Prediction of Sensitivity to STI571 among Chronic Myeloid Leukemia Patients by Genome-wide cDNA Microarray Analysis

Yasuyuki Kaneta,1 Yoshitoyo Kagami,2,12 Toyomasa Katagiri,1,12 Tatsuhiko Tsunoda,3 Itsuro Jin-nai,1 Hirokuni Taguchi,5 Hisamaru Hirai,6 Kazunori Ohnishi,7 Takanori Ueda,9 Nobuhiko Emi,9 Akihiro Tomida,10 Takashi Tsuruo,10 Yusuke Nakamura1, 13 and Ryuzo Ohno11

1Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, 2Department of Hematology and Chemotherapy, Aichi Cancer Center Hospital, Kanokoden, Chikusa-ku, Nagoya 464-8681, 3Laboratory of Medical Informatics, SNP Research Center, RIKEN (Institute of Physical and Chemical Research), 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, 4Department of Hematology, Atomic Bomb Disease Institute, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852-8523, 5Third Department of Internal Medicine, Kochi Medical School, 185-1 Kohasu, Okok-cho, Nankoku, Kochi 783-8505, 6Third Department of Internal Medicine, Faculty of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, 7Department of Internal Medicine III, Hamamatsu University School of Medicine, 3600 Handacho, Hamamatsu 431-3192, 8First Department of Internal Medicine, Fukui Medical School, 23-3 Shimoaizuki, Matsuoka-cho, Yoshida-gun, Fukui 910-1104, 9The First Department of Internal Medicine, Nagoya University School of Medicine, 65 Tsurumai, Showa-ku, Nagoya 466-0064, 10Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032 and 11Aichi Cancer Center Hospital, Kanokoden, Chikusa-ku, Nagoya 464-8681

One of the most critical issues to be solved in regard to cancer chemotherapy is the establishment of ways to predict the efficacy of anti-cancer drugs for individual patients. To develop a prediction system based on expression of specific genes, we analyzed expression profiles of mononuclear cells from 18 chronic myeloid leukemia (CML) patients who were treated with the tyrosine kinase inhibitor STI571. cDNA microarrays representing 23,040 genes identified 79 genes that were expressed differentially between responders and non-responders to STI571. On the basis of the expression patterns of 15 or 30 of these genes among the patients, we developed a “Prediction Score” system that could clearly separate the responder group from the non-responder group. Verification of this system using four additional (“test”) cases succeeded in predicting the response of each of those four patients to the drug. These results provide the first evidence that gene-expression profiles can predict sensitivity of CML cells to STI571, and may eventually lead to the achievement of “personalized therapy” for this disease.

Key words: cDNA microarray — Chronic myeloid leukemia — STI571 — Prediction score — Chemosensitivity

Chronic myeloid leukemia (CML) is a clonal disorder arising from neoplastic transformation of hematopoietic stem cells, most of which are characterized by the presence of a Philadelphia chromosome (Ph) and by constitutive activation of BCR-ABL tyrosine kinase.31 CML progresses through three phases; chronic phase, accelerated phase and invariably fatal blast crisis. Conventional therapeutic options include interferon-α and allogeneic stem cell transplantation (SCT). Interferon-α prolongs overall survival, but has considerable adverse effects. SCT is the only curative treatment, but is associated with substantial morbidity and is limited to patients with suitable donors. Thus, the prognosis of CML is still poor.

Development of the ABL-selective tyrosine kinase inhibitor STI571 (imanitib; Glivec; Novartis Pharmaceuticals, Basel, Switzerland) was an important advance in the management of CML.2, 3) With this drug, around 90% of CML patients are induced into hematological complete remission, and in more than 60% of patients Ph chromosome-positive leukemia cells are completely or partially reduced without severe adverse effects.5) Thus, STI571 has become the first choice drug for the treatment of CML, and its promising effects make it difficult to decide the timing for SCT.7) Moreover, time and medical cost are wasted if the drug is ineffective, and non-responsive patients risk losing a chance for alternative chemotherapy. Therefore, accurate prediction regarding effectiveness of a specific therapy is of critical importance for CML patients. Recent studies have demonstrated that information generated by cDNA microarray analysis of gene expression in
human tumors can provide more accurate data as to the nature of cancer cells than traditional histopathological methods are able to supply.6–8 The promise of such information lies in its potential for improving clinical strategies for treating neoplastic diseases.

