Identifying and Validating a Combined mRNA and MicroRNA Signature in Response to Imatinib Treatment in a Chronic Myeloid Leukemia Cell Line

Steven Bhutra[1], Divya Lenkala[2], Bonnie LaCroix[2], Meng Ye[3], R. Stephanie Huang[2,3]*

1. Pritzker School of Medicine, University of Chicago, Chicago, Illinois, United States of America, **2.** Department of Medicine, University of Chicago, Chicago, Illinois, United States of America, **3.** The Affiliated Hospital, School of Medicine, Ningbo University, Ningbo, Zhejiang, China

*rhuang@medicine.bsd.uchicago.edu

Abstract

Imatinib, a targeted tyrosine kinase inhibitor, is the gold standard for managing chronic myeloid leukemia (CML). Despite its wide application, imatinib resistance occurs in 20–30% of individuals with CML. Multiple potential biomarkers have been identified to predict imatinib response; however, the majority of them remain externally uncorroborated. In this study, we set out to systematically identify gene/ microRNA (miRNA) whose expression changes are related to imatinib response. Through a Gene Expression Omnibus search, we identified two genome-wide expression datasets that contain expression changes in response to imatinib treatment in a CML cell line (K562): one for mRNA and the other for miRNA. Significantly differentially expressed transcripts/miRNAs post imatinib treatment were identified from both datasets. Three additional filtering criteria were applied 1) miRbase/miRanda predictive algorithm; 2) opposite direction of imatinib effect for genes and miRNAs; and 3) literature support. These criteria narrowed our candidate gene-miRNA to a single pair: *IL8* and miR-493-5p. Using PCR we confirmed the significant up-regulation and down-regulation of miR-493-5p and *IL8* by imatinib treatment, respectively in K562 cells. In addition, *IL8* expression was significantly down-regulated in K562 cells 24 hours after miR-493-5p mimic transfection (p=0.002). Furthermore, we demonstrated significant cellular growth inhibition after *IL8* inhibition through either gene silencing or by over-expression of miR-493-5p (p=0.0005 and p=0.001 respectively). The *IL8* inhibition also further sensitized K562 cells to imatinib cytotoxicity (p<0.0001). Our study combined expression changes in transcriptome and miRNA after imatinib exposure to identify a potential gene-miRNA pair that is a critical target in imatinib response.

Experimental validation supports the relationships between *IL8* and miR-493-5p and between this gene-miRNA pair and imatinib sensitivity in a CML cell line. Our data suggests integrative analysis of multiple omic level data may provide new insight into biomarker discovery as well as mechanisms of imatinib resistance.

Introduction

Chronic myelogenous leukemia (CML) is a clonal myeloproliferative neoplasm of mature and maturing granulocytes resulting from the translocation of chromosomes 9 and 22 t(9;22)(q34:q11) [1]. This fusion generates the shortened 22q known as the Philadelphia (Ph) chromosome and the new fusion oncogene *BCR-ABL1,* a constitutively active tyrosine kinase that can be selectively targeted by imatinib and other tyrosine kinase inhibitors (TKIs) [2, 3]. Imatinib is a small molecule inhibitor that semi-competitively binds the ATP-binding site of BCR-ABL, thus preventing the conformational switch to its active form and inhibiting enzymatic activity, interfering with downstream signal transduction [4]. Treatment with imatinib alone in the early-chronic phase of CML shows an 88% overall survival rate after five years compared to 57% with previous nonspecific treatments including interferon and hydroxyurea, with far fewer side effects [5, 6]. Although, imatinib prolongs survival, it does not cure the disease. The success of the drug as a front-line therapy in CML has been tempered by latent problems such as disease persistence or relapse in nearly 20–30% of all CML patients [7]. Moreover, imatinib has been cited as one of the first exceptionally expensive cancer drugs, costing nearly $100,000 per year per patient [8]. Given the huge financial burden and sporadically temporary management, it is crucial to identify molecular mechanisms of imatinib resistance in order to individually tailor imatinib therapy and spare those non-responders from unnecessary side effects and economic burden.

There have been dozens of studies which predict imatinib response based on molecular components in both *in vitro* and *in vivo* models [9–27]; however, the majority of these studies remain externally uncorroborated [9–12, 26]. This lack of reproducibility is in part due to the lack of comprehensive evaluation of biomarkers on a global scale [28]. Over the past decade, increasing accessibility of high-throughput sequencing and mass spectroscopy has made it possible to profile a multitude of molecular components in a cost-effective and timely way [29, 30]. The National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) provides a platform to increase data sharing in the research community and to allow subsequent data mining [31]. With the advent of expansive molecular component data, the ability to integrate these data may provide a better understanding of the underlying biology of human complex traits [32].

