CANCER

# Comment on "PP2A inhibition sensitizes cancer stem cells to ABL tyrosine kinase inhibitors in BCR-ABL human leukemia"



**Danilo Perrotti[1,2]*†, Anupriya Agarwal[3]*, Claire M. Lucas[4]*, Goutham Narla[5]*, Paolo Neviani[6]*, Maria D. Odero[7]*, Peter P. Ruvolo[8]*, Nicole M. Verrills[9]***

**LB100 does not sensitize CML stem cells to tyrosine kinase inhibitor–induced apoptosis.**

When trying to identify inhibitors of Abelson helper integration site–1 (AHI-1), a scaffold protein involved in the maintenance of Janus kinase 2 (JAK2)–β-catenin survival signals in chronic myeloid leukemia (CML), Lai *et al.* found that the nonspecific protein phosphatase 2A (PP2A) inhibitor cantharidin inhibited growth of K562 CML cells by 93% (*1*). Despite PP2A being already described as a key regulator of leukemia stem cell (LSC) and progenitor cell survival and proliferation in CML (*2*, *3*), Lai and co-workers (*1*) claim that they identified PP2A as a CML LSC regulator and that, in agreement with LB100 clinical trial results (*4*), the PP2A inhibitor LB100 sensitizes CML LSCs/progenitors to ABL1 tyrosine kinase inhibitor (TKI)–induced apoptosis by suppressing PP2A-B55α–dependent JAK2–AHI-1–β-catenin–driven survival signals. However, given that only cell lines and not primary TKI-resistant normal and CML stem cells were examined and that LB100 is primarily a cytostatic drug that does not sensitize CML LSCs to TKI-mediated killing, this claim is speculative at best.

PP2A is a tumor suppressor inactivated in nearly all types of cancer, mostly by increased expression of its inhibitors SET (I2PP2A, PP2A inhibitor 2) and CIP2A, and preclinical studies have shown strong anticancer effects and very low toxicity profiles of SET-sequestering and $PP2A_A$-interacting PP2A-activating drugs (PADs) (*3*). Conversely, PP2A-inhibiting drugs (PIDs; such as LB100), developed on the basis of the highly toxic and tumor-promoting phosphatase inhibitors okadaic acid and cantharidin, induced Akt activation- and PP2A-B55δ inhibition-dependent mitotic catastrophe in tumor lines and reduced tumor growth but did not induce regression while causing grade 3 toxicity in patients enrolled in the first LB100 trial (*4*).

BCR-ABL1 inhibits PP2A activity in BCR-ABL1$^+$ cells, including K562 (*3*); hence, tyrosine kinase inhibitors (TKIs) should antagonize and not synergize with PIDs' activity. To claim that PIDs synergize with TKIs to inactivate PP2A and trigger apoptosis of TKI nonresponder (NR) chronic myeloid leukemia (CML) leukemia stem cells (LSCs) and progenitors (*1*), apoptosis and not cell viability should have been evaluated in primary healthy and CML CD34$^+$ stem and progenitor cell fractions and not in cell lines. Moreover, PP2A activity in TKI- and TKI + LB100–treated cells must also be shown. In addition, vector control and PP2Ac-shRNA (short hairpin RNA) variants of K562-IMR cells are not suitable as genetic proof of principle because expression of various proteins in parental compared to scramble-shRNA cells is not similar and is increased in scramble- or PP2Ac-shRNA–expressing K562-IMR cells. Moreover, a direct comparison of K562-IMR with K562 cells is needed to determine whether expression of factors such as AHI-1, β-catenin, and phosphorylated signal transducer and activator of transcription 5 (pSTAT5) is also altered in drug-sensitive cells.

Thirteen studies from 11 independent groups (*3*) reported that PP2A activity is reduced up to 80% in a SET-dependent but BCR-ABL1 kinase-independent (LSCs) and -dependent (progenitors) manner in TKI-sensitive and -resistant (T315I included) chronic (CP) and blastic crisis (BC) phase CML and that PP2A reactivation triggers apoptosis of nearly all CML but not healthy stem/progenitor cells in vitro and in animals (Fig. 1A). Notably, 15 Gene Expression Omnibus profiles (GDS3042/210231_x_at, GDS3042/213048_s_at, GDS3043/210231_x_at, GDS3043/213048_s_at, GDS3044/210231_x_at, GDS3044/213048_s_at, GDS3045/210231_x_at, GDS3045/213048_s_at, GDS3046/210231_x_at, GDS3046/213048_s_at, GDS3047/213048_s_at, GDS3047/210231_x_at, GDS3048/210231_x_at, GDS3048/213048_s_at, and GDS5406/213048_s_at) of untreated and TKI-treated CML cells showing that BCR-ABL1 induces SET expression also contradict the authors' findings, and the statement that unchanged SET expression in K562-IMR–PP2Ac-shRNA cells contrasts with Neviani *et al.* (*2*) is incorrect because the latter did not show that PP2A directly inhibits SET. Likewise, their claim that forskolin-induced PP2A activation depends on adenylate cyclase/cyclic adenosine monophosphate induction (*3*) is incorrect; in fact, Perrotti's group demonstrated exactly the contrary.

