Associazione Italiana Sindrome di Shwachman-Diamond (AISS)
Via Pioveghetto 15, - 35136 Padova
Tel - FAX +39 049 8736130
E-mail: aiss@shwachman.it

# Shwachman-Diamond Syndrome Italian Research Grant

**Maximum Amount euro 10.000,00**

**Firm Deadline for Receipt of Applications: 3rd March 2018**

Eligibility: Persons applying for these grants if not in a faculty position need to provide a declaration by a supervisor with a position in the department (not a training position) and with authority to hold an independent research grant.

Terms of Support: Support may be provided for one (1) year in an amount not to exceed E 10,000. Indirect costs are permitted and are not to exceed 10% of the total costs.
The AISS will provide preference to those applications in which funds are used for supplies, equipment, technicians and other expenses and not for support of the salary of the PI or co-PIs.

Review: All applications will be reviewed by the AISS Scientific Committee (AISS-SC) or its designees.

Application: The application contains two sections.

Section 1, forms attached. The applicant and co-applicants must also include a current curriculum vitae.
Section 2: Research Plan, divided as indicated below. Parts A through D should not exceed 6 pages, using a font no smaller than 10 point.

Part A. Specific aims
Part B. Significance and background
Part C. Preliminary studies
Part D. Experimental design and methods
Part E. References (not included in the 6 page limit)
Part F. Relevance of the research to Shwachman-Diamond Syndrome
Part G. For junior faculty separate letter from supervisor or department head confirming commitment to project, and to provision of space and facilities
Part K. If human subjects and animals are involved, a statement by the PI or supervisor overseeing human or animal studies is compulsory. If considered as necessary by the AISS-SC, more information about ethical committee study approval may be asked.

Submission: by email to the AISS: aiss@shwachman.it

**1. Title of Proposal:**

Molecular insights into the translational regulation in SDS: Ataluren-driven modifications of translational initiation complex and control in lymphoblastoid cell lines.

2**. Applicant Information:**

Name: Jacopo Morini

Title and Degree(s): PhD In Genetics, Molecular And Cellular Biology; MSc in Biology

Work Address: Laboratory of Radiobiology and Radiation Biophysics, Physics Department, via Bassi 6, I-27100 Pavia (PV), Italy.

Phone: +39 - 0382 - 987644 (lab) / +39 - 0382 - 987906 (office)

Fax: +39 - 0382 - 987752

Email: jacopo.morini@unipv.it

3. **Applicant Curriculum Vitae**: beginning on the next page, with 2 page limit. This will form application pages 3 and 4.

**CV of Dr. Jacopo Morini, MSc PhD -** PostDoc Fellow at the Physics Department, University of Pavia (Italy).

**Curriculum Vitae et Studiorum**

January 2015: PhD in Genetics, Molecular and Cellular Biology (XXVII cycle), University of Pavia, Italy.
November 2012: Granted access to the Italian Biologist Council in virtue of state examination.
July 2011: Master Degree *cum laude* in Experimental Applied Biology, curriculum "Human Biology and Biomedical Sciences".

**Schools and Courses (selection)**

From March, 21st, 2017 to March, 22nd, 2017: Training Course in Cytofluorimetry "Attune NxT Acoustic Focusing Cytometer Basic Training", ThermoFisher Scientific, Darmstadt, Germany.

From June, 3rd, 2013 to June, 21st, 2013: Selected for the "2013 NASA Space Radiation Summer School" at the U.S. Department of Energy - Brookhaven National Laboratory, Upton NY, USA.

**Awards**

Best Poster Award: "*Radiosensitivity and DNA damage repair in lymphoblastoid cell lines derived from Shwachman-Diamond syndrome patients*". 8th International Congress on Shwachman-Diamond Syndrome, Verona (Italy), 17-20 April 2016.

**Working Activity**

From January 2015 - ongoing: Post-Doc Fellowship, Radiobiology and Radiation Biophysics Group, Department of Physics, University of Pavia.

