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

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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'.

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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

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-

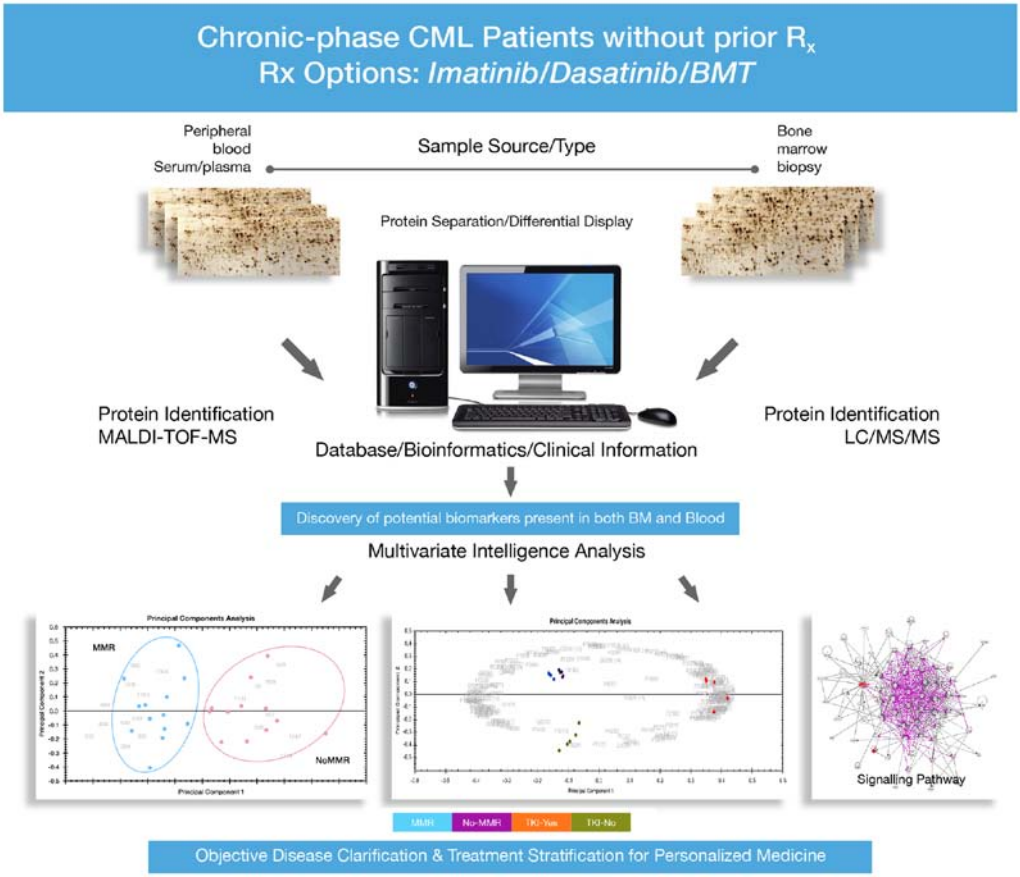

Figure 1. Overview of our biomarker discovery proteomics approach. Bone marrow and peripheral blood samples were analyzed by 2-DE and LC/MS/MS. Identified proteins were subjected to statistical analysis and evaluated for early treatment response and prediction of individualized treatment options. Potential markers would be validated for clinical use.

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). Currently, there is no recognized prognostic value for the baseline BCR-ABL level, furthermore, there are variations in sensitivity or dependability of RQ-PCR assays across different laboratories (13). There is therefore a need to develop molecular markers for selection of choice of therapy at the time of diagnosis and to identify patients that are more likely to achieve a sustained remission, and patients who are more likely to develop resistance to imatinib therapy.

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

Table I. Clinical characteristics of analyzed samples.

			TKI-fail		MMR at 6 months		MMR at 12 months		MMR at 18 months		MMR at 24 months	
Samples	Gender	Age (years)	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
CML1	Female	14	√			√		√		√		√
CML2	Female	14	√			√		√		√		√
CML3	Female	26		√	√		√					
CML4	Male	18		√		√	√		√			√
CML5	Male	50	√			√		√		√		√
CML6	Female	50	√			√		√		√		√
CML7	Male	41		√		√		√		√		√
CML8	Female	64		√	√		√		√		√	
CML10	Male	27	√			√	√			√		√
CML13	Male	44	√			√		√		√		√
CML15	Male	21	√			√		√		√		√
CML16	Male	44	√			√		√		√		√
CML17	Female	18		√	√		√		√		√	
CML18	Female	65	√		√		√		√		√	
CML19	Male	26		√	√		√		√		√	
CML21	Male	39		√	√		√			√		√
CML22	Female	67	√			√		√		√		√
CML23	Male	47	√			√		√		√		√
CML24	Male	18		√	√		√		√		√	
CML25	Male	40	√			√		√		√		√
CML26	Female	30		√	√							
CML27	Female	36	√		√			√		√		√
CML28	Female	37	√			√		√		√		√
CML29	Female	33		√	√		√		√			√
CML30	Female	44	√			√		√		√		√
CML31	Female	48										
CML32	Female	38		√	√							
CML33	Female	32		√	√		√					
CML34	Male	52		√	√		√		√		√	
CML38	Male	37										
CML40	Male	61		√	√		√		√			
CML41	Male	47	√			√		√		√		√
CML43	Female	51		√	√		√		√		√	
CML44	Female	14	√			√		√		√		√
CML45	Female	45	√			√		√		√		√
CML46	Female	45	√		√		√			√		√
CML47	Male	32		√	√		√		√		√	
Total			19	16	17	18	16	17	11	20	8	22

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

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 for qualitative and quantitative differences. In addition, the protein expression profiles were used to assess the level of individual variability and only samples with similar phenotypic changes were used for sample pools for LC/MS/MS (due to low through-put analysis) as detailed below. Polypeptide quantities were calculated based on the normalized total integrated density volume.