Here, we propose a pilot integrative approach that combines gene and microRNA (miRNA) expression changes in response to imatinib in a CML cell line K562 to identify potential markers and pathways that are important in imatinib response. The rationale for focusing on K562 is based on the rich publicly available transcriptome and miRNA expression data post imatinib treatment, which provides us a tool to conduct this proof-of-concept study. Gene and miRNA expression change were used over baseline expression in order to improve the molecular level resolution. Although imatinib was designed to specifically target BCR-ABL, little is understood about the drug's downstream effects. By focusing on expression change we hoped to uncover gene/miRNA networks that are specifically responsive to imatinib treatment. Furthermore, functional experiments were conducted to validate the role of the putative mRNA and miRNA markers in response to imatinib treatment.

Materials and Methods

Identify imatinib responsive gene/miRNA

Whole genome gene and miRNA expression levels in the K562 cell line with and without imatinib treatment were obtained from GEO. Dataset GSE1922 contains whole genome mRNA expression data after 24 hours of 1 μM imatinib treatment [33] and dataset GSE28825 contains whole genome miRNA expression after 48 hours of 0.15 μM imatinib treatment [13]. Both datasets contain appropriate no treatment controls. Detailed workflow can be found in Fig. 1. From dataset GSE1922, 19 mRNAs were selected which showed a greater than 1.5-fold change in expression level after imatinib treatment as compared to control (p value threshold $<2.2 \times 10^{-6}$). Similarly, 69 miRNAs were selected from dataset GSE28825 by filtering for human miRNA probes with expression levels greater than background (307 miRNA probes with expression level >0.1), removing any probes with higher than average standard deviation (239 miRNA probes), and selecting only those miRNAs which showed a greater than 1.5-fold change in expression level after imatinib treatment as compared to control. The 19 differentially expressed genes were examined for their predicted miRNA binding partners using the miRanda algorithm built on miRBase annotation (http://www.scandb.org/apps/microrna/search.html). In total, the 19 unique imatinib affected genes have 384 potential miRNA partners. These potential partners were then compared to the 69 differentially expressed miRNA from dataset GSE28825 to identify overlapping miRNA. Additional filtering criteria were applied to select only those differentially expressed mRNA and miRNA, which are negatively correlated, leaving 9 unique mRNA and 11 unique miRNA. In addition, we used literature support to further narrow down our list of gene-miRNA pairs for functional validation. Criteria used include gene/miRNA's biological role in cancer and potential interaction with imatinib.
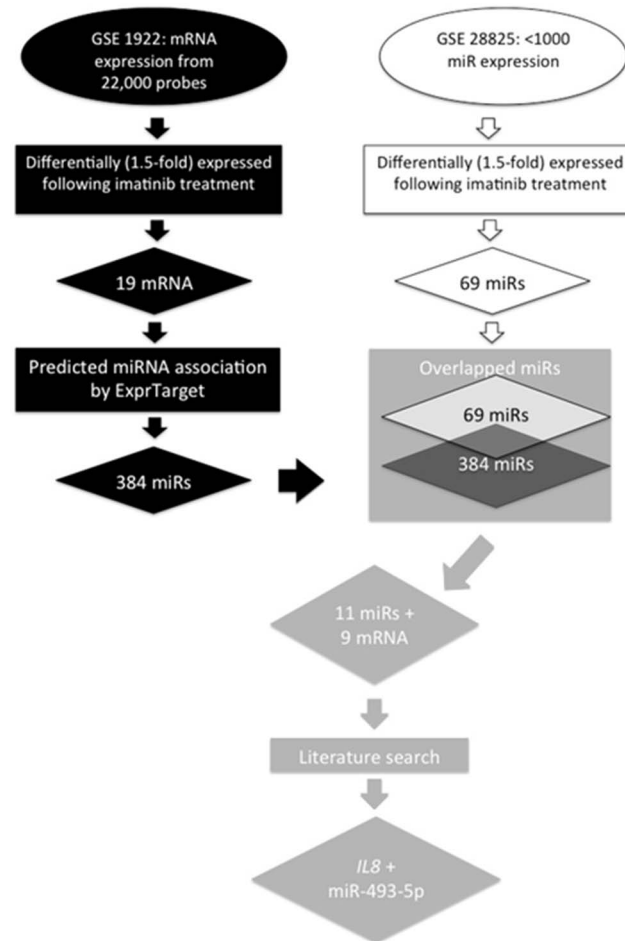
Fig. 1. Work flow for identifying imatinib response biomarkers through an informatics approach in K562 cells.

Cells and materials

CML cell line K562 was obtained from ATCC (cat # CCL-243). Cryopreserved cells were thawed and grown at 37℃ and 5% CO_2 in RPMI medium supplemented with 15% fetal bovine serum, and 2% L-glutamine. Cells were passaged 3 times per week to maintain exponential growth. Imatinib mesylate (Enzo Life Sciences cat # ALX-270-492) was dissolved in DMSO to 100 mM master stock, aliquoted, and stored at −20℃.