PADs and PIDs have dissimilar effects in CML (Fig. 1A); thus, the decision on whether it is better to restore or further reduce PP2A activity in CML trials for patients with NR-CML-CP and CML-BC and for LSC eradication in patients with durable complete molecular remission (CMR) or deep molecular remission ($MR^{4,5}$) CML rests on the ability of these drugs to effectively and selectively kill TKI-resistant quiescent LSCs (qLSCs) without inducing adverse effects. Notably, PID translatability into CML clinical settings remains speculative because LB100 and LB100 + TKI effects on normal hematopoiesis and quiescent leukemic and normal hematopoietic stem cells (HSCs) were evaluated neither in vitro nor in vivo.

Analysis of LB100/LB102 activity (*1*) indicates that these are primarily cytostatic drugs with almost null and very modest sensitizing

[1]University of Maryland School of Medicine, Baltimore, MD 21201, USA. [2]Department of Haematology, Imperial College London, London W12 0HS, UK. [3]Knight Cancer Institute, Oregon Health and Science University, Portland, OR 97239, USA. [4]University of Chester Medical School, Chester CH2 1BR, UK. [5]Department of Medicine, University of Michigan, Ann Arbor, MI 48109, USA. [6]Keck School of Medicine, University of Southern California, Los Angeles, CA 90027, USA. [7]University of Navarra, Pamplona 31009, Spain. [8]Department of Leukemia, MD Anderson Cancer Center, Houston, 77054 TX, USA. [9]School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, NSW 2308, Australia.
*These authors contributed equally to this work.
†Corresponding author. Email: dperrotti@som.umaryland.edu

**A**

| HSC and progenitor activity | PADs[1,2,3] | | PIDs[4] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CML | NBM | CML | | | PID sensitization to TKI-induced apoptosis | NBM | |
| | PAD[2] (SET-sequestering ) | | TKI (DAS) | PID (LB100) | TKI (DAS) + PID | | PID | TKI + PID |
| CFC (% decrease) | ↓ 80–95% Apoptotic 95% | N.S. (0%) | ↓50% Apoptotic 20% | ↓25% Apoptotic 30–50% | ↓60–75% Apoptotic 40–55% | 25% None | 0% | ↓ 35–50% |
| LTC-IC (%) | ↓ 70% | ↑40–50% [5] | ↓70–85% | ↓ 80% | ↓80–90% | <10% | NR | NR |
| CFC/replating (%) | ↓ 75–90% | N.S. (0%) | NR | NR | NR | | NR | NR |
| % CFSEmax (%) | ↓ 60% (h), 90% (m) | ↑ 50–80%[5] | NR | NR | NR | | NR | NR |
| In vivo survival (% alive) | 80% at 30 wk | N.S. (0%) | 0% | 0% | 20–30% at 25 wk | | NR | NR |
| In vivo hCD34+ (% recovery) | ↓ 85% | N.S. (0%) | NR | NR | NR | | NR | NR |
| In vivo hLSCs (% recovery) | ↓ 97% CD34+CD38− | N.S. (0%) | NR | NR | NR | | NR | NR |
| BCR-ABL(dTg) LT-HSC (% ) | ↓ 80% | N/A | ↑79% | ↓38.6% | ↓34.5% | None | NR | NR |
| Other signs | BCR-ABL− RT-PCR, 30 wk | None | GR1+Mac1+ ↓49% | ↑Splenomegaly GR1+Mac1+ ↑611% BCR-ABL+ infiltration | GR1+Mac1+ ↑25% | Desensitization | NR | NR |

