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Plasma proteomics of biomarkers for inflammation or cancer cannot predict relapse in chronic myeloid leukaemia patients stopping tyrosine kinase inhibitor therapy

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Abstract

Several studies have now shown that chronic myeloid leukaemia (CML) patients in deep molecular remission may discontinue tyrosine kinase inhibitor (TKI) treatment with a treatment free remission (TFR) rate of approximately 40-60%. Some factors influencing the possibility of TFR have been described but better tools are needed for individual prediction of long-term TFR. Herein, two multiplex panels were utilised to analyse a total of 162 different plasma proteins from 56 patients included in the TKI stopping trial EURO-SKI.[1] The purpose was to identify possible plasma protein markers for prediction of successful TKI discontinuation and to evaluate effects of TKI discontinuation on plasma protein profiles. No protein biomarkers sampled before TKI discontinuation could separate relapse cases from non-relapse cases but some plasma proteins differed between patients who relapsed and those who remained in TFR when followed over time after TKI cessation. In conclusion, the plasma protein markers in this study could not predict relapse after TKI discontinuation but may be of use to understand the mechanisms involved in maintenance of TFR.

Keywords

chronic myeloid leukemia; tyrosine kinase inhibitor; treatment free remission; proteomics; immunology

1. Introduction

Chronic myeloid leukaemia (CML) is a chronic myeloproliferative disorder arising due to the formation of a constitutively active tyrosine kinase (Bcr-Abl) through a translocation between chromosomes 9 and 22 in hematopoetic stem or progenitor cells.[2] Treatment with inhibitors of the Bcr-Abl (tyrosine kinase inhibitors, TKIs) has during the last two decades drastically improved CML prognosis.[3-6] However, long term TKI treatment is associated with high costs and severe adverse effects such as cardiovascular events.[7] During recent years, several studies have shown that a proportion of TKI-treated patients who achieve optimal treatment responses (MR[4], i.e. ≥4 log reduction in *BCR-ABL1*; *BCR-ABL1* ≤0.01%, or better) may discontinue treatment and remain in treatment-free remission (TFR) (reviewed in[8]). It is not fully known what factors influence the probability of TFR but so far, reports suggest that duration of TKI treatment and deep molecular response (DMR) before TKI stop influence the chances of remaining in TFR.[1] Also, factors related to the patients´ immune status seem to be of importance.[9-11] Recently described factors of potential significance for TFR are genetic variants discovered by whole-exome sequencing[12] and telomere-

length shortening.[13] However, there is still need for better prediction tools for choosing the right patients for stopping attempts. Easily accessible biomarkers such as plasma proteins would be a valuable complement for guiding decision making when TKI stopping attempts are moved in to clinical routine in the management of CML patients.
We have previously shown that plasma proteomics are feasible and useful in CML patients, both for studying patterns of protein expression and for identifying single proteins differentially expressed before and after TKI treatment initiation.[14] In the present paper, we use a proximity extension assay (PEA)-based proteomic platform to study protein expression in a patient cohort from the large TKI stopping study EURO-SKI.[1] We studied a total of 162 plasma proteins in TKI-treated patients (n=56) before, at one and six months after TKI cessation, and at relapse with the primary aim of finding plasma protein markers predicting a successful treatment stop by comparing the markers between patients with and without relapse.

2. Materials and methods

2.1. Patients and samples

The study was conducted as an exploratory sub-study of the European Stop Tyrosine Kinase Inhibitor Study (EURO-SKI) (ClinicalTrials.gov identifier: NCT01596114). The patients included (n=56, age range 18-78 years) were from Finland, Norway and Sweden. All were in chronic phase and had been treated with TKIs for at least three years prior to inclusion in the study. As outlined in the EURO-SKI inclusion criteria, all patients had sustained DMR ($MR^{4.0}$) for at least one year before TKI cessation. Relapse was defined as loss of major molecular response (MMR; *BCR-ABL* ≤0.1% on the international scale). Peripheral blood samples were obtained at baseline (immediately prior to TKI cessation), at one and six months after stopping TKIs, and at relapse. All patients gave written informed consent to study inclusion. The study was approved by the local ethics committees in each country and conducted in accordance with the Declaration of Helsinki.

