Exploiting DNA Repair Defect in Triple Negative Brest Cancer Using CDK Inhibition Strategy

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Background

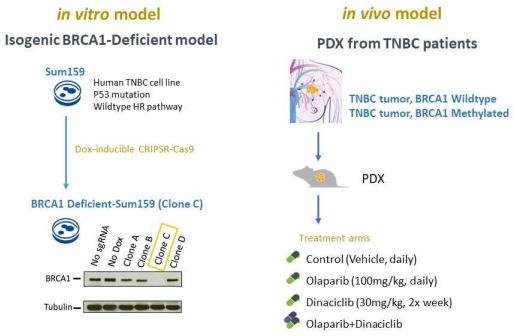
Triple-negative breast cancer (TNBC), representing 15% of breast carcinomas, is an aggressive breast cancer subtype with a high probability of metastasis and limited treatment options.

Noticeably, BRCA-deficiency occurs in 25% of the TNBCs and results in deficient homologous recombination (HR) repair. Interestingly, PARP inhibitors (PARPi) have shown synthetic lethality in a BRCA-deficient context, however, their efficacy is frequently hampered by intrinsic or acquired resistance mechanisms involving restoration of the HR. In that regard, the role of some CDKs proven to regulate key HR actors was of interest to find synergist therapeutical combinations.

Material & Methods

We used *in vitro* and *in vivo* (PDX) TNBC-models to explore the effect of CDK-inhibitors (CDKi).

We analysed different key HR-players using immunofluorescence microscopy and RT-PCR to characterise the effect of two CDK inhibitors, dinaciclib and SR-4835, both in BRCA1 proficient and BRCA1 deficient TNBC-cells. Moreover, we tested dinaciclib, alone and in combination with olaparib, in the PDX from two TNBC patients, one BRCA1 wiltype and other BRCA1 deficient.



Results

First, we tested the different drugs in the BRCA1 proficient and BRCA1 deficient TNBC-cell models. The cell viability assay showed that olaparib is more efficient in BRCA1-defiecient cells, according to the synthetic lethality phenomenon (Figure 1a). Interestingly, dinaciclib performance depended on BRCA1 status while SR-4835 did not (Figure 1b,1c).

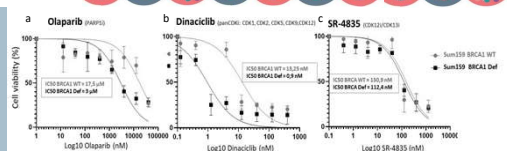


Figure 1. BRCA-proficient and BRCA-deficient TNBC cell viability under a)olaparib; b)dinaciclib; c) SR4835 treatment. The IC50 values for each cell line and treatment are indicated

Second, we assesed the BRCA1 foci and RAD51 foci formation to explore how the different treatments, alone and in combination, affected these HR-response markers. Dinaciclib, alone and in combination with olaparib strongly reduced both RAD51 and BRCA1 foci formation, suggesting that dinaciclib could sensitize HR-proficient cell to PARP inhibition through an induced-BRCAness (Figure 2a). Cells treated with the CDKi SR-4835 in combination with Olaparib presented as well a substantial reduction of RAD51 and BRCA1 foci (Figure 2b).

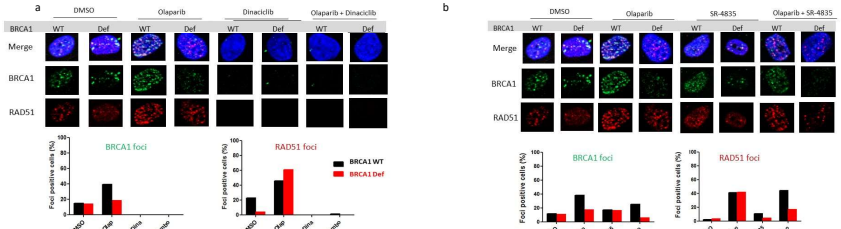


Figure 2. Cells were treated with DMSO, olaparib, dinaciclib and olaparib + dinaciclib (a) or DMSO, olaparib, SR-4835 and olaparib + SR-4835 (b) andanalyzed by immunofluorescence for RAD51 and BRCA1 foci. Graphs show quantification of cells with > 5 foci in vehicle and drug-treated cells.