Effect of imatinib on selected gene and miRNA expression

K562 cells were seeded in 6-well plates at 2×10^6 cells per well. After 24 hours, the cells were treated with 1 μM imatinib. Viable cells were counted, collected and snap frozen at 0, 24 and 48 hours after drug treatment. Cells were homogenized using QiaShredders and total RNA was isolated using Qiagen miRNeasy kit (cat # 217004). Complementary cDNA was generated using Applied Biosystems High

Capactiy cDNA Reverse Transcription Kit (cat # 4368813) or Exiqon Universal cDNA Synthesis Kit II (cat # 203301) for mRNA and miRNA respectively. The relative expression of *IL8* and miR-493-5p was evaluated by real time PCR (RT-PCR). Applied Biosystems Taqman Fast Advanced Master Mix (cat # 4444557) and hs00174103_m1 primer set were used for *IL8*; while Exiqon ExiLENT SYBR Green Master Mix (cat # 203402) and Exiqon primer set (cat # 205693) were used for miR-493-5p. Gene and miRNA expression levels were normalized to *B2M* and RNU6 respectively. Relative expression was quantified using the standard curve method.

miR-493-5p effect on *IL8*

K562 cells were seeded at 5×10^5 cells per well in a 6-well plate and transfected with 15 nM miR-493-5p mimic (Qiagen MSY0002813), AllStar Negative Control siRNA (Qiagen cat # SI03650318) or water using DharmaFECT Transfection Reagent 1 (Thermo Fisher Scientific cat # T-2001) according to the manufacturer's instructions. After six hours, transfection media was removed and replaced with complete culture media. Cells were pelleted after 0, 6, 24 and 48 hours for RNA isolation, cDNA conversion and RT-PCR for miR-493-5p and *IL8*. Mean and standard deviation were calculated for the normalized quantity of each target transcript at each time point and treatment condition. Paired, two-tailed Student's t-tests were performed comparing treatment and control conditions at each time point with $p < 0.05$ considered statistically significant.

Evaluate biological function of miR-493-5p over-expression or *IL8* inhibition

K562 cells were transfected with 15 nM miR-493-5p mimic, *IL8* siRNA (Qiagen cat # SI00012236), AllStar Negative Control siRNA (Qiagen cat # SI03650318) or water independently using DharmaFECT Transfection Reagent 1 described above. Immediately after transfection, cells were plated to assess either cellular proliferation rate or imatinib sensitivity. To measure cellular proliferation rate, cells were plated at 5,000 cells per well in a 96-well plate and CellTiter-Glo Luminescent Cell Viability Assay (Promega cat # G7570) was performed after 0, 24, 48, and 72 hours. For imatinib sensitivity measurement, transfected cells were plated in 96-well plates at 10,000 cells per well. 24 hours later, cells were treated with increasing concentrations of imatinib (0, 0.3125, 0.625, 1, 1.25, 2, and 2.5 µM). 48 hours after the addition of imatinib, 100 µL of cell mixture from each well were combined with 100 µL of CellTiter-Glo reagent. For both assays luminescence was measured using a BioTEK Synergy HT plate reader. Cellular sensitivity to imatinib was presented as percent survival in comparison to control at each concentration. Two-way ANOVA was performed to evaluate the difference among different gene/miRNA modification conditions with $p < 0.05$ considered statistically significant.

Explore the role of *IL8* in CML patients

To explore the role of IL8 in CML patients, we queried another GEO dataset (GSE4170)[34]. In this study, gene expression profiling was conducted in bone marrow from 91 cases of CML in chronic, accelerated, and blast phases. Furthermore, additional cases of patients who relapsed after initially successful imatinib treatment were also profiled. We compared the *IL8* expression level from the chronic CML group to those patients who were treated with imatinib at the chronic phase and later relapsed. Student t test was performed with alpha value less than 0.05 was considered statistically significant.

Results

Identify imatinib responsive gene/miRNA

The workflow and stepwise findings is shown in Fig. 1. Two datasets were selected from the 21 unique studies garnered through GEO search results based on treatment conditions and molecular components investigated. They are GSE1922 and GSE28825. 19 distinct mRNA and 69 distinct miRNA were found to be differentially expressed in datasets GSE1922 and GSE28825 respectively. Integrating these two datasets using the methods described above identified 12 mRNA/miRNA relationships made up of 9 unique mRNA and 11 unique miRNA (Fig. 1 and Table 1). One mRNA/miRNA pair: *IL8* and miR-493-5p, was chosen for subsequent functional validation based on literature support. For example, *IL8* expression has been shown to be under expressed in patients post imatinib treatment and miR-493-5p has been implicated in colon metastasis [35, 36], bladder cancer [37], ovarian cancer [38] and triple negative breast cancer [39]; however, the regulatory connection between this gene/miRNA pair or their functional connection to imatinib sensitivity is unknown.

