SETting OP449 into the PP2A-Activating-Drug family

Running Title: Anti-leukemic activity of OP449

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SUMMARY

The PP2A tumor suppressor is inactivated in different leukemias through the activity of its endogenous inhibitors (e.g. SET), which are aberrantly regulated by oncogenic tyrosine kinases. Like other effective and non-toxic PP2A-activating-drugs (PADs), OP449 inhibits SET and impairs leukemogenesis. This, further supports the immediate use of PADs in leukemia patients.

TEXT

In this issue of Clinical Cancer Research, Agarwal and colleagues(1) identified OP449 as a new PAD (PP2A activating Drug) with anti-leukemic activity toward tyrosine kinase inhibitor (TKI) -resistant Chronic Myeloid Leukemia (CML) and Acute Myeloid Leukemia (AML) cell lines and primary patient samples. OP449 is a novel, cell-penetrating peptide able to interact with the SET oncoprotein.

SET is a potent endogenous inhibitor of the tumor suppressor protein phosphatase 2A (PP2A)(2) (Fig. 1). This phosphatase is known to be a negative regulator of several survival and proliferation pathways that are frequently activated in malignancies as a result of aberrant activation of oncogenic kinases(2). In light of several recent reports showing that PP2A is frequently functionally inhibited in numerous solid tumors and leukemias(2) there is a
considerable interest in the development of compounds that can induce the activity of PP2A and counteract oncogenic signals. Because of the complexity of the network of PP2A regulatory subunits and binding partners (reviewed in (2)), inhibition of the activity of PP2A can be achieved at multiple levels: for example through loss of its structural A subunit, mutations of one or more of its several interchangeable regulatory B subunits, or through alterations of its endogenous inhibitors and binding partners (e.g., SET, CIP2A, SETBP1)(2).

In CML, AML, JAK2V617F+ myeloproliferative disorders (MPDs), and Philadelphia-positive B-cell Acute Lymphoblastic Leukemia (Ph+ B-ALL), inhibition of PP2A is essential for leukemogenesis(2) (Fig. 1). PP2A is functionally inhibited as a consequence of the overexpression and/or post-translational modifications (e.g. phosphorylation) of SET, which results in an overall inhibition of PP2A phosphatase activity in both leukemic progenitors(3-7) and stem cells(8). Genetic (SET shRNA-mediated downregulation) or pharmacologic (i.e. PADs) restoration of PP2A activity halts malignant cell survival and proliferation both in vitro and in different animal models of leukemia(1, 3, 4, 6, 8, 9).

PADs that like the synthetic peptide OP449 directly bind SET and/or interfere with its PP2A inhibiting function have not only strong pro-apoptotic activities towards leukemic stem/progenitor cells but also a desirable non-toxic profile in ex vivo primary cells and long-term animal studies(1, 2, 9). In this regard, it is noteworthy to mention that the orally available sphingosine analog FTY720 (Fingolimod, Gilenya) is a PAD with strong anti-leukemic activity and that its adverse effects in relapsing multiple sclerosis (MS) patients (i.e. bradycardia and atrioventricular conduction block) are not only clinically manageable and observed at the time of FTY720 therapy initiation only, but they can also be avoided with the use of FTY720 non-immunosuppressive derivatives (e.g. OSU-2S and S-FTY720-regioisomer), which like FTY720 are also active against CML stem cells and CD34+ progenitors from CML patients refractory to TKIs(2, 4, 7, 8).
Although early diagnosed CML in chronic phase is currently very well manageable with TKIs (e.g. imatinib, nilotinib, dasatinib and ponatinib), a small but significant percentage of these patients still develop resistance or intolerance to one or more TKIs and, likely, progress to the still fatal blastic phase of the disease(10). Conversely, the prognosis of AML is still very dismal and the current therapeutic options are greatly limited due to the vast heterogeneity of the disease and, mostly, rely on standard chemotherapy and, ultimately, bone marrow transplantation(11). Thus, the use of PADs, which antagonize both oncogenic kinase –dependent and –independent signals while sparing normal hematopoiesis(2), represents a very promising class of anti-cancer drugs that can be used alone or in association with either kinase inhibitors or traditional chemotherapy.

PADs are very effective and selective drugs in several types of tumor demonstrated to have low PP2A activity(2). The small peptide OP449 (also known as COG449) has also been previously reported to exert anti-neoplastic potential in CLL and non-Hodgkin lymphoma cells, likely trough direct binding to SET and the release of SET-mediated PP2A inhibition(2, 9, 12). Indeed, activation of PP2A by OP449 results also in inhibition of the growth of BCR-ABL1-expressing human and mouse cell lines, and induction of apoptosis in TKI-sensitive and TKI-resistant cells (T315I and E255V/T315I BCR-ABL1). Interestingly, when used in combination with first-generation (imatinib) and second-generation (nilotinib, dasatinib) TKIs, OP449 had a synergistic effect in reducing viability and clonogenic potential of leukemia cell lines and primary CD34+ CML (harboring wild-type or mutant BCR-ABL1) progenitors with little or no effect on hematopoietic progenitors from healthy individuals(1).

