Research Article

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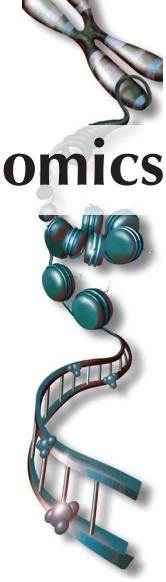
Epigenomics



Polycomb genes are associated with response to imatinib in chronic myeloid leukemia

Aim: Imatinib is a tyrosine kinase inhibitor that has revolutionized the treatment of chronic myeloid leukemia (CML). Despite its efficacy, about a third of patients discontinue the treatment due to therapy failure or intolerance. The rational identification of patients less likely to respond to imatinib would be of paramount clinical relevance. We have shown that transmembrane transporter *hOCT1* genotyping predicts imatinib activity. In parallel, Polycomb group genes (PcGs) are epigenetic repressors implicated in CML progression and in therapy resistance. **Patients & methods:** We measured the expression of eight PcGs in paired pre- and post-imatinib bone marrow samples from 30 CML patients. **Results:** BMI1, PHC3, CBX6 and CBX7 expression was significantly increased during imatinib treatment. Post-treatment levels of CBX6 and CBX7 predicted 3-month response rate. Measurement of post-treatment BMI1 levels improved the predictive power of hOCT1 genotyping. **Conclusion:** These results suggest that the expression levels of PcGs might be useful for a more accurate risk stratification of CML patients.

Keywords: BMI1 • CBX6 • CBX7 • imatinib • pharmacoepigenetics • Polycomb

Imatinib is the first tyrosine kinase inhibitor (TKI) successfully employed in the clinical setting [1]. This small molecule inhibits the constitutive kinase activity of the BCR–ABL protein, thereby inducing apoptosis in Philadelphia chromosome-positive chronic myeloid leukemia (CML) cells. Clinical trials demonstrated that imatinib treatment was superior to conventional cytotoxic drugs as first-line therapy for CML [2]. A recent update reported an 89% overall survival rate in CML patients treated with imatinib for 5 years [3]. Despite these superlative results, objective response to imatinib is quite variable, and about 30% of patients must discontinue the treatment due to adverse events or unsatisfactory clinical outcome [1]. It has been shown that CML patients achieving a complete hematological response (CHR) within 3 months from the initiation of therapy are more likely to achieve complete cytogenetic response (CCyR) and better long-term clinical outcome [4]. The advent of second-generation TKIs provided clinicians with valuable therapeutic alternatives, thus having a positive impact on prognosis [5].

The identification of factors predicting imatinib response could support rational therapeutic decisions and foster therapeutic tailoring. For this reason, several groups have investigated the role of genetic variants in predicting CML patients' response to imatinib [6]. We have recently associated transmembrane transporter hOCT1 germline variants with imatinib pharmacokinetics and therapeutic activity [7]. Despite these promising results, no genetic factor has been conclusively linked to the clinical efficacy of imatinib.

Emerging evidence indicates that epigenetic factors could be at least as important as genetic ones in determining therapeutic responses [8]. In particular, Polycomb group genes (PcGs) are epigenetic effectors involved in the progression of both solid and hematological malignancies [9]. These proteins are

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organized in two multimeric polycomb repressive complexes (PRC1 and PRC2), which mediate gene silencing and control cell specification in physiological conditions. According to the most widely accepted molecular model, PRC2 catalyzes the trimethylation of histone H3-Lys27, and subsequently PRC1 binds to this epigenetic mark and catalyzes histone H2a monoubiquitination. These developmental regulatory mechanisms are deregulated in cancer cells, leading to the coordinated silencing of several tumor-suppressor genes. Notably, PRCs silence target *loci* in a context-specific manner that arises from their extensive combinatorial complexity. For example, a BMI1^{+}/PCGF2^{-} PRC1 drives breast cancer tumorigenesis and metastatic progression, while a BMI1^{-}/PCGF2^{+} complex exerts tumor-suppressive functions [10]. Selected PRC members have been implicated in chemotherapy resistance [11] and have been proposed as innovative therapeutic targets in hematological and solid malignancies [9].

In CML, the PRC1 member BMI1 has been identified as a prognostic marker [12] and as a downstream target of the BCR–ABL tyrosine kinase [13]. BMI1 expression is increasingly higher in peripheral blood samples from unaffected, chronic phase CML and blastic phase CML patients [13]. In addition, BMI1 expression is sufficient to reprogram B-lymphoid progenitors, thereby generating B-cell acute lymphoid leukemia-initiating cells [14]. Despite this evidence, no study has investigated the role of BMI1 or any other PRC1 member in CML patients' response to imatinib treatment.

Here, we describe pre- and post-treatment expression levels of eight PRC1 members, in 30 CML patients treated with imatinib in the context of the TIKlet study [15].