Protein in solution-digestion. The plasma samples were diluted and protein concentrations of all samples were normalized as previously described (16). Briefly, for analytical runs, equal amount of protein was taken from each sample to generate a pool of patient as one group. The samples within same sample cohort were pooled due to low through-put of LC/MS/MS analysis platform. However, samples were initially screened using 2-DE for homogeneity within the same analysis group. For each analysis sample group, 200 μg complex protein mixture was taken and exchanged twice with 500 μl of 0.1% RapiGest (Waters Corp., Manchester, UK). Protein concentrations of between 0.50 and 1 μg/μl was achieved at the end of digestion. Details of digestion protocols are as previously described (16,17). Briefly, proteins were denatured in 0.1% RapiGest SF at 80˚C for 15 min, reduced in 10 mM DTT at 60˚C for 30 min, and alkylated in 10 mM Iodoacetamide (IAA) for 40 min at room temperature in the dark. Samples were trypsin digested at 37˚C overnight. Samples were diluted with aqueous 0.1% formic acid prior to LC/MS analysis in order to achieve a load of ~2 μg on analytical column. All samples were spiked with yeast alcohol dehydrogenase (ADH; P00330) as internal standard to the digests in order for absolute quantitation.

Protein identification by mass spectrometry: LC-MSE analysis. The digested peptides were subjected to 1-Dimensional Nano Acquity liquid chromatography coupled with tandem mass spectrometry on Synapt G2 (Waters Corp.). Expression proteomics data were generated between sample groups using both qualitative and quantitative protein changes. The ESI-MS analysis and instrument settings were optimized on the tune page as previously reported (16).

A total of 2 μl sample injection representing ~1 μg protein digests was loaded on-column and samples were infused using the Acquity sample manager with mobile phase consisting of A1 99% water +1% acetonitrile + 0.1% formic acid and B1 acetonitrile + 0.1% formic acid with sample flow rate of 0.450 μl/min. Data acquisition using iron mobility separation 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/Swiss-Prot 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 previoudly 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 ($P \leq 0.05$) 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).

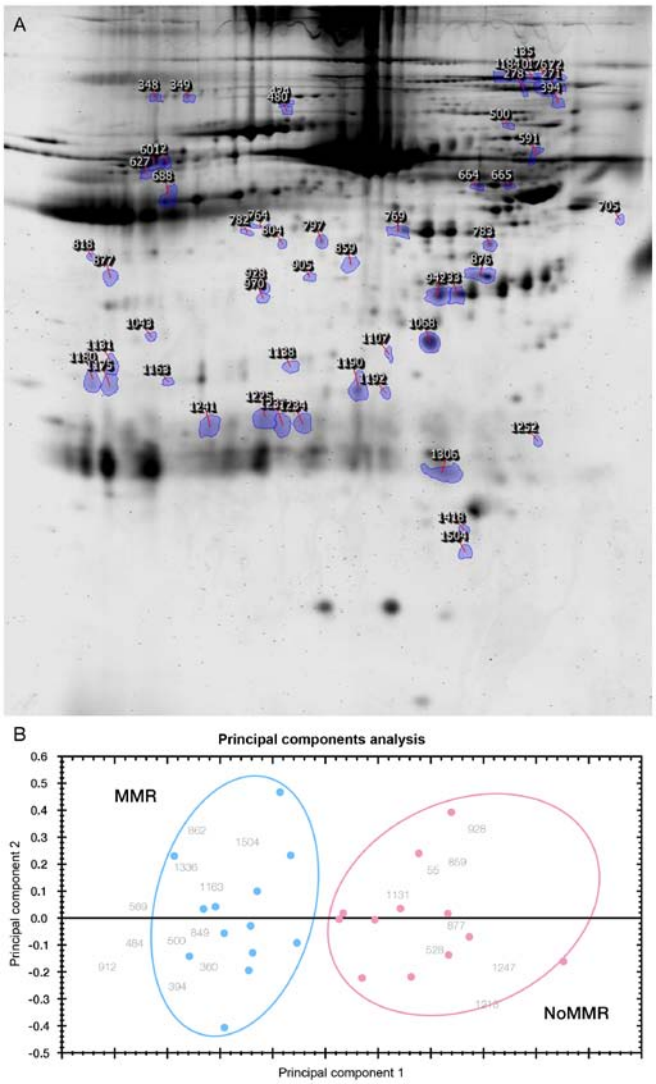

Figure 2. (A) Representative high resolution two-dimensional gel electrophoresis (2-DE) of proteins derived from CML bone marrow sample (Marked are differentially expressed protein spots between patients that achieved major molecular response from patients without major molecular response); P<0.05 and at least 1.5-fold difference. (B) Principal component analysis (PCA) using datasets of 73 differentially expressed protein spots between groups of CML samples based on MMR (blue) and No-MMR (pink) at 6 months. The letters in grey in the background represents the protein spot numbers on the 2-DE gel of all the implicated protein spots used in the analysis.

Figure 3. Unsupervised hierarchical cluster analysis of 64 identified differentially expressed proteins between patients that achieved MMR (blue) at 6 months from patients without MMR (No-MMR, red). The image was generated using J-Express Pro V 1.1 software program. (These 64 proteins used in generating this dendrogram plot are indicated by letter b in Table II).