2.2. Plasma sample analysis and data processing

Heparin blood was shipped to Helsinki from the participating centres. Heparin plasma was obtained by centrifugation at 1300 g for 10 minutes in room temperature and immediately after collection, the plasma was stored in -70°C. Patient samples were analysed with Olink Proseek Multiplex (Olink Proteomics, Uppsala, Sweden). Two different panels were used, Inflammation I (INF I) and Oncology II (ONC II), each containing 92 inflammation- and cancer-related protein biomarkers, respectively (see Supplemental Tables A1 and A2 for a complete list of all biomarkers). Olink utilizes so called proximity extension assay (PEA) which is based on pairs of antibodies linked to oligonucleotides with a slight affinity to each other. When the antibodies bind to their target protein, the oligonucleotides are brought in proximity and extended by a DNA polymerase, forming a new sequence that acts as a surrogate marker for the antigen. The new sequence is then extended by qPCR and the copy numbers formed are proportional to the concentration of the antigen in the sample.[15] Frozen aliquots of patient heparin plasma samples were shipped to Olink Proteomics and analysed there according to the laboratory's standard operating procedure. Samples were run in duplicates. The limit of detection (LOD) was defined as 3xSD above assay background and values below the LOD were replaced by the value for the LOD for statistical analyses. If many values are replaced by the

LOD value the natural variation in the data for that analyte decreases, which might have an impact on the statistical analyses. In this study, a few analytes had a large amount of values below the LOD, and the rest of the analytes had very few of these values. Therefore, analytes with ≥25% of values below the LOD were excluded from statistical analyses. The remaining analytes all had less than 10% of values below the LOD. Proseek Multiplex uses relative quantification and data are presented as normalized protein expression (NPX), which is an arbitrary unit on log2 scale. Internal and external controls were included in each run. Coefficients of variation (CV) values were calculated using linearized values (2^{NPX}) in order to investigate platform performance, for all statistical analyses the log2 scale was used.

2.3. Statistical analyses

Statistical analyses were performed on log2 data which were approximately normally distributed. Welch's unequal variances t-test was used to determine statistically significant differences between groups (relapse vs. non-relapse). Linear Mixed Effects Modelling (LMM)[16] was used to investigate differences between groups over time and the effect of time itself (longitudinal analyses) during the first 6 months. With LMM the within-individual correlations are taken into account, as well as the order of the time points, and the baseline value is allowed to vary between individuals (random intercept). The group effect relapse versus non-relapse is treated as a fixed effect, and a Toepliz within-individual correlation structure is being used which implies that the correlation between close time points may differ from the correlation between time points further apart. The mean protein levels are estimated by a linear trend over time, and these linear trends are compared between the two groups. The data does not suggest any particular trend pattern, which makes a linear trend a reasonable choice to summarize the effect of time. Analyses of the model residuals showed that the model fitted the data well for all the analytes.

All analyses were adjusted for multiple testing using the False Discovery Rate (FDR) method of correction.[17] The false discovery rate is the proportion of "discoveries" (significant results) that are actually false positives. Since we have previously explored the effects of TKI treatment on plasma protein profiles [14], we used a false discovery rate of 5% for analyses of TKI effects in treated patients. In the mixed effects model a false discovery rate of 10% was applied since this was considered an exploratory setting and positive results would have to be confirmed in follow-up studies which would reveal any false positives. Thus, using a false discovery rate of 10% is feasible in this situation. Statistical analyses were performed with R, SPSS Statistics v 23, and SAS v 9.4.

3. Results

3.1. Platform performance and output

On both panels, 98% of the samples met the quality control criteria. Intra-assay coefficient of variance (CV) values were 6% for both INF I and ONC II. Inter-assay CV values were 12% and 11% for INF I and ONC II, respectively. On the INF I panel, 76% of the analytes were detectable in >75% of the samples. On the ONC II panel, all analytes were detectable in >75% of the samples, rendering 162 evaluable proteins in total.

3.2. Patient characteristics

Patient characteristics are summarised in Table 1. The median (range) age at diagnosis was 53.3 (17.8-77.9) years. The male to female ratio was 31 to 25. Sokal risk group distribution was as follows:

52% low risk (LR), 27% intermediate risk (IR), 16% high risk (HR) and 4% unknown. For the EUTOS score, 89% were LR, 7% HR and 4% unknown. Thus, a slightly higher proportion of patients were low risk as compared to a population-based cohort.[6]

At diagnosis, patients were started on treatment with imatinib (n=42), dasatinib (n=11) or nilotinib (n=3). At TKI stop, two patients had changed treatment from imatinib to dasatinib and two had changed from dasatinib to imatinib so that the frequencies of different treatments remained unchanged. Nine patients (5 in the relapse group and 4 in the non-relapse group) had been treated with interferon (IFN) alpha prior to TKI treatment. Median (range) age at TKI stop was 62.1 (23.5-83.7) years and median TKI treatment duration was 6.1 (3.1-12.3) years. After a median (range) follow up of 22.9 (7.3-38.5) months, 32 patients had relapsed as defined in the methods section. Twenty-four relapses occurred within the first six months (early) and eight thereafter (late). At relapse, all patients were re-started on TKI treatment. At the end of follow up, all 32 patients had regained MMR and all but two had regained $MR^{4.0}$.

