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# Heterozygous *Kit* mutants with little or no apparent anemia exhibit large defects in overall hematopoietic stem cell function

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## Abstract

**Objective—** The evolutionarily conserved *Kit* receptor is vital for function of hematopoietic stem cells (HSC). $Kit^{W\text{-}41}$ *(W-41)* and $Kit^{W\text{-}42}$ *(W-42)* are single residue changes in the KIT intracellular phosphotransferase domain, while $Kit^{W\text{-}v}$ *(W-v)* is a single residue change in the ATP binding domain. This study tests how each mutation affects HSC function.

**Methods—**Cells in mutant and C57BL/6J +/+ blood and marrow were compared. Overall HSC function was measured by competitive repopulation. Functions of specific progenitor populations were tested with stage-specific competitive repopulation and standard colony forming unit assays.

**Results—**Bone marrow cells from these *Kit* mutants are severely defective at reconstituting peripheral blood lineages and bone marrow of irradiated recipients, when compared to +/+ control marrow. These defects increased with time. Marrow from *W-41*/+ and *W-v*/+ functions similarly but better than marrow from *W-41*/*W-41* and *W-42*/+, to repopulate the erythroid and lymphoid lineages. Long term (LT) - and short term (ST)-HSC from *W-v*/+, *W-41*/*W-41* and *W-42*/+ are more defective at reconstituting bone marrow than LT- and ST-HSC from *W-41*/+ and +/+. Common myeloid progenitor (CMP) cells from *W-42*/+ and *W-41*/*W-41* are more defective at producing spleen colonies than CMP from *W-v*/+ and *W-41*/+.

**Conclusion—**Heterozygous *Kit* mutants with little or no apparent anemia exhibit surprisingly large defects in overall HSC function. Multiplying the fractional defects in LT-HSC, ST-HSC and CMP can account for overall effects of *W-v/+*, but does not completely account for the defects observed with *W-41/+*, *W-42/+* and *W-41/W-41*.

## Introduction

Hematopoietic stem cells (HSC) self-renew and differentiate to maintain the hematolymphoid systems [1]. The development of HSC is hierarchical (Figure 1), whereby the most primitive long term (LT-) HSC give rise to short term (ST-) HSC, which then produce the multi-potent progenitors (MPP). These give rise to lymphoid and myeloid progenitors, which differentiate into the specific cell types of the lymphatic system, bone marrow and peripheral blood [2,3]. The cell surface markers used to distinguish these populations are listed in Figure 1.

*Kit oncogene* (*Kit*), formerly *c-Kit or W*, is an essential regulator of HSC and myeloid progenitor cells [4–6], and is also involved in melanoblastic and gonadal stem cell functions [7]. Cells at every stage of early HSC differentiation (Figure 1) are $Kit^{+}$, except common

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lymphoid progenitors (CLP), which are *Kit*$^{lo}$ [8]. *Kit* mutants are generally dominant for white spotting, and recessive for severe anemia [5,6]. The current report is the first to show severe defects in HSC repopulating abilities of heterozygous *Kit* mutants.

*Kit*, a proto-oncogene, encodes a type III transmembrane protein tyrosine kinase receptor (Figure 2); it is located on mouse Chromosome 5 [9–11]. The human homolog is located on Chromosome 4, at band position 4q11–q12 [12], and has a very similar genomic structure [13–16]. Stem cell factor (*SCF*), also called KIT ligand or mast cell growth factor, encoded by the Steel locus (Sl), is the ligand specific to *Kit* [17–19]. *Kit* has a large number of mutant alleles [5–6,9–10,20–23], causing phenotypes that vary in severity; in most cases, the molecular lesions have been identified [20–25]. *Kit* has also been linked to a wide variety of cancers [4, 26–30]. Thus understanding *Kit* function may suggest clinical applications for conditions ranging from anemia to leukemia.

The stages of HSC differentiation affected by the *Kit* mutations and the molecular mechanism by which *Kit* regulates hematopoiesis are not well understood. In this study we define 3 *Kit* heterozygous alleles — *W-42/+*, *W-41/+* and *W-v/+* — and one homozygote — *W-41*/*W-41*. Each mutation results from a different single residue change in the intracellular domain (Figure 2), and each differs in its effect on hematopoiesis, pigmentation and fertility (Table 1) [20–23]. The residues altered by the *W-v* and *W-41* alleles are highly conserved among several receptor tyrosine kinases, while that of the *W-42* allele is conserved in all tyrosine and serine-threonine kinases [20,23]. Our results demonstrate large HSC functional defects in heterozygous *Kit* mutants with little or no apparent anemia and variations, among the mutants, in effects specific to the CMP, LT-and ST- stages of HSC differentiation.