From April, 16th, 2014 to October, 16th, 2014: Six-month contract "*Uso della radiobiologia per ottimizzare l'esposizione in radiologia: risposta multiscala dei sistemi biologici* ", EU-funded EUTEMPE-RX project, Department of Physics, University of Pavia.

From March, 1st 2013 to September, 1st 2013: Six-month contract "*Biological responses and pathway activities activated by ionizing radiation induced damage*", EU-funded DoReMi (Initium) project, Department of Physics, University of Pavia.

**Projects**

Jacopo Morini has contributed to several National- and EU-funded research projects, with research activities on biological effects of radiation exposure (e.g. inflammation pathways and radio-immunotherapy) and individual radiosensitivity. Furthermore, he was PI of two research projects on Shwachman-Diamond syndrome.

*EU-funded projects*: Andante - *Multidisciplinary evaluation of the cancer risk from neutrons relative to photons using stem cells and the induction of second malignant neoplasms following paediatric radiation therapy*; EpiRadBio - *Combining epidemiology and radiobiology to assess cancer risks in the breast, lung, thyroid and digestive tract after exposures to ionizing radiation with total doses in the order of 100mSv or below*; DoReMi - *Low Dose Research towards Multidisciplinary Integration*.

*National projects*: *Mechanism underpinning the DNA-damage response in lymphoblastoid cell lines from Shwachman-Diamond syndrome patients*, funded by AISS - Italian Society for Shwachman Syndrome; MERIDIAN - *Measuring the Effects of Radiation on Immunity and DIfferentiAtioN*, funded by INFN - Nuclear Physics National Institute; *Susceptibility to oxidative stress caused by ionizing radiation exposure in Shwachman-Diamond Syndrome affected patients lymphocytes*, funded by AISS - Italian Society for Shwachman Syndrome; RADIOSTEM - *Meccanismi di risposta RADIObiologica a fotoni e a particelle cariche di cellule STaMinali tumorali e derivanti da tessuto sano*, funded by INFN - Nuclear Physics National Institute.

**International Publication *in extenso*:**

I. G. Babini, **J. Morini**, S. Barbieri, G. Baiocco, G. B. Ivaldi, M. Liotta, P. Tabarelli de Fatis, A. Ottolenghi. A *co-culture method to investigate the crosstalk between X-ray irradiated Caco-2 cells and PBMC*. Journal of Visualized Experiments. (131), e56908, doi:10.3791/56908 (2018).

II. S. Plumitallo, L. Ruiz-Llorente, C. Langa, **J. Morini**, G. Babini, D. Cappelletti, L. Scelsi, A. Greco, C. Danesino, C. Bernabeu, C. Olivieri. *Functional analysis of a novel ENG variant in a patient with Hereditary Hemorrhagic Telangiectasia (HHT) identifies a new Sp1 binding-site*. Gene. 647:85-92. doi: 10.1016/j.gene.2018.01.007. (2018) [Epub ahead of print].

III. V. Bezzerri, D. Bardelli, **J. Morini**, A. Vella, S. Cesaro, C. Sorio, A. Biondi, C. Danesino, P. Farruggia, B. M. Assael, G. D'Amico, M. Cipolli. *Ataluren-driven restoration of Shwachman-Bodian-Diamond Syndrome protein function in Shwachman-Diamond Syndrome bone marrow cells.* American Journal of Hematology. doi: 10.1002/ajh.25025. (2017). [Epub ahead of print].

*IV.* G. Baiocco, S. Barbieri, G. Babini, **J. Morini**, W. Friedland, P. Kundràt, E. Schmitt, M. Puchalska, U. Giesen, R. Nolte, A. Ottolenghi. *At the physics-biology interface: the neutron affair*. Radiation Protection Dosimetry. doi.org/10.1093/rpd/ncx222. (2017).

