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GAS6 expression identifies high-risk adult AML patients: potential implications for therapy

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Abstract

Emerging data demonstrate important roles for the TYRO3/AXL/MERTK receptor tyrosine kinase (TAM RTK) family in diverse cancers. We investigated the prognostic relevance of *GAS6* expression, encoding the common TAM RTK ligand, in 270 adults (n=71 aged <60 years; n=199

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Author contributions S.P.W., M.A.C., G.M., C.D.B designed the study and analyzed the data. S.P.W., J.K., K. Maharry, K. Mrózek, M.A.C., G.M. and C.D.B wrote the manuscript, and all authors agreed to the final version. J.K., K. Maharry, D.N. and S.V. performed statistical analyses. S.P.W., K.H.M., S.W., H.B., J.M., A.-K.E., A.J.C. and I-K.P. generated, compiled and interpreted lab data. B.L.P., T.H.C., M.R.B., J.E.K., R.M.S., M.A.C., G.M., C.D.B. were involved directly or indirectly in care of patients and/or sample procurement.

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aged ≥60 years) with *de novo* cytogenetically normal acute myeloid leukemia (CN-AML). Patients expressing *GAS6* (*GAS6+*), especially those aged ≥60 years, more often failed to achieve a complete remission (CR). In all patients, *GAS6+* patients had shorter disease-free (DFS) and overall (OS) survival than patients without *GAS6* expression (*GAS6−*). After adjusting for other prognostic markers, *GAS6+* predicted CR failure (P=0.02), shorter DFS (P=0.004) and OS (P=0.04). To gain further biologic insights, we derived a *GAS6*-associated gene-expression signature (P<0.001) that in *GAS6+* patients included overexpressed *BAALC* and *MN1*, known to confer adverse prognosis in CN-AML, and overexpressed *CXCL12*, encoding stromal cell-derived factor, and its receptor genes, *CXCR4* and *CXCR7*. This study reports for the first time that *GAS6* expression is an adverse prognostic marker in CN-AML. Although GAS6 decoy receptors are not yet available in the clinic for *GAS6+* CN-AML therapy, potential alternative therapies targeting *GAS6+*-associated pathways, e.g., CXCR4 antagonists may be considered for *GAS6+* patients to sensitize them to chemotherapy.

Keywords

GAS6; acute myeloid leukemia; prognosis

INTRODUCTION

Constitutive activity of the receptor tyrosine kinase (RTK) family has been observed in malignant blasts from patients with acute myeloid leukemia (AML). Members of the RTK family include FLT3 and KIT, whose constitutive kinase activity can occur through several mechanisms, such as mutationally-induced autophosphorylation, receptor overexpression and/or aberrant expression of the receptors' ligands.[1-3] The constitutive activity of FLT3 and KIT has been associated with poor clinical outcomes, and therapeutic targeting of activated RTKs is currently an area of intense investigation.[4-8]

In cancer, another RTK family, the TAM RTKs (i.e., TYRO3, AXL and MERTK), has been shown to support survival, proliferation, migration, invasion, angiogenesis, metastasis and chemoresistance,[9-12] and TAM RTK inhibitors are already in pre-clinical and clinical development for several solid tumors.[11-13] Although TAM RTKs are aberrantly expressed in AML,[11,14,15] to date, only *AXL* expression has been reported to adversely impact outcome in adults with cytogenetically normal AML (CN-AML).[16]

No *AXL* mutations have been described in AML, suggesting activation of *AXL* may occur at least in part via aberrant autocrine expression of GAS6, that binds AXL with high affinity and is also the common ligand for all three TAM RTKs.[17] *GAS6* was shown to be aberrantly expressed in AML cell lines.[18] These data point to a possible role for the GAS6/TAM RTK signaling axis in AML and prompted us to test the clinical impact of *GAS6* expression in a molecularly characterized cohort of chemotherapy-treated adults with *de novo* CN-AML.

METHODS

Patients

Available pretreatment bone marrow or blood samples were obtained from 270 patients with *de novo* CN-AML (aged 18 to 83 years; median, 66 years; n=71 aged <60 years; n=199 aged ≥60 years) enrolled on Cancer and Leukemia Group B (CALGB)/Alliance companion protocols 8461 (cytogenetic analyses), 20202 (molecular analyses) and 9665 (tissue banking). Patients were treated on CALGB/Alliance protocols 8525, 8923, 9420, 9720, 10201, or 19808.[19-25] The treatment protocols included cytarabine/daunorubicin-based induction but differed with regard to consolidation therapy (for details see Supplemental Material). Per protocols, no patient received allogeneic stem-cell transplantation in first complete remission (CR). All protocols were in accordance with the Declaration of Helsinki and approved by institutional review boards at each center, and all patients provided written informed consent.

