Preclinical study of Dbait, an inhibitor of three DNA repair pathways, in Breast Cancer treatment

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Abstract

DBT is the 1st drug candidate of family of DNA repair inhibitor already in a phase 1 trial in local metastatic melanoma. This study demonstrates the efficiency of DBT in BC treatment. Twelve BC cell lines were characterized for DNA repair gene expression and activation of the DNA damage response (DDR) by western blot analysis of the proteins and classified according to their BRCA status. Clonal survival to Dbait (the DBT) in vitro survival) was analyzed 10 days after treatment. Four xenografted models derived from cell lines or patient samples were assessed for sensitivity to DBT. This study provides the evidence that: i) Dbait induces death in vitro in BC/RCA cells but also in BC/CAR cells having a high level of spontaneous DDR; ii) Dbait controls tumor growth in all the model tested with or without BRCA mutations; iii) Dbait cured 30% BRCA1 and 50% BRCA2 tumors; iv) Dbait was more efficient that ABT-888. Dbait is a promising investigational medicinal product to treat Breast Cancer. Its association with radiotherapy is under investigation.

DNA repair inhibition by Dbait molecules

Dbait are short and stabilized DNA molecules which remiss DSBs and bind DNA Protein Kinase [DNA-PK] and Poly ADP Ribose Polymerase (PARP). This results in the activation of these enzymes and an extensive phosphorylation on PARylation of their target proteins which last for 24 hours. This “false” damage signal leads to the high levels of DNA damage repair proteins and the inhibition of several DNA repair pathways resulting in a p53-mediated and radio-sensitization of tumors.

1. Dbait induces Breast Cancer cells death

Twelve breast cancer (BC) cell lines were classified according to their BRCA expression, and activation of the “DNA Damage Response” (DDR) by hHAX, PARP and Poly-ADP-Ribose (PAR) detection. BRCA1AX and BRCA2AX are hXca cells line respectively silenced for BRCA1 or BRCA2.

All BRCA- cell lines BRCA1S, BRCA2S, HCC1937, MDAMB468 have a high level of PARP, PAR and hHAX (noted BRCA+/DDR+) but also in BRCA+/DDR- cells, having a high level of spontaneous DDR; i) Dbait induces death in vitro in BC/RCA cells but also in BC/CAR cells having a high level of spontaneous DDR; ii) Dbait controls tumor growth in all the model tested with or without BRCA mutations; iii) Dbait cured 30% BRCA1 and 50% BRCA2 tumors; iv) Dbait was more efficient that ABT-888. Dbait is a promising investigational medicinal product to treat Breast Cancer. Its association with radiotherapy is under investigation.

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Inhibition of DNA repair molecules recruitment by Dbait treatment:

Repair proteins are trapped on PARP complex (or) modified after binding damage and/or diluted on all the modified chromatin. SSB and DSB repair pathways are inhibited

To determine cell sensitivity to Dbait we performed clonal survival tests (9 d) and trypan blue cell analysis at 24h and 48h after treatment. We found that 0.1 mM Dbait gave similar lethality to 10 mM ABT-888 PARP inhibitor in BRCA BC cells

DDR and Micronuclei are good markers of Dbait sensitivity in vitro

2. Characterization of Breast Cancer tumor models

Four mouse xenograft models : BC227 and BC173 tumours (xenograft derived from patient surgical sample) and MDAMB468 and MDAMB231 (xenograft from cell lines). All tumors are Triple Negative Breast Cancer (HER2-;PR-;). Structure (HES), vasculization (CD31), proliferation (Ki67), BRCA status (BRCA1 and BRCA2) and DDR status (PAR and hHAX) level were determined on tumor sections by immunodetection.

3. DT01 controls tumor growth in all models tested independently of BRCA status

DT01 molecules are clinical Dbaits molecules

DT01 is a Dbaits molecule conjugated to a heavy cholesterol chain at the 5’ free end strand.

BRCA- tumors (BC227) are sensitive to DT01

DT01 molecules were administered by intra-tumoral and subcutaneous injection during 1 week (5d) or 3 weeks (3x). PARP inhibitor, ABT-888, was also administered by oral gastric.

Recent studies on human melanoma xenograft model (SK-Br: BRCA+/DDR+) DT01+ is a high-sensitization tool by DT01 treatment

DT01 shows a standalone antitumoral activity in tumors with constitutive DNA damage revealed by Micronuclei and DDR markers.

4. DRIIM clinical trials

DNA Repair Inhibitor and Irradiation on Melanoma DRIIM trial (NC30: 888/DBT/BRCA)

Since November 2011, DRIIM trial has investigated the combination of DT01 with radiotherapy in patients suffering from melanoma in transit. Protocol: During 2 weeks, DT01 is administered by local injections within and/or around tumors (5x per week), and associated with 3 Gy irradiation (5x per week).

Preliminary results (4/5 doses achieved):

- Low toxicity and tolerability concern in the healthy skin exposed to DT01 and irradiation
- Dose-dependent antitumoral activity of DT01

Conclusion

- DT01 molecules have a stand alone effect in Breast Cancer cell lines and tumor models.
- Analysis of DDR response markers and micronuclei frequency on biopsies could help to identify “good responder” patients.
- Association with Radiotherapy is well tolerated and improve tumor control.