Protein signatures as potential surrogate biomarkers for stratification and prediction of treatment response in chronic myeloid leukemia patients

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Received April 8, 2016; Accepted May 4, 2016

DOl: 10.3892/ijo.2016.3618

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Abbreviations: CML, chronic myeloid leukemia; CMR, complete molecular response; CP, chronic phase; DAS, dasatinib; IM, imatinib mesylate; MCyR, major cytogenetic response; MMR, major molecular response; No-MMR, no-major molecular response; LT-MMR, long-term-MMR; P-No-MMR, persistent-no-MMR; TKI, tyrosine kinase inhibitors; 2-DE, two-dimensional gel electrophoresis; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry

Key words: proteomics, chronic myeloid leukemia, treatment response, biomarkers, tyrosine kinase inhibitor, imatinib

Abstract. There is unmet need for prediction of treatment response for chronic myeloid leukemia (CML) patients. The present study aims to identify disease-specific/disease-associated protein biomarkers detectable in bone marrow and peripheral blood for objective prediction of individual's best treatment options and prognostic monitoring of CML patients. Bone marrow plasma (BMP) and peripheral blood plasma (PBP) samples from newly-diagnosed chronic-phase CML patients were subjected to expression-proteomics using quantitative two-dimensional gel electrophoresis (2-DE) and label-free liquid chromatography tandem mass spectrometry (LC-MS/MS). Analysis of 2-DE protein fingerprints preceding therapy commencement accurately predicts 13 individuals that achieved major molecular response (MMR) at 6 months from 12 subjects without MMR (No-MMR). Results were independently validated using LC-MS/MS analysis of BMP and PBP from patients that have more than 24 months followed-up. One hundred and sixty-four and 138 proteins with significant differential expression profiles were identified from PBP and BMP, respectively and only 54 proteins overlap between the two datasets. The protein panels also discriminates accurately patients that stay on imatinib treatment from patients ultimately needing alternative treatment. Among the identified proteins are TYRO3, a member of TAM family of receptor tyrosine kinases (RTKs), the S100A8, and MYC and all of which have been implicated in CML. Our findings indicate analyses of a panel of protein signatures is capable of objective prediction of molecular response and therapy choice for CML patients at diagnosis as ‘personalized-medicine-model’.

Introduction

Chronic myeloid leukemia (CML) is unequivocally distinguishable from other myeloproliferative disorders by the presence of a reciprocal translocation of chromosomes 9 and 22 (1-3). Although the Philadelphia chromosome is detected in 90-95% of CML patients, evidence of the BCR-ABL rearrangement is also usually detected in the subgroup of Philadelphia chromosome-negative CML patients (4-6). The presence of BCR-ABL in CML patients and the requirement of kinase activity for BCR-ABL function make this an attractive target for selective kinase inhibitors.

The old traditional therapy of newly diagnosed chronic phase-CML patients includes busulfan and hydroxyurea and most of the patients will stay in a chronic phase for approximately 3-5 years (7,8). Treatment of CML later evolved to where the goal was prolongation of the chronic phase through induction of karyotypic remission and possibly molecular remission using Alfa-interferon therapy with or without cyto-
sine arabinoside. Thereafter, imatinib mesylate (IM) a tyrosine kinase inhibitor (TKI) was introduced as potential molecular therapy for CML (7,9). IM is capable of inhibiting BCR-ABL kinase activity by blocking ABL tyrosine kinase action through the binding and subsequent inactivation of the ATP-binding sites of ABL tyrosine kinase in leukemic cells (9,10). Since its introduction, several clinical trials have demonstrated the efficacy of IM and new generation TKIs in the treatment of CML, including patients with interferon-refractory CP-CML, as well as patients with CML in blast crisis (11).

Approximately more than 50% of CML patients treated with imatinib achieve a complete cytogenetic response (11,12). CML progression while on imatinib is usually due to the emergence of imatinib-resistant BCR-ABL mutant cells.

The relatively unpredictable biological behavior is a major challenge in its management as the chronic phase of CML is less aggressive and has very favorable prognosis with an excellent 5-year survival rate. By contrast, the biologically aggressive blast phase of CML is often rapidly fatal (2).

New analytical tools in proteomics are emerging that give new insights into biological processes that may speed up the discovery of potential biomarkers. Quantitative molecular variations may be used for the development of methods for tumor classification based on large amounts of gene expression data generated by 2-DE analysis of proteins (14,15).

The main aim of the present study is towards discovery of objective markers that predict patients' response status and selection of appropriate choice of therapy at the onset of disease diagnosis. It focuses on the analysis of global peripheral blood plasma and bone marrow plasma protein expression profiles among CP-CML patients who achieved LT-MMR on imatinib compared with patients without MMR as well as whether or not they remain on TKI or switch to second generation TKI or requiring alternative therapy.

The endpoint is to identify disease-specific/disease-associated protein biomarkers seen in bone marrow tissue as well as in peripheral blood plasma. This would subsequently allow monitoring of such biomarker proteins in peripheral blood, rather than bone marrow, demanding less invasive procedures for objective prediction of individual's best treatment options and prognostic monitoring of CML patients.

Materials and methods

All bone marrow samples were obtained by aspiration procedure via posterior iliac crest under local anesthesia. Because
of limited amount of materials for analysis, the cells were not flow cytometry sorted, rather unsorted bone marrows as well as unsorted peripheral blood plasma were collected and prepared for analysis.

Bone marrow and plasma, samples obtained at diagnosis and prior to initiation of treatment from 37 patients with newly diagnosed CP-CML were subjected to expression proteome analysis using combined gel-based 2-DE and label-free in-solution quantitative liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Patients selections into those that achieved or did not achieve MMR was based on patients with serial positive or negative responses to treatment at different time-points (3, 6, 12 and 24 months, respectively). Patients that responded at a time-point but failed to respond at the next time-point were not included in the analysis. However, patients that did not achieve MMR at 3 months, but subsequently achieved MMR at 6, 12 and 24 months were included. Because there was fewer number of patients with MMR at 3 months, the focus of our analyzed time-points were at 6, 12 and 24 months.

Twenty-five patients consisting 13 with major molecular response and 12 without major molecular response were analyzed. In addition, patients that failed tyrosine kinase inhibitor (TKI) were analyzed. Four additional patients

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Table I. Clinical characteristics of analyzed samples.

| Samples  | Gender | Age (years) | TKI-fail | MMR at 6 months | MMR at 12 months | MMR at 18 months | MMR at 24 months |
|----------|--------|-------------|----------|-----------------|-----------------|-----------------|-----------------|
| CML1     | Female | 14          | No       | Yes             | Yes             | Yes             | Yes             |
| CML2     | Female | 14          | No       | Yes             | Yes             | Yes             | Yes             |
| CML3     | Female | 26          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML4     | Male   | 18          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML5     | Male   | 50          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML6     | Female | 50          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML7     | Male   | 41          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML8     | Female | 64          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML10    | Male   | 27          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML13    | Male   | 44          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML15    | Male   | 21          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML16    | Male   | 44          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML17    | Female | 18          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML18    | Female | 65          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML19    | Male   | 26          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML21    | Male   | 39          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML22    | Female | 67          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML23    | Male   | 47          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML24    | Male   | 18          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML25    | Male   | 40          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML26    | Female | 30          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML27    | Female | 36          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML28    | Female | 37          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML29    | Female | 33          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML30    | Female | 44          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML31    | Female | 48          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML32    | Female | 38          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML33    | Female | 32          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML34    | Male   | 52          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML38    | Male   | 37          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML40    | Male   | 61          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML41    | Male   | 47          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML43    | Female | 51          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML44    | Female | 14          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML45    | Female | 45          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML46    | Female | 45          | Yes      | Yes             | Yes             | Yes             | Yes             |
| CML47    | Male   | 32          | Yes      | Yes             | Yes             | Yes             | Yes             |

Total 19 16 17 18 16 17 11 20 8 22
samples not included in the proteomics analysis were used in the western blot analysis. The overview of experimental design is shown in Fig. 1 and the clinical characteristics of all patients were as indicated in Table I.

Sample preparation protocols for proteomic analysis. All the patients with primary diagnosis of CML were recruited in Oncology Center at KFSh&RC. From each of the patients, 10 ml of peripheral EDTA-anti-coagulated blood (plasma) was taken. Where possible, bone marrow aspirations were obtained from the same patients in addition to peripheral blood samples.

All samples were subjected to extensive pre-analysis cleanup using human albumin removal protocols (Agilent Technologies). Written and signed informed consents were obtained from all patients and the Institution's Research Advisory Council, under the Office of Research Affairs, approved the study (RAC# 2050-040).