Assess relationships among imatinib treatment, *IL8* and miR-493-5p expression

We treated K562 cells with imatinib and measured both *IL8* and miR-493-5p expression after 24 and 48 hours. As expected, *IL8* expression was significantly down-regulated; while miR-493-5p expression was significantly up-regulated post imatinib treatment both at 24 and 48 hours (respectively p=0.02 and p=0.0001 for miR-493-5p, p=0.001 and p=0.001 for *IL8*) (Fig. 2A). In addition, after transfection with miR-493-5p mimic, significant down-regulation of *IL8* was observed at 24 hours (p=0.002) when compared to scramble treated control (Fig. 2B). However, the inhibitory effect of miR-493-5p mimic on *IL8* expression was not observed at 48 hours post mimic transfection (p>0.05, data not shown).

Table 1. 12 predicted imatinib response gene-miRNA pairs based on GEO data.

Gene name	mRNA expression fold change	miR name	miRNA expression fold change
ALAS2	1.73	miR-500	−2.23
ALAS2	1.73	miR-886-3p	−1.74
CD69	−1.68	miR-323-3p	1.52
COL18A1	1.69	miR-933	−1.67
EGR1	−1.54	miR-493-5p	1.52
IL8	**−1.51**	**miR-493-5p**	**1.52**
PDE4DIP	−1.54	miR-877*	1.54
PDE4DIP	−1.54	miR-483*	1.71
PHLDA2	−1.6	miR-134	1.71
RHD	1.54	miR-19b	−1.6
SLC30A10	1.52	miR-335*	−4.01
SLC30A10	1.52	miR-193b	−1.91

Functional experiments were conducted on the bolded gene/miR pair.

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Evaluate biological function of expression modulation of miR-493-5p or *IL8*

In order to investigate the consequences of *IL8* inhibition, we transfected K562 cells with siRNA for *IL8* and miR-493-5p mimic and measured cell viability. Both *IL8* inhibition and miR-493-5p over-expression resulted in significant decrease in *IL8* mRNA expression ($p < 0.05$ at 6, 24, and 48 hours post si-IL8; and at 6, and 24
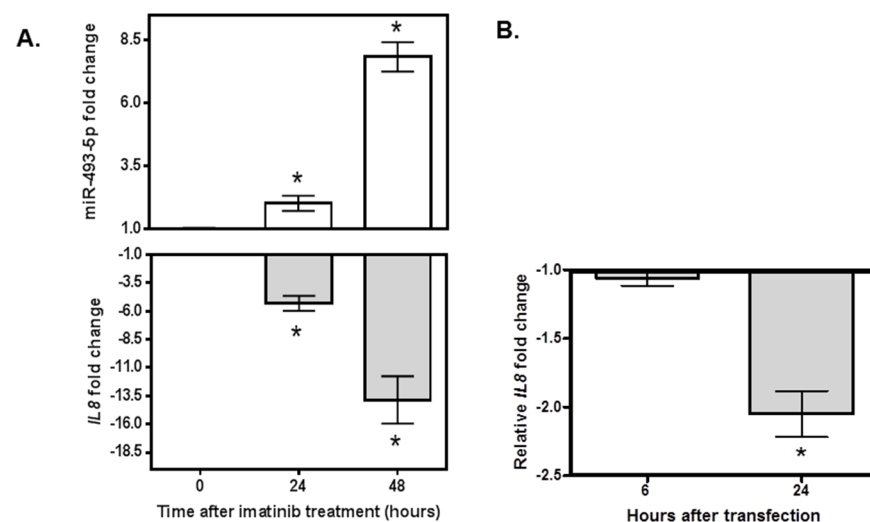

Fig. 2. Relationships among imatinib treatment, and the expression of miR-493-5p and *IL8* in K562 cells. (**A**) Imatinib treatment (1μM for 24 and 48 hours) significantly up-regulated miR-493-5p expression and significantly down-regulated *IL8* expression in K562 cells (*$p < 0.05$, N=6). (**B**) *IL8* is significantly down-regulated by miR-493-5p mimic at 24 hours after transfection (*$p = 0.002$, N=6). Relative fold change was calculated by first normalizing *IL8* to *B2M* and miR-493-5p to *RNU6* and then comparing 6 and 24 hours data to 0 hour data.

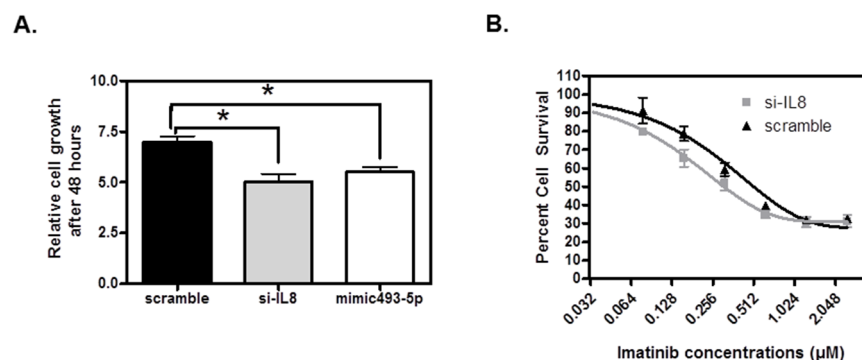doi:10.1371/journal.pone.0115003.g002

A.

