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A pathway-based gene signature correlates with therapeutic response in adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia

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Biomarkers to predict response to therapy in adults with Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) are not yet established. In this study, we performed a meta-analysis of earlier genome-wide gene expression studies to identify pathway-based genes that are associated with therapeutic response. The predictive power of these genes was validated by transcript profiling in diagnostic bone marrow samples from Ph+ ALL patients using a quantitative real-time PCR array. Gene expression was correlated with cytogenetic and molecular characteristics, including presence of *ABL1* mutations and *IKZF1* deletion. A total of 43 *de novo* Ph+ ALL patients treated uniformly with tyrosine kinase inhibitors combined with chemotherapy were selected to validate 46 identified genes. A 9-gene signature was established to distinguish optimal responders from patients with persistent residual disease and early molecular recurrence. The signature was subsequently validated with 87% predictive accuracy in an independent validation set of patients. When initially optimal responders relapsed, their gene expression patterns also shifted. Optimal responders showed upregulation of genes involved in proliferation and apoptosis pathways, whereas poor responders had higher expression of genes that facilitate tumor cell survival in hypoxic conditions as well as development of drug resistance. This unique 9-gene signature may better enable stratification of patients to proper therapeutic regimens and provides new insights into mechanisms of Ph+ ALL response to therapy.
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Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) is characterized by the presence of t(9;22)(q34;q11) that results in the creation of the *BCR–ABL1* fusion protein. Ph+ ALL accounts for up to 30% of adult and 3% of pediatric ALL.[1] Approximately one-third of ALL cases afflict adults, therefore there are <600 newly diagnosed adult Ph+ ALL cases each year in the United States.[2] Ph+ ALL has a poor prognosis in both children and adults compared with other ALL types. Chemotherapy alone can achieve complete remission (CR) in 50% of these patients compared with 80–90% of patients with Ph− ALL.[1] As a result, stem cell transplantation (SCT) in first CR has become the standard strategy in treating adult Ph+ ALL patients. With the introduction of tyrosine kinase inhibitors (TKI), such as imatinib, the CR rate has increased significantly, but 30% of the patients

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relapse in a short time interval. The 5-year overall survival (OS) also has remained low, ranging from 5% (>65 years old) to 30% (20–44 years old).[3] Identification of predictive and prognostic factors for patient stratification is therefore of crucial importance in management. Owing to the rarity and rapid progression of this disease, however, relatively few studies related to therapeutic response and prognostic factors in adults with Ph+ ALL have been published.[4,5] Most of the available information is scattered in studies of ALL of all types.[1] Moreover, as Ph+ ALL can be derived from blast phase of chronic myelogenous leukemia (CML) or biphenotypic leukemia, and the inclusion criteria of some earlier studies are unclear, some of the published data are not easily interpreted. Nevertheless, these results imply that Ph+ ALL may be a heterogeneous group of diseases with different clinical course and response to therapy.

To further explore the nature of this heterogeneity at the molecular level, genomic and gene expression profiling studies have been performed in very small cohorts of adult Ph+ ALL patients, or in mixed cohorts of patients with other types of ALL.[6–10] Except for the common finding that most Ph+ ALL cases have deletions on chromosome 7p spanning the *IKZF1* gene,[11] the gene expression profiling results have varied considerably in different studies, and have been of limited clinical utility.

The goal of this study was to identify a robust gene signature that predicts therapeutic response in adult Ph+ ALL patients. We initially selected adults with *de novo* Ph+ ALL in our files for this study, all of whom were uniformly treated with a TKI and combination chemotherapy. Following our review of earlier studies, we selected a representative set of genes that enabled us to globally assess the activities of disease-related pathways. We examined a broad spectrum of factors, including clinical characteristics, cytogenetic data, and gene mutations to correlate with the gene expression profile to illustrate a thorough picture of the gene effects. Our findings suggest that Ph+ ALL is a heterogeneous group of diseases with significantly different pathogenesis, clinical manifestations, therapeutic response, and survival. The gene signature we report can accurately predict patient response to therapy, enabling better stratification of patients for assignment to optimal therapeutic regimens.

Materials and methods

Patient Selection

The study group included previously untreated adults with *de novo* Ph+ ALL who came to our institution between 1 September 2001 and 30 June 2008. No patient had received previous therapy and all were treated with the HyperCVAD regimen (hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone, alternating with high-dose cytarabine and methotrexate) plus either imatinib or dasatinib.[12] We reviewed patient records and excluded those patients who had history of CML or acute biphenotypic leukemia. Patients who did not have adequate RNA from diagnostic bone marrow (BM) aspirates were also excluded. As a second phase to this study, additional smaller group of patients seen at our institution from 1 May 2008 to 31 January 2009 was selected based on the same criteria to serve as an independent validation set. The study was performed according to an approved laboratory protocol and in accordance with the Declaration of Helsinki.

The diagnostic workup on all patients included a complete blood count, BM aspiration and biopsy, conventional cytogenetic analysis (Giemsa-banded karyotype), fluorescence *in situ* hybridization (FISH) using a dual-fusion *BCR–ABL1* probe,[13] and quantitative reverse transcription polymerase chain reaction (qRT-PCR) for *BCR–ABL1* fusion transcript levels, as described previously.[14,15] Complete molecular response (CMR) was signified by undetectable levels of *BCR–ABL1* transcripts (representing at least 4.5-log reduction from baseline levels).[15] *ABL1* kinase domain (KD) mutations were assessed in relapsed patients using a nested PCR strategy covering codons 221 to 500 and a screening strategy as previously described.[13,16]

Selection of Genes for Transcript Profiling

Genes for the test panel were selected by data mining and network analysis.[17,18] Briefly, gene expression profiling data from previous studies[7–10,19,20] comparing therapeutic response with various reagents in ALL patients were pooled and normalized. Relative expression levels were calculated, and associated with outcomes by analysis of variance (ANOVA). The top associated genes were mapped to gene ontology (GO) pathways (www.geneontology.org). The GO classifications of interest in this study included: cell growth and proliferation, cell communication, metabolism and development, cell motility, response to stress, and cell death. Final selection was based on network analysis of the pathways using Ingenuity Pathway Analysis software (Ingenuity, Redwood City, CA, USA), in combination with expert knowledge of the disease mechanism. Forty-six target genes were selected for profiling study. Gene symbols and their full names are listed in Supplementary Table 1.