## Materials and methods

### Strains of mice

All experiments were performed using age-matched mice of the C57BL/6J (B6) background reared in specific pathogen free colonies at The Jackson Laboratory [31]. The *Kit* mutations are maintained on the B6 background. Gender- and age-matched B6 +/+ siblings served as controls. The mice were reared on a 4% fat diet of NIH-31 food (breeders were reared on a 6% fat diet) and acidified water *ad libitum*.

### Peripheral blood analysis

Peripheral blood was collected via orbital sinus bleeding from 3 to 5-month-old males and females into BD Microtainer® EDTA coated tubes (BD, Franklin Lakes, NJ). Hematopoietic parameters were measured, and analysis was conducted with the Advia 120 Hematology System (Bayer) in the Diagnostic Division of The Jackson Laboratory [32].

### Competitive repopulation

Bone marrow cells (BMC) were extracted [33] from 2- to 6-month-old adult males: *Kit* mutants and B6 controls provided donor BMC, while B6.Cg-Gpi1$^{a}$ mice provided competitor BMC. Five million donor and 1 million competitor cells were injected into the tail veins of 3-month-old, lethally irradiated (11 Gy) male B6 recipients (Shepard Mark 1 $^{137}$Cesium gamma source, J.L. Shepherd & Associates, Glendale, CA). The cell numbers were determined with a model ZBI Coulter Counter (Coulter Electronic, Inc., Hialeah, FL). After 2, 6 and 10 months, peripheral blood was collected via orbital sinus bleeding and analyzed to compare the repopulating abilities of donor vs. competitor HSC. Each RU is equivalent to the function of 100,000 standard competitor cells, where the number of donor RU in the mixture is equal to (% of donor cells /[100 -% of donor cells]) x 10, as previously described [33,34].

### HSC and progenitor numbers

Flow cytometry (FACS Calibur, BD Biosciences, San Jose, CA) was used to characterize bone marrow populations at different stages of hematopoietic differentiation [32]. We used lineage low ($lin^-$):Sca-$1^+$:$Kit^+$ and CD34 markers to define the multipotent progenitor and precursor populations, staining 2.5 x $10^6$ cells/ml with cell marker antibodies. All antibodies were purchased from BD Biosciences/PharMingen (San Diego, CA). Anti-CD117 labeled with allophycocyanin (APC) detected KIT expression (clone 2B8), anti-Ly6A/E labeled with phycoerythrin (PE) identified Sca-1, and anti-CD34 labeled with fluorescein-isothiocyanate (FITC) identified the CD34 positive cells. The lineage marker cocktail (lin) consisted of several hematopoietic cell lineage markers, labeled with phycoerythrin-cyanine 7 (PE-Cy7); it included markers for granulocytes (Gr-1), macrophages (Mac-1), B cells (B220), T cells (CD4), and red blood cells (Ter119).

### Colony assays (CFU-E, BFU-E, CFU-S)

Bone marrow was extracted from un-manipulated B6 and *Kit* mutant males and females, 8–12 weeks of age. For BFU-E (burst forming units-erythroid) and CFU-E (colony-forming unit-erythroid) assays, whole bone marrow was cultured in MethoCult Media (StemCell Technologies, BC, Canada) at a concentration of 1.0 x $10^6$ cells/ml. The cultures were incubated at 37° C with 5% $CO_2$. CFU-E were indicated by clusters of 8– 20 cells, manifested 2 days after incubation. Seven days of incubation produced BFU-E in clusters of > 30 cells. The StemCell Technologies manual, “Mouse colony-forming cell assays using MethoCult,” suggested the guidelines for CFU-E and BFU-E sizes. For spleen colony forming unit (CFU-S) assays, 1.0 x $10^5$ cells from each donor were injected into tail veins of irradiated B6 recipients (9 Gy). Donors and recipients were 10- to 15-week-old males. After 9 days, recipient spleens were removed and fixed in Bouin’s, and numbers of macroscopic colonies counted [35]. Media samples containing no donor cells were used for the negative control; no colonies developed on the spleens of these recipients.