The primary aims of the present study were to demonstrate that PcG expression can be measured in bone marrow samples obtained during routine clinical procedures and PcG expression levels in bone marrow samples are associated with imatinib clinical activity. Our analysis identified the coordinated regulation of four PcGs upon TKI treatment, and suggested for the first time that post-therapeutic levels of PcGs might be used as predictors of therapeutic efficacy. We believe that our preliminary analysis paves the way for more extensive studies examining the utilization of PcGs as predictors of imatinib response in CML.

Patients & methods

Patients

Thirty consecutive CML patients observed at the Hematology Division of the Pisa University, Italy, between January 2011 and June 2013 were enrolled in the TIKlet trial [15] and then assessed for PcG expression prior to, and 3 months following, imatinib treatment. Twenty milliliter of EDTA-anticoagulated bone marrow was used for total RNA extraction after buffy coat preparation. Bone marrow was employed for cytogenetic analysis, as stated by the international guidelines [16], and for PcG expression analysis. Concomitantly, 20 ml of peripheral blood was employed for RNA extraction, banking and for analysis of the BCR–ABL1/ABL1 International Scale (IS) ratio by the GeneXpert Technology (Cepheid, Maurent-Scopont, France) after 3 and 6 months of therapy, as indicated by European guidelines [17]. For 23 patients, we obtained paired pre- and post-imatinib bone marrow samples harvested 3 months after start of imatinib treatment.

The study was approved by the Ethics Committee of Pisa University Hospital and by the University of British Columbia/British Columbia Cancer Agency REB. All patients gave informed consent to the study. Clinical outcome data were recorded throughout the whole duration of the follow-up. The clinical characteristics of the patients are reported in Supplementary Table 1 (see online at www.futuremedicine.com/doi/full/10.2217/epi.15.35).

Quantitative PCR

Gene-expression levels of PRC1 components were assayed using reverse transcribed cDNA and SYBR green primers following a previously described procedure for amplification and relative gene-expression quantification [16]. GAPDH was used as a reference gene. Expression values were calculated using the 2-ΔΔCt method and normalizing the measurement to the highest Ct in the cohort. Primers sequences are reported in Supplementary Table 2.

Statistical analyses

All statistical analyses were performed using SPSS 21.0 (SPSS Inc, Chicago, IL, USA) or GraphPad Prism 6.0 software.

At the third month of treatment, all cases were scored for hematological, cytogenetic and molecular response (MR). CHR was defined as white blood cells (WBC) $<10 \times 10^9$/l, platelets (PLT) $<450 \times 10^9$/l and absence of immature cells and palpable splenomegaly. Major cytogenetic response (MCyR) corresponded to <35% Philadelphia-positive metaphases, while CCyR identified cases without any Philadelphia-positive metaphases. The MR was scored according to the logarithmic reduction of the BCR–ABL1/ABL1 ratio in respect of the 100% value of the diagnosis (MR1 = reduction of 1 log; MR3 = reduction of 3 logs).

In order to ensure our measurements took into account differences in cell populations pre- and post-treatment, both basal and post-treatment PcG expression levels values have been divided by the number

of Philadelphia-positive metaphases detected by conventional cytogenetics.

Epigenetic data were analyzed to find any possible correlation with the achievement of CHR at 3 months, early molecular response (EMR; *BCR–ABL1/ABL1* ratio <10% IS at 3 months), CCyR at 6 months and MR3 (*BCR–ABL1/ABL1* ratio <0.1% IS) at 12 months. The same analyses were performed in combination with *hOCT1* genotyping, because our previous work demonstrated that the *hOCT1* c.480C>G polymorphism significantly influences both drug disposition and event-free survival (EFS) [7]. Therefore, the inclusion of these variables within the analysis could improve the identification of possible markers of clinical outcome.

The EFS was defined as time elapsed from treatment beginning and the occurrence of one of the following events: therapy discontinuation for any reason, loss of CCyR or MR3, or unacceptable toxicity. Survival curves were calculated using the Kaplan–Meier method, and statistical comparisons between curves were made using the log-rank test. The Wilcoxon matched-pairs test, chi-squared test, Fisher's exact test and Kruskal–Wallis test were used to compare variables, when appropriate. Time to CCyR and to MR3 values were not normally distributed, as demonstrated by the Shapiro–Wilk test, hence data were rank-transformed to perform a nonparametric test (Spearman's) correlation analysis.

Diagnostic accuracy of tests was evaluated by using the ROC analysis and area under curve (AUC) analyzed by a nonparametric test. All statistical comparisons were two-sided.

