

A Critical Appraisal of PP2A Inhibition and ABL Tyrosine Kinase Targeting in Leukemia Stem Cells

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Abstract. The paper by Lai et al. [1] showed that inhibition of PP-2A with LB100 or LB102 in conjunction with Tyrosine kinase Inhibitors (TKIs) was effective at targeting BCR-ABL⁺ blast cells and insensitive leukemic stem cells (LSCs). Perotti et al. [2] has severely criticized the paper by Lai et al [2]. However, Perotti et al. [2] has failed to appreciate an important principle of the role of PP-2A as a tumor suppressor and its regulation in the cell, namely that in general, PP-2A dephosphorylates and inactivates enzymes and proteins that upregulate cell proliferation, cell survival, tumorigenic and metastasizing pathways but with a limited number of enzymes and proteins, PP-2A acts to upregulate them. Thus, PP-2A is a double-edge sword and modulation of its activity must be calibrated empirically. Also, Perotti et al. [2] has misunderstood an important principle of enzyme regulation, namely that PP-2A is regulated by inhibitory and activating molecules and not through "targeting proteins" which has not been scientifically verified. It is submitted that the paper by Lai et al. [1] has strong merit as it shows for the first time that an inhibitor of PP-2A can synergize with a TKI.

In their paper, Lai et al [1] shows convincingly that less toxic derivatives of PP-2A inhibitor, Cantharidin, namely the water soluble (LB-100) and the lipid-soluble (LB102) which are in clinical trials together with Tyrosine kinase Inhibitor (TKI), Imatinib mesylate (IM) caused (i) synergistic cytotoxicity in IM-resistant cells . (ii) an increase of G2-M cell

cycle cycle arrest with key features of mitotic catastrophe in IM-resistant cells, (iii) a decrease in protein phosphorylation and expression of several key signaling proteins including, BCR-ABL, JAK2 and STATS (iv) a slight reduction in AHI-1 and β -catenin expression, (v) reduction in phosphorylation of Y204 of ERK, and Y86 of β -catenin,

component of PI3K and RAS/MAPK pathways (vi) decrease in total β -catenin with related resuction in transcripts of several β -catenin sownstream target genes, including CCND1, MYC, LEF1 and TCF1. (vii) reduction in phosphorylation of BCR-ABL, JAK2, AKT and STAT5 with knockdown of the catalytic subunit of PP-2A in the absence of LB100. Lai et al. [1] also showed that both IM or DA with LB100/LB2102 (i) significantly decreased the viability and colony growth of CD34⁺ stem/progenitor cells obtained at diagnosis from patients classified retrospectively as IM non-responders in comparison to CD34⁺ healthy bone marrow cells, (ii) significantly eliminated primitive leukemic cells, (iii) decreased the engraftment of BCR-ABL⁺ blast cell in sublethally irradiated nonobese diabetic (NOD)/severe combined immunodeficient (SCID) interleukin-2 receptor γ -chain-deficient (NSG) mice., (iii) prolonged the survival of leukemic in a transgenic mouse model of CML.

The paper by Lai et al. [1] was severely criticized by Perotti et al. [2] who argued that the claim of Lai et al. [2] was "speculative at best" on the grounds that "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). 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-B55 δ 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" [3,4].

The statements of Perotti et al. [3] are quite misleading. There is currently no scientific evidence that proves that SET (I2-PP2A, PP2A inhibitor 2) and CIP2A are specific inhibitors of PP-2A. SET has been reported to interact with PP-1 and cause the activation of PP-1 [5,6]. There is in fact very few studies that have shown that SET is a specific inhibitor of PP-2A. Preventing the interaction of SET could also result in the activation of PP-1 which is involved in large number of cellular processes, including the control of cell metabolism, cell proliferation, cell cycle, cell death, tumorigenecity and metastasis.

That CIP2A is a specific inhibitor of PP-2A is also not proven and quite speculative.

