Evolution of tumor cells under Dbait (AsiDNA) treatment results in "autosensitization"

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#2851

Abstract

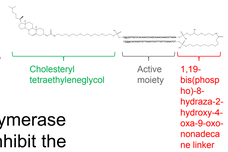
Purpose: Purpose: The Achille's hill of all conventional and targeted anticancer treatments is intrinsic or acquired resistance. The era of precision medicine where tumors are selected for specific treatments based on their genetic alterations is revolutionary, but unfortunately prolonged responses are rarely observed due to rapid emergence of resistant clones. We recently developed a new concept of DNA repair inhibitor (Dbait) acting by activating enzymes involved in DNA damage signaling. We tested how such agonist activity would be prone to induce resistance to Dbait treatment.

Experimental design: We performed repeated cycles of treatment with various targeted therapy agents (Imatinib, Olaparib, 6-thioguanineand the clinical form of Dbait, AsiDNA™. We analyzed the specific sensitivity of independent cultures after each treatment. The study was performed in different tumor cell lines ((MDAMB231, MDAMB468, HCC1143, THP1, U937, KBM7) and in two non-malignant breast cell lines (MCF10A, MCF12A). Extensive transcriptome and genome studies were performed on MDAMB231 breast cancer cell lines.

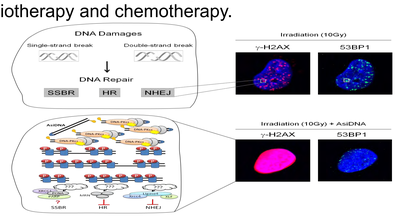
Results: Resistant clones appeared at a frequency of 1.45% for Olaparib, 0.62% for imatinib, and 1.66% for 6-thioguanine. Similar protocol did not select for resistance to AsiDNA, the clinical form of Dbait. Unexpectedly, tumor cells became more sensitive to AsiDNA after each cycle of treatment. This behavior was specific of AsiDNA and was not observed with other treatments. The six tumor cell lines tested developed AsiDNA sensibility and no resistance after cyclic treatments. Non tumoral cells were not affected by repeated treatments. The acquired sensitivity of the treated tumor populations was conserved for month after end of treatment Transcriptional and genetic analysis of independently treated MDAMB-231 populations reveals that all evolved similarly. They display a few conserved genome modifications and deregulation of 1160 genes involved in cell cycle and DNA repair.

Conclusion: Our results indicate a phenomenon of "autosensitisation", along treatment which has never been described for anticancer treatment and could prevent development of resistance during treatment.

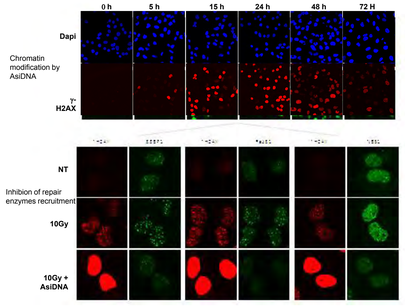
DNA Repair inhibition by AsiDNA

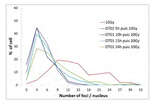


AsiDNA are short and stabilized DNA molecules that mimic DSBs. They act by hijacking and hyper-activating the DNA-dependent protein kinase (DNA-PK), and the poly-ADP-ribose polymerase which modify the chromatin and consequently inhibit the recruitment of many proteins involved in the BER, HR and NHEJ pathways at the damage sites. This strategy sensitizes tumors to DNA damaging therapies such as radiotherapy and chemotherapy.



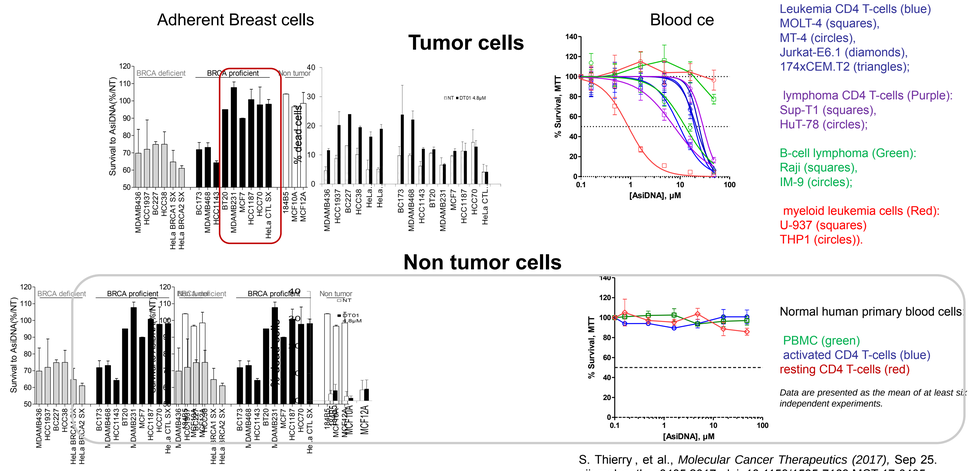
→Inhibition of DNA repair proteins recruitment to damage sites → no repair → DNA damage accumulation→ mitotic/necrotic death





Inhibition of recruitment at damage site of:
53BP1
XRCC4
Mre11
Nbs1
Rad51

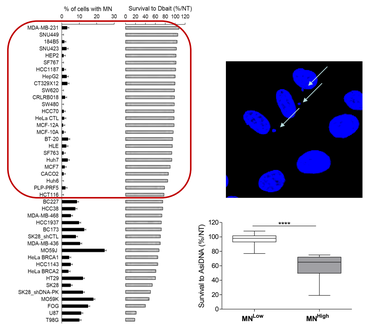
1. Standalone effect of AsiDNA is specific for tumor cells



S. Thierry , et al., *Molecular Cancer Therapeutics (2017)*, Sep 25. pii: molcanther.0405.2017. doi: 10.1158/1535-7163.MCT-17-0405.

