tested (n)	Positive (n)	%	Tested	Positive	%	Tested	Positive	%
CD123	23	22	95	11	1	9	29	1	3
CD25	25	24	96	11	0	0	28	18	64
CD11c	25	25	100	11	11	100	26	10	38
CD103	25	25	100	11	4	36	25	0	0
Smlg $\kappa$	25	12	48	11	5	45	29	18	62
Smlg $\lambda$	25	13	52	11	6	55	29	11	38
FMC7	25	25	100	11	11	100	29	28	96
CD22	25	25	100	11	11	100	28	27	96
CD5	25	0	0	11	1	9	29	4	14
CD23	24	3	12.5	10	0	0	28	11	39
CD19	25	25	100	11	11	100	29	28	96
CD79b	24	23	96	7	4	57	28	26	93
CD20	25	25	100	7	7	100	29	29	100

polyclonal antibodies following lysis of erythrocytes and washing the cells in phosphate-buffered saline (PBS). Thereafter, cells were washed once with PBS containing bovine serum albumin (BSA) and sodium-azide and resuspended in 500  $\mu$ L of Isoton. Before data acquisition, thawed cells were stained with propidium iodide (PI), added at a concentration of 2  $\mu$ g/mL to each tube and incubated at room temperature for 15 minutes.

Data were acquired and analyzed on a FACSCalibur flow cytometer (Becton Dickinson) using a Cell Quest software program (Becton Dickinson) on 5000 lymphocytes per sample. An electronic gate was applied in the lymphoid area of the FSC/SSC dot plot for the analysis of CLL and SLVL samples. An enlarged lymphoid gate including the monocyte area was applied to HCL and HCL-variant samples.

A two-gate strategy was used to analyze the thawed cells. First, viable cells were identified setting up a gate on PI-negative cells in a PI vs SSC dot plot and following this, a gate on the FSC/SSC dot plot was applied as described above. Thresholds for markers' positivity were based on isotype negative controls. A marker was considered positive when expressed in more than 30% B-cells, defined by positivity with CD19.

In four typical HCL cases, no differences were found in the percentage of positivity or intensity of expression of CD123 between fresh and frozen cells.

### Statistics

We analyzed the sensitivity and specificity of CD123 and the three HCL-associated markers CD25, CD11c and CD103 individually and in combination in their ability to predict the diagnosis of typical HCL. Sensitivity and specificity were expressed as proportions with 95% exact confidence intervals. Only samples for

which the expression of all 4 markers was known were included. In the analysis focused on HCL-variant and SLVL the differences between the CD25/CD11c patterns were assessed using logistic regression (with HCL-variant/SLVL as the dependent variables and CD25/CD11c positivity or negativity as the independent variables). A *p* value of <0.05 was considered statistically significant.

