Medicina Traslazionale e Molecolare – DIMET Molecular and Translational Medicine – DIMET	
Progetto di ricerca **Research project**	*"Sviluppo di un protocollo analitico per il monitoraggio della Tireoglobulina in pazienti con carcinoma tiroideo con campionamento da remoto mediante sangue capillare essiccato"* – **DIMET. 1** *"Development of an analytical protocol for monitoring thyroglobulin in patients with thyroid cancer by remote sampling using dried capillary blood"* - **DIMET. 1**
Tipo **Type**	Borsa cofinanziata ex D.M. 118/2023 - Scholarship co-funded ex D.M. 118/2023 -
Borse **Scholarships**	1
Abstract	**ITA** Il cancro della tiroide è la più comune neoplasia endocrina e rappresenta circa il 90% dei casi. Una volta diagnosticata, la tiroidectomia totale è il primo passo nel trattamento del DTC. Generalmente, questo tipo di tumori rimane localizzato nella ghiandola tiroidea. Tuttavia, le metastasi più comuni sono quelle linfonodali e raramente anche metastasi a distanza. Il follow-up standard comprende il trattamento con radioiodio Iodio 131 per l'ablazione del tumore residuo e le misurazioni dei livelli sierici di tireoglobulina (Tg) per monitorare possibili recidive. Tuttavia, le valutazioni immunometriche standard dei livelli di Tg potrebbero essere falsate dalla presenza di anticorpi anti-tireoglobulina, producendo livelli sierici di Tg falsamente alti, bassi o non rilevabili e quindi mascherando lo stato della malattia. Recentemente è stata proposta la misurazione della tireoglobulina sierica con approcci di spettrometria di massa come valida alternativa ai saggi immunometrici. Questo progetto mira a sviluppare un nuovo metodo di spettrometria di massa con cromatografia liquida (LC-MS/MS) per la determinazione dei livelli di tireoglobulina utilizzando campioni di di sangue capillare essiccato (DBS) raccolti direttamente dai pazienti in totale autonomia, su carta da filtro e successivamente inviati direttamente al laboratorio di analisi. Le misurazioni ottenute con DBS verranno confrontate con le quelle ottenute da prelievo di sangue venoso convenzionale. **ENG** Thyroid cancer is the most common endocrine malignancy account for approximately the 90% of cases. Once diagnosed, total thyroidectomy is the first step in the treatment of DTC. Generally, these type of cancers remains localized in the thyroid gland and their evolution is very slow with high survival rate. However, lymph nodes metastasis is the most common and rarely also distant metastasis (e.g. lung, bone, brain) can occur. The standard follow-up comprises ^{131}I radioiodine treatment for the ablation of thyroid

	remnant or residual tumour, the whole body scan scintigraphy and the measurements of serum levels of thyroglobulin (Tg) to monitor the recurrence of disease. However, Tg levels measures by standard immunometric assays could be misrepresented by the presence of anti-thyroglobulin antibodies, by producing falsely high, low or undetectable serum Tg levels and thus masking the disease status. Recently the measurements of serum thyroglobulin with mass spectrometry approaches has been proposed has valuable alternative to immunometric assays. This project aims to develop a novel liquid chromatography mass spectrometry (LC-MS/MS) method for the determination of thyroglobulin levels using dried blood spot (DBS) samples. Samples-drops of whole-blood will be collected on filter papers directly from fingers at home, hence shipping the samples directly to the laboratory of analysis. Dried blood spot (DBS) will be compared with conventional venous blood sampling.
Tutor	Prof. Fulvio Magni
Mesi previsti in azienda **Expected months at the company**	**Laboratorio di Patologia Clinica, Policlinico Riuniti di Foggia e Dipartimento d i Medicina Clinica e Sperimentale. Università degli Studi di Foggia** 6
Mesi previsti all'estero **Expected months abroad**	6
Specific IPR rules: standard	