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

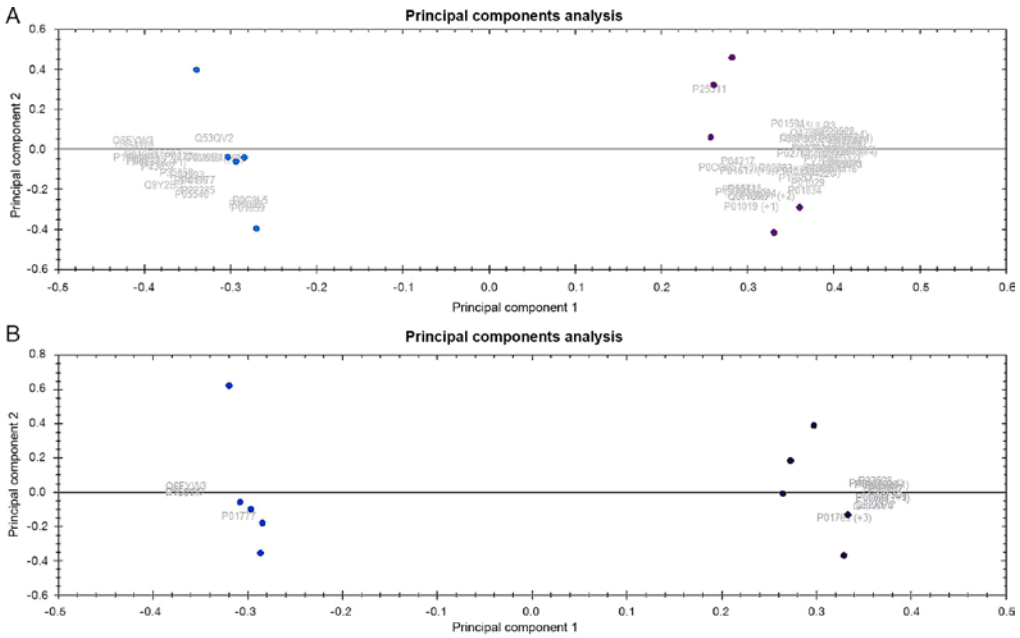

Figure 4. (A) Principal component analysis (PCA) plot of CML peripheral blood samples using the expression dataset of 164 identified proteins that were significantly differentially expressed (>1.5- to ∞-fold change; P<0.05) between LT-MMR and P-No-MMR sample groups. The expression profiles of these proteins correctly predict patients with major molecular response (LT-MMR, blue) vs. no-major molecular response (P-No-MMR, purple) using principal component analysis. (B) Principal component analysis (PCA) plot of CML bone marrow samples using the expression dataset of 138 identified proteins that were significantly differentially expressed (>1.5- to ∞-fold change; P<0.05) between LT-MMR and P-No-MMR sample groups. The expression profiles of these proteins correctly predict patients with long-term major molecular response (LT-MMR, blue) vs. persistent no-major molecular response (P-No-MMR, purple) using principal component analysis. The letters in grey color in the background represents the accession numbers of all the implicated proteins in the analysis. [Both images were generated using Progenesis QI for proteomics (Progenesis QIfp version 2.0.5387) (Nonlinear Dynamics/Waters)].

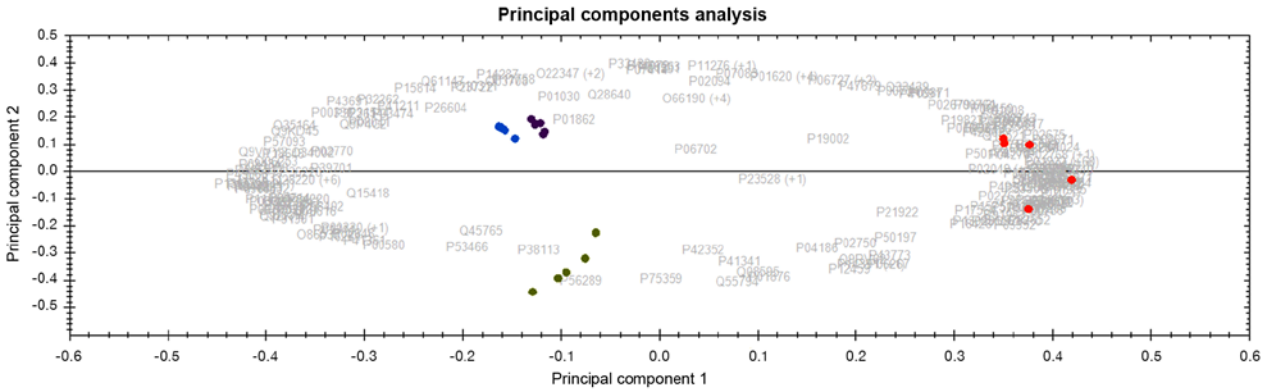

Figure 5. The same dataset from Fig. 4B (i.e. the expression of 138 identified bone marrow proteins that were significantly differentially expressed (>1.5- to ∞-fold change; P<0.05) between LT-MMR and P-No-MMR sample groups) separate all four sample groups including patients that stays on TKI after 1 year of imatinib Rx from patients ultimately requiring alternative treatment using principal component analysis. Long-term major molecular response (LT-MMR, blue), persistently no-major molecular response (P-No-MMR, purple, patients that stays on TKI after 1 year of imatinib Rx, green and patients ultimately requiring alternative treatment, red). The letters in grey color in the background represents the accession numbers of all the implicated proteins in the analysis. [The image was generated using Progenesis QI for proteomics (Progenesis QIfp version 2.0.5387) (Nonlinear Dynamics/Waters)]. Some of the identified proteins were implicated in hematological diseases as potential biomarkers using ingenuity pathway analysis as detailed in Fig. 6.

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.

