Published in final edited form as:

Mutat Res. 2009 December 1; 671(1-2): 1–5. doi:10.1016/j.mrfmmm.2009.03.009.

Spontaneously arising red cells with a McLeod-like phenotype in normal donors

David J. Araten^{(1),(2)}, Katie J. Sanders⁽¹⁾, Jeffrey Pu^{(3),(4)}, and Soohee Lee⁽³⁾

⁽¹⁾Division of Hematology, Department of Medicine, NYU School of Medicine, New York, NY

⁽²⁾New York Veteran's Hospital, New York, NY

⁽³⁾New York Blood Center, New York, NY

⁽⁴⁾Johns Hopkins School of Medicine, Baltimore, MD

Abstract

Very few human genes can be used to identify spontaneous inactivating somatic mutations. We hypothesized that because the *XK* gene is X-linked, it would be easy to identify spontaneously arising red cells with a phenotype resembling the McLeod syndrome, which results from inherited *XK* mutations. Here, by flow cytometry, we detect such phenotypic variants at a median frequency of 9×10^{-6} in neonatal cord blood samples and 39×10^{-6} in healthy adults ($p = 0.004$). It may be possible to further investigate the relationship between aging, mutations, and cancer using this approach.

Introduction

Spontaneous inactivating somatic mutations are a key process in malignant transformation. The vast majority of mutations, however, will not be detectable because there is either no phenotype or, conversely, the mutations are lethal. Furthermore, for most human genes there are two active copies, and the chance of identifying a cell that has lost the function of both alleles would probably be so low as to preclude detection. Due to these considerations, there are only a handful of genes in which somatic mutations can be identified in normal human cells.

The most commonly used gene, *HPRT*, is on the X-chromosome; therefore, only a single mutation is required to produce the mutant phenotype, due to hemizyosity in males and X-inactivation in females. *HPRT* mutant lymphocytes, which have impairment in the purine salvage pathway, are detected by their ability to grow in the presence of 6-thioguanine in limiting dilution cloning [1,2]. A different approach is based on *GPA* or *HLA-A*. These genes are autosomal, but they are polymorphic, and in individuals with certain genotypes, phenotypic variants with spontaneous loss of a single allele can be detected [3-10]. The assay for spontaneously arising *GPA* variants is particularly robust due to the ease of obtaining red cells. However, it is suitable only for compound heterozygotes carrying both the M and N alleles.

© 2009 Elsevier B.V. All rights reserved.

Corresponding Author: David J. Araten, MD, NYU Cancer Center, 160 East 34th Street, 7th floor, New York, NY 10016; 212-731-5186 (o); 212-731-5540 (f) David.Araten@nyumc.org.

Conflict of Interest Disclosures: The authors do not have any conflicts of interest to disclose

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Our previous work [11-13] has been based on the *PIG-A* gene [14], which is X-linked, like *HPRT*. As for *HLA* and *GPA*, *PIG-A* mutations affect the surface of the cell, in this case, resulting in a lack of glycosylphosphatidylinositol (GPI) linked proteins [15]. As a result, mutants can be detected by flow cytometry, which provides a very rapid screen for rare GPI (–) leucocytes. Red cells can be analyzed as well [11], but unlike for leucocytes, complement-mediated hemolysis will select against GPI (–) red cells *in vivo* [15].

Hoping to find other examples of genes with the same favorable characteristics as *PIG-A*, we performed a literature search for other X-linked genes that affect the red cell membrane, and *XK* (Xp21.1) emerged as a prime candidate. Like *HPRT*, much is known about the *XK* gene because inherited mutations are associated with a known human genetic condition, the McLeod syndrome [16-18]. Affected males with this condition are noted to have some acanthocytes on the peripheral blood smear [18], and they have a mild compensated hemolysis with generally normal hemoglobin levels [18,19]. There is an absence of XK antigens on the red cell surface, and there is a great reduction in Kell protein levels [16]. This occurs because the Kell protein is covalently linked to the XK protein by a disulfide bond, which is required for the expression of Kell proteins on the surface [16]. [Although there are polymorphic determinants on the Kell protein that are important in blood banking, including the “Kell” (Met193) and “Cellano” (Thr193) blood group antigens, here “*KEL*” refers to the gene on chromosome 7, and “Kell protein” refers to its 732 amino acid gene product, regardless of these polymorphisms.] Obligate female heterozygote carriers have two populations of red cells with respect to this phenotype, due to random X-chromosome inactivation [18,20]. Some affected males will also develop muscular atrophy, neuropathy, and psychiatric changes [20,21], presumably due to loss of an unknown function of XK on the surface of myocytes and neurons.