Transcript Profiling by Low-Density Quantitative PCR Array

Expression profiling of the selected genes was performed on BM samples. We used a custom-designed TaqMan qRT-PCR array, which is a 384-well microfluidic card containing gene-specific forward and reverse primers and fluorescence-labeled probe in

each well (Applied Biosystems, Foster City, CA, USA). Total RNA was extracted from BM aspirate specimens using the guanidium solubilization method (Trizol, Invitrogen, Carlsbad, CA, USA) and complementary DNA (cDNA) was synthesized using Superscript III reverse transcriptase (Invitrogen) and random hexamers for priming. qRT-PCR was performed on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems) with 1 μg of cDNA from each sample according to the manufacturer's recommendation. Thermal cycling conditions were as follows: 2 min at 50 °C, 10 min at 94.5 °C, 40 cycles of denaturation at 97 °C for 30 s, and annealing and extension at 59.7 °C for 1 min.

Statistical Analysis

The relative expression level of each gene was calculated by the delta delta (Δ)Ct method.[21] The ΔCt value of each gene was calculated as the difference between its Ct value and that of the control gene, *GAPDH*, as previously described.[22] Another control gene, glucuronidase β (*GUSB*), was used to monitor variations between experiments. ANOVA and Kruskal–Wallis tests were applied to calculate differential expression of these genes among therapeutic response groups. A feature-specific *P*-value was obtained for each gene based on permutation test. The false discovery rate was also calculated to control the multiple testing error rate.[23] Unsupervised hierarchical clustering and principle component analysis (PCA) were performed to visualize gene expression patterns among therapeutic response groups. A support vector machines (SVMs) model was applied to assess the predictive power of the gene signature (genepattern.broadinstitute.org). The discriminative abilities of the SVM models for therapeutic response groups were determined according to the area under the receiver operating characteristic (ROC) curve.

Clinical parameters were analyzed using the χ^2 test for categorical variables and the Kruskal–Wallis test for numerical variables. Patient survival was plotted by Kaplan–Meier method and differences were compared by the log-rank test. For the purpose of calculating event-free survival (EFS) in this study, an event was defined as *BCR–ABL1* fusion transcript detection by qRT-PCR in a follow-up sample after a patient had achieved CMR, which may or may not correlate with relapse defined clinically.

Results

Pathway-Based Gene Expression Panel Reveals Distinct Therapeutic Response Groups

A total of 43 adult *de novo* Ph+ ALL patients met our selection criteria and were selected as the study group. There were 23 men and 20 women with a median age of 43 years (range, 19–84 years). The median follow-up time was 16 months. All patients were confirmed to harbor the t(9;22)(q34;q11) by conventional cytogenetics and/or FISH analysis and 81% had the e1a2 fusion transcript encoding the $p190^{BCR-ABL1}$ detected by qRT-PCR.

We initially assessed the pathway status of the leukemic cells in these patients before therapy using our pathway-based gene expression panel of the 46 selected genes. Expression profiling was performed on BM samples collected at time of diagnosis before the initiation of therapy with TKI and combination chemotherapy using the low-density qRT-PCR array. Four genes, *NR3C1*, *GSTT1*, *ONECUT1*, and *POU2AF1* failed to amplify consistently and therefore were excluded from further analyses. An unsupervised hierarchical clustering analysis of the relative expression levels of the remaining 42 genes resulted in two major clusters with distinct expression patterns, which can be further divided into three subgroups as shown in Figure 1a.

By following these patients' post-therapy *BCR–ABL1* fusion transcript levels, we found that the two major clusters from the gene expression profile correlated with whether or not patients achieved CMR after therapy. Furthermore, three gene expression pattern subgroups enabled us to identify three therapeutic response groups characterized as: group A, patients who achieved CMR within 3 months of therapy and maintained undetectable *BCR–ABL1* transcript levels during the following 6 months; group B, patients who did not achieve CMR within 3 months of therapy; and group C, patients who achieved CMR within 3 months of therapy but had recurrent disease detected by qRT-PCR during the following 6 months. Using these criteria, the 43 patients were further divided into groups A, B, or C and their characteristics are summarized in Table 1.

A 9-Gene Signature Predicts Therapeutic Response

As evidenced in Figure 1a, the 42-gene expression profiles did not perfectly match the response groups defined by *BCR–ABL1* fusion transcript levels. To further identify genes that had the highest distinguishing power among the three response groups, we calculated and ranked each gene by its feature-specific *P*-value from ANOVA analysis and permutation testing. Nine genes were identified that most significantly correlated with response to therapy (Table 2). Application of this 9-gene signature to unsupervised hierarchical clustering analysis of the 43 cases resulted in three distinct gene expression clusters that had a 97.7% correlation with therapeutic response groups (Figure 1b). The only misclassified case was a patient that clinically manifested with persistent minimal residual disease (group B) but had a gene expression pattern closer to that of patients with recurrent disease (group C). To examine the robustness of this 9-gene signature, we further applied the data to a SVM model to