### Stage-specific competitive repopulation (SSCR)

We developed a modified competitive repopulation assay, in which the relative functional abilities of donor and competitor cells were compared at specific stages of HSC differentiation. Donor and competitor cells were distinguished using two alleles of Ly5 [36] — Ly5.1 and Ly5.2— that do not affect cell functions, but that are readily distinguished by flow cytometry [37]. From 3 donors of each genotype, 6 million BMC were intravenously transplanted with 6 million competitor BMC into each of 4 irradiated recipients per donor. All donors, +/+ and *Kit* mutants, were Ly5.2, as were the irradiated recipients, while the competitors were Ly5.1. Some irradiated recipients received only competitor cells (Ly5.1) to assure that recipient contributions were minimal. All mice were 2- to 3-month-old males. We used cell surface markers (Figure 1) to define stages of HSC differentiation [2,38–40]. After 1 and 4 months, LT-HSC and ST-HSC were identified in recipient marrow cell populations by their cell surface markers (Figure 1). Propidium iodide exclusion focused on viable stem cells, which were further identified by expression of Kit and Sca-1, and by the absence of the lineage markers (lin), also referred to as KSL [39]. The fluorochrome used for CD117 was APC-Cy7; APC was used for Sca-1, FITC for Ly5.1 and Pacific blue for the lineage cocktail. Long term and short term HSC were distinguished with Flk2, CD135 labeled with phycoerythrin [40]. LT-HSC are KSL $Flk2^-$ while ST-HSC are KSL $Fllk2^+$. Within the KSL or KSL Flk population, percentages of donor and competitor cells were determined by Ly5 type, and RU values were calculated for each of the 4 mutants and the +/+ control donors. Because the competitor RU was set at 60 values of donor RU in the 6 million donor marrow cells equal (% of donor cells/[100 - % of donor cells]) x 60.

### Statistical analysis

Effects of genotype on all assays conducted were analyzed through ANOVA using JMP software. Data are presented as means ± SEM; $P < 0.05$ is statistically significant.

## Results

### Peripheral blood lineages

In peripheral blood, the red blood cell (RBC) mean corpuscular volume in all 4 mutant groups was significantly increased compared to +/+, reflecting the expected macrocytic anemias of *Kit* mutants. RBC numbers were significantly reduced in *W-v*/+, *W-41*/*W-41* and *W-42*/+ mice, while hematocrit percentages were significantly lower only for *W-41*/*W-41* and *W-42*/+ mice. Details are shown online in supplemental Table 1. Importantly, no hemoglobin values differed significantly from normal, indicating that the mutants are, at most, only mildly anemic.

### Competitive repopulation

In competitive repopulation studies, HSC from +/+ mice repopulated significantly better than HSC from the *Kit* heterozygous mutants (Table 2). Peripheral blood was sampled after 2, 6 and 10 months. Effects of *Kit* mutants tended to become more severe with time. At 2 months, relative repopulating abilities for *W-42*/+ BMC were < 1% of normal in erythrocytes; they were 6% in lymphocytes. Repopulating ability for *W-42*/+ lymphocytes declined to < 1% after 6 and 10 months. Functions of *W-41*/*W-41* BMC were similar or slightly worse when compared to *W-42*/+. BMC from *W-41*/+ and *W-v*/+ donors functioned better than *W-42*/+ and *W-41*/*W-41*, but still showed severe defects compared to normal controls. *Kit* mutants consistently produced lymphocytes more effectively than erythrocytes (Table 2). These data emphasize the surprisingly severe degree of HSC dysfunction in these *Kit* mutants, despite only small effects on steady state peripheral blood lineages and normal values for circulating hemoglobin.

### HSC and progenitor numbers

Similar numbers of stem cells were observed for bone marrow isolated from controls and the 4 *Kit* mutants; there were no significant differences ($P > 0.05$) in these or any of the progenitor populations, as defined in Figure 1 (Supplemental Table 2). These data indicate that the *Kit* mutations do not interfere with the quantitative expression of KIT on the cell surface of the cell, which is consistent with prior studies using mast cells from *W-41* and *W-42* [20–23], and with the molecular nature of these mutations. The CD117 antibody recognizes the extracellular domain, and the molecular lesions for these mutants are in the intracellular region. CD34 [41–42] was used along with the KSL markers, and no differences were observed between mutants and controls. These data support the hypothesis that KIT defects are caused by decreased function of KIT positive cells in the HSC differentiation pathway, and not by decreased HSC or progenitor numbers.

