weeks of treatment with atorvastatin. Serum alanine aminotransferase and creatine kinase levels remained unchanged from baseline. Adverse events during the study included 4 acute pain episodes, 3 of which required hospitalization, equivalent to 1.59 hospitalizations per patient-year during enrollment on the study, this is the same as in SCA population statistics. One of these episodes involved transient mental status changes, a small pulmonary infiltrate and mild transient renal insufficiency, all of which resolved after transfusion of two units of packed red blood cells. This patient was removed from the study due to an exclusion criterion of transfusion within two weeks of entry or end point.

Much has been written about the pleiotropic effects of statins in the general population to improve clinical outcomes related to vasculopathies such as atherosclerosis. Part of this activity derives from statin activation of NOS activity. It has been speculated that these vasculoprotective effects might be useful in SCA.[3-5] Our SCA clinical trial of atorvastatin at moderate doses with physiological outcome measures is a negative study for its primary purpose. However, there are still several findings of scientific value that emerge from this project: 1) reproducing previous baseline findings of nitric oxide resistance in SCA; 2) developing an enrichment strategy for recruitment that resulted in 100% of the SCA subjects having characteristics of nitric oxide resistance; 3) secondary outcome variables indicating some evidence that endothelial function is being impacted, albeit to a smaller degree than expected. This last finding raises the question as to whether higher doses of atorvastatin or earlier intervention for longer duration should be considered for future investigation to delay vascular dysfunction in SCA patients.

Candice Bereal-Williams,[1] Roberto F. Machado,[2] Vicki McGowan II,[1] Amy Chi,[1] Christian J. Hunter,[1] and Gregory J. Kato[1]

[1]Hematology Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD; [2]Division of Pulmonary, Critical Care, Sleep and Allergy, University of Illinois College of Medicine at Chicago, Chicago, IL, USA.

Correspondence: Gregory J. Kato, 9000 Rockville Pike, MSC 1476, Building 10CRC, Room 5-5140, Bethesda, MD 20892-1476, USA. Phone: international +3014518497; Fax: international +3014517091; E-mail: gkato@mail.nih.gov.

Key words: atorvastatin, sickle cell anemia, nitric oxide resistance, vascular fuction.

The online version of this article has a Supplementary Appendix.

Acknowledgments: this research was funded by the Division of Intramural Research at the National Institutes of Health (1 ZIA HL006023-03).
Additional Contributing Authors: Lori Hunter, Carole K. Dalby, Kristine Partovi Hauser, Anitaben Tailor, Richard O. Cannon III (National Heart, Lung and Blood Institute, Bethesda, MD).

The authors thank Dr. Mark Gladwin for much helpful discussion. They also gratefully acknowledge the contributions of Mary K. Hall for expert protocol management, to James Nichols, Marlene Peters-Lawrence, and Wynona Coles for additional research coordination, the NIH Clinical Center Procedure Service, and to Britny Hall for clerical support. The authors thank the sickle cell patients and healthy volunteers whose participation made this study possible.

Citation: Bereal-Williams C, Machado RF, McGowan V II, Chi A, Hunter CJ, and Kato GJ. Atorvastatin reduces serum cholesterol and triglycerides with limited improvement in vascular function in adults with sickle cell anemia. Haematologica 2012;97(11):1768-1770.doi:10.3324/haematol.2011.054957