The *XK* gene was cloned in 1994 [22], it is about 12 MB centromeric to the *PIG-A* gene, 4 MB centromeric to *DMD*, and immediately upstream of *CYBB*, and its gene product is a 444 amino acid surface protein, which spans the membrane 10 times [17,22-24]. Among over 150 reported cases of the McLeod syndrome [25], at least 28 mutations have been identified [26,27]. These include frameshift, nonsense, missense and splice site mutations, partial deletions, and large deletions of over 5.5 MB [20,26-28]. Neighboring genes on the X-chromosome can be simultaneously affected by large deletions involving *XK*, resulting in muscular dystrophy [29] or chronic granulomatous disease [29,30].

We have postulated that just as *XK* mutations can be present in the germline of affected individuals, somatic *XK* mutations might be present in erythropoietic cells in normal individuals. Unlike the case for *PIG-A* mutant red cells, hemolysis is mild in the McLeod syndrome [18,19], so we predicted that spontaneously arising red cells with the *XK* null phenotype would not be significantly selected against *in vivo*. Using an antibody that is specific for a non-polymorphic Kell protein determinant, we have now detected McLeod-like red cells in normal individuals. Here we compare the frequency of these phenotypic variants in adults and umbilical cord blood samples as well as with results from the other assay systems.

2. Materials and Methods

Umbilical cord blood samples from placentas were collected, and whole blood samples from healthy consenting adults were obtained, in accordance with IRB protocols. K14 antibody recognizes a non-polymorphic antigen (Arginine 180) on the Kell protein that is expressed on red cells from all donors [31]. Thawed aliquots of red cells from a patient with the McLeod syndrome and an obligate heterozygote carrier female served as controls. Approximately 10 to 15 million red cells were first incubated on ice with undiluted K14 hybridoma supernatant (New York Blood Center) for 30 minutes at 10^8 cells/ml and washed twice. The cells were then incubated with a 1:5 dilution of PE-conjugated rabbit-anti-mouse antibody (Dako-

Cytomation), washed twice again, followed by incubation with a FITC-conjugated antibody specific for a non-polymorphic glycophorin A determinant (Dako-Cytomation), at a 1:10 dilution. Cells were washed again and passed through a 35 μ M filter prior to analysis on a Becton-Dickinson FACScan. Forward and side scatter were acquired on a log-log scale, and only glycophorin A (+) events were gated. At least 1.2 million gated events were collected.

3. Results

Normal red cells expressed glycophorin A as well as the Kell protein (figure 1A, left panel). Red cells from a patient with the McLeod syndrome expressed significantly lower levels of Kell proteins but normal levels of glycophorin A (figure 1A, middle panel). As expected, given random X-chromosome inactivation, a female obligate carrier of the McLeod syndrome had two populations, in about a 1:1 ratio, suggesting lack of significant selection against the mutants *in vivo* (figure 1A, right panel).

In 8 adult normal donors, there was a small population of red cells with a McLeod -like phenotype (figure 1B), at a median frequency of 39×10^{-6} (range 26 to 61×10^{-6}). As a comparison, we also measured the frequency of cells with a *PIG-A* mutant phenotype by staining red cells with a mixture of anti-CD59 and anti-CD55 antibodies, followed by rabbit anti-mouse-PE and then anti-glycophorin A antibodies as previously described [11]. In 5 adults, the frequency of red cells with the *PIG-A* mutant phenotype (range 0.8 to 6.3×10^{-6} , mean 3.3×10^{-6}) was considerably lower than the frequency of McLeod-like cells, which is likely accounted for due to lysis of GPI (–) red cells. We hypothesized that somatic *XK* mutations in erythrocyte progenitors and stem cells are lowest at birth and accumulate with age. We therefore performed a parallel analysis of red cells derived from umbilical cord blood samples. Indeed, in 5 cord blood samples there was ~4 fold lower frequency of McLeod-like red cells-- a median of 9×10^{-6} , (range 6.9 to 16.3×10^{-6} ; $p = 0.004$, two-sided Mann Whitney test, figure 1D, table 1).