With this in mind we applied a microarray of human cDNAs consisting of 23,040 transcribed elements to analyze gene-expression profiles in CML cells, with a view to developing a novel system for predicting responsiveness of an individual CML patient to treatment with STI571. We identified a group of genes as differentially expressed among 12 patients belonging to the “good responder” category and six who showed no response to the drug, and established a “Prediction Score” system that correctly predicted the responsiveness or non-responsiveness among four additional test cases. Our results suggest that the expression levels of a set of genes selected in this way can determine the fate of CML exposed to STI571, and that such information may lead to “personalized therapy” and thereby improve the quality of life and prognosis of CML patients.

We obtained peripheral blood samples with informed consent from 22 Japanese adult CML patients prior to treatment with STI571. Each patient was then enrolled into a phase II study of STI571. mRNA from eighteen samples in which more than 65% of cells had been positive for the Ph chromosome prior to treatment, by means of a FISH analysis detecting a bcr/abl fusion gene,9 were analyzed on our cDNA-microarray system. Fabrication of our cDNA-microarray system containing 23,040 cDNAs

![Fig. 1. Cytogenetic responses to STI571 treatment among 22 CML patients. Each line represents the response of an individual patient; blue lines indicate non-responders and red lines indicate responders. Black lines indicate test cases.](image)

Table I. Clinicopathological Features of Patients Examined

| Patient’s ID | Age | Sex | Response      | Prediction | Phase    |
|--------------|-----|-----|---------------|------------|----------|
| CML003       | 66  | M   | Responder     | Learning   | Chronic  |
| CML004       | 55  | F   | Responder     | Learning   | Chronic  |
| CML008       | 61  | F   | Responder     | Learning   | Chronic  |
| CML009       | 68  | M   | Responder     | Test       | Chronic  |
| CML010       | 56  | M   | Responder     | Learning   | Chronic  |
| CML013       | 59  | F   | Non-responder | Learning   | Chronic  |
| CML014       | 47  | M   | Responder     | Learning   | Chronic  |
| CML015       | 63  | F   | Responder     | Test       | Chronic  |
| CML018       | 57  | M   | Non-responder | Learning   | Chronic  |
| CML019       | 23  | M   | Non-responder | Learning   | Chronic  |
| CML021       | 57  | M   | Responder     | Learning   | Chronic  |
| CML025       | 44  | M   | Non-responder | Learning   | Chronic  |
| CML027       | 35  | M   | Non-responder | Learning   | Chronic  |
| CML030       | 61  | M   | Responder     | Learning   | Chronic  |
| CML033       | 56  | M   | Responder     | Learning   | Chronic  |
| CML036       | 48  | M   | Responder     | Learning   | Chronic  |
| CML047       | 32  | F   | Responder     | Learning   | Chronic  |
| CML050       | 38  | M   | Non-responder | Learning   | Blast crisis |
| CML054       | 32  | M   | Responder     | Learning   | Chronic  |
| CML056       | 46  | F   | Responder     | Learning   | Blast crisis |
| CML080       | 59  | F   | Non-responder | Test       | Accelerated |
| CML197       | 30  | F   | Non-responder | Test       | Accelerated |