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

References

1. Perticone F, Ceravolo R, Maio R, Cloro C, Candigliota M, Scozzafava A, et al. Effects of atorvastatin and vitamin C on endothelial function of hypercholesterolemic patients. Atherosclerosis. 2000;152(2):511-8.
2. Laufs U, Wassmann S, Hilgers S, Ribaudo N, Bohm M, Nickenig G. Rapid effects on vascular function after initiation and withdrawal of atorvastatin in healthy, normocholesterolemic men. Am J Cardiol. 2001;88(11):1306-7.
3. Solovey A, Kollander R, Shet A, Milbauer LC, Choong S, Panoskaltsis-Mortari A, et al. Endothelial cell expression of tissue factor in sickle mice is augmented by hypoxia/reoxygenation and inhibited by lovastatin. Blood. 2004;104(3):840-6.
4. Canalli AA, Proença RF, Franco-Penteado CF, Traina F, Sakamoto TM, Saad ST, et al. Participation of Mac-1, LFA-1 and VLA-4 integrins in the in vitro adhesion of sickle cell disease neutrophils to endothelial layers, and reversal of adhesion by simvastatin. Haematologica. 2011;964:526-33.
5. Hoppe C, Kuypers F, Larkin S, Hagar W, Vichinsky E, Styles L. A pilot study of the short-term use of simvastatin in sickle cell disease: effects on markers of vascular dysfunction. Br J Haematol. 2011;153(5):655-63.
6. Kato GJ, Martyr S, Blackwelder WC, Nichols JS, Coles WA, Hunter LA, et al. Levels of soluble endothelium-derived adhesion molecules in patients with sickle cell disease are associated with pulmonary hypertension, organ dysfunction, and mortality. Br J Haematol. 2005;130(6):943-53.
7. Gladwin MT, Barst RJ, Castro OL, Gordeuk VR, Hillery CA, Kato GJ, et al. Pulmonary hypertension and NO in sickle cell. Blood. 2010;116(5):852-4.
8. Gladwin MT, Schechter AN, Ognibene FP, Coles WA, Reiter CD, Schenke WH, et al. Divergent nitric oxide bioavailability in men and women with sickle cell disease. Circulation. 2003;107(2):271-8.
9. Belhassen L, Pelle G, Sediame S, Bachir D, Carville C, Bucherer C, et al. Endothelial dysfunction in patients with sickle cell disease is related to selective impairment of shear stress-mediated vasodilation. Blood. 2001;97(6):1584-9.
10. Kaul DK, Liu XD, Fabry ME, Nagel RL. Impaired nitric oxide-mediated vasodilation in transgenic sickle mouse. Am J Physiol Heart Circ Physiol. 2000;278(6):H1799-806.
11. Nath KA, Shah V, Haggard JJ, Croatt AJ, Smith LA, Hebbel RP, et al. Mechanisms of vascular instability in a transgenic mouse model of sickle cell disease. Am J Physiol Regul Integr Comp Physiol. 2000;279(6):R1949-55.
12. Eberhardt RT, McMahon L, Duffy SJ, Steinberg MH, Perrine SP, Loscalzo J, et al. Sickle cell anemia is associated with reduced nitric oxide bioactivity in peripheral conduit and resistance vessels. Am J Hematol. 2003;74(2):104-11.

Predictors and short-term outcomes of hyperleukocytosis in children with acute myeloid leukemia: a report from the Children's Oncology Group

Adults and children with acute myeloid leukemia (AML) who present with a high initial white blood cell count (WBC) have poor outcomes, and a consistent association with induction death has been observed when the initial WBC is 100×10^9/L or over.[1] However, rates of induction death have varied between trials.[2,3] Furthermore, major advances in supportive care have been incorporated into routine clinical care such as prompt transfusion of platelets, leukapheresis, hydroxyurea, urate oxidase and hemodialysis. With these advances in supportive care, it is unclear whether poor outcomes with hyperleukocytosis in pediatric AML continue to be problematic.

The Children's Oncology Group (COG) adopted a modified AML Medical Research Council backbone and

accrued patients to trials AAML03P1 and AAML0531. In this study, we analyzed risk factors for, and outcomes associated with, hyperleukocytosis for children and adolescents with AML.

