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(54) Title: COMPOSITIONS AND METHODS FOR TREATING ACUTE MYELOID LEUKEMIA

(57) Abstract: A method of treating acute myeloid leukemia in a mammal in need of such treatment by inhibiting G-CSF activity or reducing G-CSF protein level in the mammal. This may be accomplished, for example, by administering to the mammal an effective amount of an G-CSF antagonist or by inhibiting expression of a G-CSF gene. Compositions and polynucleotide constructs useful in the invention are also disclosed.



WO 2004/043353 A2

COMPOSITIONS AND METHODS FOR TREATING ACUTE MYELOID LEUKEMIA

FIELD OF THE INVENTION

This invention relates to novel compositions and methods for the treatment of acute myeloid leukemia (AML). Specifically, it relates to compositions that inhibit or antagonize the activities of granulocyte colony-stimulating factor (G-CSF) and uses thereof.

BACKGROUND OF THE INVENTION

Acute myeloid leukemia (AML), also referred to as non-lymphoid, myeloblastic, granulocytic or myelocytic leukemia, affects various white blood cells including granulocytes, monocytes and platelets. Leukemic cells accumulate in the bone marrow, replace normal blood cells and spread to the liver, spleen, lymph nodes, central nervous system, kidneys and gonads.

AML is the predominant form of neonatal leukemia, but represents only a small proportion of childhood and adolescent cases. Nonetheless, approximately 500 children are diagnosed with AML in the United States each year being the second most common malignancy in children. AML accounts 80 percent of acute leukemias in adults. It is slightly more common in males, but occurs with roughly equal frequency in persons of European or African descent at any age. There is a greater incidence of leukemia among people exposed to large amounts of radiation and certain chemicals (e.g. benzene). See Lichtman et al., "Acute Myelogenous Leukemia" in Beutler et al. eds., Williams Hematology, 6th ed., McGraw-Hill, New York (2001). The mortality rate from AML increases from only about 0.5 per 100,000 for patients under age 10 to about 20 per 100,000 among octogenarians.

Chemotherapy is the most common form of therapy for children with AML. Autologous and analogous blood stem cell transplantation may be performed as part of treatment. Allogeneic blood stem transplantation is a

preferred treatment for those patients with AML who are at a high-risk of relapse or who have disease that is resistant to other treatments.

Although approximately 80 to 90 percent of children with AML attain remissions, some of those patients have later recurrences. Between 40 to 50 percent of children with AML achieve long-term remissions with chemotherapy.

In adults, 50-90% of patients attain a remission with modern chemotherapy regimens that include anthracycline and cytarabine. The median survival is about 12 months overall; of those patients who enter remission, 24-month survival is 30% and 60-month survival is 10% (Lichtman et al., "Acute Myelogenous Leukemia" in Beutler et al. eds., Williams Hematology, 6th ed., McGraw-Hill, New York (2001), page 1066)

Acute promyelocytic leukemia (APL) is a subtype of AML according to the French-American-British (FAB) Cooperative Group classification. This variant is associated with an exaggerated hemorrhagic syndrome and can occur at any age. Approximately 7 percent of adult AML is APL (Lichtman et al., "Acute Myelogenous Leukemia" in Beutler et al. eds., Williams Hematology, 6th ed., McGraw-Hill, New York (2001), page 1054; TR Golub, "The genetics of AML: an update" in Schechter GP et al. Eds., "Hematology 1999" (American Society of Hematology Education Program Book), page 102-111). Acute promyelocytic leukemia is specifically associated with a chromosome translocation which involves the retinoic acid receptor (RAR) alpha ($RAR\alpha$) gene on chromosome 17, and which results in an oncogenic, abnormal protein. $RAR\alpha$ variably translocates and fuses to several distinct loci. One such locus is the promyelocytic leukemia (*PML*) gene on chromosome 15 [t(15;17)]. This translocation leads to PML- $RAR\alpha$, a nonfunctional RAR that does not respond to normal physiologic levels of circulating retinoic acid. About 95 percent of APL is the t(15;17)(q31;q22) variety, i.e. PML- $RAR\alpha$ (Jansen et al., 1999, Blood. 94:39-45). Cell transcription and differentiation are thus arrested by the inability of the RAR to activate normal target genes. The *PML* gene seems to function as a histone deacetylase, which also serves to suppress gene transcription and cellular maturation. The administration of all-*trans*-retinoic

acid (ATRA) allows the histone deacetylation complex to dissociate from the RAR-PML fusion protein, enabling the RAR to function appropriately, which, in turn, leads to cellular differentiation. Retinoic acid forces terminal maturation of the malignant cells and this application represents the first generally accepted differentiation-based therapy in leukemia.

RAR α translocations may also include t(11;17) and other variant translocations involving chromosome 17 (Grignani et al., 1994, Blood. 83:10-25; Kalantry et al., 1997, J. Cell Physiol. 173:288-96; He et al., 1998, Nature Genetics, 18: 126-135; Zelent et al., Oncogene 20: 7186-7203). Fusions involving t(11;17) translocate the RAR α gene on chromosome 17 to the promyelocytic leukemia zinc finger (PLZF, also known as ZNF145) gene on chromosome 11, creating the PLZF-RAR α fusion. About 5 percent of APL is the t(11;17)(q32;q21) variety, i.e., PLZF-RAR α (Janssen et al., 1999, Blood. 94:39-45). In less common APL variants, such as t(11;17), administration of ATRA enables removal of the histone deacetylase complex from RAR α , but does not dislodge it from the fusion protein. Consequently, gene transcription remains suppressed.

Patients with t(15;17) APL generally have a favorable prognosis when treated with all-trans retinoic acid (ATRA) and anthracycline-based chemotherapy (Frankel et al., 1994, Ann Intern Med. 20:278-86; Kanamaru et al., 1995, Blood 85:1220-6; Fenaux et al., 1993, Blood 82:3241-9). Five-year survival rates of 70-80% have been reported with the most impressive results being seen with addition of maintenance therapy (Fenaux et al., 1999, Blood 94:1192-200). Arsenic trioxide also is an effective anti-leukemic drug in t(15;17) APL, and As₂O₃ therapy or As₂O₃ and retinoic acid (RA) combination therapy may be beneficial for treating t(15;17) APL (Rego et al., 2000, PROC NATL ACAD SCI USA. 97(18):10173-78).

Patients with the t(11;17) translocation, however, have a poor prognosis and respond less well to conventional APL therapies such as all-trans retinoic acid and/or arsenic trioxide (Licht et al., 1995, Blood 85:1083-1094; Fenaux et al., 1999, Blood. 94:1192-1200; Rego et al, 2000, PROC NATL ACAD SCI USA. , 97(18):10173-78). In these patients, resistance to RA could be

conferred by the PLZF-RAR α protein itself, or by the RAR α -PLZF fusion protein which could function as an aberrant transcription factor because it retains part of the PLZF DNA binding domain and still binds to PLZF target sites (Li et al., 1997, J. Biol. Chem. 272: 22447-22455). There is, therefore, a need for an improved treatment for this type of APL.