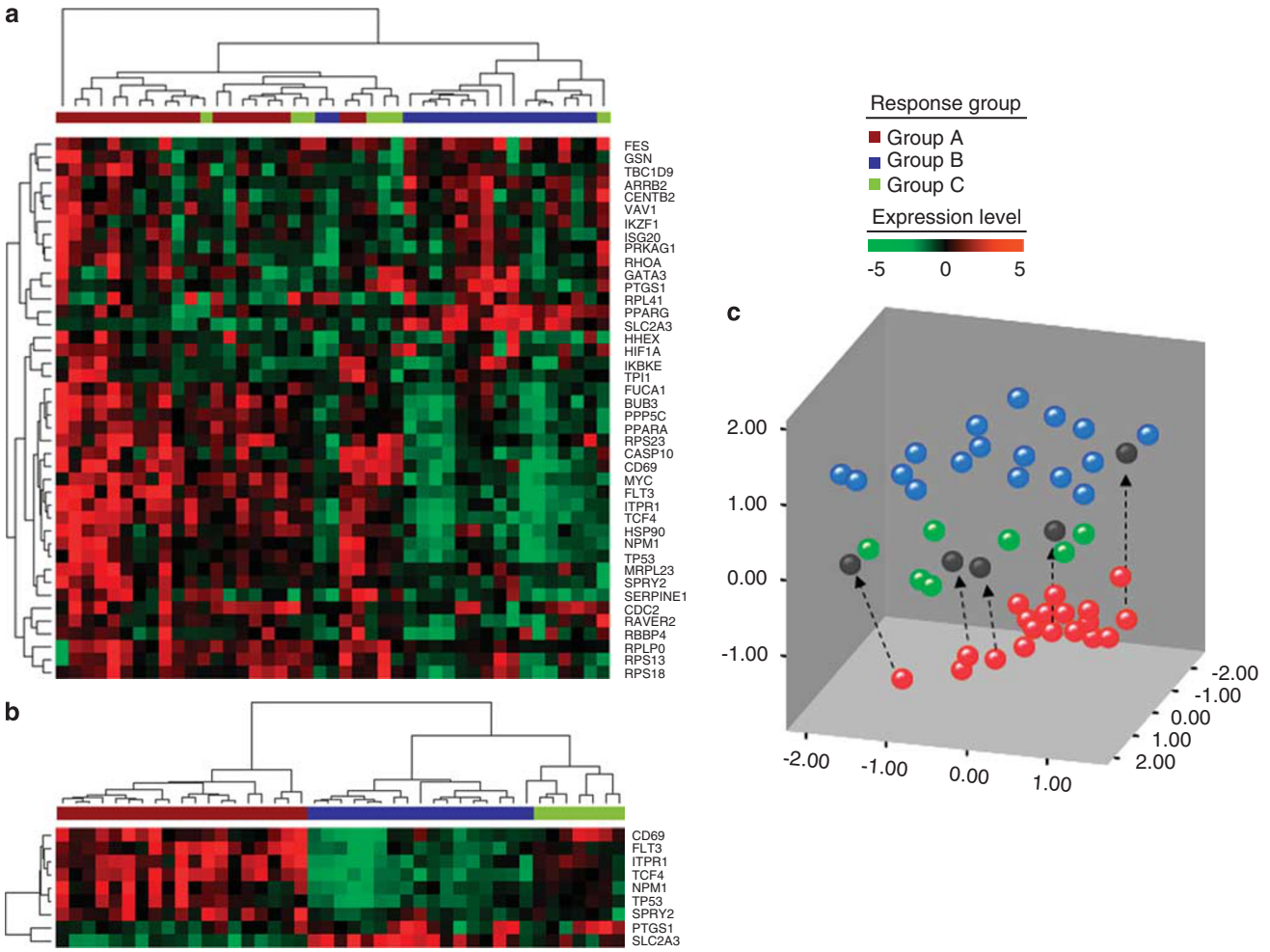

Figure 1 Correlation of gene expression with therapeutic response. (**a**) Unsupervised two-way hierarchical clustering of the 43 cases using expression levels of 42 genes. (**b**) Clustering of the cases using expression levels of the nine signature genes. (**c**) Different therapeutic response can be determined by their distinct expression pattern of the signature genes. Colors in the heatmaps (**a** and **b**) represent normalized gene expression levels, with black representing 0, pure red representing +5, and pure green representing −5. Color codes for therapeutic response groups are shown in the legend. In panel **c**, black dots with black arrows represent gene expression pattern of the relapsed bone marrow samples (at variable time interval) of initially optimal responders.

predict response to therapy using the leave-one-out cross validation algorithm. The overall predictive accuracy was 92.9%.

Figure 1c shows a schematic of the three distinct clusters according to this 9-gene signature in three-dimensional space using PCA with color codes representing the response groups to which they belong. In five patients from group A who eventually relapsed at different time points, we also examined the expression levels of these nine genes in their relapse BM samples. As demonstrated by the black dots in Figure 1c, in all five patients their expression pattern shifted from the original optimal responder pattern toward the recurrent or persistent disease pattern. This confirmed that our 9-gene signature is highly robust and closely reflects disease res ponsiveness. These findings also illustrate the highly dynamic nature of gene expression in Ph+ ALL cells.

After completion of the study, we similarly tested this 9-gene signature in an independent set of eight adult patients who were recently diagnosed with *de novo* Ph+ ALL who had clinical follow-up as a validation set. As shown in Table 3, the SVM model produced correct predictions in seven of the eight (87%) patients, with an absolute error = 0.125 and ROC error = 0.167.