Medicina Traslazionale e Molecolare – DIMET Molecular and Translational Medicine – DIMET	
Progetto di ricerca **Research project**	*"Patologia digitale per la caratterizzazione molecolare del carcinoma tiroideo"* - **DIMET. 2** *"Digital pathology for a multiomics characterization of thyroid cancer"* - **DIMET. 2**
Tipo **Type**	Borsa PNRR cofinanziata ex D.M. 118/2023 - Scholarship PNRR co-funded ex D.M. 118/2023 -
Borse **Scholarships**	1
Abstract	**ITA** La caratterizzazione molecolare delle neoplasie tiroidee si avvale dell'uso sempre più importante della digital pathology come strumento di realizzazione del workflow operativo all'interno del Dipartimento di Anatomia Patologica o come tool di partenza per l'applicazione delle scienze omiche. Il presente progetto si inserisce nella creazione di un network capace di collezionare neoplasie follicolari di diverso spettro biologico e complessità analitica attraverso una rete di digital pathology. Questa premessa sarà il punto di partenza per l'applicazione di livelli multiomici di approfondimento (NGS, MALDI-IMaging e lipidomica) per l'identificazione di nuovi targets molecolari possibilmente traslabili in strumenti diagnostici additivi. Inoltre approcci di computational pathology arricchiranno il periodo del dottorato, per ulteriormente incentivare l'identificazione di criteri aggiuntivi all'occhio umano nella diagnostica istopatologica del cancro tiroideo. **ENG** The molecular characterization of thyroid neoplasms makes use of the digital pathology as a tool for implementing the operational workflow within the Department of Pathological Anatomy or as a starting tool for the application of omics sciences.The present project is part of the creation of a network capable of collecting follicular neoplasms of different biological spectrum and analytical complexity through a digital pathology platform.This premise will be the starting point for the application of in-depth multiomics levels (NGS, MALDI-IMaging and lipidomics) for the identification of new molecular targets possibly translatable into additive diagnostic tools. Furthermore, computational pathology approaches will enrich the PhD period, to further encourage the identification of additional criteria to the human eye in the histopathological diagnosis of thyroid cancer.
Tutor	Prof. Fabio Pagni
Mesi previsti in azienda	**IRCCS IEO, Anatomia Patologica** Da definire

Expected months at the company	To be defined
Mesi previsti all'estero **Expected months abroad**	6
Specific IPR rules: standard	

Medicina Traslazionale e Molecolare - DIMET Molecular and Translational Medicine – DIMET	
Progetto di ricerca Research project	*"Investigation of the co-mutational landscape of naïve and drug-resistant ALK+ tumors: prognostic and therapeutic value"* **- DIMET. 3**
Tipo/Type	Borsa Dipartimentale Scholarship Department
Borse/Scholarships	1
Abstract	**ENG** The project aims to comprehensively analyze the mutational landscape of ALK+ ALCL and NSCLC samples by sequencing: (1) the whole exome; (2) a panel of selected genes at high depth; (3) the whole transcriptome at single-cell level; (4) the circulating tumor DNA. We aim to develop a molecular signature classifier to stratify patients for risk of failing TKI therapy, to identify novel prognostic markers and to characterize the mechanisms of resistance. We plan to apply these findings to the development of combinatorial therapies to treat refractory disease or prevent the rise of TKI resistance in ALK+ cancers. Genomic DNA will be collected from tumor biopsies and normal tissue of 100 ALK+ patients. Matched tumor/normal samples will be analyzed by whole-exome and targeted sequencing using state-of-the-art bioinformatic tools, to detect possible additional co-mutations that may influence treatment outcome. The identified mutations will be weighted using various available in silico resources. Recurrent lesions will be studied in cellular models in vitro, as well as in animal models. Tumor heterogeneity will be investigated by single-cell RNA sequencing. A molecular classifier will be developed by similarity analyses and different clustering methods. Molecular residual disease and recurrence will be monitored by circulating free DNA ultradeep sequencing. Finally, a polypharmacology approach will be designed based on the results obtained, and tested in vitro and in vivo, to preventor tackle TKI resistance. We expect to identify and characterize the somatic events that may co-exist, along with the primary fusion oncogene, in ALK+tumors, thus influencing TKI treatment outcome. Specific genetic signatures will be correlated to the response to TKI therapyand used to design preventive drug combinations. A tool to predict in advance the outcome of TKI therapy in ALK+ patients may lead to an optimization of patients' allocation and the possible development of personalized drug combinations for high-risk patients.
Tutor	Da definire/o be defined
Mesi previsti in azienda/Expected months at the company	/
Mesi previsti all'estero/Expected months abroad	Da definire To be defined
Specific IPR rules: standard	