Granulocyte colony-stimulating factor (G-CSF) is a glycoprotein that influences the survival, proliferation, differentiation and function of mature neutrophil granulocytes and their precursors. Experiments with G-CSF knock out mice have shown that G-CSF is a primary physiological regulator of neutrophil production. (Lieschke, "CSF-deficient mice - what have they taught us?" in The Molecular Basis of Cellular Defence Mechanisms, Wiley, chichester (CIBA Foundation Symposium 204) pp. 60-77, 1997). Other possible physiological roles remain unknown (Metcalf, 1993, Blood. 82:3513).

U.S. Patent No. 5,777,193, incorporated herein by reference, discloses transgenic animal in which expression of at least one of GM-CSF, G-CSF and CSF-1 is disrupted.

Although the role of GM-CSF in juvenile myelomonocytic leukemia has been evaluated *in vivo* (Birnbaum et al., 2000, Mol Cell. 5:189-95.), the role of endogenous hematopoietic growth factor production on the development and progression of acute myeloid leukemia has not been fully elucidated. However, one case report of G-CSF treatment of a t(11;17) APL reported a favourable outcome (Jansen et al., Blood 1999:94:39-45) indicating that t(11;17) APL cells are responsive and susceptible to alterations in G-CSF concentrations *in vivo*.

A murine model of acute myeloid leukemia based on transgenic overexpression of PLZF has been generated (He et al., 1998, Nature Genetics 18:126-135). Wild-type (WT) mice carrying the PLZF-RAR α transgene develop a myeloproliferative syndrome culminating in lethal acute myeloid leukemia with 100% penetrance. These mice are a useful animal model for evaluating APL therapies (Rego et al., 2000, PROC NATL ACAD SCI USA. . 97(18):10173-78).

SUMMARY OF THE INVENTION

The invention provides novel compositions and methods for the treatment of acute myeloid leukemia (AML). Specifically, this invention provides methods and compositions that inhibit or antagonize the activities of granulocyte colony-stimulating factor (G-CSF) and uses thereof for the treatment of acute promyelocytic leukemia.

According to one aspect, the invention provides a method for the treatment of acute myeloid leukemia in a mammal, comprising inhibiting G-CSF activity or reducing G-CSF protein level or concentration in the mammal.

A further embodiment of the invention utilizes a composition for treating leukemia comprising a G-CSF antagonist and a pharmaceutically acceptable carrier or adjuvant.

In one embodiment, the present invention provides a method for the treatment of acute myeloid leukemia in a mammal in need of such a treatment. The inventive method comprises inhibiting G-CSF activity or reducing G-CSF protein level in the mammal.

Preferably, the G-CSF protein level in the mammal is reduced by inhibiting the expression of G-CSF gene in the mammal.

According to a preferred embodiment, an antisense G-CSF polynucleotide molecule is administered to the mammal, whereby the G-CSF gene expression is inhibited.

As readily recognized by those ordinarily skilled in the art, the antisense G-CSF polynucleotide is preferably operatively linked to a suitable regulatory element, preferably as a component of a vector.

According to another embodiment of the present invention, the G-CSF activity may be inhibited by administering to the mammal an antagonist of G-CSF. Suitable antagonists include an anti-G-CSF antibody, preferably a humanized antibody or a monoclonal antibody. The invention may also use chimeric antibodies.

According to still another embodiment, gene therapy methods may be used whereby the G-CSF gene in the mammal is disrupted.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing survival curves for six mice genotypes, namely, normal wild-type mice, wild-type mice with the PLZF-RAR α translocation, G-CSF deficient mice, G-CSF deficient mice with the PLZF-RAR α translocation, GM-CSF deficient mice, and GM-CSF deficient mice with the PLZF-RAR α translocation.

Figures 2A through 2F are graphs of serial white cell counts of the six mice genotypes, respectively.

Figures 3A and 3B are plots of the initial and final white cell counts, respectively, of the six mice genotypes.

Figures 4A and 4B are plots of the initial and final granulocyte counts, respectively, of the six mice genotypes.

Figures 5A and 5B are plots of the initial and final red cell counts, respectively, of the six mice genotypes.

Figures 6A and 6B are plots of the initial and final platelet counts, respectively, of the six mice genotypes.

DETAILED DESCRIPTION

As discussed above, the PLZF-RAR α fusion oncogene is associated with acute promyelocytic leukemia in humans, and a murine model of acute myeloid leukemia based on transgenic overexpression of PLZF-RAR α has been generated (He et al., 1998, Nature Genetics. 18:126-135). The PLZF-RAR α transgene acts dominantly, and wild-type (CSF-replete) mice carrying the PLZF-RAR α transgene develop a myeloproliferative syndrome culminating in lethal acute myeloid leukemia with 100% penetrance.

To determine if endogenous G-CSF or GM-CSF played a role in modifying the leukaemia driven by PLZF-RAR α , an experiment was set up that exploited the absolute genetically-determined CSF deficiency of G-CSF and GM-CSF knockout mice. The PLZF-RAR α transgene was bred onto mouse strains with absolute hematopoietic growth factor deficiency due to targeted

gene disruption, either of G-CSF (G-/- mice) or GM-CSF (GM-/- mice). See U.S. Patent No. 5,777,193.

G-CSF-deficient mice (Lieschke et al., 1994, Blood. 84:1737-1746), GM-CSF-deficient mice (Stanley et al., 1994, Proc Natl Acad Sci USA. 91:5592-5596) and PLZF-RAR α mice (He et al., 1998, Nature Genetics. 18:126-135) were interbred to generate 6 cohorts of 14-21 mice of the following genotypes: wild-type (WT); wild-type carrying the PLZF-RAR α transgene (WT- PLZF-RAR α +); G-CSF-deficient (G-/-); G-CSF-deficient carrying the PLZF-RAR α transgene (G-/- PLZF-RAR α +); GM-CSF-deficient (GM-/-); GM-CSF-deficient carrying the PLZF-RAR α transgene. The breeding program ensured that mice carrying the PLZF-RAR α transgene were hemizygous for the transgene (i.e. carried only one copy of the transgenic locus). All mice were housed in positive-pressure micro-isolators in the Ludwig Institute for Cancer Research Animal Facility. The experimental protocol was approved by the LICR/DOS Animal Ethics Committee.

To separate mice into their cohorts, mice were genotyped at the G-CSF, GM-CSF and PLZF-RAR α transgene loci by PCR of DNA prepared from tail clips. PCR-genotyping was carried out as described: G-CSF locus (Lieschke et al, 1994, Blood. 84:1737-1746); GM-CSF locus (Stanley et al, 1994, Proc Natl Acad Sci USA. 91:5592-5596); PLZF-RAR α transgene locus (He et al., 1998, Nature Genetics. 18:126-135). Approximately 20 months into the experiment, all surviving mice in the G-CSF-deficient cohorts were again genotyped on DNA prepared from a second tail clip, to confirm their presumed genotype at the G-CSF and PLZF-RAR α transgene loci.

The study was a parallel group longitudinal cohort study. Details of the cohorts are summarised in Table 1 below. Mice were monitored daily by animal house staff.