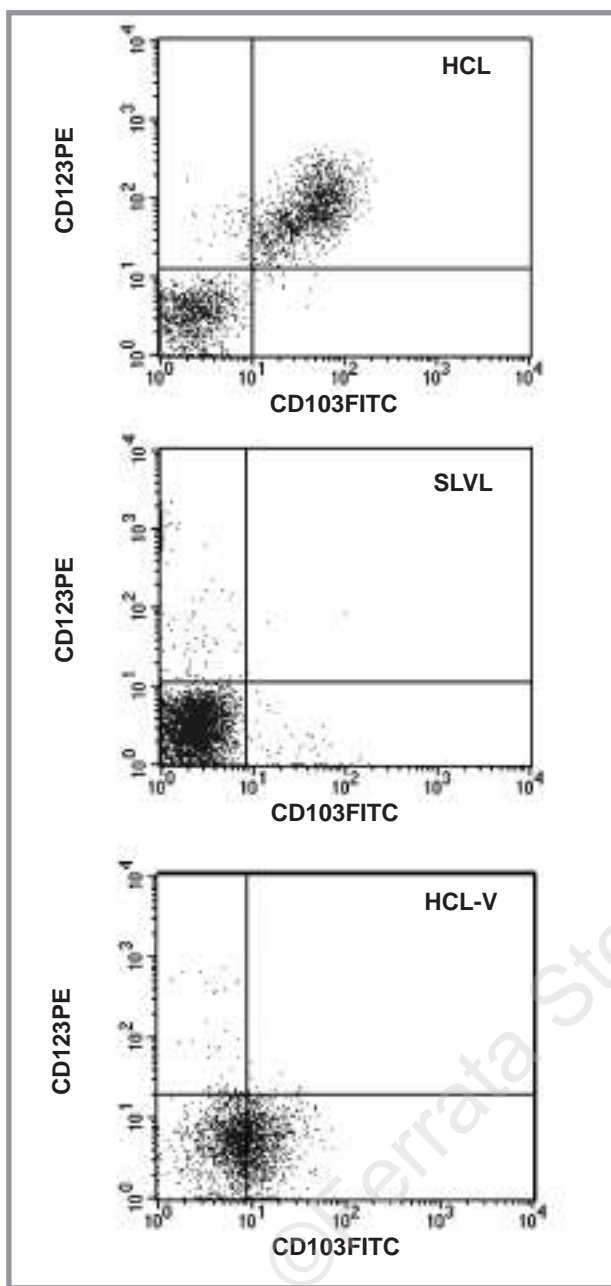
### Results

The median number of clonal B-cells in the gated population was 70% (range 10–93%) in HCL, 89% (range 56–98%) in HCL-variant and 72% (range 38–95%) in SLVL; the median number of T-cells was 14% (range 1–85%), 6% (range 2–45%) and 20% (range 6–64%), respectively.

Leukemic cells from all the cases studied were CD19<sup>+</sup>, CD20<sup>+</sup>, CD22<sup>+</sup>, FMC7<sup>+</sup> and expressed Smlg with moderate to strong intensity (Table 1). CD22 expression was strong in HCL and HCL-variant but weak/moderate in 70% of SLVL. CD79b was moderately to strongly positive in the majority of SLVL (93%) and typical HCL (96%). Four out of 7 HCL-variant cases evaluable for CD79b expression were positive with a moderate/weak expression.

The numbers and proportion of cases with typical HCL, HCL-variant and SLVL positive with CD123 and with the other HCL-associated markers CD11c, CD25 and CD103 are shown in Table 1. In the control group, cells from all CLL cases were CD123 and CD103 negative.

Cells from all but one typical HCL were CD123 positive. The intensity of CD123 expression was strong in 14 cases, moderate in 6 and weak in 2 (Figure 2). Cells



**Figure 2.** Dot plots illustrating the flow cytometry patterns in a case of HCL, SLVL and HCL-V.

from 10/11 HCL-variant cases were CD123 negative; spleen cells from only one case expressed CD123 but with weak intensity in 39% of cells. All SLVL cases were CD123 negative, except one out of the 29 (3%), which was CD123 positive with moderate intensity.

Regarding the expression of the other markers, all HCL were CD103<sup>+</sup> and CD11c<sup>+</sup> and CD25 was positive in the majority of them. All HCL-variant cases were CD11c<sup>+</sup>, CD25<sup>-</sup> and one-third CD103<sup>+</sup>. All SLVL cases were CD103 negative, up to two thirds were CD25<sup>+</sup>

and one-third CD11c<sup>+</sup>. For the distinction between HCL-variant and SLVL the combination of CD25 and CD11c was the most helpful as CD25 was consistently negative in HCL-variant and positive in 60% SLVL cases while CD11c was always positive in HCL-variant and negative in two thirds of SLVL cases. A CD25<sup>-</sup>/CD11c<sup>+</sup> phenotype was seen almost exclusively in the HCL-variant (100%) and rarely in SLVL (20%) ( $p < 0.001$ ).

We also analyzed the statistical value of CD123 and the three other HCL-associated markers (CD25, CD11c, and CD103) individually and in combination to discriminate between typical HCL versus HCL-variant and SLVL (Table 2). For the distinction between HCL and HCL-variant/SLVL, CD123 showed a sensitivity of 95.7% and all the four markers individually analyzed showed similar sensitivities in predicting the diagnosis of HCL. CD123 and CD103 individually showed a significantly higher specificity than CD25 and CD11c for HCL, with no significant difference between them. The combination of two positive markers (i.e. CD25<sup>+</sup>/CD123<sup>+</sup>; CD25<sup>+</sup>/CD103<sup>+</sup>; CD103<sup>+</sup>/CD123<sup>+</sup>) predicted the diagnosis of typical HCL with a sensitivity of 91–96% and specificity of 97–100%; the addition of a third or fourth marker did not increase the diagnostic power of these tests as the overlap of all the confidence intervals demonstrated the lack of any significant difference.

