Clinical outcome and gene- and microRNA-expression profiling according to the Wilms tumor 1 (*WT1*) single nucleotide polymorphism rs16754 in adult *de novo* cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study

Heiko Becker,[1] Kati Maharry,[1,2] Michael D. Radmacher,[1,2] Krzysztof Mrózek,[1] Klaus H. Metzeler,[1] Susan P. Whitman,[1] Sebastian Schwind,[1] Jessica Kohlschmidt,[1,2] Yue-Zhong Wu,[1] Bayard L. Powell,[3] Thomas H. Carter,[4] Jonathan E. Kolitz,[5] Meir Wetzler,[6] Andrew J. Carroll,[7] Maria R. Baer,[8] Joseph O. Moore,[9] Michael A. Caligiuri,[1] Richard A. Larson,[10] Guido Marcucci,[1] and Clara D. Bloomfield[1]

[1]Division of Hematology, Department of Internal Medicine, Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA; [2]The Cancer and Leukemia Group B Statistical Center, Duke University Medical Center, Durham, NC, USA; [3]Comprehensive Cancer Center, Wake Forest University, Winston-Salem, NC, USA; [4]University of Iowa, Iowa City, IA, USA; [5]North Shore University Hospital, Manhasset, NY, USA; [6]Roswell Park Cancer Institute, Buffalo, NY, USA; [7]University of Alabama at Birmingham, Birmingham, AL, USA; [8]University of Maryland, Baltimore, MD, USA; [9]Duke University Medical Center, Durham, NC, USA, and [10]University of Chicago, Chicago, IL, USA

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Supplementary Data

Treatment

Patients enrolled on Cancer and Leukemia Group B (CALGB) protocol 19808 (n=102) were randomly assigned to induction chemotherapy consisting of cytarabine, daunorubicin, and etoposide with or without the multidrug resistance protein modulator PSC-833 (valspodar).[1] Upon achievement of complete remission, patients received high-dose cytarabine and etoposide for stem-cell mobilization followed by myeloablative treatment with busulfan and etoposide supported by autologous peripheral blood stem-cell transplantation. Patients not eligible for stem-cell transplantation received high-dose cytarabine.

Patients enrolled on CALGB 9621 (n=89) were treated similarly to those on CALGB 19808.[2,3] Patients on CALGB 8525 (n=24) received induction chemotherapy consisting of cytarabine with daunorubicin and were randomly assigned to consolidation with different doses of cytarabine followed by maintenance treatment.[4] Patients on CALGB 8923 (n=22) were treated with induction chemotherapy consisting of cytarabine and daunorubicin and were randomly assigned to post-remission therapy with cytarabine alone or in combination with mitoxantrone.[5] Patients on CALGB 9420 (n=6) and 9720 (n=112) received induction chemotherapy consisting of cytarabine in combination with daunorubicin and etoposide, with (CALGB 9420) or with/without (CALGB 9720) PSC-833[6-8] Patients on CALGB 9420 received post-remission therapy with cytarabine (2 g/m^2/day) alone, and those on CALGB 9720 received a single cytarabine/daunorubicin consolidation course and were randomly assigned to low-dose recombinant interleukin-2 maintenance therapy or none.[6-8] Patients on CALGB 10201 (n=78) received induction chemotherapy consisting of cytarabine and daunorubicin, with or without the *BCL2* antisense oblimersen sodium (Genasense, G3139). The consolidation regimen included two cycles of cytarabine (2 g/m^2/day) with or without oblimersen.[9]

Sample preparation

Patients enrolled on the treatment protocols gave written informed consent to participate in the companion protocols CALGB 8461 (prospective cytogenetic companion), CALGB 9665 (leukemia tissue bank) and CALGB 20202 and 20502 [(molecular studies in acute myeloid leukemia (AML)], which involved pretreatment bone marrow and peripheral blood collection and their use for research. Samples were subjected to Ficoll-Hypaque gradient separation and cryopreserved until use.