Cytogenetic and molecular analyses

For the patient's karyotype to be considered normal, ≥20 metaphases from short-term cultures of the bone marrow specimens obtained at diagnosis had to have been analyzed and the normal result confirmed by central karyotype review.[26] Tissue samples were cryopreserved after mononuclear cell enrichment through a Ficoll gradient. The presence or absence of *FLT3* internal tandem duplication (*FLT3*-ITD),[27] *FLT3* tyrosine kinase domain mutations (*FLT3*-TKD),[28] *MLL* partial tandem duplication (*MLL*-PTD),[29,30] mutations in the *NPM1*,[31] *CEBPA*,[32] *WT1*,[33] *TET2*,[34] *IDH1/2*,[35] *RUNX1*,[36] *ASXL1*[36] and *DNMT3A*[37] genes, and *BAALC*,[38] *ERG*,[38] and *MN1*[39] expression levels were assessed centrally as previously described. Patients were also categorized according to the European LeukemiaNet (ELN) reporting system.[40] CN-AML patients with *CEBPA* mutation and/or *NPM1* mutation without *FLT3*-ITD were classified in a Favorable genetic group and those with wild-type *CEBPA*, *FLT3*-ITD and/or *NPM1* mutation, or wild-type *NPM1* in an Intermediate-I genetic group

Expression analysis of *GAS6* and TAM RTKs

GAS6, *TYRO3*, *AXL* and *MERTK* transcript expression levels measured with Affymetrix U133 plus 2.0 array (Affymetrix, Santa Clara, CA, USA) assays. The GeneAnnot chip definition file was used to derive a single expression value for each gene per patient sample.[41] For array normalization and expression value computation, the robust multichip average method was implemented separately for samples from older and younger patients.[42]

Patients were categorized as either expressing *GAS6* (yes or *GAS6*-positive, hereafter denoted *GAS6*+) if the probe-set fluorescence intensity (PFI) was greater than background fluorescence intensity (BFI) and not expressing *GAS6* (no or *GAS6*-negative denoted *GAS6*−) if the *GAS6* PFI was less than or equal to the BFI. Similarly, patients were categorized as either *TYRO3*+ or *AXL*+ (if the PFIs were greater than BFI) and *TYRO3*− or *AXL*− (if the PFIs were less than or equal to BFI). The *MERTK* PFI was above the BFI in all samples and, based on an optimal cutpoint analysis (see Supplemental Material),[43] patients

were grouped into high expressers (*MERTK*+) or lower expressers (*MERTK*−) if they were in the upper two tertiles or in the lowest tertile groups, respectively.

Affymetrix-microarray gene expression profiling analysis

To establish a signature of genes differentially expressed between *GAS6*+ and *GAS6*− patients, we evaluated the aforementioned Affymetrix gene-expression profiles. Normalized expression values were compared between *GAS6*+ and *GAS6*− patients and a univariable significance level of $P<0.001$ was used to identify differentially expressed genes. A global test of significance based on a permutation procedure was performed to determine whether or not the number of differentially expressed genes was more than expected by chance. The false discovery rate (FDR) was used to assess multiple testing errors. A permutation test was computed based on 1 000 random permutations.

The Ingenuity Pathway Analysis tool (IPA Tool; Ingenuity H Systems, Redwood City, CA, USA; http://www.ingenuity.com) was used to identify enriched biological networks, global functions and functional pathways. Genes with altered expression profile associated with *GAS6* expression status were imported into the IPA Tool. As a second means for identifying enriched ontologies, the web-based Database for Annotation, Visualization, and Integrated Discovery (DAVID) tool (DAVID Bioinformatics resources 6.7 http://david.abcc.ncifcrf.gov/) was used.

Clinical endpoints and statistical analyses

Baseline characteristics were compared between *GAS6*+ and *GAS6*− patients using the Fisher's exact test for categorical variables and the Wilcoxon rank-sum test for continuous variables. Definitions of clinical endpoints [i.e., CR, disease-free (DFS) and overall (OS) survival] and details of outcome analyses are provided in the Supplemental Material. Briefly, for time-to-event analyses, we calculated survival estimates using the Kaplan-Meier[44] method, and compared groups by the log-rank test. We constructed age group-adjusted multivariable logistic regression models to analyze factors associated with the achievement of CR, and age group-adjusted multivariable Cox proportional hazards models[45] for factors associated with survival endpoints. All analyses were performed by the Alliance for Clinical Trials in Oncology Statistics and Data Center.

