Myotonic dystrophy type 2 (DM2) and type 1 (DM1) are the most common forms of muscular dystrophy in adults. These are human neurological-neuromuscular disorders associated with genetic instability and mutational expansions of simple repetitive sequences called microsatellites. These are progressive and fatal diseases and the most commonly affected systems are the skeletal muscle, heart and central nervous system. The mutation responsible for DM type 2 is a tetranucleotide CCTG expansion in the first intron of *CNBP* gene. Whereas DM type 1 is associated with a CTG trinucleotide repeat expansion in the 3' untranslated region of *DMPK* gene. In DM2 patients the CCTG repeat is expanded beyond several thousands of copies while unaffected individuals have up to 26 repeats. In DM1 patients the triplet repeats reach often hundreds or even thousands of copies. DM2 and DM1 are RNA-related disorders and the CCUG and CUG mutation harbouring transcripts (mutRNAs) play a major role in their development. The expanded repeats of DM2 and DM1 when transcribed, become abnormally processed. This is manifested through nuclear accumulation of mutant repeats in characteristic foci which has adverse impact on a cell function and appears to be a key mediator of pathogeneses. The presence of mutRNA triggers series of abnormal events which include binding and sequestering of various proteins and one of them are the *muscleblind*-like (MBNL) family proteins. The functional depletion of MBNLs by the expanded repeats leads to abnormalities in many pathways of RNA metabolism including aberrant alternative splicing which is a hallmark of pathogenesis in DM2 and DM1. Currently, there is no cure and no effective treatment to halt or reverse the progression of these diseases. However, the most recent study published in Science Translation Medicine aimed at developing therapy for DM1 identified cyclin-dependent kinase 12 (CDK12) as a druggable target for DM1. Treatment of DM1 human cells and mouse model of the disease with CDK12-specific kinase inhibitors reduced some abnormalities associated with DM1. Remarkably, preliminary tests of small-molecule compounds screening in DM2 cells also indicated mitigation of associated symptoms. Considering this novel results it is essential to perform systematic drugs screening in DM2 to select the most potent molecules. Determination of the spectrum of molecular changes alleviating DM2 molecular symptoms will be a starting point in developing therapy for DM2.

Primary aim of the project: High-throughput screening of various libraries of compounds in cultured DM2 human fibroblasts and selection of molecules most potent in reducing CCUG RNA foci.

Research objectives: a) primary screening of small molecule compounds in DM2 cells for their effect in CCUG RNA foci reduction; b) secondary screen of selected compounds supplemented with analysis of their cytotoxicity in DM2 cells; c) tertiary screening and collection of most potent and least toxic compounds for further analysis; d) characteristics of molecular biomarkers of DM2 pathogenesis in cells exposed to most effective molecules.

Justification for tackling a specific scientific problem: This proposed study represents part of a bigger project aimed at deciphering the efficacy of small-molecule chemicals in human neurodegenerative disorders with pathogenesis linked to mutational expansions of simple repetitive sequences. Preliminary results indicated that in vitro treatment of DM2 cells with random small-molecules induced molecular changes expressed as mitigation of some of the symptoms of pathogenesis. Thus, it is essential to pursue the study and decipher the magnitude of molecular changes and the most importantly a target for the conditions.

Expected results: The outcome of this project will have multidimensional and far-reaching impact for human neurological disorders and drug discovery fields. In particular: a) it will help in finding effective therapies for repeat-associated disorder such as DM2; b) it will deliver a new basic knowledge and will increase understanding of the causes and fundamental mechanisms of DM2 and other neurodegenerative diseases; c) it will support discovering new biomarkers which will help the broad scientific community working on human neurological disorders.