Medicina Traslazionale e Molecolare - DIMET Molecular and Translational Medicine – DIMET	
Progetto di ricerca **Research project**	*"FANTOM - Future of ALCL: Novel Therapies, Origins, Bio-Markers and Mechanism of resistance"* - **DIMET. 4**
Tipo/Type	Borsa Dipartimentale Scholarship Department
Borse/Scholarships	1
Abstract	**ENG** We plan to run single-cell RNA (scRNA) sequencing to prospectively analyze the gene expression profile of ALK+ ALCL single cells from 20 newly diagnosed patients at presentation. Mononuclear cells will be isolated from bone marrow using a Ficoll gradient, according to a standard protocol. To enrich the samples for lymphoma cells, CD30+ tumour cells will be isolated by fluorescence activated cell sorting (FACS) using an anti-CD30 antibody. CD30+ cells will be loaded onto a Chromium 10x microfluidic instrument to generate CD30+ enriched scRNA libraries. On average 8000 cells per sample will be sequenced at a read-depth of 30,000 reads/cell. The libraries will be sequenced on a Novaseq 6000 instrument (Illumina). Gene expression signatures will be obtained from scRNAseq data and used to identify subgroups of patients with different prognoses. Gene-set enrichment and pathway analyses will be performed, starting from gene expression data, using off-the-shelf bioinformatic tools. Signatures will be correlated to the outcome of treatment. At the same time, single-cell cDNA genotyping will be used to match mutational data to the gene expression profile from the same cell. This information will also allow a study of the clonal evolution of the disease. These data will be used to build molecular signatures of ALCL as a tool for stratification and treatment of relapsed patients: scRNAseq data will be employed to identify subpopulations associated with relapse or with long-term remission in single samples and across patients, through similarity analyses. Single-cell clustering methods, such as SIMLR or the Louvain algorithm, will be used to identify distinct cell clusters and visualize them in a lower-dimension space (e.g., t-SNE, UMAP). The genetic and transcriptomics data generated by scRNAseq analyses will be integrated into bioinformatics pipelines to develop a score to predict therapy outcome for individual patients.
Tutor	Da definire/To be defined
Mesi previsti in azienda/Expected months at the company	/
Mesi previsti all'estero/Expected months abroad	Da definire To be defined
Specific IPR rules: standard	

Medicina Traslazionale e Molecolare – DIMET Molecular and Translational Medicine – DIMET	
Progetto di ricerca **Research project**	*"Modeling neurodevelopmental deficits of the Prr12-dependent syndrome in mice and human pluripotent stem cells"* **– DIMET. 5**
Tipo/Type	Borsa finanziata da ente esterno /Scholarship funded by external body **Consiglio Nazionale delle Ricerche (CNR-IN)**
Borse/Scholarships	1
Abstract	**ENG** Neurodevelopmental disorders affect about 3% of the younger population worldwide and include a large variety of rare diseases with heterogeneous etiology. Because of the dramatic impact that these pathologies have on our society, understanding their pathomolecular mechanisms is of crucial importance. Recently, the widespread application of advanced sequencing technologies has enabled the identification of neglected genes responsible for such disorders. PRR12 is one of these genes and, although its molecular function remains unknown, its haploinsufficiency has recently emerged as the cause of a syndrome associated with developmental delay, intellectual disabilities and eye abnormalities. This project will investigate the molecular mechanisms of action underlying the pathogenic effects of PRR12 haploinsufficiency. Furthermore, as we have demonstrated a direct interaction between PRR12 and SOX2, whose neurodevelopmental syndromes share many similarities, and PRR12 is a paralog of QSER1, a potent regulator of DNA demethylation, we will test if PRR12 might play a key role in promoting DNA demethylation on Sox2 target genes. Genome-wide changes in gene expression, chromatin accessibility and DNA methylation will be analyzed in wild type versus PRR12-mutated human neural progenitor cells derived from induced pluripotent stem cells. In the same cells we will evaluate PRR12 and SOX2 chromatin occupancy and the ability of PRR12 to form condensates, as predicted by our protein structure analysis. To address the role of PRR12 in vivo, we will analyze the molecular, cellular and behavioral phenotypes in a mouse line harboring heterozygous loss-of-function Prr12 mutations, also testing selected data obtained from human neural progenitor cells. Overall, this work is expected to gain molecular insights on the disease and to establish human pluripotent stem cells and mouse models of PRR12 haploinsufficiency that will provide complementary information in the proposed and in future experiments.
Tutor	Da definite/To be defined
Mesi previsti in azienda **Expected months at the company**	/
Mesi previsti all'estero **Expected months abroad**	Max 12
Specific IPR rules: standard	