Protein separation by high resolution two dimensional gel electrophoresis, (2-DE) scanning and image analysis. Equivalent amount of 50 mg total proteins for each analyzed sample was dissolved in 350 ml volume of rehydration buffer [2% (v/v) IPG-buffer 4-7 linear] and loaded onto an 11-cm IPG-strip 4-7 linear (Bio-Rad Laboratories). This gave better overview of gel separated protein spots across the entire chosen pH window and gel images were visualized by SYPRO Ruby fluorescent staining. Stained gels were scanned using a Typhoon Trio Imager (GE) and data were analyzed using the Progenesis SameSpots software (version 7.1.0; Nonlinear Dynamics, Ltd., Newcastle, UK). Gel images were compared to low through-put analysis experiments (hDMSE) were performed and data were acquired over a range of m/z 50-2000 Da with a total acquisition time of 115 min. All samples were analyzed in triplicate runs (triplicate runs were repeated on two different occasions as a measure of reproducibility) and data were acquired using the MassLynx programs (version 4.1, SCN833; Waters) operated in resolution and positive polarity modes. ProteinLynx Global Server (PLGS) 2.2 and Progenesis QI for proteomics (Progenesis QIfp version 2.0.5387) (Nonlinear Dynamics/ Waters) were used for all automated data processing and database searching. The generated peptide masses were searched against two-unified non-redundant databases (Uniprot/SwissProt Human protein sequence database) using the PLGS 2.5 and Progenesis QIfp for protein identification (Waters).

Data analysis and informatics. Progenesis QI v.2.0.5387 for proteomics was used to process and search the data to accurately quantify and identify proteins that are significantly changing between sample groups. The human database containing thousands of reviewed non-redundant entries were downloaded from UniProt/Swiss-Prot and search algorithm was applied as previously described (18). The criteria used for the database search were as previously described (16). Normalized label-free quantification was achieved using Progenesis QI software. The generated differentially expressed data was filtered to show only statistically (ANOVA), significantly regulated proteins (P<0.05) and a fold change >1.5. In addition, ‘Hi3‘ absolute quantification was performed using ADH as an internal standard to give an absolute amount of each identified protein. These options are available as incorporated into the Progenesis QIfp (Nonlinear Dynamics/Waters).

Results

Changes in protein expression between patients with/without major molecular response at 6 months. A total of 73 protein spots on 2-DE gels differed significantly between patients that achieved MMR from those who did not achieve MMR (P<0.05 and at least 1.5-fold difference). The locations of these protein spots are shown as marked on a representative 2-DE map derived from a sample with MMR in Fig. 2A. Even though the identifications of these protein spots were not done, their quantitative expression fingerprints from 2-DE analysis pattern accurately predicts 13 individuals that achieved MMR at 6 months from 12 subjects without MMR (No-MMR) using principal component analysis (PCA) (Fig. 2B).
These findings are similar to what was observed with PCA plot generated from non-gel LC/MS/MS analysis platform, as some of the results were independently validated using the label free quantitative liquid chromatography tandem mass spectrometry as detailed below.

**LC/MS/MS analysis of peripheral blood for prognostic monitoring of early CML treatment response.** Peripheral blood samples were evaluated for early treatment response at 6 month and prediction of treatment options towards personalized medicine. Approximately 115 protein species were identified, of which only 64 were significantly differentially expressed between MMR and No-MMR sample groups. (>1.5- to ∞-fold change, p<0.05). These proteins predict accurately patients with MMR vs. No-MMR patients using unsupervised Hierarchical Cluster Analysis (Fig. 3).

**Evaluation of bone marrow and peripheral blood protein profiles for prognostic monitoring of prolonged and sustained treatment response vs. persistent no-major molecular response.** Some of the patients have been followed for more than 24 months. Patients who have been consistent over a long-term in achieving and maintaining MMR from 6 months until 24 months were labeled as LT-MMR, while patients that have been persistent with No-MMR from 6 months until 24 months were called P-No-MMR. We believe that the ability to select early responders from 6 months all through 24 months would be very helpful to identify markers that would accurately predict patients with risk of delayed or suboptimal response further than 6 months. These cohorts of patients were considered as important in an effort to provide the possibility to identify surrogate biomarkers to evaluate long-term treatment response and discovery of disease-specific/disease-associated proteins for objective prognostic monitoring of CML patients.

Equal amounts of total peripheral blood plasma proteins from 10 LT-MMR patients were pooled and compared for their protein expressions among 10 other samples from P-No-MMR.
patients using quantitative label-free LC/MS/MS expression proteome analysis.

Approximately 700 proteins representing 280 unique protein species were identified (due to different protein isoforms). Only 164 of the 280 proteins were significantly differentially expressed between LT-MMR and P-No-MMR sample groups (>1.5- to ∞-fold change; P<0.05) and accurately predict patients with major molecular response (LT-MMR) vs. No-major molecular response (P-No-MMR) using unsupervised principal component analysis (Fig. 4A). The list of identified differentially expressed proteins in PBP is described in Table IIA.

Similar to peripheral blood samples, >700 proteins representing 250 unique protein species were identified when similar analysis was done on bone marrow pooled samples from 8 LT-MMR patients and 8 P-No-MMR patients. One hundred and thirty-eight of the total identified proteins were significantly differentially expressed between LT-MMR and P-No-MMR bone marrow sample groups (>1.5- to ∞-fold change, P<0.05; Table IIB). These proteins predict accurately
Table II. The identified differentially expressed proteins in peripheral blood plasma (PBP) and bone marrow plasma (BMP) from CML patients with major molecular response (MMR), No-MMR, On-tyrosine kinase inhibitor (On-TKI) and NOT-on-TKI.

| Accession | Peptide count | Anova (p)   | Max fold change | Highest mean condition | Lowest mean condition | Description                                                                 |
|-----------|---------------|-------------|-----------------|------------------------|-----------------------|----------------------------------------------------------------------------|
| P50197    | 2             | 0.00534     | 2.41067         | CML-PBP-TKI-Y          | CML-PBP-MMR           | 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase                      |
| P16281    | 4             | 9.90E-08    | 2.92498         | CML-PBP-TKI-N          | CML-PBP-TKI-Y         | 23 kDa protein                                                              |
| P49313    | 4             | 1.97E-07    | 9.09421         | CML-PBP-TKI-Y          | CML-PBP-No-MMR        | 30 kDa ribonucleoprotein, chloroplast precursor                             |
| O86535    | 3             | 4.48E-12    | 22.9885         | CML-PBP-TKI-N          | CML-PBP-TKI-Y         | 3-isopropylmalate dehydratase small subunit                                 |
| P42352    | 1             | 2.83E-12    | 12.8902         | CML-PBP-TKI-N          | CML-PBP-MMR           | 30S ribosomal protein L9.                                                   |
| O66190    | 3             | 0.001921    | 15.3266         | CML-PBP-No-MMR        | CML-PBP-TKI-N         | 60 kDa chaperonin (Protein Cpn60) (groEL protein)                           |
| P50174    | 1             | 0.000148    | 2.33176         | CML-PBP-TKI-Y          | CML-PBP-MMR           | Acetyl-CoA acetyltransferase                                                 |
| P41341    | 5             | 1.37E-09    | 3.82215         | CML-PBP-TKI-N          | CML-PBP-No-MMR        | Actin 11                                                                    |
| P53458    | 4             | 2.59E-10    | 25.5243         | CML-PBP-TKI-Y          | CML-PBP-No-MMR        | Actin 5 (Fragment)                                                          |
| P53505    | 4             | 1.85E-06    | 6.06449         | CML-PBP-TKI-N          | CML-PBP-No-MMR        | Actin, cytoplasmic type 8                                                   |
| P53466*   | 4             | 0.000178    | 4.16358         | CML-PBP-TKI-N          | CML-PBP-TKI-Y         | Actin, cytoskeletal 2 (LPC2)                                                |
| P07326    | 1             | 1.50E-14    | 33782.8         | CML-PBP-TKI-Y          | CML-PBP-MMR           | Allophycocyanin beta chain                                                 |
| P72505    | 1             | 1.97E-11    | 50.0172         | CML-PBP-TKI-Y          | CML-PBP-TKI-N         | Allophycocyanin beta chain                                                 |
| P02763    | 9             | 8.94E-05    | 2.16961         | CML-PBP-TKI-Y          | CML-PBP-MMR           | Alpha-1-acid glycoprotein 1 precursor (AGP 1)                               |
| P19652    | 7             | 8.07E-10    | 3.8292          | CML-PBP-TKI-Y          | CML-PBP-MMR           | Alpha-1-acid glycoprotein 2 precursor (AGP 2)                               |
| P01009    | 35            | 7.33E-06    | 2.57662         | CML-PBP-TKI-Y          | CML-PBP-TKI-N         | Alpha-1-antitrypsin precursor                                               |
| P04217*   | 17            | 4.44E-11    | 2.21378         | CML-PBP-TKI-Y          | CML-PBP-MMR           | Alpha-1B-glycoprotein                                                      |
| P01023    | 71            | 4.34E-09    | 3.03669         | CML-PBP-TKI-Y          | CML-PBP-No-MMR        | Alpha-2-macroglobulin precursor (Alpha-2-M)                                 |
| P39701    | 2             | 0.001857    | 17.1724         | CML-PBP-MMR            | CML-PBP-TKI-Y         | Alpha-ribazole-5'-phosphate phosphatase                                    |
| P41361ab  | 6             | 2.78E-07    | 2.68159         | CML-PBP-TKI-N          | CML-PBP-TKI-Y         | Antithrombin-III (ATIII)                                                   |
| P10008    | 15            | 1.56E-12    | 5.19919         | CML-PBP-TKI-N          | CML-PBP-TKI-Y         | Antithrombin-III precursor (ATIII) (PRO0309)                               |
| P32262*   | 6             | 2.65E-06    | Infinity        | CML-PBP-MMR            | CML-PBP-TKI-Y         | Antithrombin-III precursor (ATIII)                                          |
| P32261    | 8             | 7.40E-06    | Infinity        | CML-PBP-No-MMR         | CML-PBP-TKI-Y         | Antithrombin-III precursor (ATIII)                                          |
| P15497    | 4             | 8.88E-16    | 32.5405         | CML-PBP-MMR            | CML-PBP-TKI-Y         | Apolipoprotein A-I precursor (Apo-A1)                                      |
| P18648    | 3             | 6.73E-08    | 2.76435         | CML-PBP-MMR            | CML-PBP-TKI-Y         | Apolipoprotein A-I precursor (Apo-A1)                                      |
| P02648    | 12            | 7.81E-06    | 2.49354         | CML-PBP-TKI-N          | CML-PBP-TKI-Y         | Apolipoprotein A-I precursor (Apo-A1)                                      |
| P02652    | 6             | 4.96E-10    | 3.48432         | CML-PBP-TKI-Y          | CML-PBP-No-MMR        | Apolipoprotein A-II precursor (Apo-AII)                                   |
| P06727*   | 12            | 0.00063     | 2.06242         | CML-PBP-TKI-Y          | CML-PBP-TKI-N         | Apolipoprotein A-IV precursor (Apo-AIV)                                   |
| P02655    | 2             | 3.46E-11    | 7.42195         | CML-PBP-TKI-Y          | CML-PBP-No-MMR        | Apolipoprotein C-II precursor (Apo-CII)                                   |
| P02649    | 10            | 8.01E-08    | 3.13115         | CML-PBP-TKI-Y          | CML-PBP-No-MMR        | Apolipoprotein E precursor (Apo-E)                                         |
| P43773    | 1             | 1.03E-08    | 3.21196         | CML-PBP-TKI-N          | CML-PBP-MMR           | ATP-dependent hsl protease ATP-binding subunit                              |
### Table II. Continued.