This report used data collected from AAML03P1[4] and AAML0531.[5] Both studies were approved by each institutional review board and all parents/participants provided informed consent. Common eligibility were aged one month to 21 years with *de novo* AML, and infants aged under one month with progressive disease and isolated chloromas. Hydroxyurea was excluded in both. Induction I consisted of cytarabine 100 mg/m^2/dose intravenous (i.v.) every 12 h on Days 1-10; daunorubicin 50 mg/m^2/dose i.v. on Days 1, 3 and 5; and etoposide 100 mg/m^2/dose i.v. on Days 1-5. All patients received gemtuzumab (GMTZ) on AAML03P1 whereas administration was randomized on AAML0531 (3 mg/kg/dose once on Day 6). Uniform guidelines for supportive care were provided. It was recommended that before initiation of chemotherapy, the best possible control of coagulopathy, metabolic derangement, and institution of treatment for fever and/or infection be established. Specific management for hyperleukocytosis consisted of cautious administration of packed red blood cell transfusions and liberal use of platelets as clinically indicated. Both studies suggested using allopurinol while AAML0531 also suggested considering rasburicase.

Hyperleukocytosis was defined as an initial WBC count of 100×10^9/L or over. All data concerning non-hematologic grade 3 or higher toxicities were obtained prospectively by clinical research associates of participating institutions and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. We did not describe outcomes according to GMTZ administration as the outcomes on AAML0531 are still blinded.

We examined the following potential predictors of initial WBC 100×10^9/L or over: age, race, ethnicity, body mass index (BMI) category at diagnosis, FAB AML classification and cytogenetics. BMI (>2 years) or weight-for-length (1-≤2 years) were used to delineate underweight as 10th percentile or under, overweight as 95th percentile or over, and normal weight as more than 10th to less than 95th percentile.[6] We also determined whether patients underwent leukapheresis using data from the Pediatric Health Information System (PHIS), which is an administrative database that includes inpatient data from 43 tertiary children's hospitals. Data from COG and PHIS were merged using a probabilistic merge based on ICD9 diagnosis code, treatment center and date of birth. Leukapheresis was defined by a leukapheresis procedure code (9972).

To determine predictors of hyperleukocytosis, univariate

Table 1. Demographic variables and disease characteristics in children with *de novo* acute myeloid leukemia enrolled on AAML03P1 or AAML0531 who had or did not have hyperleukocytosis.

Characteristic	WBC < 100×10^9/L (n=1108) N	%	WBC ≥ 100×10^9/L (n=256) N	%	Odds Ratio	95% CI	P
Gender							
Female	542	49	129	50	1.06	0.81, 1.39	0.671
Male	566	51	127	50	REF		
Study							
AAML03P1	282	25	58	23	REF		
AAML0531	826	75	198	77	1.17	0.84, 1.61	0.352
Age (years)							
Median, range	9.8	0.02, 29.8	9.1	0.003, 20.9	0.97	0.95, 0.99	0.014
0-1	219	20	66	26	1.37	0.99, 1.89	0.057
2-16	771	70	170	66	REF		
≥ 17	118	11	20	8	0.77	0.47, 1.27	0.305
Race							
Caucasian	805	80	176	80	REF		
Asian	57	6	13	6	1.04	0.56, 1.95	0.893
African American	133	13	30	14	1.03	0.67, 1.58	0.886
Other	11	1	2	1	0.83	0.18, 3.79	0.812
Unknown	102	-	35	-			
Ethnicity							
Hispanic or Latino	194	18	53	22	1.25	0.89, 1.75	0.207
Not Hispanic or Latino	871	82	191	78	REF		
Unknown	43	-	12	-			
BMI percentile*							
Underweight	110	11	31	15	1.32	0.85, 2.04	0.213
Normal weight	693	71	148	70	REF		
Overweight	173	18	32	15	0.87	0.57, 1.31	0.499
Unknown	132	-	45	-			
FAB classification							
M0	25	3	6	3	2.35	0.89, 6.26	0.086
M1	110	11	37	17	3.3	1.91, 5.71	<0.001
M2	255	26	26	12	REF		
M4	197	20	80	36	3.98	2.46, 6.44	<0.001
M5	195	20	59	27	2.97	1.80, 4.88	<0.001
M6	21	2	0	0	No convergence		
M7	75	8	2	1	0.26	0.06, 1.13	0.072
AML NOS	106	11	12	5	1.11	0.54, 2.28	0.776
Unknown	124	-	34	-			