In patients with the McLeod syndrome, about 25% of the red cells have membrane projections [32], and we tried two approaches to isolate these cells for visualization: sorting to collect the K14 negatives, and MiniMacs bead depletion of the K14 positive cells. In some cases, we were able to identify membrane projections in the enriched population by light microscopy (figure 1E). We also incubated red cells with K14 antibody, performed bead depletion, followed by sequential staining with rabbit anti-mouse PE and anti-glycophorin A-FITC, followed by Compucyte iCyte analysis. The gated FITC-positive, PE-negative (McLeod-like) population appeared as deeply green staining discocytes, whereas incidentally visualized neighboring cells and gated FITC (+) PE (+) cells produced a merged yellow fluorescence (figure 1F). While membrane projections were not seen in this experiment, these abnormalities might not be preserved during the incubation and selection steps, perhaps as a result of changes in phospholipids, which can affect the acanthocyte phenotype [32].

4. Discussion

Here we have shown that it is possible to identify in normal individuals phenotypic variant red cells resembling cells from patients with the McLeod syndrome. As for assays of the frequency of *GPA* variants, the analysis of McLeod-like cells has as a disadvantage the fact that DNA can not be extracted from normal red cells for sequencing. We believe that it may be possible to address this question in the future using samples from individuals who, as a result of pathological conditions, have large numbers of circulating nucleated red cells. As for analyses based on *GPA*, an advantage of this assay is the ease of isolation of red cells, the large number of cells that can be screened by flow cytometry, and their uniform size distribution, which facilitates flow cytometry. An additional advantage of the analysis for McLeod-like cells is

that it can be performed on samples derived from any individual, whereas only compound heterozygotes carrying both the M and N alleles can be studied in the *GPA* analysis. We do not yet know whether cells from laboratory animals can be studied in this way. However, it is notable that Xk is on the mouse X-chromosome. As in humans, the mouse Xk protein is covalently linked to the gene product of the mouse *KEL* homologue, and both are expressed on red cells [33-35].

The median frequency of phenotypic variants for normal adults in this study, 39×10^{-6} , is higher than corresponding values for 6-thioguanine resistant lymphocytes (1.9 to 8.7×10^{-6} [1-3]), GPI (-) granulocytes (22×10^{-6} , [11]), GPI (-) marrow precursors (14.7×10^{-6} [36]), GPI (-) lymphocytes (18×10^{-6} , [37]), *GPA* MN variant red cells (7 to 10×10^{-6} [8,10]), and *HLA-A* variant lymphocytes (24×10^{-6} [5]) in normal adults. A possible explanation for this difference would be if red cell progenitors could tolerate larger deletions involving *XK* compared with these other genes. It is also possible that mutations in both copies of the *KEL* gene (which is autosomal) could produce the same phenotype, but we believe that this is less likely to occur than a single mutation in *XK*. We can not rule out that in some cells, global abnormalities in the synthesis of membrane proteins would mimic the McLeod phenotype. However, we believe that gating to include only glycophorin-A positive cells would likely exclude such events.

Others have observed that the frequency of phenotypic variants is higher in adults than in neonates [3,5,38-40]. In one study, the median frequency of lymphocytes with the *HPRT* mutant phenotype was about 1×10^{-6} in neonates and it increased with a slope of about 0.2×10^{-6} per year through adulthood [38]. In a separate study, the log of the frequency of phenotypic variants appeared to be increasing linearly, with a multiplicative increase of 1.6% per year [40]. In a study of spontaneous *HLA-A* allele loss, the frequency of phenotypic variants increased from a geometric mean of 7×10^{-6} in cord blood samples to 24×10^{-6} in 20 to 60 year olds, and 65×10^{-6} in those over the age of 60, with a suggestion of exponential increases in the oldest subjects [5]. In contrast, no changes were seen in the frequency of spontaneous *GPA* allele loss variants between cord blood samples and adolescents [10].

As in these previous studies using *HPRT* and *HLA-A*, our data demonstrates that the frequency of spontaneously arising *XK* phenotypic variants is higher in adults than neonates. This could be due to accumulation of mutations or an increase in the rate of mutations with age, as we do not yet know whether the increase is linear or exponential. A second question is whether differences in fetal and adult hematopoiesis, including migration of stem cells and fetal to adult hemoglobin gene switching, could somehow be responsible for the lower frequency of phenotypic variants in neonates. We believe that a detailed study of elderly individuals compared with those of different age ranges will address these two questions. Furthermore, it may be possible to investigate the relationship between aging and cancer risk with this approach.