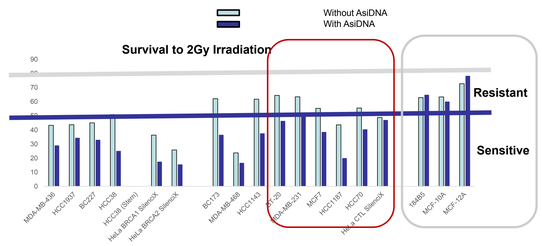
2. Cell lines with micronuclei are sensitive to AsiDNA

Micronuclei (MN) result from chromosomal breakage or spindle damage. It can be in the nuclei of the daughter cells following cell division and form single, or two micronuclei in the cytoplasm of these cells. A good way to quantify cytogenetic damage and chromosomal stability.



Only cell lines with high spontaneous level of micronuclei are highly sensitive to AsiDNA

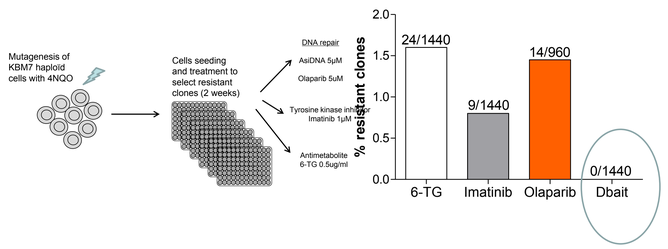
Most cell lines become sensitive when DNA damaging treatment is associated to AsiDNA



W. Jdey et al., *(2017) Cancer Research,* Jun 6. pii: canres.2693.2016. doi: 10.1158/0008-5472.CAN-16-2693.

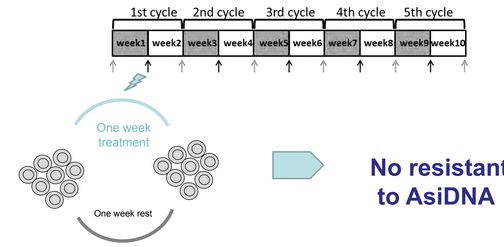
3. No resistance acquired after continuous treatment or cycle treatments

Targeted treatments rapidly give rise to resistant clone emergence



No resistant clone to AsiDNA

4. Cyclic treatments do not favor resistance emergence

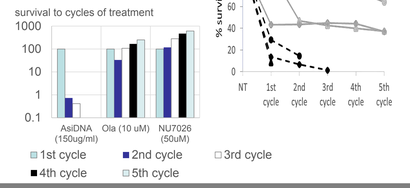


No resistant clone to AsiDNA

5. Increase of sensitivity with treatments

Cell sensitivity to AsiDNA increases with treatments independently of initial sensitivity (first cycle survival).

AsiDNA
(not observed with other repair inhibitors)

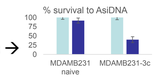


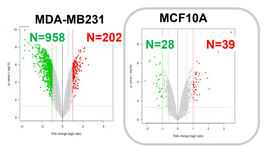
sensitisation is specific of **non tumor cells** treatments
MCF10A(square),
MCF12A (triangle)

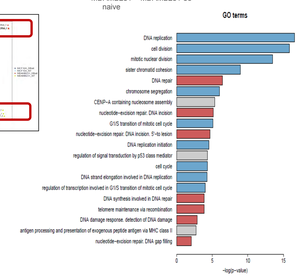
tumor cells
THP1 (circle),
MDAMB-231 (triangle),
HCC1143 (diamond);
MDAMB-468 (circle),
KBM7 (diamond),
U937 (triangle).

6. Transcriptomes change after 3 cycles AsiDNA treatment

Transcriptomes were analyzed
3 weeks after treatment (sensitivity is conserved) →







Transcriptomes are strongly modified by AsiDNA treatment in tumor cells but not in normal cells. Modifications are essentially down regulation of genes involved in cell cycle and repair.

Conclusion

AsiDNA sensitizes tumors to its own treatment, thereby enabling its continuous use for cancer treatment without emergence of resistance. Such behavior is unique and probably linked to the original mechanism of action of AsiDNA. which acts as an agonist and a multi-DNA repair pathway inhibitor. AsiDNA counteracts the ability of tumor cells to bypass specific inhibition or genetic silencing by the reversion of existing mutations or the use of alternative pathways.

For further information

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See other Poster #2818: *board 8 session 37* **4/16/2018**