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PARP1 inhibitor eliminated imatinib-refractory chronic myeloid leukemia cells in bone marrow microenvironment conditions

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Treatment with tyrosine kinase inhibitors (TKIs) results in remission and prolongation of survival but fails to eliminate the leukemia stem cells (LSCs) responsible for disease development and propagation in most chronic myeloid leukemia (CML) patients [1]. In concordance discontinuation of TKI therapy usually leads to rapid leukemia relapse. Most patients require continued treatment to prevent relapse, with associated risk of relapse, toxicity, teratogenic effects, financial burden, and noncompliance.

Bone marrow niche microenvironment (BMM) is known to play an important role in regulating LSC maintenance and in protecting LSCs from anti-leukemia treatment [2]. It has been reported that CML-CP cells survive TKI treatment in bone marrow niche, which may contribute to persistent presence of residual CML-CP cells in TKI-treated patients. Therefore development of strategies eradicating LSCs from BMM is a critical area of investigation in CML to increase the proportion of CML patients achieving a cure.

We reported before that CML-CP LSCs and LPCs accumulate high numbers of potentially lethal DNA double-strand breaks (DSBs) [3,4]. Moreover, BCR-ABL1 kinase causes downregulation of BRCA1 and DNA-PKcs proteins, which play key roles in two major DSB repair pathways: homologous recombination (HR) and classical non-homologous end-joining (cNHEJ), making TKI-naïve CML cells susceptible to synthetic lethality triggered by PARP1 inhibitors [5–9]. On the other hand, TKI-mediated inhibition of BCR-ABL1 kinase caused downregulation of RAD51 (key role in HR) and LIG4 (key role in cNHEJ) were associated with PARP1 inhibitor-mediated synthetic lethality [8]. Altogether, TKI-naïve and TKI-treated CML cells display specific defects in DSB repair and are sensitive to synthetic lethality induced by PARP1 inhibitors in standard in vitro liquid culture conditions in normoxia [9].

To test if PARP1 inhibitor is effective against CML cells in the conditions mimicking BMM HS-5 human bone marrow stromal cell line cells (secrete significant levels of G-CSF, GM-

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CSF, M-CSF, SCF, MIP1 α , IL-6, IL-8, and IL-11 to support the growth of hematopoietic stem cells [10]) were co-cultured for 24 hrs under hypoxia (1% O₂) with imatinib-sensitive primary Lin⁻CD34⁺ CML-CP cells collected at diagnosis (1:1 cell ratio). Then BCR-ABL1 kinase inhibitor imatinib and/or PARP1 inhibitor talazoparib were added for 3 days followed by evaluation of DSBs by γ -H2AX immunofluorescence and neutral comet assay, proliferation by clonogenic activity, and of leukemia engraftment in humanized immunodeficient NRGS mice (Fig. 1).

As expected [11,12] Lin-CD34⁺ CML-CP cells were protected from imatinib in BMM-like conditions (Fig. 2A-C). Talazoparib modestly increased the number of DSBs and decreased clonogenic activity of these cells in concordance with our report that CML cells are BRCA1 and DNA-PKcs -deficient [9] and other report [13] that hypoxia promotes HR-deficiency and sensitivity to PARP1 inhibitor-mediated synthetic lethality.

Remarkably, talazoparib increased DSBs and inhibited clonogenic potential of imatinib-refractory CML-CP cells. Moreover, talazoparib reduced the number of imatinib-refractory CML-CP cells (Fig. 2D), including Lin-CD34⁺ cells (Fig. 2E) capable to engraft in immunodeficient mice. However, the effect of PARP1 inhibitor against imatinib-treated CML-CP cells in BMM-like conditions appears less profound than that previously reported in the absence of stromal cells in normoxia [9].

In conclusion, we postulate that while BMM protects CML-CP cells from TKIs, it provides limited protection against PARP1 inhibitors. Therefore PARP1 inhibitors could be applied together with TKIs to target TKI-refractory CML-CP cells in BMM, which should reduce the risk of disease relapse. The anti-CML effect of imatinib + olaparib in BMM conditions, however, is not as robust as that observed in normoxia implicating niche-specific protective impact, which requires further investigation.

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Figure 1. Experimental protocol.

CML-CP Lin-CD34+ cells were plated on HS-5 cells monolayer (1:1 ratio) and incubated in hypoxia (1% O₂) with diluents (Control), imatinib (I, 1 μ M), talazoparib (T, 100 nM), and imatinib + talazoparib. Leukemia cells were then collected and tested. DSBs were detected in CML-CP cells by neutral comet assay and γ -H2AX immunofluorescence as described before [4,14]. CML-CP cells were plated in methylcellulose and colonies were counted after 7 days as described before [4]. In addition, 2×10^6 CML-CP cells were injected i.v. in to the total body irradiated (600 cGy) mice and leukemia engraftment was assessed 8 weeks later by immunofluorescence [human CD45 (hCD45+), hCD45+Lin-CD34+] in peripheral blood leukocytes (PBL), splenocytes (SPL) and bone marrow cells (BMC) as described before [9].

Figure 2. Talazoparib induced DSBs and eliminated imatinib-refractory CML-CP primary cells in the presence of hematopoietic stromal cells and hypoxia.

(A) DNA damage examined by comet assay after 24h of treatment as described before [14]. Olive tail moment was determined in 3 CML-CP samples (each sample in triplicate) using ImageJ/Open Comet software. Results represent mean value of olive tail moment presented as % of control $\pm$ SD. (B) Level of γ -H2AX in live Ki67+ cells was analyzed after 24h of treatment and is presented as mean fluorescence intensity $\pm$ SD from 3 CML-CP samples (each sample in triplicate) as described before [4]. (C) Clonogenic potential determined by 7 day colony formation assay as described before [9]. Number of colonies calculated for 6 different patient samples (each in triplicate) is presented as mean % of control $\pm$ SD. (D) CML-CP cells were detected by immunofluorescent staining with anti-human CD45, anti-lineage markers (Lin), and anti-CD34 antibodies. Mean number $\pm$ SD of hCD45+ cells per 10^6 PBL, SPL and BMC from 5 mice/group. (E) Mean number $\pm$ SD of hCD45+Lin-CD34+ cells in BMC from 5 mice/group; representative dot plots are shown. Student's two tailed t-test was used to compare results from Imatinib and Talazoparib+Imatinib treated cells; * $p < 0.05$.