Definition of clinical end points

Complete remission required an absolute neutrophil count of 1.5×10^9/L or more, a platelet count of 100×10^9/L or more, no leukemic blasts in the blood, bone marrow cellularity greater than 20% with maturation of all cell lines, no Auer rods, less than 5% bone marrow blast cells, and no evidence of extramedullary leukemia, all of which had persisted for at least 1 month.[10] Relapse was defined by 5% or more bone marrow blasts, circulating leukemic blasts, or the development of extramedullary leukemia. Disease-free survival was measured from the date of complete remission until the date of relapse or death; patients alive and relapse-free at last follow-up were censored. Overall survival was measured from the date on study until the date of death, and patients alive at last follow-up were censored.

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Appendix

Participating Institutions

The following Cancer and Leukemia Group B (CALGB) institutions, principal investigators, and cytogeneticists participated in this study: Wake Forest University School of Medicine, Winston-Salem, NC: David D. Hurd, P. Nagesh Rao, Wendy L. Flejter and Mark J. Pettenati (grant no. CA03927); The Ohio State University Medical Center, Columbus, OH: Clara D. Bloomfield, Karl S. Theil, Diane Minka and Nyla A. Heerema (grant no. CA77658); North Shore–Long Island Jewish Health System, Manhasset, NY: Daniel R. Budman and Prasad R. K. Koduru (grant no. CA35279); University of Iowa Hospitals, Iowa City, IA: Daniel A. Vaena and Shivanand R. Patil (grant no. CA47642); Roswell Park Cancer Institute, Buffalo, NY: Ellis G. Levine and AnneMarie W. Block (grant no. CA02599); Duke University Medical Center, Durham, NC: Jeffrey Crawford, Sandra H. Bigner, Mazin B. Qumsiyeh, John Eyre and Barbara K. Goodman (grant no. CA47577); Washington University School of Medicine, St. Louis, MO: Nancy L. Bartlett, Michael S. Watson, Eric C. Crawford, Peining Li, and Jaime Garcia-Heras (grant no. CA77440); Dana Farber Cancer Institute, Boston, MA: Harold J. Burstein, Ramana Tantravahi, Leonard L. Atkins, Paola Dal Cin and Cynthia C. Morton (grant no. CA32291); University of Chicago Medical Center, Chicago, IL: Hedy L. Kindler, Diane Roulston, Katrin M. Carlson, Yanming Zhang and Michelle M. Le Beau (grant no. CA41287); University of North Carolina, Chapel Hill, NC: Thomas C. Shea and Kathleen W. Rao (grant no. CA47559); University of Massachusetts Medical Center, Worcester, MA: William V. Walsh, Vikram Jaswaney, Michael J. Mitchell and Patricia Miron (grant no. CA37135); Vermont Cancer Center, Burlington, VT: Steven M. Grunberg, Elizabeth F. Allen and Mary Tang (grant no. CA77406); Dartmouth Medical School, Lebanon, NH: Konstantin Dragnev, Doris H. Wurster-Hill and Thuluvancheri K. Mohandas (grant no. CA04326); Weill Medical College of Cornell University, New York, NY: John Leonard, Ram S. Verma, Prasad R. K. Koduru, Andrew J. Carroll and Susan Mathew (grant no. CA07968); Ft. Wayne Medical Oncology/Hematology, Ft. Wayne, IN: Sreenivasa Nattam and Patricia I. Bader; Eastern Maine Medical Center, Bangor, ME: Harvey M. Segal and Laurent J. Beauregard (grant no. CA35406); Minneapolis VA Medical Center, Minneapolis, MN: Vicki A. Morrison and Sugandhi A. Tharapel (grant no. CA47555); Mount Sinai School of Medicine, New York, NY: Lewis R. Silverman and Vesna Najfeld (grant no. CA04457); University of Puerto Rico School of Medicine, San Juan, PR: Eileen I. Pacheco, Paola Dal Cin, Leonard L. Atkins and Cynthia C. Morton; Christiana Care Health Services, Inc., Newark, DE: Stephen S. Grubbs, Digamber S. Borgaonkar and Jeanne M. Meck (grant no. CA45418); University of California at San Diego: Barbara A. Parker, Renée Bernstein and Marie L. Dell'Aquila (grant no. CA11789); SUNY Upstate Medical University, Syracuse, NY: Stephen L. Graziano and Constance K. Stein (grant no. CA21060); Rhode Island Hospital, Providence, RI: William Sikov, Teresita Padre-Mendoza, Hon Fong L. Mark, Shelly L. Kerman and Aurelia Meloni-Ehrig (grant no. CA08025); Long Island Jewish Medical Center CCOP, Lake Success, NY: Kanti R. Rai and Prasad R. K. Koduru (grant no. CA11028); Massachusetts General Hospital, Boston, MA: Jeffrey W. Clark, Leonard L. Atkins, Paola Dal Cin and Cynthia C. Morton (grant no. CA 12449); University of Maryland Cancer Center, Baltimore, MD: Martin J. Edelman, Joseph R. Testa, Maimon M. Cohen, Judith Stamberg and Yi Ning (grant no. CA31983); Western Pennsylvania Hospital, Pittsburgh, PA: John Lister and Gerard R. Diggans; University of Minnesota, Minneapolis, MN: Bruce A. Peterson, Diane C. Arthur and Betsy A. Hirsch (grant no. CA16450); University of Missouri/Ellis Fischel Cancer Center, Columbia, MO: Michael C. Perry and Tim H. Huang (grant no. CA12046); University of Nebraska Medical Center, Omaha, NE: Anne Kessinger and Warren G. Sanger (grant no. CA77298); University of Illinois at Chicago: David J. Peace, Maureen M. McCorquodale and Kathleen E. Richkind (grant no. CA74811); Walter Reed Army Medical Center, Washington, DC: Brendan M. Weiss, Rawatmal B. Surana and Digamber S. Borgaonkar (grant no. CA26806); Georgetown University Medical Center, Washington, DC: Minnetta C. Liu and Jeanne M. Meck (grant