Clinical and Molecular Manifestations of Patients with Different Responses to Therapy

Given the fact that the therapeutic response groups defined by either *BCR–ABL1* transcript levels or the 9-gene signature were essentially identical except in one patient, and this did not cause significant differences in any aspect in this study (data not shown), we present further results using groups

Table 1 Patient characteristics among therapeutic response groups

Characteristic	*Group A*	*Group B*	*Group C*	P-*value*
Total number of patients	19	17	7	
Gender				
Male/female	9/10	11/6	3/4	0.481
Age, median (range)	53 (19–84)	42 (21–67)	55 (29–68)	0.220
Presenting WBC $\times 10^9$/l, median	11.7	6.7	5.3	0.401
Presenting BM blast count (%), median	89	74	58	0.002
Presenting BM *BCR–ABL1* to *ABL1* ratio (%), median	82.69	24.37	53.50	0.034
BCR–ABL1 *fusion transcript type*				
$p190^{BCR-ABL1}/p210^{BCR-ABL1}$	17/2	14/3	4/3	0.183
ABL1 KD *mutations,* n				
Any mutation[a]	9/10	4/8	3/5	0.162
T315I[b]	4/9	2/4	1/3	0.906
Cytogenetic findings, n *(%)*				
t(9;22) as sole abnormality	4 (21%)	13 (76%)	3 (43%)	0.004 (0.001)[c]
Aneuploidy	9 (47%)	2 (12%)	2 (29%)	0.067 (0.021)[c]
der(22)	7 (37%)	2 (12%)	2 (29%)	0.223 (0.083)[c]
+21	4 (21%)	0 (0%)	0 (0%)	0.062 (0.045)[c]
Abn(9p)	5 (26%)	1 (6%)	2 (29%)	0.221 (0.101)[c]
−7	4 (21%)	0 (0%)	2 (29%)	0.091 (0.045)[c]
Median survival, months (range)				
Event-free survival	10 (6–17)	0	4 (2–5)	<0.001
Overall survival	30 (9–33)	14 (4–47)	16 (2–18)	0.005

[a]Number of cases positive for mutations/number of cases tested.
[b]Number of cases positive for T315I mutation/number of cases with mutation.
[c]Comparing between group A and B only.

Table 2 The list of genes that highly correlate with therapeutic response in adult Ph+ ALL patients

Symbol	*Gene name*	*Group*	*Major pathways*	*Upregulated*	P-*value**
CD69	CD69 molecule	Transmembrane receptor	Cytotoxicity, apoptosis	Group A	0.002
FLT3	fms-related tyrosine kinase 3	Protein kinase receptor	Growth, apoptosis	Group A	0.002
ITPR1	Inositol 1,4,5-triphosphate receptor, type 1	Ion channel, cation transporter	Apoptosis, growth	Group A	0.002
NPM1	Nucleophosmin	Chaperone, transcription regulator	Apoptosis, growth	Group A	0.002
PTGS1	Prostaglandin-endoperoxide synthase 1	Synthase	Apoptosis, drug resistance	Group B	0.029
SLC2A3	Solute carrier family 2, member 3	Carbohydrate transporter	Apoptosis, Hif1a signaling	Group B	0.002
SPRY2	Sprouty homolog 2	Signaling molecule	Growth, migration	Group A	0.002
TCF4	Transcription factor 4	Helix-loop-helix transcription factor	Growth, acute phase response	Group A	0.002
TP53	Tumor protein p53	Transcription factor	Apoptosis, growth	Group A	0.002

**P*-values were calculated by permutation tests.

determined by *BCR–ABL1* levels. After TKI-combined chemotherapy, 19 patients achieved optimal response (group A), with a median EFS of 10 months and a median OS of 30 months (Figure 2). Seventeen patients had persistent residual disease and failed to achieve CMR within 3 months of therapy (group B). This patient group had a median OS of 14 months. Another seven patients showed early recurrence of disease detected by qRT-PCR after achieving CMR (group C), with a median EFS and OS of 4 and 16 months, respectively. There were no statistical differences in age, gender, or white blood cell counts at the time of diagnosis among these groups (Table 1). However, patients in group A presented with higher BM blast count ($P=0.002$) and higher *BCR–ABL1* fusion transcript levels ($P=0.034$). There was no

Table 3 Prediction of therapeutic response in an independent set of adult Ph+ ALL patients using the 9-gene signature-based support vector machines model

Samples	*True group*	*Predicted group*	*Confidence*	*Correct*
1	B	B	0.843	True
2	B	B	0.898	True
3	A	A	0.863	True
4	C	C	0.803	True
5	A	A	0.916	True
6	B	B	0.872	True
7	C	A	0.531	False
8	B	B	0.887	True