Acknowledgments

Supported in part by NIH grants RO1 CA-109258 (DJA) and RO1 HL075716 (SL). We are indebted to Dr. Marion Reid of the New York Blood Center for assistance in obtaining control red cell samples, Gregory Halverson of the New York Blood Center for assistance in obtaining K14 hybridoma supernatant, and to Bridget Lane of the NYU Cancer Center for assistance in coordinating the collection of blood samples from healthy donors. We would like to thank Dr. Ed Luther for assistance with the iCyte analysis.

References

- [1]. Albertini R, Castle K, Borchering W. T-cell cloning to detect the mutant 6-thioguanine-resistant lymphocytes present in human peripheral blood. *Proc Natl Acad Sci, USA* 1982;79:6617–6621. [PubMed: 6983072]
- [2]. Morley A, Trainor K, Seshadri R, Ryall R. Measurement of in vivo mutations in human lymphocytes. *Nature* 1983;302:155–156. [PubMed: 6828165]
- [3]. Albertini R, Nicklas J, O'Neill J, Robison S. In vivo somatic mutations in humans: measurement and analysis. *Annu Rev Genet* 1990;24:305–326. [PubMed: 2088171]
- [4]. Bigbee W, Langlois R, Swift M, Jensen R. Evidence for an elevated frequency of in vivo somatic cell mutations in ataxia telangiectasia. *Am.J.Hum.Genet* 1989;44:402–408. [PubMed: 2916583]
- [5]. Grist S, McCarron M, Kutlaca A, Turner D, Morley A. In vivo human somatic mutation: frequency and spectrum with age. *Mutation Research* 1992;266:189–196. [PubMed: 1373828]
- [6]. Kavathas P, Bach F, DeMars R. Gamma ray-induced loss of expression of HLA and glyoxalase I alleles in lymphoblastoid cells. *Proc Natl Acad Sci USA* 1980;77:4251–4255. [PubMed: 6933474]
- [7]. Kyoizumi S, Nakamura N, Hakoda M, Awa A, Bean M, Jensen R, Akiyama M. Detection of Somatic Mutations at the Glycophorin A Locus in Erythrocytes of Atomic Bomb Survivors Using a Single Beam Flow Sorter. *Cancer Research* 1989;49:581–588. [PubMed: 2910480]
- [8]. Langlois R, Bigbee W, Jensen R. Measurements of the frequency of human erythrocytes with gene expression loss phenotypes at the glycophorin A locus. *Human Genetics* 1986;74:353–362. [PubMed: 3793098]
- [9]. Pious D, Hawley P, Forest G. Isolation and Characterization of HL-A Variants in Cultured Human Lymphoid Cells. *Proc Natl Acad Sci USA* 1973;70
- [10]. Vickers MA, Hoy T, Lake H, Kyoizumi S, Boyse J, Hewitt M. Estimation of Mutation Rate at Human Glycophorin A Locus In Hematopoietic Stem Cell Progenitors. *Env Mol Mut* 2002;39:333–341.
- [11]. Araten D, Nafa K, Pakdeesuwan K, Luzzatto L. Clonal populations of hematopoietic cells with paroxysmal nocturnal hemoglobinuria genotype and phenotype are present in normal individuals. *Proc Natl Acad Sci, USA* 1999;96:5209–5214. [PubMed: 10220445]
- [12]. Araten DJ, Golde DW, Zhang RH, Thaler HT, Gargiulo L, Notaro R, Luzzatto L. A Quantitative Measurement of the Human Somatic Mutation Rate. *Cancer Res* 2005;65:8111–8117. [PubMed: 16166284]
- [13]. Araten DJ, Luzzatto L. The mutation rate in PIG-A is normal in patients with paroxysmal nocturnal hemoglobinuria (PNH). *Blood* 2006;108:734–736. [PubMed: 16543465]
- [14]. Miyata T, Takeda J, Iida Y, Yamada N, Inoue N, Takahashi M, Maeda K, Kitani T, Kinoshita T. The cloning of PIG-A, a component in the early step of GPI-anchor biosynthesis. *Science* 1993;259:1318–1320. [PubMed: 7680492]
- [15]. Rosse WF, Ware RE. The Molecular Basis of Paroxysmal Nocturnal Hemoglobinuria. *Blood* 1995;86:3277–3286. [PubMed: 7579428]
- [16]. Allen F, Krabbe S, Corcoran P. A new phenotype (McLeod) in the Kell blood-group system. *Vox Sanguinis* 1961;6:555–560. [PubMed: 13860532]
- [17]. Lee S, Russo D, Redman C. The Kell Blood Group System: Kell and XK Membrane Proteins. *Seminars in Hematology* 2000;37:113–121. [PubMed: 10791880]
- [18]. Wimer B, Marsh W, Taswell H, Galey W. Haematological Changes Associated with the McLeod Phenotype of the Kell Blood Group System. *British Journal of Haematology* 1977;36:219–224. [PubMed: 871435]
- [19]. Ballas S, Bator S, Aubuchon J, Marsh W, Sharp D, Toy E. Abnormal membrane physical properties of red cells in McLeod syndrome. *Transfusion* 1990;30:722–727. [PubMed: 2219261]
- [20]. Singleton B, Green C, Renaud S, Fuhr P, Poole J, Daniels G. McLeod syndrome resulting from a novel XK mutation. *British Journal of Haematology* 2003;122:682–685. [PubMed: 12899725]
- [21]. Jung H, Hergersberg M, Kneifel S, Alkadhi H, Schiess R, Weigell-Weber M, Daniels G, Kollias S, Hess K. McLeod syndrome: a novel mutation & predominant psychiatric manifestations and distinct striatal imaging findings. *Annals of Neurology* 2001;49:384–392. [PubMed: 11261514]

