Associazione Italiana Sindrome di Shwachman-Diamond (AISS)
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Shwachman-Diamond Syndrome Italian Research Grant

Maximum Amount euro 10.000,00

Firm Deadline for Receipt of Applications: 31 December 2016

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

version history 16/11/2016

1. Title of Proposal: ROLE OF THE HUMAN NICHE IN INDUCING AND SUPPORTING MYELODYSPLSTIC SYNDROME AND/OR LEUKAEMIA EVOLUTION IN SHWACHMAN-DIAMOND SYNDROME PATIENTS

2**. Applicant Information:**

Name: Giovanna D'Amico

Title and Degree(s): Researcher, PhD

Work Address: Centro Ricerca "M. Tettamanti", Clinica Pediatrica Università Milano- Bicocca, Ospedale San Gerardo, via Pergolesi, 33, 20900 Monza.

Telephone: +39-039-2333661
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Email: giovanna.damico@hsgerardo.org

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

GIOVANNA D'AMICO: Biologist

Date and place of birth: August 7th 1972, Milan

Education

2001-2002: European Master in "Advanced Immunology" University of Milano-Bicocca, Italy, Institute Pasteur and M. Curie University of Paris, France.
1998-2000: Specialist in Biotecnology application, University of Milano-Bicocca. 50/50 with honours.
1996- 1999: Specialist in Pharmacology Research at the "Mario Negri" Institute, Milan, Italy
1996: Biological Science degree at the University of Milan, Italy.
110/110 with honours.

Research Support

2005-Present Permanent position as head of "Immunology and Cell therapy" Unit at the "M.Tettamanti" Research Center.
2002-2005 Fellowship from Federazione Italiana Ricerca sul Cancro (FIRC)
2000-2001 Fellowship from Centro Ricerche "M. Tettamanti"
1998-2000: Fellowship from University of Milan
1997-1998 Fellowship from "Mario Negri" Institute

Research Experience

2005-now Senior scientist at the "M. Tettamanti" Research Center, Monza, Italy. Head of the "Immunology and Cell therapy" Unit.
2000-2005 : Researcher at the "M. Tettamanti" Research Center, for leukemia and hematological diseases of children, Pediatric Clinic, University of Milan-Bicocca, Ospedale San Gerardo, Monza, Italy.
1997-2000: Researcher at the department of immunology and Cellular Biology of Prof. Alberto Mantovani, "M. Negri" Research Institute, Milan, Italy.
1995-1996: Research internship at the department of immunology and Cellular Biology of Prof. Alberto Mantovani "M. Negri" Research Institute, Milan, Italy.

Awards

Sep 2008 Award from Italian Society of Pediatric Oncology for the communication: "Potenziale ruolo dei linfociti T Helper (TH)-17 nell'induzione della malattia di trapianto verso l'ospite nei pazienti sottoposti a trapianto allogenico di cellule staminali".

Grants

2014-2017 Italian Association for Cancer Research (IG), Italy. Principal Investigator. Subject: Analysis of ALL niche.
2013-2015 Italian Association for Cancer Research (AIRC5X1000). Renewal. Component, Subject: innate immunity of cancer. Molecular targeting and cellular therapy
2009-2012 Italian Association for Cancer Research (Progetto Regionale), Italy. Component. Subject: Molecular and Cellular Imaging in Cancer
2011-2013 Italian Association for Cancer Research (AIRC5X1000). Component, Subject: innate immunity of cancer. Molecular targeting and cellular therapy
2011-2012 Ministero della Salute- Giovani Ricercatori. Co-Pricipal investigator. Subject: Immunomodulatory properties of MSC
2011-2012 Ministero della Salute-Bando staminali. Component. Subject: Immunomodulatory properties of MSC

The applicant has a long lasting experience in immunology and cell biology in particular applied to the hematopoietic system and to human leukemias. Her interests include the phenotypical and functional characterization of MSC derived from patients with Shwachman-Diamond Syndrome (SDS). The applicant has

worked for almost 15 years in a group of excellence on childhood leukemia leaded by Prof Andrea Biondi. The scientific contributions are demonstrated by 48 international peer reviewed publications.

Selected reference (last 5 years)