Medicina Traslazionale e Molecolare – DIMET Molecular and Translational Medicine – DIMET	
Progetto di ricerca **Research project**	*"Gene therapy for Dravet syndrome by CRISPR/Cas9 tools"* **– DIME. 6**
Tipo/Type	Borsa finanziata da ente esterno Scholarship funded by external body **Consiglio Nazionale delle Ricerche (CNR-IN)**
Borse/Scholarships	1
Abstract	**ENG** DS is a severe encephalopathy appearing as febrile seizures in the first year of life in otherwise normal babies and evolving in chronic epilepsy associated with cognitive and behavioral deficits. It is mainly caused by heterozygous loss-of-function mutations in the SCN1A gene, which encodes for the alpha-subunit of the voltage-gated sodium channel Nav1.1, responsible for GABAergic interneuron excitability. To date, no effective cure is available for Dravet patients. Several approaches based on CRISPR/Cas9 mediated gene correction are moving into a pre-clinal phase and one CRISPR/Cas9 therapy has been already administrated to patients with a genetic form of retinal dystrophy. Repairing the endogenous gene will ensure to regain its complete functionality and to re-establish the correct levels of Nav1.1 protein in any neuronal cell type. However, two critical challenges are currently preventing the development of this approach to treat Dravet syndrome. First, the CRISPR/Cas9 system can work only with an error-prone modality in neurons. Second, it is currently lacking a viral vector capable to transduce neurons in large brain areas through an unharmful and non-invasive administration route. We have previously showed the efficacy of the CRISPR/dCas9 activatory system to boost the expression of Scn1a gene in primary neurons derived from a DS murine model (Colasante et al., Molecular Therapy 2020). However, the large size of this activatory tools did not allow its packaging in a single AAV vector and, consequently, the delivery in vivo resulted inefficient and insufficient to rescue the severe phenotype of Scn1a mutant mice. This project aims to generate new dCas9A-activatory systems using smaller Cas9 (SaCas9 and cjCas9) fused to different activatory domains (VPR, P300, VP64) to assess the combination promoting the best activation of Scn1a to possibly achieve physiological levels in neurons. With its reduced size these Cas9 transactivator systems will be packaged together with the sgRNA cassette in a single AAV vector that will be delivered in neonatal Scn1 mutant mice by intraventricular injections in perinatal mouse pups or systemically in symptomatic Dravet mice. Assessment of the efficacy of the treatments in rescuing or ameliorating seizures and cognitive/social alterations will be performed by video-EEG recordings and behavioral tests between 2 and 3 months. For behavioral studies, animals will be tested for 3 different paradigms: learning (Barnes circular maze), social interactions (three chamber test) and hyperactivity (open field). The effect of the treatment on neuronal activity will be assessed by patch-clamp experiments during different stages of the pathology (onset and chronic).
Tutor	Da definire/To be defined

Mesi previsti in azienda/Expected months at the company	Da definite To be defined
Mesi previsti all'estero Expected months abroad	Max 12
Specific IPR rules: standard	