A. The identified differentially expressed proteins in PBP of CML patients

| Accession | Peptide count | Anova (p)   | Max fold change | Highest mean condition | Lowest mean condition | Description                                                                 |
|-----------|---------------|-------------|-----------------|------------------------|-----------------------|----------------------------------------------------------------------------|
| P01884    | 1             | 2.13E-09    | Infinity        | CML-PBP-TKI-Y          | CML-PBP-MMR           | Beta-2-microglobulin precursor                                               |
| P31625    | 1             | 4.44E-16    | 29.2811         | CML-PBP-TKI-Y          | CML-PBP-MMR           | Bifunctional protease/dUTPase [Includes: Aspartic]                           |
| Q08595    | 2             | 5.42E-07    | 2.36202         | CML-PBP-TKI-N          | CML-PBP-No-MMR        | BRI protein                                                                 |
| P06702a   | 3             | 2.35E-12    | 5.10685         | CML-PBP-No-MMR         | CML-PBP-MMR           | Calgranulin B (Migration inhibitory factor-related)                          |
| P07090    | 2             | 9.28E-09    | 4.35593         | CML-PBP-TKI-Y          | CML-PBP-MMR           | Calretinin (CR)                                                             |
| P00450    | 33            | 6.96E-10    | 2.07132         | CML-PBP-TKI-Y          | CML-PBP-TKI-N          | Ceruloplasmin precursor (EC 1.16.3.1) (Ferroxidase)                         |
| P13635    | 19            | 3.89E-07    | 2.06575         | CML-PBP-TKI-N          | CML-PBP-No-MMR        | Ceruloplasmin precursor (EC 1.16.3.1) (Ferroxidase)                         |
| Q61147    | 19            | 6.29E-05    | 5.77271         | CML-PBP-No-MMR         | CML-PBP-TKI-N          | Calgranulin B (Migration inhibitory factor-related)                          |
| O34002    | 1             | 0.000137    | 68.1783         | CML-PBP-MMR            | CML-PBP-TKI-Y          | Complement C1r component precursor                                          |
| P23528    | 1             | 6.64E-09    | 17.4873         | CML-PBP-No-MMR         | CML-PBP-MMR           | Complement C1s component precursor                                          |
| Q03708    | 2             | 2.25E-07    | Infinity        | CML-PBP-MMR            | CML-PBP-TKI-N          | Complement C3 precursor (HSE-MSF)                                            |
| P00736    | 4             | 1.06E-11    | 3.2943          | CML-PBP-TKI-Y          | CML-PBP-No-MMR        | Complement C3 precursor [Contains: C3a anaphylatox]                          |
| P09871    | 4             | 2.38E-07    | 2.92284         | CML-PBP-TKI-N          | CML-PBP-TKI-N          | Complement C4 precursor [Contains: C4a anaphylatox]                          |
| P01027a   | 22            | 1.58E-11    | 8.12844         | CML-PBP-TKI-N          | CML-PBP-TKI-N          | Complement factor B precursor (C3/C)                                          |
| P01024    | 83            | 5.80E-10    | 2.95796         | CML-PBP-TKI-N          | CML-PBP-TKI-N          | Complement factor I precursor (EC 3.4.21) (C3B/)                             |
| P01030a   | 20            | 0.001479    | 2.42252         | CML-PBP-No-MMR         | CML-PBP-TKI-N          | Cytochrome c oxidase polypeptide I                                           |
| P04186    | 7             | 0.000166    | 2.2386          | CML-PBP-TKI-N          | CML-PBP-MMR           | Cytochrome C551 peroxidase precursor                                         |
| P05156    | 3             | 4.54E-07    | 3.80805         | CML-PBP-TKI-Y          | CML-PBP-No-MMR        | DAG protein, chloroplast precursor                                          |
| Q33439    | 1             | 6.13E-11    | 76.1488         | CML-PBP-TKI-Y          | CML-PBP-TKI-N          | Endoplasmic reticulum protein ERp29 precursor                                |
| P14532    | 1             | 8.42E-08    | 11.1984         | CML-PBP-TKI-N          | CML-PBP-TKI-Y          | Excisionase                                                                 |
| Q38732    | 1             | 5.73E-08    | 16.099          | CML-PBP-TKI-N          | CML-PBP-TKI-Y          | Ferric uptake regulation protein                                            |
| P57759    | 3             | 5.60E-13    | 5.45666         | CML-PBP-TKI-N          | CML-PBP-TKI-Y          | Fibrinogen alpha-alpha-E chain precursor                                    |
| P20710    | 1             | 1.19E-08    | 24.9012         | CML-PBP-TKI-N          | CML-PBP-TKI-Y          | Fibrinogen beta chain precursor                                             |
| Q45765    | 1             | 0.000582    | 13.2686         | CML-PBP-TKI-N          | CML-PBP-No-MMR        | Fibrinogen gamma chain precursor                                            |
| P02671    | 23            | 0           | 5.53907         | CML-PBP-TKI-N          | CML-PBP-TKI-N          | Fibrinogen precursor (FN) (Fragments)                                       |
| P02675    | 36            | 1.52E-09    | 2.8323          | CML-PBP-TKI-Y          | CML-PBP-TKI-N          | Gas vesicle protein C                                                       |
| P02679    | 26            | 6.02E-06    | 3.07718         | CML-PBP-TKI-Y          | CML-PBP-TKI-N          | Gastrulation specific protein G12                                            |
| P11276    | 11            | 0.000201    | 2.27101         | CML-PBP-No-MMR         | CML-PBP-TKI-N          | Gelsolin (Actin-depolymerizing factor)                                      |
| P08041    | 1             | 4.74E-05    | 4.36822         | CML-PBP-MMR            | CML-PBP-TKI-Y          |                                     |
Table II. Continued.