For the survival analysis, mice were found dead in cages were deemed to have died on that day. Mice were monitored daily for signs of clinical distress (including rapid respiration, ruffled coat, abnormal respiration rate, abnormal behaviour), and when clinical distress was recognized, mice were sacrificed and treated as deaths on the day of sacrifice. Two groups of mice

were treated as censored events in the survival analysis: mice for whom no death date was recorded were censored on the date of their last bleed for a haematological analysis; mice that were sacrificed at the end of the experiment were censored on the date of their being culled.

Haematological endpoints were determined as follows. Mice were bled by saphenous vein puncture, initially at approximately 6 weekly intervals, but when survival was observed to be longer than had been anticipated in the second year of the experiment, this was changed to 3 monthly intervals. For determination of red cell, total leukocyte, and platelet counts, samples were diluted 1:4 or 1:7 in dilution buffer, and counted on a Sysmex-K1000 automated blood counter (Toa Medical Electronics Co, Kobe, Japan) as previously described (Lieschke et al., 1994, *Blood*. 84:1737-1746). For differential white cell counts, blood smears of undiluted blood were prepared, stained by May-Grunwald/Giemsa, and 100 nucleated cells were scored under light microscopy at X100-X400 magnification. Haematological data were displayed as serial data for all individual mice per group (e.g. Figure 2), and comparative analyses were performed on the data from the first bleed and last bleed on the experiment, irrespective of the age of the mice at the time of first and last bleeds.

All data were tabulated in spreadsheets, and statistical analyses were carried out using GraphPad Prism software (Version 3.0, GraphPad Software Inc, San Diego, CA). The graphing function of the statistical analysis software was used to generate graphs, which are shown in the accompanying drawing figures. Survival data were compared by the Logrank test. All two-group comparisons reported employed the non-parametric Mann Whitney test. A two-tailed p-value of less than 0.05 was regarded as significant.

Figure 1 depicts Kaplan-Meier survival curves of wild-type, G-CSF-deficient and GM-CSF-deficient mice with and without the PLZF-RAR α transgene. Cohorts of mice as detailed in Table 1 were followed until death, sacrificed due to clinical signs of illness, or culled at the end-of-experiment. Mice found dead or culled because of clinical illness are treated as deaths; where the death date was unknown, mice are censored at the date of their last

blood sampling. Mice culled at end-of-experiment are also censored. Although CSF-replete (WT) mice and GM-CSF-deficient (GM-/-) mice carrying the PLZF-RAR α transgene died with median survivals of 408 and 390 days respectively, G-CSF-deficient (G-/-) mice carrying the transgene survived normally with a median survival of >731 days, not significantly different to that of their non-transgenic G-/- controls (692 days) and non-transgenic WT and GM-/- peers (>716 days and 674 days respectively).

Figure 1 shows the survival curves for the 6 cohorts of mice. Wild-type and GM-CSF-deficient mice carrying the leukaemogenic PLZF-RAR α transgene died with a median survival of 408 and 390 days, which was significantly shorter than that of their non-transgene carrying peers (median survivals >716 and 674 days respectively, $p<0.0001$ and $p<0.0001$). In contrast, G-CSF-deficient mice carrying the leukaemogenic PLZF-RAR α transgene survived indistinguishably from their non-transgenic peers, with median survivals of >731 and 692 days respectively. The survival of G-/-PLZF-RAR α + mice was also significantly longer than either WTPLZF-RAR α + and GM-/-PLZF-RAR α + mice ($p<0.0001$, $p<0.001$), indicating that G-CSF-deficiency, but not GM-CSF deficiency, prolonged survival of PLZF-RAR α + mice. The statistical comparison of the survival curves is summarized in Table 2.

Figures 2-6 show that the WTPLZF-RAR α + and GM-/-PLZF-RAR α + mice developed haematological features of myeloid leukaemia in their pre-terminal peripheral blood haematological analysis, but that G-/-PLZF-RAR α + did not.

Figure 2 depicts the serial peripheral blood total white cells counts for individual WT (panels A,B), G-CSF-deficient (panels C,D), and GM-CSF-deficient (panels E,F) mice, without the PLZF-RAR α transgene (panels A,C,E) and carrying the PLZF-RAR α transgene (panels B,D,F). Wild-type (i.e. CSF-replete) mice maintained a normal total white cell count over their lifespan (Figure 2A), as did non-transgenic G-/- and GM-/- mice. Elevated total white cells counts characterized the preterminal white cell count of WT and GM-CSF-/- mice carrying the PLZF-RAR α transgene (panels B and F): e.g. 6/16 WTPLZF-RAR α + mice developed supra-physiological white cell counts in their

pre-terminal analysis (Figure 2A&B). In contrast, G-CSF^{-/-} mice carrying the transgene maintained a stable total white cell count throughout their lives (Figure 2D).

Figures 3A and 3B present scattergraphs of the peripheral blood total white cell counts for mice of the 6 genotypes as shown. Figure 3A shows data for the first haematological analysis (at a median age of approximately 3 months as detailed in Table 1), and Figure 3B shows data for the last haematological analysis before death or culling. (P-values are given for the comparisons bracketed; NS = not significantly different). Comparison of the baseline (Figure 3A) and final (Figure 3B) total white cell counts showed no significant differences at baseline (Figure 3A). However, comparison of the final total white cell counts showed supra-physiological levels in WTPLZF-RAR α ⁺ and GM^{-/-}PLZF-RAR α ⁺ mice compared with their respective controls (p=0.2 and p=0.002). In contrast, G-CSF-deficient mice had normal total white cell counts at the start and end of the experiment, and no G^{-/-}PLZF-RAR α ⁺ mouse developed a supraphysiological total white cell count. In summary, although all mice started with comparable total white cell counts, non-physiological elevated end-of-experiment total white cell counts occurred only in WTPLZF-RAR α ⁺ and GM^{-/-} PLZF-RAR α ⁺ mice, but not in G^{-/-} PLZF-RAR α ⁺ mice.

Examination of the total granulocyte counts provided further evidence as to whether mice developed a granulocytosis typical of leukaemia or not. Figures 4A and 4B are scattergraphs of the peripheral granulocyte (neutrophil) counts for mice of the 6 genotypes shown. Figures 4A and 4B show first and last peripheral blood total neutrophil (granulocyte) counts, respectively (P-values are given for the comparisons bracketed; NS = not significantly different.) WT, WTPLZF-RAR α ⁺, GM^{-/-} and GM^{-/-}PLZF-RAR α ⁺ mice had equivalent neutrophil counts at their first blood analysis. As expected, G^{-/-} and G^{-/-}PLZF-RAR α ⁺ mice were neutropenic relative to the other genotypes (Lieschke et al, 1994, Blood. 84:1737-1746), but had comparable neutrophil counts to each other (Figure 4A). At their pre-terminal peripheral blood analysis, WTPLZF-RAR α ⁺ and GM^{-/-}PLZF-RAR α ⁺ mice had developed a

significant granulocytosis relative to their respective controls, consistent with the development of leukaemia, and recapitulating the previously described effects of the PLZF-RAR α transgene (Figure 4B) ($p=0.05$, $p=0.002$ respectively) (He et al., 1998, Nature Genetics. 18:126-135). Although the final granulocyte counts of the G $^{-/-}$ -PLZF-RAR α^{+} were statistically significantly higher than those of their G $^{-/-}$ controls at the end of the experiment ($p=0.04$), counts remained in the physiological range. The granulocyte counts of the G $^{-/-}$ -PLZF-RAR α^{+} mice were significantly lower than those of both the WTPLZF-RAR α^{+} and GM $^{-/-}$ -PLZF-RAR α^{+} ($p=0.0002$, $p<0.0001$), evidencing that the G $^{-/-}$ -PLZF-RAR α^{+} mice had not developed leukemia, whereas the WTPLZF-RAR α^{+} and GM $^{-/-}$ -PLZF-RAR α^{+} mice had. In summary, non-physiological elevated end-of-experiment granulocyte counts occurred only in WTPLZF-RAR α^{+} and GM $^{-/-}$ -PLZF-RAR α^{+} mice, but not in G $^{-/-}$ -PLZF-RAR α^{+} mice. G $^{-/-}$ -PLZF-RAR α^{+} mice had statistically significantly elevated end-of-experiment granulocyte counts relative to their non-transgenic G $^{-/-}$ controls, but these remained within the physiological range.