*V.* **J. Morini**, G. Babini, S. Barbieri, G. Baiocco, A. Ottolenghi. *The interplay between radio-resistant Caco-2 cells and the immune system increases epithelial layer permeability and alters signaling protein spectrum*. Frontiers in Immunolology. 8:223. doi: 10.3389/fimmu.2017.00223 (2017).

VI. L. Nacci, R. Valli, R.M. Pinto, M. Zecca, M. Cipolli, **J. Morini**, S. Cesaro, E. Boveri, V. Rosti, P. Corti, M. Ambroni, F. Pasquali, C. Danesino, E. Maserati, A. Minelli. *Parental origin of del(20q) in Shwachman-Diamond patients and loss of the paternally derived allele of the imprinted L3MBTL1 gene*. Genes Chromosomes and Cancer. 56(1):51-58. doi: 10.1002/gcc.22401 (2017).

VII. G. Baiocco, S. Barbieri, G. Babini, **J. Morini**, D. Alloni, W. Friedland, P. Kundrát, E. Schmitt, M. Puchalska, L. Sihver, A. Ottolenghi. *The origin of neutron biological effectiveness as a function of energy*. Scientific Report 6:34033. doi: 10.1038/srep34033. (2016).

VIII. G. Babini, **J. Morini**, G. Baiocco, L. Mariotti, A. Ottolenghi. *In vitro γ-ray-induced inflammatory response is dominated by culturing conditions rather than radiation exposures*. Scientific Reports 5:9343. doi: 10.1038/srep09343. (2015).

IX. **J. Morini**, G. Babini, M. Ferrari, C. Maccario, L. Mariotti, A. Minelli, M. Savio, A. Guertler, U. Kulka, U. Roessler, A. Ottolenghi, C. Danesino. *Radiosensitivity in lymphoblastoid cell lines derived from Shwachman-Diamond syndrome patients*. Radiation Protection Dosimetry vol. 166, p. 95-100 (2015).

X. G. Babini, V. Bellinzona, **J. Morini**, L. Mariotti, K. Unger, A. Ottolenghi. *Mechanisms of the induction of apoptosis mediated by radiation-induced cytokine release*. Radiation Protection Dosimetry vol. 166, p. 165-169 (2015).

XI. G. Babini, M. Ugolini, **J. Morini**, G. Baiocco, L. Mariotti, P. Tabarelli de Fatis, M. Liotta, A. Ottolenghi. *Investigation of radiation-induced multilayered signalling response of the inflammatory pathway*. Radiation Protection Dosimetry vol. 166, p. 157-160 (2015).

XII. Minelli, L. Nacci, L. Sainati, D. Longoni, F. Poli, M. Cipolli, S. Perobelli, E. Nicolis, Z. Cannioto, F. Pasquali, **J. Morini**, C. Danesino. *Acquired copy number neutral loss of heterozygosity (CN-LOH) of chromosome 7 is not a common mechanism in patients with Shwachman-Diamond syndrome*. British Journal of Haematology. vol. 165, p. 573-575 (2014).

4. **Applicant's Commitment as Investigator of the Project:**

I agree as the applicant to accept responsibility for the scientific management of this project as outlined in this application. I further agree to submit a report at the end of the granting period.

5. **Applicant's Affirmation:**

I certify that the investigations involving human subjects to be carried out in the application will have approval of the applicant's Institutional Ethical Committee

Approvals from the Institutional Ethical Committee must be included with the application.

6. **Research Results:**

Results of research may be made available to the public through appropriate scientific channels. All publications will bear the statement: "This work was supported by a grant from Associazione Italiana Sindrome di Shwachman - AISS"

Signature of Applicant

01/03/2018
Date

**7. Applicant's Institution Certification and Commitment:**

I certify that the statements herein and the Applicant's Affirmation are true, complete and accurate to the best of my knowledge and I agree to accept responsibility for the fiscal management of this project as outlined in this application. I further agree to commit this institution to comply with the Associazione Italiana Sindrome di Shwachman-Diamond (AISS) terms and conditions if a grant is awarded as a result of this application.