Results

Pre- & post-treatment expression levels of PcGs

In order to investigate whether expression of PRC1 components is affected by imatinib, we quantified pre- and post-treatment gene-expression levels on bone marrow samples. All mRNAs were reproducibly detectable (average Ct values <28) indicating that target genes were robustly expressed. Possible correlations between PcG expression values and Philadelphia metaphase percentages were explored using the Spearman's rho test; results showed that the observed variation in PcG expression was totally independent of the Philadelphia-positive residual component ($p = 0.305$). Our analysis revealed that 4/8 PcGs were significantly upregulated after 3 months of treatment (Figure 1). In particular, BMI1 was the most highly upregulated gene (fold change = 4.9; $p < 0.0001$; the Wilcoxon matched-pairs test). The median value of BMI1 expression was 21.16 (range: 1.0–55.8), while the mean value was 21.20 ± 13.98. These measurements are in line with previously reported data [13]. PHC3 and CBX6 showed greater than twofold upregulation ($p < 0.01$). Notably, no PRC1 member showed significant downregulation upon treatment and we found no significant correlation between pretreatment levels and fold-change value.

In order to investigate gene-expression correlations between each PRC1 member, we calculated Spearman's rank coefficients (Supplementary Table 3). Analysis of pretreatment levels showed relevant correlation ($R > 0.5$; $p < 0.01$) between CBX6–CBX7 and PCGF2–CBX8. Notably, after imatinib treatment, the number of significant correlations increased to 14. No significant negative correlation ($R < -0.5$) was observed either before or after treatment.

With the exception of CBX6–CBX7, the four genes that were significantly upregulated by imatinib (Figure 1) were only loosely correlated before treatment. Strikingly, the correlation of BMI1 with PHC3, CBX6 and CBX7 showed a significant increase in post-treatment samples. In keeping with this observation, the correlation between pairs of PHC3, CBX6 and CBX7 was significantly amplified upon treatment ($p < 0.01$; Spearman's correlation). When we analyzed only cases with a concomitant increase of BMI1 and CBX6 or CBX7, we observed that in about 60% of them the upregulation of BMI1 after imatinib treatment was higher than that measured for CBX6 and CBX7 ($p < 0.01$; Spearman's rho test).

Taken together, those results indicate that imatinib is able to trigger the coordinated activation of PRC1 members, with a stronger effect on BMI1, compared with the other concomitantly upregulated PcGs.

Predictive value of PcGs

In order to test the predictive value of PcGs, we investigated the correlation between their expression levels, clinical features at diagnosis and clinical outcome. The four genes, showing significant upregulation following imatinib treatment (Figure 1), were selected for this analysis. In our patient series, Sokal risk score was low in 35%, intermediate in 38% and high in 27% of cases (for further details, see Supplementary Table 1); no patient had an additional Philadelphia chromosome. The median time to CHR was 1.7 months. Within 3 months of treatment start, 76% of patients achieved CHR. At the same timepoint, 50% of patients showed a BCR–ABL1/ABL1 ratio lower than 10% IS, thereby achieving an EMR. The median time to CCyR was 8.2 months. Within 12 months of treatment start, 80% of cases achieved a CCyR. PcG expression levels (before or after treatment) were not significantly associated with sex, age or risk score (Hasford, Sokal, EUTOS). Analogously, we found no significant correlation between PcG expression values and EMR.

