AsiDNA™ and HDAC inhibitors: A cross-potentiation team work to kill tumor cells

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Abstract

Purpose: While HDAC inhibitors (HDACi) are registered in restricted subset of T-cell lymphoma, in monotherapy, recent studies have shown an effect of HDACi on DNA damage accumulation, rationalizing their combination with DNA repair inhibitors. In the current study, we propose a novel therapeutic strategy, based on drug combination of HDACi with the pan-DNA break repair inhibitor AsiDNA to promote their antitumor activity.

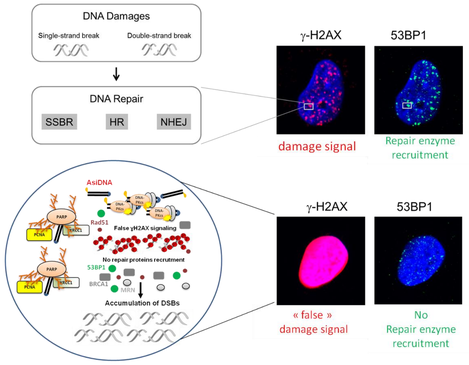
Experimental design: AsiDNA™ is a double stranded DNA molecule (decoy oligo-nucleotide) that mimics double stranded DNA breaks (DSBs) and interfere with DNA repair cellular mechanism, redirecting repair enzymes away from sites of tumor DNA damages. Belinostat is a pan-HDACi displaying a better safety profile compared to other HDACi. We characterized the effects of each drug on DNA break accumulation and genetic instability by γH2AX analysis, COMET assay and micronuclei detection. We further studied the antitumor efficacy of the combination of the two drugs. Finally, we assessed the effect of AsiDNA on the occurrence of acquired resistance after long term treatment with belinostat.

Results: Molecular analyses of DNA damage after treatment demonstrate that belinostat paves the way for AsiDNA efficacy by inducing DNA DSBs as measured by γH2AX accumulation and tail moment increase on COMET assay. Moreover, continuous treatment with belinostat induced an increase of basal genetic instability in tumor cells measured by micronuclei accumulation, a prerequisite for AsiDNA antitumor efficacy. On the other hand, AsiDNA enhances the effects of belinostat on histone acetylation, demonstrating a high potentiation of belinostat activity on its targets by AsiDNA. This mechanism-based cross-potentiation between AsiDNA and belinostat results in a high synergistic antitumor efficacy of the combined treatment in different tumor models. This synergistic effect was further confirmed with several HDACi belonging to different classes. Importantly, the combined treatment do not induce any DNA damage increase and/or lethality in non-tumor cells. Finally, repeated treatments induce the emergence of resistance to belinostat, which is abrogated in presence of AsiDNA, indicating an unlikely tumor escape to this combined therapy.

Introduction

Multiple DNA Repair pathways inhibition by AsiDNA

Dbait are short and stabilized DNA molecules that mimic DSBs. AsiDNA, a molecule of Dbait family, acts by hijacking and hyper-activating the DNA-dependent protein kinase (DNA-PK), and Poly(ADP-Ribose) Polymerase (PARP), which modify the chromatin and consequently inhibit the recruitment at the damage sites of many proteins involved in the DSB (HR and NHEJ) and SSB (BER) repair pathways. This strategy sensitizes tumors to DNA damaging therapies such as radiotherapy and chemotherapy.



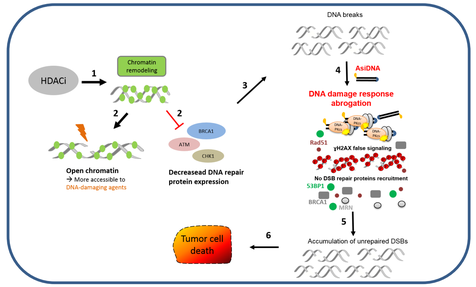
→Inhibition of DNA repair proteins recruitment to damage sites
→no repair → DNA damage accumulation → **Cell death**

DNA targeting by HDAC inhibitors (HDACi)

DNA damage resulting from HDAC inhibition[1] has been attributed to profound changes in chromatin structure, exposing DNA normally protected to intracellular and extracellular DNA-damaging agents[2]. Moreover, it has been shown that HDACi transcriptionally down-regulate a large number of DNA DSB repair proteins[2].

Rational for combining AsiDNA and HDACi

All the DNA repair-related effects of HDACi induce DNA breaks[3], that would not be repaired if cells are treated by AsiDNA[4]. The combined treatment would lead to the accumulation of toxic DSBs[5] and tumor cell death[6].