Name of Institution Official: Prof. Alberto Rotondi

Title: Head of the Physics Department

Address: Physics Department, via Bassi 6, I-27100 Pavia (PV), Italy

Phone: +39 - 0382 - 987626

Fax: +39 - 0382 - 987752

E-mail Address: alberto.rotondi@unipv.it / dipartimento.fisica@unipv.it / fisica@certunipv.it

Università degli Studi di Pavia
DIPARTIMENTO DI FISICA
IL DIRETTORE
Prof. Alberto Rotondi

Signature of Institution Official

01/03/2018
Date

**ABSTRACT OF RESEARCH PLAN**

Within the space provided, summarize the long-term objectives, scientific aims and methodology of the proposal.

**TITLE: "**Molecular insights into the translational regulation in SDS: Ataluren-driven modifications of translational initiation complex and control in lymphoblastoid cell lines"

Shwachman-Diamond Syndrome (SDS) is a rare autosomic recessive ribosomopathy caused by mutations in *SBDS*. Only very recently, mutations in *DNACJ21*, *EFL1* and *SRP54* were also reported as causative. SDS features include exocrine pancreatic insufficiency, neutropenia, skeletal and hematologic abnormalities.

To date, therapeutic strategies for SDS rely on symptomatic control, while hematopoietic stem cell (HSC) transplantation could be required in case of severe bone marrow (BM) dysfunction or MDS/AML. Ribosomes are the fundamental machinery able to translate the RNA sequence into the amino acids' alphabet. After a pre-assembly process which is located within the nucleus, ribosome subunit precursors pre-60S and pre- 40S are exported to the cytoplasm where maturation is completed. In this framework, SBDS is involved in the final step of pre- 60S ribosomal subunit maturation. Furthermore, SBDS acts by uncoupling GTP hydrolysis from eIF6 release during late cytoplasmic maturation of pre-60S subunits. Pathogenic SBDS mutations have been found to compromise the ability of SBDS to promote ribosomal subunit association, and cells from SDS patients exhibited lower 80S levels. Moreover, it has been shown that the mechanism of eIF6 release from pre-60S ribosomal subunits is independent of eIF6 phosphorylation, but required GTP binding and hydrolysis by EFL1.

A promising pharmacological approach has been recently proposed: the drug Ataluren (already used in the management of Duchenne Muscular Dystrophy) has been demonstrated to restore SBDS expression in hematopoietic stem cells and lymphoblastoid cell lines from SDS patients. New mechanistic studies of translation initiation and control modification in SDS and their alterations after treatment with Ataluren are now mandatory, to shed light on mechanisms underpinning the observed effect.

To this aim, we propose to investigate the main players involved in the regulation of translation process in presence/absence of Ataluren. The experiments will be performed by means of western blot analysis and flow cytometry.

This project will contribute to elucidate translation mechanisms involved in SDS and the mechanisms of pharmacological-driven restoration.

**BUDGET**

List below a budget by categories for the support. The review committee will carefully consider the appropriateness of your budget. It must be well defined, justified, and realistic to complete the work proposed. The first column defines the total expenses that are expected to be necessary to realistically complete the project. The second column indicates the expenses requested from the AISS. Applicants **will not** be penalized in funding considerations for requiring additional funds beyond what is requested from the Foundation (AISS); however, the true costs of the project must be acknowledged. [This and the section on page 1 re: Other Funding need to be consistent]

**EURO Amount Requested for:**

| | **TOTAL COSTS REQUIRED TO COMPLETE PROJECT:** | **COSTS REQUESTED FROM AISS**: (not to exceed E 10,000) |
|---|---|---|
| Personnel (including fringe benefits):<br>PI: Name:<br>Co-I Name:<br>Additional personnel (identify role):<br>Name: | 0<br><br><br>0 | 0<br><br><br>0 |
| Equipment: | 0 | 0 |
| Supplies: | 0 | E. 7.500 |
| Other Expenses: | 0 | E. 1.000 |
| Indirect Costs (not to exceed 10% of total) | 0 | 0 |
| **TOTAL COSTS:** | 0 | E. 8.500 |

**Justification**: Define and justify expenses in each category. Explain the role of each of the individuals named in the Personnel section. The justification must include an explanation of what each category contributes to the project. Also explain any marked differences between the first- and second-year expenses in a particular category. The AISS will provide preference to those applications in which funds are used for supplies, equipment, technicians and other expenses and not for support of the salary of the PI or co-PIs. The AISS-SC may ask for further expense details.