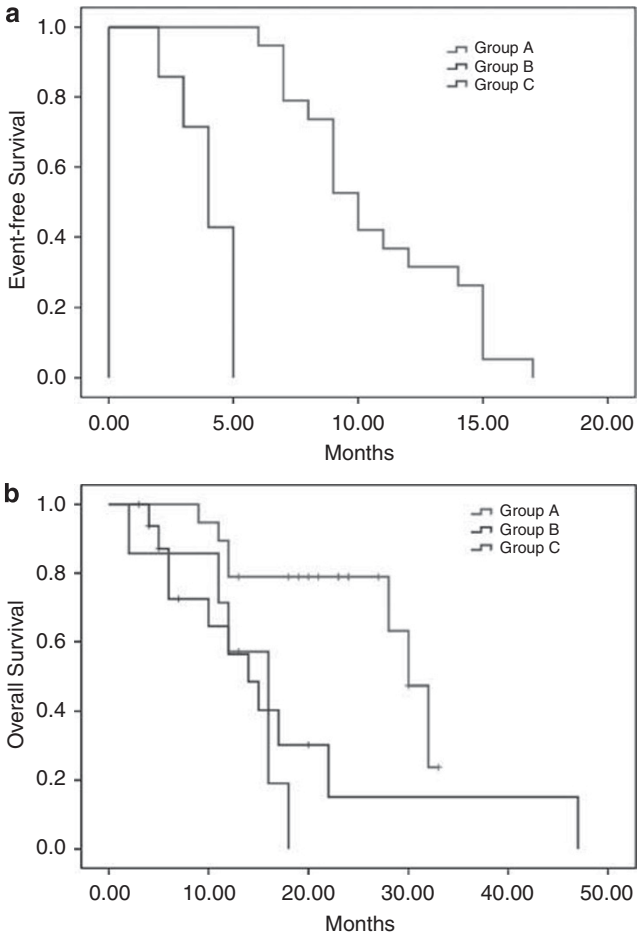

Figure 2 Comparison of (**a**) event-free survival (EFS) and (**b**) overall survival (OS) of patients from therapeutic response groups. $P<0.001$ and $P=0.005$, respectively by the log-rank test.

difference in the types of the fusion transcript ($P=0.183$) between groups and fusion transcript type had no significant impact on either EFS or OS ($P=0.192$ and 0.326, respectively).

The relative expression levels of the 9-gene signature among the three response groups are shown in Figure 3, with higher ΔCt level indicating lower gene expression. The cellular functions of these genes include regulation of cell growth, proliferation, apoptosis, hypoxia signaling, and drug metabolism pathways (Table 2 and Figure 4). Genes that regulate the key proliferation and apoptosis pathways (*CD69*, *FLT3*, *ITPR1*, *NPM1*, *SPRY2*, *TCF4*, and *TP53*) were upregulated in tumors of optimal responders (group A), consistent with the higher blast counts and higher *BCR–ABL1* fusion transcript levels observed in group A patients. In poor responders (group B), on the other hand, the glucose transporter gene, *SLC2A3*, which protects cell death from hypoxia, and the prostaglandin-endoperoxide synthase 1 (*PTGS1*) gene, involved in drug resistance, were upregulated.

Interaction of Gene Profiles with ABL1 KD Mutations at Relapse

Mutations of the *ABL1* KD have been implicated as a major cause of resistance to TKI therapy. *ABL1* KD mutation status was available in relapsed samples of 23 patients in this study cohort. Nearly 70% of these samples were positive for one or more mutations, and about half of these mutations were T315I (Table 1). The frequency was particularly high in group A where 9 in 10 (90%) of patients harbored mutations at relapse. This number, however, may not accurately reflect the true frequency of mutations because patients that had been selected for mutation testing were clinically suspected to be resistant to therapy. The presence of ABL1 KD mutations, even T315I, did not significantly impact on OS ($P=0.967$ and 0.657, respectively).

When comparing the gene expression patterns at relapse with the patterns in corresponding patient samples at time of diagnosis, we found that patients who developed ABL1 KD mutations at relapse had higher expression levels of growth-promoting genes, including *SPRY2, BUB3, MYC*, and *HHEX* ($P=0.006$, 0.012, 0.016, and 0.018, respectively), whereas *SLC2A3* expression was higher in patients who did not develop *ABL1* KD mutation ($P=0.029$). This suggests that *ABL1* mutations tend to develop in tumors with a proliferative phenotype, possibly producing larger numbers of tumor cells from which mutations can emerge. This hyperproliferative phenotype also would be expected to be more sensitive to cytotoxic chemotherapeutic agents, perhaps explaining their increased frequency in group A.

Correlation of Gene Signatures with Unique Cytogenetic Features

To assess the effects of specific cytogenetic alterations on therapeutic response and gene expression in Ph+ ALL, we further examined gene expression patterns in patients with various cytogenetic aberrations including: der(22), abnormalities involving chromosome 9p, −7, +21, aneuploidy, and t(9;22) as a sole abnormality (Table 1). Patients with t(9;22) as a sole abnormality showed significant shorter EFS when compared with patients with other karyotypic