no. CA77597); McGill Department of Oncology, Montreal, Quebec: J. L. Hutchison and Jacqueline Emond (grant no. CA31809); Virginia Commonwealth University MB CCOP, Richmond, VA: John D. Roberts and Colleen Jackson-Cook (grant no. CA52784); Medical University of South Carolina, Charleston, SC: Mark R. Green, G. Shashidhar Pai and Daynna J. Wolff (grant no. CA03927); University of Cincinnati Medical Center, Cincinnati, OH: Orlando J. Martelo and Ashok K. Srivastava (grant no. CA47515); Columbia-Presbyterian Medical Center, New York, NY: Rose R. Ellison and Dorothy Warburton (grant no. CA12011); SUNY Maimonides Medical Center, Brooklyn, NY: Sameer Rafla and Ram S. Verma (grant no. CA25119); University of California at San Francisco: Charles J. Ryan and Kathleen E. Richkind (grant no. CA60138); Southern Nevada Cancer Research Foundation CCOP, Las Vegas, NV: John A. Ellerton and Marie L. Dell'Aquila (grant no. CA35421).

Online Supplementary Table S1. Outcome according to the single nucleotide polymorphism rs16754 in cytogenetically normal *de novo* acute myeloid leukemia patients shown separately for those with *WT1* wild-type and those with a *WT1* mutation.

End Point	*WT1*^{AA}	*WT1*^{AG}	*WT1*^{GG}	*P* *WT1*^{AA} v *WT1*^{AG}	*P* *WT1*^{AA} v *WT1*^{GG}	*P* *WT1*^{AG} v *WT1*^{GG}
***WT1* wild-type patients (n=394), n.**	**286**	**97**	**11**			
Complete remission, n.(%)	222 (78)	70 (72)	8 (73)	0.27	0.72	1.0
Disease-free survival				0.72	0.24	0.24
Median (years)	1.3	1.1	Not reached			
% Disease-free at 3 years, % (95% CI)	33 (27-39)	29 (19-39)	50 (15-77)			
% Disease-free at 5 years, % (95% CI)	28 (23-35)	27 (17-38)	50 (15-77)			
Overall survival				0.35	0.82	0.69
Median (years)	1.6	1.4	1.1			
% Alive at 3 years, % (95% CI)	37 (32-43)	29 (20-38)	45 (17-71)			
% Alive at 5 years, % (95% CI)	31 (26-37)	25 (17-34)	34 (9-62)			
***WT1*-mutated patients (n=39), n.**	**23**	**15**	**1**			
Complete remission, n. (%)	15 (65)	11 (73)	-*	0.73	-*	-*
Disease-free survival				0.84	-*	-*
Median (years)	0.6	0.6	-*			
% Disease-free at 3 years, % (95% CI)	20 (5-42)	9 (1-33)				
% Disease-free at 5 years, % (95% CI)	13 (2-35)	9 (1-33)				
Overall survival				0.95	-*	-*
Median (years)	0.8	0.7	-*			
% Alive at 3 years, % (95% CI)	13 (3-30)	13 (2-35)				
% Alive at 5 years, % (95% CI)	13 (3-30)	7 (0-26)				