Personnel: Not Requested.

Supplies: costs requested from AISS includes all the materials for cell culturing of lymphoblastoid cell lines, for the analysis of translational initiation complex and control analysis by flow cytometry.
In particular, expenses comprises the purchase of reagent for cell culture (RPMI 1640, fetal bovine serum (FBS), additives (antibiotics, L-Glutamine)); consumable for cell culturing (flasks, tubes); reagent and antibodies (eIF4A, eIF4B, p-eIF4B, eIF4E, p-eIF4E, eIF4G, p-eIF4G, eIF4H, etc…).

Other: costs requested from AISS for “other expenses” include expenses for possible publication of results.

Indirect Costs: Not Requested.

**Other Support for this Project:**
Applicants are allowed to receive funding from other sources for parts of the project not funded by the AISS. Please, list all other funding sources.

A project on Shwachman-Diamond Syndrome and Ataluren effects has been submitted to "Fondazione Cariplo" as an answer to the call " Ricerca Biomedica Giovani Ricercatori - 2018".

**Research Plan**

**Part A. Specific aims**

The aim of the project: "*Molecular insights into the translational regulation in Shwachman-Diamond Syndrome: Ataluren-driven modifications of translational initiation complex and control in lymphoblastoid cell lines*" is to investigate the mechanisms of translation disruption due to SBDS deficiency and the effects of its pharmacological-driven restoration by the drug Ataluren. To this aim, we will exploit lymphoblastoid cell lines (LCLs) from patients affected with Shwachman-Diamond Syndrome (SDS), already established in the laboratory of Radiobiology and Radiation Biophysics, Physics Department, University of Pavia, in collaboration with the Unit of Medical Genetics of University of Pavia. As main endpoint, we will focus on the analysis of the principal proteins involved in the above mentioned pathways, trying to elucidate their roles in SDS, with an approach based on flow cytometry analysis.

**Part B. Significance and background**

***i) Shwachman-Diamond Syndrome (SDS)***

Shwachman-Diamond Syndrome (SDS), a rare disease affecting 1/168000 newborns in Italy (Minelli, 2012), is caused by mutations in *SBDS* (Boocock *et al.*, 2003). From a clinical point of view, the symptoms of SDS consist in exocrine pancreas insufficiency, skeletal problems and bone marrow failure, the latter leading to neutropenia, and a risk of developing bone marrow failure and myelodysplastic syndrome (MDS) / acute myeloid leukemia (AML).

The most frequent mutations affecting SBDS are c.[183-184TA>CT] and c.[258+2T>C], although others less frequent mutations are described in literature. The c.[183-184TA>CT] mutation causes an in-frame insertion of a premature stop codon which abolishes completely protein synthesis; on the other hand, c.[258+2T>C] causes an amino acid change resulting in the disruption of a splice donor site, with the following reduction in the protein synthesis. Since c.[183-184TA>CT] has never been observed in homozygosis, and since SBDS is very conserved during evolution, it has been supposed that a little amount of SBDS is mandatory for life; for these reasons, c.[183-184TA>CT] is considered as a lethal mutation when present in homozygosis.

In 2017, biallelic mutations in *DNAJC21* and *EFL1* and a dominant mutation in *SRP54* were described as causative of SDS (Carapito *et al.*, 2017; Dhanraj *et al.*, 2017; Stepensky *et al.*, 2017), leading to a renewed attention to SDS research, concerning in particular the evidence for a genotype-phenotype correlation.

