References

1. Niemeyer CM, Locatelli F. Chronic myeloproliferative disorders. In: Pui CH, editor. Childhood leukemias. 3rd ed. New York: Cambridge University Press; 2012. p. 444–502.

2. Flotho C, Valcamonica S, Mach-Pascual S, Schmahl G, Corral L, Ritterbach J, et al. RAS mutations and clonality analysis in children with juvenile myelomonocytic leukemia (JML). Leukemia. 1999;13:32–37.

3. Locatelli F, Niemeyer CM. How I treat juvenile myelomonocytic leukemia. Blood. 2015;125:1083–90.

4. Olk-Batz C, Poetsch AR, Nöllke P, Claus R, Zucknick M, Sandrock I, et al. Aberrant DNA methylation characterizes juvenile myelomonocytic leukemia (JMLM) with poor outcome. Blood. 2011;117:4871–80.

5. Lipka DB, Witte T, Toth R, Yang J, Wiesenfarth M, Nöllke P, et al. RAS-pathway mutation patterns define epigenetic subclasses in juvenile myelomonocytic leukemia. Nat Commun. 2017;8:2126.

6. Stieglitz E, Mazor T, Olshen AB, Geng H, Gelston LC, Akutagawa J, et al. Genome-wide DNA methylation is predictive of outcome in juvenile myelomonocytic leukemia. Nat Commun. 2017;8:2127.

7. Murakami N, Okuno Y, Yoshida K, Shiraishi Y, Nagae G, Suzuki K, et al. Integrated molecular profiling of juvenile myelomonocytic leukemia. Blood. 2018;131:1576–86.

8. Cseh A, Niemeyer CM, Yoshimi A, Dworzak M, Hasle H, van den Heuvel-Eibrink MM, et al. Bridging to transplant with azacitidine in juvenile myelomonocytic leukemia: a retrospective analysis of the EWOG-MDS study group. Blood. 2015;125:2311–3.

9. Krombholz CF, Aumann K, Kolleck M, Bertle D, Fluhr S, Kunze M, et al. Long-term serial xenotransplantation of juvenile myelomonocytic leukemia recapitulates human disease in Rag2−/−gammac−/− mice. Haematologica. 2016;101:597–606.

10. Flotho C, Sommer S, Lübbert M. DNA-hypomethylating agents as epigenetic therapy before and after allogeneic hematopoietic stem cell transplantation in myelodysplastic syndromes and juvenile myelomonocytic leukemia. Semin Cancer Biol. 2018;51:68–79.

11. Roulois D, Loo YH, Singhania R, Wang Y, Danesh A, Shen SY, et al. DNA-demethylating agents target colorectal cancer cells by inducing viral mimicry by endogenous transcripts. Cell. 2015;162:961–73.

12. Chiappinelli KB, Strissel PL, Desrichard A, Li H, Henke C, Akman B, et al. Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses. Cell. 2015;162:974–86.

13. Brocks D, Schmidt CR, Daskalakis M, Jang HS, Shah NM, Li D, et al. DNMT and HDAC inhibitors induce cryptic transcription start sites encoded in long terminal repeats. Nat Genet. 2017;49:1052–60.

14. Goodyear O, Agathanggelou A, Novitzky-Basso I, Siddique S, Akman B, et al. Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses. Cell. 2015;162:974–86.

15. Li H, Chiappinelli KB, Guzzetta AA, Easwaran H, Yen RW, Vatapalli R, et al. Immune regulation by low doses of the DNA methyltransferase inhibitor 5-azacitidine in common human epithelial cancers. Oncotarget. 2014;5:587–98.

To the Editor:

Nilotinib, a second-generation tyrosine kinase inhibitor (TKI), has been described to be a superior drug in the frontline treatment of patients with Philadelphia chromosome–positive (Ph+) chronic myeloid leukemia (CML) [1]. However, with more mature follow-up, it has become clear that nilotinib is associated with impaired glucose and lipid metabolism [2–4] and an excess in arterial thrombotic events in comparison to imatinib [5]. The 5-year safety update of the ENESTnd trial [3] provided further confirmation; it reported significant elevations in fasting glucose and serum lipids and an increased
Gene expression of with isopropyl alcohol and absorbance was measured at 450 nm. 1A Lipid droplets were stained with Oil Red O on day 10 following treatment assays (Life Technologies) on a 7900HT Fast Real-Time PCR system Srebp1. Accumulation of drugs in adipose tissue could result in metabolic disturbances [8], was used as positive control. We have only investigated these two TKIs in the current study. Statistical analyses were conducted by one-way ANOVA with Dunnett’s Test. All in vitro experiments were repeated three times in triplicate. A p value ≤ 0.05 was considered significant.