Mimicking the anti-leukemic effect observed in CML, and confirming the notion that PP2A activity is reduced in a SET-expression dependent manner also in AML cells(2, 5, 6), OP449 efficiently induced PP2A activity and impacted the survival of several AML cell lines with different genetic lesions, and of primary AML blasts characterized by SET upregulation(1). Notably, OP449 showed the greatest killing activity toward MOLM-14 cells, which harbors the FLT3-ITD
mutation, a notorious poor prognostic factor in AML(1). Similarly, OP449 also suppressed leukemia cell growth in mouse xenografts model of AML(1). As described for other PADs (e.g. 1,9-dideoxy-forskolin, FTY720)(3, 4), the OP449 anti-leukemic activity correlated with inhibition of major signal transducers (e.g. STAT5, AKT, ERK) found activated in AML cells(1) and also described as direct and/or indirect targets of PP2A phosphatase activity(2) (Fig. 1).

Finally, the authors assessed the effect of combinations of OP449 with known FLT3 and JAK kinase inhibitors in MOLM-14 and CMK cells, harboring FLT3-ITD and JAK3A572V mutations, respectively, and found that the drug combination (TKI+PAD) had a synergistic cytotoxic effect on these leukemic cells(1), further indicating that the combination of kinase inhibitors and PP2A-activating drugs may be a valid therapeutic option for these acute leukemias (Fig. 1). Consistent with this observation, it has been reported that the combination of imatinib and FTY720 exerts a more powerful pro-apoptotic effect toward primary CD34+ progenitors from CML chronic phase and blast crisis patients(8). The ability of PADs to potentiate the pro-apoptotic effect of TKIs, and vice versa, is not surprising. In fact, PADs-induced PP2A reactivation not only results in silencing and/or degradation of oncogenic tyrosine kinases (e.g. BCR-ABL1, Jak2, KIT)(2, 7), but it can also permanently switch-off signaling pathways found aberrantly activated in all acute myeloid and lymphoid leukemias(2, 11).

Thus, the data presented in the current issue of Clinical Cancer Research by Agarwal and colleagues(1) reinforces the central role played by PP2A as a central regulator of cell homeostasis capable of restraining aberrant proliferating and survival signals generated by the activity of different oncogenic kinases. Moreover, their observation that the SET antagonist OP449 impairs myeloid leukemogenesis(1) totally support both the immediate recognition of this and other PADs as clinically relevant anticancer drugs that need to be introduced into therapeutic protocols for patients with hematopoietic and non-hematopoietic malignancies characterized by functional loss of the PP2A tumor suppressor.
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REFERENCES.


**Figure Legend**

**Figure 1.** *PP2A and SET-binding PADs in leukemias.*

Regulation of the PP2A tumor suppressor in leukemias (i.e. CML, AML, MPN, Ph⁺ ALL) and possible use of PP2A Activating Drugs (PADs; e.g. OP449, FTY720, OSU-2S) in combination with tyrosine kinase inhibitors (TKIs) or conventional chemotherapy for treating leukemias characterized by SET-dependent inactivation of PP2A. OP449 and other PADs exert their anti-leukemic activity upon interaction with SET and inhibition of its ability to interact with PP2A catalytic subunit (PP2Ac) and inhibit PP2A phosphatase activity.
Figure 1:

Leukemia

- MPN
- Jak2V617F
- AML
- FLT3-ITD
- JAK34572V
- NRASQ61L
- KITD816V

Combination therapy

- CML, Ph+ALL
- BCR-ABL1

Oncogene-dependent activation of proliferation, self-renewal and survival pathways

Chemotherapy /TKIs

- FLT3-ITD
- JAK34572V
- NRASQ61L
- KITD816V

- TKIs
- TKIs

- BCR-ABL1
- Jak2V617F

PP2A-activating drugs (e.g., OP449, FTY720, OSU-2S)

PP2A-dependent inhibition of oncogenic signaling pathways

AKT
ERK
β-catenin
STAT5
Myc
Jak2
t wt & mut.
Bcl2
MNK
eIF4E
BCR-ABL1
t wt & mut.
Lyn

Combination therapy

- FLT3-ITD
- JAK34572V
- NRASQ61L
- KITD816V

- BCR-ABL1
- Jak2V617F

PP2A ON

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