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
P50197	2	0.000534	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	50S 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)
P53506	4	1.85E-05	6.06449	CML-PBP-TKI-Y	CML-PBP-No-MMR	Actin, cytoplasmic type 8
P53466[a]	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[a]	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
P41361[a,b]	6	2.78E-07	2.68159	CML-PBP-TKI-N	CML-PBP-TKI-Y	Antithrombin-III (ATIII)
P01008	15	1.56E-12	5.19919	CML-PBP-TKI-Y	CML-PBP-TKI-N	Antithrombin-III precursor (ATIII) (PRO0309)
P32262[a]	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-AI)
P18648	3	6.73E-08	2.76435	CML-PBP-MMR	CML-PBP-TKI-Y	Apolipoprotein A-I precursor (Apo-AI)
P02648	12	7.81E-06	2.49354	CML-PBP-TKI-N	CML-PBP-TKI-Y	**Apolipoprotein A-I precursor (Apo-AI)**
P02652	6	4.96E-10	3.48432	CML-PBP-TKI-Y	CML-PBP-No-MMR	**Apolipoprotein A-II precursor (Apo-AII) (ApoA-II)**
P06727[a]	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	**BR1 protein**
P06702[a]	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-Y	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	Ceruloplasmin precursor (EC 1.16.3.1) (Ferroxidase)
O34002	1	0.000137	68.1783	CML-PBP-MMR	CML-PBP-TKI-Y	Citrate synthase (EC 4.1.3.7)
P23528	1	6.64E-09	17.4873	CML-PBP-No-MMR	CML-PBP-MMR	Cofilin, non-muscle isoform (18 kDa phosphoprotein)
Q03708	2	2.25E-07	Infinity	CML-PBP-MMR	CML-PBP-TKI-N	**Colicin E7 immunity protein (ImmE7)**
P00736	4	1.06E-11	3.2943	CML-PBP-TKI-Y	CML-PBP-No-MMR	Complement C1r component precursor
P09871	4	2.38E-07	2.92284	CML-PBP-TKI-Y	CML-PBP-TKI-N	Complement C1s component precursor
P01027[a]	22	1.58E-11	8.12844	CML-PBP-TKI-N	CML-PBP-TKI-Y	Complement C3 precursor (HSE-MSF)
P01024	83	5.80E-10	2.95796	CML-PBP-TKI-Y	CML-PBP-TKI-N	Complement C3 precursor [Contains: C3a anaphylatox]
P01030[a]	20	0.001479	2.42252	CML-PBP-No-MMR	CML-PBP-TKI-N	Complement C4 precursor [Contains: C4A anaphylatox]
P04186	7	0.000166	2.2386	CML-PBP-TKI-N	CML-PBP-MMR	**Complement factor B precursor (C3/C)**
P05156	3	4.54E-07	3.80805	CML-PBP-TKI-Y	CML-PBP-No-MMR	**Complement factor I precursor (EC 3.4.21) (C3B/)**
Q33439	1	6.13E-11	76.1488	CML-PBP-TKI-Y	CML-PBP-TKI-N	Cytochrome c oxidase polypeptide I
P14532	1	8.42E-08	11.1984	CML-PBP-TKI-N	CML-PBP-TKI-Y	Cytochrome C551 peroxidase precursor
Q38732	1	5.73E-08	16.099	CML-PBP-TKI-N	CML-PBP-TKI-Y	**DAG protein, chloroplast precursor**
P57759	3	5.60E-13	5.45666	CML-PBP-TKI-N	CML-PBP-TKI-Y	Endoplasmic reticulum protein ERp29 precursor
P20710	1	1.19E-08	24.9012	CML-PBP-TKI-N	CML-PBP-TKI-Y	**Excisionase**
Q45765	1	0.000582	13.2686	CML-PBP-TKI-N	CML-PBP-No-MMR	**Ferric uptake regulation protein**
P02671	23	0	5.53907	CML-PBP-TKI-Y	CML-PBP-TKI-N	Fibrinogen alpha/alpha-E chain precursor
P02675	36	1.52E-09	2.8323	CML-PBP-TKI-Y	CML-PBP-TKI-N	**Fibrinogen beta chain precursor**
P02679	26	6.02E-06	3.07718	CML-PBP-TKI-Y	CML-PBP-TKI-N	Fibrinogen gamma chain precursor
P11276	11	0.000201	2.27101	CML-PBP-No-MMR	CML-PBP-TKI-N	**Fibronectin precursor (FN) (Fragments)**
P08041	1	4.74E-05	4.36822	CML-PBP-MMR	CML-PBP-TKI-Y	Gas vesicle protein C
P47805	2	0.005106	6.89488	CML-PBP-TKI-Y	CML-PBP-MMR	Gastrulation specific protein G12
P13020	3	2.96E-06	2.44545	CML-PBP-TKI-Y	CML-PBP-MMR	Gelsolin (Actin-depolymerizing factor)

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
P19002[a]	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
P02134	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
P02049	5	3.19E-05	976.807	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
P02094[a]	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-TKI-N	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)
P09631[a]	1	8.27E-14	6.74686	CML-PBP-MMR	CML-PBP-TKI-Y	Homeobox protein Hox-A9 (Hox-1.7)
Q10521[a]	1	2.13E-05	3.30175	CML-PBP-TKI-Y	CML-PBP-TKI-N	**Hypothetical 16.9 kDa protein Rv2239c**
P37506[a]	1	8.12E-10	3.91542	CML-PBP-TKI-Y	CML-PBP-MMR	Hypothetical 20.4 kDa protein in COTF-T**ETB**
Q10616	**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-nrdG 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
P42962[a]	2	0.000554	9.91114	CML-PBP-TKI-Y	CML-PBP-TKI-N	Hypothetical protein ycsE