### Measures of precursor cell function: colony assays

Numbers of BFU-E and CFU-E were not significantly different between *Kit* mutants and controls (mean numbers of BFU-E ranged from 300–500 and CFU-E ranged from 100–180), which is consistent with previous results on some of the same mutants [43]. Once the *Kit* mutant cells achieve these stages of differentiation (Figure 1), they function normally. CFU-S assays measure ability of the common myeloid precursor (CMP) to produce macroscopic colonies on spleens of irradiated hosts [35]. Concentrations of CMP from the BMC of each mutant, except *W-41*/+, were significantly less than in controls, and significantly higher in *W-41*/+ and *W-v*/+ than in *W-42*/+ or *W-41*/*W-41* ($P < 0.05$; Figure 3). Weights of spleens from each group were not significantly different. The colonies produced by the *Kit* mutants, including some from *W-41*/+, were smaller than those of the B6 controls, suggesting that CMP numbers may

underestimate the *Kit* mutant defects. The numbers of CMP for *W-41*/*W-41* and *W-42*/+ are consistent with previous reports [43,44].

### Measures of precursor cell function: SSCR

The stage-specific competitive repopulation (SSCR) was designed to measure the function of donor LT- and ST-HSC. ST-HSC are those stem cells that self-renew and produce the differentiated cells of the bone marrow and peripheral blood for 4 to 6 weeks while LT-HSC self-renew and produce the ST-HSC, progenitors and differentiated cells for the life of the mouse (45). At 1 and 4 months post transplantation bone marrow was extracted from the recipients and percentages of donor and competitor stem cells –LT-HSC ($Flk2^{-}$) and ST-HSC ($Flk2^{+}$) –were quantified.

When recipients received +/+ donor marrow, the percentages of donor type stem cells at 1 and 4 months post transplantation were close to the expected 50%, however percentages of donor-derived stem cells were significantly reduced for each *Kit* mutant, at both time points (Supplemental Figure 1A). The LT-HSC from each *Kit* mutant showed greater defects because the percentages of donor derived stem cells decreased between 1 and 4 months post-transplantation. We calculated the repopulating unit (RU) values for each donor (Supplemental Figure 1B) from percentages of donor-derived cells. RU values for control donors at 1 month (ST-HSC) and 4 months (LT-HSC) were standardized at 100% and used to calculate the relative repopulating ability of the STand LT-HSC from each donor (Table 3).

## Discussion

This is the first study to measure the effects of *W-41*, *W-42* and *W-v* heterozygous mutants on the overall HSC differentiation pathway. Competitive repopulation shows that these mutations greatly reduce relative HSC repopulating abilities for the peripheral blood (Table 2) and the bone marrow (Table 3), despite having only small effects on steady state hematologic parameters (Supplemental Table 1). This is also the first study to define the quantitative differences between the *W-41* and *W-42* mutants, and to test mutation specific effects on well-defined stages of HSC differentiation.

*Kit* mutant HSC and other precursors must have defective intrinsic functions, because cell numbers are normal for the progenitor stages of HSC differentiation in the 4 *Kit* mutants (Supplemental Table 2). Thus in these mutants repopulating and differentiating function per cell must be defective to produce the severe defects observed with competitive repopulation.

Relative repopulating abilities of the *Kit* mutants were significantly reduced compared to normal B6 controls. These functional defects increased over time (Table 2), suggesting that the primary effects of these *Kit* mutations are on the most primitive hematopoietic stem cells. Percentages of ST-HSC derived from the *W-v*/+, *W-41*/*W-41* and *W-42*/+ decrease from 1 to 4 months post-transplantation (Table 3), suggesting that the LT-HSC which were originally transplanted into the irradiated recipients have a differentiation defect. Percentages of LT-HSC derived from these donors also decreased over time, suggesting that the originally transplanted *Kit* LT-HSC also have a self-renewal defect. The *W-41*/+ and +/+ donor cells did not exhibit these defects, as the percentages of LT- and ST-HSC remained constant or increased slightly (Table 3). Together these results support the hypothesis that the *Kit* gene is involved in HSC self-renewal and differentiation, at the primitive stem cell stage.

LT- and ST-HSC were distinguished solely on phenotypes and were not tested for self-renewal and differentiation activity. While transplanted cells with HSC phenotypes do not have the expected repopulating ability (46), we recently showed that these HSC phenotypes predict repopulating abilities after transplantation in B6 mice (34). Thus these phenotypes provide a

way to compare precursors at defined stages of HSC differentiation. Of course, it is possible that a sizable subpopulation of HSC was excluded by not including CD4 or CD34 [41], however we used a standard, widely accepted definition of HSC [2,38–40,45]. As markers for HSC phenotypes are improved, they should be used in stage specific competitive repopulation.

The similar numbers of CFU-E and BFU-E in marrow from the *Kit* mutants and controls indicate that, once the mutated *Kit* cells progress this far in the hematopoietic differentiation pathway, they can produce enough peripheral blood lineages for nearly normal values of hematopoiesis (Supplemental Table 1). However, the reduced number and size of spleen colonies (Figure 3), indicates that *Kit* mutations also affect this stage of differentiation. The much more severe effects of the *W-42* mutation at this stage may explain why it diminishes erythrocyte production much more than the *W-v* and *W-41* mutations.

