NMR Characterization of a Multiple Myeloma-Associated lncRNA G-Quadruplex and Its Ligand Interactions

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Multiple myeloma (MM) is an incurable haematological malignancy that affects plasma cells in the bone marrow. The long non-coding RNA (lncRNA) RP11-350G8.5 has recently been identified as a new therapeutic target, as it is selectively required for the survival of MM cells. Bioinformatic and biophysical analyses revealed that a G-rich region of this lncRNA, beginning at nucleotide 1051 (hereafter 1051), displays a marked propensity to fold into a G-quadruplex (G4), although its three-dimensional architecture and druggability remained unestablished. By nuclear magnetic resonance (NMR) spectroscopy, we determined the solution structure of the 1051 RNA G4, revealing a parallel topology comprising a canonical G-tetrad stacked on an adenine-expanded G(:A):G:G(:A):G hexad; two such units further self-associate into a dimer through hexad-hexad stacking. We then characterised the molecular recognition of this scaffold by a novel indole-based cationic ligand, MLC2507, synthesised via the Nenitzescu reaction. MLC2507 binds the 1051 G4 with low-micromolar affinity, forming a well-defined 2:1 (ligand:dimer) complex in which two ligand molecules interact with the external G-tetrads through tilted end-stacking interactions, leaving the tetrad-hexad architecture unperturbed, as established by the NMR structure of the complex. Furthermore, MLC2507 reduced cell viability and induced apoptosis in an RP11-350G8.5-dependent, bortezomib-resistant MM cell model, while sparing healthy peripheral blood mononuclear cells. Altogether, this study provides the first solution NMR structure of a small-molecule complex with a disease-associated lncRNA-derived G4, establishing a structural framework for the rational design of ligands and chemical probes targeting lncRNA G-quadruplexes in multiple myeloma.

a) K. Grillone, S. Ascrizzi, P. Cremaschi, J. Amato, N. Polerà, R. Rocca, S. Alcaro, B. Pagano, A. Randazzo, P. Tagliaferri, F. Iorio, P. Tassone, *An Unbiased LncRNA Dropout CRISPR-Cas9 Screen Reveals RP11-350G8.5 as a Novel Therapeutic Target for Multiple Myeloma*, Blood, 144(16) (2024), https://doi.org/10.1182/blood.2023021991
b) G. Satta, J. Brčić, J. Plavec, *Targeting G-Rich LncRNA and Its Structural Polymorphism With Selective G-Quadruplex Ligands: An NMR Study*, Chemistry – A European Journal, 31(72) (2025), https://doi.org/10.1002/chem.202502472
c) V. J. Sahayasheela, H. Sugiyama, *RNA G-Quadruplex in Functional Regulation of Noncoding RNA: Challenges and Emerging Opportunities*, Cell Chem. Biol., 31(1) (2024), https://doi.org/10.1016/j.chembiol.2023.08.010
d) F. Malard, P. Neri, N. J. Bahlis, E. Terpos, N. Moukalled, V. T. M. Hungria, S. Manier, M. Mohty, *Multiple Myeloma*, Nat. Rev. Dis. Primers, 10(1) (2024), https://doi.org/10.1038/s41572-024-00529-7

Short bio:

Giuseppe Satta, PhD, is a postdoctoral researcher at the National Institute of Chemistry in Ljubljana (SloNMR department). He received his PhD in Chemical Sciences and Technologies, specialising in Organic Chemistry, from the University of Sassari in 2024. His research project focused on the synthesis of tetracationic porphyrin derivatives for use as ligands for G-quadruplexes. Thereafter, he spent two years as a researcher at CERIC, in collaboration with SloNMR at the National Institute of Chemistry in Ljubljana, working on high-resolution nuclear magnetic resonance (NMR) studies involving guanine-rich nucleic acids forming G-quadruplexes. His current research applies NMR, circular dichroism and molecular modelling to the structural and biophysical characterisation of G-quadruplex structures found in long non-coding RNAs associated with diseases, and their interactions with ligands and peptides.