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The use of Plk1 inhibitors in ovarian cancer and molecular understanding of the pathological role of its coactivator Bora

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Introduction: Polo-like kinase 1 (Plk1) is an attractive mitotic target to treat cancer. A variety of anti-Plk1 agents are presently in clinical trials with successful results. Nonetheless, the advantages of Plk1-based monotherapy versus the standard chemotherapy has proven limited in ovarian cancer (NCT01121406). Attractive possibilities would be to combine Plk1 inhibitors with DNA damaging agents or with DNA-repair or homologous recombination machinery proteins such as PARP inhibitors. To date, the use of Plk1 ATP-competitive inhibitors have been associated to dose-limiting toxicity and low specificity (related for example to the Plk3 tumor suppressor-related function, Yang *et al.,* 2008). Hence, to find alternative strategies to treat Plk1 with less adverse effects is paramount. An appealing approach is to target the polo-box domain (PDB) as it is a structurally unique domain, optimizing the selectivity of the inhibitor. However, the exploit of the geometrical and shape arrangement of this part of the structure is still needed. As an alternative, we propose to target the C-terminal part of Plk1 by its main coactivator, Bora (*Aurora Borealis*) since Bora binds to the Plk1-PDB in a Cdk-dependent manner (Bruinsma et al., 2015), to change the conformational status of Plk1 and activate the kinase. In addition, Bora has been reported to be essential in G2/M DNA damage checkpoint recovery to activate Plk1 and promote mitosis (Parrilla et al., 2016).

Objectives: The objectives of the work include (i) the potential use of Plk1 inhibitors in combination with other classical drugs to achieve a better clinical outcome in ovarian cancer patients; (ii) to provide new insights into the mechanistic role of Bora in cancer and explore whether it could represent a promising novel therapeutic approach for cancer treatment.

Methods*: In silico* data was obtained from The Cancer Genome Atlas (TCGA) database. *In vitro* studies were carried out using a wide panel of ovarian cancer cell lines with different histology and mutational profile. Human tissue-samples were obtained from benign and ovarian cancer patients from the Vall d'Hebron Hospital in Barcelona, Spain.

Results: Here, we report that the combination of different Plk1 inhibitors with other chemotherapeutic drugs (namely, Cisplatin and Olaparib) increased the apoptosis and reduced the proliferation of the ovarian cancer cells lines, enhancing the role of the chemotherapy in a wide panel of ovarian cancer cell lines. On the other hand, i*n silico* data revealed that Bora is overexpressed in several kind of cancers compared to normal tissues, including ovarian cancer. Using human tissue-samples from ovarian cancer patients, we showed that Bora is overexpressed in these tumours compared to benign samples both at mRNA and protein level. The upregulation was significantly higher in advance stages of the disease and correlated with poor survival. *In vitro*, the downregulation of Bora using lentiviral particles led to a reduction in proliferation associated with an increase in the apoptosis rate in the ovarian cancer cell lines tested. In addition, our results indicated that Bora is essential for cell survival since we were unable to obtain Bora knock-out cells was through CRISPR/Cas 9 technology. Nevertheless, SK-OV-3 knock-down clones reproduced the effects in lowering cell proliferation and delaying the cell cycle, observed by other means (siRNA-based approaches for instance).

Conclusions: Combinational therapy of Plk1 inhibitors with other classical chemotherapeutic agents currently used in the clinics show a potential benefit *in vitro*, although further characterization of the synergistic effects is still needed. Furthermore, targeting Plk1 activity through its main co-activator Bora could represent a novel approach as it is also overexpressed in cancer and its downregulation reduces the proliferation of the ovarian cancer cell lines. Future steps are directed to perform high throughput studies by gene expression profiling to shed light on the

underlying mechanisms of Bora depletion and its possible role as therapeutic tool.

References

NCT01121406: *BI 6727 (Volasertib) Randomised Trial in Ovarian Cancer*. Clinicaltrial.gov

Yang et al., *Polo-like kinase 3 functions as a tumor supressor and is a negative regulator of hypoxia-inducible factor-1 alpha under hypoxic conditions.* Cancer Res. 2008 Jun 1;68(11):407785

Bruinsma et al., *Spatial separation of Plk1 phosphorylation and activity.* Front Oncol. 2015 Jun 10;5:132.

Parrilla et al., *Mitotic entry: The interplay between Cdk1, Plk1 and Bora.* Cell cycle. 2016 Dec;15(23):3177-3182

TCGA Data Portal – NIH *https://tcga-data.nci.nih.gov*