Since the discovery of *SBDS* as causative gene, a plethora of studies aimed to understand the molecular pathogenesis of this complex disease. Different studies highlighted that SBDS is involved in many different pathways: i) SBDS absence was observed to cause mitotic spindle destabilization (Austin *et al.*, 2008); ii) SBDS influences the cellular response to several stressors, both chemical and physical: absence of SBDS was described to modify the response to endoplasmic reticulum drugs like tunicamycin and thapsigargin, UV light and X-rays (Ball *et al.*, 2009; Morini *et al.*, 2015); iii) apoptosis was observed to be influenced by SBDS absence (Rujkijyanont *et al.*, 2009; Watanabe *et al.*, 2009).

***ii) Shwachman-Diamond Syndrome as a Ribosomopathy***

Nowadays, due to the strong involvement of SBDS in ribosome biogenesis, SDS is commonly considered as a ribosomopathy caused by uncoupling GTP hydrolysis from eIF6 release during late cytoplasmic maturation of pre-60S subunits.

The first evidence of correlation between SBDS protein and 60S ribosomal subunit biogenesis and functioning was obtained from studies in *Saccharomices cerevisiae* (Menne *et al.*, 2007). In fact, the yeast SBDS orthologue SDO1 has a critical function in a late cytoplasmic step of 60S ribosomal subunits maturation. SDO1 is involved in a pathway together with the GTPase elongation factor-like 1 (EFL1), to facilitate the release and recycling of Tif6 from pre-60S subunits. Tif6, the mammalian homolog of eIF6, is

implicated in the maturation and nuclear export of the 60S subunit (Basu *et al.*, 2001; Senger *et al.*, 2001) and sterically prevents premature joining of the 60S subunits to the 40S subunits. Indeed, 80S assembly can occur only after dissociation of Tif6, because Tif6 and the 40S subunit recognize the same binding region on the 60S subunit (Gartmann *et al.*, 2010). Multiple gain-of-function Tif6 alleles were found to suppress the slow-growth phenotype observed in cells deleted for SDO1 (Menne *et al.*, 2007) or EFL1 (Senger *et al.*, 2001), recovering the pre-60S nuclear export defect and cytoplasmic mislocalization of Tif6.

Based on these results, the model of eIF6 release by SBDS and EFL1 was revised in an evolutionarily conserved one. In all Eukaryotes, SBDS and EFL1 directly catalyze eIF6 removal from pre-60S ribosomal subunits, leading to the translational activation of ribosomes. A direct interaction between recombinant SBDS and EFL1 alters the conformation of the interacting domain of EFL1 and supports the model wherein SBDS conformational changes couple ELF1 activity to eIF6 release from the pre-60S subunits (Asano *et al.*, 2014).

***iii) Ataluren and its effects in SBDS restoration***

Ataluren is a drug approved by both European Medicines Agency (EMA) and American Food and Drug Administration (FDA) for the management of patients affected with Duchenne Muscular Dystrophy. The molecule actively promotes the nonsense mutation suppression (Peltz *et al.*, 2013) and the insertion of a near-cognate tRNA at the nonsense codon site, in a process comparable to the physiological read-through (Roy *et al.*, 2016).

Ataluren was demonstrated to restore the expression of SBDS in different experimental SDS set-ups. In their work, Bezzerri and colleagues (Bezzerri *et al.*, 2017) showed the restoration of SBDS in lymphoblastoid cell lines (LCLs), mononuclear cells (MNCs) and mesenchymal stromal cells (MSCs) from peripheral venous blood or bone marrow samples from patients carrying the c.[183-184TA>CT] mutation. In particular, treatment with Ataluren was shown to promote myeloid differentiation of hematopoietic progenitor cells, reducing the apoptosis rate in PBMCs and the phosphorylation of different proteins (*i.e.* mTOR) associated with the onset of different types of leukemia and lymphoma.