RESULTS

Association of *GAS6* expression status with clinical characteristics, TAM RTK expression status and molecular markers at diagnosis

Of the 270 patients, 26% of patients were *GAS6*+, (n=69) and 74% *GAS6*− (n=201). At diagnosis, *GAS6*+ patients had higher platelet counts ($P=0.03$), lower percentages of blood blasts ($P=0.01$), more often hepatomegaly ($P=0.006$) and co-expression of *AXL* (28% vs 5%, $P<0.001$) compared with *GAS6*− patients. There was no association between *GAS6* and *TYRO3* expression ($P=0.74$), whereas more *GAS6*− than *GAS6*+ patients were *MERTK*+ ($P=0.02$, Table 1). Compared with *GAS6*− patients, *GAS6*+ patients were more often wild-type for *NPM1* ($P<0.001$) and *CEBPA* ($P=0.02$) and therefore more often in the ELN

Intermediate-I Genetic Group (*P*<.001), and had mutations in *RUNX1* (*P*<0.001) and *ASXL1* (*P*=0.002), and high *BAALC* (*P*=0.02) and *MN1* (*P*=0.05; Table 1) expression.

Impact of *GAS6* expression on clinical outcomes of *de novo* CN-AML patients

In age group-adjusted analyses, *GAS6+* expression associated with lower odds of achieving CR (*P*<0.001; Table 2), with CR rates significantly different in patients ≥60 years of age [46% *GAS6+* (n=54) vs 74% *GAS6−* (n=145); *P*<0.001]. None of the TAM RTKs impacted on CR (Table S1). To assess whether *GAS6* expression independently affects clinical outcomes when other known clinical and molecular prognostic features are considered, we performed multivariable analyses (MVAs). For CR, *GAS6+* status predicted lower probability of achieving CR [(*P*=0.02; odds ratio (OR), 0.46; 95% confidence interval (CI), 0.23-0.89)], after adjusting for the ELN CN-AML Genetic Group[45] status, *BAALC* expression status, white blood cell (WBC) count and age group (Table 3).

GAS6+ expression associated with shorter DFS (*P*=0.03) and OS (*P*=0.004) compared with *GAS6−* patients (Table 2 and Figure 1). As single markers, neither *AXL* nor *MERTK* influenced DFS or OS, whereas *TYRO3* expression adversely impacted on both endpoints (Table S1). In multivariable modeling for DFS and OS, we noted a significant interaction (DFS, *P*=0.01; OS, *P*=0.04) between *GAS6* expression and the combined *TYRO3* and *AXL* expression status. In the dual receptor-positive patients, i.e., positive for one or both *TYRO3* and *AXL* expression, *GAS6* expression did not independently impact outcome, which may be reflective of the interplay between the GAS6 ligand and the TYRO3 and AXL receptors (Table 3). In the dual receptor-negative patients, i.e., negative for both *TYRO3* and *AXL* expression, *GAS6* expression remained an independent, adverse prognostic marker. Within the subgroup of dual receptor-negative patients, *GAS6+* expression was a predictor of shorter DFS (*P*=0.004; hazard ratio (HR)=2.12; 95% CI, 1.27-3.56) and after adjusting for *WT1* and *DNMT3A* R882 mutations, *BAALC* expression and age group; and shorter OS (*P*=0.04; HR=1.55; 95% CI, 1.01-2.38) after adjusting for ELN group, *WT1* and *DNMT3A* R882 mutations, *BAALC* expression, WBC and age group.