A. The identified differentially expressed proteins in PBP of CML patients

| Accession | Peptide count | Anova (p)       | Max fold change | Highest mean condition          | Lowest mean condition          | Description                                                                 |
|-----------|---------------|-----------------|-----------------|---------------------------------|--------------------------------|------------------------------------------------------------------------------|
| P06396    | 3             | 0.000102        | 4.00281         | CML-PBP-TKI-Y                   | CML-PBP-TKI-N                  | Gelsolin precursor, plasma (Actin-depolymerizing)                            |
| P06228    | 2             | 5.86E-07        | 2.30924         | CML-PBP-TKI-Y                   | CML-PBP-TKI-N                  | **Gene 27 protein**                                                          |
| P15751    | 1             | 1.74E-07        | 2.52369         | CML-PBP-TKI-Y                   | CML-PBP-MMR                    | General secretion pathway protein L                                           |
| P23722    | 4             | 0.004817        | 3.55572         | CML-PBP-MMR                     | CML-PBP-TKI-N                  | Glyceraldehyde 3-phosphate dehydrogenase                                     |
| P55042    | 2             | 1.22E-08        | 4.00025         | CML-PBP-TKI-Y                   | CML-PBP-TKI-N                  | **GTP-binding protein RAD (RAS associated)**                                  |
| P00739    | 13            | 3.99E-11        | 5.55201         | CML-PBP-TKI-Y                   | CML-PBP-TKI-N                  | Haptoglobin-related protein precursor                                        |
| P91953    | 1             | 1.37E-07        | 4.42879         | CML-PBP-TKI-Y                   | CML-PBP-No-MMR                 | **Hatching enzyme precursor (HE) (HEZ)**                                      |
| P01922    | 6             | 6.01E-14        | 10.9884         | CML-PBP-TKI-Y                   | CML-PBP-MMR                    | Hemoglobin α chain                                                          |
| P07414    | 2             | 0.001548        | 22.3314         | CML-PBP-No-MMR                  | CML-PBP-TKI-N                  | Hemoglobin α chain                                                          |
| P19002a   | 2             | 2.15E-05        | 2.87378         | CML-PBP-No-MMR                  | CML-PBP-MMR                    | **Hemoglobin α-1, α-2, and α-3 chains**                                       |
| P02054    | 4             | 8.10E-15        | 54.1252         | CML-PBP-TKI-Y                   | CML-PBP-MMR                    | Hemoglobin β chain                                                          |
| P14391    | 5             | 4.48E-11        | 5.10044         | CML-PBP-TKI-N                   | CML-PBP-No-MMR                 | Hemoglobin β chain                                                          |
| P18985    | 8             | 1.04E-09        | 2.8812          | CML-PBP-TKI-Y                   | CML-PBP-No-MMR                 | Hemoglobin β chain                                                          |
| P20134    | 2             | 2.66E-09        | 19.544          | CML-PBP-MMR                     | CML-PBP-TKI-Y                  | Hemoglobin β chain                                                          |
| P18984    | 5             | 4.21E-09        | 3.66515         | CML-PBP-TKI-Y                   | CML-PBP-MMR                    | Hemoglobin β chain                                                          |
| P20499    | 5             | 3.19E-05        | 9.76807         | CML-PBP-TKI-Y                   | CML-PBP-No-MMR                 | Hemoglobin β chain                                                          |
| P11758    | 6             | 0.002277        | 13.0218         | CML-PBP-MMR                     | CML-PBP-TKI-N                  | Hemoglobin β chain                                                          |
| P02094a   | 2             | 0.004366        | 7.02752         | CML-PBP-MMR                     | CML-PBP-TKI-N                  | Hemoglobin β-major chain                                                     |
| Q28220    | 4             | 0.000235        | 30.7953         | CML-PBP-TKI-N                   | CML-PBP-TKI-Y                  | Hemoglobin ε chain                                                          |
| P05546    | 13            | 0.005774        | 2.11422         | CML-PBP-TKI-Y                   | CML-PBP-MMR                    | Heparin cofactor II precursor (HC-II)                                         |
| P33433    | 5             | 0.000577        | 3.03464         | CML-PBP-MMR                     | CML-PBP-TKI-N                  | Histidine-rich glycoprotein (Histidine-proline rich)                         |
| Q28640    | 5             | 0.001028        | 6.73632         | CML-PBP-MMR                     | CML-PBP-TKI-N                  | Histidine-rich glycoprotein precursor                                        |
| P11457    | 1             | 2.09E-10        | 43.477          | CML-PBP-TKI-N                   | CML-PBP-TKI-Y                  | Histone-like protein HLP-1 precursor (DNA-binding)                           |
| P09631a   | 1             | 8.27E-14        | 6.74686         | CML-PBP-MMR                     | CML-PBP-TKI-Y                  | Homeobox protein Hox-A9 (Hox-1.7)                                            |
| Q10521a   | 1             | 2.13E-05        | 3.30175         | CML-PBP-TKI-Y                   | CML-PBP-MMR                    | **Hypothetical 16.9 kDa protein Rv2239c**                                     |
| P37506a   | 1             | 8.12E-10        | 3.91542         | CML-PBP-TKI-Y                   | CML-PBP-MMR                    | Hypothetical 20.4 kDa protein in COTF-TETB                                    |
| Q10616a   | 1             | 1.93E-06        | 2.87092         | CML-PBP-TKI-N                   | CML-PBP-TKI-Y                  | Hypothetical 56.0 kDa protein Rv1290c                                         |
| P07083    | 1             | 0.000415        | 11.8324         | CML-PBP-No-MMR                  | CML-PBP-TKI-N                  | Hypothetical 9.8 kDa protein in Gp55-ndG intergenic region                   |
| Q9KD45    | 2             | 1.21E-10        | 3.97407         | CML-PBP-MMR                     | CML-PBP-TKI-Y                  | Hypothetical protein BH1374                                                  |
| P47679    | 2             | 0.000507        | 4.0852          | CML-PBP-TKI-Y                   | CML-PBP-TKI-N                  | Hypothetical protein MG441                                                   |
| P42962a   | 2             | 0.000554        | 9.91114         | CML-PBP-TKI-Y                   | CML-PBP-TKI-N                  | Hypothetical protein ycsE                                                    |
Table II. Continued.

| Accession | Peptide count | Anova (p) | Max fold change | Highest mean condition | Lowest mean condition | Description |
|-----------|---------------|-----------|-----------------|------------------------|-----------------------|-------------|
| P56651    | 1             | 3.39E-11  | 18.9887         | CML-PBP-MMR            | CML-PBP-TKI-Y         | Inter-alpha-trypsin inhibitor heavy chain H2 precursor |
| P19823    | 17            | 0.000377  | 2.02663         | CML-PBP-TKI-N          | CML-PBP-TKI-Y         | Inter-alpha-trypsin inhibitor heavy chain H2 precursor |
| P56289    | 2             | 0.000279  | 4.88405         | CML-PBP-TKI-Y          | CML-PBP-TKI-N         | Mating pheromone 3 precursor |
| P01591    | 5             | 0.000549  | 13.799           | CML-PBP-No-MMR         | CML-PBP-TKI-N         | Osteopontin precursor (Bone sialoprotein 1) |
| P28717    | 1             | 8.32E-07  | 2.27719         | CML-PBP-TKI-N          | CML-PBP-MMR           | Osteopontin precursor (Bone sialoprotein 1) |
| P97085    | 2             | 2.31E-06  | 2.01262         | CML-PBP-MMR            | CML-PBP-MMR           | Outer membrane protein U precursor (Porin ompU) |
| P31544    | 2             | 0.000651  | 49.286          | CML-PBP-MMR            | CML-PBP-MMR           | PhoH protein (Phosphate starvation-inducible protein) |
Table II. Continued.