## Discussion

Our study focused on the expression of CD123 in three B-cell disorders characterized by the presence of circulating hairy or villous lymphocytes. We have shown that cells from the majority of typical HCL express CD123 with strong intensity while this molecule is absent in circulating cells from HCL-variant and SLVL. Therefore, CD123 is a novel characteristic marker of the typical form of HCL and allows this to be differentiated from its variant form and SLVL with very high sensitivity and specificity. However, this marker is not useful for distinguishing SLVL from HCL-variant as it is consistently negative in the majority of patients with these two conditions.

The combination of CD25 and CD11c seemed more helpful for discriminating between cases of HCL-variant and SLVL; for example, a CD25<sup>-</sup>/CD11c<sup>+</sup> phenotype was seen almost exclusively in HCL-variant and rarely in SLVL ( $p < 0.001$ ).

Immunophenotype has not so far been shown to be discriminative between HCL-variant and SLVL. Peripheral blood lymphocyte morphology and histology of bone marrow and spleen specimens remain the keystones to the differentiation of HCL-variant and SLVL. Nevertheless, our study shows that in single difficult

**Table 2. Distinction between HCL and HCL-variant/SLVL: sensitivity and specificity in predicting the diagnosis of typical HCL of the markers analyzed individually and in combination.**

Disease	Single markers				Combinations					
	CD123 <sup>+</sup>	CD25 <sup>+</sup>	CD11c <sup>+</sup>	CD103 <sup>+</sup>	CD25 <sup>+</sup> CD103 <sup>+</sup>	CD25 <sup>+</sup> CD123 <sup>+</sup>	CD103 <sup>+</sup> CD123 <sup>+</sup>	CD25 <sup>+</sup> CD103 <sup>+</sup> CD11c <sup>+</sup>	CD25 <sup>+</sup> CD103 <sup>+</sup> CD123 <sup>+</sup>	CD25 <sup>+</sup> CD103 <sup>+</sup> CD11c <sup>+</sup> CD123 <sup>+</sup>
HCL	22/23	22/23	23/23	23/23	22/23	21/23	22/23	22/23	21/23	21/23
HCL-V	1/11	0/11	11/11	4/11	0/11	0/11	1/11	0/11	0/11	0/11
SLVL	1/25	15/25	10/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25
Sensitivity*	95.7% (78-99.9)	95.7% (78-99.9)	100% (85.2-100)	100% (85.2-100)	95.7% (78-99.9)	91.3% (72-98.9)	95.7% (78-99.9)	95.7% (78-99.9)	91.3% (72-98.9)	91.3% (72-98.9)
Specificity*	94.4% (81.3-99.3)	58.3% (40.8-74.5)	41.7% (25.5-59.2)	88.9% (73.9-96.9)	100% (90.2-100)	100% (90.2-100)	97.2% (85.4-99.9)	100% (90.2-100)	100% (90.2-100)	100% (90.2-100)

\*Sensitivity and specificity expressed as proportions with 95% exact confidence interval.

cases immunophenotype can be helpful by supporting one or the other diagnosis (i.e. HCL-variant CD25/CD11c<sup>+</sup>/CD103<sup>+</sup>, SLVL CD25<sup>+</sup>/CD103<sup>-</sup>/CD11c<sup>-</sup>). On the other hand, CD123 is not useful for distinguishing between CLL and CD5<sup>+</sup> SLVL, as it is negative in both conditions.

There is little information on the diagnostic and/or possible pathogenetic role of CD123 expression in lymphoproliferative disorders. A single study has documented that CD123 is absent in cells from CLL, mantle cell and follicular lymphomas while it is strongly expressed in HCL<sup>16</sup> and widely expressed in all B-cell lineage ALL and AML.<sup>16,22-26</sup> Although its role in leukemogenesis is uncertain, one study<sup>26</sup> suggested that the IL3-R deregulated overexpression in AML may give the leukemic cells a proliferative advantage, resistance to *in vitro* apoptosis and associates with poor prognostic features such as high white cell count, low complete remission rate and shorter overall survival. Its functional role in HCL has not, however, been clarified.