In all cases, stage-specific defects (Table 3) were far smaller than overall defects (Table 2). The defects for the erythroid and lymphoid lineages of the peripheral blood are referred to as the overall defects. This difference cannot be due to transplantation damage, as cells were only transplanted once for the assay, while damage is only shown after serial transplantation [47]. We propose a model to explain the cumulative defects observed with each mutant (Table 4). We tested the hypothesis that the overall defect (total observed) is the product of all the fractional defects in each stage of differentiation (total predicted): (the relative functional defects measured in LT-HSC [Table 3]) x (relative defects in ST-HSC [Table 3]) x (defects in CMP [Figure 3]). With this hypothesis, two patterns emerge. First, the *W-v* mutation, in the ATP binding domain, causes a greater defect in the LT-HSC (the most primitive HSC stage tested) than does the far more severe, *W-42* mutation, suggesting that the ATP binding domain may be especially important in the LT-HSC stage of differentiation. Second, the product of effects at these 3 cell differentiation stages predicts the quantitative competitive repopulating difference observed between *W-v*/+ and +/+ for erythrocytes (lymphyocytes are less severely affected), but overall effects of the other mutants are still more severe in both erythrocytes and lymphocytes than predicted. Perhaps the ATP binding domain is important only in these 3 stages of differentiation, while an untested cell stage, possibly a more primitive stage responsible for LT-HSC production, is affected by mutations in the KIT phosphotransferase domain.

*W-41*/+ cells are less severely affected than *W-42*/+ cells at all stages, although both are similar types of mutations in the KIT phosphotransferase domain. The residue altered in *W-42* may have a much greater effect on the same pathways than the residue altered in *W-41*, or *W-42* may alter a separate, more important pathway. Unfortunately, neither of these residues was tested in extensive molecular studies of *Kit* actions *in vitro* [4,7].

Overall, our findings quantify the vital and sensitive role *Kit* plays in HSC differentiation and self-renewal *in vivo*. The cellular stages of HSC repopulation and differentiation affected by the *Kit* mutants differ in specific patterns. By defining effects of these mutants in the critical stages of HSC differentiation, we have begun to identify molecular regulatory mechanisms in different portions of the *Kit* receptor.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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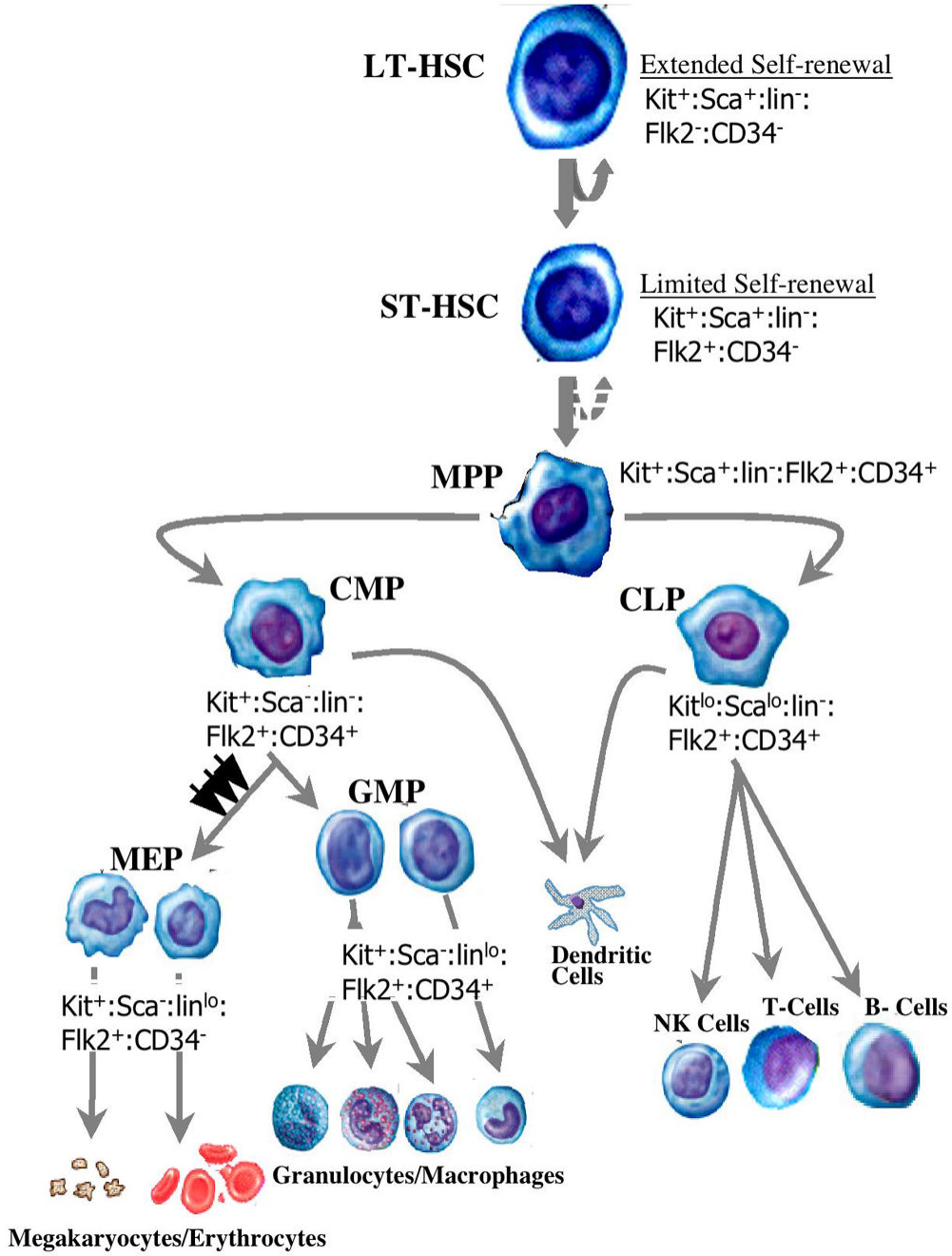