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
P54462	2	2.28E-13	60.8113	CML-PBP-MMR	CML-PBP-TKI-Y	Hypothetical protein yqeV
P01876[b]	14	1.04E-12	4.48826	CML-PBP-TKI-N	CML-PBP-MMR	**Ig alpha-1 chain C region**
P01862[a]	2	0.001527	Infinity	CML-PBP-No-MMR	CML-PBP-TKI-N	**Ig gamma-2 chain C region**
P01860	11	0.000542	4.16369	CML-PBP-TKI-Y	CML-PBP-No-MMR	Ig gamma-3 chain C region (Heavy chain)
P01861	14	3.90E-09	2.35422	CML-PBP-TKI-Y	CML-PBP-No-MMR	Ig gamma-4 chain C region
P19181[a]	4	0.005572	2.28883	CML-PBP-MMR	CML-PBP-TKI-N	**Ig heavy chain V region 5A precursor**
P01765[a]	2	4.91E-09	5.63765	CML-PBP-TKI-N	CML-PBP-TKI-Y	**Ig heavy chain V-III region TIL**
P01620[a]	5	0.000589	11.6515	CML-PBP-No-MMR	CML-PBP-TKI-N	**Ig kappa chain V-III region SIE**
P01842	6	0.000394	2.20304	CML-PBP-TKI-Y	CML-PBP-TKI-N	Ig lambda chain C regions
P01714	2	5.10E-12	3.83063	CML-PBP-No-MMR	CML-PBP-TKI-Y	Ig lambda chain V-III region SH
P04220	12	7.49E-06	3.79369	CML-PBP-TKI-N	CML-PBP-TKI-Y	Ig MU heavy chain disease protein (BOT)
P01591	5	0.000549	5.43077	CML-PBP-No-MMR	CML-PBP-TKI-N	Immunoglobulin J chain
P15814	2	9.08E-06	5.19282	CML-PBP-MMR	CML-PBP-TKI-Y	Immunoglobulin lambda-like polypeptide 1
P36228	1	0.000179	3.92057	CML-PBP-MMR	CML-PBP-TKI-Y	Infection structure-specific protein 56
P56289	3	3.29E-07	2.32089	CML-PBP-TKI-N	CML-PBP-No-MMR	Initiation factor EIF-5A-1
P01314	1	2.90E-09	5.68794	CML-PBP-TKI-N	CML-PBP-TKI-Y	Insulin
O02833	6	2.32E-09	183.422	CML-PBP-MMR	CML-PBP-TKI-Y	Insulin-like growth factor binding protein complex
P19827[a]	13	2.04E-07	2.19294	CML-PBP-TKI-N	CML-PBP-TKI-Y	Inter-alpha-trypsin inhibitor heavy chain H1 precursor
P56651	1	5.41E-11	18.9887	CML-PBP-MMR	CML-PBP-TKI-Y	**Inter-alpha-trypsin inhibitor heavy chain H2**
P19823	17	0.001377	2.02663	CML-PBP-TKI-Y	CML-PBP-TKI-N	**Inter-alpha-trypsin inhibitor heavy chain H2**
P02750	7	1.91E-12	2.51124	CML-PBP-TKI-N	CML-PBP-MMR	Leucine-rich alpha-2-glycoprotein (LRG)
P06267	2	1.32E-12	4.06168	CML-PBP-TKI-N	CML-PBP-No-MMR	Light-independent protochlorophyllide reductase
P18428	2	7.86E-08	2.56066	CML-PBP-TKI-Y	CML-PBP-MMR	Lipopolysaccharide-binding protein precursor (LBP)
P13796[a]	4	9.06E-13	7.72276	CML-PBP-No-MMR	CML-PBP-TKI-Y	L-plastin (Lymphocyte cytosolic protein 1) (LCP-1)
P28717	1	2.95E-07	4.88405	CML-PBP-TKI-Y	CML-PBP-TKI-N	**Mating pheromone 3 precursor**
Q9RV62	1	8.32E-07	2.27719	CML-PBP-TKI-N	CML-PBP-MMR	**NADH pyrophosphatase (EC 3.6.1.-)**
P41211	1	2.57E-06	2.48053	CML-PBP-MMR	CML-PBP-TKI-Y	Neuron specific calcium-binding protein
P70563	1	0.000537	13.799	CML-PBP-No-MMR	CML-PBP-TKI-N	**Nucleoside diphosphate-linked moiety X motif 6**
P14287	1	5.51E-05	142.537	CML-PBP-MMRs	CML-PBP-TKI-N	**Osteopontin precursor (Bone sialoprotein 1)**
P97085	2	2.31E-06	2.01262	CML-PBP-TKI-Y	CML-PBP-MMR	Outer membrane protein U precursor (Porin ompU)
P31544	2	0.000651	49.286	CML-PBP-MMR	CML-PBP-TKI-Y	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	3.76097	CML-PBP-TKI-Y	CML-PBP-No-MMR	**Plasma kallikrein precursor**
P02753[a]	4	5.90E-13	3.91711	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	Precorrin-4 C11-methyltransferase
Q06253	2	1.39E-09	4.17508	CML-PBP-MMR	CML-PBP-TKI-Y	Prevent host death protein
P07737[a]	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	6.67068	CML-PBP-TKI-N	CML-PBP-TKI-Y	Protein translation factor SUI1 homolog.
P00734	15	0.000479	3.44209	CML-PBP-TKI-Y	CML-PBP-TKI-N	Prothrombin precursor (EC 3.4.21.5)
Q55794	2	2.35E-13	8.13328	CML-PBP-TKI-N	CML-PBP-MMR	Putative arsenical pump-driving ATPase
Q15418	4	0.004805	6.05567	CML-PBP-TKI-N	CML-PBP-TKI-Y	Ribosomal protein S6 kinase alpha 1
P00580	3	2.27E-09	4.02263	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	9.67406	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[a]	53	2.49E-05	2.63861	CML-PBP-TKI-Y	CML-PBP-TKI-N	**Serotransferrin precursor (Siderophilin)**
P49064[a]	4	5.43E-05	Infinity	CML-PBP-TKI-Y	CML-PBP-MMR	Serum albumin precursor (Allergen Fel d 2)
Q28522	43	5.22E-11	5.61756	CML-PBP-TKI-Y	CML-PBP-No-MMR	Serum albumin precursor (Fragment)
P02768	120	1.15E-09	2.87802	CML-PBP-TKI-Y	CML-PBP-No-MMR	Serum albumin precursor
P02743	1	1.17E-12	6.80911	CML-PBP-TKI-Y	CML-PBP-TKI-N	Serum amyloid P-component precursor (SAP)
P27169	5	2.21E-05	2.43474	CML-PBP-TKI-Y	CML-PBP-MMR	Serum paraoxonase/arylesterase 1
P04278	2	8.55E-09	4.0875	CML-PBP-TKI-Y	CML-PBP-No-MMR	Sex hormone-binding globulin precursor (SHBG)
P95340[a]	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	6.96573	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	11.1468	CML-PBP-TKI-N	CML-PBP-TKI-Y	Tail assembly protein K
P43691	3	9.61E-11	3.55237	CML-PBP-No-MMR	CML-PBP-TKI-Y	**Transcription factor GATA-4 (GATA binding factor-4)**
O22347	1	0.002132	12.1326	CML-PBP-MMR	CML-PBP-TKI-N	Tubulin alpha-1 chain (Alpha-1 tubulin)
P12459	1	8.40E-14	9.68647	CML-PBP-TKI-N	CML-PBP-No-MMR	Tubulin beta-1 chai
P02774[a]	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	2.12057	CML-PBP-TKI-Y	CML-PBP-MMR	**Vitronectin precursor (Serum spreading factor)**

Table II. Coninued.