A. The identified differentially expressed proteins in PBP of CML patients

| Accession | Peptide count | Anova (p)   | Max fold change | Highest mean condition | Lowest mean condition | Description                                                                 |
|-----------|---------------|-------------|-----------------|------------------------|-----------------------|-----------------------------------------------------------------------------|
| P57093    | 1             | 4.74E-10    | 5.0011          | CML-PBP-No-MMR         | CML-PBP-TKI-Y         | Phytanoyl-CoA dioxygenase, peroxisomal                                     |
| P03952    | 2             | 5.36E-10    | 3.7609          | CML-PBP-TKI-Y         | CML-PBP-No-MMR        | Plasma kallikrein precursor                                                |
| P02753*   | 4             | 5.90E-13    | 3.9171          | CML-PBP-TKI-Y         | CML-PBP-No-MMR        | Plasma retinol-binding protein precursor (PRBP)                            |
| P21922    | 1             | 0.000235    | 36.2475         | CML-PBP-TKI-Y         | CML-PBP-No-MMR        | Prenorin-4 C11-methyltransferase                                           |
| Q06253    | 2             | 1.39E-09    | 4.1750          | CML-PBP-MMR           | CML-PBP-TKI-Y         | Prevent host death protein                                                 |
| P07737*   | 3             | 3.18E-14    | 14.753           | CML-PBP-TKI-Y         | CML-PBP-MMR           | Profilin I                                                                 |
| P26604    | 1             | 0.001614   | Infinity         | CML-PBP-No-MMR        | CML-PBP-TKI-Y         | Protein hdeA precursor (10K-S protein)                                    |
| Q9SM41    | 1             | 5.77E-08    | 6.6706          | CML-PBP-TKI-N         | CML-PBP-TKI-Y         | Protein translation factor SUI homolog.                                    |
| P00734    | 15            | 0.000479    | 3.4420          | CML-PBP-TKI-Y         | CML-PBP-TKI-N         | Prothrombin precursor (EC 3.4.21.5)                                       |
| Q55794    | 2             | 2.35E-13    | 8.1332          | CML-PBP-TKI-N         | CML-PBP-MMR           | Putative arsenical pump-driving ATPase                                    |
| Q15418    | 4             | 0.004805    | 6.0557          | CML-PBP-TKI-N         | CML-PBP-TKI-Y         | Ribosomal protein S6 kinase alpha 1                                       |
| P00580    | 3             | 2.27E-09    | 4.0226          | CML-PBP-TKI-N         | CML-PBP-TKI-Y         | RNA polymerase sigma-32 factor (Heat shock regulator)                     |
| P14072    | 1             | 0.000233    | 168.597         | CML-PBP-No-MMR        | CML-PBP-TKI-N         | Rubredoxin (Rd)                                                           |
| P58402    | 2             | 9.27E-06    | 9.6740          | CML-PBP-TKI-N         | CML-PBP-TKI-Y         | Sensor protein evgS precursor                                             |
| Q9ZK14    | 2             | 6.65E-12    | 18.9567         | CML-PBP-TKI-N         | CML-PBP-TKI-Y         | Serine acetyltransferase (SAT)                                            |
| P02787*   | 53            | 2.49E-05    | 2.3681          | CML-PBP-TKI-Y         | CML-PBP-TKI-N         | Serotransferrin precursor (Siderophilin)                                  |
| P49064*   | 4             | 5.43E-05    | Infinity         | CML-PBP-TKI-Y         | CML-PBP-MMR           | Serum albumin precursor (Allergen Fel d 2)                                |
| Q28522    | 43            | 5.22E-11    | 5.6175          | CML-PBP-TKI-Y         | CML-PBP-No-MMR        | Serum albumin precursor (Fragment)                                        |
| P02768    | 120           | 1.15E-09    | 2.8780          | CML-PBP-TKI-Y         | CML-PBP-No-MMR        | Serum albumin precursor                                                   |
| P02743    | 1             | 1.17E-12    | 6.8091          | CML-PBP-TKI-Y         | CML-PBP-TKI-N         | Serum amyloid P-component precursor (SAP)                                  |
| P27169    | 5             | 2.21E-05    | 2.4347          | CML-PBP-TKI-Y         | CML-PBP-MMR           | Serum paraoxanase/arylesterase 1                                          |
| P04278    | 2             | 8.55E-09    | 4.0875          | CML-PBP-TKI-Y         | CML-PBP-No-MMR        | Sex hormone-binding globulin precursor (SHBG)                              |
| P95340*   | 1             | 3.77E-15    | 16.6343         | CML-PBP-TKI-Y         | CML-PBP-No-MMR        | Shikimate 5-dehydrogenase                                                 |
| P57675    | 1             | 1.56E-07    | 24.6905         | CML-PBP-TKI-Y         | CML-PBP-MMR           | Stanniocalcin 2 (STC-2) (Fragments)                                       |
| Q9R0K8    | 2             | 2.68E-10    | 6.9657          | CML-PBP-TKI-Y         | CML-PBP-MMR           | Stanniocalcin 2 precursor (STC-2)                                         |
| P41691    | 3             | 4.82E-11    | 19.1566         | CML-PBP-TKI-Y         | CML-PBP-TKI-N         | Superfast myosin regulatory light chain 2 (MYLC2)                         |
| P03729    | 1             | 2.18E-12    | 11.4468         | CML-PBP-TKI-Y         | CML-PBP-TKI-Y         | Tail assembly protein K                                                   |
| P43691    | 3             | 9.61E-11    | 3.5523          | CML-PBP-No-MMR        | CML-PBP-TKI-Y         | Transcription factor GATA-4 (GATA binding factor-4)                      |
| Q02374    | 1             | 0.002132    | 12.1326         | CML-PBP-MMR           | CML-PBP-TKI-N         | Tubulin alpha-1 chain (Alpha-1 tubulin)                                  |
| P12459    | 1             | 8.40E-14    | 9.6864          | CML-PBP-TKI-N         | CML-PBP-No-MMR        | Tubulin beta-1 chai                                                      |
| P02774*   | 17            | 2.45E-07    | 2.6983          | CML-PBP-TKI-Y         | CML-PBP-No-MMR        | Vitamin D-binding protein precursor (DBP) (Group-s)                       |
| P04004    | 9             | 6.06E-09    | 2.1205          | CML-PBP-TKI-Y         | CML-PBP-MMR           | Vitronectin precursor (Serum spreading factor)                            |
| Accession | Peptide count used for quantification | Anova (p) | Max fold change | Highest mean condition | Lowest mean condition | Description |
|-----------|-------------------------------------|-----------|-----------------|------------------------|----------------------|-------------|
| Q9ZEY8    | 2                                   | 0.00866   | 1.5676          | CMR-N                  | TKI-N                | 2-isopropylmalate synthase (EC 4.1.3.12) |
| P49313b   | 1                                   | 0.00086   | 2.8992          | TKI-N                  | CMR-Y                | 30 kDa ribonucleoprotein, chloroplast precursor |
| P02578b   | 1                                   | 0.00023   | 2.4784          | TKI-N                  | CMR-Y                | Actin 1     |
| Q03341b   | 1                                   | 0.00033   | 19.7447         | CMR-N                  | TKI-Y                | Actin 2     |
| P02580b   | 2                                   | 0.00001   | 16.5471         | CMR-Y                  | CMR-N                | Actin 3     |
| P07829    | 1                                   | 0.01832   | 3.2349          | CMR-Y                  | TKI-N                | Actin 3-SUB1 |
| P93584    | 1                                   | 0.01376   | 1.5206          | CMR-N                  | CMR-Y                | Actin 82 (Fragment) |
| P53460    | 1                                   | 0.00928   | 8.5151          | TKI-N                  | CMR-N                | Actin, muscle 1A |
| P50138b   | 1                                   | 0.00431   | 88.6922         | CMR-Y                  | TKI-Y                | Actin       |
| Q9P441d   | 1                                   | 0.01099   | 3.7590          | CMR-Y                  | TKI-Y                | Actin       |
| P43652b   | 13                                  | 0.00003   | 2.0878          | CMR-Y                  | TKI-N                | Afamin precursor (Alpha-albumin) (Alpha-Alb) |
| P19652b   | 6                                   | 0.00163   | 1.5175          | CMR-Y                  | TKI-N                | Alpha-1-acid glycoprotein 2 precursor (AGP 2) |
| P01010b   | 1                                   | 0.00421   | 2.2484          | CMR-Y                  | CMR-N                | Alpha-1-antitrypsin precursor (Alpha-1 protease inhibitor) |
| P01009    | 27                                  | 0.02049   | 1.7589          | CMR-Y                  | TKI-N                | Alpha-1-antitrypsin precursor (Alpha-1 protease inhibitor) |
| P08697b   | 7                                   | 0.00231   | 2.7616          | CMR-Y                  | TKI-N                | Alpha-2-antiplasmin precursor (Alpha-2-plasmin inhibitor) |
| Q9N2D0    | 1                                   | 0.03147   | 4.9779          | CMR-Y                  | TKI-N                | Alpha-2-HS-glycoprotein precursor (Fetuin-A) |
| P01023a   | 67                                  | 0.00130   | 1.5666          | CMR-Y                  | TKI-N                | Alpha-2-macroglobulin precursor (Alpha-2-M) |
| P01019    | 11                                  | 0.02295   | 1.4615          | CMR-Y                  | CMR-N                | Angiotensinogen precursor [Contains: Angiotensin I |
| P00896b   | 1                                   | 0.00001   | 5.0581          | CMR-N                  | TKI-N                | Anthranilate synthase component I (EC 4.1.3.27) |
| P01008a   | 9                                   | 0.00320   | 1.4376          | CMR-Y                  | TKI-Y                | Antithrombin-III precursor (ATIII) (PRO0309) |
| P32261b   | 2                                   | 0.00084   | 5.0712          | CMR-N                  | CMR-N                | Antithrombin-III precursor (ATIII) |
| P09809    | 2                                   | 0.02421   | 1.5680          | TKI-N                  | CMR-Y                | Apolipoprotein A-I precursor (Apo-AI) |
| P15497b   | 2                                   | 0.03898   | 4.7003          | CMR-Y                  | CMR-N                | Apolipoprotein A-I precursor (Apo-AI) |
| P06727    | 14                                  | 0.01399   | 2.0475          | CMR-Y                  | TKI-Y                | Apolipoprotein A-IV precursor (Apo-AIV) |
| P02655b   | 2                                   | 0.00001   | 2.0801          | CMR-Y                  | TKI-N                | Apolipoprotein C-II precursor (Apo-CII) |
| P41697    | 1                                   | 0.00423   | 1.9243          | TKI-Y                  | CMR-N                | Bud site selection protein BUD6 (Actin interacting protein) |
| P05109    | 2                                   | 0.04617   | 9.0518          | TKI-Y                  | CMR-N                | Calgranulin A (Migration inhibitory factor-related protein) |
| P25854    | 2                                   | 0.01368   | 1.5390          | TKI-Y                  | CMR-N                | Calmodulin-1 (Fragment) |
| Q9NZT1    | 1                                   | 0.00088   | 1.9462          | CMR-N                  | TKI-Y                | Calmodulin-like skin protein |
| Q0037ib   | 1                                   | 0.0002    | 23.1103         | TKI-N                  | CMR-N                | CAP22 protein |
| P00915b   | 6                                   | 0.00072   | 5.4236          | CMR-N                  | TKI-N                | Carbonic anhydrase I (EC 4.2.1.1) (Carbonate dehydrase) |
Table II. Continued.