continued on the next column

continued from the previous column

Cytogenetic group**							
Favorable	251	24	58	24	1.01	0.73, 1.40	0.967
Standard	767	72	176	74	REF		
Unfavorable	42	4	4	2	0.42	0.15, 1.17	0.097
Unknown	48	-	18	-			
Cytogenetic change							
Normal	248	23	55	23	REF		
t(8;21)	159	15	10	4	0.28	0.14, 0.57	<0.001
inv(16)	92	9	48	20	2.35	1.49, 3.71	<0.001
Monosomy 5/5q-	12	1	2	1	0.75	0.16, 3.45	0.714
Monosomy 7	30	3	2	1	0.30	0.07, 1.30	0.107
t(9;11)/11q23	213	20	57	24	1.21	0.80, 1.82	0.373
Other	306	29	64	27	0.94	0.63, 1.40	0.773
Unknown	48	-	18	-			
FLT3-ITD							
Positive	113	11	57	25	2.55	1.78, 3.64	<0.001
Negative	883	89	175	75	REF		
Unknown	112	-	24	-			
High allelic ratio***	72	64	42	74	1.69	0.79, 3.22	0.194
Low allelic ratio	41	36	15	26	REF		
NPM-1							
Positive	73	8	12	6	0.70	0.37, 1.31	0.265
Negative	837	92	197	94	REF		
Unknown	198	-	47	-			

** BMI percentile at diagnosis: underweight - BMI <10th percentile; overweight - BMI >95th percentile; and normal weight - BMI >10th to <95th percentile. See Design and Methods for more details. ** Cytogenetic risk group: favorable - t(8;21) or inv(16); unfavorable - monosomy 7/5 or 5q-; and standard - all others. *** FLT3-ITD high allelic ratio defined as greater than 0.4. BMI: body mass index; FAB: French-American-British; CI: confidence interval; NOS: not otherwise specified; REF: reference; FLT3-ITD: fms-like tyrosine kinase 3-internal tandem duplication; NPM-1:nucleophosmin-1.*

Table 2. Grades 3, 4 or 5 metabolic, pulmonary, and central nervous system toxicities during Induction I by initial white blood cell counts.

	WBCx10⁹/L										
	<100		≥ 100 to < 200		≥ 200 to < 300		≥ 300 to < 400		≥ 400		
	N	%	N	%	N	%	N	%	N	%	P
Total patients for whom Induction 1 data available	1,101	100	147	100	65	100	25	100%	19	100%	-
Metabolic											
Hyperkalemia	22	2.0	6	4.1	0	0.0	0	0.0	2	10.5	0.059
Hyperphosphatemia	36	3.3	3	2.0	7	10.8	2	8.0	0	0.0	0.023
Hypercreatinemia	8	0.7	2	1.4	0	0.0	1	4.0	0	0.0	0.303
Hyperuricemia	6	0.5	1	0.7	0	0.0	0	0.0	2	10.5	0.023
Pulmonary											
Hypoxia	52	4.7	14	9.5	7	10.8	1	4.0	6	31.6	<0.001
Pulmonary hemorrhage	3	0.3	2	1.4	1	1.5	1	4.0	1	5.3	0.003
CNS											
Seizure	7	0.6	2	1.4	0	0.0	0	0.0	0	0.0	0.665
CNS ischemia or hemorrhage	6	0.5	3	2.0	0	0.0	1	4.0	1	5.3	0.021
Induction I death	14	1.3	5	3.4	1	1.5	2	8.0	2	10.5	0.004
Median time to death (days) (range)	- (1, 56)	17 (1, 17)	-	4	-	1	-	19.5 (3, 36)	-	4.5 (1, 8)	-

CNS: central nervous system; WBC: white blood cell.

and multivariable logistical regression analyses were performed. Associations between toxicities and initial WBC groups were compared using χ^2 / Fisher's exact test. The Mann-Whitney test was used to determine differences in medians for continuous variables. All statistical analysis was performed using the SAS statistical program (SAS-PC, version 9.2; SAS Institute Inc., Cary, NC, USA). All tests of significance were two-sided.