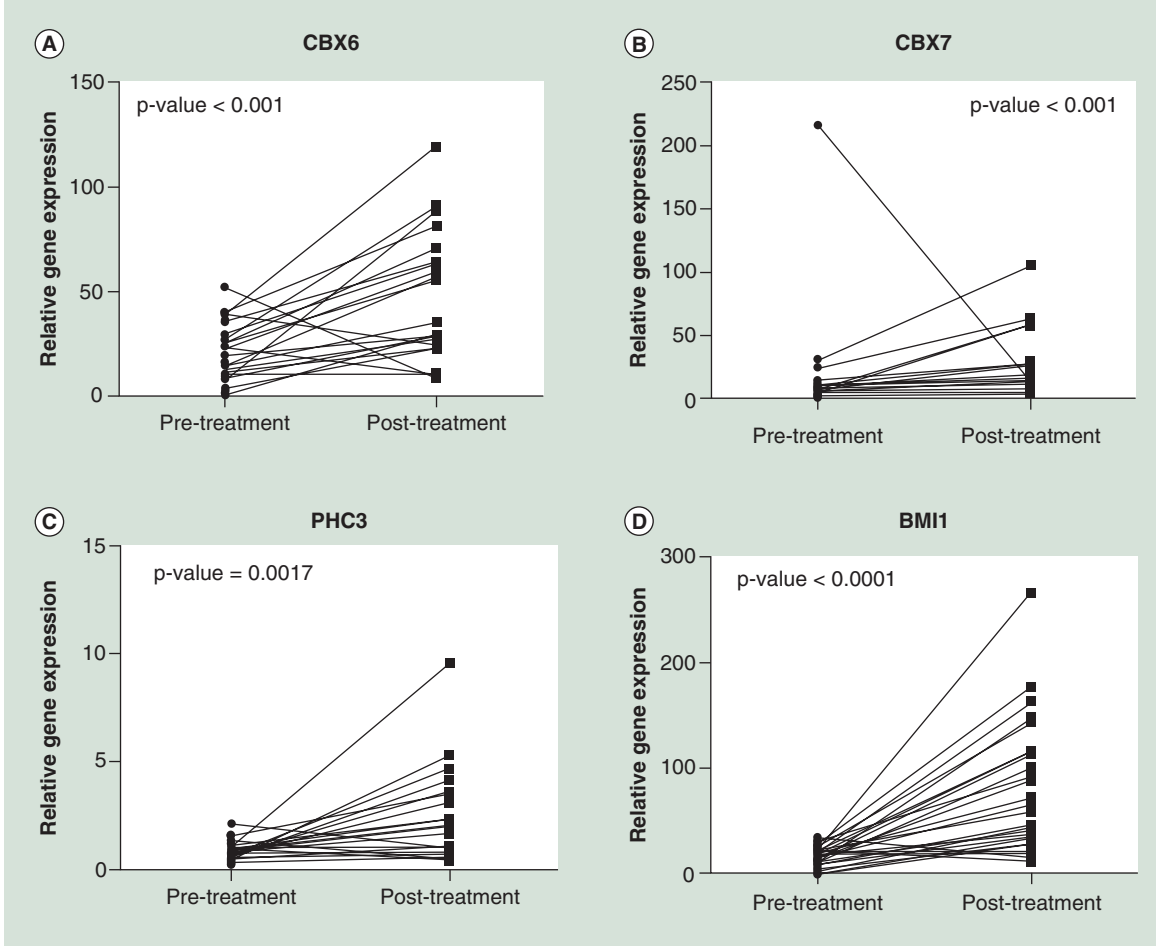

Figure 1. Modulation of Polycomb gene expression in imatinib-treated patients. Relative gene expression in **(A)** CBX6; **(B)** CBX7; **(C)** PHC3 and **(D)** BMI1. p-values refer to the Wilcoxon matched-pairs test. Mean fold changes with respect to pretreatment values: CBX6 = 2.1; CBX7 = 1.4; PHC3: 2.6; BMI1 = 4.9.

We found no correlation between pretreatment PcG mRNA levels and response to imatinib. As shown in Figure 2A & B, post-treatment levels of CBX6 and CBX7 were able to discriminate patients achieving CHR at or after 3 months of treatment (ROC AUC = 0.811 and 0.833; p = 0.029 and 0.020, respectively). Optimal cutoff values based on likelihood ratios and Youden index [18] revealed that a CBX6 value higher than 29.26 (arbitrary units, see 'Quantitative PCR' section in the 'Patients & methods' section) identified responders with 73.3% sensitivity and 100% specificity. Similarly, we found that a CBX7 value higher than 14.74 allowed us to identify responders with 73.3% sensitivity and 100% specificity. We therefore investigated if CBX6 and CBX7 modulation upon imatinib treatment was different in responders versus nonresponders (groups defined based on the achievement of CHR at or after 3 months of treatment). We found no significant differences in CBX6 modulation (data not shown). On the contrary, CBX7 modulation was sharply different in the two groups: responders elicited a significant post-treatment upregulation, while nonresponders showed a trend toward a reduced expression (Figure 2C).

When we computed PcG values after 3 months of treatment, we found that patients with higher upregulation of BMI1 during imatinib treatment required a longer time to achieve MR3 (22 vs 11 months; p = 0.06; t-test). Analogously, patients with BMI1 plus CBX6 upregulation required a significantly longer time to achieve MR3 (23.66 ± 9.77 vs 14.04 ± 7.17 months; p = 0.04; t-test). These same cases showed a lower probability of remaining free from events (disease progression, loss of CCyR and/or MR3) at 36 months (24% for patients with upregulation of BMI1 and CBX6 vs 80% for those with no or low BMI1 increase or stable levels of CBX6; p = 0.09).

Overall, these data demonstrate that upregulation of CBX6 and CBX7 predicts an optimal hematological response and that an increase of BMI1 is associated with longer time to achieve the satisfying molecular response.

To further dissect the role of BMI1 in CML progression, we analyzed three patients who switched to

second-generation TKIs upon unsatisfactory response to imatinib (Figure 2D). The first patient achieved MR3 (BCR–ABL1 transcript decreased 16-fold) after 3 months of dasatinib treatment; in this case, we measured a tenfold reduction in BMI1 levels. The second patient obtained a tenfold reduction in BCR–ABL levels after 3 months of nilotinib treatment; in this patient, post-therapy BMI1 levels halved. Finally, the third patient did not respond to second-generation TKI treatment. In this case, BMI1 downregulation was less than 1.5-fold.

When we analyzed the c.480 C>G hOCT1 polymorphism, we found that the wild-type CC genotype had a favorable impact on 36 months-EFS (87.5 vs 30% of individuals carrying the G allele; p = 0.04), hence confirming our previous results in this subset of patients [7]. In our series, 50% of cases showed a wild-type genotype. In this subset, the median EFS for cases with stable levels of BMI1 after short-term treatment was 50.40 ± 0.70 months versus 30.60 ± 8.60 months for patients with a higher increase of BMI1 and/or polymorphic genotype (p = 0.001; t-test). Unfortunately, the small sample size did not allowed us to perform further comparisons. Taken together, these results indicate that BMI1 expression profiling increases the prognostic power of hOCT1 genotyping.