B.

Fig. 3. Biological consequences of *IL8* inhibition. (**A**) *IL8* silencing through siRNA and miR-493-5p mimic resulted in significantly slower cellular proliferation as measured by CellTiter-Glo at 48 hours. (*p<0.05, N=6). Relative cell growth was calculated by normalizing fluorescence indexes of each time point to the 0 hour control. (**B**) Cellular response to imatinib treatment curves. Cellular response to imatinib was measured using CellTiter-Glo. p<0.0001 by two way ANOVA, N=6. Percent survival was calculated by normalizing fluorescence indexes of imatinib treated cells to cells treated with vehicle and plotting on a log(2) scale.

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hours post miR-493-5p mimic). Furthermore, inhibition of *IL8* resulted in marked decrease in K562 cell proliferation measured by CellTiter-Glo at 48 hours (Fig. 3A, p=0.0005 and p=0.001 respectively). Moreover, cytotoxicity experiments showed that K562 cells transfected with *IL8* siRNA were more sensitive to imatinib treatment (Fig. 3B, two-way ANOVA p<0.0001). Imatinib IC_{50} is 0.30 and 0.50 μM for *IL8* siRNA and scrambled control transfected K562 cells respectively. The effect of miR-493-5p overexpression did not result in a change in imatinib sensitivity in comparison to scrambled control.

Explore the role of *IL8* in CML patients

We obtained another GEO dataset (GSE4170) [34] and compared the *IL8* expression level from 23 chronic phase CML patients to 9 of those patients who were treated with imatinib at the chronic phase and later relapsed. A significant difference in *IL8* expression was observed between these 2 groups ($p=1 \times 10^{-4}$).

Discussion

Imatinib and other TKI implementation has resulted in a major breakthrough for the treatment of CML, but resistance remains a significant clinical problem. Although it is clear that *BCR-ABL* fusion gene is a key target of imatinib, little else is known about the downstream effects of BCR-ABL or other molecular targets of imatinib in CML. Indeed, recent recognition of imatinib's other on and off target effects have led to the testing of imatinib treatment in many other disease settings including Gastointestinal stromal tumor [40], AIDS associated Kaposi's sarcoma [41], and systemic sclerosis [42]. Molecular response signatures based on expression change can provide both a snapshot of the downstream targets specific to a drug and clinical insight into drug efficacy and resistance mechanisms that

can be used to guide patient care. Taking advantage of existing large omic datasets from GEO, we set out to develop an approach that integrates both transcriptome and epigenome changes to decipher the underlying biology of imatinib response.

By thoroughly examining two independent datasets along with incorporation of an mRNA-miRNA relationship prediction tool, we identified a set of 12 pairs of gene/miRNA relationships (consisting of 9 genes and 11 miRNAs), all of which are affected by imatinib treatment. Among them, *SLC30A10* is a known zinc binding protein imperative in autophagy [43] and has been implicated in colorectal cancer [44]. *PHLD2* is one of several genes in the imprinted gene domain of 11p15.5, which is considered to be an important tumor suppressor gene region. Alterations in this region have been associated with Wilms tumor, osterosarcoma, rhabdomyosarcoma, lung, ovarian, and breast cancer [45]. *PDE4DIP* is part of the PDGFR family and is known to mark eosinophilia and myeloproliferative disorders [46]. All these gene/miRNA pairs warrant further evaluation in relation to imatinib sensitivity in CML.

Our study followed up on one gene/miRNA pair (*IL8*/miR-493-5p) both of which are affected by imatinib treatment. *IL8* is a known proinflammatory cytokine important in the NFKB survival pathway [47]. In a previous clinical study imatinib treatment response was shown to down-regulate *IL8* expression in patients with CML [48]. Our study confirmed similar effect of imatinib in K562 cells with treatment effects lasting at least 48 hours. miR-493-5p has been reported to be differentially expressed in multiple cancers [34]. In particular, its expression is known to decrease cell growth and migration in bladder cancer by inhibiting targets *RhoC* and *FDZ4* [37]. In our study, we identified miR-493-5p as a potential mediator for *IL8* down-regulation as evidenced by 1) the miRbase miRanda algorithm prediction [49, 50]; and 2) observed significant *IL8* down-regulation after addition of miR-493-5p mimic. We speculated that imatinib treatment may up-regulate miR-493-5p expression which subsequently inhibits *IL8* expression and led imatinib sensitivity, although these sequential events remain to be tested.

By integrating two distinct high throughput expression datasets we were able to hone in on a gene/miRNA pair - *IL8*/miR-493-5p – critical for imatinib sensitivity. Our work suggests that differential inhibition of *IL8* among individuals may be a good marker for imatinib sensitivity. Furthermore, we speculate a directional relationship where imatinib can induce miR-493-5p expression which then subsequently inhibits *IL8* expression and leads to biological consequences such as cellular growth inhibition. We also demonstrate that inhibition of *IL8* in addition to imatinib may result in better disease control, which supports the need for further evaluation of IL8 inhibition as a tool to overcome imatinib resistance.