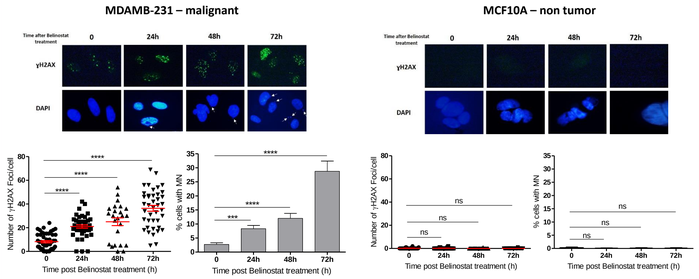


AsiDNA would stimulate HDACi efficacy at least by inhibiting the repair of HDACi-induced DNA damage

Molecular mechanisms underlying the combination of AsiDNA and HDACi

Induction of DSBs by belinostat

Belinostat is a pan-HDACi FDA approved (Beleodaq®) in refractory and relapsed PTCL, with recognized efficacy and superior safety profile compared to other HDACi. We first tested if belinostat could induce DNA damage, and especially DSBs.

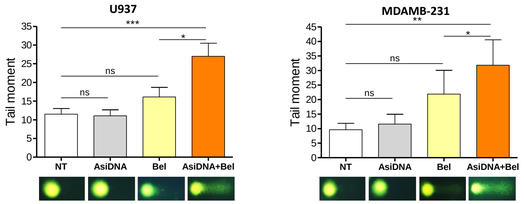


→ Belinostat induces DNA DSBs/genetic instability as revealed by γH2AX foci and micronuclei (MN)
→Belinostat acts specifically on tumor cells

AsiDNA potentiates the effect of belinostat

- **Effect on DNA damage**

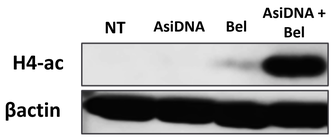
To test the hypothesis that AsiDNA would inhibit the repair of Belinostat-induced DNA damage, we performed a COMET assay after single and double-treatment.



→ AsiDNA+belinostat combined treatment show more accumulation of DNA damage compared to belinostat alone.

- **Effect on belinostat-induced acetylation**

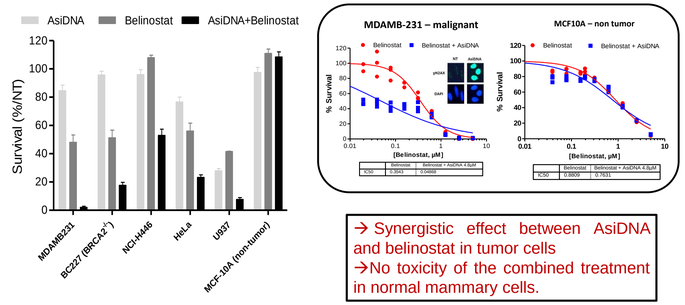
Belinostat, like all HDACi, is known to induce histone acetylation. We performed western blot analysis to check if AsiDNA has an impact in this acetylation.



→ AsiDNA highly potentiates the belinostat-induced acetylation, which could also increase the antitumor efficacy of belinostat.

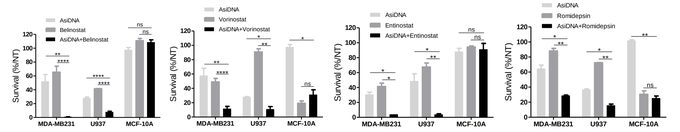
AsiDNA and HDACi: Synergistic antitumor efficacy

The combined treatment AsiDNA and belinostat is toxic in all tested tumor cells



→ Synergistic effect between AsiDNA and belinostat in tumor cells
→No toxicity of the combined treatment in normal mammary cells.

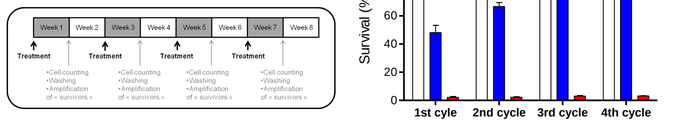
Similar effect is found with all HADCis, showing a class effect



→ Synergistic effects are observed with different classes of HDACi

AsiDNA abrogates the acquired resistance observed during belinostat repeated treatment

Repeated cycles of treatment/release to select acquired resistance



Conclusion: Altogether these results indicate a cross potentiation between AsiDNA and belinostat, and support the rational to investigate the clinical activity of this novel synergistic combination in different tumor types. As belinostat is already approved by FDA and AsiDNA is already tested in a first-in-man clinical trial, clinical confirmation of the interest of this new combination could be rapidly obtained.

Related publications:

- C. Le Tourneau, et al. (2016) *Br J Cancer. May 3. doi: 10.1038/bjc.2016.120.* First-in-human phase I study of the DNA repair inhibitor DT01 in combination with radiotherapy in patients with skin metastases from melanoma.
- M. Quanz, et al. (2009) *PloS ONE* 4(7) e6398. Hyperactivation of DNA-PK by Double-Strand Break Mimicking Molecules Disorganizes DNA Damage Response.
- A. Bodiford et al. OncoTargets and Therapy 2014:7 1971-1977. Profile of belinostat for treatment of relapsed or refractory peripheral T-cell lymphoma.
- W. Paul Roos and A. Krumm. NAR 2016. The multifaceted influence of histone deacetylases on DNA damage signaling and DNA repair.
- J.-H. Lee et al. PNAS 2010. Histone deacetylase inhibitor induces DNA damage, which normal but not transformed cells can repair.

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