**B**

| Figure 6 | CML LSC | | | CML-NR progenitors | | | |
|---|---|---|---|---|---|---|---|
| | DA | LB100 | LB +TKI | DA | LB100 | LB +TKI | Sensitization to TKIs |
| Viability (% alive cells) | | | | 50 | 20–30 | 10 | 15% |
| Apoptosis (% increase) | | ? | ? | 20 | 30–50 | 40–55 | Null |
| LTC-IC (% colonies) | 15–30 | 20 | 10–22 | | | | Null |
| CFC (% colonies) | | | | 50 | 75 | 25–40 | 25% |
| **Figure 8 and S8** | | | | | | | |
| LT-HSCs/LSKs (%) | 179 | 69 | 65.5 | | | | Null |
| GR1+Mac+ (% *t*1/*t*0) | | | | 51 | 711 | 125 | Null |
| GMPs/CD45.2 LK (%) | | | | 67.1 | 112.3 | 72.6 | Desensitization |
| CMPs/CD45.2 LK (%) | | | | 204 ? | 73.9 ? | 194 | Desensitization |

**Fig. 1. Effects of protein phosphatase 2A–activating drug and protein phosphatase 2A–inhibiting drug in chronic myeloid leukemia.** (**A**) Hematopoietic stem cell (HSC) and progenitor activity after protein phosphatase 2A–activating drug (PAD) or protein phosphatase 2A–inhibiting drug (PID) treatment. (1) Eight PADs were independently tested in nine laboratories. (2) Average of data from Perrotti group's publications. (3) PAD-induced apoptosis also in BCR-ABL1$^{T315I}$ chronic myeloid leukemia (CML). (4) PID values are from Lai *et al.* (*1*). (5) FTY720 and derivatives increase bone marrow (BM) homing of normal but not tumor HSCs. (**B**) Effects of LB100 on CML cell proliferation and survival. Data are from figures 6 and 8 and figure S8 of Lai *et al.* (*1*). TKI, tyrosine kinase inhibitor; CFC, colony-forming cell; LTC-IC, long-term culture-initiating cell; NR, nonresponder; N/A, not applicable; N.S, nonsignificant differences; wk, weeks; DAS, dasatinib; RT-PCR, reverse transcription polymerase chain reaction. NBM, normal bone marrow; CFSEmax, maximum staining with carboxyfluorescein succinimidyl ester; hLSC, human leukemia stem cell; CMPs, common myeloid progenitors; LSK, lineage-negative Sca+ Kit+; DA, dasatinib.

activity toward TKI-induced CML LSC and progenitor cell apoptosis, respectively. In fact, LB100/LB102 induced apoptosis in only ~40% of cells without affecting the survival of leukemic animals (*1*), and their combination with TKIs only increased apoptosis by 0 to 10% and survival of leukemic animals by 0 to 30%. Colony-forming cells (CFCs) were decreased by 25 and 70%, and long-term culture-initiating cells (LTC-ICs) were decreased by 50 to 80% and 80 to 98% in CML cells treated with LB100/LB102 alone or combined with TKIs, respectively (Fig. 1B). Conversely, leukemic TKI-resistant quiescent long-term HSC (LT-HSC) numbers remained similar in LB100 and LB100 + TKI–treated animals (Fig. 1A). This likely depends on LB100-induced cell cycle arrest and impaired in vitro and in vivo LSC and progenitor proliferation and not on LB100 sensitization to TKI-induced apoptosis. In fact, TKI-induced K562-IMR apoptosis was only marginally affected by shRNA-mediated PP2Ac inhibition, TKI increased apoptosis of LB100-treated CML progenitors by <10% (Fig. 1B), and TKI activity and not LB100 sensitization reduced granulocyte macrophage precursors (GMPs) in LB100 + TKI–treated mice (Fig. 1B), and reduced CFCs/LTC-ICs and LT-HSCs in LB100- and LB100 + TKI–treated cells and animals.

In addition, safety concerns emerge from Lai *et al.* (*1*). LB100/LB102 reduced CFCs of TKI-treated healthy progenitors by ~50% and increased leukemia-induced splenomegaly associated with aberrant infiltration of BCR-ABL1$^+$ cells in the bone marrow and liver, indicating that PIDs may enhance leukemogenesis in TKI-resistant patients and induce leukopenia in TKI-responding CML patients. Furthermore, reduced leukemia infiltration and BCR-ABL1 activity in LB100 + TKI–treated animals are not LB100-sensitizing effects but were dependent on the well-known TKI sensitivity of BV173 and SCL-tTA–BCR-ABL cells.