A *GAS6*-associated gene expression signature in *de novo* CN-AML

To gain additional molecular insights into *GAS6+* CN-AML, an Affymetrix microarray-based gene expression signature was derived. The signature contained 1 238 genes that were significantly differentially expressed between *GAS6+* and *GAS6−* CN-AML blasts at diagnosis (Table S2). Within this signature, genes for which high expression level is an established adverse prognosticator in CN-AML were *BAALC* and *MN1*. These were overexpressed, respectively, 2.57-fold and 2.41-fold in the *GAS6+* subgroup. Although hitherto not validated in large, independent patient sets, overexpression of the following four genes was reported to have an impact on outcome of AML patients. These were *APP*, which encodes the amyloid precursor protein and whose overexpression was associated with shorter survival than that of AML patients without *APP* overexpression[46] (overexpressed 3.37-fold in *GAS6+* patients); *SETBP1*, whose overexpression leads to PP2A inhibition, promoting proliferation of leukemic cells[47] (overexpressed 1.73-fold); *SPARC*, which is overexpressed in patients with *IDH2*-R172 mutations and contributes to AML aggressiveness[48] (overexpressed 2.05-fold); and *CD74*, whose lower surface protein levels

associated with achievement of CR/partial CR in AML patients ages 60-75 years treated with bortezomib in combination with chemotherapy (overexpressed 2.51-fold).[49] Moreover, overexpressed in *GAS6+* CN-AML diagnostic samples were *CXCL12* (3.2-fold), encoding for stromal cell-derived factor-1, and genes encoding both of its receptors, *CXCR4* (1.52-fold) and *CXCR7* (1.72-fold). Overexpression of *CXCR4* has been previously associated with adverse clinical outcome in patients with CN-AML.[50,51]

CEBPA was among the 611 genes underexpressed in *GAS6+* patients. Mutations in the *CEBPA* gene that encodes a transcription factor are associated with better outcome of AML patients (Table S2).[32] *CD33*, encoding an immunotherapeutic target in AML, was also underexpressed (1.67-fold) in *GAS6+* patients.

Gene expression signatures were not identified for any of the TAM RTKs (less than 10 genes, with FDRs of 10%; data not shown). Indeed, there was no apparent contribution from *TAM* RTKs in profiling analyses combining *GAS6* with each of the TAM RTKs. This indicates that it is *GAS6* expression status that drives the differential gene expression we observed.

Pathway analysis revealed that the *GAS6*-associated gene expression signature contained the following overrepresented molecular and cellular functions: a) cell cycle, b) cellular growth and proliferation, c) cell death and survival, d) cellular assembly and organization and, e) DNA replication, recombination and repair (Table 4). The top canonical pathways included a) IL-8 signaling, b) growth hormone signaling, c) mitotic roles of Polo-like kinase, d) CXCR4 signaling and e) Tec kinase signaling (Table 4). Of the top upstream regulators, colony stimulating factor 2 (granulocyte-macrophage), CSF2, was predicted by Ingenuity to be activated, while the cyclin dependent kinase inhibitor, CDKN1A, was predicted by Ingenuity to be inhibited (Table 4). A second analysis using DAVID also identified enriched clusters of genes, with the most highly enriched cluster (score of 9.66; Benjamini corrected *P*-values ranging from 2.8E-9 to 3.5E-6) containing genes involved in the cell cycle (data not shown).

DISCUSSION

We report herein that *GAS6* expressed by AML blasts is a marker of poor clinical outcomes in adults with CN-AML, independent of other established prognostic markers in this cytogenetic subset. Not only does *GAS6* expression predict CR failure, albeit driven by older age, it has also a negative impact on DFS and OS in the studied cohort. The MVA revealed the negative prognostic impact of *GAS6* was in patients whose leukemic blasts did not express *TYRO3* and *AXL*. This suggests that *GAS6* may contribute to a more aggressive disease through signaling mechanisms that are not dependent on *AXL* and *TYRO3* expression within the AML cells. Perhaps it is the third GAS6 receptor, MERTK that together with GAS6 has a role within the *TYRO3−/AXL−* patient subgroup. Based on recently published data related to MERTK function in leukemia, *MERTK*, even when expressed at relatively low levels appears to contribute to a leukemic phenotype. [14] However, in the current study, too few numbers of patients prohibited a reliable *GAS6/MERTK* subgroup analyses.

Given that *GAS6* expression has an adverse impact on CR achievement, mainly in older patients, and on DFS and OS in all patients, this warrants development of novel, less toxic and perhaps more personalized therapies targeting GAS6. We recently reported that blocking the engagement of GAS6 to the AXL receptor with soluble AXL-Fc chimeric protein inhibits downstream signal transduction, inducing differentiation and apoptosis in human AML cell lines and patient samples with activated AXL.[54] Consistent with our work,[54] a recent study by Ben-Batalla et al,[55] showed that pharmacologic inhibition of GAS6/AXL signaling induces leukemic cell death. We did not find an impact of *AXL*-positive expression as a sole marker on outcomes in our study, whereas *AXL* expression above the median was associated with shorter OS in the Ben-Batalla and colleagues study.[55] This may be explained in part by therapy differences and differences in patient cohort characteristics. Their study exclusively analyzed adult patients ≤60 years of age, whereas 73% of patients in our study were 60 years of age or older.