Author Contributions

materials/analysis tools: RSH. Contributed to the writing of the manuscript: SB DL RSH.

References

1. **Rowley JD** (1973) A New Consistent Chromosomal Abnormality in Chronic Myelogenous Leukaemia identified by Quinacrine Fluorescence and Giemsa Staining. Nature 243: 290–293. doi:10.1038/243290a0.

2. **Daley GQ, Etten RV, Baltimore D** (1990) Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science 247: 824–830. doi:10.1126/science.2406902.

3. **Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, et al.** (2001) Efficacy and Safety of a Specific Inhibitor of the BCR-ABL Tyrosine Kinase in Chronic Myeloid Leukemia. N Engl J Med 344: 1031–1037. doi:10.1056/NEJM200104053441401.

4. **Mauro MJ, Druker BJ** (2001) STI571: a gene product-targeted therapy for leukemia. Curr Oncol Rep 3: 223–227.

5. **Von Bubnoff N, Duyster J** (2010) Chronic myelogenous leukemia: treatment and monitoring. Dtsch Ärztebl Int 107: 114–121. doi:10.3238/arztebl.2010.0114.

6. **Allan NC, Richards SM, Shepherd PC** (1995) UK Medical Research Council randomised, multicentre trial of interferon-alpha n1 for chronic myeloid leukaemia: improved survival irrespective of cytogenetic response. The UK Medical Research Council's Working Parties for Therapeutic Trials in Adult Leukaemia. Lancet 345: 1392–1397.

7. **Frame D** (2007) New strategies in controlling drug resistance in chronic myeloid leukemia. Am J Health Syst Pharm 64: S16–21. doi:10.2146/ajhp070483.

8. **Pollack A (25 April 2013) Cancer Physicians Attack High Drug Costs.** The New York Times. Available: http://www.nytimes.com/2013/04/26/business/cancer-physicians-attack-high-drug-costs.html?pagewanted=2&google_editors_picks=true&_r=1. Accessed 17 July 2014.

9. **Gromicho M, Magalhães M, Torres F, Dinis J, Fernandes AR, et al.** (2013) Instability of mRNA expression signatures of drug transporters in chronic myeloid leukemia patients resistant to imatinib. Oncol Rep 29: 741–750. doi:10.3892/or.2012.2153.

10. **Nardinelli L, Sanabani SS, Didone A, Ferreira P de B, Serpa M, et al.** (2012) Pretherapeutic Expression of the hOCT1 Gene Predicts a Complete Molecular Response to Imatinib Mesylate in Chronic-Phase Chronic Myeloid Leukemia. Acta Haematol 127: 228–234. doi:10.1159/000336610.

11. **Chen W, Meng F, Zhong J, Yin C, Wang Z** (2012) [Effects of hOCT1 and ABCB1 gene on the efficacy of imatinib mesylate in chronic myelocytic leukemia]. Zhonghua Yi Xue Za Zhi 92: 1405–1408.

12. **Dickens D, Owen A, Alfirevic A, Pirmohamed M** (2013) ABCB1 single nucleotide polymorphisms (1236C>T, 2677G>T, and 3435C>T) do not affect transport activity of human P-glycoprotein. Pharmacogenet Genomics 23: 314–323. doi:10.1097/FPC.0b013e328360d10c.

13. **Turrini E, Haenisch S, Laechelt S, Diewock T, Bruhn O, et al.** (2012) MicroRNA profiling in K-562 cells under imatinib treatment: influence of miR-212 and miR-328 on ABCG2 expression. Pharmacogenet Genomics 22: 198–205. doi:10.1097/FPC.0b013e328350012b.

14. **Shibata M, Ezoe S, Oritani K, Matsui K, Tokunaga M, et al.** (2011) Predictability of the response to tyrosine kinase inhibitors via in vitro analysis of Bcr-Abl phosphorylation. Leuk Res 35: 1205–1211. doi:10.1016/j.leukres.2011.01.012.

15. **Lucas CM, Harris RJ, Giannoudis A, Copland M, Slupsky JR, et al.** (2011) Cancerous inhibitor of PP2A (CIP2A) at diagnosis of chronic myeloid leukemia is a critical determinant of disease progression. Blood 117: 6660–6668. doi:10.1182/blood-2010-08-304477.

16. **Zhang J, Jin Z, Du Q, Li R, Yao F, et al.** (2012) Analysis of altered proteins related to blast crisis in chronic myeloid leukemia by proteomic study. Int J Lab Hematol 34: 267–273. doi:10.1111/j.1751-553X.2011.01389.x.

17. **Jalkanen SE, Lahesmaa-Korpinen A-M, Heckman CA, Rantanen V, Porkka K, et al.** (2012) Phosphoprotein profiling predicts response to tyrosine kinase inhibitor therapy in chronic myeloid leukemia patients. Exp Hematol 40: 705–714.e3. doi:10.1016/j.exphem.2012.05.010.