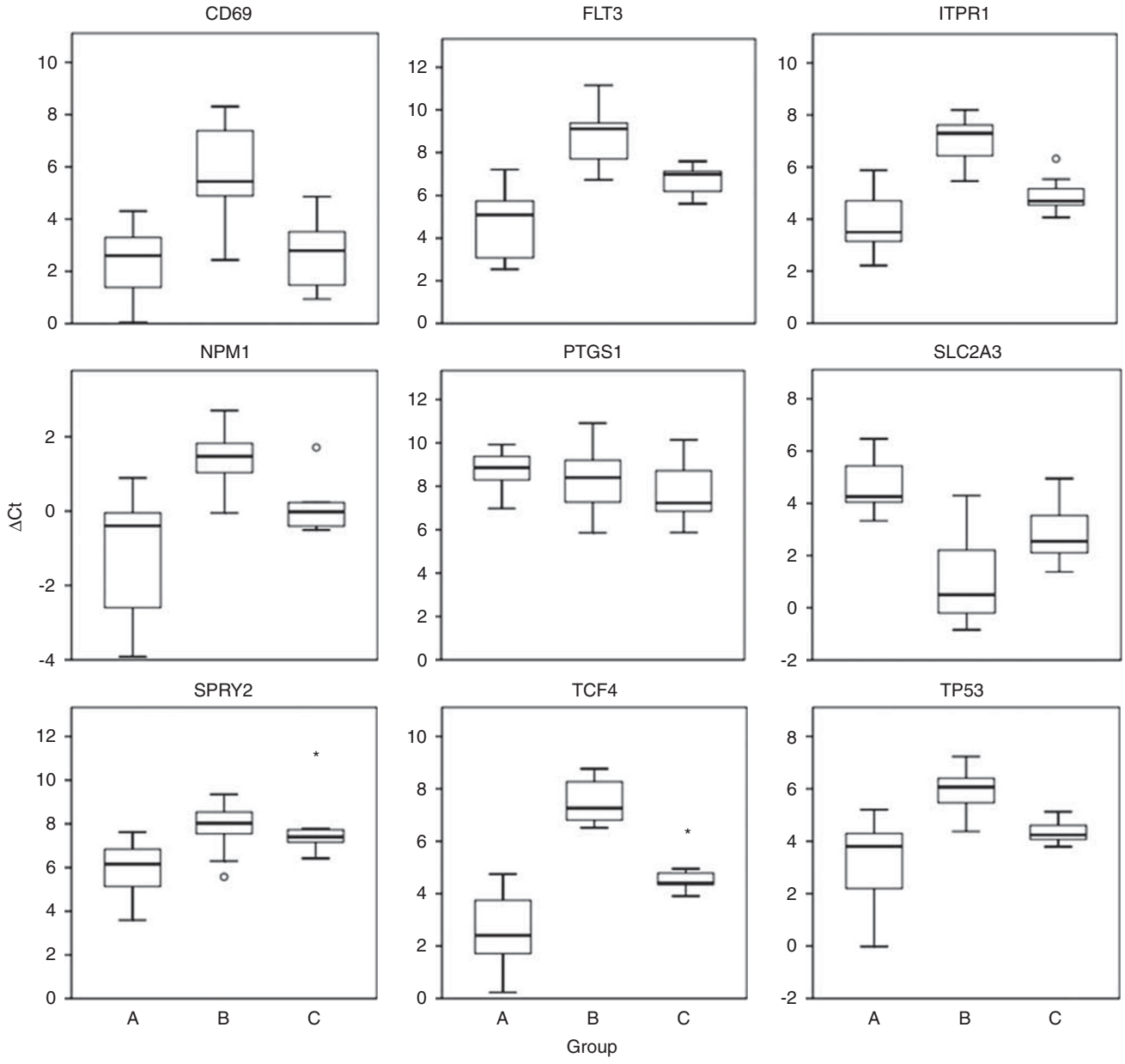

Figure 3 Expression levels of the nine signature genes among therapeutic response groups. Higher ΔCt level indicates lower gene expression. Refer to Table 2 for *P*-values.

abnormalities ($P=0.001$), although there was no significant difference in OS ($P=0.085$). t(9;22) as a sole abnormality was also found significantly more often in group B ($P=0.001$), whereas significantly higher frequencies of −7, +21, and aneuploidy were observed in group A ($P=0.045$, 0.045, and 0.021, respectively).

The gene expression pattern of Ph+ ALL cases with t(9;22) as a sole abnormality was characterized by higher levels of *SLC2A3* ($P=0.006$) and lower levels of *FLT3* ($P=0.004$). The other common cytogenetic finding that showed significant impact on survival was +21, which was associated with better EFS ($P=0.027$). Trisomy 21 also appeared to correlate with improved OS, but this was not statistically significant, possibly because of small sample size ($P=0.097$). Patients with +21 also expressed higher levels of *FLT3* and *TCF4* ($P=0.014$ and 0.029, respectively).

As the *IKZF1* gene resides on chromosome 7p13-p11.1, we further correlated monosomy 7 and *IKZF1* mRNA expression levels in our study group. We used qRT-PCR primers to amplify the transcribed sequence from exon 3 of the *IKZF1* gene, which is the most commonly deleted region in Ph+ ALL.[11] The average *IKZF1* ΔCt in the −7 group was 5.69 ± 0.6 *versus* 4.80 ± 0.69 in the remaining cases ($P=0.005$), which translates into approximately a