We investigated whether nilotinib and/or imatinib (0.01–100 μM) caused cytotoxicity in both undifferentiated and differentiating adipocytes using the MTT assay. Neither nilotinib nor imatinib reduced cell viability in these cell types at clinically relevant concentrations (Supplementary Figure 1). We hypothesised that nilotinib may interfere with adipocyte lipid accumulation and alter mRNA levels of key adipogenic regulatory genes (Ppary, Lpin1, Srebp1). Lipid accumulation in differentiated adipocytes was assessed on day 10 using Oil Red O staining [8] and gene expression was assessed by Real-Time PCR using Taqman assays (Life Technologies). Nilotinib (4 μM) caused cytotoxicity in both undifferentiated and differentiating adipocytes, leading to the development of insulin resistance [10]. Nilotinib, but not imatinib, also resulted in dose-dependent downregulation of all three adipogenic regulatory genes, with the effect evident at therapeutic concentrations (4 μM nilotinib: Ppary: 48% downregulation, Lpin1: 40% downregulation, Srebp1: 29% downregulation; all p < 0.05; Fig. 1B). PPARY is a master regulator of adipogenesis and mediates adipogenic gene expression and insulin sensitivity [11]; lipin1, a gene that
encodes a magnesium-ion-dependent phosphatidic acid phosphohydrolase enzyme, is involved in triglyceride synthesis [12]; SREBP1 plays a role in cholesterol homeostasis [13]. We then assessed the effect of these two TKIs on Glut4, the principal glucose transporter in the adipocyte; both nilotinib (p = 0.01), and to a lesser extent imatinib (p = 0.02), significantly downregulated Glut4 mRNA expression in differentiating adipocytes (Fig. 1B). Downregulation of Glut4 by nilotinib could result in reduced glucose uptake into the adipocyte and may lead to insulin resistance observed in CML patients. Downregulation of Glut4 by imatinib, a drug that has been consistently suggested to improve insulin sensitivity in CML patients [14], is interesting; this suggests the need to assess other mechanisms involved in the regulation of whole body insulin sensitivity, such as the role of liver and skeletal muscle. We then assessed whether telmisartan can reverse nilotinib-induced adipocyte toxicity; co-incubation of telmisartan (5 µM) with 4 µM nilotinib resulted in significant reversal of nilotinib-mediated inhibition of adipocyte lipid accumulation (NILO + TEL: 0.52 ± 0.01, in comparison to NILO 4 µM: 0.46 ± 0.02, p = 0.01; Fig. 1A) and adipogenic mRNA downregulation (p = 0.02; Fig. 1B).

Next, we investigated whether TKIs affect adiponectin in vitro and in plasma samples obtained from CML patients.

Fig. 2 Effect of nilotinib and imatinib on adiponectin in vitro and in vivo. Effect of nilotinib (with and without telmisartan) and imatinib on secreted adiponectin in differentiating 3T3-F442A adipocytes (A); plasma adiponectin levels at baseline, 3 months and 12 months in CML patients treated with imatinib (B); first-line nilotinib (C) and second-line nilotinib (D). Telmisartan was co-incubated with only one concentration of nilotinib (4 µM). Lopinavir (LPV), an anti-HIV drug, was used as a positive control in vitro. All in vitro experiments were repeated three times in triplicate. One-way ANOVA with Dunnett’s Test was used for in vitro statistical analysis; Repeated measures ANOVA with Dunnett’s Test was used to compare plasma adiponectin levels at different time points in CML patients. Data represent Mean ± SD; p ≤ 0.05. *Vehicle vs NILO/LPV/IMA; † NILO4µM vs NILO4µM + TEL5µM (in vitro). Mean adiponectin levels in each patient group at different time points were compared against the baseline value. NILO: nilotinib, IMA: imatinib, TEL: telmisartan, LPV: lopinavir.
Adiponectin is a protein exclusively secreted by the adipocyte and is a key mediator of systemic insulin sensitivity and glucose homeostasis [6]. Total adiponectin in the conditioned media collected from drug-treated and control adipocytes were measured using a standard ELISA. Nilotinib induced a dose-dependent reduction in adiponectin secretion (4 μM: 20% reduction, \( p = 0.02 \)); however, the effect of imatinib was only marginal (9.9% reduction, \( p = 0.04 \)). Interestingly, co-incubation of telmisartan with nilotinib reversed the inhibitory effect of nilotinib on adiponectin secretion in vitro (\( p = 0.001 \); Fig. 2A).