WT1^{AA}: homozygous for nucleotide A in rs16754; *WT1*^{AG}: heterozygous A and G in rs16754; *WT1*^{GG}: homozygous for the nucleotide G in rs16754; CI: confidence interval.

*sample size too small

Online Supplementary Table S2. Pretreatment characteristics and outcome according to the single nucleotide polymorphism rs16754 in 285 cytogenetically normal *de novo* acute myeloid leukemia patients with *FLT3*-ITD and/or *NPM1* wild-type.

Characteristic	*WT1*^{AA} (n=204)	*WT1*^{AG} (n=71)	*WT1*^{GG} (n=10)	*P* *WT1*^{AA} v *WT1*^{AG}	*P* *WT1*^{AA} v *WT1*^{GG}	*P* *WT1*^{AG} v *WT1*^{GG}
Age (years)				0.31	0.93	0.61
Median	63	59	63			
Range	19-83	18-83	24-79			
Age ≥60 years, n.(%)	119 (58)	34 (48)	7 (70)	0.13	0.53	0.31
Male sex, n.(%)	104 (51)	38 (54)	5 (50)	0.78	1.0	1.0
Race, n.(%)				0.49	0.10	0.055
Caucasian	181 (89)	66 (93)	7 (70)			
Non-Caucasian	22 (11)	5 (7)	3 (30)			
Hemoglobin (g/dL)				0.63	0.21	0.16
Median	9.5	9.9	8.9			
Range	4.6-15.0	4.9-13.6	8.1-10.5			
Platelet count (x10^9/L)				0.20	0.61	0.29
Median	57	67	54			
Range	4-850	11-510	30-156			
WBC count (x10^9/L)				0.45	0.62	0.44
Median	24.9	28.6	24.3			
Range	0.9-450.0	1.0-261.6	1.8-273.0			
% Blood Blasts				0.91	0.82	0.75
Median	56	60	62			
Range	0-99	0-95	0-89			
% Bone Marrow Blasts				0.26	0.61	0.36
Median	64	73	70			
Range	7-98	26-99	17-83			
FAB*, n.(%)				---	---	---
M0	4 (3)	3 (6)	1 (13)			
M1	40 (28)	15 (31)	1 (13)			
M2	50 (35)	13 (27)	2 (25)			
M4	31 (22)	13 (27)	3 (38)			
M5	15 (11)	3 (6)	1 (13)			
M6	2 (1)	2 (4)	0 (0)			
Extramedullary Involvement, n. (%)	45 (22)	16 (23)	3 (30)	0.87	0.70	0.70
NPM1, n.(%)				1.0	1.0	1.0
Mutated	82 (40)	29 (41)	4 (40)			
Wild-type	122 (60)	42 (59)	6 (60)			
FLT3-ITD, n.(%)				0.78	1.0	1.0
Positive	108 (53)	36 (51)	5 (50)			
Negative	96 (47)	35 (49)	5 (50)			
FLT3-TKD, n.(%)				0.77	1.0	1.0
Positive	13 (6)	3 (4)	0 (0)			
Negative	190 (94)	67 (96)	10 (100)			
WT1, n.(%)				0.27	1.0	1.0
Mutated	19 (9)	10 (14)	1 (10)			
Wild-type	185 (91)	61 (86)	9 (90)			
CEBPA, n.(%)				0.49	0.46	0.38
Mutated	44 (22)	12 (17)	3 (30)			
Wild-type	160 (78)	59 (83)	7 (70)			

