Increased sensitivity to olaparib by BRCA1/2 knockdown using a CRISPR/Cas9-mediated knock-in method in pancreatic cancer cell lines.

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Introduction
Pancreatic cancer is one of the most incurable disease. Different chemotherapy regimens can be effective, but all treatments showed no real benefits without toxicity in both adjuvant and metastatic settings (Lambert et al., Ther Adv Med Oncol, 2019). Olaparib, a PARP inhibitor, is used as maintenance treatment in patient with metastatic pancreatic adenocarcinoma bearing a germline BRCA1/2 mutation and did not undergo progression during at least 16 weeks of a prior platinum-based chemotherapy regimen (Golan et al., N Engl J Med, 2019). However, germline BRCA mutations have been described in less than 10% of patients with pancreatic adenocarcinoma (Wong et al., Cancer Manag Res, 2020).

The objective of our study was to determine whether inducing a BRCA1/2 mutation with a CRISPR/Cas9 method in pancreatic cells may restore sensitivity to olaparib.

Methods
We developed a CRISPR/Cas9-mediated knock-in technique to induce deleterious BRCA1 or BRCA2 mutations in pancreatic cancer cell lines. The c.7650T>C (p.Glu2553*) and c.2133C>A (p.Cys711*) mutations were selected to obtain truncated and non-functional BRCA1 and BRCA2 proteins respectively. A CRISPR/Cas9 system as a ribonucleoprotein (RNP) complex was assembled for each mutation with two gRNAs (guide RNAs) (for BRCA1: TCTAGGCAACGCGCTCTCATCG and CTTAAGGATAGGGTCGCTG; for BRCA2: TCTCTCCCCTCTCTCTCTCTC and TCTCTCATCATCATGACCGA) and was transfected into two PDAC cell lines (Capan-2 and T3M4) and into a breast cancer cell line (MC7) as control. Off-target mutations were confirmed by ddPCR and NGS. Off-target effects were predicted by the CrispGold tool and the CRISPR LIFEPi® tool. A crystal violet assay was conducted to obtain olaparib IC50 (µM). Early and late apoptosis and necrosis were detected by flow cytometry after staining with Annexin V and propidium iodide.

Results

1. Efficacy of CRISPR/Cas9-mediated knock-in to induce target mutations.

Alleric frequencies (AF) obtained by ddPCR analysis and given for pools of BRCA-knockdown (KD) cells after transfection by each CRISPR/Cas9 system designed.

2. Off-target sites predicted

No off-target sites were predicted by the CRISPR LIFEPi® tool and no off-target in functional sites were identified by the CrispGold tool.

3. Increased apoptosis after treatment by olaparib for BRCA-KD cells.

Actual flow cytometry data of early apoptosis, late apoptosis and necrosis, without treatment (a) and after 72 hours of treatment with 40µM olaparib (b), for Capan-2 WT cells (A), Capan-2 BRCA-KD cells (B), T3M4 WT cells (C), T3M4 BRCA-KD cells (D), MCF7 WT cells (E), and MCF7 BRCA-KD cells (F), after staining with Annexin V and propidium iodide.

4. Increased sensitivity of BRCA-KD cell lines to olaparib.

Cytotoxic effect of olaparib on transfected cells with a CRISPR/Cas9 system (BRCA1 or BRCA2 KD cells), non-transfected (wild-type (WT) cells), and transfected cells with a CRISPR/Cas9 system without donor sequences (control cells) for Capan-2 (A), T3M4 (B), and MCF7 cell lines (C) after 72h of exposure.

Conclusion
We designed and validated a CRISPR/Cas9 system to induce in vitro deleterious BRCA1 or BRCA2 mutations by knock-in in pancreatic cancer cell-lines. The olaparib sensitivity of modified cells was increased compared to wild-type cells. This strategy might offer an attractive therapeutic alternative for the management of patients with pancreatic cancer. Further investigations are needed to resolve CRISPR addressing issues, yield, and toxicity in in vivo models.

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The authors declare no conflict of interest.