Anaemia is a common clinical accompaniment of acute leukaemia (Lichtman et al., "Acute Myelogenous Leukemia" in Beutler et al. eds., Williams Hematology, 6th ed., McGraw-Hill, New York (2001), page 1049) and is also typical of the leukaemia in PLZF-RAR α transgenic mice (He et al., 1998, Nature Genetics, 18: 126-135). Figures 5A and 5B are scattergraphs of the peripheral blood red cell counts for mice of the 6 genotypes. Figure 5A shows the first and Figure 5B shows the last red cell counts, respectively (P-values are given for the comparisons bracketed; NS = not significantly different.). Mice of all 6 cohorts had comparable peripheral blood red cell counts at their first blood analysis (Figure 5A). At their final blood analysis (Figure 5B), WTPLZF-RAR α^{+} and GM $^{-/-}$ -PLZF-RAR α^{+} had developed significant declines in their red cell counts indicative of anaemia relative to their respective controls ($p<0.0001$, $p=0.0009$), whereas G $^{-/-}$ -PLZF-RAR α^{+} mice had comparable red cell counts to their controls and were not anaemic. The red cell counts of G $^{-/-}$ -PLZF-RAR α^{+} mice were also significantly higher than those of WTPLZF-RAR α^{+} and GM $^{-/-}$ -PLZF-RAR α^{+} peers ($p=0.0011$ and $p<0.0001$ respectively). In summary, all

mice started with comparable red cell counts; significantly reduced end-of-experiment red cell counts indicative of anaemia occurred only in WTPLZF-RAR α + and GM-/-PLZF-RAR α + mice, but not in G-/-PLZF-RAR α + mice.

Thrombocytopenia is also a usual accompaniment of myeloid leukaemia (Lichtman et al., "Acute Myelogenous Leukemia" in Beutler et al. eds., Williams Hematology, 6th ed., McGraw-Hill, New York (2001), page 1050), and also accompanies the leukaemia of PLZF-RAR α + transgenic mice (He et al., 1998, Nature Genetics. 18: 126-135). Figure 6 presents scattergraphs of the peripheral blood platelet counts for mice of the 6 genotypes: panels 6A and 6B show first and last blood platelet counts respectively (P-values are given for the comparisons bracketed; NS = not significantly different.). Mice of all 6 cohorts had had comparable peripheral blood platelet counts at their first blood analysis (Figure 6A). At their final blood analysis, WTPLZF-RAR α + and GM-/-PLZF-RAR α + had developed significant declines in their platelet counts indicative of thrombocytopenia relative to their respective controls (p=0.0003, p=0.0008), whereas G-/-PLZF-RAR α + mice had comparable platelet counts to their controls and were not thrombocytopenic. In summary, all mice started with comparable platelet counts; significantly reduced end-of-experiment platelet counts indicative of thrombocytopenia occurred only in WTPLZF-RAR α and GM-/-PLZF-RAR α mice, but not in G-/-PLZF-RAR α mice.

Although all genotypes were diagnosed at the start of the experiment by PCR genotyping analysis of tail DNA, fresh tail samples from surviving G-/-PLZF-RAR α + mice were obtained in their second year of life, DNA extracted, and PCR genotyping analysis undertaken. This verified that these surviving mice indeed carried the PLZF-RAR α -transgene. Their G-CSF (-/-) genotype was also re-confirmed. This indicated that the surviving G-/-PLZF-RAR α + mice were indeed carrying the leukaemogenic transgene, and that loss of the PLZF-RAR α transgene or mistaken mouse identity were not spurious causes of the phenomenon observed.

The following Table 1 details the characteristics of the mice in the experiment on which the present invention is based. It shows the size of the cohorts, the sex ratio of the mice in the groups, the age at the first (baseline)

haematological analysis, the birthdates of the cohorts (which document that the experiment was a parallel group study). It includes a breakdown of how many mice in each group fell into the 4 survival endpoints, which is important for the interpretation of the survival curves in Figure 1. It also provides the median survival data in a tabulated form.

Table 1. Characteristics of the mice.

Genotype	WT	WT PLZF- RAR α +	G-/-	G-/- PLZF- RAR α +	GM-/-	GM-/- PLZF- RAR α +
n	20	16	14	17	21	13
male:female	11:9	7:9	5:9	14:3	14:7	6:7
Median survival (range)	>716 (292 ^d - 716 ^c)	408 (203 ^d -489 ^d)	692 (296 ^d - 737 ^c)	>731 (283 ^d - 731 ^c)	674 (403 ^d - 717 ^d)	390 (260 ^d - 528 ^d)
Median age at first bleed (range) d	88 (53-367)	82 (53-312)	114 (72-507)	114 (69-320)	216.5 (69-389)	115.5 (69-325)
Survival end-point						
-found dead	4	11	3	2	6	8
-sacrificed due to illness	2	1	4	4	6	4
-censored @ end-of- experiment	12	0	4	8	1	0
-censored at last bleed	2	3 ¹	3	3	8 ²	1

Notes: 1. 1 death date recorded but no bleed or information (treated as death).

2. 1 mouse died during last bleed (censored @ LB).

3. d = death; c= end-of-experiment culled/censored.

Table 2 shows the p-values for the statistical comparisons necessary to interpret Figure 1. WT PLZF-RAR α and GM-/- PLZF-RAR α mice have significantly different (shorter, see Figure 1) survival compared with WT and GM-/- mice respectively, and G-/-PLZF-RAR α mice have significantly different (longer, see Figure 1) survival than WTPLZF-RAR α and GM-/-PLZF-RAR α mice.

Table 2. Survival statistical comparison

	WT	WT PLZF- RAR α	G-/-	G-/- PLZF- RAR α	GM-/-	GM-/- PLZF- RAR α
Wild Type(WT)		<0.0001	NS	n/a	NS	n/a
WT PLZF-RAR α			n/a	<0.0001	n/a	NS
G-/-				NS	NS	n/a
G-/- PLZF-RAR α					n/a	<0.0001
GM-/-						<0.0001
GM-/- PLZF-RAR α						

The foregoing tests show that the PLZF-RAR α transgene resulted in shortened survival of mice when the transgene was carried on CSF-replete and GM-CSF deficient backgrounds. These mice died with the haematological hallmarks of myeloid leukaemia: leukocytosis, granulocytosis, anaemia and thrombocytopenia. In marked contrast, G-CSF-deficient mice carrying the same leukaemogenic PLZF-RAR α transgene survived normally, and their haematological profile did not suggest any features of myeloid leukaemia: they remained relatively granulocytopenic (as is typical of G-CSF-deficient mice), and did not develop anaemia or thrombocytopenia. We conclude that G-CSF deficiency suppressed the leukaemogenic potential of the PLZF-RAR α transgene.