**Figure 1. HSC differentiation pathway**

The LT-HSC (curved arrow) self-renew and produce differentiated lineages (straight arrow) for > 6 months, while the ST-HSC have limited self-renewal (dotted curved arrow). KIT is expressed on LT- and ST-HSC, as well as the multipotent progenitor (MPP), the common myeloid progenitor (CMP), the megakaryocyte/erythrocyte progenitor (MEP), and the granulocyte/macrophage progenitor (GMP). KIT expression is low on the common lymphoid progenitor (CLP). The black arrows between MEP and erythrocytes indicate the stages tested with the BFU-E and CFU-E assays. Studies conducted by several groups determined the cell surface markers used to define each population (2, 38–40, 45). Image modified from Mader ’04 (48).

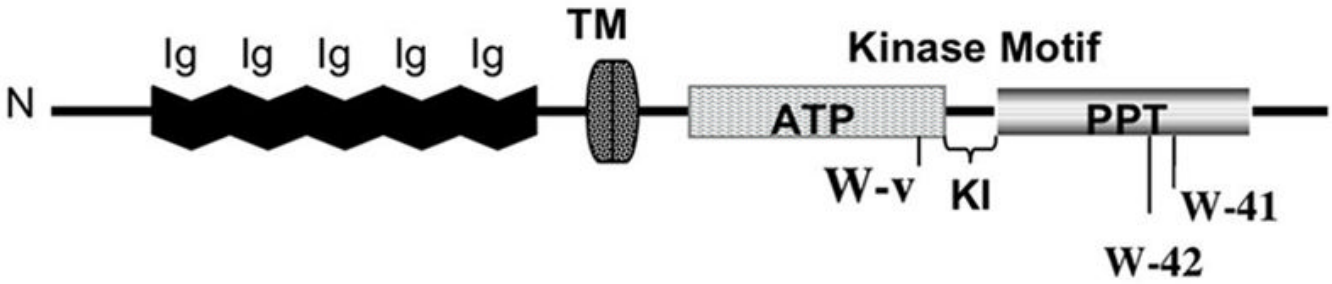


**Figure 2. KIT receptor**

Five Ig domains are located in the extracellular domain (residues 23–518). Residues 1–22 define the signal peptide. SCF binding occurs at the third Ig motif [18]. The cytoplasmic region (residues 543–975) contains 2 kinase domains, separated by the kinase insert (KI): one is involved in ATP binding (residues 543–975) while the other is a phosphotransferase (PPT) domain (residues 763–925). TM refers to the transmembrane spanning region (residues 519–542). The affected residues for this study are indicated as follows: *W-v* (residue 660), *W-42* (residue 790) and *W-41* (residue 831). This drawing was modified from published material [14,20].