3.3. *Relapse versus non-relapse*

At baseline, immediately prior to TKI discontinuation, no significant differences in protein levels were found by Welch's t-test between patients who later relapsed and those who remained in TFR. However, when analysing data over time after TKI discontinuation with the linear mixed model approach, some differences between groups could be seen. The analytes that differed between relapse and non-relapse cases were leukaemia inhibitory factor receptor (LIF-R; LMM estimate for relapse -0.1838, p=0.0106), IL-15 receptor alpha (IL-15R alpha; LMM estimate for relapse -0.2136, p=0.0235), colony stimulating factor-1 (CSF-1; LMM estimate for relapse -0.18679, p=0.0014), transforming growth factor beta receptor type 2 (TGFR-2; LMM estimate for relapse -0.2672, p=0.0218), stem cell factor (SCF; LMM estimate for relapse -0.2221, p=0.0254) and folate receptor (FR) alpha (LMM estimate for relapse -0.2208, p=0.0257) (Figure 1). Thus, a significant difference between group means could be seen over time, with non-relapse cases having higher group means for all analytes mentioned above. Individual patients' values were also compared to group means and related to relapse status. At the 1 month-time point, many relapse cases had values higher than the group means (between 31 and 55% for the different analytes) and no clear cut off-value could be found to distinguish between relapse and non-relapse cases. At the 6 month-time point, a smaller proportion of relapse cases had values higher than the group means (between 13 and 38%). For the LIF-R and TGFR-2, only one relapse case per analyte had a value above the group mean. However, no clear cut-off value separating relapse and non-relapse cases could be found at 6 months either.

Since most relapses after TKI discontinuation occur during the first 6 months, we were interested in finding possible biological differences between early (within the first six months) and late relapses. Results from baseline and month 1 for patients who relapsed were therefore compared between the two groups but no significant differences could be seen at any time point.

3.4. *Effects of TKI treatment*

Since imatinib and dasatinib are known to affect partly different signalling pathways [18], we compared baseline values between these two groups. However, only a few proteins (monocyte chemotactic protein (MCP) 4, glypican-1 (GPC1), Erb B2, Erb B4, CD207; adjusted p-values <0.05; Figure 2) were significantly different between imatinib- and dasatinib-treated patients with values being higher in imatinib-treated patients for all differing analytes.

After TKI discontinuation, an increase or decrease over time could be seen for a total of 39 proteins with the LMM analysis (Table 2), while the remaining analytes did not significantly change over the course of six months.

Since prior treatment with IFN alpha has been implicated to increase the probability of TFR[19], we evaluated possible differences in protein levels between patients who had received IFN pre-treatment at any time point and those who had not. With reservation for unequal group sizes, no significant differences in protein levels could be found at any time point between patients who had been previously treated with IFN alpha and those who had not received such treatment.

4. Discussion

Despite increasing knowledge about clinical and biological factors influencing the probability of TFR after TKI discontinuation, there is still a need for better prediction tools to guide clinical decision making when choosing patients for TKI stopping attempts. In the present study, we performed plasma proteomics on 56 patients discontinuing TKIs within the EURO-SKI trial with the aim of identifying potential plasma protein biomarkers for relapse after TKI discontinuation.

No plasma proteins were significantly different between relapse and non-relapse patients before stopping TKI treatment and thus, no predictive plasma protein markers for successful TKI discontinuation could be identified. Also after TKI discontinuation, only a few plasma proteins were significantly different between relapse and non-relapse cases, probably reflecting that the levels of disease at molecular relapse are low with a low mutational profile that does not evoke T-cell responses or profound effects on plasma cytokine levels. The fact that NK cell-based immunity, which is independent of the formation of new tumour antigens, has been shown to be important for TFR[10] supports this theory. Nevertheless, six plasma proteins differed significantly between relapsing and non-relapsing patients after TKI discontinuation: LIF-R, IL-15R alpha, CSF-1, TGFR-2, SCF and FR alpha. The mean protein levels were higher in non-relapse cases for all analytes.

The LIF-R, upon binding of its ligand LIF, activates JAK/STAT and other signalling pathways.[20] LIF-R has been shown to suppress metastasis of hepatocellular carcinoma and pancreatic cancer.[21,22] However, in CML, STAT signalling has been connected to leukaemia initiation and maintenance[23] as well as TKI resistance.[24] Thus, the mechanisms behind our findings remain to be elucidated but might involve other signalling pathways than the JAK:STAT pathway in residual malignant cells or signalling in other cell types such as immune cells since LIF:LIF-R signalling plays an important role in T-cell immunity.[20]

IL-15R alpha is a sub-unit of the IL-15 receptor that binds IL-15 to the cell surface and thereby supports NK-cell development.[25,26] Clinical trials using IL-15 alone or in combination with NK cells have shown beneficial effects in both solid tumours and haematological malignancies.[27] We have previously shown that an increased proportion of mature NK cells was associated with successful imatinib discontinuation[10] and our current finding of higher levels of IL-15R alpha in the non-relapsing patients might offer support for the importance of NK cells in successful TFR.