Since the clinical management of symptoms in SDS relies only on a symptomatic therapy (*e.g.* pancreatic enzymes supplementation), and since hematopoietic stem cell transplantation could be the only therapeutic strategy in the case of bone marrow failure or MDS/AML, the characterization of translation impairment in SDS is essential to understand the pathogenesis of the different disease-related features. Research into the underlying mechanisms could lead to practical outputs for the development of specific therapies, at least for the potentially fatal outcomes. Results from this project will clearly represent a step forward, both in the knowledge about SDS pathogenesis and its clinical management. For these reasons, we strongly believe that this project has great potential to ensure benefits for patients.

**Part C. Preliminary studies**

As preliminary study, we already assessed the effect of Ataluren in SDS-derived LCLs viability and mortality. We performed MTT and Trypan Blue assays to test a range of Ataluren concentrations, looking for a possible threshold above which a cytotoxic effect starts to manifest: we treated two LCLs with Ataluren for 24-48h, with concentrations ranging from 0 to 20 μM, and compared the results to those obtained from non-treated cells and cells treated only with vehicle (DMSO). Our results revealed no cytotoxic effects in the tested concentration range.

As a follow-up of the study, we then performed viability and mortality assays in fresh Peripheral Blood Mononuclear Cells (PBMC) isolated from peripheral venous blood and stimulated with Phytohaemoagglutinin (PHA): results obtained in freshly collected PHA-stimulated PBMC were completely comparable with those obtained in LCLs. Moreover, if either 5 or 10 μM of Ataluren were able to increase SBDS protein level was assessed through western blot analysis; results confirmed the ability of the drug to improve SBDS synthesis. These results were published in 2017, as part of the dataset in the peer-reviewed

article by Bezzerri et al. (Bezzerri *et al.*, 2017).

**Part D. Experimental design and methods**

The main focus of this project will be the analysis of the proteins involved in the regulation of translation initiation complex and in the translation control; to this aim, we will culture LCLs in presence or absence of Ataluren.

Several LCLs from SDS patients carrying different *SBDS* mutation are already available in our laboratory: in detail, thanks to the collaboration with the Unit of Medical Genetics of University of Pavia (Prof. Cesare Danesino), we collected 8 LCLs carrying different mutations, namely c.[183_184TA>CT], c.[258+2T>C], c.[101A>T].

For the purposes of our project, we will select only those LCLs carrying nonsense mutations. The LCLs carrying nonsense mutation will be cultured at 37°C, 5% $CO_2$.

Our experimental plan foresees the evaluation of the activity of translation initiation complex through the analysis of the eIF4 family proteins (eIF4A, eIF4A1, eIF4B, eIF4E, eIF4G, eIF4H) and their phosphorylated form (p-eIF4B, p-eIF4E, p-eIF4G). The role of the different proteins is specified in Tab. 1.

| TRANSLATION INITIATION COMPLEX | |
|---|---|
| **PROTEIN** | **FUNCTION** |
| eIF4A | ATP-dependent RNA helicase, unwinds the secondary structure of the 5' mRNA UTR. |
| eIF4B | Assists the eIF4F complex in translation initiation. |
| eIF4E | Binds 5'$m^7$GTP cap and unwinds mRNA secondary structure; allows 40S subunit binding. |
| eIF4G | Scaffold protein that promotes assembly of eIF4E and eIF4A into the eIF4F complex. |
| eIF4H | Induces the RNA-dependent ATP hydrolysis catalyzed by the initiation factors eIF4A and eIF4B. |

Tab. 1 - Principal proteins involved in the Translation Initiation Complex and their functions.

Moreover, we will focus also on those proteins involved in translation control. The roles of the different proteins are reported in Tab. 2.

| TRANSLATION CONTROL | |
|---|---|
| **PROTEIN** | **FUNCTION** |
| 4E-BP1 | Activated by mTOR. |
| p70 S6 kinases | Phosphorylates the 40S ribosomal subunit protein S6 and stimulates the translation of 5' oligopyrimidine tract containing mRNAs. |
| Akt | Regulates mTOR. |
| S6 ribosomal protein | Correlates with an increase in translation of mRNA transcripts that contain an oligopyrimidine tract in their 5' UTRs. |
| eIF2α | Down-regulates protein synthesis under a variety of stress conditions. |

Tab. 2 - Principal proteins involved in the Translation Control and their functions.