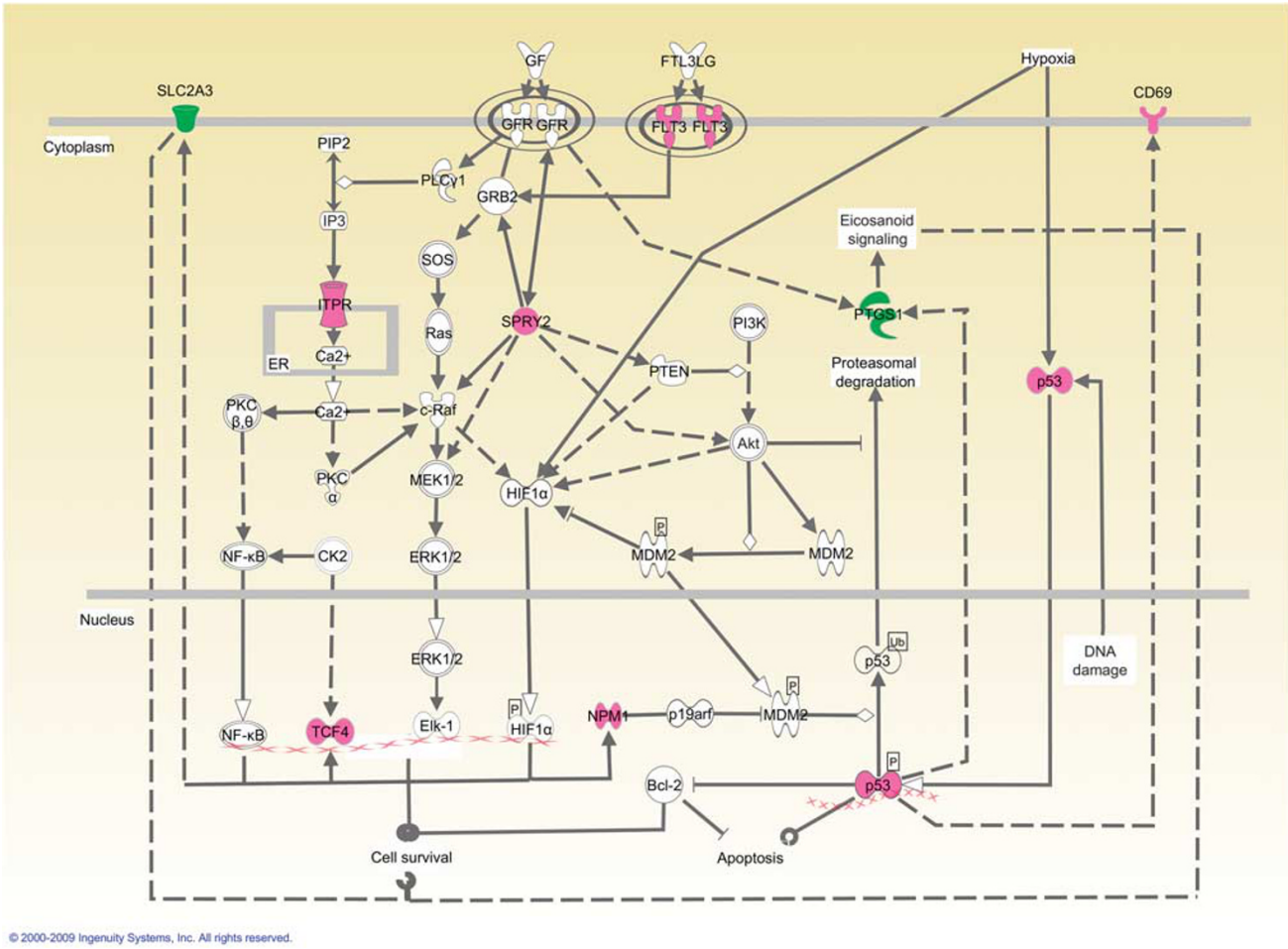

Figure 4 The nine signature genes in the context of their representing pathways. The pathways are displayed graphically as nodes (genes/gene products) and edges (the biologic relationships between the nodes). Genes that are color coded are the signature genes and their expression pattern in patients with persistent residual disease (group B), with red representing upregulation and green representing downregulation.

1.9-fold difference in mRNA levels. Patients with −7 expressed higher levels of *RPLP0, CASP10*, *MYC*, *SPRY2*, *PPARA*, *RPS18*, *RPS23*, *ITPR1*, and *TP53* ($P = 0.002$, 0.006, 0.016, 0.019, 0.026, 0.028, 0.029, 0.032, and 0.032, respectively) and lower levels of SLC2A3 ($P = 0.008$). However, neither −7 nor *IKZF1* expression was significantly associated with therapeutic response or outcome.

Discussion

The Ph+ subset is the most common genetic type of ALL in adults and conveys a poor prognosis in both pediatric and adult patients. Although Ph+ ALL shares the t(9;22)(q34;q11) with CML, progression and therapeutic response in these two diseases differ substantially. TKI therapies that have achieved remarkable success in treating CML patients have improved the complete response (CR) rate to over 90% in Ph+ ALL patients, but are less effective in maintaining a long-lasting CR.[12] With the current standard of therapy being TKI and combination chemotherapy, 5-year OS in adult Ph+ ALL patients is approximately 30% or less.[24] SCT in first CR is potentially curative,[25,26] and TKI regimens have increased the number of patients who are able to proceed to transplant. However, morbidity and mortality associated with SCT also complicate its use, especially in elderly patients. Moreover, studies have found that optimal responders to therapy may do just as well without SCT as those who received SCT.[27,28] To date, there is no effective model that can predict response to therapy at the time of diagnosis in patients with Ph+ ALL.

In this study, we systematically analyzed clinical, hematological, cytogenetic, and molecular data derived from 43 *de novo* adult Ph+ ALL patients and identified a 9-gene signature for predicting therapeutic response. Owing to the rarity of this disease, we were only able to validate the result in a small independent cohort of eight patients seen at our institution recently. Nevertheless, the prediction accuracy for patients therapeutic response was high, 87%. This highly

robust gene signature will enable more effective stratification of patients for therapy, design of personalized treatment plans, and a more accurate assessment of the risk and benefit when it comes to evaluating aggressive procedures, such as SCT.

The unique gene expression patterns observed in our study among three different therapeutic response groups suggest that rapidly growing tumors with high expression levels of genes involved in cell growth, proliferation, and apoptosis regulation tend to respond well to chemotherapy, while the poor responders overexpress hypoxia-induced glucose transporter (*SLC2A3*) and the *PTGS1* drug metabolism gene that facilitate tumor cell survival in the harsh environment created by chemotherapy. This was consistent with patient clinical presentation, as patients in the optimal response group (group A) presented with higher blast counts and *BCR–ABL1* levels compared with patients who had persistent residual disease group (group B).

The pathogenesis of Ph+ ALL is incompletely understood, other than the presence of its signature translocation, t(9;22). The growth-promoting effects of the constitutively activated tyrosine kinase activity in the *BCR–ABL1* fusion gene have been widely attributed to be the major driver in this disease.[1] Two aspects of this translocation have been implicated in affecting disease outcome: type of fusion transcript and gene dosage. Unlike in CML, where most cases express the $p210^{BCR–ABL1}$ fusion protein, the $p190^{BCR–ABL1}$ isoform is most common in Ph+ ALL, and has been associated with favorable prognosis.[29] In our cohort, 35 of 43 (81%) patients had the $p190^{BCR–ABL1}$ isoform. However, we did not observe a correlation between fusion transcript isoform and either therapeutic response or survival. In terms of a gene dosage effect, earlier studies have suggested a correlation between an additional copy of the fusion gene with worse prognosis.[30] However, we did not observe a significant correlation between addition of der(22) and either DFS or OS. This finding is consistent with a more recent study in a large cohort of patients.[31] Interestingly, in this study patients with t(9;22) as a sole abnormality responded poorly to therapy, suggesting that mechanisms involving regulation of other genes may have an important role in response to therapy.