CSF-1 (or M-CSF; macrophage-colony stimulating factor) is important for innate immunity by its regulation of the development of tissue macrophages.[28] In solid cancers, the expression of CSF-1 and presence of tumour infiltrating macrophages have been linked to poor outcome[29] while less is known about the role of CSF-1 in leukaemia. Imatinib has off-target effects on the receptor for CSF-1, the c-fms.[30] Thus, our findings are of unclear significance but could have potential explanations both in immunological functions and treatment effects.

TGFR-2 is the receptor for TGFβ, which is a transcriptional regulator in hematopoietic cells and induces cell cycle arrest in primitive bone marrow progenitors.[31] A study comparing the inhibitory

effects of TGFβ on primitive hematopoetic cells from CML patients and healthy donors found that the CML progenitors were more sensitive to TGFβ mediated inhibition than their normal counterparts.[32] Low levels of TGFR-2 have been found in both CML and other myeloproliferative neoplasms.[33] Thus, one can speculate that the lower levels of TGFR-2 that we found in relapse cases might give a proliferative advantage to cells that escape the negative regulation of TGFβ.

SCF, which is the ligand for receptor c-kit that is targeted by imatinib, has previously been implied in the pathogenesis of CML by inducing proliferation of CD34+ progenitors *in vitro.*[34] However, SCF levels in serum from untreated CML patients are not different from healthy controls.[35] Imatinib treatment has been shown to increase SCF in patients treated for gastrointestinal stromal tumours (GIST)[36] and, in a pilot study evaluating the effects of TKI treatment on multiple plasma proteins in CML patients, we also found that SCF increased after treatment initiation.[14] It is thus possible that the effect of SCF on CML cells is different *in vitro* and *in vivo.* The increase in SCF described after imatinib treatment might represent a compensatory mechanism to maintain normal c-kit related functions which might somehow be of importance for TFR.

FR alpha is increased in tumours of epithelial origin and might confer a growth advantage to the tumour by modulating folate uptake from serum.[37] FR alpha expression has not been extensively studied in CML or other haematological malignancies and its effects in CML are to our knowledge unknown. However, in our previous study referred above, FR alpha increased in CML patients after TKI treatment initiation.

In conclusion, only a few differences in protein levels were seen between non-relapsing and relapsing cases. No predictive protein biomarkers for successful TKI discontinuation could be found. It could be of interest to confirm the differences between relapse and non-relapse cases in an experimental setting or in a bigger cohort of patients, but it is likely that plasma protein markers alone are not sensitive enough to predict molecular relapse. It is plausible that several different clinical and biological factors will need to be combined for the best prediction of which patients will benefit most from TKI cessation attempts.

Acknowledgements
The authors would like to thank participating EURO-SKI centres and investigators.

Funding
Funding for this sub-study was supplied by the Nordic Cancer Union and Lions Uppsala.

Appendix A. Supplementary data

Supplemental tables A1 and A2.

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Tables and figures

Table 1. Patient characteristics (N=56)

Age at diagnosis (years)	53.3 (17.8-77.9)
Age at stop (years)	62.1 (23.5-83.7)
Sex (N (%))	
Female	25 (45)
Male	31 (55)
Sokal score (N (%))	
LR	29 (52)
IR	15 (27)
HR	9 (16)
Missing	2 (4)
EUTOS score (N (%))	
LR	50 (89)
HR	4 (7)
Missing	2 (4)
First TKI (N (%))	
Ima	42 (75)
Dasa	11 (20)
Nilo	3 (5)
TKI at stop (N (%))	
Ima	42 (75)
Dasa	11 (20)
Nilo	3 (5)
Total TKI duration at stop (years)	6.1 (3.1-12.3)
Relapse, total (N (%))	32 (57)
Relapse, early (≤6 months) (N (%))	24 (75)
BCR-ABL1 ≥1% at relapse (N (%))	3 (9)
Regained MMR after TKI re-initiation (N (%))	32 (100)
Regained MR4.0 after TKI re-initiation (N (%))	30 (94)

Abbreviations: LR=low risk, IR=intermediate risk, HR=high risk; ima=imatinib, dasa=dasatinib, nilo=nilotinib; MMR=major molecular response - BCR-ABL1^{IS} ≤0.1%, MR4.0=BCR-ABL1^{IS} <0.01%.

Table 2. Plasma protein changes over time after TKI discontinuation.