B. The identified differentially expressed proteins in BMP of CML patients

| Accession | Peptide count used for quantification | Anova (p)   | Max fold change | Highest mean condition | Lowest mean condition | Description                                                                 |
|-----------|--------------------------------------|-------------|-----------------|------------------------|---------------------|----------------------------------------------------------------------------|
| P25773b   | 1                                    | 0.00000     | 6.6740          | CMR-Y                  | CMR-N               | Cathepsin L (EC 3.4.22.15) (Progesterone-dependent)                         |
| P00450    | 20                                   | 0.00727     | 1.5284          | CMR-Y                  | CMR-N               | Ceruloplasmin precursor (EC 1.16.3.1) (Ferroxidase)                        |
| P13635    | 6                                    | 0.02286     | 1.5201          | TKI-N                  | TKI-Y               | Ceruloplasmin precursor (EC 1.16.3.1) (Ferroxidase)                        |
| Q61147    | 5                                    | 0.03054     | 2.4399          | TKI-N                  | TKI-Y               | Ceruloplasmin precursor (EC 1.16.3.1) (Ferroxidase)                        |
| P10909    | 6                                    | 0.00012     | 1.5866          | CMR-Y                  | CMR-N               | Clusterin precursor (Complement-associated protein)                        |
| P25958    | 3                                    | 0.00747     | 1.9061          | TKI-Y                  | TKI-N               | ComG operon protein 6                                                      |
| P02747    | 2                                    | 0.04052     | 28.8755         | CMR-Y                  | TKI-Y               | Complement C1q subcomponent, C chain precursor                             |
| P01026    | 10                                   | 0.00001     | 1.8285          | TKI-N                  | CMR-Y               | Complement C3 precursor [Contains: C3A anaphylatox]                        |
| P12387    | 7                                    | 0.00010     | 1.8101          | CMR-N                  | CMR-Y               | Complement C3 precursor [Contains: C3a anaphylatox]                        |
| P01024A   | 68                                   | 0.00088     | 1.6430          | CMR-Y                  | TKI-N               | Complement C3 precursor [Contains: C3a anaphylatox]                        |
| P01028b   | 42                                   | 0.00020     | 2.0579          | CMR-Y                  | TKI-Y               | Complement C4 precursor [Contains: C4A anaphylatox]                        |
| P10643    | 3                                    | 0.04712     | 1.4974          | CMR-Y                  | CMR-N               | Complement component C7 precursor                                          |
| P02748b   | 7                                    | 0.00131     | 2.5543          | CMR-Y                  | TKI-N               | Complement component C9 precursor                                          |
| P08603    | 30                                   | 0.00365     | 1.4060          | CMR-Y                  | TKI-N               | Complement factor H precursor (H factor 1)                                 |
| P48416b   | 3                                    | 0.00000     | 3.5184          | TKI-N                  | CMR-Y               | Cytochrome P450 10 (EC 1.14.-.-) (CYPX)                                    |
| Q92125b   | 1                                    | 0.00007     | 2.6454          | TKI-Y                  | CMR-N               | Dihydroadipicoline synthase (EC 4.2.1.52) (DHDPS)                          |
| P31073b   | 1                                    | 0.00010     | 2.2735          | TKI-N                  | CMR-N               | Dihydrofolate reductase (EC 1.5.1.3)                                       |
| P20861b   | 1                                    | 0.00000     | 16.7020         | TKI-N                  | CMR-Y               | Fan G protein precursor                                                   |
| P02671ab  | 21                                   | 0.00003     | 2.2257          | CMR-Y                  | TKI-Y               | Fibrinogen alpha/alpha-E chain precursor                                  |
| P02675ab  | 24                                   | 0.00010     | 2.4767          | CMR-Y                  | CMR-N               | Fibrinogen beta chain precursor [Contains: Fibrinogen]                     |
| Q02020b   | 2                                    | 0.00461     | 2.5361          | CMR-Y                  | CMR-N               | Fibrinogen beta chain precursor [Contains: Fibrinogen]                     |
| P14480    | 7                                    | 0.00542     | 2.0499          | CMR-N                  | CMR-Y               | Fibrinogen gamma chain precursor                                          |
| P02679ab  | 13                                   | 0.00110     | 2.1792          | CMR-Y                  | CMR-N               | Glucokinase (EC 2.7.1.2) (Glucose kinase)                                 |
| Q92T27    | 2                                    | 0.00030     | 1.5959          | TKI-N                  | CMR-N               | Glutamyl-tRNA(Gln) amidotransferase subunit C                              |
| Q92J74    | 1                                    | 0.00712     | 2.6314          | CMR-Y                  | CMR-N               | Glutaryl-CoA dehydrogenase, mitochondrial precursor                        |
| Q60759    | 4                                    | 0.00301     | 1.8431          | TKI-N                  | CMR-Y               | Glyceraldehyde 3-phosphate dehydrogenase                                  |
| P23722a   | 3                                    | 0.00380     | 1.5602          | TKI-N                  | CMR-N               | Glycerol-3-phosphate dehydrogenase [NAD(P)+]                              |
| Q9ZKP0ab  | 2                                    | 0.00292     | 2.4902          | CMR-Y                  | TKI-N               | Guanyl nucleotide-binding protein G(1)/G(S)/G(O)                           |
| P50150    | 1                                    | 0.03327     | 5.9505          | TKI-N                  | CMR-Y               | Guanyl-specific ribonuclease U1 (EC 3.1.27.3) (Rna)                        |
| P07736b   | 1                                    | 0.00189     | 2.7741          | TKI-N                  | CMR-Y               | Haptoglobin precursor                                                    |
Table II. Continued.

B. The identified differentially expressed proteins in BMP of CML patients

| Accession    | Peptide count used for quantification | Anova (p) | Max fold change | Highest mean condition | Lowest mean condition | Description                                                                 |
|--------------|---------------------------------------|-----------|-----------------|------------------------|-----------------------|-----------------------------------------------------------------------------|
| P00738       | 4                                     | 0.04834   | 2.6291          | CMR-Y                  | TKI-Y                 | Haptoglobin-2 precursor                                                    |
| P07414       | 2                                     | 0.00753   | 8.8724          | CMR-N                  | TKI-N                 | Hemoglobin alpha chain                                                    |
| P01932       | 1                                     | 0.04336   | Infinity        | CMR-Y                  | TKI-Y                 | Hemoglobin alpha chain                                                    |
| P01948       | 1                                     | 0.00432   | 2.0401          | TKI-Y                  | CMR-Y                 | Hemoglobin alpha-1 and alpha-2 chains                                      |
| Q9XSN3       | 1                                     | 0.00834   | 1.3880          | CMR-Y                  | TKI-N                 | Hemoglobin alpha-1 chain                                                  |
| P19002       | 2                                     | 0.00000   | 3.9434          | CMR-N                  | CMR-Y                 | Hemoglobin alpha-1, alpha-2, and alpha-3 chains                           |
| P02037       | 1                                     | 0.00166   | 5.3495          | CMR-N                  | TKI-Y                 | Hemoglobin beta chain                                                     |
| P11758       | 2                                     | 0.03762   | 3.2576          | CMR-Y                  | TKI-Y                 | Hemoglobin beta chain                                                     |
| P02027       | 1                                     | 0.04456   | 16.1529         | CMR-N                  | CMR-Y                 | Hemoglobin beta chain                                                     |
| P02064       | 1                                     | 0.02202   | 2.3093          | CMR-N                  | CMR-N                 | Hemoglobin beta-1 chain (Major)                                           |
| P02074       | 1                                     | 0.00000   | 4.1199          | CMR-N                  | CMR-Y                 | Hemoglobin beta-III chain                                                 |
| P19886       | 2                                     | 0.00008   | 2.0278          | CMR-N                  | CMR-Y                 | Hemoglobin delta chain                                                    |
| P20058       | 2                                     | 0.03619   | 1.8809          | TKI-N                  | CMR-N                 | Hemopexin precursor                                                      |
| P45965       | 1                                     | 0.04029   | 13.7398         | CMR-Y                  | CMR-N                 | Hypothetical 19.4 kDa protein T09A5.5 in chromosome                       |
| Q05107       | 1                                     | 0.02505   | 2.0311          | CMR-Y                  | CMR-N                 | Hypothetical 23.6 kDa protein                                              |
| O34717       | 2                                     | 0.01355   | 1.4268          | TKI-Y                  | CMR-Y                 | Hypothetical oxidoreductase ykuF (EC 1)                                   |
| P44030       | 1                                     | 0.00000   | 4.4405          | TKI-Y                  | CMR-Y                 | Hypothetical protein H10659                                               |
| P42968       | 1                                     | 0.00003   | 4.3060          | TKI-N                  | CMR-N                 | Hypothetical transcriptional regulator yesO                               |
| P01876       | 1                                     | 0.00013   | 3.1121          | CMR-Y                  | CMR-N                 | Ig alpha-1 chain C region                                                |
| P01859       | 8                                     | 0.00015   | 1.8808          | TKI-Y                  | TKI-N                 | Ig gamma-2 chain C region                                                 |
| P01860       | 3                                     | 0.00018   | 1.4555          | TKI-Y                  | TKI-N                 | Ig gamma-3 chain C region (Heavy chain disease protein)                  |
| P01861       | 5                                     | 0.02495   | 1.4049          | CMR-Y                  | TKI-N                 | Ig gamma-4 chain C region                                                 |
| P01779       | 2                                     | 0.02052   | 2.4688          | CMR-Y                  | TKI-N                 | Ig heavy chain V-III region TUR                                           |
| P01617       | 1                                     | 0.00016   | 1.9790          | CMR-Y                  | TKI-N                 | Ig kappa chain V-II region TEW                                            |
| P01625       | 3                                     | 0.01464   | 1.8173          | CMR-Y                  | CMR-N                 | Ig kappa chain V-IV region Len                                           |
| P01842       | 5                                     | 0.00763   | 1.4632          | CMR-N                  | CMR-N                 | Ig lambda chain C regions                                                |
| P01591       | 5                                     | 0.03430   | 2.1773          | CMR-Y                  | TKI-Y                 | Immunoglobulin J chain                                                   |
| P01335       | 1                                     | 0.00514   | 2.4827          | TKI-N                  | CMR-Y                 | Insulin precursor                                                        |
| O02668       | 1                                     | 0.01041   | 13.1392         | CMR-Y                  | TKI-Y                 | Inter-alpha-trypsin inhibitor heavy chain H2 precursor                    |
| P097279      | 2                                     | 0.03423   | 2.0472          | TKI-Y                  | TKI-N                 | Inter-alpha-trypsin inhibitor heavy chain H2 precursor                    |
| Q42891       | 1                                     | 0.00002   | 2.2505          | TKI-N                  | CMR-N                 | Lactoylglutathione lyase (EC 4.4.1.5) (Methylglyoxal)                      |
Table II. Continued.