As our study measured *GAS6* transcript levels, the relationship between *GAS6* mRNA, protein and secretion in AML is not yet clear. However, there are several studies of various solid cancers that report *GAS6* mRNA and protein are present *within* the tumor cells. For example, Buehler and coworkers recently reported that *GAS6* mRNA and the translated protein are both elevated *within* ovarian cancer.[56] Additionally, *GAS6* mRNA and protein levels were found in 81% and 74% of glioblastoma multiforme tissue samples, suggesting a close 1:1 relationship between transcription and translation of GAS6.[57] As for secretion, Ben-Batalla, and co-workers performed immunohistochemistry for GAS6 on five AML patients' bone marrows and concluded that stromal cells are the primary source of secreted GAS6 ligand (their Figure 1 and Supplement Table 3)[55]

Interaction of the GAS6/TAM RTK signaling axis with the stromal microenvironment in solid tumors has been associated with poor progression-free survival,[58,59] A similar mechanism may be active in chemotherapy-resistant AML patients that express *GAS6*. This suggests a potential for novel therapies targeting *GAS6*+ leukemic blasts that could also simultaneously inhibit negative effects of GAS6 in the microenvironment, ultimately improving patient survival. Promising studies using decoy receptors showed significant activity against the growth of lung carcinoma cells in a xenograft model,[13] and the absence of toxicity in normal murine tissues or hematopoiesis is encouraging.[60] Given our current findings, GAS6-targeted therapeutic agents could lead to higher CR rates, particularly, as our data indicate, benefiting older patients, and possibly prolonging survival of the *GAS6*+ subset of CN-AML patients. Furthermore, while our results await independent validation, development of GAS6 decoy receptors, in addition to selective small molecule TAM inhibitors, appears warranted.

Meanwhile, in the short term, one possibility for improving outcomes of *GAS6*+ patients is alluded to by the *GAS6*-associated gene expression signature we identified. Overexpression of *CXCR4* and its ligand was detected in pretreatment samples from patients expressing *GAS6*. In separate reports, overexpression of this signaling axis associated with increased risk of relapse and shorter overall survival in AML.[50,51] CXCR4 is expressed on normal hematopoietic stem cells and regulates stem cell homing and retention in the BM when CXCL12, produced by BM stroma, engages the receptor.[61] The CXCR4 antagonist,

Plerixafor, currently has FDA approval in combination with G-CSF as a stem cell mobilizing agent for patients with multiple myeloma and non-Hodgkin lymphoma who undergo autologous hematopoietic stem cell transplantation.[62] Uy and co-workers[63] recently reported their results of a phase I/II clinical trial that demonstrated antagonizing the CXCL12/CXCR4 axis with Plerixafor induces chemosensitization of relapsed or refractory AML blasts. Thus, although GAS6 decoy receptors are not yet available in the clinic for *GAS6+* CN-AML therapy, potential alternative therapies such as CXCR4 antagonists should be considered for *GAS6+* patients to sensitize them to chemotherapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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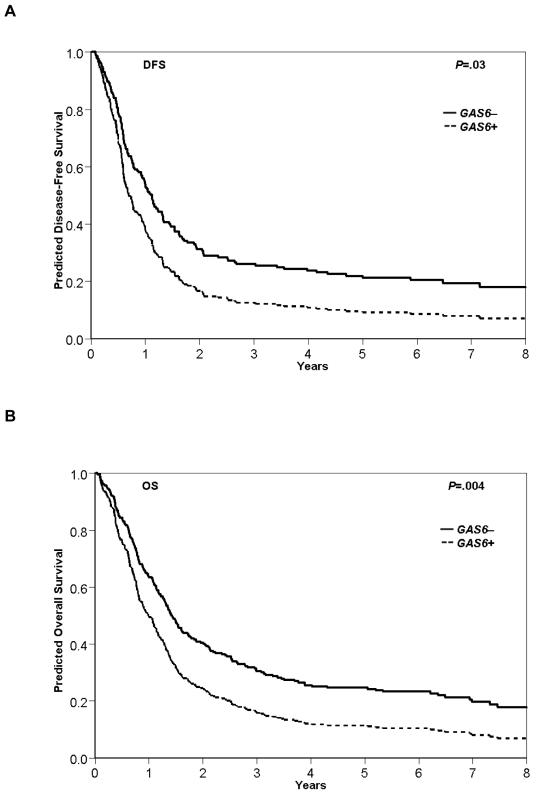

Figure 1.