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Characteristic	*WT1*^{AA} (n=204)	*WT1*^{AG} (n=71)	*WT1*^{GG} (n=10)	*P* *WT1*^{AA} v *WT1*^{AG}	*P* *WT1*^{AA} v *WT1*^{GG}	*P* *WT1*^{AG} v *WT1*^{GG}
MLL-PTD, n. (%)				1.0	0.48	0.51
Positive	13 (7)	5 (8)	1 (13)			
Negative	162 (93)	61 (92)	7 (88)			
IDH1, n. (%)				0.09	1.0	0.35
Mutated	14 (7)	10 (14)	0 (0)			
Wild-type	185 (93)	61 (86)	10 (100)			
IDH2, n. (%)				0.22	1.0	0.44
Mutated	34 (17)	17 (24)	1 (10)			
Wild-type	165 (83)	54 (76)	9 (90)			
TET2, n. (%)				0.01	0.46	0.04
Mutated	51 (26)	8 (11)	4 (40)			
Wild-type	147 (74)	62 (89)	6 (60)			
ERG expression group**, n. (%)				0.62	0.31	0.48
High	74 (53)	24 (49)	3 (33)			
Low	65 (47)	25 (51)	6 (67)			
BAALC expression group***, n. (%)				0.74	1.0	1.0
High	92 (66)	33 (62)	6 (67)			
Low	48 (34)	20 (38)	3 (33)			
Complete remission, n. (%)	145 (71)	46 (65)	7 (70)	0.37	1.0	1.0
Disease-free survival				0.97	0.04	0.06
Median (years)	0.8	0.8	Not reached			
% Disease-free at 3 years, % (95% CI)	23 (17-31)	20 (10-32)	57 (17-84)			
% Disease-free at 5 years, % (95% CI)	18 (12-25)	20 (10-32)	57 (17-84)			
Overall survival				0.69	0.12	0.13
Median (years)	1.2	1.1	2.8			
% Alive at 3 years, % (95% CI)	26 (20-32)	20 (11-30)	50 (18-75)			
% Alive at 5 years, % (95% CI)	20 (15-26)	17 (9-26)	38 (10-66)			

WT1^{AA}: homozygous for nucleotide A in rs16754; *WT1*^{AG}: heterozygous A and G in rs16754; *WT1*^{GG}: homozygous for nucleotide G in rs16754; WBC: white blood count; FAB: French-American-British classification; *FLT3*-ITD: internal tandem duplication of the *FLT3* gene; *FLT3*-TKD: tyrosine kinase domain mutations of the *FLT3* gene; *MLL*-PTD: partial tandem duplication of the *MLL* gene.

* FAB are centrally reviewed.
** For patients on CALGB 9621, cut point was the same as in Marcucci *et al.* (J Clin Oncol. 2005;23(36):9234-42). For patients on all other protocols, median *ERG* expression value was used as the cut point.
*** Median expression was used as cut point.

Online Supplementary Table S3. Selected pretreatment characteristics and outcome of cytogenetically normal *de novo* acute myeloid leukemia patients with the *WT1*^{GG} genotype.

Pt	Race	Age (years)	*NPM1*	*FLT3*-ITD	*FLT3*-TKD	*WT1*	*CEBPA*	*MLL*-PTD	*IDH1*	*IDH2*	*TET2*	*ERG* *** expression	*BAALC* **** expression	Achieved complete remission	Disease free (months)
1	Asian	≥60	mutated	negative	positive	wild-type	wild-type	negative	wild-type	wild-type	mutated	unknown	unknown	no	n/a
2	Caucasian	≥60	mutated	positive	negative	wild-type	wild-type	negative	wild-type	wild-type	mutated	high	high	no	n/a
3	Caucasian	<60	mutated	positive	negative	wild-type	wild-type	negative	wild-type	wild-type	mutated	high	low	no	n/a
4	Caucasian	≥60	wild-type	positive	negative	mutated	mutated*	negative	wild-type	wild-type	wild-type	high	high	no	n/a
5	Caucasian	≥60	wild-type	negative	negative	wild-type	wild-type	positive	wild-type	wild-type	wild-type	unknown	unknown	yes	4
6	Asian	<60	mutated	negative	negative	wild-type	wild-type	negative	wild-type	wild-type	mutated	low	low	yes	5
7	Caucasian	≥60	wild-type	negative	negative	wild-type	wild-type	negative	wild-type	mutated**	wild-type	low	high	yes	10
8	Caucasian	≥60	wild-type	negative	negative	wild-type	wild-type	unknown	wild-type	wild-type	mutated	low	low	yes	32
9	Asian	≥60	wild-type	negative	negative	wild-type	mutated*	negative	wild-type	wild-type	wild-type	low	high	yes	46+
10	Hispanic	<60	mutated	positive	negative	wild-type	wild-type	negative	wild-type	wild-type	wild-type	low	high	yes	92+
11	Native American	<60	wild-type	negative	negative	wild-type	mutated*	negative	wild-type	wild-type	wild-type	low	high	yes	119+
12	Caucasian	≥60	mutated	positive	negative	wild-type	wild-type	unknown	wild-type	wild-type	mutated	low	low	yes	130+