Examples

The following examples describe G-CSF antagonists which could be used to treat leukemia.

Example 1: Antagonists based on modification of the G-CSF ligand.

Although no antagonist of G-CSF has been reported that is a modification of the ligand, a competitive GM-CSF antagonist (E21R) has been developed that has a single amino acid modification of the GM-CSF polypeptide chain (Hercus et al, 1994, PROC NATL ACAD SCI USA. , 91:5838-42,) E21R is devoid of agonist activity. E21R has been evaluated in human studies (Olver et al., 2002, Can Chemother Pharmacol. 50:171-8). This antagonist of GM-CSF is

proof-of-principle that a competitive antagonist of G-CSF could be developed by an analogous approach, since the binding domains of G-CSF to its receptor have now been well characterised (Layton et al., 2001, J Biol Chem. 276:36779-87)

Example 2: Administration of G-CSF or G-CSFR antibodies.

Neutralizing monoclonal antibodies to G-CSF exist (Layton et al., 1991, J Biol Chem. 266:23815-23823). The antibodies have only modest affinity, but are proof-of-principle that antibodies with specificity for G-CSF can be made. It is a standard procedure to humanize such antibodies once the high-affinity binding moiety is obtained. It is also a straight-forward mechanistic application of an existing technology to search out direct antibodies with high G-CSF affinity. See, for example, U.S. Patent Nos. 5,969,108; 5,885,793 and 5,565,332, the disclosures of which are incorporated herein by reference.

The generation of hG-CSF receptor antibodies is disclosed in U.S. Patent No. 5, 902,584. This particular patent covers the use of the G-CSFR mAb to inhibit JAK phosphorylation (ie receptor signalling). Australian Patent No. AU 695,001 also discloses such antibodies and their production. Antibodies produced by analogous techniques could be used to combat leukemia.

Example 3: Administration of a soluble G-CSF receptor "Ligand Trap".

Soluble forms of the receptors for growth factors act to suppress growth factor activity by acting as binding sumps for ligand. Several soluble forms of the G-CSF receptor exist. (Aritomi et al., 2000, Acta Crystallogr D Biol Crystallogr. 56:751-3; Iwasaki et al., 1999, J Immunol. 163:6907-11; Asano et al., 1997, Cancer Res. 57:3395-7). One of these is an engineered form (Asano et al 1997). It is also a simple matter to engineer the production new forms of soluble receptor by molecular biological manipulation of the G-CSFR cDNA. Soluble forms of the G-CSF receptor inhibit G-CSF-dependent leukaemia cell growth in vitro (Asano et al., 1997, Cancer Res. 57:3395-7).

Example 4: Immunization to G-CSF

The absolute genetically-based G-CSF-deficiency phenotype of G-/- mice has been phenocopied in mice by immunizing mice against mG-CSF (Coccia et al., 2001, *Exp Hematol.* 29:59-67), and also in dogs treated with HuG-CSF that developed antibodies that cross-reacted with canine G-CSF (Hammond et al., 1991, *J Clin Invest.* 87:704-10). High-titre autologous G-CSF-neutralizing IgG autoantibodies were induced, resulting in a durable recapitulation of the murine G-CSF-deficiency phenotype. This provides proof-of-principle for this approach in mammals. Particularly if ongoing suppression of G-CSF is important for the ongoing suppression of a leukemic clone, this approach may have clinical potential despite the risk that would accompany a prolonged G-CSF-deficiency phenotype in humans.

Example 5: Small molecule G-CSF antagonist

A small non-peptidyl molecule (called SB 247464) that is a murine G-CSF agonist has been described (Tian et al., 1998, *Science.* 281:257-9). It was identified in a screening approach that could be modified to find a G-CSF antagonist. The antagonist screen would look for suppression of the G-CSF-stimulated phenotype in the reporter assay cell line. SB 247464 is proof-of-principle that non-peptide small molecules can be found that interact with a mammalian G-CSF receptor. An appropriate screen would be expected to find an antagonist as well. Such an antagonist could be used to inhibit leukemia.

The foregoing description and examples have been set forth merely to illustrate the invention and are not intended to be limiting. Since modifications of the disclosed embodiments incorporating the spirit and substance of the invention may occur to persons skilled in the art, the invention should be construed broadly to include all variations falling within the scope of the appended claims and equivalents thereof.

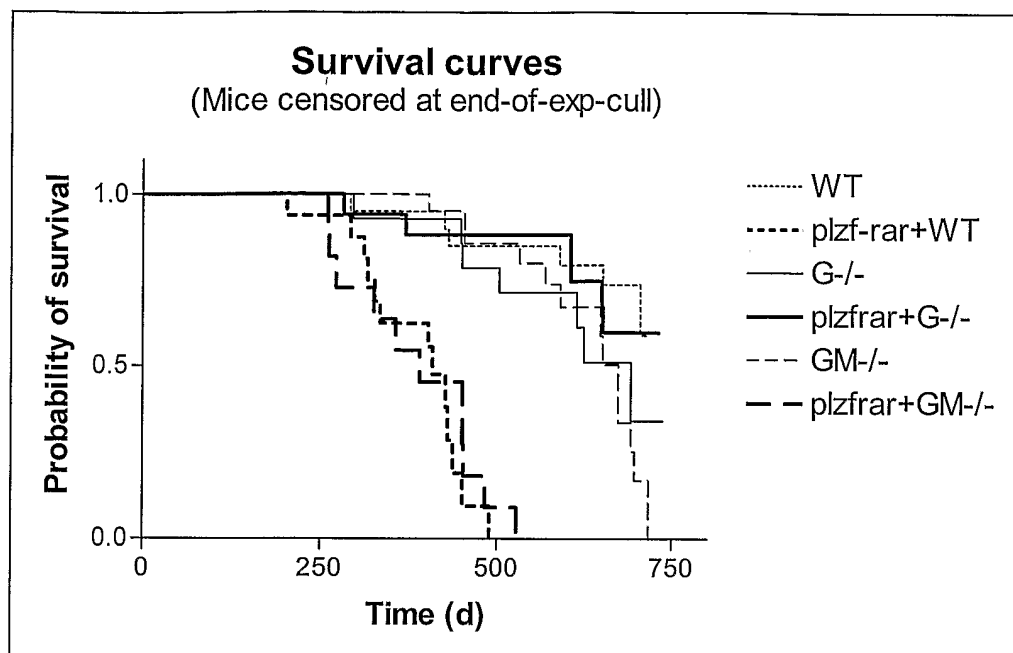
WHAT IS CLAIMED IS:

1. A method for the treatment of acute myeloid leukemia in a mammal, comprising inhibiting G-CSF activity or reducing G-CSF protein level or concentration in the mammal.
2. A method according to claim 1, wherein the G-CSF protein level/concentration in the mammal is reduced by inhibiting the expression of G-CSF gene in the mammal.
3. A method according to claim 2, wherein an antisense G-CSF polynucleotide molecule is administered to the mammal, whereby the G-CSF gene expression is inhibited.
4. A method according to claim 3, wherein the antisense G-CSF polynucleotide is operatively linked to a suitable regulatory element.
5. A method according to claim 4, wherein a vector comprising an antisense G-CSF polynucleotide is administered to the mammal.
6. A method according to claim 1, wherein the G-CSF activity is inhibited by administering to the mammal an antagonist of G-CSF.
7. A method according to claim 6, wherein the antagonist is an anti-G-CSF antibody.
8. A method according to claim 6, wherein the anti-G-CSF antibody is a humanized antibody.
9. A method according to claim 6, wherein the anti-G-CSF antibody is a monoclonal antibody.