Whether CIP2A could directly inhibit PP-2A₂ at the molecular level is debatable. In order to show that CIP2A is a specific inhibitor of PP-2A₂, the effects of CIP2A on the dephosphorylation of at least five physiological substrates of PP-2A₂ and the IC₅₀ of the inhibitions must be determined. Determining the IC₅₀ if any of the inhibition of PP-2A₂ is a crucial prerequisite to determining whether the effects of CIP2A on PP-2A₂ at the molecular level is enzyme or substrate directed or both. An important question that must be answered is whether CIP2A binds and inhibits PP-2A₂ or PP-2A₀ or PP-2A₁ or all of them. Another important question that must be answered is whether CIP2A binds PP-2A₀ and/or PP-2A₁ and displaces the B' and/or B proteins. CIP2A was initially identified as a protein that binds to the A subunit of PP-2A₂ [7]. Immunoprecipitation with anti-CIP2A antibodies brought down the A, C, and B α proteins. However, subsequent studies showed that CIP2A was also bound to the B'56 and B56 proteins [8]. It is not clear why the B'56 proteins were not found by the A subunit bait in [7]. The binding of CIP2A to PP-2A that contains the B protein must be revisited. The finding that CIP2A could bind

to c-Myc amino terminus [7] suggests that the effect of CIP2A on PP-2A if any was substrate directed. However, since there was no quantification of the K_a for the interaction between CIP2A and c-Myc and IC₅₀ for the inhibition of PP-2A by CIP2A, it is impossible to determine whether the effect of CIP2A was substrate directed or enzyme directed or both. As it stands now, all one can conclude is that CIP2A interacted with the B' proteins but cannot say whether CIP2A is an inhibitor of PP-2A or not.

The concept of PP-2A [9] being permanently inhibited by inhibitory proteins as opposed to a periodic activating mechanism is difficult to reconcile with the tumor suppressor activity of PP-2A₂ as logic would dictate that permanent inhibition of PP-2A₂ would essentially lead to cell transformation and tumorigenic competency, as has been shown with the inhibition of PP-2A₂ by SV 40 small t antigen [10]. To account for the multiplicity of PP-2A substrates in the cell, the concept of B' and B proteins as targeting proteins have been generally accepted as a dogma. However, the concept of B' and B proteins as targeting proteins cannot explain all the paradoxical effects of PP-2A as a key regulator of signaling pathways in normal cells nor can it explain all the paradoxical effects as a key

controller of enzymes and proteins that are components of the cell transformation and tumorigenic competency pathways. On the other hand, alternative mechanism of PP-2A regulation involving B' inhibitory proteins and other inhibitory proteins and B activating proteins and other activating proteins can explain the paradoxical effects of PP-2A and the multiplicity of PP-2A substrates. In the Inhibitory and Activating (IAA) mechanism of PP-2A regulation, PP-2A in the form of PP-2A₂ which consists of the A and C subunits is like any other enzymes that are regulated by inhibitory and activating molecules. Inhibitory molecules like the B' proteins can be regulated by phosphorylation and activating molecules like the B proteins can also be regulated by phosphorylation. While it may seem paradoxical that PP2A₂ is involved in both the activation and inhibition of antagonistic cell signaling pathways, the presence of an inhibitory or activating protein will determine whether PP-2A can dephosphorylate its substrates or not (Figure 1). For example, PKA is activated by cAMP and inhibited by PKI, and Cdk1 is activated by the Cyclins and inhibited by INKs. PP-1_I is inhibited by phosphorylation of PP-1-I₁ and activated by phosphorylation of PP-1-I-2. The principles of protein kinase and Protein phosphatase regulations by inhibitory and

activating molecules are well established whereas the concept of PP-2A regulation by targeting proteins does not have a scientific foundation.

In conclusion, it is submitted that the declarations of Perotti et al [2] are misplaced and misleading because they were based on the assumption that SET and CIP2A are specific inhibitors of PP-2A which is incorrect. Because PP-2A is a double edge sword that in general acts as a tumor suppressor but in a few instances can act as an oncogene, exploration of both its activation and inhibition in human cancer is warranted. A thorough understanding of PP-2A regulation by inhibitory and activating molecules as opposed to the concept of PP-2A regulation by "targeting proteins" which has no scientific foundation must be revisited in order to account for the paradoxical functions of PP-2A as a tumor suppressor in most instances but also as an oncogene in a few cases [Tung, H.Y.L., Manuscript in preparation].