For the in vivo analysis of adiponectin, nonfasted plasma samples at three different time points (baseline, 3 and 12 months) were obtained from 30 CML patients who received either nilotinib (\( n = 14 \)) or imatinib (\( n = 16 \)) for at least 12 months. Relevant ethics approval and patient consent were obtained. All patients were in first chronic phase throughout. In the nilotinib-treated group, six patients received the drug as first-line therapy and eight as second line following initial treatment with imatinib. Five out of the eight second-line nilotinib patients were imatinib-resistant and showed higher BCR-ABL1 transcript levels at the time of the switch; the remaining three were switched due to imatinib intolerance. In all second-line nilotinib patients, the sample collected at the time of initiation of nilotinib therapy was considered as the baseline sample. All patients in the imatinib-treated group received the drug as first-line. We did not have baseline sample for one of the imatinib-treated patients, therefore we excluded that patient from any analysis (i.e. imatinib, final \( n = 15 \)). The median ages of imatinib and nilotinib-treated CML patients were 39 and 49 years, respectively; both drug groups had eight female subjects each. None of the patients recruited had a medical history of diabetes. Total adiponectin was measured using an electrochemiluminescence-based sandwich immunoassay (Meso Scale Discovery, USA). Repeated measures ANOVA with Dunnett’s Test was used to compare adiponectin levels at different time points. Imatinib resulted in a significant increase in plasma adiponectin levels at 3 (38.4 ± 7.1 mg/l; \( p < 0.01 \)) and 12 month (36.7 ± 7.2 mg/l; \( p < 0.01 \)) time points compared with baseline values (27.3 ± 5.7 mg/l; \( p < 0.05 \); Fig. 2B). By contrast, in both first-line (Fig. 2C) and second-line (Fig. 2D) nilotinib patients, there was no change in adiponectin concentrations; however, with second-line nilotinib, there was a non-significant decrease at both 3 (15.2 ± 1.8 mg/l; \( p = \text{NS} \)) and 12 months (14.6 ± 1.7 mg/l; \( p = \text{NS} \); Fig. 2D) when compared to baseline levels (21.3 mg/l).

Nilotinib-induced reduction in adiponectin in vitro was, to a certain extent, mirrored in the CML plasma samples obtained from second-line nilotinib-treated CML patients, but this was non-significant. However, it should be noted that our sample size was small (\( n = 14 \) or 15 per drug group) and therefore lacked sufficient power to detect a statistically significant difference in adiponectin. On the other hand, the increase in plasma adiponectin observed with imatinib correlates with what has been previously reported for imatinib in CML patients [14]. Adiponectin expression is directly regulated by PPARγ [6]; it is possible that nilotinib-induced reduction in adiponectin could be a direct result of the downregulation of PPARγ by nilotinib. The molecular mechanism(s) by which imatinib increases adiponectin secretion is not clear; it is also possible that the increase in adiponectin levels observed with imatinib in vivo could be a mere reflection of improvement in general health in CML patients.

Here we have shown that repeated exposure of nilotinib and imatinib has contrasting effects on adipocyte lipid accumulation, adipogenic mRNA expression and secretion of adiponectin. Together, these mechanisms may explain the impaired glucose and lipid metabolism observed in nilotinib-treated CML patients. Although aggressive screening for cardiovascular risk factors and cardiometabolic surveillance in CML patients has been suggested to reduce nilotinib-related cardiometabolic events [15], there is also a need for therapeutic preventive strategies. The reversal of nilotinib-induced adipocyte toxicity by telmisartan in vitro is important in this context. The metabolic beneficial effects of telmisartan have been suggested to be due to both PPARγ agonism [8] and angiotensin receptor blockade; the potential therapeutic utility of telmisartan to counter the deleterious cardiometabolic adverse effects caused by nilotinib in CML patients will now need to be evaluated by observational, as well as randomised studies. Our in vivo study has some major limitations, such as small sample size, non-availability of fasting plasma samples and lack of complete concurrent clinical data; future studies will need to address these limitations and validate these results to obtain a better understanding of nilotinib-induced metabolic adverse effects.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Peripheral blood minimal/measurable residual disease assessed in flow cytometry in acute myeloblastic leukemia

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