Clinical outcome by *GAS6* expression status. Survival curves for (**a**) disease-free survival (DFS) and (**b**) overall survival (OS) are displayed for *GAS6*+ and *GAS6*− patient groups. Data were age adjusted (<60 years of age, n=71; ≥60 years of age, n=199).

Table 1

Comparison of clinical and molecular characteristics of *de novo* cytogenetically normal AML patients according to *GAS6* expression status

Variable	GAS6+[a] (n=69)	GAS6−[a] (n=201)	p[b]
Age, years			0.02
Median	68	65	
Range	37-81	18-83	
Age group, n (%)			0.35
<60 years	15 (22)	56 (28)	
≥60 years	54 (78)	145 (72)	
Male sex, n (%)	35 (51)	101 (50)	1.00
Race, n (%)			0.81
White	63 (93)	181 (91)	
Non-white	5 (7)	19 (9)	
Hemoglobin, g/dL			0.56
Median	9.4	9.4	
Range	6.4-12.5	4.8-15.0	
Platelet count, $\times 10^9$/L			
Median	84	66	
Range	11-309	4-850	
White blood cell count, $\times 10^9$/L			
Median	21.1	26.5	
Range	1.0-434.1	1.0-450.0	
Blood blasts (%)			0.01
Median	40	59	
Range	0-96	0-99	
Bone marrow blasts (%)			0.87
Median	70	67	
Range	7-97	4-97	
Extramedullary involvement, n (%)	20 (29)	45 (23)	0.33
Hepatomegaly	8 (12)	5 (3)	0.006
TYRO3 expression group [a], n (%)			0.74
Positive	16 (23)	43 (21)	
Negative	53 (77)	158 (79)	
AXL expression group [a], n (%)			<0.001

Variable	GAS6+[a] (n=69)	GAS6−[a] (n=201)	P[b]
Positive	19 (28)	11 (5)	
Negative	50 (72)	190 (95)	
MERTK expression [c]*, n (%)*			0.02
Positive	38 (55)	143 (71)	
Negative	31 (45)	58 (29)	
TYRO3/AXL dual receptor [d] *, n (%)*	30 (43)	51 (25)	0.006
NPM1, n (%)			<0.001
Mutated	20 (29)	141 (70)	
Wild-type	48 (71)	60 (30)	
FLT3-ITD, *n (%)*			0.56
Present	26 (38)	68 (34)	
Absent	42 (62)	133 (66)	
CEBPA, n (%)			0.02
Mutated	4 (6)	35 (17)	
Single mutated	4	19	
Double mutated	0	16	
Wild-type	64 (94)	166 (83)	
ELN Genetic Group [e] *, n (%)*			<0.001
Favorable	12 (18)	115 (57)	
Intermediate-I	56 (82)	86 (43)	
FLT3-TKD, *n (%)*			1.00
Present	7 (10)	23 (11)	
Absent	61 (90)	178 (89)	
WT1, n (%)			0.25
Mutated	2 (3)	15 (7)	
Wild-type	66 (97)	186 (93)	
TET2, n (%)			0.43
Mutated	15 (22)	55 (28)	
Wild-type	52 (78)	143 (72)	
MLL-PTD, n (%)			1.00
Present	4 (6)	13 (7)	
Absent	64 (94)	182 (93)	
IDH1, n (%)			0.83
R132	5 (7)	24 (12)	
V71I	2 (3)	0 (0)	

Variable	GAS6+[a] (n=69)	GAS6−[a] (n=201)	P[b]
Wild-type	60 (90)	173 (88)	
IDH2, n (%)			0.72
IDH2	14 (21)	37 (19)	
R140	8	33	
R172	6	4	
Wild-type	53 (79)	160 (81)	
RUNX1, n (%)			<0.001
Mutated	27 (44)	9 (5)	
Wild-type	35 (56)	173 (95)	
ASXL1, n (%)			0.002
Mutated	16 (24)	16 (8)	
Wild-type	51 (76)	179 (92)	
DNMT3A, n (%)			0.76
Mutated	21 (32)	66 (35)	
R882	17	40	
Non-R882	4	26	
Wild-type	44 (68)	124 (65)	
ERG expression group [f] *, n (%)*			0.33
High	30 (43)	102 (51)	
Low	39 (57)	99 (49)	
BAALC expression group [f] *, n (%)*			0.02
High	44 (64)	93 (46)	
Low	25 (36)	108 (54)	
MN1 expression group [f] *, n (%)*			0.05
High	34 (65)	65 (48)	
Low	18 (35)	70 (52)	