Mechanistically, the authors indicated that LB100-sensitive PP2A-B55α activity is essential for β-catenin–dependent induction of cell cycle and survival regulators [cyclin D1 (CCND1) and Myc] in LSCs and progenitors. However, they do not provide data indicating that PP2A-B55α activity, but not JAK2-SET–induced PP2A inhibition (*2*), is essential for β-catenin–dependent survival of CML qLSCs and proliferating progenitors. In addition, it remains unknown whether RNA interference– and LB100-induced PP2A-B55α inhibition impairs proliferation and self-renewal and induces apoptosis of CML cells through inhibition of β-catenin and CCND1 and induction of mitotic catastrophe without affecting normal hematopoiesis. Furthermore, the authors' concept that LB100 activates essential BCR-ABL1(AHI-1)–JAK2–AKT–glycogen synthase kinase-3β (GSK3β)–β-catenin–mediated CML mitogenic and survival pathways is unrealistic because inactivation of these essential CML survival signals requires PP2A activity (*2*).

Because LB100 increases Akt$^{S473/T308}$ and β-catenin$^{S45/T41}$ and reduces total β-catenin, Lai *et al.* (*1*) suggested that LB100 decreases β-catenin stability through inhibition of PP2A-B55α–dependent S45/T41 dephosphorylation. However, data showing that LB100 neither increased β-catenin$^{S45/T41}$ in LB100-treated (48 hours) K562-IMR cells nor reduced total β-catenin in K562, K562-IMR, and untreated and LB100-treated shRNA-PP2Ac–K562-IMR cells argue against the existence of an LB100-sensitive PP2A-B55α–β-catenin pathway. Thus, reduced β-catenin expression and stability in TKI- and TKI + LB100–treated progenitors depend on BCR-ABL1 activity and not PP2A inhibition. Accordingly, unchanged Akt$^{S473}$ expression in untreated and LB100-treated K562-IMR cells indicates that LB100 does not inactivate PP2A-B55α.

Notably, reduced B55α was detected in TKI- and in LB100-treated and shRNA-PP2Ac–expressing K562-IMR cells. If, as stated, PP2A-B55α–β-catenin pathway is essential for CML LSC survival (*1*), then it is expected that TKI-induced PP2A-B55α inhibition would induce LSC mitotic arrest and β-catenin inactivation and reduce CML qLSC numbers. Instead, CML qLSCs are TKI-resistant, and TKIs expand the $G_0$ cell cycle fraction without inducing M phase arrest or mitotic catastrophe. This and the notions that B55α inhibition is a dismal prognostic factor in leukemias and that PP2Ac down-regulation is dispensable for TKI-induced B55α inhibition suggest that PP2A-B55α activity is not critical for CML survival.

The correct analysis of LB100 and LB100 + TKI effects is also compromised by the unexpected response of known BCR-ABL1 and PP2A targets to TKI and LB100 treatment, respectively. This raised a series of concerns. Reportedly, wild-type and mutated BCR-ABL1, JAK2, and Akt suppress GSK3β and PP2A activities to prevent their inactivation and that of extracellular signal–regulated kinase (ERK), STAT5, SET, AHI-1, MYC, CCND2, and β-catenin. If PP2A-B55α was essential for CML, why did LB100 and/or PP2Ac down-modulation not affect BCR-ABL1, JAK2$^{Y1007/8}$, Akt$^{S473}$, β-catenin, and ERK$^{Y204}$ expression (or activity) and induce apoptosis in only 15% of cells? Why are the amounts of pJAK2, pAkt, and pERK unchanged in TKI-treated (imatinib-sensitive) K562 cells when BCR-ABL activity (4G10) and STAT5 phosphorylation are inhibited? Last, why did the expression of AHI-1 correlate with BCR-ABL expression and activity in previous work from the same group (*5*) but not in this one (*1*)?

In conclusion, the evidence that PIDs arrest proliferation of TKI-resistant CML progenitors but do not sensitize them to TKI-induced apoptosis (*1*) and that genetic PP2A inactivation or LB100 treatment does not affect CML leukemogenic potential (*6*) indicates that PP2A functions in CML as a tumor suppressor and that its inhibition is of scarce therapeutic relevance because the residual PP2A activity is required for cell cycle progression but dispensable for CML pathogenesis. Moreover, the existence and therapeutic relevance of a JAK2–AHI-1–PP2A-B55α–β-catenin survival pathway in CML LSCs is a mere speculation at this stage.

