Aminoglycosides as suppression agents of nonsense mutations in *BRCA1*

Abreu, R. B. V. 1, Fuchshuber-Moraes, M. 1, Monteiro, A. N. A. 3, Suarez-Kurtz, G. 1, Carvalho, M. A. 1,2

1 Divisão de Farmacologia, Instituto Nacional de Câncer, Rio de Janeiro, Brazil; 2 Departamento de Biotecnologia, Instituto Federal do Rio de Janeiro, Rio de Janeiro, Brazil; 3 H. Lee Moffitt Cancer Center and Research Institute, Tampa, USA.

Mutations in *BRCA1* are responsible for most cases of hereditary breast and ovarian cancer syndrome (HBOC). Nonsense variants account for ~ 12% of mutations in *BRCA1* gene; they are characterized by the appearance of a premature stop codon, generating a truncated protein. Different studies have shown that aminoglycosides are able to induce readthrough of premature stop codons. The suppression of premature stop codon potentially restores the function of the protein. Given the lack of alternatives to the treatment of HBOC, the use of aminoglycosides may represent an important strategy for the prevention of hereditary breast and ovarian cancer, and also another forms of hereditary cancer associated with nonsense mutations. Our study intends to evaluate the use of aminoglycosides on the restoration of tumor suppressor activity of nonsense variants of the gene *BRCA1*. Four variants coding premature stop codons in the C-terminus of the protein were selected for the study (S1457X, Q1785X, E1836X and Y1853X) and generated by PCR routine. Variants were cloned in pCR2.1-TOPO vector and then subcloned into pQCXIH, in a fusion with EGFP or fused with GAL4DBD. HeLa cells transfected with the constructions will have expression levels evaluated by quantitative real-time PCR and immunoblotting. Cells will be treated with different aminoglycosides (amikacin, G418, gentamicin, kanamycin and puromycin) and the expression of full-length protein will be examined by flow cytometry, immuno-blotting and fluorescence microscopy. The functional activity of the translated protein will be evaluated by its transcriptional transactivation property and also its ability to interact with CtIP protein.

Keywords: BRCA1, aminoglycosides, readthrough, premature stop codon.