Abbreviations: *FLT3*-ITD, internal tandem duplication of the *FLT3* gene; ELN, European LeukemiaNet; *FLT3*-TKD, tyrosine kinase domain mutation in the *FLT3* gene; *MLL*-PTD, partial tandem duplication of the *MLL* gene.

[a] All patients with *GAS6* probe-set fluorescence intensity greater than the background fluorescence intensity (BFI) are defined as *GAS6*-positive (GAS6+) and those with *GAS6* probe-set intensity less than or equal to the BFI as *GAS6*-negative (*GAS6*−). Similarly, patients were categorized as either *TYRO3*+ (*TYRO3* expression greater than BFI) or *TYRO3*− (if *TYRO3* expression was less than or equal to BFI) and *AXL*+ (expression greater than BFI) or *AXL*− (expression less than or equal to BFI).

[b] *P*-values for categorical variables are from Fisher's exact test, *P*-values for continuous variables are from Wilcoxon rank sum test.

[c] All patients in the upper 2/3 of the values of *MERTK* are defined as *MERTK*+. All patients in the lower 1/3 of the values of *MERTK* are defined as *MERTK*−.

[d] If patient has *AXL*+ and *TYRO3*+ expression, *AXL*+ and *TYRO3*− expression, or *AXL*− and *TYRO3*+ expression then *TYRO3/AXL* dual receptor is defined to be positive. If a patient has *AXL*− and *TYRO3*− expression then *TYRO3/AXL* dual receptor is defined to be negative.

[e] According to the ELN recommendations,[40] Favorable Genetic Group is defined as *CEBPA*-mutated or *FLT3*-ITD-negative and *NPM1*-mutated. Intermediate-I Genetic Group is defined as *CEBPA* wild-type and *FLT3*-ITD-positive and *NPM1*-mutated, *FLT3*-ITD-negative and *NPM1*-wild-type, or *FLT3*-ITD-positive and *NPM1*-wild-type.

[f] The median expression value was used as the cutoff for high and low values.

Table 2

Age group-adjusted analyses of outcomes by *GAS6* positive expression versus no expression in *de novo* cytogenetically normal AML patients

Outcome Endpoint	OR/HR (95% CI)	P
CR	0.35 (0.20, 0.63)	<0.001
DFS	1.55 (1.05, 2.26)	0.03
OS	1.55 (1.16, 2.09)	0.004

Abbreviations: CR, complete remission; DFS, disease-free survival; OS, overall survival; OR, odds ratio; HR, hazard ratio; CI, confidence interval.

Note: ORs < 1.0 means a lower CR rate, and HRs > 1.0 mean higher risk

Table 3

Multivariable models

Complete remission		
Variable	*OR (95% CI)*	*P*
GAS6 expression (+ vs −)	0.46 (0.23-0.89)	0.02
ELN Genetic Group (Favorable vs Intermediate-I)[a]	2.13 (1.07-4.23)	0.03
BAALC expression (high vs low) [b]	0.26 (0.13-0.50)	<0.001
WBC count (continuous, 50 unit increase)	0.57 (0.42-0.77)	<0.001
Age group (≥60 years vs <60 years)	0.35 (0.16-0.75)	0.007
Disease-free survival		
Variable	*HR (95% CI)*	*P*
GAS6 expression (+ vs −)		
TYRO3/AXL dual receptor− patients	2.12 (1.27-3.56)	0.004
TYRO3/AXL dual receptor+ patients	0.73 (0.38-1.38)	0.33
Interaction between *GAS6* expression status and *TYRO3/AXL* dual receptor status		0.01
WT1 (mutated vs wild-type)	2.62 (1.24-5.52)	0.01
DNMT3A (R882 mutated vs non-R882 mutated and wild-type)	1.95 (1.29-2.93)	0.001
BAALC expression (high vs low)[b]	1.58 (1.10-2.25)	0.01
Age group (≥60 years vs <60 years)	2.62 (1.72-3.99)	<0.001
Overall survival		
Variable	*HR (95% CI)*	*P*
GAS6 expression (+ vs −)		
TYRO3/AXL dual receptor− patients	1.55 (1.01-2.38)	0.04
TYRO3/AXL dual receptor+ patients	0.78 (0.47-1.30)	0.34
Interaction between *GAS6* expression status and *TYRO3/AXL* dual receptor status		0.04
ELN Genetic Group (Favorable vs Intermediate-I)[a]	0.64 (0.46-0.89)	0.008
WT1 (mutated vs wild-type)	2.59 (1.47-4.55)	0.001
DNMT3A (R882 mutated vs non-R882 mutated and wild-type)	1.45 (1.02-2.05)	0.04
BAALC expression (high vs low)[b]	1.74 (1.29-2.34)	<0.001
WBC count (continuous, 50 unit increase)	1.14 (1.04-1.25)	0.008
Age group (≥60 years vs <60 years)	2.90 (2.00-4.22)	<0.001

