The DNA binding polyamine moiety in the vectorized DNA topoisomerase II inhibitor F14512 alters reparability of the consequent enzyme-linked DNA double strand breaks

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\textbf{Purpose:}
Poisons of Topoisomerase II (TOP2) kill cancer cells by preventing religation of intermediate DNA breaks during the enzymatic process and thus by accumulating enzyme-drug-DNA complexes called TOP2 cleavage-complex (TOP2cc). F14512 is a highly cytotoxic polyamine-vectorized TOP2 inhibitor derived from etoposide and currently in clinical trials. The molecule F14512 aimed at exploiting the overexpression of the polyamine transport system in tumours through the addition to the epipodophyllotoxin core of a spermine moiety intended to change its cellular uptake properties. Interestingly, F14512 exhibited a marked increase in cytotoxic potency compared to etoposide in cell proliferation assays and showed also potent anti-tumour activity in various in vivo mice models, including acute myeloid leukemia. It was shown \textit{in vitro} that F14512 has acquired DNA binding properties and that the stability of TOP2cc was strongly increased. Paradoxically, at equitoxic concentrations in cells, F14512 induced less DNA breaks than etoposide.

\textbf{Experimental Design:}
To understand the basis of F14512 cytotoxicity, we compared etoposide and F14512 for the rates of TOP2cc production and resolution in human cells, for their susceptibility to repair by TDP2-dependent or endonucleolytic processes and the susceptibility of the associated DSB to repair by end-joining or resection-based processes.

\textbf{Results:}
We report that targeting of TOP2\textalpha{} and not \beta{} impacts cell killing by F14512, contrarily to etoposide that kills cells through targeting both isoforms. Then we show that despite being more cytotoxic, F14512 is less efficient than etoposide at producing TOP2cc in cells. Finally, we report that compared to TOP2cc mediated by etoposide, those generated by F14512 persist longer in the genome, are not dependent on TDP2 for cleaning break ends from TOP2\textalpha{}, are channelled to a larger extent to resection-based repair processes relying on CtIP and BRCA1 and promote RAD51 recruitment to damaged chromatin.

\textbf{Conclusion:}
In addition to the addressing of F14512 to the polyamine transport system, the properties uncovered here would be particularly valuable for a therapeutic usage of this new anticancer compound. More generally, the concept of increasing drug cytotoxicity by switching the repair mode of the induced DNA lesions \textit{via} addition of a DNA-binding moiety deserves further developments.

\textbf{Reference:}