*Modulo richiesta assegno*

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| **TUTOR** | **CATERINA GARONE**  |
| **TITOLO DEL PROGETTO** |
| Development of treatment strategies for mitochondrial dNTP unbalancerelated disorders |
| ASSEGNO FINANZIATO DA PROGETTO COMPETITIVO*(barrare la casella corrispondente)* | X SI | □ NO |  |
| SE IL FINANZIAMENTO È COMPETITIVO L’ENTE FINANZIATORE  | Charlie Gard Foundation |
| PROGETTO/ATTIVITÀ A SCOPO COMMERCIALE*(es. sperimentazione profit)* | □ SI | XNO |
| CARATTERISTICHE DEL PROGETTO (*biomedico/osservazionale/clinico-interventistico/multidisciplinare*) | Biomedico  |
| STATO DI APPROVAZIONE DEL PROGETTO DA PARTE DEL COMITATO ETICO (*se necessario per il tipo di studio barrare o evidenziare la casella corrispondente*) | □ Ottenuto | XDa ottenere |
| **DESCRIZIONE DEL PROGETTO** *(max 800 parole)* |  |
| **Background:** mitochondria are eukaryotic intracellular organelles that play a central role in cellular metabolism by performing oxidative phosphorylation (OXPHOS). Unique features of mitochondria are the presence of multiple copies of a 16.6 kb circular DNA molecule (mtDNA) and a dedicated intra-mitochondrial replication machinery. The mitochondrial replisome consists of DNA polymerase-γ, the replicative helicase Twinkle, the mitochondrial single-stranded DNA binding protein (SSBP1) and other factor including RNA polymerase (POLRMT) that produces replication primers, mitochondrial transcription factor A (TFAM) and various mtDNA-processing enzymes. In addition to proteins that functions in mtDNA synthesis, the maintenance of mtDNA depends on the balanced supply of four mitochondrial deoxyribonucleoside triphosphates (dNTPs) that constitute the nucleotide pool. A complex biochemical pathway regulates the synthesis and exchange of the dNTPs between the cytosolic and mitochondrial compartments via *de novo* synthesis or salvage route in a tissue specific and ontogenic manner. Defects in mtDNA replication represent the most common cause of multiple mitochondrial respiratory chain deficiencies. Clinically, they present as a spectrum of disorders ranging from severe infantile multi-systemic diseases, rapidly progressing to death, to tissue-specific disorders, with brain and muscle the most affected tissues, and variable age of onset. The hallmark of defects of mtDNA maintenance is the accumulation of mtDNA multiple deletions or profound reduction of mtDNA copy number (depletion) in critical tissues. RRM2B is a nuclear gene coding for a small, p53-inducible subunit of the ribonucleotide-reductase complex known as p53R2. Ribonucleotide reductase is responsible of cytosolic dNTPs pool synthesis and contribute to the maintenance of mitochondrial dNTPs pool in post-mitotic cells thanks to the activation of p53R2 subunit. Similarly to other mtDNA replication defect, RRM2b deficiency cause a spectrum of disorders that goes from infantile and fatal renal failure with multisystem involvement to late onset progressive external ophthalmoplegia. *In vitro* studies have hypothesized mitochondrial dNTPs pool unbalance as the disease mechanism for this disorder suggesting the potential benefit of nucleoside supplementation. However, the mitochondrial dNTP pool has not been evaluated *in vivo* and the exact beneficial nucleosides supplementation has not been defined yet. **Aims:** in this project we will further investigate the disease mechanism and tissue specificity by generating an RRM2b knockout (KO) and RRM2b knock-in (KI) mouse models (Aim 1) and to evaluate the efficacy of nucleosides supplementation (Aim 2). **Methods:** We will develop the murine models by using CRISPR/Cas9 technologies with the aim to inactivate (RRM2B-knckout) or site-specifically mutate (RRM2B-knockin) the gene of interest. The generation of the two murine models will go through two phases: the first step will consist in the purchase of a murine embryonic stem cell (mESC) line, which will be the platform for genetic engineering experiments (both knockout and knockin). Following each experiment, single-cell cloning will be performed in order to genotype a significant number of clones and find the ones with the desired mutation. The second phase of this project starts with the delivery of the same selected clones to an outsourced facility for the production of the mice through injection of RRM2B-edited cells in murine blastocysts which will be transplanted in recipient females. The RRM2B-knockout