Abbreviations: OR, odds ratio; CI, confidence interval; WBC, white blood cell; HR, hazard ratio.

[a] According to the European LeukemiaNet (ELN) recommendations,[40] Favorable Genetic Group is defined as *CEBPA*-mutated or *FLT3*-ITD-negative and *NPM1*-mutated. Intermediate-I Genetic Group is defined as *CEBPA* wild-type and *FLT3*-ITD-positive and *NPM1*-mutated, *FLT3*-ITD-negative and *NPM1*-wild-type, or *FLT3*-ITD-positive and *NPM1*-wild-type.

[b] Median cut was used to determine whether patients were in the high or low expression group.

Note: ORs > (<) 1.0 mean a higher (lower) complete remission rate, and HRs > (<) 1.0 mean higher (lower) risk for the higher values of the continuous variables and the first category listed for the categorical variables. Variables considered were those significant at α=.20 in univariable models, ie, for complete remission, GAS6 (+ vs −), ELN (Favorable vs Intermediate-I), *WT1* (mutated vs wild-type), ASXL1 (mutated vs wild-type), *ERG* (high vs low), *BAALC* (high vs low), platelet counts (50×10^9/L increase), WBC count (50×10^9/L increase), extramedullary involvement (present vs absent), age group (≥60 years vs <60 years); for disease-free survival, *GAS6* (+ vs −), *TYRO3/AXL* dual receptor status (+ vs −),ELN (Favorable vs Intermediate-I), *WT1* (mutated vs wild-type), *MLL*-PTD (present vs absent), *DNMT3A* (R882 mutated vs non-R882 mutated and wild-type), *ERG* (high vs low), *BAALC* (high vs low), age group (≥60 years vs <60 years); for overall survival, *GAS6* (+ vs −), *TYRO3/AXL* dual receptor status (+ vs −), ELN (Favorable vs Intermediate-I), *WT1* (mutated vs wild-type), *MLL*-PTD (present vs absent), *RUNX1* (mutated vs wild-type), *DNMT3A* (R882 mutated vs non-R882 mutated and wild-type), *ERG* (high vs low), *BAALC* (high vs low), WBC count (50×10^9/L increase), age group (≥60 years vs <60 years).

Table 4

Biological pathways over-represented in the *GAS6* expression signature in cytogenetically normal AML

Molecular and cellular functions (number of genes) [a]	*P* [b]
Cell cycle (202)	7.75E-17 to 2.15E-03[a]
Cellular growth and proliferation (360)	3.48E-15 to 2.41E-03
Cell death and survival (363)	5.28E-15 to 2.44E-03
Cellular assembly and organization (195)	2.76E-11 to 2.33E-03
DNA replication, recombination and repair (136)	2.76E-11 to 2.15E-03
Top canonical pathways [a]	
Interleukin-8 signaling	5.43E-06
Growth hormone signaling	2.28E-05
Mitotic roles of Polo-like kinase	7.24E-05
CXCR4 signaling	1.23E-04
Tec kinase signaling	1.62E-04
Top upstream regulators [a]	
Colony stimulating factor 2 (granulocyte-macrophage), CSF2	9.17E-16
Cyclin-dependent kinase inhibitor, CDKN1A	2.55E-15

[a]These data were obtained from Ingenuity's Pathway Analysis program (see Methods)

[b]Significance values shown indicate the range of *P*-values for each of the genes that were identified within each of the annotated functions listed