Protein	LMM estimate month	Lower 95% CL	Upper 95% CL	P-value (unadjusted)
Hepatocyte growth factor (HGF)	0.05009	0.007848	0.09233	0.0016
Eucaryotic translation initiation factor 4E-binding protein 1 (4E-BP1)	0.03773	-0.02801	0.1035	0.0131
Delta and notch-like epidermal growth factor-related receptor (DNER)	0.00803	-0.00618	0.02224	0.0057
CD40	0.03106	-0.00372	0.06585	0.0111
5’ nucleotidase (5-NT)	0.03020	-0.00573	0.06613	0.0320
Adenosine Deaminase (ADA)	0.04524	0.01808	0.07240	<.0001
Annexin 1 (ANXA1)	0.04215	-0.02057	0.1049	0.0089
C-X-C motif chemokine 13 (CXCL13)	0.05079	0.006012	0.09557	0.0142
Carbonic anhydrase 9 (CA9)	-0.06850	-0.1178	-0.01925	0.0002
Carboxypeptidase E (CPE)	-0.02186	-0.04436	0.000638	0.0007
Disintegrin and metalloproteinase domain-contacting protein 8 (ADAM-8)	0.03020	-0.00573	0.06613	0.0320
Epidermal growth factor (EGF)	0.03004	-0.03263	0.09272	0.0017
FC-receptor-like B (FCRLB)	-0.01600	-0.03981	0.007811	0.0022
Fasantigen ligand (FasL)	0.02967	0.01057	0.04877	<.0001
Fibroblast growth factor-binding protein 1 (FGF-BP1)	-0.02240	-0.04886	0.004050	0.0008
Folate receptor alpha (FR alpha)	-0.02230	-0.04214	-0.00247	0.0036
Fractalkine (CX3CL1)	-0.03501	-0.05676	-0.01327	0.0096
Furin	0.02235	0.000552	0.04414	0.0023
Galectin 1 (Gal-1)	0.01812	0.005558	0.03069	0.0234
Hepatocyte growth factor (HGF)	0.05009	0.007848	0.09233	0.0016
ICOS ligand (ICOSLG)	0.01673	0.001090	0.03237	0.0040
Integrin beta-5 (ITGB5)	0.01451	-0.00780	0.03681	0.0203
Interleukin 10 receptor subunit beta (IL10 RB)	0.02328	0.006953	0.03961	<.0001
Interleukin 18 receptor 1 (IL18 R1)	0.02000	-0.00229	0.04229	0.0008
Kallikrein 14 (hK14)	-0.01090	-0.04234	0.02053	0.0139
Matrix metalloproteinase 10 (MMP10)	-0.05329	-0.08039	-0.02620	0.0004
Proto-oncogene tyrosine-protein kinase receptor (RET)	0.13607	0.09343	0.1787	<.0001

Protein	LMM estimate month	Lower 95% CL	Upper 95% CL	P-value (unadjusted)
Receptor tyrosine-protein kinase (Erb-B2)	0.00834	-0.00691	0.02358	0.0068
S100-A12 (EN-RAGE)	0.07587	-0.00169	0.1534	0.0027
TNF-related apoptosis-inducing ligand (TRAIL)	0.02462	0.009219	0.04001	<.0001
TNFRSF1919	0.05662	0.03045	0.08278	<.0001
Tissue factor pathway inhibitor 2 (TFPI2)	-0.01954	-0.04471	0.005625	0.0062
Toll-like receptor 3 (TLR3)	0.04275	0.01960	0.06589	<.0001
Transforming growth factor alpha (TGF alpha)	0.07339	0.000477	0.1463	0.0075
Transforming growth factor beta receptor type 2 (TGFR2)	0.02914	0.008093	0.05019	0.0226
Tumour necrosis factor (TNF) receptor superfamily member 9 (TNFRSF9)	0.03568	0.008146	0.06322	0.0001
Vascular endothelial growth factor A (VEGF-A)	0.02176	-0.00999	0.05352	0.0047
WNT1-inducible signalling pathway protein 1 (WISP-1)	0.10061	0.07561	0.1256	<.0001
s100-A11	0.03298	-0.03280	0.09876	0.0006

The estimate for month denotes the average slope of the linear trend over time, i.e. how much the average protein value changes over one month (a positive value indicates an increase over time). A false discovery rate of 10% was applied. All p-values presented in the table remained significant after FDR correction.