Our findings suggest that CD123 is an additional useful diagnostic marker to be evaluated in B-cell disorders with circulating villous lymphocytes in cases in which the differential diagnosis from HCL arises. It allows typical HCL to be distinguished from its variant form and SLVL, but does not distinguish between these latter two conditions, as both are CD123 negative.

The scoring system reported by Matutes *et al.*<sup>18</sup> for the differential diagnosis of these three disorders combined four markers (CD25, CD11c, CD103, and HC2) and suggested the possibility of distinguishing

between typical HCL and HCL-variant/SLVL. Scores were consistently high (3 or 4) in typical HCL and low (0 to 2) in HCL-variant and SLVL. However, because HC2 is not commercially available the combination of these four markers cannot easily be used in a routine diagnostic work-up. Our analysis combining the remaining McAbs (CD25, CD11c, and CD103) and CD123 shows that the combination of at least two positive markers (i.e. CD25 and CD123; CD25 and CD103; or CD103 and CD123) predicts the diagnosis of typical HCL with high sensitivity and specificity. The addition of a third or fourth marker did not significantly increase the statistical power of the test (Table 2). However, the size of our series could not allow us to highlight possible further differences in sensitivity and specificity.

In the sometimes difficult differential diagnosis among lymphoid disorders with villous or hairy lymphocytes, the pattern of expression of the four markers is helpful. Therefore, the use of all four markers is desirable rather than a selection of them.

IDG, RM, AM, KO-A, FR, MBB contributed to the analysis and interpretation of the immunophenotypic data. JD and RA'H contributed to the design and analysis of the statistical data. IDG, EM, DC contributed to the design of the study, revising the morphology of the cases, drafting the article, and revising it critically. All the authors approved the final version of the paper to be published, after revising it critically. The authors reported no potential conflicts of interest.

IDG was supported by a fellowship from the British Journal of Haematology.

Manuscript received September 8, 2003. accepted December 18, 2003.