Medicina Traslazionale e Molecolare – DIMET Molecular and Translational Medicine – DIMET	
Progetto di ricerca **Research project**	*"Generazione e caratterizzazione di modelli di Caenorhabditis elegans per malattie neurodegenerative causate da tossicità proteica e da difetti del controllo di qualità delle proteine"* – **DIMET. 7** *"Generation and characterization of Caenorhabditis elegans model(s) for neurodegenerative disorders caused by protein toxicity and defects of protein quality control"* - **DIMET. 7**
Tipo **Type**	Borsa finanziata da ente esterno Scholarship funded by external body **Fondazione IRCCS Istituto Neurologico "Carlo Besta"**
Borse **Scholarships**	1
Abstract	**ITA** La tossicità proteica, definita come accumulo/oligomerizzazione aberrante di proteine tossiche associate a malattia, e i difetti nel controllo di qualità delle proteine sono meccanismi patogenetici chiave in molte malattie neurodegenerative come la malattia di Alzheimer (AD), la malattia di Parkinson (PD), la sclerosi laterale amiotrofica (SLA), la demenza frontotemporale (FTD), la malattia di Huntington (HD) e le atassie spinocerebellari (SCA) (Chung et al., 2018). Nelle forme neurodegenerative ereditarie, la tossicità delle proteine può essere causata da mutazioni genetiche che modificano direttamente le proteine associate alla malattia aumentandone la tossicità o da varianti che causano difetti nell'elaborazione delle proteine/RNA e nel controllo cellulare di qualità. La tossicità da poliglutamine (polyQ) è causata da un'espansione anomala della regione che codifica i tratti poliglutaminici nei geni responsabili delle malattie polyQ (ATXN1/SCA1, ATXN2/SCA2 e HTT/HD). Le espansioni di ripetute non codificanti possono anche provocare la produzione di peptidi tossici mediante il meccanismo della traduzione ATG-indipendente associata alle ripetute (traduzione RAN), come nella patogenesi delle malattie neurodegenerative associate al gene C9ORF72 (SLA e FTD). Nei neuroni, l'accumulo e l'aggregazione di proteine tossiche gravano inevitabilmente sul sistema di controllo di qualità delle proteine (PQC), costituito dal sistema ubiquitina-proteasoma (UPS), dall'autofagia mediata da chaperonine (CMA), compresa l'autofagia selettiva mediata da chaperonine (CASA), e dalla degradazione associata al reticolo endoplasmico (ERAD). Numerose mutazioni genetiche nei componenti della PQC sono associate a malattie neurodegenerative: La E3 ligasi parkina è associata a PD (PARK2), l'enzima deubiquitinante ubiquitina carbossi-terminale idrolasi L1 (UCHL1) è associato a HSP e a PD (SPG79/PARK5), la proteina ATPasi contenente valosina (VCP) è associata a SLA, FTD, miopatia a corpi inclusi e malattia di Charcot-Marie-Tooth (FTDALS6/IBMPFD1/CMT2Y), mentre CHIP, la E3 ligasi del complesso CASA, è associata ad una forma di atassia spinocerebellare (SCAR16). Coerentemente con questi scenari patologici, il potenziamento dei complessi PQC e CASA migliora il fenotipo patologico nei neuroni in molti modelli di malattie neurodegenerative (tra esse, LRRK2, ATXN3 e HD). Recentemente, è stato inoltre dimostrato che CHIP, codificato da STUB1, ha un ruolo cruciale nella patogenesi dell'atassia SCA17 come partner digenico dell'espansione polyQ nel gene TBP. La cosegregazione di una mutazione di STUB1 è infatti necessaria affinché gli alleli intermedi di TBP (41-46 ripetizioni) causino un fenotipo SCA17 completo (Magri et al., 2022). Le basi molecolari della neurodegenerazione causata da tossicità

