

ABSTRACT

DESIGN OF NUCLEOLIN'S INHIBITORS

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In the sixties, when quadruplexes were discovered by Dr. David Davies, nobody expected that these DNA structural motifs would play such a significant role in cell function, as well as have multiple applications in medicine. These four-stranded species though appeared to be very diverse, and they remain to this day not well understood. As a consequence, there are some assumptions and false dogma that arose over time and are still commonly used in literature. Out of this group of nucleic acids originated a novel drug candidate, which is currently undergoing the second phase of clinical trials in cancer treatment. Despite its anticancer activity and lack of noticeable side effects, its structure and character was not fully understood, providing a great opportunity for vast research. Also nucleolin, AS1411 putative target, appeared to be very attractive for rational drug discovery.

Here we present our approach to the rational discovery of an anticancer drug, which would mimic the AS1411 mode of action through inhibiting the nucleolin.

Indeed, we identified at least two diverse chemical structural templates exhibiting anticancer activity in the cancer cell lines that were studied. We also demonstrated that a relatively small modification introduced to the AS1411 sequence may increase its anticancer activity, and that some of the AS1411 conformers are more active in selected cancer cell lines.

We were able to bring a whole new understanding to the AS1411 system by revealing its remarkable complexity. We also designed a sequence modification allowing, for the first time, to control the quadruplex fold.

We challenged some assumptions and practices commonly met in literature. We designed a set of experiments which allowed for rebutting persistent for decades dogma on structural classification of quadruplexes based on CD spectra alone. We also provided evidence that biophysical techniques alone are not reliable in revealing the full content and structural information about the quadruplex systems, for which they are commonly used.

Overall, we created a great platform for accelerating the studies on these still mysterious and fascinating DNA motifs. We also provided a direct basis for discovering novel anticancer drugs.

Selected publications:

1. Bates, P.J., et al., *Discovery and development of the G-rich oligonucleotide AS1411 as a novel treatment for cancer*. *Exp Mol Pathol*, 2009. **86**(3): p. 151-64.
2. Barnhart K.M., et al., *AGRO100: The translation from lab to clinic of a tumor-targeted nucleic acid aptamer* *Journal of Clinical Oncology*, 2004 ASCO Annual Meeting Proceedings (Post-Meeting Edition), 2004. **22**(14S): p. 3126.
3. Bates, P.J., et al., *Antiproliferative activity of G-rich oligonucleotides correlates with protein binding*. *J Biol Chem*, 1999. **274**(37): p. 26369-77.
4. Dapic, V., et al., *Biophysical and biological properties of quadruplex oligodeoxyribonucleotides*. *Nucleic Acids Res*, 2003. **31**(8): p. 2097-107.
5. Xu, X., et al., *Inhibition of DNA replication and induction of S phase cell cycle arrest by G-rich oligonucleotides*. *J Biol Chem*, 2001. **276**(46): p. 43221-30.
6. Dailey, M.M., et al., *Resolution and characterisation of the structural polymorphism of a single quadruplex-forming sequence*. *Nucleic Acids Res* - accepted, 2010.
7. Dailey, M.M., et al., *Structure-based drug design: from nucleic acid to membrane protein targets*. *Exp Mol Pathol*, 2009. **86**(3): p. 141-50.
8. Dailey, M.M., et al., *Structural studies of G-quadruplexes*. Second International Meeting on Quadruplex DNA; oral presentation by J.O. Trent, April, 2009.
9. Miller, C.M., et al., *Size Exclusion HPLC Separation of Quadruplex DNA*. Second International Meeting on Quadruplex DNA; poster session, April, 2009.
10. Podlaska, M.M., et al., *Introducing dSpacer modification to facilitate a monomeric fold of GRO26B quadruplex*. First International Meeting on Quadruplex DNA; poster session, 2008.