B, The identified differentially expressed proteins in BMP of CML patients with MMR, No-MMR, On-TKI and NOT-on-TKI

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)
P49313[a,b]	1	0.00086	2.8992	TKI-N	CMR-Y	30 kDa ribonucleoprotein, chloroplast precursor
P02578[b]	1	0.00023	2.4784	TKI-N	CMR-Y	Actin 1
Q03341[b]	**1**	**0.00033**	**19.7447**	**CMR-N**	**TKI-Y**	**Actin 2**
P02580[b]	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	8.5512	TKI-N	CMR-N	Actin, muscle 1A
P50138[b]	1	0.00431	88.6922	CMR-Y	TKI-Y	Actin
Q9P4D1	1	0.01099	3.7590	CMR-Y	TKI-Y	Actin
P43652[b]	**13**	**0.00003**	**2.0878**	**CMR-Y**	**TKI-N**	**Afamin precursor (Alpha-albumin) (Alpha-Alb)**
P19652[b]	6	0.00163	1.5175	CMR-Y	TKI-N	Alpha-1-acid glycoprotein 2 precursor (AGP 2)
P01010[b]	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)**
P08697[b]	**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)
P01023[a]	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
P00896[b]	1	0.00001	5.0581	CMR-N	TKI-N	Anthranilate synthase component I (EC 4.1.3.27)
P01008[a]	9	0.00320	1.4376	CMR-Y	TKI-Y	Antithrombin-III precursor (ATIII) (PRO0309)
P32261[b]	2	0.00084	5.0712	TKI-N	CMR-N	Antithrombin-III precursor (ATIII)
P09809	2	0.02421	1.5680	TKI-N	CMR-Y	Apolipoprotein A-I precursor (Apo-AI)
P15497[a]	**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)
P02655[a,b]	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	CMR-Y	TKI-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**
Q00371[b]	1	0.00002	23.1103	TKI-N	CMR-N	CAP22 protein
P00915[b]	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
P25773[b]	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	CMR-Y	TKI-N	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]
P01024[a]	**68**	**0.00088**	**1.6430**	**CMR-Y**	**TKI-N**	**Complement C3 precursor [Contains: C3a anaphylatox]**
P01028[b]	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**
P02748[b]	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)**
P48416[b]	**3**	**0.00000**	**3.5184**	**TKI-N**	**CMR-Y**	**Cytochrome P450 10 (EC 1.14.-.-) (CYPX)**
Q92I25[b]	1	0.00007	2.6454	TKI-Y	CMR-N	Dihydrodipicolinate synthase (EC 4.2.1.52) (DHDPS)
P31073[b]	1	0.00010	2.2735	TKI-N	CMR-N	Dihydrofolate reductase (EC 1.5.1.3)
P20861[b]	1	0.00000	16.7020	TKI-N	CMR-Y	Fan G protein precursor
P02671[a,b]	21	0.00003	2.2257	CMR-Y	TKI-Y	Fibrinogen alpha/alpha-E chain precursor
P02675[a,b]	24	0.00010	2.4767	CMR-Y	CMR-N	Fibrinogen beta chain precursor [Contains: Fibrinogen]
Q02020[b]	**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 beta chain precursor [Contains: Fibrinogen]**
P02679[a,b]	13	0.00110	2.1792	CMR-Y	CMR-N	Fibrinogen gamma chain precursor
Q92T27	2	0.00030	1.5959	TKI-N	CMR-N	Glucokinase (EC 2.7.1.2) (Glucose kinase)
Q92J74	**1**	**0.00712**	**2.6314**	**CMR-Y**	**CMR-N**	**Glutamyl-tRNA(Gln) amidotransferase subunit C**
Q60759	4	0.00301	1.8431	TKI-N	CMR-Y	Glutaryl-CoA dehydrogenase, mitochondrial precursor
P23722[a]	**3**	**0.00380**	**1.5602**	**TKI-N**	**CMR-Y**	**Glyceraldehyde 3-phosphate dehydrogenase**
Q9ZKP0[a,b]	**2**	**0.00292**	**2.4902**	**CMR-Y**	**TKI-N**	**Glycerol-3-phosphate dehydrogenase [NAD(P)+]**
P50150	1	0.03327	5.9505	TKI-N	CMR-Y	Guanine nucleotide-binding protein G(I)/G(S)/G(O)
P07736[b]	**1**	**0.00189**	**2.7741**	**TKI-N**	**CMR-Y**	**Guanyl-specific ribonuclease U1 (EC 3.1.27.3) (Rna)**
P50417	**1**	**0.00764**	**5.7455**	**CMR-Y**	**TKI-N**	**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[b]	**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[b]	2	0.00000	3.9434	CMR-N	CMR-Y	Hemoglobin alpha-1, alpha-2, and alpha-3 chains
P02037[b]	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	TKI-N	CMR-N	Hemoglobin beta-1 chain (Major)
P02074[b]	1	0.00000	4.1199	CMR-N	CMR-Y	Hemoglobin beta-III chain
P19886[b]	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[b]	**1**	**0.00000**	**4.4405**	**TKI-Y**	**CMR-Y**	**Hypothetical protein HI0659**
P42968[b]	**1**	**0.00003**	**4.3060**	**TKI-N**	**CMR-N**	**Hypothetical transcriptional regulator ycsO**
P01876[a,b]	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[a]	3	0.00018	1.4555	TKI-Y	TKI-N	Ig gamma-3 chain C region (Heavy chain disease protein)
P01861[a]	5	0.02495	1.4049	CMR-Y	TKI-N	Ig gamma-4 chain C region
P01779	2	0.02052	2.4688	CMR-Y	CMR-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[a]	**5**	**0.00763**	**1.4632**	**CMR-Y**	**CMR-N**	**Ig lambda chain C regions**
P01591[a]	**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
P97279	2	0.03423	2.0472	TKI-Y	TKI-N	Inter-alpha-trypsin inhibitor heavy chain H2 precursor
Q42891[b]	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
P02750[a]	9	0.01798	1.3841	TKI-Y	CMR-N	Leucine-rich alpha-2-glycoprotein (LRG)
P06267[a,b]	**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
P48310[b]	1	0.00024	2.4866	CMR-Y	TKI-N	Minor capsid protein VI precursor
O03698[b]	**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
P39115[b]	1	0.00000	3.4012	CMR-N	CMR-Y	Nucleotide binding protein ExpZ
P32119[b]	3	0.00000	4.3238	CMR-N	CMR-Y	Peroxiredoxin 2 (Thioredoxin peroxidase 1)
Q42858[b]	**1**	**0.00007**	**4.2693**	**CMR-N**	**TKI-N**	**Phenylalanine ammonia-lyase (EC 4.3.1.5)**
O07125[b]	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	**2**	**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	TKI-N	CMR-Y	Probable lysozyme from lambdoid prophage Qin
O67024	**1**	**0.03110**	**Infinity**	**CMR-Y**	**TKI-N**	**Probable peroxiredoxin**
P07737	2	0.00870	1.8459	CMR-Y	CMR-N	Profilin I
P00536	**2**	**0.00697**	**1.5076**	**TKI-N**	**CMR-N**	**Proto-oncogene serine/threonine-protein kinase mos**
P45604	1	0.00021	1.9033	CMR-N	CMR-Y	PTS system, N-acetylglucosamine-specific EIIABC component
Q59482	**1**	**0.00519**	**4.2028**	**CMR-Y**	**TKI-N**	**Purine nucleoside phosphorylase (deoD)**
P55429[b]	**1**	**0.00004**	**2.5979**	**CMR-N**	**CMR-Y**	**Putative integrase/recombinase Y4EF**
Q9AB80	3	0.00001	1.5354	TKI-Y	CMR-Y	Putative outer membrane protein CC0351 precursor
Q9X480	2	0.00113	1.8668	CMR-N	CMR-Y	Putative signal peptide peptidase sppA
P34443	3	0.02905	2.3131	CMR-Y	TKI-Y	Ras-like protein F54C8.5
P34295	2	0.02474	1.4695	TKI-Y	CMR-N	Regulator of G protein signaling rgs-1
Q9CG17[a]	1	0.00003	1.7092	CMR-Y	TKI-N	Ribonuclease HII (EC 3.1.26.4) (RNase HII)
P56566[b]	**2**	**0.00478**	**3.4601**	**TKI-N**	**CMR-N**	**S100 calcium-binding protein A3 (S-100E protein)**