10. A method according to claim 6, wherein the antibody is a chimeric antibody.
11. A method according to claim 1, wherein the G-CSF gene in the mammal is disrupted.
12. A composition for treating leukemia comprising a G-CSF antagonist and a pharmaceutically acceptable carrier or adjuvant.
13. A composition according to claim 12, wherein said G-CSF antagonist is a modified G-CSF ligand which binds to G-CSF receptors but fails to exhibit G-CSF activity.
14. A composition according to claim 12, wherein said G-CSF antagonist is a G-CSF specific antibody.
15. A composition according to claim 12, wherein said G-CSF antagonist is a G-CSFR specific antibody.
16. A composition according to claim 12, wherein said G-CSF antagonist is a soluble G-CSF receptor ligand trap.
17. A composition according to claim 12, wherein said G-CSF antagonist is a high-titre autologous G-CSF neutralizing IgG autoantibody.
18. A composition according to claim 12, wherein said G-CSF antagonist is a small non-peptidyl molecule.
19. A composition according to claim 12, wherein said G-CSF antagonist is a vector comprising anti-sense G-CSF DNA.

1/8

Figure 1



2/8

Figure 2A - serial white cell counts (wild-type)

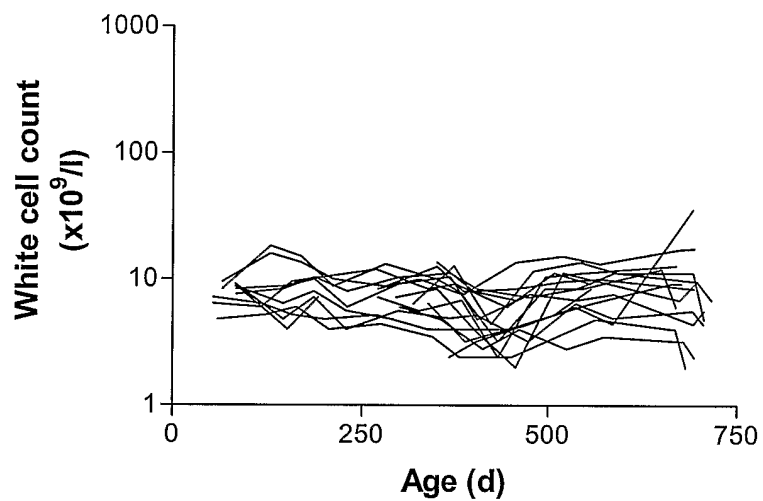
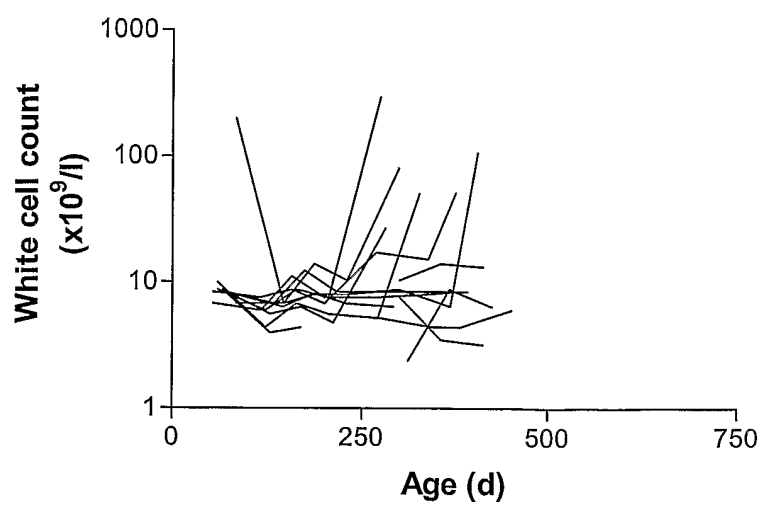


Figure 2B - serial white cell counts (WT PLZF-RAR α +)



3/8

Figure 2C - serial white cell counts (G-/-)

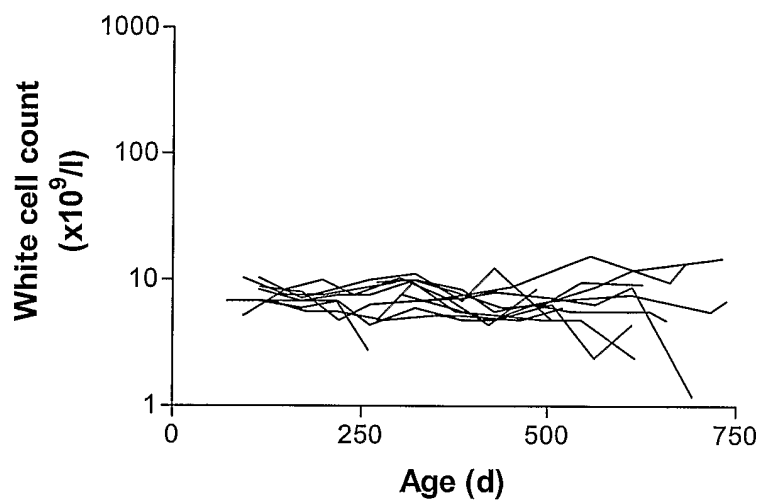
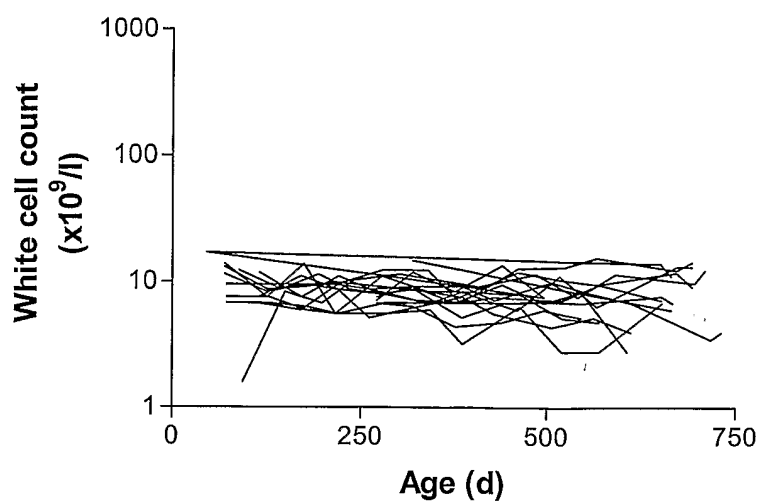