A total of 1,364 children with *de novo* AML were included in the study; 256 (18.8%, 95% CI: 16.7-20.9%) had an initial WBC of 100×10⁹/L or over. Characteristics are illustrated by the presence or absence of initial hyperleukocytosis (Table 1). Multivariable regression analysis showed that age one year or under, FAB M1, M4 and M5, inv(16) and FLT3-ITD⁺ were independently associated with an increased risk of hyperleukocytosis (*Online Supplementary Table S1*).

The prevalence of grade 3 or higher metabolic, pulmonary and CNS toxicities by initial WBC count are presented in Table 2; 7 children were excluded due to lack of or conflicting data. Hyperkalemia, hyperphosphatemia, hypercreatinemia and hyperuricemia were rare. Hyperphosphatemia and hyperuricemia were significantly associated with an increased initial WBC. Elevated initial WBC was significantly associated with hypoxia (P<0.001) and pulmonary hemorrhage (P=0.003). CNS ischemia or hemorrhage was significantly associated with initial WBC count, but no seizures occurred in patients with an initial WBC of 200×10⁹/L or over. Induction I death rate was significantly associated with an increased initial WBC (P=0.004) and occurred in 4 of 44 (9.1%, 95% CI: 2.5-21.7%) patients with an initial WBC of 300×10⁹/L or over.

Timing of Induction I deaths and severe toxicities by increasing WBC categories, with timing of toxicities being restricted to patients enrolled on AAML0531, are described in Table 2 and in the Online Supplementary Table S2. Although there were few events, those occurring in patients with the highest initial WBC (≥ 400×10⁹/L) tended to occur early. There were 89 children available in PHIS with an initial WBC of 100×10⁹/L or over, representing 35% of the hyperleukocytosis cohort. Sixteen (18%) underwent leukapheresis: initial WBC ≥100-<200×10⁹/L (3 of 49); WBC ≥200-<300×10⁹/L (7 of 24); WBC ≥300-<400×10⁹/L (5 of 10); and WBC ≥400×10⁹/L (one of 6). Among the children who received leukapheresis, one of 16 (6.3%) experienced an Induction I death compared to 3 of 73 (4.1%) for those who did not receive leukapheresis.

We found that almost 20% of children with *de novo* AML had hyperleukocytosis at presentation, and that patients at higher risk were infants (≤ 1 year) and those with FAB M1, M4 and M5, inv(16) and FLT3-ITD⁺. Tumor lysis syndrome was uncommon and hyperleukocytosis was not associated with acute renal failure. However, hyperleukocytosis continues to be associated with pulmonary toxicities in terms of hypoxia and pulmonary hemorrhage as well as CNS ischemia/hemorrhage, with increasing risk in those with higher initial WBC counts.

Our report suggests that pulmonary and CNS injuries are still the major issues associated with hyperleukocytosis in spite of current supportive care. Our report also suggests that leukapheresis does not reduce induction mortality.

We conclude that outcomes remain poor in children with extremely high initial WBC in spite of the supportive care currently available. Future supportive care trials focused on reducing pulmonary and CNS toxicities should be a priority for children with AML.