Table II. Continued.

B, The identified differentially expressed proteins in BMP of CML patients with MMR, No-MMR, On-TKI and NOT-on-TKI

Accession	Peptide count used for quantification	Anova (p)	Max fold change	Highest mean condition	Lowest mean condition	Description
P12346[b]	2	0.00000	2.4638	TKI-Y	TKI-N	Serotransferrin precursor (Siderophilin) (Beta-1-metal-binding globulin)
P19134[b]	**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	Serotransferrin precursor (Siderophilin) (Beta-1-m-b-g)
P02769[b]	**5**	**0.00003**	**2.4650**	**TKI-Y**	**CMR-N**	**Serum albumin precursor (Allergen Bos d 6)**
Q28522	**7**	**0.04108**	**2.6927**	**CMR-Y**	**TKI-N**	**Serum albumin precursor (Fragment)**
P49065[a,b]	**2**	**0.00016**	**6.1150**	**CMR-N**	**TKI-Y**	**Serum albumin precursor**
P27169[a,b]	3	0.00416	2.1032	TKI-Y	TKI-N	Serum paraoxonase/arylesterase 1 (EC 3.1.1.2)
Q9CES7[b]	1	0.00006	2.0972	TKI-Y	TKI-N	Shikimate 5-dehydrogenase (EC 1.1.1.25)
P29950[b]	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**

[a]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. [b]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.