	proteica possono essere studiate in vivo utilizzando il nematode Caenorhabditis elegans (C. elegans). C. elegans è un piccolo nematode trasparente che è stato impiegato con successo come modello geneticamente manipolato per diverse malattie genetiche associate a ripetizioni (HD, SCA3, DM1 e le malattie legate a C9ORF72) (Rudich e Lamitina, 2018). L'obiettivo di questo progetto è la generazione e la caratterizzazione di modelli di Caenorhabditis elegans per studiare malattie neurodegenerative causate da tossicità proteica e/o da difetti del controllo di qualità delle proteine. Il progetto avrà un focus specifico sul ruolo dell'ereditarietà digenica e dei geni modificatori del fenotipo nel determinare la ridotta penetranza e l'espressività variabile osservata in alcune malattie neurologiche rare ereditarie. I modelli generati saranno utilizzati per la ricerca di nuovi modificatori di malattia e di nuovi bersagli terapeutici mediante screening genetici e farmacologici su larga scala. **ENG** Protein toxicity, defined as aberrant accumulation/oligomerisation of toxic disease-associated proteins, and defects in protein quality control are key pathogenic mechanisms in many neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), amyo-trophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Huntington's disease (HD), and Spinocerebellar Ataxias (SCAs) (Chung et al., 2018). In hereditary neurodegenerative forms, protein toxicity may be caused by genetic mutations that directly affect disease-associated proteins by increasing their toxicity or variants that cause defects in protein/RNA processing and quality control. Polyglutamine (polyQ) protein toxicity is caused by an abnormal expansion of the region encoding polyglutamine stretches in genes responsible for polyQ diseases (e.g., ATXN1/SCA1, ATXN2/SCA2 and HTT/HD). Moreover, noncoding repeat expansions may also result in toxic peptide production by repeat-associated non-ATG translation (RAN translation) contributing to the pathogenesis of neurodegenerative diseases such as C9ORF72-related diseases ALS and FTD. In neurons, accumulation and aggregation of toxic proteins inevitably burden the protein quality control (PQC) system which consists of chaperones and the ubiquitin–proteasome system (UPS), chaperone-mediated autophagy (CMA) including chaperone-assisted selective autophagy (CASA), and ER-associated degradation (ERAD). Interestingly, genetic mutations in PQC components are associated with neurodegenerative diseases: E3 ligase Parkin is associated with PD (PARK2) , deubiquitinating enzyme ubiquitin carboxy-terminal hydrolase L1 (UCHL1) is associated with HSP and PD (SPG79/PARK5), ATPase valosin-containing protein (VCP) is associated with ALS, FTD, and Inclusion body myopathy and Charcot-Marie-Tooth disease (FTDALS6/IBMPFD1/CMT2Y), and the CASA complex component E3 ligase C-terminus of HSP70-interacting protein (CHIP) is associated with spinocerebellar ataxia SCAR16. Consistently, PQC and CASA complex enhancement ameliorate the disease phenotypes in neurons in many neurodegenerative disease models (e.g., LRRK2, ATXN3 and HD mouse and drosophila models). Furthermore, CHIP, encoded by STUB1, was recently demonstrated to have a crucial role in the pathogenesis of SCA17 as a digenic partner of TBP polyQ expansion, since coinheritance of a STUB1 mutation is necessary for TBP intermediate alleles (41-46 repeats) to cause a full-blown SCA17 phenotype (Magri et al., 2022). Relevant information on the molecular basis of protein toxicity-driven neurodegeneration in vivo can be obtained using the nematode Caenorhabditis elegans (C. elegans). C. elegans is a small, transparent nematode that has been successfully employed as a genetic model system for several repeat-associated genetic disorders such as HD, SCA3, DM1 and C9ORF72-related diseases (Rudich and Lamitina, 2018). The aim of the project is the generation and characterization of Caenorhabditis elegans models for neurodegenerative disorders caused by protein toxicity and/or

	defects of protein quality control, with a specific focus on the role of digenic inheritance and phenotype-modifying genes in determining the reduced penetrance and variable expressivity observed in some hereditary rare neurological diseases. The generated models will be used to search for new disease-modifiers and new therapeutic targets by large scale genetic and pharmacological screenings.
Tutor	Da definite To be defined
Mesi previsti in azienda **Expected months at the company**	Da definite To be defined
Mesi previsti all'estero **Expected months abroad**	Max 12
Specific IPR rules: standard	