Response, response to STI571 treatment; Learning, samples used to develop the prediction system; Test, samples used for test cases.
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Fig. 2. Expression patterns of the 79 discriminating genes among 18 CML patients. The mean ($\mu$) and standard deviation ($\sigma$) were calculated from the log-transformed relative expression ratios of each gene in responder (r) and non-responder (n) patients. A discrimination score (DS) for each gene was defined as follows: $DS = (\mu_r - \mu_n)/(\sigma_r + \sigma_n)$. We carried out permutation tests to estimate the ability of individual genes to distinguish between responders and non-responders; samples were randomly permuted between the two classes 10,000 times. Since the DS dataset of each gene showed a normal distribution, we calculated a $P$ value for the user-defined grouping.7) Horizontal rows represent individual genes; vertical columns represent individual samples. Each cell in the matrix represents the expression level of a single transcript in single sample, with red and green indicating transcript levels respectively above and below the median for that gene across all samples. Black represents unchanged expression; gray indicates no or slight expression (intensities of both Cy3 and Cy5 under the cut-off value). Color saturation is proportional to the magnitude of the difference from the median.
### Table II. A List of 79 Candidate Genes for Development of the Prediction System

| Rank | Permutation P-value | GenBank ID | Symbol | Gene name |
|------|---------------------|------------|--------|-----------|
| 1    | 0.0003              | AI086871   | HN1    | Humanin   |
| 2    | 0.0003              | D17793     | AKR1C3 | aldo-keto reductase family 1, member C3 |
| 3    | 0.0013              | X76013     | QARS   | glutaminyl-tRNA synthetase |
| 4    | 0.0015              | AA136180   | KIAA1105 | KIAA1105 protein |
| 5    | 0.0019              | AA506972   | KIAA0668 | KIAA0668 protein |
| 6    | 0.0020              | AF053470   | BLCAP  | bladder cancer associated protein |
| 7    | 0.0021              | X97324     | ADFP   | adipose differentiation-related protein |
| 8    | 0.0029              | AA894587   | FLJ10422 | hypothetical protein FLJ10422 |
| 9    | 0.0038              | L07033     | HMGCL  | 3-hydroxymethyl-3-methylglutaryl-coenzyme A lyase |
| 10   | 0.0040              | AI051454   | EST    | EST |
| 11   | 0.0063              | AI290876   | KLF4   | Kruppel-like factor 4 |
| 12   | 0.0083              | M11354     | H3F3A  | H3 histone, family 3A |
| 13   | 0.0094              | V00478     | ACTB   | actin, beta |
| 14   | 0.0094              | AA401318   | DFKZP566D193 | DFKZP566D193 protein |
| 15   | 0.0101              | U79268     | APEX   | APEX nuclease |
| 16   | 0.0107              | U88047     | DRIL1  | dead ringer (*Drosophila*)-like 1 |
| 17   | 0.0113              | AF001383   | BIN1   | bridging integrator 1 |
| 18   | 0.0114              | AA495984   | EST    | EST |
| 19   | 0.0123              | N41902     | CLTH   | Clathrin assembly lymphoid-myeloid leukemia gene |
| 20   | 0.0127              | X179832    | M6PR   | mannose-6-phosphate receptor |
| 21   | 0.0133              | D14662     | KIAA0106 | anti-oxidant protein 2 |
| 22   | 0.0139              | J05528     | IGF2R  | insulin-like growth factor 2 receptor |
| 23   | 0.0151              | AA330014   | IDH1   | isocitrate dehydrogenase 1 (NADP+), soluble |
| 24   | 0.0154              | AI333449   | EST    | EST |
| 25   | 0.0156              | AA365986   | SDHB   | succinate dehydrogenase complex, subunit B |
| 26   | 0.0165              | AI743134   | TNRC3  | trinucleotide repeat containing 3 |
| 27   | 0.0171              | AA156488   | MGP    | KIAA1008 protein |
| 28   | 0.0178              | U26710     | CBLB   | Cas-Br-M ectropic retroviral transforming sequence b |
| 29   | 0.0187              | AA055355   | EST    | EST |
| 30   | 0.0191              | T70782     | FLJ10803 | hypothetical protein FLJ10803 |
| 31   | 0.0193              | J05272     | IMPDH1 | IMP (inosine monophosphate) dehydrogenase 1 |