- [22]. Ho M, Chelly J, Carter N, Danek A, Crocker P, Monaco A. Isolation of the gene for McLeod syndrome that encodes a novel membrane transport protein. *Cell* 1994;77:869–880. [PubMed: 8004674]
- [23]. Russo D, Redman C, Lee S. Association of XK and Kell Blood Group Proteins. *Journal of Biological Chemistry* 1998;273:13950–13956. [PubMed: 9593744]
- [24]. Carbonnet F, Hattab C, Collec E, LevanKim C, Cartron J, Bertrand O. Immunochemical analysis of the Kx protein from human red cells of different Kell phenotypes using antibodies raised against synthetic peptides. *British Journal of Haematology* 1997;96:857–863. [PubMed: 9074432]
- [25]. Hewer E, Danek A, Schoser B, Miranda M, Reichard R, Castiglioni C, Oechsner M, Goebel H, Heppner F, Jung H. McLeod myopathy revisited: more neurogenic and less benign. *Brain* 2007;130:3285–3296. [PubMed: 18055495]
- [26]. Danek A, Rubio J, Rampoldi L, Ho M, Dobson-Stone C, Tison F, Symmans W, Oechsner M, Kalckreuth W, Watt J, Corbett A, Hamdalla H, Marshall A, Sutton I, Dotti M, Malandrini A, Walker R, Daniels G, Monaco A. McLeod neuroacanthocytosis: genotype and phenotype. *Annals of Neurology* 2001;50:755–764. [PubMed: 11761473]
- [27]. Walker R, Danek A, Uttner I, Offner R, Reid M, Lee S. McLeod phenotype without the McLeod syndrome. *Immunohematology* 2007;47:299–305.
- [28]. Peng J, Redman C, Wu X, Song X, Walker R, Westhoff C, Lee S. Insights into extensive deletions around the XK locus associated with McLeod phenotype and characterization of two novel cases. *Gene* 2007;392:142–150. [PubMed: 17300882]
- [29]. Bertelson C, Pogo A, Chaudhuri A, Marsh W, Redman C, Banerjee D, Symmans W, Simon T, Frey D, Kunkel L. Localization of the McLeod Locus (XK) Within XP21 by Deletion analysis. *American Journal of Human Genetics* 1988;42:703–711. [PubMed: 3358422]
- [30]. Frey D, Machler M, Seger R, Schmid W, Orkin S. Gene deletion in a patient with chronic granulomatous disease and McLeod syndrome: fine mapping of the Xk gene locus. *Blood* 1988;71:252–255. [PubMed: 3334897]
- [31]. Russo DCW, Lee S, Reid M, Redman C. Topology of Kell Blood Group Protein and the Expression of Multiple Antigens by Transfected Cells. *Blood* 1994;85:3518–3523. [PubMed: 7949106]
- [32]. Redman C, Huima T, Robbins E, Lee S, Marsh W. Effect of Phosphatidylserine on the Shape of McLeod Red Cell Acanthocytes. *Blood* 1989;74:1826–1835. [PubMed: 2790207]
- [33]. Collec E, Colin Y, Carbonnet F, Hattab C, Bertrand O, Cartron J, Kim CV. Structure and expression of the mouse homologue of the XK gene. *Immunogenetics* 1990;50:16–21. [PubMed: 10541802]
- [34]. Lee S, Russo D, Pu J, Ho M, Redman C. The mouse Kell blood group gene (Kel): cDNA sequence, genomic organization, expression, and enzymatic function. *Immunogenetics* 2000;52:53–62. [PubMed: 11132157]
- [35]. Lee S, Sha Q, Caldenda G, Peng J. Expression Profiles of Mouse Kell, XK, XPLAC mRNA. *Journal of Histochemistry & Cytochemistry* 2007;55:365–374. [PubMed: 17189525]
- [36]. Hu R, Mukhina GL, Piantadosi S, Barber JP, Jones RJ, Brodsky RA. PIG-A mutations in normal hematopoiesis. *Blood* 2005;105:3848–3854. [PubMed: 15687243]
- [37]. Ware RE, Pickens CV, DeCastro CM, Howard TA. Circulating PIG-A mutant T lymphocytes in healthy adults and patients with bone marrow failure syndromes. *Exp Hematol* 2001;29:1403–1409. [PubMed: 11750098]
- [38]. Green MHL, O'Neill JP, Cole J. Suggestions concerning the relationship between mutant frequency and mutation rate at the *hprt* locus in human peripheral T-lymphocytes. *Mutation Research* 1995;334:323–339. [PubMed: 7753096]
- [39]. Morley A, Cox S, Holliday R. Human Lymphocytes Resistant to 6-Thioguanine Increase with Age. *Mechanisms of Ageing and Development* 1982;19:21–26. [PubMed: 7109703]
- [40]. Trainor K, Wigmore D, Chrysostomou A, Dempsey J, Seshadri R, Morely A. Mutation frequency in human lymphocytes increases with age. *Mechanisms of Ageing and Development* 1984;27:83–86. [PubMed: 6492889]