Figure 1
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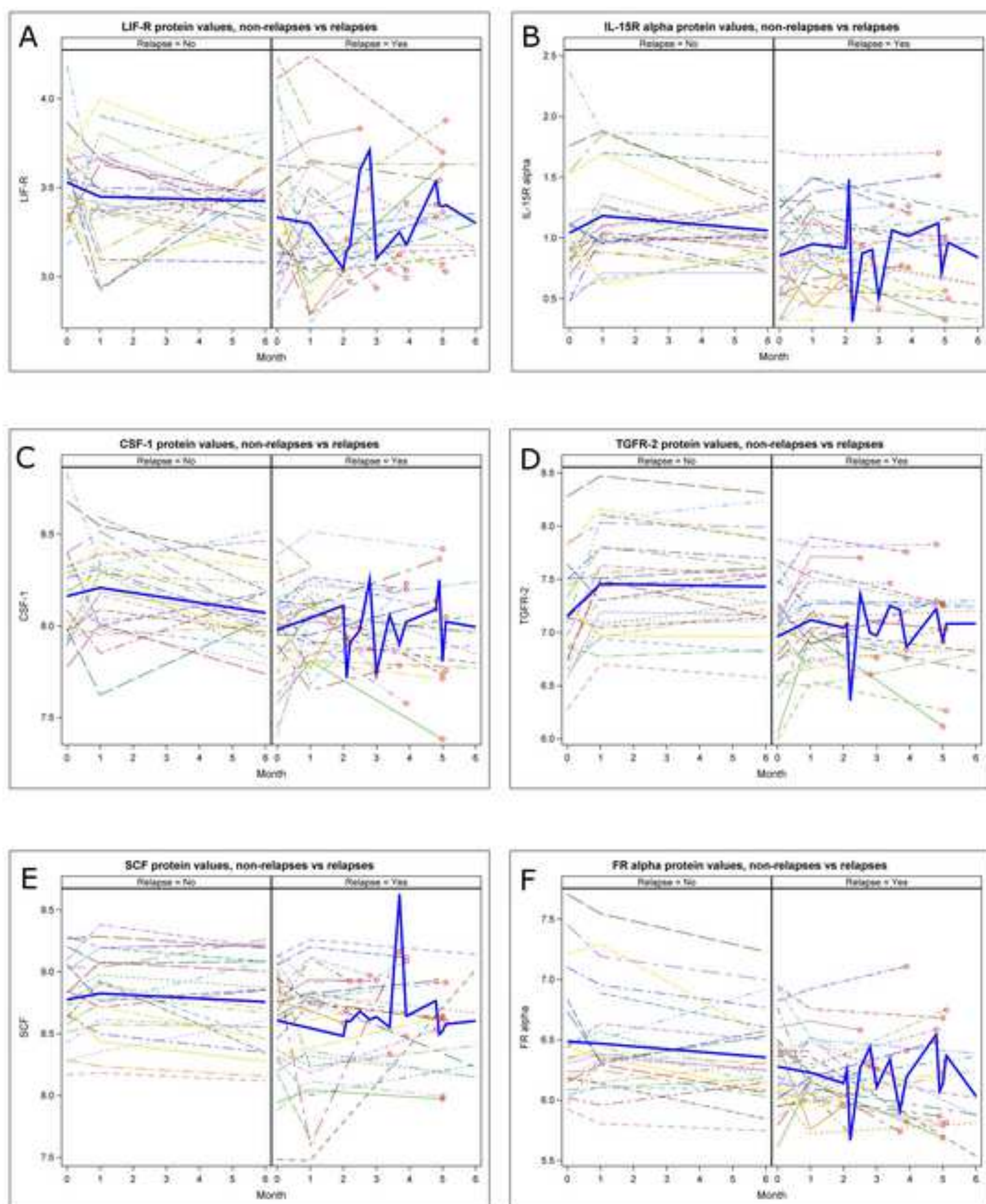

Figure 2

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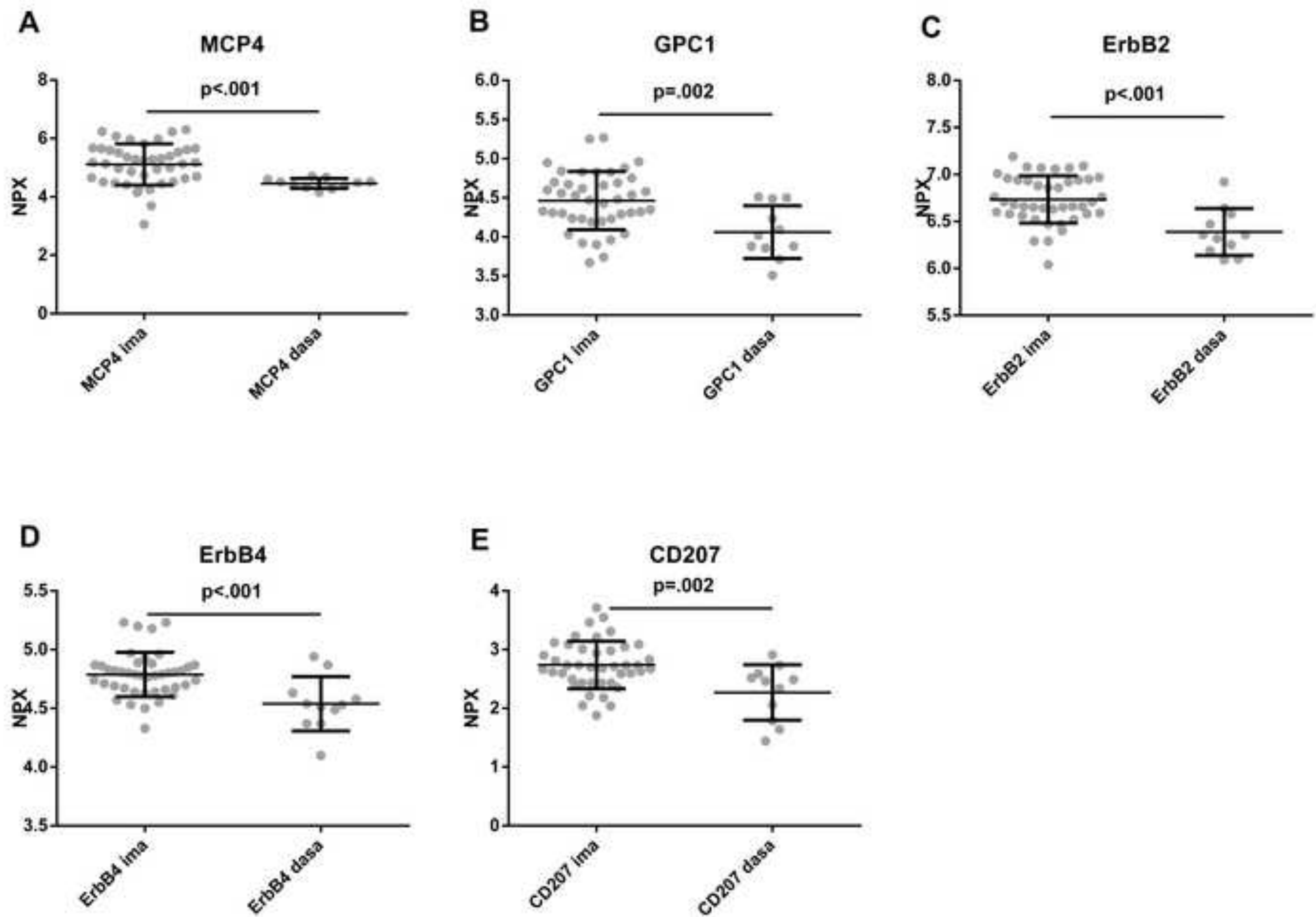