| 32   | 0.0197              | AI919459   | FLJ20489 | hypothetical protein FLJ20489 |
| 33   | 0.0200              | U77948     | GTF2I  | major histocompatibility complex, class I, B |
| 34   | 0.0217              | U31906     | GOLGA4 | golgi autoantigen, golgin subfamily a, 4 |
| 35   | 0.0272              | AA743462   | EST    | EST |
| 36   | 0.0279              | U46767     | SCYA13 | small inducible cytokine subfamily A, member 13 |
| 37   | 0.0281              | D29805     | B4GALT1 | beta 1,4-galactosyltransferase, polypeptide 1 |
| 38   | 0.0290              | AA1433048  | DFKZP564O0463 | DFKZP564O0463 protein |
| 39   | 0.0299              | V00478     | ACTB   | actin, beta |
| 40   | 0.0314              | X63368     | HSJ1   | heat shock protein, neuronal DNAJ-like 1 |
| 41   | 0.0315              | X06323     | MRPL3  | mitochondrial ribosomal protein L3 |
| 42   | 0.0320              | D80005     | C9orf10 | C9orf10 protein |
| 43   | 0.0327              | X70649     | DDX1   | DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 1 |
| 44   | 0.0350              | AA421326   | EST    | Homo sapiens cDNA: FLJ21918 fis, clone HEP04006 |
| 45   | 0.0353              | AF055066   | HLA-A  | major histocompatibility complex, class I, A |
| 46   | 0.0359              | AA101834   | STIM1  | stromal interaction molecule 1 |
| 47   | 0.0360              | M91029     | AMPD2  | adenosine monophosphate deaminase 2 |
| 48   | 0.0361              | U26648     | STX5A  | syntaxin 5A |
| 49   | 0.0366              | M25460     | IFNB1  | interferon, beta 1, fibroblast |
| 50   | 0.0370              | AI291745   | MAEA   | macrophage erythroblast attacher |
| 51   | 0.0372              | L25941     | LBR    | lamin B receptor |
| 52   | 0.0373              | D45906     | LIMK2  | LIM domain kinase 2 |
| 53   | 0.0387              | AI365683   | EST    | Homo sapiens PAC clone RP4-751H13 from 7q35-pter |
was described previously. We prepared mononuclear cells using Ficoll (Amersham Biosciences, Buckinghamshire, UK) and extracted total RNA using TRIzol (Life Technologies, Inc., Grand Island, NY) according to the manufacturer’s instructions. After treatment with DNase I (Nippon Gene, Tokyo), T7-based RNA amplification was carried out as described previously. Two rounds of amplification using 2 \( \mu \)g of total RNA as starting material yielded 40–100 \( \mu \)g of amplified RNA (aRNA). For control samples we also performed two rounds of T7-based RNA amplification to obtain sufficient amounts of aRNA. RNA amplified by this method accurately reflects the proportions in the original RNA source, as we had confirmed earlier by semi-quantitative RT-PCR experiments, in which data from microarrays were consistent with results from total RNA or aRNA was used as the template. Labeling, hybridization, washing, scanning, and quantification of signals were performed as described previously except that all processes were carried out with an Automated Slide Processor. Sixteen patients with CML in the chronic phase were treated with 400 mg/day of STI571 and two patients in blast crisis were treated with 600 mg/day. We determined the clinical response to STI571 by cytogenetic criteria; that is, by the percentage of peripheral blood cells positive for Ph chromosome by the FISH analysis. The 12 patients who showed major cytogenetic responses (less than 35% of cells remaining positive for the Ph chromosome) were classified as responders (red lines in Fig. 1), whereas the six patients with more than 65% of cells still positive for the Ph chromosome after 5 months of STI571 treatment were considered non-responders (blue lines in Fig. 1). The remaining four were reserved to test the predictive scoring system later (black lines in Fig. 1). Of the 22, two “learning” cases were in blast crisis phase and two “test” cases were in accelerated phase (Table I), and their cytogenetic responses were analyzed within 12 weeks after the start of treatment, because STI571 was clinically ineffective and was discontinued within 12 weeks (Table I, Fig. 1). As controls we used a mixture of mononuclear cells from peripheral blood of 11 healthy volunteers.