Clinical significance of PcGs in myeloid leukemias

Albeit PcGs have been widely investigated in many neoplasms, evidence of their role in myeloid neopla-

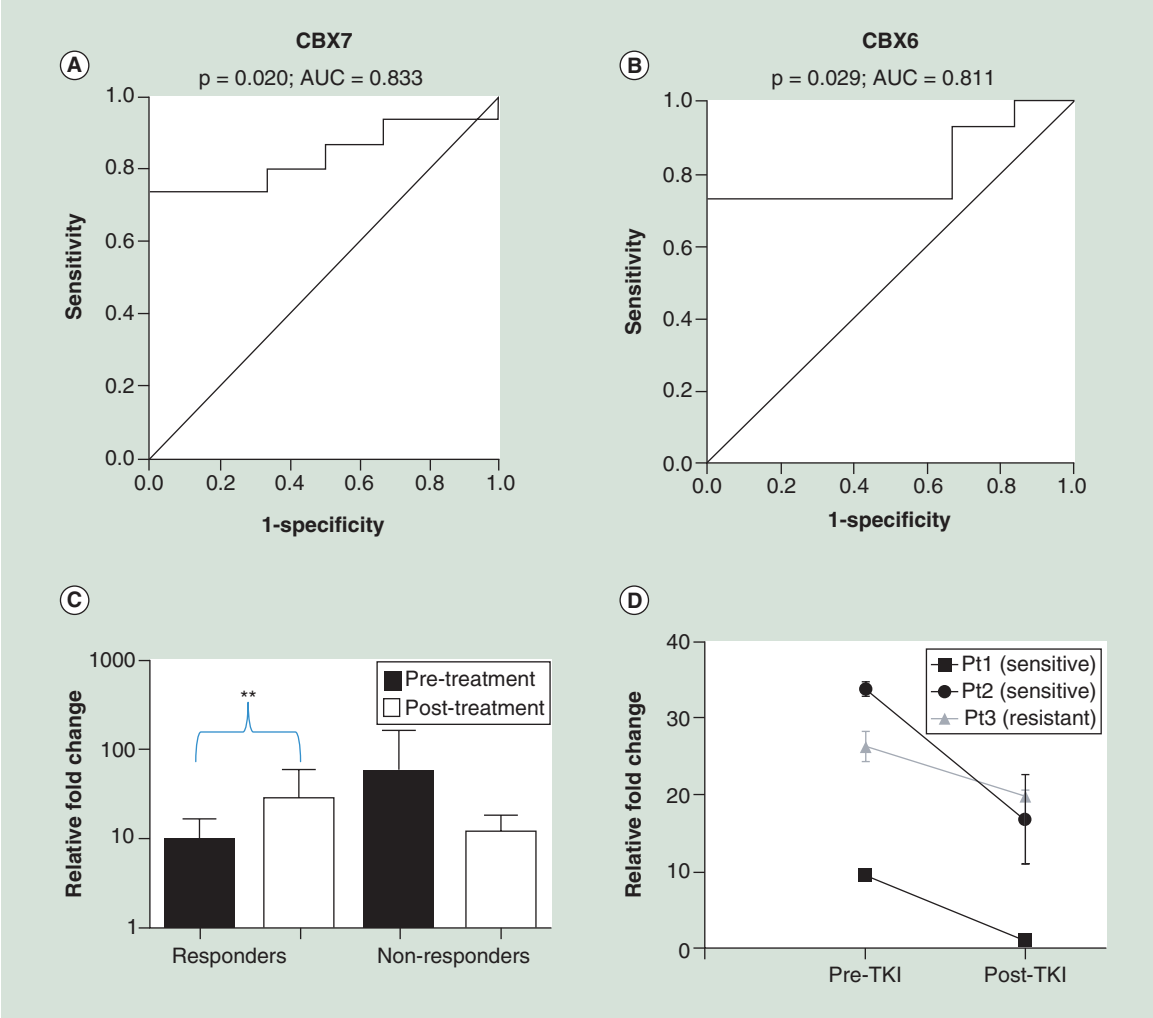

Figure 2. Clinical relevance of *Polycomb* genes in chronic myeloid leukemia patients. **(A & B)** Predictive role of post-therapy levels of CBX6 and CBX7 genes according to ROC curves. Cutoff values of 29.26 and 14.74 for CBX6 and CBX7, respectively, correspond to 73.3% sensitivity and 100% specificity for both genes. **(C)** Pre- and post-treatment levels of CBX7 in responders versus nonresponders. **p < 0.01; t-test. **(D)** Pre- and post-treatment BMI1 levels in three chronic myeloid leukemia patients treated with second-generation TKIs.
AUC: Area under the curve; TKI: Tyrosine kinase inhibitor.