Figure 2D - serial white cell counts (G-/-PLZF-RAR α +))



4/8

Figure 2E - serial white cell counts (GM-/-)

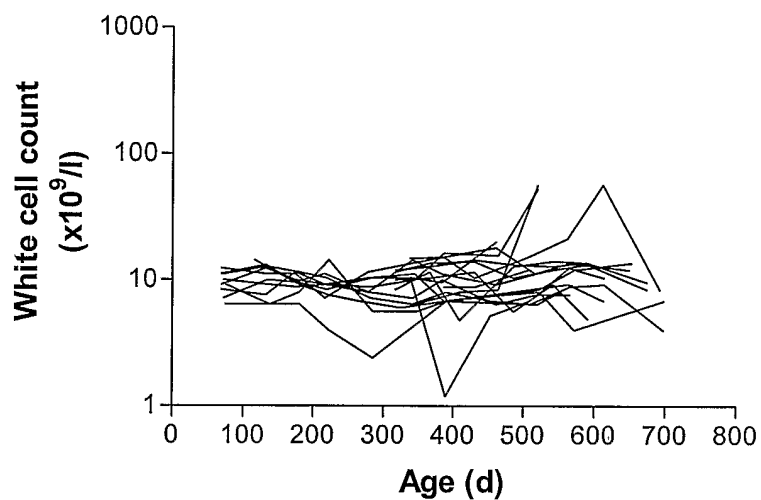


Figure 2F - serial white cell counts (GM-/-PLZF-RAR α +)