Using this information we attempted to establish a scoring system to predict the efficacy of STI571 treatment. We calculated the prediction score according to procedures described previously. Each gene \( g \) votes for either

Table II. (Continued)

| Rank | Permutation P-value | GenBank ID | Symbol | Gene name |
|------|---------------------|------------|--------|-----------|
| 62   | 0.0391              | AA778161   | RPL26  | ribosomal protein L26 |
| 63   | 0.0395              | AL137271   | FLJ10209 | hypothetical protein FLJ10209 |
| 64   | 0.0407              | AA132519   | FAAH   | EST |
| 65   | 0.0415              | Y07572     | C21ORF33 | ES1 (zebrafish) protein, human homolog of |
| 66   | 0.0427              | Z44513     | EST    | EST |
| 67   | 0.0432              | X07767     | PRKACA | protein kinase, cAMP-dependent, catalytic, alpha |
| 68   | 0.0433              | L19713     | EPB49  | erythrocyte membrane protein band 4.9 (dematin) |
| 69   | 0.0439              | M91029     | AMPD2  | adenosine monophosphate deaminase 2 |
| 70   | 0.0439              | U51712     | EST    | EST |
| 71   | 0.0442              | AI334396   | CRSP9  | cofactor required for Sp1 transcriptional activation |
| 72   | 0.0442              | AA600323   | EST    | EST |
| 73   | 0.0442              | L77564     | STK22B | serine/threonine kinase 22B |
| 74   | 0.0444              | X01410     | TRB@   | T cell receptor beta locus |
| 75   | 0.0446              | Z21507     | EEF1D  | eukaryotic translation elongation factor 1 delta |
| 76   | 0.0446              | U57629     | RPGR   | retinitis pigmentosa GTPase regulator |
| 77   | 0.0454              | AA918725   | ARRB1  | arrestin, beta 1 |
| 78   | 0.0458              | AA602490   | NOP5/NOP58 | nucleolar protein NOP5/NOP58 |
| 79   | 0.0461              | M87790     | IGL@   | immunoglobulin lambda locus |

Information was retrieved from Unigene database in National Center for Biotechnology Information (NCBI) (build#131).
responder or non-responder depending on whether the expression level ($x_i$) in the sample is closer to the mean expression level of responders or non-responders in reference samples. The magnitude of the vote ($v_i$) reflects the deviation of the expression level in the sample from the average of the two classes:

$$V_i = |x_i - (\mu_r + \mu_n)/2|.$$  

We summed the votes to obtain total votes for the responder ($V_r$) and non-responder ($V_n$), and calculated PS values as follows: $PS = ((V_r - V_n)/(V_r + V_n)) \times 100$, reflecting the margin of victory in the direction of either responder or non-responder. PS values range from $-100$ to $100$; a higher absolute value of PS reflects a stronger prediction. Next we rank-ordered the 79 candidate genes on the basis of the magnitude of their permutation $P$-values (Table II) and calculated the prediction score by the leave-one-out test for cross-validation using the top 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, and 79 genes on the rank-ordered list. For the leave-one-out test, one sample is withheld, the permutation $P$-value and mean expression levels are calculated using the remaining samples, and the class of the withheld sample is subsequently evaluated by calculating its prediction score. We repeated this procedure for each of the 18 samples. Then, to determine the number of discriminating genes that provided the best separation of the two groups, we calculated a classification score (CS) for each gene set (see the legend of Fig. 3A).

