

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

Andréa Witz^{1*}, Julie Dardare¹, Marie Husson¹, Aurélie Francois¹, Pauline Gilson¹, Jean-Louis Merlin¹, Alexandre Harlé¹

¹ Université de Lorraine, CNRS UMR 7039 CRAN, Service de Biopathologie, Institut de Cancérologie de Lorraine, F-54519, Vandœuvre-lès-Nancy, France.

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).

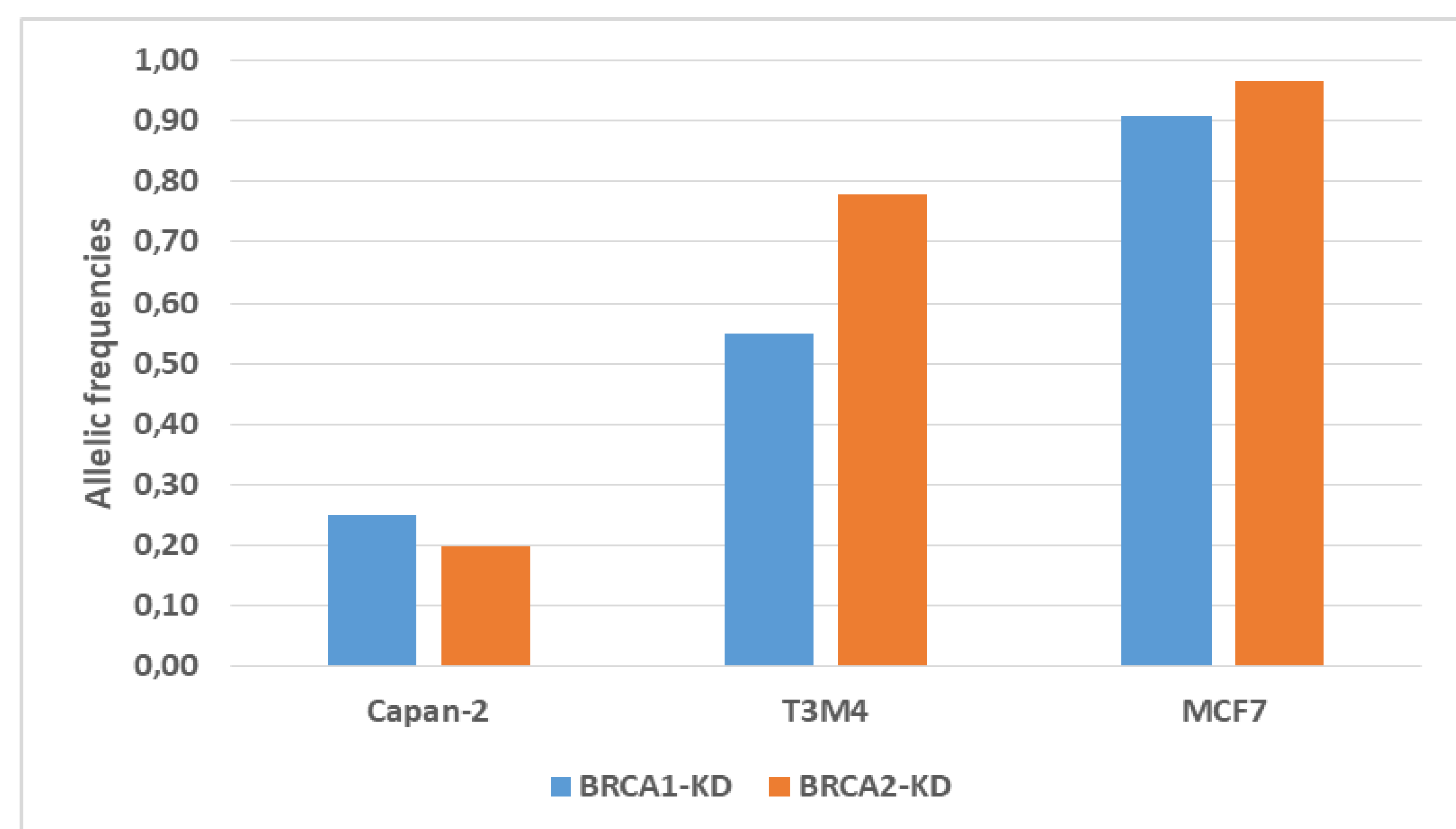
The objective of our study was to determine whether inducing a *BRCA1/2* mutation with a CRISPR/Cas9-method in pancreatic cells may restore sensitivity to olaparib.

Methods

We developed a CRISPR/Cas9-mediated knock-in technique to induce deleterious *BRCA1* or *BRCA2* mutations in pancreatic cancer cell lines. The c.763G>T p.(Glu255*) 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*: CTGAGAAGCGTGCAGCTGAG and CTCAGTGCACGCTTCTCAG; for *BRCA2*: TGATTCTCTGTCATGCCTGC and TCTCTGTCATGCCTGCAGGA) and was transfected into two PDAC cell lines (Capan-2 and T3M4) and into a breast cancer cell lines (MCF7) as control. On-target mutations were confirmed by ddPCR and NGS. Off-target effects were predicted by the CrispRGold tool and the CRISPR LIFEPIPE® 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.