Figure Legend

Figure 1. ***Proteins significantly different between non-relapse and relapse cases.*** The mean protein levels were estimated by a linear trend over time, and these linear trends were compared between the two groups in a linear mixed effects model. The mean protein levels are displayed as a solid blue line while individual patient values are shown in dotted coloured lines. Red circles indicate where relapses occurred. A false discovery rate of 10% was applied. A) Leukaemia inhibitory factor receptor (LIF-R), B) IL-15 receptor alpha C) Colony stimulating factor 1 (CSF-1), D) Transforming growth factor beta receptor type 2 (TGFR-2), E) Stem cell factor (SCF) and F) Folate receptor alpha (FR alpha).

Figure 2. ***Proteins significantly different between imatinib and dasatinib treated patients.*** Thin vertical lines represent mean values and error bars represent the standard deviation. A) Monocyte chemotactic protein 4 (MCP4), B) Glypican-1 (GPC-1), C) ErbB2, D) ErbB4, E) CD207.

Acknowledgements

The authors would like to thank participating EURO-SKI centres and investigators. Funding for this sub-study was supplied by the Nordic Cancer Union and Lions Uppsala.

***Statement of conflict of interest**

Declaration of interests:

Stina Söderlund: none

Inger Persson: none

Mette Ilander: none

Joëlle Guilhot: none

Henrik Hjorth-Hansen: none

Perttu Koskenvesa: reports receiving lecture fees from Incyte

Johan Richter: none

Susanne Saussele: reports honoraria from Novartis, BMS, Incyte, Pfizer and research support from BMS, Novartis and Incyte

Satu Mustjoki: reports research funding from BMS, Novartis and Pfizer and honoraria from BMS

Ulla Olsson-Strömberg: none

Appendix A. Supplementary data

Supplemental Table A1. All analytes, Inflammation I.

Adenosine Deaminase (ADA)	FGF-23	Monocyte chemotactic protein (MCP)-1
Artemin (ARTN)	Fms-like tyrosine kinase 3 ligand (Flt3L)	MCP-2
Axin-1	Glial cell line-derived neurotropic factor (GDNF)	MCP-3
Brain-derived neurotropic factor (BDNF)	Hepatocyte growth factor (HGF)	MCP-4
Beta-nerve growth factor (Beta-NGF)	Interferon (IFN)-gamma	Macrophage inflammatory protein (MIP)-1 alpha
Caspase (CASP)-8	Interleukin (IL)-1-alpha	Matrix metalloproteinase (MMP)-1
C-C motif chemokine (CCL)4	IL-2	MMP-10
CCL11	IL-4	Neurturin (NRTN)
CCL19	IL-5	Neurotrophin (NT)-3
CCL20	IL-6	Osteoprotegerin (OPG)
CCL23	IL-7	Oncostatin-M (OSM)
CCL25	IL-8	Programmed death ligand 1 (PD-L1)
CCL28	IL-10	Stem cell factor (SCF)
Cluster of differentiation (CD)5	IL-12B	SIR2-like protein 2 (SIRT2)
CD6	IL-13	Signaling lymphocytic activation molecule 1 (SLAMF1)
CD40	IL-17A	STAM-binding protein (STAMPB)
CD244	IL-17C	Sulfotransferanse (ST)1A1
CUB domain-containing protein 1 (CDCP1)	IL-18	S100-A12 (EN-RAGE)
Colony stimulating factor (CSF)-1	IL-20	Transforming growth factor (TGF)-alpha
Cystatin D (CST5)	IL-24	Tumour necrosis factor (TNF)
C-X-C motif chemokine(CXCL)1	IL-33	TNFB
CXCL5	IL-2 receptor (R)B	TNF-receptor superfamily member 9 (TNFRSF9)
CXCL6	IL-10RA	TNF-ligand superfamily member 14 (TNFSF14)
CXCL9	IL-10RB	TNF-related apoptosis-inducing ligand (TRAIL)
CXCL10	IL-15RA	TNF-related activation-induced cytokine (TRANCE)
CXCL11	IL-18R1	Thymic stromal lymphopoietin (TSLP)
Fractalkine (CX3CL1)	IL-20RA	TNF-ligand superfamily member 12 (TWEAK)
Delta- and notch-like epidermal growth factor-	IL-22-RA1	Urokinase-type plasminogen activator (uPA)