Lillian Sung,[1] Richard Aplenc,[2]* Todd A. Alonzo,[3,4] Robert B. Gerbing,[3] Alan S. Gamis,[5] and the AAML0531/PHIS Group*

**LS and RA contributed equally to this manuscript.*

[1]The Hospital for Sick Children, Toronto, ON, Canada; [2]Children's Hospital of Philadelphia, Philadelphia, PA, USA; [3]Children's Oncology Group, Arcadia, CA, USA; [4]University of Southern California, CA, USA; [5]Children's Mercy Hospitals and Clinics, Kansas City, MO, USA

Correspondence: Lillian Sung, Division of Haematology/Oncology, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, M5G 1X8, Canada. Phone: international +416.8135287; Fax: international + 416.8135327; E-mail: lillian.sung@sickkids.ca

Key words: hyperleukocytosis, pediatric cancer, acute myeloid leukemia, toxicity, leukapheresis.

The online version of this article has a Supplementary Appendix.

Acknowledgments: the authors would like to thank Vani Shanker for editorial assistance.

Funding: this work was supported by research funding in the form of a New Investigator Award from the Canadian Institutes of Health research to LS (Grant n. 87719). This research also was supported by the Chair's Grant U10 CA98543-08 of the Children's Oncology Group from the National Cancer Institute, National Institutes of Health, Bethesda, MD, USA. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NCI or the NIH.

AAML0531/PHIS Group: Laura Burden from the Children's Oncology Group, Arcadia, CA; Susana C.Raimondi from St. Jude Children's Research Hospital Memphis, TN; Betsy A. Hirsch from the University of Minnesota Medical Center, Minneapolis, MN; Soheil Meshinchi from Fred Hutchinson Cancer Research Center, Seattle, WA; Matt Hall and David Bertoch from Child Health Corporation of America, Shawnee Mission, KS; Yuan-Shung V. Huang, Yimei Li, and Brian T. Fisher from Children's Hospital of Philadelphia, Philadelphia, PA, USA.

Citation: Sung L, Aplenc R, Alonzo TA, Gerbing RB, Gamis AS and the AAML0531/PHIS Group. Predictors and short-term outcomes of hyperleukocytosis in children with acute myeloid leukemia: a report from the Children's Oncology Group. Haematologica 2012;97(11):1770-1773. doi:10.3324/haematol.2012.065490

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

References

1. Dutcher JP, Schiffer CA, Wiernik PH. Hyperleukocytosis in adult acute nonlymphocytic leukemia: impact on remission rate and duration, and survival. J Clin Oncol. 1987;5(9):1364-72.
2. Creutzig U, Zimmermann M, Ritter J, Reinhardt D, Hermann J, Henze G, et al. Treatment strategies and long-term results in paediatric patients treated in four consecutive AML-BFM trials. Leukemia. 2005;19(12):2030-42.
3. Greenwood MJ, Seftel MD, Richardson C, Barbaric D, Barnett MJ, Bruyere H, et al. Leukocyte count as a predictor of death during remission induction in acute myeloid leukemia. Leuk Lymphoma. 2006;47(7):1245-52.
4. Cooper TM, Franklin J, Gerbing RB, Alonzo TA, Hurwitz C, Raimondi SC, et al. AAML03P1, a pilot study of the safety of gemtuzumab ozogamicin in combination with chemotherapy for newly diagnosed childhood acute myeloid leukemia: a report from the children's oncology group. Cancer. 2011;118(3):761-9.
5. Sung L, Aplenc R, Alonzo TA, Gerbing RB, Meshinchi S, Burden L, et al. Abstract: High mortality in extreme hyperleukocytosis in pediatric acute myeloid leukemia: a report from the Children's Oncology Group. American Society of Hematology, Orlando, Florida. 2010;116:1072.
6. Ogden CL, Kuczmarksi RJ, Flegal KM, Mei Z, Guo S, Wei R, et al. Centers for Disease Control and Prevention 2000 growth charts for the United States: improvements to the 1977 National Center for Health Statistics version. Pediatrics. 2002;109(1):45-60.