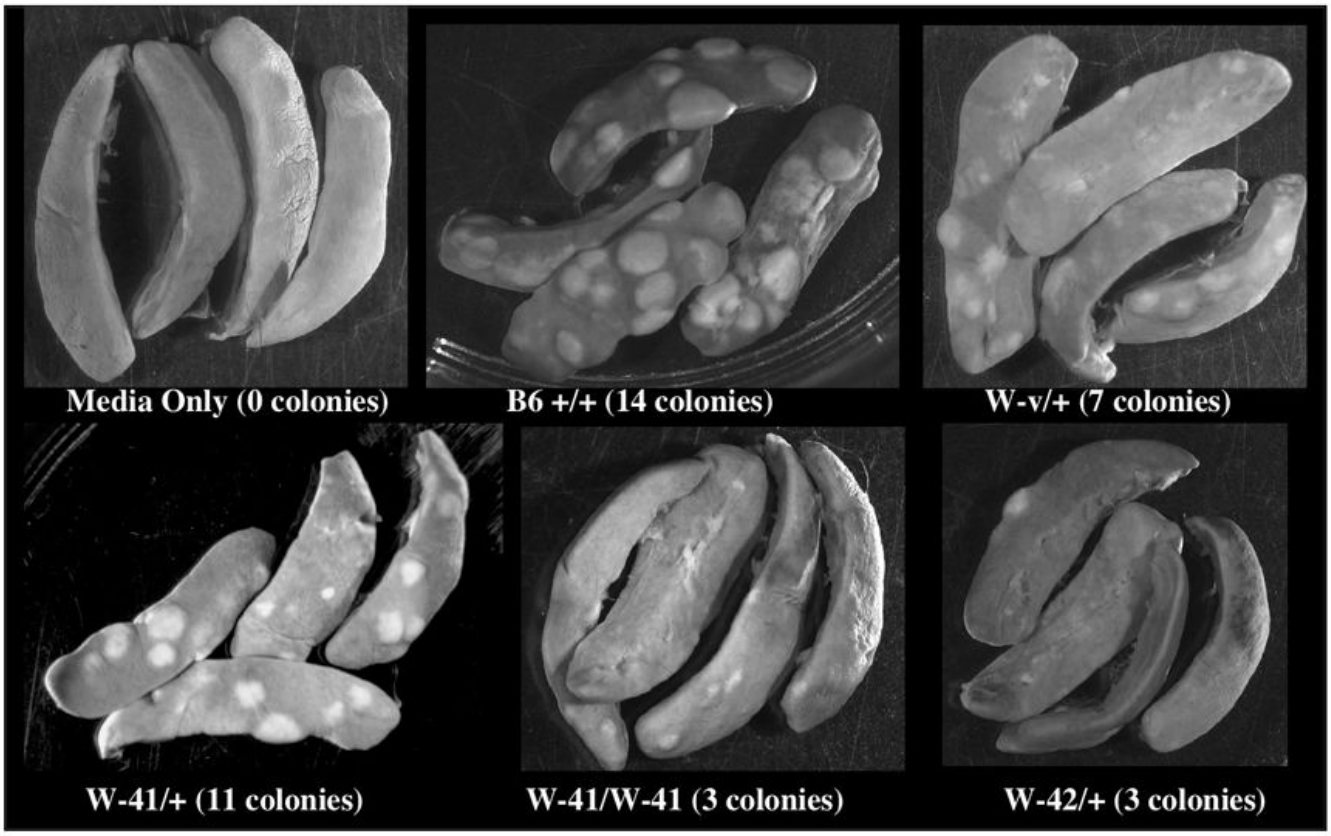


**Figure 3. Spleen colony forming unit (CFU-S) assay**

Mean numbers of colonies per $10^5$ donor cells are shown, with representative spleens for each genotype, for which 12 spleens were tested. Values for all mutants, except *W-41*/+, are significantly lower than values for +/+ ($P < 0.05$). Values for *W-41*/+ and *W-v*/+ are significantly higher than for *W-42*/+ or *W-41/W-41* ($P < 0.05$).

**Table 1**

Background data for *Kit* mutant alleles in this study

| ***Kit* alleles** | **Degree of anemia** | **Coat color** | **Fertility** | **Affected domain** | **Kinase activity** |
|---|---|---|---|---|---|
| *W-v/-W-v* | Severe | Pure white | Sterile | ATP: T660M | Greatly reduced |
| *W-v/+* | Mild | White spot | Normal | | Reduced |
| *W-41/W-41* | Moderate | Mottled | Reduced | PPT: V831M | Reduced |
| *W-41/+* | Mild | White spot | Normal | | Reduced |
| *W-42/W-42* | Fetal death | *NA* | *NA* | PPT: D790N | *NA* |
| *W-42/+* | Moderate | Pure white | Normal | | None |

Kinase activities were obtained from mast cell cultures. Data for this table are from [20,22,23].*NA* indicates not applicable, due to lethality.

