Abstract: S152

Title: COMBINATORY THERAPY OF ASCIMINIB AND PONATINIB WAS EFFECTIVE IN THE TREATMENT OF BLAST PHASE CML CDX MODEL

Abstract Type: Oral Presentation

Session Title: CML biology and translational research

Background:

CML blast phase (BC-CML) is a heterogeneous disease carrying more lesions than *BCR::ABL1*. Although patients who progressed to BC-CML during tyrosine kinase inhibitor (TKI) therapy optimally undergo intensive treatment and transplantation their outcome is unsatisfactory. Therefore, there is a need for personalised therapy with novel drugs.

Aims:

To create CDX model of clonal evolution in BC-CML and to test combinatory therapy with the most potent TKI ponatinib (PON), STAMP-inhibitor asciminib (ASC), and BCL-2 inhibitor venetoclax (VEN).

Methods:

Five imatinib/ponatinib cross-resistant KCL-22 clones were established and characterized for mutations in *BCR::ABL1* and other cancer-related genes. IC_{50} values for each tested drug were measured. For *in vivo* experiments equal number of cells of individual clones were mixed and subcutaneously xenotransplanted into NOD-SCID-gamma mice ($5x10^6$ cells/mouse; n=42). Mice were randomly divided into 7 groups according to the treatment: 1) control; 2) PON 25 mg/kg b.w.; 3) ASC 30 mg/kg b.w.; 4) VEN 50 mg/kg b.w.; 5) PON+VEN; 6) ASC+VEN; 7) PON+ASC. Treatment was administered perorally once a day after tumor detection (diameter 5 mm in one direction). At the end of the experiment (tumor diameter ≥20 mm in one direction or skin necrosis) mice were euthanised and tumors were homogenised using 70μm cell strainer. Nucleic acids were isolated and NGS mutational analysis of *BCR::ABL1* kinase domain and panel sequencing were performed.

Results:

In the control group of mice, the most proliferated clone during tumor growth was F5 (BCR::ABL1-T315I, -G250E, SETD1B-G1963fs) followed by clones with proliferation fitness in a descendent order E72 (BCR::ABL1-T315I, -Y253H, ZRSR2-R451H) > C7 (BCR::ABL1-T315I, -H396R) > D9 (BCR::ABL1-T315I, -E255V, IKZF1-E383G). Clone D8 (BCR::ABL1-T315I, -I418T, -H396R) did not proliferate. *In vitro* IC_{50} values were of predictive value for ASC and PON treatment of the CDX model. Proliferation of clones during ASC therapy *in vivo* were in descendent order C7>F5>E72>D8>D9, while inversed effect on proliferation was found on PON D9>E72>D8>F5>C7. During combinatory PON+ASC therapy, tumors were unmeasurable during daily drugs administration because of a synergy effect (clones D9 and D8 were sensitive to ASC; clones C7 and F5 were sensitive to PON) and an additive effect of both drugs on the clone E72. Clonal proliferation *in vivo* during VEN therapy was in the descendent order C7>E72>F5>D8>D9 and tumor growth was significantly delayed compared to monotherapy with ASC and PON, respectively ($p>0.001$). Combination of PON+VEN also delayed the tumor growth with strong additive effect on the clone D8, which did not grow.

Summary/Conclusion: All five established imatinib/ponatinib cross-resistant clones originated from T315I clone of the KCL-22 BC-CML cell line acquired additional mutations in *BCR::ABL1* and in 3 clones also in *IKZF1*, *SETD1B*, and *ZRSR2*. Although artificially prepared mixture of cross-resistant clones represented highly resistant model of BC-CML, combinatory therapy with asciminib and ponatinib was potent to suppress tumor growth to immeasurability. The IC_{50} values of asciminib and ponatinib for each clone were highly predictive and corresponded to *in vivo* observation of monotherapy.

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Keywords: CML blast crisis, Animal model, Asciminib, Venetoclax