sias remains scant. As mentioned in the introduction, PRC1 can play an oncogenic or oncosuppressive role, depending on its composition and cellular context. In order to elucidate the meaning of our findings in the specific context of myeloid malignancies, we analyzed the expression of CBX6, CBX7, BMI1 and PHC3 in cancer cells compared with their normal counterparts using the Oncomine database [19]. Our results indicate that BMI1 is highly upregulated, whereas CBX6 and CBX7 are downregulated, in AML samples compared with normal blood cells (Figure 3A–C). These results indicate that CBX6 and CBX7 are likely to play an oncosuppressive role in myeloid malignancies, and confirm the putative oncogenic role of BMI1 in the same context. Our results also suggest that post-therapy CBX6 and CBX7 upregulation is a partial, but not complete return to normal expression levels (average fold change [FC] in normal vs leukemic samples was 3.7–3.9; post- vs pre-therapy upregulation was 1.4–2.1-fold).

The Oncomine database also included a list of genes specifically downregulated in clinical CML samples exposed to imatinib. The main pathways inactivated by imatinib involved mitochondrial function, protein synthesis and DNA replication (Supplementary Table 4). These results confirm recent preclinical findings on imatinib mechanisms of action [20]. In addition, we identified a significant overlap between PcG targets and genes downregulated by imatinib ($p < 1E^{-5}$; odds ratio: >2). The PcG target list was obtained from a genome-wide mapping study conducted on human embryonic fibroblasts [21]. These results are in line with previous evidence showing that PcGs are activated by antineoplastic therapies [9,22]. Notably, better short-term response to imatinib in CML patients was predicted by PcG target gene silencing (Figure 3D). Our results also showed that imatinib-suppressed PcG targets mediate key cellular functions, including DNA replication and energy production (Supplementary Table 5). Therefore, it is conceivable that imatinib effects are at least in part mediated by PcG activation, resulting in selected PcG target silencing.

Discussion

In this report, we show for the first time that selected PRC1 genes are coordinately upregulated in CML patients treated with the TKI imatinib. More importantly, we find significant correlations between post-treatment PcG levels and imatinib activity. In particular, higher post-treatment CBX6 and CBX7 levels are associated with faster hematological response rates. CBX6 and CBX7 bind to the PRC1 core complex, thereby directing its gene silencing activity to specific targets [23]. Both CBX6 and CBX7 have been suggested to play an oncosuppressive role in solid tumors [24] and, as shown in the Oncomine database, in myeloid leukemias (Figure 3). Notably, CBX7 oncosuppressive activity is predominant in hematologic neoplasms [25]. Taken together, this evidence suggests that CBX6 and CBX7 could play a tumor suppressive role in CML also, and that their concurrent activation upon imatinib treatment might lead to the silencing of prosurvival genes, thereby inducing neoplastic cell death and better short-term responses to imatinib.

Our analysis also indicates that the post-treatment activation of other PRC1 members is correlated with worse long-term clinical outcome. Notably, higher BMI1 post-treatment levels are associated with worse prognosis. The negative impact of increased BMI1 expression is reinforced by the observation that its upregulation could overcome the positive value of the CBX6 increase in patients that showed a BMI1 upregulation higher than that of CBX6.

Moreover, the negative predictive role of BMI1 is retained also in cases with wild-type hOCT1, which is essential for imatinib intake into the neoplastic cell. These results are in agreement with the previously described oncogenic role of BMI1 in CML [26], and with the evidence that BMI1 is able to silence proapoptotic and oncosuppressive genes, thereby mediating therapy resistance in several neoplasms [9]. It is worth noting that BMI1 expression is significantly upregulated in CML patients' $CD34^+$ cells, compared with healthy controls [13]. This finding is particularly evident during a blastic crisis [27]. Most notably, *in vitro* results demonstrated that BCR–ABL upregulation increases BMI1 expression, thus suggesting that BMI1 is positively regulated by BCR–ABL [13], and that BMI1 cooperates with BCR–ABL during disease progression [28]. These data might provide a mechanistic explanation to our clinical findings: the greater the inhibitory effect of imatinib against BCR–ABL, the lower the upregulation of BMI1, the higher the therapeutic benefit for patients.

Furthermore, our results demonstrate that a model aimed at predicting treatment efficacy can combine pharmacodynamic and pharmacokinetic biomarkers (i.e., post-therapy BMI1 gene expression and hOCT1 genotyping), thereby achieving an enhanced predictive power.

As indicated in the introduction, PRC1 can play an oncogenic or oncosuppressive role, depending on its composition and on cellular context. It has been shown that $CBX7^+/BMI1^-$ and $CBX7^-/BMI1^+$ PRC1 complexes play opposite roles in controlling embry-

onic stem cell self-renewal and differentiation [23]. Our data indicate that both oncosuppressive and oncogenic PcGs are upregulated upon imatinib treatment, but that the expression levels of the first and the latter types of genes have opposite implications on imatinib activity. A unified model to explain this phenomenon is illustrated in Figure 4. We propose that the imatinib-dependent activation of CBX7 and other oncosuppressive PcGs plays a crucial role in triggering apoptosis in most CML cells, thereby resulting in better short-term treatment response. However, a small fraction of cells survive this pro-apoptotic response due to concurrent BMI1 upregulation that sustains self-renewing capabilities [28]. This subpopulation may eventually trigger-delayed neoplastic progression. As a consequence, higher BMI1 expression is associated with worse long-term clinical outcome. The observation of a further BMI1 modulation in cases exposed to second-generation TKIs supports the hypothesis that the expression of this gene is mainly associated with neoplastic progression and BCR–ABL1 activity.