In addition to t(9;22)(q34;q11), other cytogenetic features that are commonly found in other subtypes of ALL can often coexist in Ph+ ALL and correlate with prognosis.[31] In this study, aneuploidy, −7, and +21 were found to correlate with better response to therapy. Aneuploidy, especially hyperdiploidy, is common in pediatric ALL and is associated with a good outcome. From our results, and those of another study,[31] this effect seems to be true in adult patients with Ph+ ALL as hyperdiploidy occurred most often in the optimal response patient group. Abnormalities of chromosome 9p, such as deletion of *CDKN2A* at 9p21, have or have not correlated with prognosis in previous studies,[30,31] suggesting that different regions on this chromosome may have different effects. No correlation was observed in the small number of patients with abnormal chromosome 9p in our study.

Chromosome 7 contains the *IKZF1* gene, which has been found to be deleted in up to 84% of Ph+ ALL patients.[32] This deletion is believed to be associated with poor prognosis.[32] *IKZF1* deletion was not a focus of our study. However, in six (14%) patients with −7, where deletion of *IKZF1* is implied, we found no significant correlation with either therapeutic response or survival. One possible explanation for this lack of correlation is the small number of cases in our study. In another recent study of Ph+ ALL patients, however, *IKZF1* deletion was found to be associated with short disease-free survival and high relapse rate, but there was no difference in OS.[33]

An additional chromosome 21 was found in four patients, all of whom were optimal responders (group A). There is a folate transporter gene located on chromosome 21, and the gene dosage effect of +21 could account for an influx of methotrexate, thereby providing a rationale for optimal response.[34]

Therapy refractoriness and relapse is a major concern in managing Ph+ ALL patients. In our cohort, 56% patients responded poorly to therapy or had early recurrence of the fusion transcript, as detected by qRT-PCR. The mechanisms involved in the development of therapeutic resistance or relapse in these patients are largely unclear. *ABL1* KD mutations, especially T315I mutation, have been reported as a mechanism for developing resistance to TKI therapy in both CML and Ph+ ALL patients.[16,35] Compared with CML, mutations develop much faster in ALL after therapy, and a significant number of patients harbor mutations. As we did not have the *ABL1* KD mutation status for all patients in our study, the frequencies of the mutations we observed may not be representative. Nevertheless, our observation of 70% mutations in relapsed patients is consistent with published studies.[36] The significance of these mutations in therapeutic response and prognosis is still debatable.[37,38] In our study, the presence of *ABL1* KD mutations, including the highly resistant T315I mutation, was not associated with survival, suggesting that additional mechanisms such as gene regulation may be involved. When comparing the gene expression patterns among patients with or without *ABL1* KD mutation at relapse, we found that tumors with overexpression of growth-promoting genes but not hypoxia survival genes, such as *SLC2A3*, tend to develop mutations. This finding suggests that the development of resistant mutations may result from the combined effects of high proliferation and survival, thereby promoting clonal evolution under the selective pressure of cytotoxic chemotherapeutic agents.

Integration of these findings with the current knowledge of Ph+ ALL may lead to novel insights

into the mechanisms by which leukemia cells respond to therapy. First, optimal patient responders tend to have neoplasm that focus on growth/proliferation, as indicated by their high blast counts and *BCR–ABL1* transcript levels and activation of growth pathways. The rapid growth and lack of strategic regulation of the tumor cells cause their genomes to become highly disorganized and unstable, supported by the high frequency of aneuploidy or mutations found in this group. The high growth rate and genomic instability also have a role in the leukemic cells being highly sensitive to chemotherapy. A similar phenomenon is also seen in other types of ALL.[1] In patients with therapy-resistant tumors, on the other hand, the leukemic cells are more focused on survival, and tend to have a more stable genome, with *BCR–ABL1* translocation as the only karyotypic change. These neoplasms tend to exhibit a moderate growth rate, and have strategically developed 'survival mechanisms' to counter therapeutic effects, such as overexpression of the hypoxia-induced glucose transporter gene, *SLC2A3*, and the cell signaling and drug metabolism gene, *PTGS1*. The end result is a synergic effect of low growth demand and high survival protection status that enables the leukemic cells to survive therapy.

Downregulation of *TP53* and *NPM1* in therapy-resistant tumors found in our study is also consistent with the reported high rate of inactivation of *CDKN2A/B* by deletion, mutation, aberrant repression of epigenetic silencing in such patients.[11] As illustrated in Figure 4, the leukemic cells can adjust the regulation of complex signal pathways to achieve a balance between growth and survival. Moreover, our observation of the shift in gene expression pattern when a tumor relapses suggests that this is a constantly evolving process. Under the selective pressure from chemotherapy, Ph + ALL cells can either adjust their gene expression pattern or develop therapy-resistant mutations, such as *ABL1* mutations, to ensure survival. Recent evidence suggests that *ABL1* is a direct target of the tumor suppressor microRNA, miR-203, which is silenced in leukemic cells by loss of heterozygosity and CpG hypermethylation.[39] These data support the concept that t(9;22) may be only one part of a much larger, regulatory network of the leukemogenesis.

In conclusion, we have identified a unique 9-gene signature that predicts therapeutic response in adults with Ph + ALL. Our results support the concept that current TKI and genotoxic chemotherapy regimens are effective in eliminating rapidly growing and genomically unstable tumors. These agents are less effective, however, in leukemias that have developed adaptive survival mechanisms. Additional studies with a larger patient cohort will be valuable to further validate this gene signature.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Modern Pathology website (http://www.nature.com/modpathol)