## References

- Moretti S, Lanza F, Dabusti M, Tieghi A, Campioni D, Dominici M, et al. CD123 (Interleukin 3 receptor  $\alpha$  chain). *J Biol Regul Homeost Agents* 2001;15:98-100.
- Kitamura T, Sato N, Arai K, Miyajima A. Expression cloning of the human IL-3 receptor cDNA reveals a shared  $\beta$  subunit for the human IL-3 and GM-CSF receptors. *Cell* 1991;66:1165-74.
- Barry SC, Korpelainen E, Sun Q, Stomski FC, Moretti PAB, Wakao H, et al. Roles of the N and C terminal domains of the interleukin-3 receptor  $\alpha$  chain in receptor function. *Blood* 1997;89:842-52.
- Hara T, Miyajima A. Function and signal transduction mediated by the Interleukin 3 receptor system in hematopoiesis. *Stem Cells* 1996;14:605-18.
- Korpelainen EI, Gamble JR, Smith WB, Goodall GJ, Qiyu S, Woodcock JM, et al. The receptor for interleukin 3 is selectively induced in human endothelial cells by tumor necrosis factor  $\alpha$  and potentiates interleukin 8 secretion and neutrophil transmigration. *Proc Natl Acad Sci USA* 1993;90:11137-41.
- Sato N, Caux C, Kitamura T, Watanabe Y, Arai K, Banchereau J, et al. Expression and factor-dependent modulation of the interleukin-3 receptor subunits on human hematopoietic cells. *Blood* 1993;82:752-61.
- Sun Q, Woodcock JM, Rapaport A, Stomski FC, Korpelainen EI, Bagley CJ, et al. Monoclonal antibody 7G3 recognizes the N-terminal domain of the human interleukin-3 (IL-3) receptor  $\alpha$ -chain and functions as a specific IL-3 receptor antagonist. *Blood* 1996;87:83-92.
- Elliott MJ, Vadas MA, Eglinton JM, Park LS, To LB, Cleland LG, et al. Recombinant human interleukin-3 and granulocyte-macrophage colony-stimulating factor show common biological effects and binding characteristics on human monocytes. *Blood* 1989;74:2349-59.
- Lopez AF, Eglinton JM, Gillis D, Park LS, Clark S, Vadas MA. Reciprocal inhibition of binding between interleukin-3 and granulocyte-macrophage colony-stimulating factor to human eosinophils. *Proc Natl Acad Sci USA* 1989;86:7022-6.
- Lopez AF, Lyons AB, Eglinton JM, Park LS, To LB, Clark SC, et al. Specific binding of human interleukin-3 and granulocyte-macrophage colony-stimulating factor to human basophils. *J Allergy Clin Immunol* 1990;85:99-102.
- Park LS, Friend D, Price V, Anderson D, Singer J, Prickett KS, et al. Heterogeneity in human interleukin-3 receptors. *J Biol Chem* 1989;264:5420-7.
- Huang SH, Chen Z, Yu JF, Young D, Bashesy A, Ho AD, et al. Correlation between IL-3 receptor expression and growth potential of CD34<sup>+</sup> hematopoietic cells from different tissues. *Stem Cells* 1999;17:265-72.
- Muench MO, Roncarolo MG, Namikawa R. Phenotypic and functional evidence for the expression of CD4 by hematopoietic stem cells isolated from human fetal liver. *Blood* 1997;89:1364-75.
- Olweus J, BitMansour A, Warnke R, Thompson PA, Carballido J, Picker LJ, et al. Dendritic cell ontogeny: a human dendritic cell lineage of myeloid origin. *Proc Natl Acad Sci USA* 1997;94:12551-6.
- Sorg RV, Kogler G, Wernet P. Identification of cord blood dendritic cells as an immature CD11c<sup>-</sup> population. *Blood* 1999;93:2302-7.
- Munoz L, Nomdedeu JF, Lopez O, Carnicer MJ, Bellido M, Aventin A, et al. Interleukin-3 receptor  $\alpha$  chain (CD123) is widely expressed in hematologic malignancies. *Haematologica* 2001;86:1261-9.
- Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. World Health Organization. Classification of Tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid tissues. IARCpress: Lyon 2001. p. 138-41.
- Matutes E, Morilla R, Owusu-Ankomah K, Houliham A, Meeus P, Catovsky D. The immunophenotype of hairy cell leukemia (HCL). Proposal for a scoring system to distinguish HCL from B-cell disorders with hairy or villous lymphocytes. *Leuk Lymphoma* 1994;14:57-61.
- Matutes E, Morilla R, Owusu-Ankomah K, Houliham A, Catovsky D. The immunophenotype of splenic lymphoma with villous lymphocytes and its relevance to the differential diagnosis with other B-cell disorders. *Blood* 1994;83:1558-62.
- Matutes E, Wotherspoon A, Brito-Babapulle V, Catovsky D. The natural history and clinico-pathological features of the variant form of hairy cell leukemia. *Leukemia* 2001;15:184-6.
- Serke S, Schwaner I, Yordanova M, Szczepek A, Huhn D. Monoclonal antibody FMC7 detects a conformational epitope on the CD20 molecule; evidence from phenotyping after Rituxan therapy and transfectant cell analyses. *Cytometry* 2001;46:98-104.
- Uckun FM, Gesner TG, Song CW, Myers DE, Mufson A. Leukemic B-cell precursors express functional receptors for human interleukin-3. *Blood* 1989;73:533-42.
- Park LS, Waldron PE, Friend D, Sassenfeld HM, Price V, Anderson D, et al. Interleukin-3, GM-CSF and G-CSF receptor expression on cell lines and primary leukaemia cells: receptor heterogeneity and relationship to growth factor responsiveness. *Blood* 1989;74:56-65.
- Budel LM, Touw IP, Delwel R, Clark SC, Löwenberg B. Interleukin-3 and granulocyte-monocyte colony-stimulating factor receptors on human acute myelocytic leukemia cells and relationship to the proliferative response. *Blood* 1989;74:565-71.
- Jordan CT, Upchurch D, Szilvassy SJ, Guzman ML, Howard DS, Pettigrew AL, et al. The interleukin-3 receptor  $\alpha$  chain is a unique marker for human acute myelogenous leukemia stem cells. *Leukemia* 2000;14:1777-84.
- Testa U, Riccioni R, Militi S, Coccia E, Stellacci E, Samoggia P, et al. Elevated expression of IL-3R  $\alpha$  in acute myelogenous leukemia is associated with enhanced blast proliferation, increased cellularity and poor prognosis. *Blood* 2002;100:2980-8.