The purpose of this study was to identify an easy-to-perform molecular test to predict response to imatinib. Ideally, the test should be performed on the same biological material used for the cytogenetic analysis, thereby avoiding any further discomfort for patients. For these reasons, we could not perform sample purifications and proteomic analyses. Notwithstanding those limitations, our results suggest that post-therapy BMI1 levels can improve the predictive power of hOCT1 genotyping. Larger prospective studies are needed to confirm our preliminary findings.

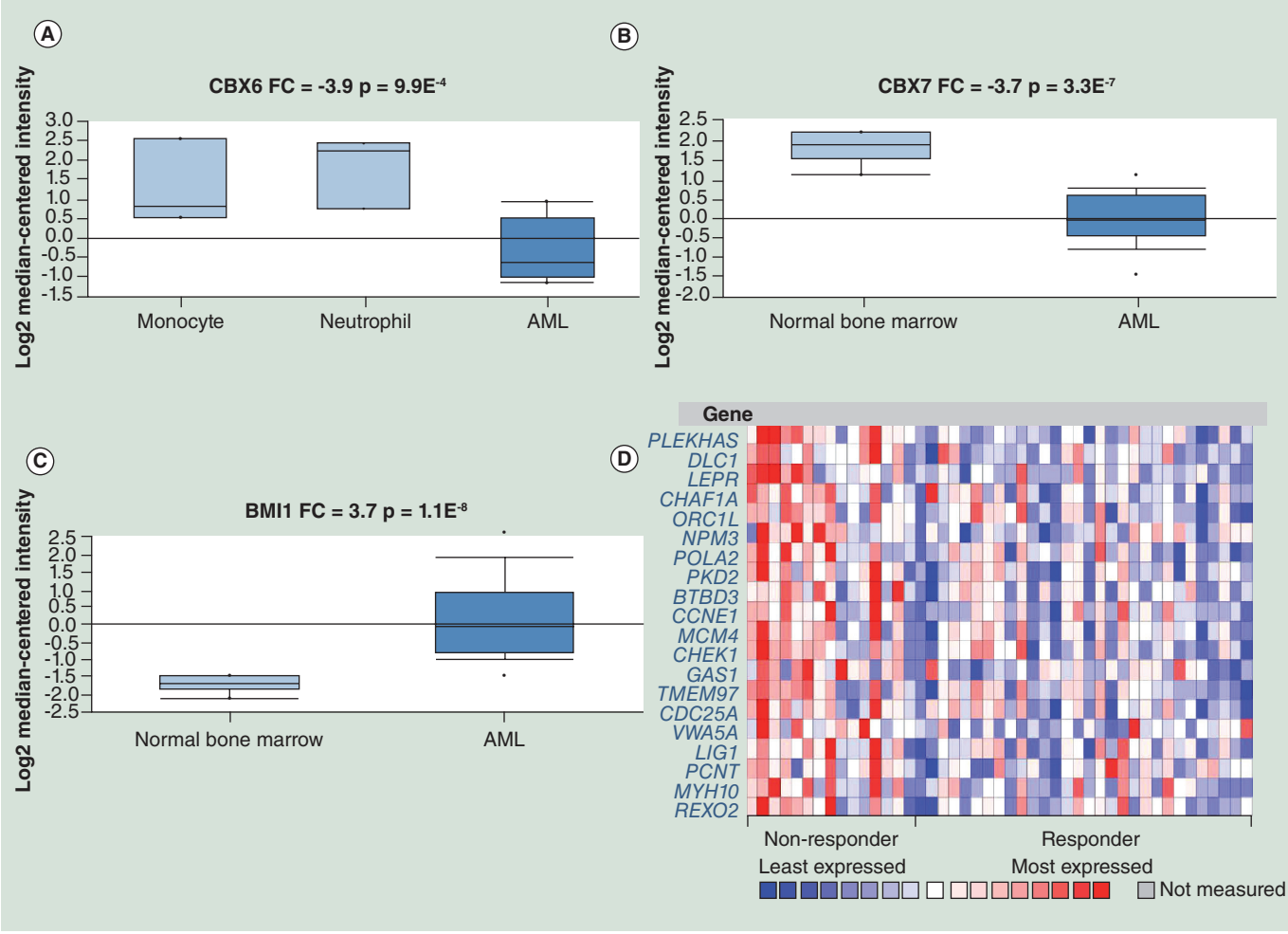

Figure 3. Expression pattern of Polycomb group genes in myeloid malignancies. Results were obtained using the following cutoff values: **(A–C)** $p < 0.01$; fold change >2; **(D)** odds ratio >2. **(D)** PcG targets are significantly downregulated in imatinib responders (30) vs nonresponders (15). Data are from 'Frank, Leukemia' study (blood or bone marrow samples obtained before the start of imatinib treatment; response was determined based on cytogenetic analysis performed on samples obtained within the first 12 months of imatinib treatment). We report noncontradictory results obtained from more than one cohort.
AML: Acute myeloid leukemia; FC: Fold change.