B. The identified differentially expressed proteins in BMP of CML patients

| Accession | Peptide count | Used for quantification | Anova (p) | Max fold change | Highest mean condition | Lowest mean condition | Description |
|-----------|---------------|-------------------------|-----------|-----------------|------------------------|-----------------------|-------------|
| P02750a   | 9             |                         | 0.01798   | 1.3841          | TKI-Y                  | CMR-N                 | Leucine-rich alpha-2-glycoprotein (LRG) |
| P06267b   | 1             |                         | 0.00005   | 3.9296          | CMR-N                  | TKI-N                 | Light-independent protochlorophyllide reductase iron-sulfur ATP-binding protein |
| Q61233    | 2             |                         | 0.01594   | 3.5492          | CMR-Y                  | TKI-Y                 | L-plastin (Lymphocyte cytosolic protein 1) (LCP-1) |
| P52162    | 1             |                         | 0.01027   | 25.2703         | CMR-Y                  | TKI-N                 | MAX protein |
| P48310b   | 1             |                         | 0.00024   | 2.4866          | CMR-Y                  | TKI-N                 | Minor capsid protein VI precursor |
| O03698b   | 1             |                         | 0.00041   | 2.9113          | CMR-N                  | CMR-Y                 | NADH-ubiquinone oxidoreductase chain 4 (EC 1.6.5.3) |
| Q43875    | 1             |                         | 0.01342   | 4.0047          | CMR-Y                  | CMR-N                 | Nonspecific lipid-transfer protein 4.2 precursor |
| P23051    | 1             |                         | 0.00002   | 3.3474          | TKI-Y                  | TKI-N                 | Nucleocapsid protein |
| P39115b   | 3             |                         | 0.00000   | 3.4012          | CMR-N                  | CMR-Y                 | Nucleotide binding protein ExpZ |
| P32119b   | 0             |                         | 0.00000   | 4.3238          | CMR-N                  | CMR-Y                 | Peroxiredoxin 2 (Thioredoxin peroxidase 1) |
| Q42858b   | 1             |                         | 0.00007   | 4.2693          | CMR-N                  | TKI-N                 | Phenylalanine ammonia-lyase (EC 4.3.1.5) |
| O07125b   | 1             |                         | 0.00099   | 2.7853          | CMR-N                  | TKI-N                 | Phosphocarrier protein HPr (ptsH) |
| P09411    | 1             |                         | 0.01886   | 1.5949          | TKI-Y                  | TKI-N                 | Phosphoglycerate kinase 1 (EC 2.7.2.3) |
| Q9KDM4    | 3             |                         | 0.00513   | 1.6582          | TKI-N                  | CMR-N                 | Phosphoserine aminotransferase (serC) (PSAT) |
| P02753    | 3             |                         | 0.01195   | 1.5216          | CMR-N                  | TKI-N                 | Plasma retinol-binding protein precursor (PRBP) |
| P76159    | 1             |                         | 0.00538   | 1.7156          | CMR-N                  | TKI-N                 | Probable lysozyme from lambdoid phage Qin |
| O67024    | 1             |                         | 0.03110   | Infinity        | CMR-N                  | TKI-N                 | Probable peroxiredoxin |
| P07737    | 2             |                         | 0.00871   | 1.8459          | CMR-N                  | TKI-N                 | Proto-oncogene serine/threonine-protein kinase mos |
| P00536    | 2             |                         | 0.00697   | 1.5076          | TKI-N                  | CMR-N                 | PTS system, N-acetylglucosamine-specific EIIABC component |
| P45604    | 1             |                         | 0.00211   | 1.9033          | CMR-N                  | CMR-Y                 | Purine nucleoside phosphorylase (deoD) |
| Q59482b   | 1             |                         | 0.00519   | 4.2028          | CMR-Y                  | TKI-N                 | Putative integrase/recombinase Y4EF |
| P55429b   | 1             |                         | 0.00004   | 2.5979          | CMR-N                  | CMR-Y                 | Putative outer membrane protein CC0351 precursor |
| Q9AB80    | 3             |                         | 0.00001   | 1.5354          | TKI-Y                  | CMR-Y                 | Putative signal peptide peptidase sspA |
| Q9X480    | 2             |                         | 0.00113   | 1.8668          | CMR-N                  | CMR-Y                 | Ras-like protein F54C8.5 |
| P34443    | 3             |                         | 0.02905   | 2.3131          | CMR-Y                  | TKI-Y                 | Regulator of G protein signaling rgs-1 |
| P34295    | 2             |                         | 0.02474   | 1.4695          | TKI-Y                  | CMR-N                 | Ribonuclease HII (EC 3.1.26.4) (RNase HII) |
| Q9CG17b   | 1             |                         | 0.00003   | 1.7092          | CMR-Y                  | TKI-N                 | S100 calcium-binding protein A3 (S-100E protein) |
| Accession   | Peptide count used for quantification | Anova (p) | Max fold change | Highest mean condition | Lowest mean condition | Description                                                                 |
|-------------|--------------------------------------|-----------|-----------------|------------------------|-----------------------|----------------------------------------------------------------------------|
| P12346b     | 2                                    | 0.00000   | 2.4638          | TKI-Y                  | TKI-N                 | Serotransferrin precursor (Siderophilin) (Beta-1-metal-binding globulin)    |
| P19134b     | 11                                   | 0.00347   | 2.2136          | TKI-N                  | CMR-N                 | Serotransferrin precursor (Siderophilin) (Beta-1-metal-binding globulin)    |
| P02787      | 44                                   | 0.00574   | 1.4954          | CMR-Y                  | CMR-N                 | Serum albumin precursor (Allergen Bos d 6)                                  |
| P02769b     | 5                                    | 0.00003   | 2.4650          | TKI-Y                  | CMR-N                 | Serum albumin precursor (Fragment)                                         |
| Q28522      | 7                                    | 0.04108   | 2.6927          | CMR-Y                  | TKI-N                 | Serum albumin precursor (Fragment)                                         |
| P49065b     | 2                                    | 0.00016   | 6.1150          | CMR-N                  | TKI-Y                 | Serum albumin precursor                                                     |
| P27169b     | 3                                    | 0.00416   | 2.1032          | TKI-Y                  | TKI-N                 | Serum paraoxonase/arylesterase 1 (EC 3.1.1.2)                              |
| Q9CES7b     | 1                                    | 0.00006   | 2.0972          | TKI-Y                  | TKI-N                 | Shikimate 5-dehydrogenase (EC 1.1.1.25)                                    |
| P29950b     | 2                                    | 0.00297   | 2.6116          | CMR-Y                  | TKI-Y                 | Uracil-DNA glycosylase (EC 3.2.2.-) (UDG) (Fragment)                       |
| P02774      | 24                                   | 0.00013   | 1.9884          | CMR-Y                  | TKI-N                 | Vitamin D-binding protein precursor (DBP) (VDB)                            |
| P73069      | 1                                    | 0.00765   | 1.8377          | CMR-N                  | CMR-Y                 | Ycf48-like protein                                                        |

*Fifty-four differentially expressed proteins that were common between the two body fluid compartments (i.e. the 164 and 138 datasets from PBP and BMP respectively) as described in Fig. 4. This set of 54 proteins was then used in the unsupervised hierarchical clustering analysis as shown in Fig. 7. The proteins that are in bold in part A are also identified in BMP samples. *Sixty-four significantly differentially expressed proteins (>1.5- to ∞-fold change, P<0.05) between MMR and No-MMR sample groups used for the generation of dendrogram in Fig. 3. These proteins predict accurately patients with MMR vs. No-MMR patients using unsupervised Hierarchical Cluster Analysis. (Due to resolution problem, the list was cropped from the dendrogram plot). The proteins that are in bold in part B are also identified in PBP samples.
Figure 6. (A) Pathway analysis of network signaling of some of the identified proteins as represented in the ingenuity pathway analysis database. The analysis of the identified proteins is composed of 2 hematological disease related networks with over 100 associated molecules that were merged into one as shown above. The connections and the expression profiles of some of the identified proteins are as indicated. Red indicates an upregulated protein, and pink color is indicative of downregulation. A direct connection is by solid line and broken lines indicate an indirect interaction between different molecules. Other molecules outside the identified in this study are in grey color. (B) The functional characteristics and disease relatedness of some of the identified proteins were mapped in Ingenuity database. The majority of these molecules are located mostly in the plasma membrane, cytoplasm and extracellular space, while only a few are located in the nucleus. Some these molecules functions as enzymes, transporters, transcription regulator, or G-protein coupled receptor. Others act as kinases, peptidase or growth factor. Furthermore, some of these molecules as represented in multiple sub-signaling networks mostly regulate among others: Cell-To-Cell Signaling and Interaction, Hematological System Development and Function. Other implicated functional annotations include, aggregation of blood cells, coagulation, quantity of aggregate cells as well as quantity of granulocytes. [The network analysis was done and figure generated in ingenuity pathway analysis program (IPA v8.7)].
LT-MMR patients vs. P-No-MMR patients using unsupervised principal component analysis (Fig. 4B). These results were subsequently evaluated for comparisons with the patterns obtained in early treatment response at 6 months. Notably, the pattern and accuracy of clustering of samples is very similar to that observed with the hierarchical cluster analysis plots at 6 months (Fig. 3).