From an experimental point of view, cells will be seeded 24 hours before the experiments starts (time = 0 h): At 0 hours, cells will be treated with either 2.5, 5 and 10μM Ataluren, with vehicle or without any treatment.

Samples will be collected after 24 and 48 hours post-treatment and cells will be fixed and analyzed.

Analysis will be carried out mainly by means of flow cytometry. Multicolor analysis will assure to get high-throughput data, also exploiting the possibility, offered by this technique, to allow the analysis of a huge number of cells, improving the statistical significance of the collected data.
The project will exploit the Radiobiology and Radiation Biophysics laboratory facility and equipments (Physics Department, University of Pavia).

**Part E. References**

Asano, N., Atsuumi, H., Nakamura, A., Tanaka, Y., Tanaka, I. and Yao, M. (2014) 'Direct interaction between EFL1 and SBDS is mediated by an intrinsically disordered insertion domain', *Biochemical and Biophysical Research Communications*, 443(4), pp. 1251–1256. doi: 10.1016/j.bbrc.2013.12.143.

Austin, K. M., Jr, M. L. G., Coats, S. A., Tulpule, A., Mostoslavsky, G., Balazs, A. B., Mulligan, R. C. and Daley, G. (2008) 'Mitotic spindle destabilization and genomic instability in Shwachman-Diamond syndrome', *The Journal Clinical Investigation*, 118, pp. 1511–1518.

Ball, H. L., Zhang, B., Riches, J. J., Gandhi, R., Li, J., Rommens, J. M. and Myers, J. S. (2009) 'Shwachman-Bodian Diamond syndrome is a multi-functional protein implicated in cellular stress responses.', *Human molecular genetics*, 18, pp. 3684–3695.

Basu, U., Si, K., Warner, J. and Maitra, U. (2001) 'The Saccharomyces cerevisiae TIF6 gene encoding translation initiation factor 6 is required for 60S ribosomal subunit biogenesis.', *Mol Cell Biol*, (5), pp. 1453–1462. doi: 10.1128/MCB.21.5.1453.

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**Part F. Relevance of the research to Shwachman-Diamond Syndrome**

Over the years, several studies aimed to investigate the molecular mechanism involved in the onset of different symptoms and features observed in Shwachman-Diamond syndrome. In this framework, an important turning point was the description of SDS as a ribosomopathy, followed by structural and mechanistic studies describing how SBDS is a player in ribosome biogenesis and translation processes, with particular regards to its relation with eIF6.

Only in 2017 the idea that a pharmacological treatment could drive SBDS restoration was put forward, giving new hope for the treatment of SDS. Whether it is possible to develop drugs able to manage the spectrum of different symptoms due to a genetic syndrome is controversial, and subject of a large debate. A better understanding of the mechanisms behind SDS is then mandatory to lay the foundation for a paradigm shift. In this scenario, this projects aims to clarify some questions linked to both translation control and the effect of SBDS pharmacological restoration on such pathway. Our study represents a step forward for a possible clinical trial toward the development of new therapeutic options, with concrete benefits for patients with rare diseases such as SDS.

**Part G. For junior faculty separate letter from supervisor or department head confirming commitment to project, and to provision of space and facilities**

A separate letter from the head of the Radiobiology and Radiation Biophysics Laboratory, Prof. Andrea Ottolenghi, is attached to the proposal.

**Part K. If human subjects and animals are involved, a statement by the PI or the supervisor overseeing human or animal studies is compulsory. If considered as necessary by the AISS-SC, more information about ethical committee study approval may be asked**

No human subjects will be enrolled in this study. Experimental activity will be carried out on lymphoblastoid cell lines already available at the Radiobiology and Radiation Biophysics Lab.