related receptor (DNER)		
Fibroblast growth factor (FGF)-5	Latency associated peptide transforming growth factor (LAP TGF)-beta-1	Vascular endothelial growth factor (VEGF)-A
FGF-19	Leukemia inhibitory factor (LIF)	Eucaryotic translation initiation factor 4E-binding protein (4E-BP1)
FGF-21	LIF-R	

Supplemental Table A2. All analytes, Oncology II.

Tyrosine-protein kinase 1 (ABL1)	Folate receptor (FR)-alpha	Proto-oncogene tyrosine-protein kinase receptor Ret (RET)
A disintegrin and metalloproteinase domain-containing protein (ADAM) 8	FR-gamma	R-spondin 3 (RSPO3)
A disintegring and metalloproteinase with thrombospondin motifs (ADAM-TS) 15	Furin (FUR)	Secretory carrier-associated membrane protein 3 (SCAMP3)
Annexin A1 (ANXA1)	Galectin (Gal)-1	SCF
Amphiregulin (AR)	Glypican 1 (GPC1)	Seizure 6-like protein (SEZ6L)
Carbonic anhydrase (CA)9	Glycoprotein NMB (GPNMB)	SPARC
CD27	Granzyme (GZM)B	Syndecan 1 (SYND1)
CD48	GZMH	S100-A4
CD70	Kallikrein (hK)8	S100-A11
CD160	hK11	T cell leukemia/lymphoma protein (TCL)1A
CD207	hK14	Tissue factor pathway inhibitor (TFPI)-2
Cyclin-dependent kinase inhibitor 1 (CDKN1A)	ICOS ligand (ICOSLG)	TGF-alpha
Carcinoembryotic antigen-related cell adhesion molecule (CEACAM)1	IFN-gamma-R1	TGFR-2
CEACAM5	Insuline-like growth factor 1 receptor (IGF1R)	Toll-like receptor (TLR)3
Carboxypeptidase E (CPE)	IL-6	TNFSF13
Cornulin (CRNN)	Integrin alpha-V (ITGAV)	TNFRSF4
Cathepsin L2 (CTSV)	Integrin beta-5 (ITGB5)	TNFRSF6B
CXCL13	Kallikrein (KLK)13	TNFRSF19
CXL17	Tyrosine-protein kinase Lyn (LYN)	TRAIL
Cysteine-rich angiogenic inducer (CYR)61	Ly6/PLAUR domain-containing protein 3 (LYPD3)	Taxilin alpha (TXLNA)
Delta-like protein 1 (DLL1)	Lymphocyte antigen 9 (LY9)	VEGF-A

Epidermal growth factor (EGF)	Mothers against decapentapleic (MAD) homolog 5	VEGFR-2
Ephrin-type A receptor 2 (EPHA2)	Methionine aminopeptidase (MetAP) 2	VEGFR-3
Endothelial cell-specific molecule (ESM-)1	Melanoma-derived growth regulatory protein (MIA)	Vimentin (VIM)
Receptor protein-tyrosine kinase ErbB (ERBB)2	MHC class I polypeptide-related sequence A/B (MIC-A/B)	WAP four-disulfide core domain protein (WFDC)2
ERBB3	Midkine (MK)	Wnt inhibitory facot (WIF)-1
ERBB4	Mesothelin (MSLN)	Wnt1-inducible-signaling pathway protein (WISP)-1
Fas-associated death domain protein (FADD)	Mucin (MUC)-16	Xaa-pro aminopeptidase 2 (XPNPEP2)
Fas antigen-ligand (FASL)	Podocalyxin (PODXL)	5’ nucleotidase (5'-NT)
Fc-receptor-like B (FCRLB)	Pancreatic pro-hormone (PPY)	
Fibroblast growth factor-binding protein 1 (FGF-BP1)	Nectin-4 (PVRL4)	