**Nature and Regulation of PP-2A₂
by B' Inhibitory and B Activating Proteins**

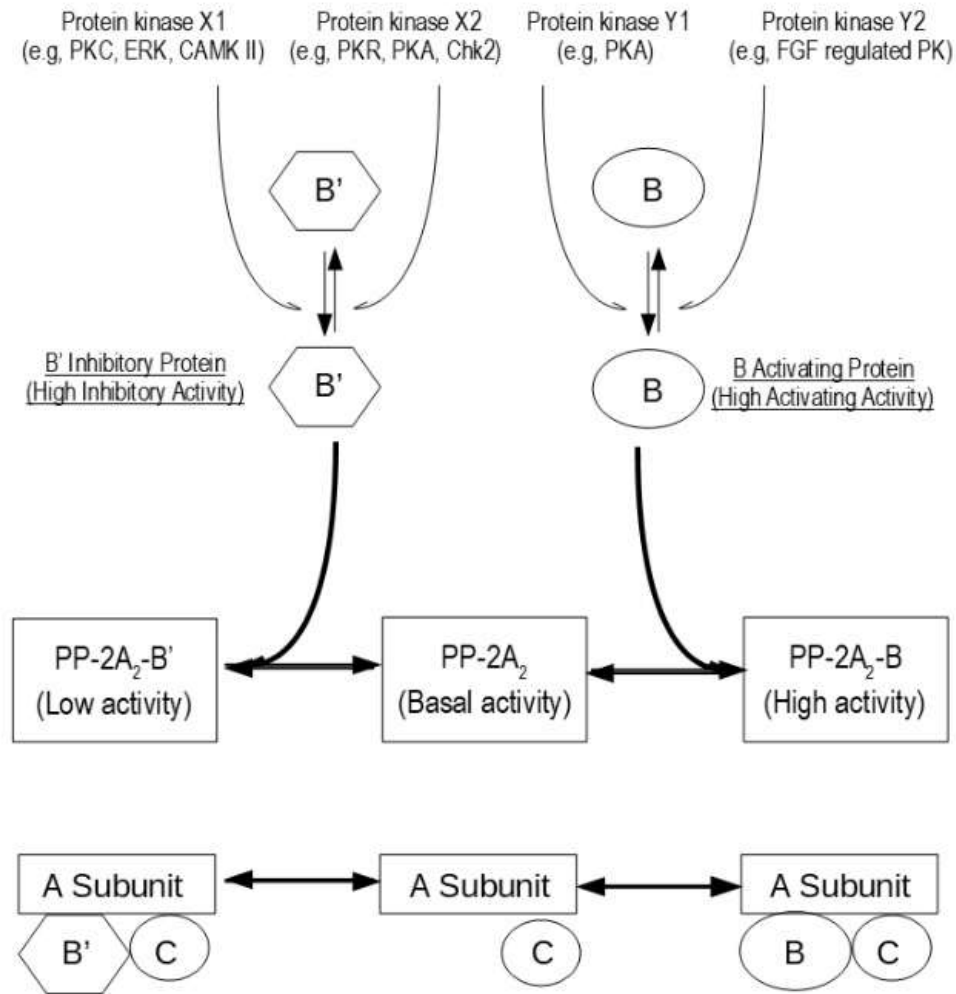


Figure 1. Model for the regulation of PP-2A₂ by B' inhibitory proteins and B activating proteins.

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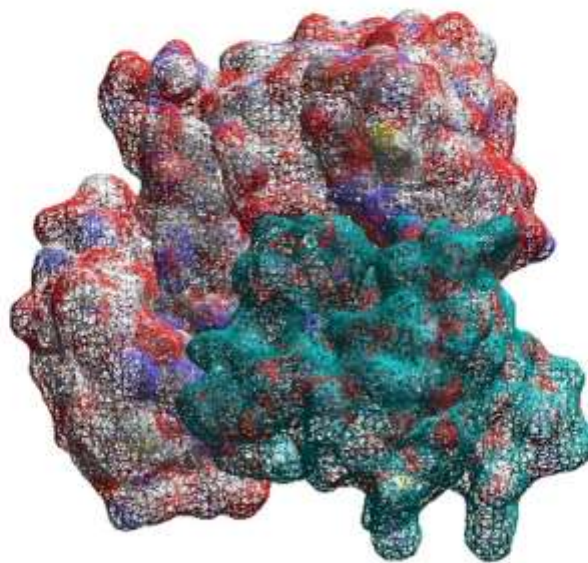
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