Figure 1. Red cells with a McLeod-like phenotype are detectable in normal donors

(A) Bivariate flow cytometry plots demonstrating that red blood cells from a patient with the McLeod syndrome express significantly lower levels of the Kell protein antigen recognized by the K14 antibody, but normal levels of glycophorin-A, compared with a normal donor. An obligate heterozygote carrier female exhibits a bimodal distribution due to random X-chromosome inactivation of either the wild type or mutant XK allele. (B) Representative analyses from three normal adult donors. In each case there is a small, distinct population of cells, in the lower right quadrant, that express much lower levels of K14 antigen. The frequency of phenotypic variants is calculated as the number of events in the lower right quadrant divided by the total number of gated events analyzed, see Table I. (C) Representative analyses from

neonatal cord blood samples. **(D)** Distribution of the frequency of phenotypic variants for adults and neonates. **(E)** Red cells from normal donors with a McLeod-like phenotype were enriched by sorting (top left, top right panels) or bead and column depletion of K14 expressing cells (bottom right, bottom left panels). Cells with acanthocyte-like projections are shown. **(F)** iCyte analysis of red cells from a normal donor that were first enriched by bead depletion of K14 expressing cells, then stained sequentially with rabbit anti-mouse PE and anti-glycophorin A-FITC antibodies. Gates were set to visualize the glycophorin A-positive, K14-negative population, which appeared as bright green discocytes. The gated K14 positive population (not shown) and incidentally visualized neighboring K14 positive cells (top left, bottom left) produce a merged yellow fluorescence.

Table I

Frequency of McLeod-likered cells in blood samples

Sample #	# Mutants	Total # red cells analyzed	frequency (x 10 ⁶)
<i>Adult donors</i>			
1	62	1791744	34.6
2	176	2874336	61.2
3	76	1968684	38.6
4	65	1181515	55.0
5	88	2244250	39.2
6	54	2063006	26.2
7	72	2768663	26.0
8	94	2439980	38.5
<i>Cord blood samples</i>			
1	21	2084890	10.1
2	17	2463413	6.9
3	14	1561238	9.0
4	23	1409899	16.3
5	13	1413783	9.2