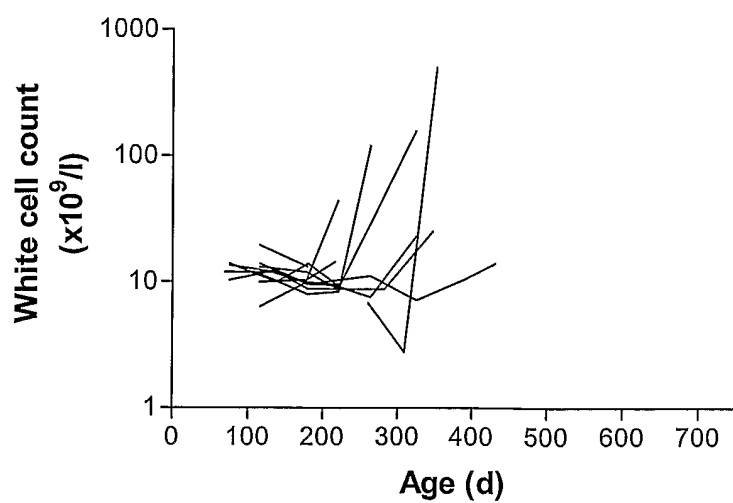


Figure 3A - First white cell count

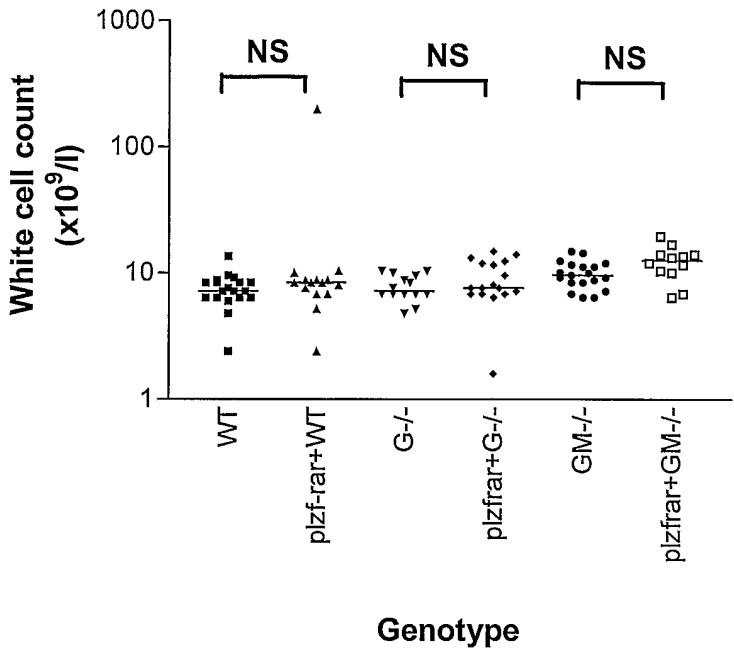


Figure 3B - last white cell count

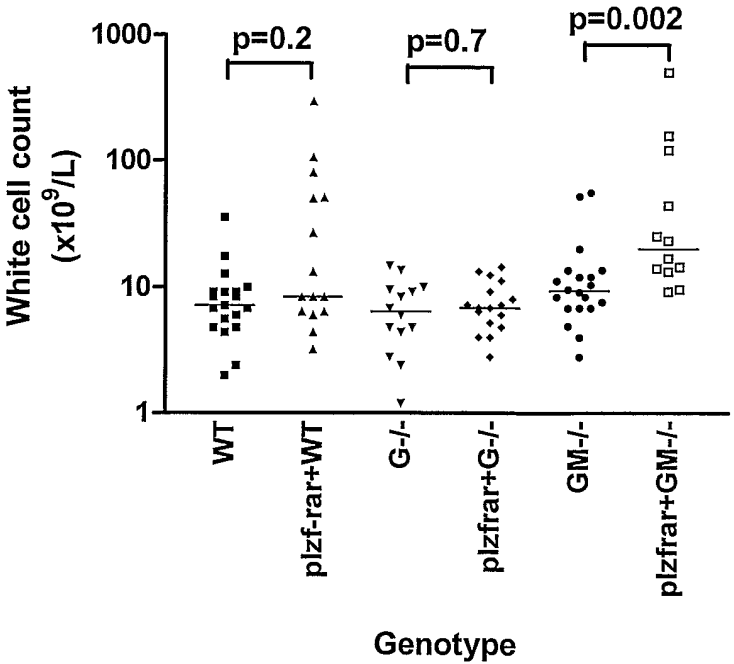


Figure 4A - first granulocyte count

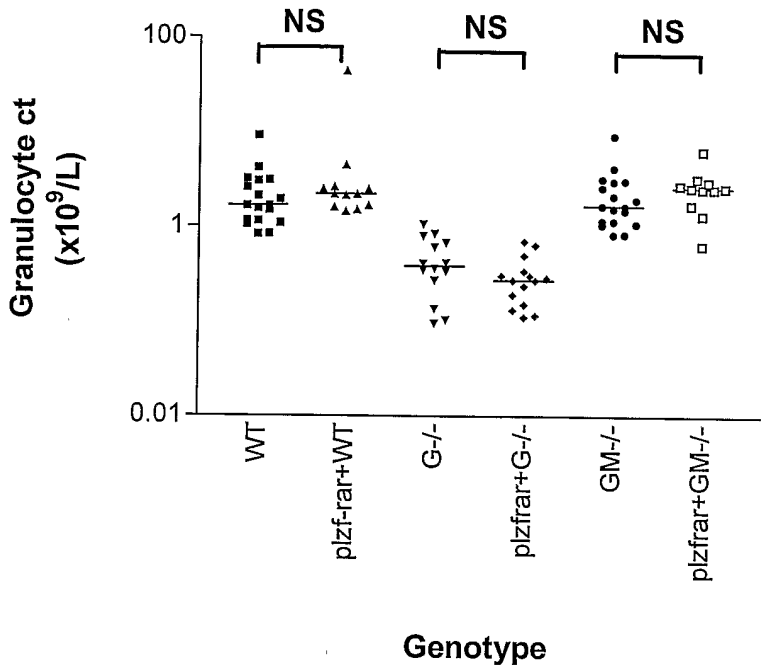


Figure 4B - last total granulocyte count

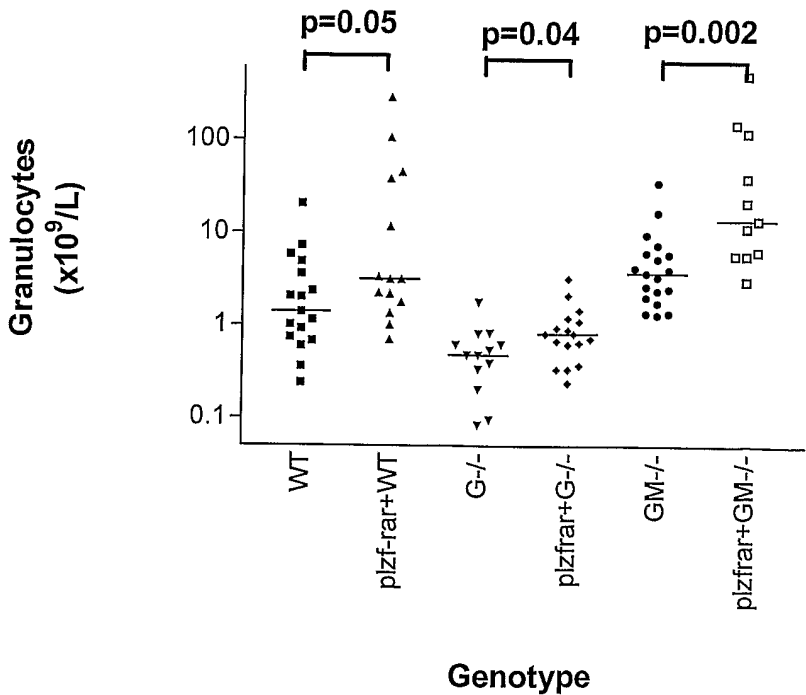


Figure 5A - First red cell count

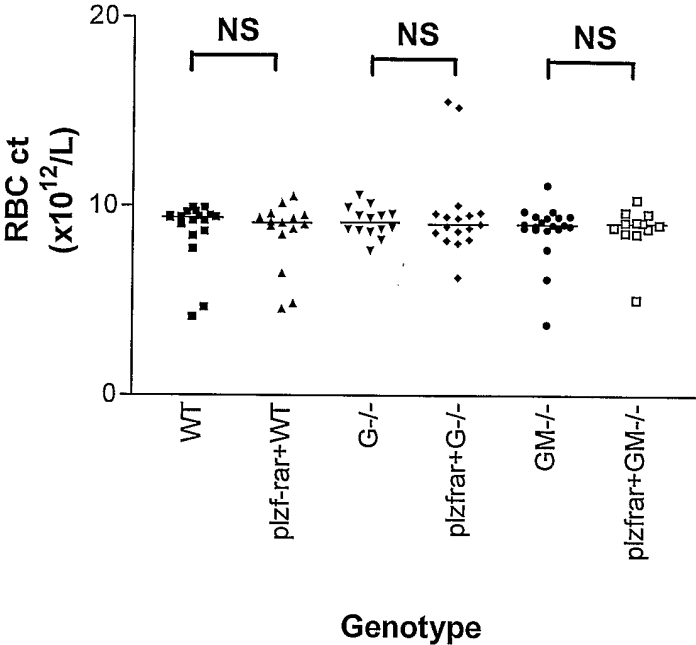


Figure 5B - Last red cell count

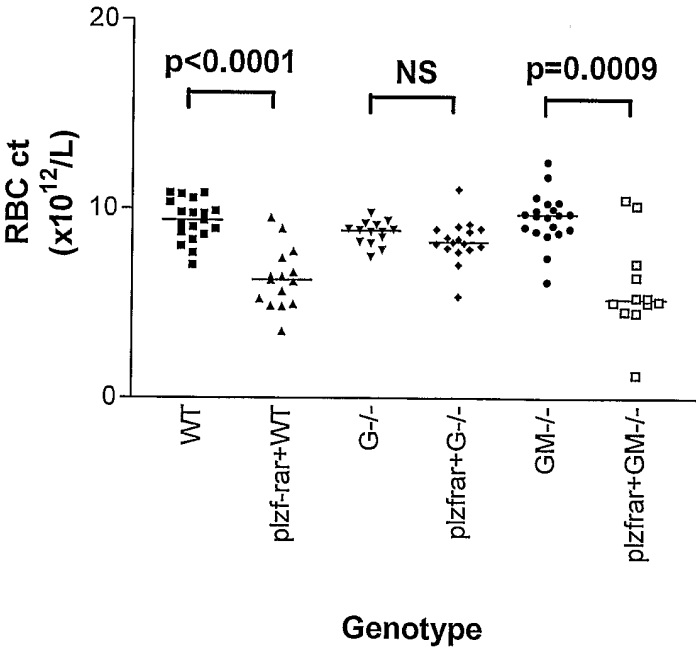


Figure 6A - first platelet count

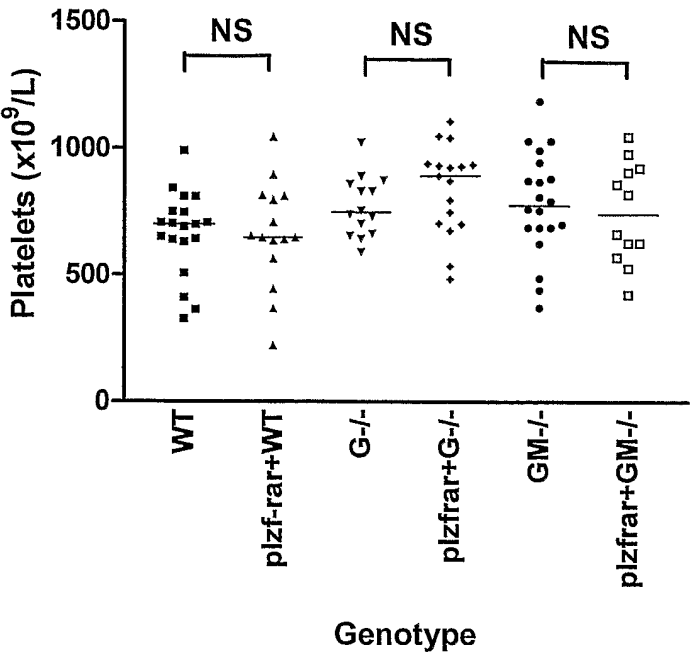


Figure 6B - last platelet count