The number of genes used for calculation influenced the power for separation of the two groups. We obtained the best separation when we used the top 15 or 30 genes in our candidate list for calculation of the scores (Fig. 3A). The “Prediction Score” system using these two sets of genes clearly separated the two patient groups (Fig. 3B).
Hierarchical clustering using the same gene sets was also able to classify the groups with regard to STI571 sensitivity (Fig. 3C). This analysis was performed using web-available software (“cluster” and “treeview”) written by M. Eisen (http://genome-www5.stanford.edu/MicroArray/SMD/restech.html). Before the clustering algorithm was applied, the fluorescence ratio for each spot was first log-transformed and then the data for each sample were median-centered to remove experimental biases.

To validate this prediction system, we investigated four additional (“test”) cases that were completely independent of the 18 “learning” cases used for establishing the system. We examined gene-expression profiles in each of these four blood samples and then calculated a prediction score for each of them using the panels of 15 or 30 discriminating genes. As shown in Fig. 3D, responsiveness of each of these four patients to STI571 was predicted accurately.

Treatment of CML patients with STI571 provides a considerable advantage over treatment with interferon-α, because the degree of cytogenetic response obtained by STI571 is clearly higher, with less severe adverse effects.\(^{13}\) However, as around 40% of CML patients fail to achieve major cytogenetic response,\(^{4}\) it is important to develop a way to predict the efficacy of STI571 before therapy is undertaken for an individual patient.

In this study we profiled the gene expression patterns of mononuclear cells from CML patients using a comprehensive cDNA-microarray system containing 23 040 genes, with a view to establishing a “Prediction Score” system. We identified 79 genes that were differentially expressed among patients who showed good response as opposed to poor response to STI571, and ranked them by the permutation test \(P\)-values of <0.05. Then we attempted to optimize the power to separate the two groups by selecting a subset of the discriminating genes on the basis of CS. We obtained the best CS using subsets of 15 or 30 discriminating genes; the scoring system based on these two subsets separated the two groups very clearly. Our scoring system was also able to predict accurately the response to STI571 of four additional cases. Although four patients who were in accelerated and blast crisis phases of CML were included, our scoring system classified all cases, as to their chemosensitivity to STI571. We believe that the use of CS is a reasonable approach to selecting appropriate indicators for predictive scores.

The 79 genes that showed different expression levels in responders versus non-responders might provide interesting insight into the biological mechanism underlying the response to STI571 in CML. Among these genes, Apex nuclease (APEX) was over-expressed in the non-responder group. APEX is the major apurinic/apyrimidinic endonuclease (Ap endo), with a key function in the DNA-repair system that confers resistance to ionizing radiation and alkylating agents in human cell lines.\(^{16}\) APEX, which stimulates the binding of MYB (v-myb myeloblastosis viral oncogene homolog) to DNA, is a known accelerator of proliferation and was up-regulated in all CML cells in our experiments.\(^{15}\)

On the other hand, Kruppel-like factor 4 (KLF4) was suppressed in the non-responder group. This gene is a zinc finger-containing transcription factor, enriched in epithelial cells, that is known to suppress cell proliferation.\(^{16}\) Thus, down-regulated expression of KLF4 in the non-responders might abrogate regulation of the cell cycle. We suggest that the 79 genes that may affect sensitivity of CML cells to STI571 could serve as molecular targets for overcoming chemo-resistance and also for development of novel drugs.

Although adverse drug reactions caused by STI571 are much less severe than with other anti-cancer drugs, its long-term use may ruin the chance of a non-responder to benefit from alternative therapies. Hence, we believe that our prediction system should provide an opportunity for potential non-responders to achieve a better prognosis and a better quality of life, although certainly a larger-scale study is warranted. Our data suggest, however, that the goal of “personalized medicine,” giving the right drug to each patient, may be achievable by selecting a set of genes for its predictive value according to the approach shown here. However, to establish optimal prediction we should consider including genotypic information such as single nucleotide polymorphisms (SNPs) that might influence the metabolism of the drug in question.

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