We reasoned that the HR impairment observed at the foci formation level in the BRCA1-proficient cells treated with CDKi could be due to the transcriptional downregulation of some HR response markers.

Our results showed that both dinaciclib and SR-4835 treatment downregulated the mRNA expression of several HR genes (Figure 3a, 3b).

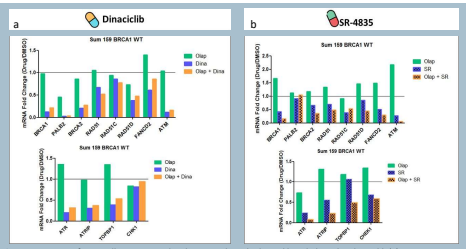


Figure 3. BRCA1 proficient cells were treated with DMSO, olaparib, dinaciclib and olaparib + dinaciclib (a) or DMSO, olaparib, SR-4835 and olaparib + SR-4835 (b) and analyzed by RT-PCR. Bars show the Fold change of the different DNA repair genes.

To further study the performance of dinaciclib treatment, we tested this pan-CDKi, alone and in combination with olaparib, in TNBC-PDXs.

In vivo, the combination of olaparib and dinaciclib resulted in a substantial tumor regression; Interestingly, treatment was effective not only in BRCA1-deficient models but also in BRCA1-proficient ones (Figure 4a, 4b). At mRNA level, dinaciclib downregulated the expression of a number of DNA damage response proteins (Figure 5a, 5b).

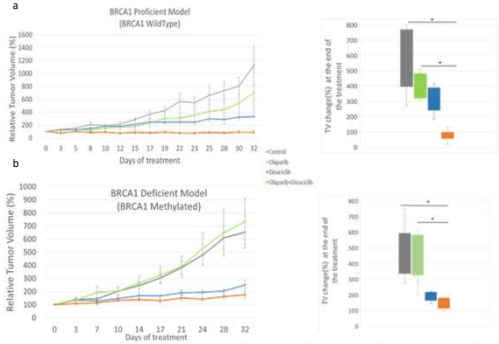


Figure 4. Mice bearing xenografts were treated with vehicle (n = 6), olaparib (n = 8), dinaciclib (n = 6) or the combination (n = 6)
a) Differentxenografts of the BRCA1 WT tumor; b) Different xenografts of the BRCA1 methylated tumor. Combination treatment produced significant tumor growth inhibition compared to vehicle or monotherapies. At day 32, combination treatment produced significant tumor growth inhibition compared to vehicle or Olaparib monotherapy.

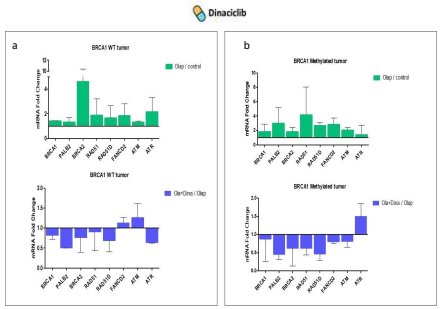


Figure 5. BRCA1-proficient (a) and BRCA1-deficient (b) tumors treated with vehicle, olaparib, dinaciclib and olaparib + dinaciclib were analyzed by RT-PCR. Bars show the Fold change of the different DNA repair genes.

Conclusions

Our results are consistent with dinaciclib sensitizing BRCA1-proficient tumors to olaparib. Interestingly, in a BRCA1-deficient context Dinaciclib as well augments the quality and degree of response.

Thus, dinaciclib may induce a pharmacological BRCAness in BRCA proficient models, leading to a synergistic effect with olaparib. Our results suggest the combination of CDKi and olaparib as a promising treatment in TNBC context.

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