Protein fingerprinting for prediction of treatment options for individualized therapy. Towards achieving the goal of personalized medicine, the above observed differentially expressed proteins between samples derived from LT-MMR patients vs. P-No-MMR patients were evaluated for their potential for objective prediction of treatment options for some of these cohorts of CML patients. Interestingly, the panel of 164 and 138 differentially expressed protein datasets derived from peripheral blood plasma (PBP) and bone marrow (BM) respectively, also discriminates patients that stay on IM after 1 year of treatment from patients that ultimately required alternative treatment options (second generation TKI/others) (Fig. 5). Following >2 years of follow-up of these patients the same dataset of potential protein biomarkers could still accurately separate all analyzed sample groups into their respective molecular response and treatment sub groups, indicating their usefulness for treatment monitoring as well as prediction of best choice of therapy for individual patient. Some of the identified proteins were implicated in hematological diseases as potential biomarkers using ingenuity pathway analysis (IPA) (Fig. 6). Functional annotations/disease affiliations of some of these proteins implicated in CML are further described under discussion below.

Identification of protein changes in BM as a reflection of detectable changes in peripheral blood. One of the main goals of this study was to identify/develop disease-specific/disease-associated protein biomarkers seen in bone marrow tissue as well as in peripheral blood plasma. This would subsequently allow monitoring of such biomarker proteins in peripheral blood, rather than bone marrow, demanding less invasive procedures for objective prediction of individual's best treatment options and prognostic monitoring of CML patients. We therefore explored the possibility whether the proteins that are significantly differentially expressed in bone marrow do also show similar expression pattern in peripheral blood. With this in mind, we calculated how many of the 164 differentially expressed proteins in peripheral blood and the 138 protein dataset in bone marrow are common to both body compartments. We found that only 54 proteins (~35%) were in common between the two 164 and 138 datasets as described above. This set of 54 proteins was then subjected to unsupervised hierarchical clustering and correspondence analyses. As shown in Fig. 7, all sample groups were distinctively separated into four response subtypes using unsupervised hierarchical cluster analysis. The common proteins between the two body fluid compartments were highlighted in bold in Table II.

Validation by western blot analysis of some of the identified proteins. In an attempt to validate some of the differentially expressed proteins, we have used immunoblotting analysis. Nine individual samples consisting of 4 samples not included in the proteomics analysis and 5 other samples from the proteomics analyzed sample groups were tested for their expression of haptoglobin and hemoglobin using specific antibodies against these proteins. The expression levels of these proteins across all sample groups were consistent with the average protein normalized levels seen with label-free quantitative LC/MS/MS analysis (Fig. 8). Large scale validation of the majority of these proteins was beyond the scope of this study in order to develop limited panel of markers for clinical trial in a later study.

Discussion

Clinical and molecular diagnosis of most hematological malignancies including CML can be accurately made; however, prediction of treatment response elude the currently available tools for patient care.
A subset of significantly differentially expressed proteins from both peripheral blood and bone marrow were selected for their ability to discriminate samples derived from CML patients that responded differently to initial first line treatment with imatinib. Our strategy of proteomics mining of BM and PBP from the same individual patient would provide unique possibility to identify biomarkers from both sources thus, entailing less invasive procedures.

Report of microarray analysis of peripheral blood and bone marrow of CML samples in blast crisis cells, has been shown with demonstrable biological changes between two bodily fluids (19). Our analysis of peripheral blood samples of 164 differentially expressed proteins show that all samples were correctly classified and similar result was observed with 138 protein changes in bone marrow samples as shown in Fig. 4. Only 54 proteins were shown to be commonly differentially expressed between blood dataset and bone marrow protein dataset in the present study, supporting our notion that it might be possible to identify significant changes in the bone marrow of CML patients that are measurable at peripheral blood compartment for routine diagnostics.

We have attempted to use both the BMP and PBP data-sets that accurately predict patients MMR status for possible prediction of patients that continue to stay on IM after 1 year of treatment vs. those that ultimately required alternative treatment options (second generation TKI/others). Thus, the expression of the 158 protein changes in BM between MMR and No-MMR were further evaluated in 16 unrelated patients that stay on TKI after 1 year of imatinib treatment from patients that ultimately required alternative treatment options (second generation TKI/others). We found four distinct clusters with samples with MMR and No-MMR being very closely separated (not as distinct as in Fig. 4), while patients that stay on TKI (i.e. after 1 year of imatinib) treatment were distantly separated from patients that ultimately required alternative treatment options (second generation TKI/others) as shown in Fig. 5, meaning that it will be challenging to construct a universal model for management of CML patients and that prognostic datasets need to be created for each specific response type.

We have used two independent proteomics analysis platforms in the present study. The expression profiles of 2-DE protein spots successfully discriminated two sample groups of CML patients with MMR and No-MMR. We recognized the inherent limitation of 2-DE based studies (20-22) hence, we have in addition used label-free quantitative protein expression using high definition liquid chromatography tandem mass spectrometry (LC/MS/MS) to extensively map the proteome of bone marrow as well as peripheral blood samples.

Previous studies have used multivariate statistical algorithms and artificial learning models to predict cancer prognosis and for grading different solid tumors (15,23-28). The majority of these studies reported varying degrees of sensitivity and specificity based on evaluation of different clinical parameters (20,24).

Gene expression studies on hematological disease have been largely carried out by analysis of DNA or RNA microarrays. These genomics studies have indicated the potentials of large scale analysis of gene expression towards better understanding the molecular basis of leukemogenesis and that this information could potentially be useful in the classification of subtypes of hematological malignancies (19,29,30). In a recent study of CLL samples, Alsagaby and colleagues used combined transcriptomics and proteomics analyses to unravel the heterogeneity of gene expression patterns as well attempting to identify proteins that are implicated in prognosis of chronic lymphocytic leukemia (31). Recent studies have attempted to evaluate protein changes between imatinib sensitive and resistance samples (32) as well as to better understand the molecular mechanism in therapy resistance at the level of bone marrow extracellular fluid in CML (33).
Our initial analysis of 64 differentially expressed proteins of peripheral blood for prognostic monitoring of early CML treatment response at 6 months was encouraging and led us into extensive analysis of samples with sustained long-term MMR against patients that persistently could not achieve MMR.

Some of the identified proteins in the bone marrow of the 138 dataset for the prolonged and sustained MMR vs. persistent No-MMR were further evaluated for their functional characteristics and their hematological disease relevance using ingenuity pathway analysis (IPA). In the canonical pathway analysis of network signaling of identified proteins, only 48 of the 138 identified differentially expressed proteins were represented in the IPA database. The analysis of the identified proteins is composed of multiple networks of which, one is implicated in hematological disorders. The cellular localization, interconnections and functional annotation as well as the expression profile of some of these 48 identified molecules are as detailed in Fig. 6A. A review of some of these molecules showed that they mostly regulate among others: cell-to-cell signaling and interaction, hematological system development and function, aggregation of blood cells, coagulation, as well as quantity of granulocytes as indicated in Fig. 6. Among the identified proteins in this study is TYRO3 protein tyrosine kinase, a member of TAM family of receptor tyrosine kinases (RTKs) and known for their role as regulator of cellular proliferation, migration and survival processes, as well as maintenance of blood coagulation equilibrium (34). We observed connection of TYRO 3 in AKT/P13K pathway; similar to that previously described (34-36).

The S100A8 is a calcium-binding protein of the S100 family and have been described to be associated with myeloid differentiation (37). We observed a more than 9-fold differential expression of S100A8 and in the network connecting with RAS, TGFβ, MAPK and MMP. The S-100 protein has been previously reported as a useful marker in juvenile chronic myeloid leukemia (JCML) as well as myeloid leukemia cutis (LC) (38,39).

Overexpression of MYC has been associated with CML with poor response to imatinib (40,41). We observed a more than 25-fold differential expression of MYC associated factor x in this study.

Altogether our findings indicate that rather than the use of a single marker, analyses of a panel of protein markers have the potential to provide better insight into complex biologic processes towards better prognostication of CML patients.

We recognize the limitation of this study as samples were prospectively collected and patients observed over the years for their treatment responses. One other issue with this study is the low number of patients enrolled in different clinical and molecular response groups; hence we have limited the analysis to evaluation of patients based on MMR and whether or not they are on IM or alternative treatment option (second generation TKI/other).

In conclusion, we have identified protein signatures capable of prediction of molecular response and choice of therapy for CML patients at 6 months and beyond using expression proteomics as objective stratification of CML patients for treatment options. Although these results were very promising, we recognized that analysis of much larger materials of patients with similar treatments and responses will be necessary to validate if clustering analysis can be used as a routine prognostic tool for CML patients.

These proteins might be valuable once validated, to complement the currently existing parameters for reliable and objective prediction of disease progression, monitoring treatment response and clinical outcome of CML patients as a model of personalized medicine.

Acknowledgements

We thank Dr Abdelilah Aboussékra for critical review, as well as Mr. Melvin Velasco, Mr. Parvez Siddiqui, Mr. Romeo Caracas and Ms. Tusneem Elhassan for technical assistant. We acknowledge the assistant and support of Mr. Faisal Al Otaibi and the logistics and purchasing department, RC, KFSH&RC. The present study was supported by the Research Center Administration, KFSH&RC, Riyadh, Saudi Arabia (RAC# 2050 040).

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