Germline and somatic *PTPN11* mutations confer diverse sensitivity to the SHP099 allosteric SHP2 inhibitor depending on the impact of individual changes on the inactive state of the enzyme

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Germline *PTPN11* variants cause Noonan syndrome (NS) and Noonan syndrome with multiple lentigines (NSML), while somatic changes occur in childhood leukemia. Based on the role of SHP2, encoded by *PTPN11*, in development and hematopoiesis, this enzyme represents an excellent target for the treatment of developmental disorders and cancer. Targeting the active site of SHP2, however, is challenging. Recently, a novel class of allosteric inhibitors have been developed but their efficacy in the presence of a *PTPN11* mutation is still unclear.

Aims of the study are (i) to characterize a panel of *PTPN11* mutations (n=14) affecting five residues in the catalytic domain, and (ii) to assess the ability of the allosteric SHP099 inhibitor to prevent catalysis.

Structural modelling started from SHP2's structure in its auto-inhibited state. Catalytic activity was measured on recombinant proteins using DiFMUP as a substrate in basal/stimulated conditions, w/wo SHP099. Substrate specificity was assessed using a phosphopeptide array. ERK activation was explored in transiently-transfected cells.

In line with structural findings, biochemical and cellular data showed that variants underlying NS and leukemia variably enhance SHP2's catalytic activity and signaling through RAS, with a more robust effect for the latter class of mutations, while substrate specificity does not change substantially. As expected, all variants causing NSML perturb catalysis. A few germline mutations underlying NS behave as somatic changes and vice versa, indicating that catalysis *per se* cannot allow associating individual variants to a specific disease. Activating variants also affect the intrinsic catalytic activity of the enzyme, indicating that counteracting effects operate to establish the overall impact of each lesion. Finally, SHP099 was shown to be largely ineffective against oncogenic mutants and only partially effective against NS mutants, except for the recurrent p.A72S change. In line with that, higher doses of inhibitor were needed to inhibit oncogenic compared to NS mutants in HEK293T cells.

Overall, our findings exemplify the concept of allelic heterogeneity and establish that allosteric inhibitors are not effective against mutations affecting the stability of the inactive state of the enzyme.

Keywords: Noonan syndrome, SHP2, allosteric inhibitors.