18. **Ouerhani S, Gharbi H, Menif S, Safra I, Douzi K, et al.** (2012) KIT mutation detection in Tunisian patients with newly diagnosed myelogenous leukemia: prevalence and prognostic significance. Cancer Genet 205: 436–441. doi:10.1016/j.cancergen.2012.05.008.

19. **Esposito N, Colavita I, Quintarelli C, Sica AR, Peluso AL, et al.** (2011) SHP-1 expression accounts for resistance to imatinib treatment in Philadelphia chromosome-positive cells derived from patients with chronic myeloid leukemia. Blood 118: 3634–3644. doi:10.1182/blood-2011-03-341073.

20. **Venturini L, Battmer K, Castoldi M, Schultheis B, Hochhaus A, et al.** (2007) Expression of the miR-17–92 polycistron in chronic myeloid leukemia (CML) CD34+ cells. Blood 109: 4399–4405. doi:10.1182/blood-2006-09-045104.

21. **Yang H, Liang H, Yan J, Tao R, Hao S, et al.** (2012) Down-regulation of hematopoiesis master regulator PU.1 via aberrant methylation in chronic myeloid leukemia. Int J Hematol 96: 65–73. doi:10.1007/s12185-012-1106-x.

22. **Stella S, Tirrò E, Conte E, Stagno F, Di Raimondo F, et al.** (2013) Suppression of survivin induced by a BCR-ABL/JAK2/STAT3 pathway sensitizes imatinib-resistant CML cells to different cytotoxic drugs. Mol Cancer Ther 12: 1085–1098. doi:10.1158/1535-7163.MCT-12-0550.

23. **Hayashi Y, Hirai H, Kamio N, Yao H, Yoshioka S, et al.** (2012) C/EBPβ promotes BCR–ABL-mediated myeloid expansion and leukemic stem cell exhaustion. Leukemia 27: 619–628. doi:10.1038/leu.2012.258.

24. **Tobin LA, Robert C, Rapoport AP, Gojo I, Baer MR, et al.** (2013) Targeting abnormal DNA double-strand break repair in tyrosine kinase inhibitor-resistant chronic myeloid leukemias. Oncogene 32: 1784–1793. doi:10.1038/onc.2012.203.

25. **Liu Y-C, Hsiao H-H, Yang W-C, Liu T-C, Chang C-S, et al.** (2013) MDM2 promoter polymorphism and p53 codon 72 polymorphism in chronic myeloid leukemia: The association between MDM2 promoter genotype and disease susceptibility, age of onset, and blast-free survival in chronic phase patients receiving imatinib. Mol Carcinog. doi:10.1002/mc.22061.

26. **Wang L, Giannoudis A, Austin G, Clark RE** (2012) Peroxisome proliferator-activated receptor activation increases imatinib uptake and killing of chronic myeloid leukemia cells. Exp Hematol 40: 811–819.e2. doi:10.1016/j.exphem.2012.05.013.

27. **Hershkovitz Rokah O, Granot G, Ovcharenko A, Modai S, Pasmanik-Chor M, et al.** (2012) Downregulation of Mir-31, Mir-155, and Mir-564 in Chronic Myeloid Leukemia Cells. PLoS ONE 7: e35501. doi:10.1371/journal.pone.0035501.

28. **Zhang W, Li F, Nie L** (2010) Integrating multiple "omics" analysis for microbial biology: application and methodologies. Microbiol Read Engl 156: 287–301. doi:10.1099/mic.0.034793-0.

29. **Grainger DC, Overton TW, Reppas N, Wade JT, Tamai E, et al.** (2004) Genomic Studies with Escherichia coli MelR Protein: Applications of Chromatin Immunoprecipitation and Microarrays. J Bacteriol 186: 6938–6943. doi:10.1128/JB.186.20.6938-6943.2004.

30. **Herring CD, Raffaelle M, Allen TE, Kanin EI, Landick R, et al.** (2005) Immobilization of Escherichia coli RNA Polymerase and Location of Binding Sites by Use of Chromatin Immunoprecipitation and Microarrays. J Bacteriol 187: 6166–6174. doi:10.1128/JB.187.17.6166-6174.2005.

31. **Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, et al.** (2013) NCBI GEO: archive for functional genomics data sets–update. Nucleic Acids Res 41: D991–995. doi:10.1093/nar/gks1193.

32. **Liu Y, Devescovi V, Chen S, Nardini C** (2013) Multilevel omic data integration in cancer cell lines: advanced annotation and emergent properties. BMC Syst Biol 7: 14. doi:10.1186/1752-0509-7-14.

33. **Auer H, Bakaletz A, Liu-Stratton Y, Sen C, Carter DE, et al.** (2004) Imatinib effect on K562 leukemia cell line. GEO DataSets BioProjects: 34039–34944. http://www.ncbi.nlm.nih.gov/bioproject/PRJNA90947. Accessed 15 June 2013.