The N676D and G697R mutations in the kinase domain of FLT3 confer resistance to the inhibitor AC220

The FLT3 receptor tyrosine kinase is constitutively activated by internal tandem duplication (ITD) in its juxtamembrane domain or tyrosine kinase domain in 30% of acute myeloid leukemia (AML) cases.[1-3] Alternatively, FLT3 can also be activated by mutations in the kinase domain (such as the D835Y mutation, which are observed in 7% of AML cases.[1,2] Both *in vitro* and *in vivo* data have demonstrated that FLT3-ITD and FLT3-D835Y encode constitutively activated kinases that drive the proliferation and survival of hematopoietic cells. Furthermore, FLT3-ITD mutations confer a bad prognosis in AML,[1,4,5] and FLT3 inhibitors are expected to improve the outcome of AML patients in this subgroup. These data provide the rationale

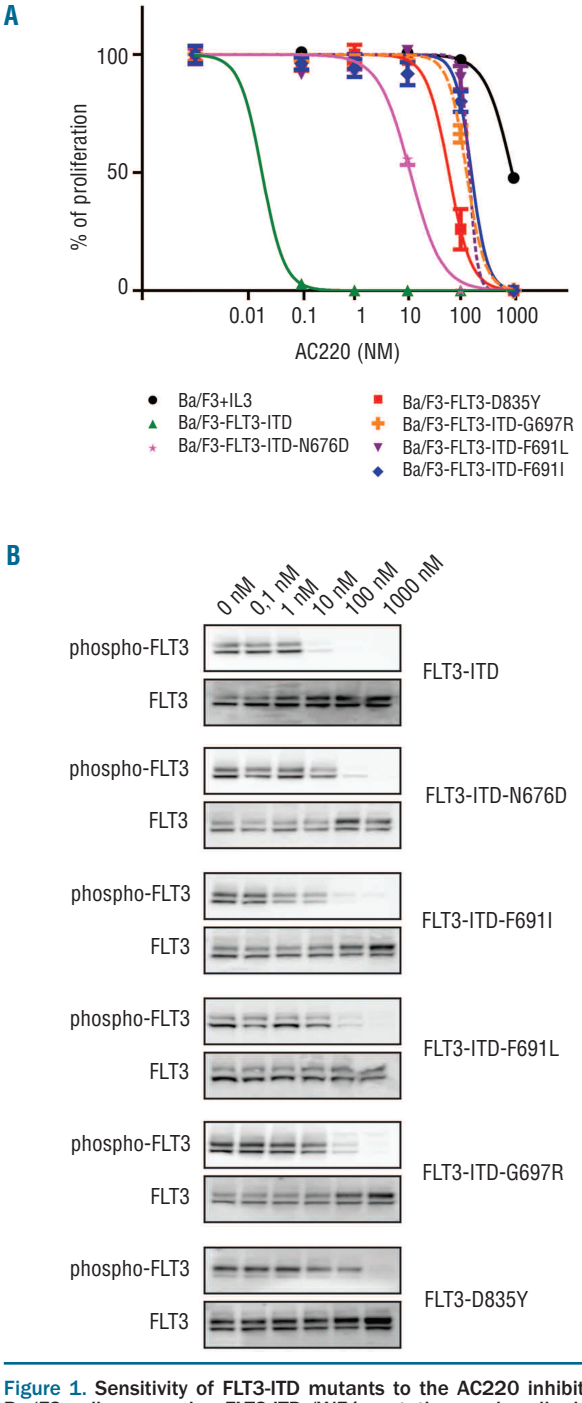

Figure 1. Sensitivity of FLT3-ITD mutants to the AC220 inhibitor. Ba/F3 cells expressing FLT3-ITD (W51 mutation as described by Kelly *et al.*[11]), FLT3-D835Y and different FLT3-ITD mutants were treated with increasing concentrations of AC220 and their proliferation was measured over a period of 24 h (A). (B) The same cells were also harvested for Western blot analysis after treatment for 90 min with different concentrations of AC220. The phosphorylation of the different FLT3 proteins was determined using a phospho-FLT3 antibody (Tyr591/3466, Cell Signaling Technology).