## REFERENCES AND NOTES


1. D. Lai, M. Chen, J. Su, X. Liu, K. Rothe, K. Hu, D. L. Forrest, C. J. Eaves, G. B. Morin, X. Jiang, PP2A inhibition sensitizes cancer stem cells to ABL tyrosine kinase inhibitors in BCR-ABL$^+$ human leukemia. *Sci. Transl. Med.* **10**, eaan8735 (2018).
2. P. Neviani, J. G. Harb, J. J. Oaks, R. Santhanam, C. J. Walker, J. J. Ellis, G. Ferenchak, A. M. Dorrance, C. A. Paisie, A. M. Eiring, Y. Ma, H. C. Mao, B. Zhang, M. Wunderlich, P. C. May, C. Sun, S. A. Saddoughi, J. Bielawski, W. Blum, R. B. Klisovic, J. A. Solt, J. C. Byrd, S. Volinia, J. Cortes, C. S. Huettner, S. Koschmieder, T. L. Holyoake, S. Devine, M. A. Caligiuri, C. M. Croce, R. Garzon, B. Ogretmen, R. B. Arlinghaus, C. S. Chen, R. Bittman, P. Hokland, D. C. Roy, D. Milojkovic, J. Apperley, J. M. Goldman, A. Reid, J. C. Mulloy, R. Bhatia, G. Marcucci, D. Perrotti, PP2A-activating drugs selectively eradicate TKI-resistant chronic myeloid leukemic stem cells. *J. Clin. Invest.* **123**, 4144–4157 (2013).
3. M. Ciccone, G. A. Calin, D. Perrotti, From the biology of PP2A to the PADs for therapy of hematologic malignancies. *Front. Oncol.* **5**, 21 (2015).
4. V. Chung, A. S. Mansfield, F. Braiteh, D. Richards, H. Durivage, R. S. Ungerleider, F. Johnson, J. S. Kovach, Safety, tolerability, and preliminary activity of LB-100, an inhibitor of protein phosphatase 2A, in patients with relapsed solid tumors: An open-label, dose escalation, first-in-human, phase I trial. *Clin. Cancer Res.* **23**, 3277–3284 (2017).
5. L. L. Zhou, Y. Zhao, A. Ringrose, D. DeGeer, E. Kennah, A. E.-J. Lin, G. Sheng, X.-J. Li, A. Turhan, X. Jiang, AHI-1 interacts with BCR-ABL and modulates BCR-ABL transforming activity and imatinib response of CML stem/progenitor cells. *J. Exp. Med.* **205**, 2657–2671 (2008).
6. G. Xiao, L. N. Chan, L. Klemm, D. Braas, Z. Chen, H. Geng, Q. C. Zhang, A. Aghajanirefah, K. N. Cosgun, T. Sadras, J. Lee, T. Mirzapoiazova, R. Salgia, T. Ernst, A. Hochhaus, H. Jumaa, X. Jiang, D. M. Weinstock, T. G. Graeber, M. Müschen, B-cell-specific diversion of glucose carbon utilization reveals a unique vulnerability in B cell malignancies. *Cell* **173**, 470–484. e18 (2018).


**Competing interests:** D.P. and/or P.N. are listed as inventors in US Patents 8,633,161, 9,220,706, and 8,318,812, which are held by The Ohio State University (Columbus, OH) and cover the use of PP2A-activating agents as a mechanism to eradicate LSC-driven myeloid and lymphoid leukemias.

Submitted 1 May 2018
Accepted 9 May 2019
Published 17 July 2019
10.1126/scitranslmed.aau0416

**Citation:** D. Perrotti, A. Agarwal, C. M. Lucas, G. Narla, P. Neviani, M. D. Odero, P. P. Ruvolo, N. M. Verrills, Comment on "PP2A inhibition sensitizes cancer stem cells to ABL tyrosine kinase inhibitors in BCR-ABL human leukemia". *Sci. Transl. Med.* **11**, eaau0416 (2019).

Science Translational Medicine

# Comment on "PP2A inhibition sensitizes cancer stem cells to ABL tyrosine kinase inhibitors in BCR-ABL human leukemia"

Danilo Perrotti, Anupriya Agarwal, Claire M. Lucas, Goutham Narla, Paolo Neviani, Maria D. Odero, Peter P. Ruvolo and Nicole M. Verrills

*Sci Transl Med* **11**, eaau0416.
DOI: 10.1126/scitranslmed.aau0416