2. Protocol Synopsis (350 words maximum) :

PROJECT TITLE: **MOLECULAR TARGETING OF INNER EAR PATHOLOGIES**
BACKGROUND: Hearing loss is highly prevalent, has a substantial morbidity and no causal therapy is available. It can be caused by numerous inner ear insults, including noise trauma, ototoxic drugs, infections, age-related degeneration and genetic diseases. The possibility to prevent, repair or reverse consequences of inner ear pathologies on hearing would bring about significant opportunities for affected individuals, treating physicians and the society. Reactive oxygen species (ROS) have been identified as the main mediators of hearing and the ROS producing enzyme, NOX3, is specifically expressed in the inner ear, where it is upregulated following ototoxic insult.
PRIMARY OBJECTIVE: The primary objective of the project is to develop vector-based molecular strategies for protection of inner ear cells from insult by manipulating molecular pathways leading to damage of critical cells.
STUDY DESIGN: RNA molecules targeting critical inner pathopathway (e.g. Nox3) will be delivered through a proprietary miRNA technology (mirGE). Experiments will be performed in rats. In-vivo efficacy of miRNAs will be assessed and functional protection will be studied in a cisplatin otoxicity model. Strategies for minimally invasive vector delivery into the inner ear, will be compared.
SAMPLE SIZE: 6 groups of 8 rats will be used. Two groups for the comparison of inner ear vector delivery methods (round window vs perilymph vector delivery) – Four groups to address the effect of Nox3 knock down (untreated / cisplatin+GFP / cis+Nox3 / cis + p22).
ENDPOINTS: Easily quantifiable read outs will be assessed 48h after vector / cisplatin treatment for both in vitro and in vivo studies. These include functional hearing assessment, cytocochleograms and quantification of Nox3 and oxidative stress markers through immunostaining and quantitative PCR.
STATISTICAL ANALYSIS: ANOVA test will be used
TIME SCHEDULE/STUDY DURATION: **First year:** Design and screening of siRNA sequences targeting Nox3, cloning into mirGE based lentivector. In vitro evaluation of the vector on hair cells derived from hearing stem cells and on explant from the organ of Corti. In vivo comparison of vector delivery through round window vs perilymph. **Second year:** in vivo evaluation of the vector efficiency upon administrated in the inner ear; investigation of cochlea protection upon cisplatin treatment.