**Table 2**

Relative repopulating ability to reconstitute peripheral blood lineages

| | Relative repopulating abilities: Percentages for erythrocytes | | | Relative repopulating abilities: Percentages for lymphocytes | | |
|---|---|---|---|---|---|---|
| **Donor genotype** | **2 months** | **6 months** | **10 months** | **2 months** | **6 months** | **10 months** |
| B6 +/+ | 100 | 100 | 100 | 100 | 100 | 100 |
| *W-v/+* | 8.0 | 5.4 | 4.1 | 21 | 9.1 | 5.2 |
| *W-41/+* | 12 | 6.0 | 3.2 | 25 | 8.0 | 4.6 |
| *W-41/W-41* | < 0.1 | 0.22 | < 0.1 | 6.0 | 0.55 | 0.22 |
| *W-42/+* | < 0.1 | 0.70 | 0.18 | 6.1 | 1.6 | 0.44 |

On the B6 strain background, *Kit* mutant and +/+ donors were $Gpi1^b$, and competitors were B6.Cg-$Gpi1^a$; all were 2–6 month old males. Mixtures of $5x10^6$ donor and $1x10^6$ competitor BMCs were iv injected into each lethally irradiated B6 recipient, whose peripheral blood was analyzed at 2, 6 and 10 months post-transplantation. Relative repopulating abilities of the *Kit* mutants and the normal control were calculated from mean percentages of donor erythrocytes or lymphocytes as RU values; values are presented as percentages of normal repopulating ability (RU donor x 100/RU normal control). All mutant values are significantly different from normal control values ($P < 0.0001$), with 6–8 donors analyzed for each group at each time point.

**Table 3**

Relative repopulating ability to reconstitute bone marrow

| Donor genotype | Relative repopulating abilities of donor ST-HSC | Relative repopulating abilities of donor LT-HSC |
|---|---|---|
| B6 +/+ | 100 | 100 |
| *W-v/+* | 48 | 19 |
| *W-41/+* | 60 | 40 |
| *W-41/W-41* | 34 | 18 |
| *W-42/+* | 41 | 26 |

On the B6 strain background, *Kit* mutant and +/+ donors were Ly5.2, and competitors were B6.Ly5.1; all were 2–3 month old males. Mixtures of $6x10^6$ donor and $6x10^6$ competitor BMCs were iv injected into each lethally irradiated B6 recipient, whose bone marrow was analyzed at 1 and 4-months post-transplantation. Relative repopulating abilities of the *Kit* mutants and the normal control were calculated from mean percentages of donor KSL cells as RU values; values are presented as percentages of normal repopulating ability (RU donor x 100/RU normal control). All mutant values are significantly different from normal control values ($P < 0.001$), with 6 donors analyzed for each genotype at each time point.

**Table 4**

Effects of *Kit* mutants at each stage of HSC differentiation*

| Differentiation stage | *W-v*/+ | *W-41*/+ | *W-41*/*W-41* | *W-42*/+ |
|---|---|---|---|---|
| LT-HSC† | 0.19 | 0.40 | 0.18 | 0.26 |
| ST-HSC† | 0.48 | 0.60 | 0.34 | 0.41 |
| CMP‡ | 0.50 | 0.79 | 0.21 | 0.21 |
| Totals predicted§ | 0.046 | 0.19 | 0.013 | 0.022 |
| Totals observed E, L‖ | 0.054, 0.091 | 0.06, 0.08 | 0.0022, 0.0055 | 0.007, 0.016 |

*Mutant effects are given as fraction of the normal control.

†Equal numbers of donor and competitor BMC were iv injected into irradiated recipients. All mice were 2- to 3-month-old males. Recipient marrow was analyzed at 1 and 4 months post-transplantation. RU values were calculated from percentages of donor type cells with antigenic markers specifying LT- or ST- HSC. Relative repopulating abilities are the RU values of the mutants divided by those of the normal control to give fractions of the normal repopulation ability. Six mice were analyzed per genotype at each time point, and all mutant values are significantly different from B6 ($P < 0.001$).

‡CMP gives the fraction of normal 9-day CFU-S.

§Totals predicted are the products of the 3 independent differentiation stage fractions.

‖Totals observed E, L are fractions of total erythrocyte and lymphocyte competitive repopulation after 6 months LT- and ST- HSC are common erythroid and lymphoid precursors. CMP contains no lymphoid cells.