Patients are sorted by duration of disease-free survival.

Pt: patient; *FLT3*-ITD: internal tandem duplication of the *FLT3* gene; *FLT3*-TKD: tyrosine kinase domain mutations of the *FLT3* gene; *MLL*-PTD: partial tandem duplication of the *MLL* gene; n/a: not applicable since patient did not achieve a complete remission.

* Patient had two mutations in *CEBPA*.
** Patient had a mutation in *IDH2* codon R140.
*** For patients on CALGB 9621, cut point was the same as in Marcucci *et al.* (J Clin Oncol. 2005;23(36):9234-42). For patients on all other protocols, median *ERG* expression value was used for cut point.
**** Median expression was used as the cut point.

Online Supplementary Table S4. Outcome according to the single nucleotide polymorphism rs16754 in younger (<60 years) and older (≥60 years) patients with cytogenetically normal *de novo* acute myeloid leukemia.

End Point	*WT1*^{AA}	*WT1*^{AG}/*WT1*^{GG}	*P*
All younger patients (n=191), n.	**132**	**59**	
Complete remission, n. (%)	115 (87)	44 (75)	0.04
Disease-free survival			0.75
Median (years)	2.5	1.7	
% Disease-free at 3 years, % (95% CI)	48 (38-57)	41 (26-55)	
% Disease-free at 5 years, % (95% CI)	44 (35-53)	41 (26-55)	
Overall survival			0.09
Median (years)	6.5	1.6	
% Alive at 3 years, % (95% CI)	55 (46-63)	41 (28-53)	
% Alive at 5 years, % (95% CI)	51 (42-59)	36 (24-48)	
Younger patients with *FLT3*-ITD and/or *NPM1* wild-type (n=125), n.	**85**	**40**	
Complete remission, n. (%)	71 (84)	28 (70)	0.10
Disease-free survival			0.26
Median (years)	1.1	1.9	
% Disease-free at 3 years, % (95% CI)	35 (24-46)	39 (22-57)	
% Disease-free at 5 years, % (95% CI)	29 (18-40)	39 (22-57)	
Overall survival			0.87
Median (years)	1.6	1.8	
% Alive at 3 years, % (95% CI)	44 (33-54)	38 (23-52)	
% Alive at 5 years, % (95% CI)	37 (27-47)	33 (19-47)	
All older patients (n=242), n.	**177**	**65**	
Complete remission, n. (%)	122 (69)	45 (69)	1.00
Disease-free survival			0.72
Median (years)	0.9	0.9	
% Disease-free at 3 years, % (95% CI)	17 (11-24)	16 (7-28)	
% Disease-free at 5 years, % (95% CI)	11 (6-18)	13 (5-25)	
Overall survival			0.74
Median (years)	1.3	1.1	
% Alive at 3 years, % (95% CI)	21 (15-27)	17 (9-27)	
% Alive at 5 years, % (95% CI)	14 (9-20)	12 (5-21)	

WT1^{AA}: homozygous for nucleotide A in rs16754; *WT1*^{AG}: heterozygous A and G in rs16754; *WT1*^{GG}: homozygous G in rs16754; CI: confidence interval.

Online Supplementary Figure S1. Disease-free survival (A) and overall survival (B) of younger (<60 years) and disease-free survival (C) and overall survival (D) of older (≥60 years) patients with cytogenetically normal de novo acute myeloid leukemia according to the genotypes of the single nucleotide polymorphism rs16754. *WT1*^{AA}: patients homozygous for nucleotide A in rs16754; *WT1*^{AG}/*WT1*^{GG}: patients with at least one G allele.