34. **Radich JP, Dai H, Mao M, Oehler V, Schelter J, et al.** (2006) Gene expression changes associated with progression and response in chronic myeloid leukemia. Proc Natl Acad Sci U S A 103: 2794–2799. doi:10.1073/pnas.0510423103.

35. **Okamoto K, Ishiguro T, Midorikawa Y, Ohata H, Izumiya M, et al.** (2012) miR-493 induction during carcinogenesis blocks metastatic settlement of colon cancer cells in liver. EMBO J 31: 1752–1763. doi:10.1038/emboj.2012.25.

36. **Sakai H, Sato A, Aihara Y, Ikarashi Y, Midorikawa Y, et al.** (2014) MKK7 mediates miR-493-dependent suppression of liver metastasis of colon cancer cells. Cancer Sci 105: 425–430. doi:10.1111/cas.12380.

37. **Ueno K, Hirata H, Majid S, Yamamura S, Shahryari V, et al.** (2012) Tumor suppressor microRNA-493 decreases cell motility and migration ability in human bladder cancer cells by downregulating RhoC and FZD4. Mol Cancer Ther 11: 244–253. doi:10.1158/1535-7163.MCT-11-0592.

38. **Lee H, Park CS, Deftereos G, Morihara J, Stern JE, et al.** (2012) MicroRNA expression in ovarian carcinoma and its correlation with clinicopathological features. World J Surg Oncol 10: 174. doi:10.1186/1477-7819-10-174.

39. **Gasparini P, Cascione L, Fassan M, Lovat F, Guler G, et al.** (2014) microRNA expression profiling identifies a four microRNA signature as a novel diagnostic and prognostic biomarker in triple negative breast cancers. Oncotarget 5: 1174–1184.

40. **Li Z-Y, Tang L, Li S-X, Shan F, Bu Z-D, et al.** (2013) Imatinib mesylate in clinically suspected gastric stromal tumors. Chin J Cancer Res 25: 600–602.

41. **Koon HB, Krown SE, Lee JY, Honda K, Rapisuwon S, et al.** (2014) Phase II Trial of Imatinib in AIDS-Associated Kaposi's Sarcoma: AIDS Malignancy Consortium Protocol 042. J Clin Oncol 32: 402–408. doi:10.1200/JCO.2012.48.6365.

42. **Bournia V-K, Evangelou K, Sfikakis PP** (2013) Therapeutic inhibition of tyrosine kinases in systemic sclerosis: a review of published experience on the first 108 patients treated with imatinib. Semin Arthritis Rheum 42: 377–390. doi:10.1016/j.semarthrit.2012.06.001.

43. **Liuzzi JP, Yoo C** (2013) Role of zinc in the regulation of autophagy during ethanol exposure in human hepatoma cells. Biol Trace Elem Res 156: 350–356. doi:10.1007/s12011-013-9816-3.

44. **Yagi K, Akagi K, Hayashi H, Nagae G, Tsuji S, et al.** (2010) Three DNA methylation epigenotypes in human colorectal cancer. Clin Cancer Res Off J Am Assoc Cancer Res 16: 21–33. doi:10.1158/1078-0432.CCR-09-2006.

45. **Kim K-P, Thurston A, Mummery C, Ward-van Oostwaard D, Priddle H, et al.** (2007) Gene-specific vulnerability to imprinting variability in human embryonic stem cell lines. Genome Res 17: 1731–1742. doi:10.1101/gr.6609207.

46. **Wilkinson K, Velloso ERP, Lopes LF, Lee C, Aster JC, et al.** (2003) Cloning of the t(1;5)(q23;q33) in a myeloproliferative disorder associated with eosinophilia: involvement of PDGFRB and response to imatinib. Blood 102: 4187–4190. doi:10.1182/blood-2003-04-1150.

47. **Sunil Y, Ramadori G, Raddatzc D** (2010) Influence of NFkappaB inhibitors on IL-1beta-induced chemokine CXCL8 and −10 expression levels in intestinal epithelial cell lines: glucocorticoid ineffectiveness and paradoxical effect of PDTC. Int J Colorectal Dis 25: 323–333. doi:10.1007/s00384-009-0847-3.

48. **Ciarcia R, Vitiello MT, Galdiero M, Pacilio C, Iovane V, et al.** (2012) Imatinib treatment inhibit IL-6, IL-8, NF-KB and AP-1 production and modulate intracellular calcium in CML patients. J Cell Physiol 227: 2798–2803. doi:10.1002/jcp.23029.

49. **Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ** (2008) miRBase: tools for microRNA genomics. Nucleic Acids Res 36: D154–D158. doi:10.1093/nar/gkm952.

50. **John B, Enright AJ, Aravin A, Tuschl T, Sander C, et al.** (2004) Human MicroRNA targets. PLoS Biol 2: e363. doi:10.1371/journal.pbio.0020363.