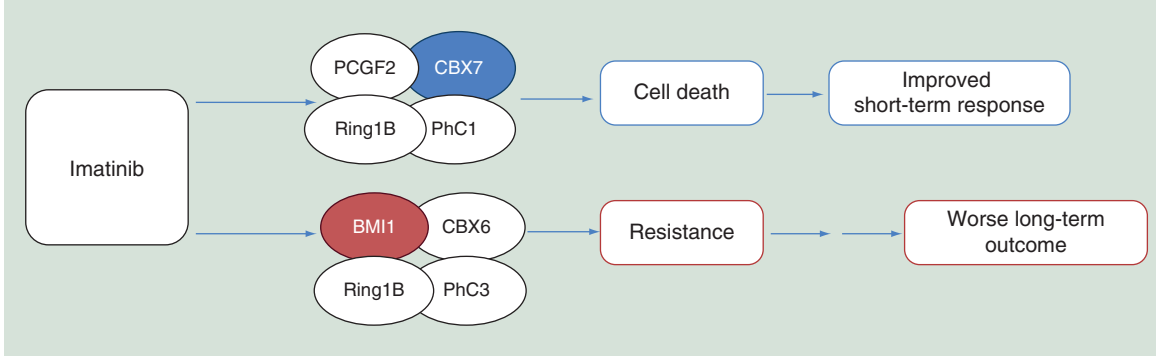

Figure 4. Proposed model of imatinib–Polycomb interaction. Imatinib is able to activate both oncosuppressive (blue) and oncogenic (red) PRC1 members. The first category triggers apoptosis on most chronic myeloid leukemia cells, thereby improving early response rate. The latter category promotes long-term survival of a fraction of chronic myeloid leukemia cells, thereby triggering delayed disease progression.
The two PRC1 multimers are adapted from [23].
For color images please see online www.futuremedicine.com/doi/full/10.2217/EPI.15.35

Conclusion

Results of the present study demonstrate that there is a significant correlation among PcG gene expression and clinical outcome in CML patients receiving imatinib. This correlation could be explained by an imatinib-dependent modulation of CBX6 and CBX7 genes, whose increased expression lead to an earlier achievement of MR3, whereas stable BMI1 mRNA levels anticipated a late benefit from imatinib. The latter phenomenon could be explained by a reciprocal interaction between BMI1 and BCR–ABL. Whether this approach could be extended to other BCR–ABL TKIs will be addressed in future studies.

Future perspective

Results from the present work could suggest that the adoption of a combined analysis of time-dependent events (i.e., variable expression of PRC1 genes in response to a BCR–ABL inhibitor) and fixed effects (patients' genotype with respect to transmembrane transporters) may help in the stratification of CML patients as early as possible, in order to adopt the best therapeutic strategy and possibly improve event-free-survival. In future studies, other TKIs directed against BCR–ABL should be evaluated for their role in modulating PcG gene expression, and this could pave the way to different epigenetic-based-tailored pharmacological strategies. The widespread use of high-throughput screening platforms, such as next-generation sequencing or digital droplet PCR, may accelerate further discoveries and application of biomarkers to real life.

Financial & competing interests disclosure

F Crea received one honorarium for expert opinion on histone methylation. AD Paolo received honoraria for expert opinions on CML. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Imatinib has been approved by the US FDA as first-line treatment for chronic myeloid leukemia (CML).
- Despite its efficacy, about 30% of patients experience a poor response or a frank resistance to imatinib.
- Currently, no molecular marker (other than the Philadelphia chromosome) has been associated with response to imatinib.
- We postulated that epigenetic factors could play a crucial role in imatinib response. We therefore collected RNA from 30 CML patients before and after treatment with imatinib.
- We selected Polycomb genes, since they have been implicated in CML progression and drug resistance and we studied their expression by quantitative PCR.
- Our results indicate that Polycomb group genes are coordinately upregulated upon imatinib treatment.
- BMI1 post-treatment levels were able to improve the predictive power of *hOCT1* genotyping, a pharmacogenetic biomarker that had been previously identified by our group.
- Overall, the variable BMI1, CBX6 and CBX7 gene expression after imatinib treatment and its association with clinical outcome may significantly influence the way how responders could be identified.
- The present strategy should be addressed to other BCR–ABL inhibitors that are used after imatinib failure, as well as nilotinib, dasatinib and the most recent ponatinib.

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