Ruthenium(III) complexes entrapped in liposomes with enhanced cytotoxic and anti-metastatic properties

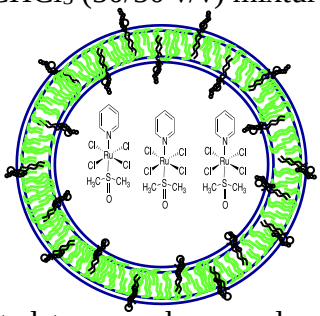
D.Tesauro[a], *M. E. Cucciolito,*[b] *R. Iannitti,*[c] *R. Palumbo,*[c] *G. Morelli,*[a] *F. Ruffo,*[b,] *A. D'Amora*[b]

[a]*Department of Pharmacy & CIRPeB, University of Naples Federico II, Via Mezzocannone, I-80134 Napoli;* [b]*Department of Chemical Sciences, University of Naples Federico II, Via Cintia, I-80126, Napoli;* [c]*Istituto di Biostrutture e Bioimmagini (IBB) CNR, Via Mezzocannone 16, 80134 Napoli,E-mail tesauro@unina.it*

Metal-based anticancer drugs are pivotal in the fight against cancer pathologies. Since 1978 *cis*-platin was licensed for medical treatment of a wide number of tumor pathologies(1). However its chemiotherapic use is strongly limited by many and severe side effects and acquired tumor resistance. Since these limitations could be overcome by other metal complexes, in the last thirty years ruthenium compounds have been tested showing a remarkable antitumoral and antimetastatic activity associated with a lower toxicity. A hexacoordinate Ru(III) complex (NAMI-A) is currently undergoing advanced clinical evaluation (2).

All data indicate that NAMI-A acts as a pro-drug, but the integrity of ruthenium complexes is essential to store the cytotoxic activity. In this scenario the condition of administration of ruthenium drugs are crucial to exploit their anticancer activity (3). In the last years innovative strategies have been produced to vehicle ruthenium ions in tumor cells like aggregates. This study aims to incorporate the ruthenium complexes in the inner aqueous compartment of liposomes and to test biological properties of two NAMI-A like pyridine derivatives. Specifically, we have investigated the pyridine derivatives of the sodium-compensated analogue of NAMI-A, Na[*trans*-$RuCl_4$(pyridine)(DMSO)] (NAMI-Pyr) and Na[*trans*-$RuCl_4$(Pytri)(DMSO)] (NAMI-Pytri).

In thelatter complex the pyridine ligand is functionalized with a sugar moiety so as to increase biocompatibility and the ability to cross the cell membrane. The stability of the complexes was studied and compared in solution at different pH following UV-VIS spectra. Lipid formulations based on Egg PC were prepared adding Cholesterol, DSPE-PEG_{2000} joining molar ratio 57/38 /5% w/w respectively in MeOH/$CHCl_3$ (50/50 v/v) mixture and hydrated with 0.9% w/w of NaCl.



This composition was selected to reproduce analog supramolecular aggregates in clinical use to vehicle doxorubicin (Doxil). Ruthenium complexes were loaded into liposomes using the passive equilibration loading method. Full drug containing liposomes were structurally characterized by dynamic light scattering (DLS) measurements. Data indicate the formation of stable aggregates with size and shape in the right range for *in vivo* applications. The amount of encapsulated ruthenium complexes was quantified by means of ICP-AES. Stability and drug release properties of ruthenium containing liposomes were confirmed in buffer. The growth inhibitory effects of both liposomal and free complexes drug were tested on prostate cancer cells (PC3).

Preliminary results show high cytotoxic effect of ruthenium complexes delivered by supramolecular aggregates with respect to free complexes drug.

References:

1. L. Kelland *Nat. Rev. Cancer* 2007, **7**:573–84.
2. E.Alessio *Eur.J. Inorg. Chem* 2017, 1549–1560,
3. B.M. Blunden, M.H. Stenzel *J.Chem.Technol.Biotechnol.* 2015, 90: 1177‑1195.