and RRM2B-knockin mice will then be transferred to the animal facility of the hosting lab, where they will be fully characterized from a phenotypic, biochemical and behavioral point of view with or without nucleosides supplementation. Treated mice will received 200 mg/kg or 400 mg/kg of standard deoxycythidine (dC), deoxyguanosine (dG), deocyadenosine (dA) and deocythymidine (dT) or modified nucleosides administrated by oral gavage. Mitochondrial DNA depletion, nucleotide pool levels and respiratory chain activities other than phenotypical observation will be used as parameter of efficacy and safety of the treatment. In the specific sub-aim 2a, we will orally supplement the RRM2b KO and KI mouse models with increasing doses (50μM, 200μM, 400μM) of deoxypurine and/or deoxypyrimidine. The dNTP pool mixture will be defined based on the results of mitochondrial dNTP pool analysis performed as in Aim1. In addition to this “canonical” dNTP mix, we will test alternative deoxynucleosides provided by Modis Therapeutics, Inc and designed to improve dNTPs bioavailability. Efficacy and safety will be characterized before and after supplementation in mutant and wild type mice with phenotypical, biochemical and molecular genetics analyses, as detailed in Aim1. **Results:** we will generate disease models for RRM2B disease and we will test the efficacy and safety of nucleosides supplementation therapies. Results from our studies will be potentially translated to patients carrying pathogenic variants in RRM2b and other defects in mitochondrial nucleotides pool balance. The two disease models will be extremely important for further studies in disease mechanism, tissue specificity and experimental therapies.  |
| **DESCRIZIONE DELLE ATTIVITÀ DELL’ASSEGNISTA** *(per i* ***nuovi*** *assegni: max 400 parole; competenze richieste, scansione temporale della formazione, scansione temporale dell’attività, obiettivi primari e secondari)**(per i* ***rinnovi****: max 600 parole – da integrare con la relazione dell’assegnista; formazione raggiunta, attività effettuata, obiettivi raggiunti/competenze acquisite, formazione ancora da acquisire (se pertinente), scansione temporale dell’attività durante il rinnovo)* |  |
| The successful candidate will create gene edited cellular and murine models using CRISPR/Cas9 technology and characterize the molecular genetics, biochemical and clinical phenotype. Candidates should have an interest in learning new skills, techniques and knowledge in mitochondrial translational medicine and applying them to understand disease mechanism and tissue specificity and identify new therapies for human use. **Essential Duties*** Design and conduct experiments in *in vitro* and *in vivo* models using CRISPR/Cas9 gene editing technologies
* Characterize phenotype of mouse models
* Perform molecular genetics and biochemical assays
* Generation and characterization of stem cells
* Pursue independent (but complementary) research interests and interact with a broad spectrum of scientists internally and externally to the Laboratory.
* Publish research results in peer-reviewed scientific or technical journals and present results at external conferences, seminars, and/or technical meetings.
* Organize, analyze, and present data from research at seminars, technical meetings, and national and international conferences

**Qualifications*** PhD in Molecular and Cellular Biology, Genetics, Bioengineering, or a related field.
* Experience with gene editing technologies (e.g., CRISPR/Cas) in mammals.
* Experience with molecular biology and cloning.
* Ability as an innovative experimentalist with a broad range of experience in experimental design, techniques, and execution.
* Ability to develop independent research projects as demonstrated through publication of peer-reviewed literature.
* Proficient verbal and written English communication
* Good interpersonal skills to collaborate effectively in a multidisciplinary team environment and present and explain technical information.

**Timeline****Months 0-6:** gene editing of RRM2b with CRISPR/CAS9 technology in ES cell lines**Months 6-12:** generation and characterization of the mouse models |

*Scheda attività assistenziale (se prevista)*

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| **ATTIVITÀ ASSISTENZIALI DELL’ASSEGNISTA/ N. ORE SETTIMANA** |
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| AZIENDA SANITARIA PRESSO CUI SI SVOLGERÀ L’ATTIVITÀ |