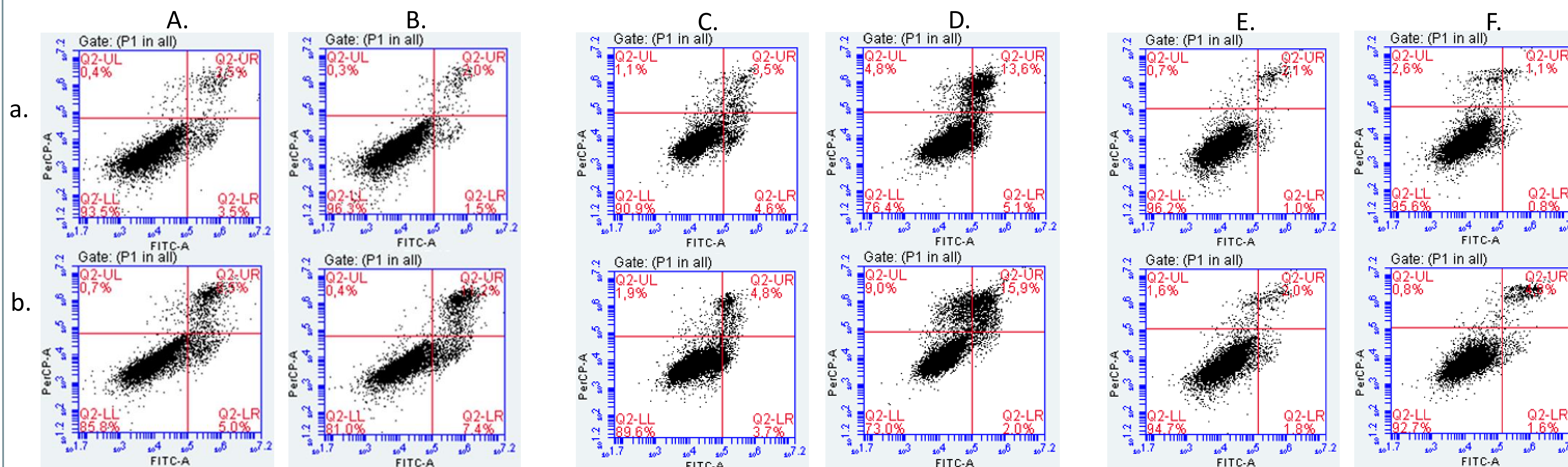


Allelic frequencies (AF) obtained by ddPCR analysis and given for pools of *BRCA*-knockdown (KD) cells after transfection by each CRISPR/Cas9 system designed.

2. Off-target sites predicted

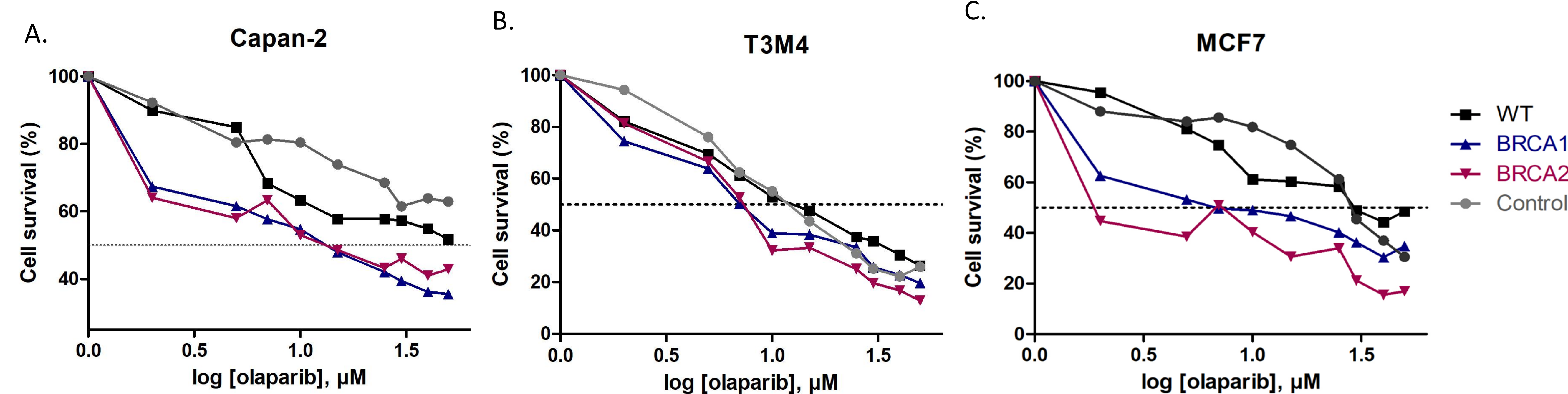
No off-target sites were predicted by the CRISPR LIFEPIPE® tool and no off-target in functional sites were identified by the CrispRGold tool.

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.

Cell lines	IC ₅₀ (μM)		
	Capan-2	T3M4	MCF7
WT	>50	13,67 ± 0,29	31,73 ± 5,53
BRCA1	14,19 ± 1,58 ***	9,04 ± 0,42 **	11,12 ± 4,28 **
BRCA2	17,51 ± 1,36 ***	8,26 ± 1,12 ***	5,92 ± 3,65 **
Control	>50 NS	12,74 ± 1,24 NS	28,29 ± 5,65 NS

Calculated IC₅₀ (μM) of olaparib for Capan-2, T3M4 and MCF7 cell lines (wild-type (WT) cells, *BRCA1* mutated cells, *BRCA2* mutated cells and control cells. Data are represented as the mean + SD of three experiments.

One-way ANOVA followed by Bonferroni's post-hoc comparisons tests were performed in statistical analyses (n=3), * p<0,05, ** p<0,01, *** p<0,001, and NS: not significant).

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.