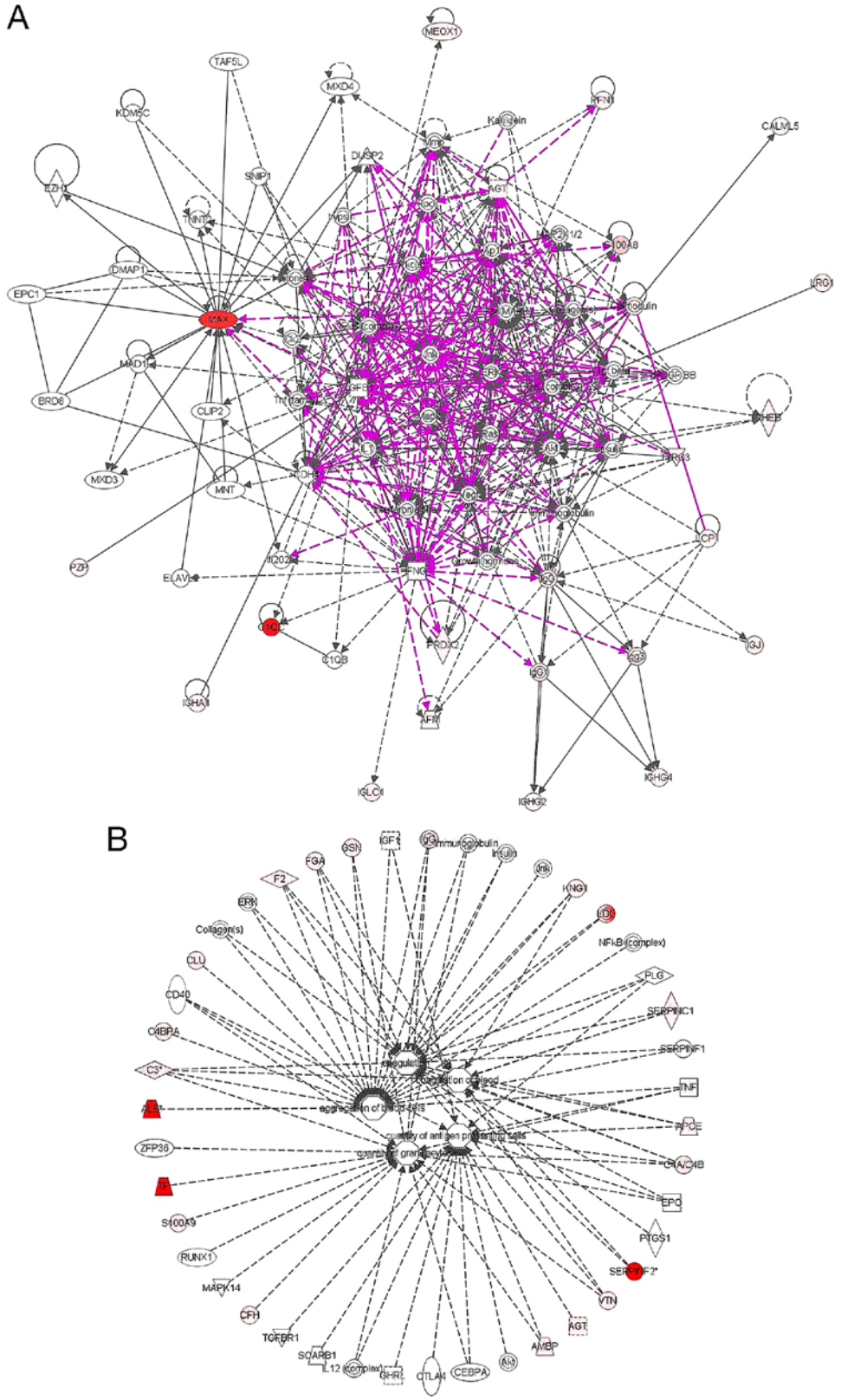

Figure 6. (A) Pathway analysis of network signaling of some of the identified proteins as represented in the ingenuity pathway analysis database. The analysis of the identified proteins is composed of 2 hematological disease related networks with over 100 associated molecules that were merged into one 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)].

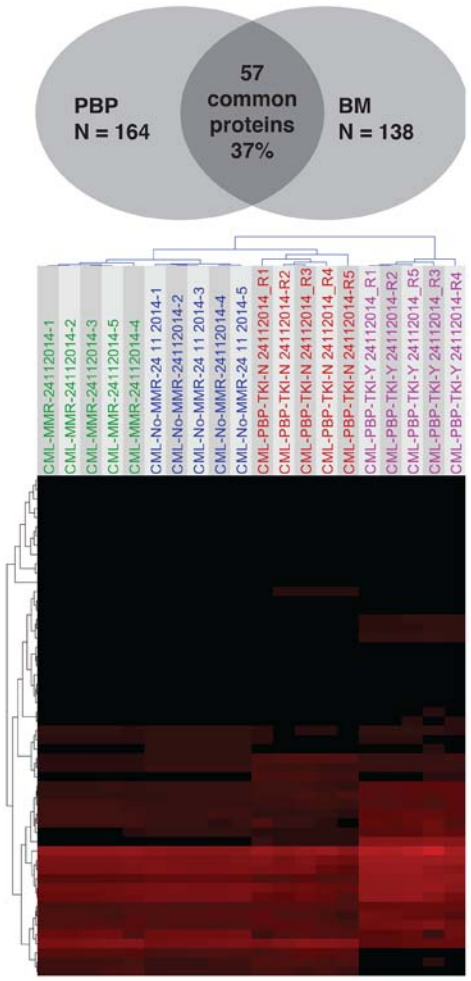

Figure 7. Unsupervised hierarchical cluster analysis of 54 identified differentially expressed proteins that are common in both bone marrow plasma (dataset of 138 proteins) and peripheral blood plasma (dataset of 164 proteins) of CML samples. The dendrogram shows correct prediction of patients with long-term major molecular response (LT-MMR, green), persistent no-major molecular response (P-No-MMR, blue), patients that stays on TKI after 1 year of imatinib Rx, purple and patients on alternative treatment outside TKI, red). The image was generated using J-Express Pro V 1.1 software program. (These 54 proteins used in generating this dendrogram plot are indicated with the letter a in Table II).

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.

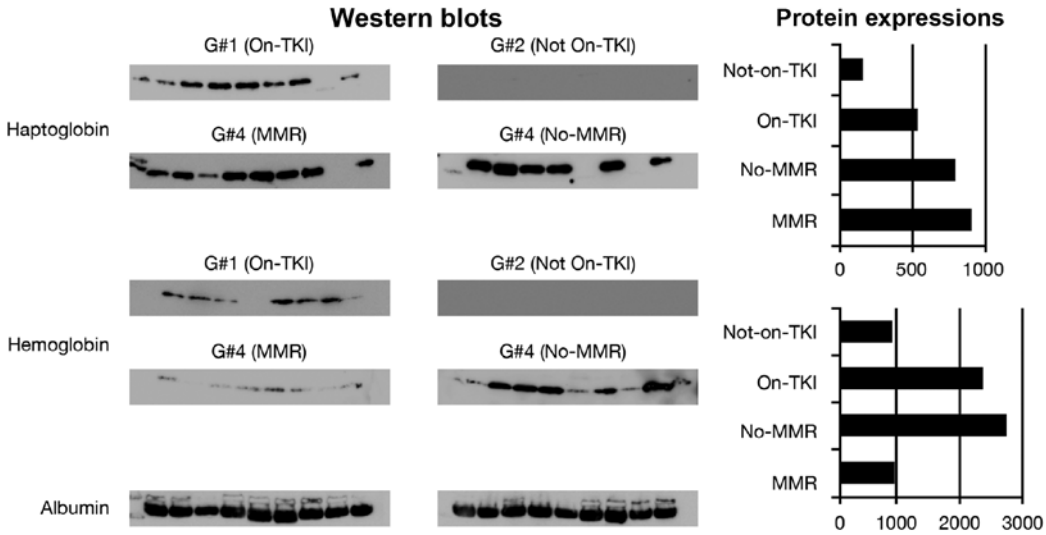

Figure 8. Western blots validation analysis abundance of 2 of the identified differentially expressed proteins. Each lane indicates the expression of 9 individual samples in each of the four sample groups representing long-term major molecular response to imatinib (LT-MMR), persistently no major molecular response (P-No-MMR), patients that stay on TKI after 1 year of imatinib treatment (On-TKI) and patients that ultimately required alternative treatment options, i.e. second generation TKI/others (Not-On-TKI). Albumin was used as internal standard for normalization. The histogram bars are the corresponding average group protein expressions of the two protein haptoglobin and hemoglobin using label-free LC/MS/MS expression analysis platform.

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 datasets 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, TGFb, 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/others).

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 are 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.

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