1. Dander E, Lorenzo P, Bottazzi B, Quarello P, Vinci P, Balduzzi A, Masciocchi F, Bonanomi S, Cappuzzello C, Prunotto G, Pavan F, Pasqualini F, Sironi M, Cuccovillo I, Leone R, Salvatori G, Parma M, Terruzzi E, Pagni F, Locatelli F, Mantovani A, Fagioli F, Biondi A, Garlanda C, Valsecchi MG, Rovelli A**, D'Amico G**. Pentraxin 3 plasma levels at graft-versus-host disease onset predict disease severity and response to therapy in children given haematopoietic stem cell transplantation. Oncotarget. 2016 Nov 21. doi: 10.18632/oncotarget.13488.
2. Vinci P, Bastone A, Schiarea S, Cappuzzello C, Del Prete A, Dander E, Biondi A, **D'Amico G** Mesenchymal stromal cell-secreted chemerin is a novel immunomodulatory molecule driving the migration of ChemR23-expressing cells. Cytotherapy. 2016 Dec 6. pii: S1465 3249(16)30585-0.
3. Bacigaluppi S, Donzelli E, De Cristofaro V, Bragazzi NL, **D'Amico G**, Scuteri A, Tredici G Human endothelial progenitor cells rescue cortical neurons from oxygen-glucose deprivation induced death..Neurosci Lett. 2016 Sep 19;631:50-5. doi: 10.1016/j.neulet.2016.08.014.
4. Doni A, D'Amico G, Morone D, Mantovani A, Garlanda C. Humoral innate immunity at the crossroad between microbe and matrix recognition: The role of PTX3 in tissue damage. Semin Cell Dev Biol. 2016 Jul 29. pii: S1084-9521(16)30230.
5. Anselmo A, Lauranzano E, Soldani C, Ploia C, Angioni R, **D'amico** G, Sarukhan A, Mazzon C, Viola A. Identification of a novel agrin-dependent pathway in cell signaling and adhesion within the erythroid niche. Cell Death Differ. 2016 Mar 18. doi: 10.1038/cdd.2016.10.
6. Zanotti L, Angioni R, Calì B, Soldani C, Ploia C, Moalli F, Gargesha M, **D'Amico**G, Elliman S, Tedeschi G, Maffioli E, Negri A, Zacchigna S, Sarukhan A, Stein JV, Viola A. Mouse mesenchymal stem cells inhibit high endothelial cell activation and lymphocyte homing to lymph nodes by releasing TIMP-1. Leukemia. 2016 May;30(5):1143-5
7. Scutera A, Ravasi M., Monfrini M., Milano A, D'Amico G, Mieloso M., Tredici G. Human Mesenchymal Stem Cells Protect Dorsal Root Ganglia from the Neurotoxic Effect of Cisplatin. 2015. Anticancer Research *35*: 5383-5390
8. Cappuzzello C, Doni A, Dander E, Pasqualini F, Nebuloni M, Bottazzi B, Mantovani A, Biondi A, Garlanda C, **D'Amico G**. Mesenchymal Stromal Cell-Derived PTX3 Promotes Wound Healing via Fibrin Remodeling. J Invest Dermatol. 2015 Sep 3. doi: 10.1038/jid.2015.346.
9. Introna M., Lucchini G., Dander E., Galimberti S., Rovelli A., Balduzzi A., Longoni D., Pavan F., Masciocchi F., Algarotti A. , Micò C., Grassi A., Deola S., Cavattoni I., Gaipa G., Belotti D., Perseghin P., Parma M., Pogliani E., Golay J., Gotti E., Capelli C., Cortelazzo S., **D'Amico G.**, Biondi A., Rambaldi A., Biagi E. Safe and effective treatment of Graft Versus Host Disease with mesenchymal stromal cells: a phase I study on 40 adult and pediatric patients. Biol Blood Marrow Transplant. 2013 Dec 7. pii: S1083-8791(13)00570-3. doi:
10. Pischiutta F, **D'Amico G**, Marchesi F, Dander E, Biondi A, Biagi E, Citerio G, De Simoni MG and Zanier ER. Long term efficacy of human bone marrow mesenchymal stromal stem cells in traumatizated mice brain is not dependent of immunosuppressive treatment. Neuropharmacology. 2013 Nov 15;79C:119-126.
11. Zanotti L, Sarukhan A, Dander E, Castor M, Cibella J, Soldani C, Trovato AE, Ploia C, Luca G, Calvitti M, Mancuso F, Arato I, Golemac M, Jonjic N, Biondi A, Calafiore R, Locati M, **D'Amico G**, Viola A. Encapsulated mesenchymal stem cells for in vivo immunomodulation. Leukemia. 2013 Feb;27(2):500-3.
12. André V, Longoni D, Bresolin S, Cappuzzello C, Dander E, Galbiati M, Bugarin C, Di Meglio A, Nicolis E, Maserati E, Serafini M, Warren AJ, Te Kronnie G, Cazzaniga G, Sainati L, Cipolli M, Biondi A, **D'Amico G**. Mesenchymal stem cells from Shwachman-Diamond syndrome patients display normal functions and do not contribute to hematological defects. Blood Cancer J. 2012 Oct 12;2:e94.
13. G. Lucchini, E. Dander, F. Pavan, I. Di Ceglie, A. Balduzzi, P. Perseghin, G. Gaipa, A. Algarotti, M. Introna, A. Rambaldi, A. Rovelli, A. Biondi, E. Biagi, **G. D'Amico.** Mesenchymal stromal cells do not increase the risk of viral reactivation nor the severity of viral events in recipients of allogeneic stem cell transplantation. Stem Cells Int. 2012;2012:690236
14. Lee Y, Chittezhath M, André V, Zhao H, Poidinger M, Biondi A, **D'Amico G**, Biswas SK. Protumoral role of monocytes in human B-cell precursor acute lymphoblastic leukemia: involvement of the chemokine CXCL10. Blood. 2012 Jan 5;119(1):227-37.

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:

_____ 19-12-2016

Signature of Applicant 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: Maria Grazia Pessina Grande

Title: General Secretary

Address: Fondazione "Matilde Tettamanti Menotti De Marchi" Onlus, via Pergolesi, 33, 20900 Monza.

Telephone: +39-039-2333661 FAX: +39-039-2332167

E-mail Address: e.agani@hsgerardo.org

_____ 19-12-2016

Signature of Institution Official Date

ABSTRACT OF RESEARCH PLAN

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

TITLE: ROLE OF THE HUMAN NICHE IN INDUCING AND SUPPORTING MYELODYSPLSTIC SYNDROME AND/OR LEUKAEMIA EVOLUTION IN SHWACHMAN-DIAMOND SYNDROME PATIENTS

SHWACHMAN-DIAMOND SYNDROME IS A RARE AUTOSOMAL RECESSIVE INHERITED DISORDER CHARACTERIZED BY BONE MARROW DYSFUNCTION AND EXOCRINE PANCREATIC INSUFFICIENCY. SIMILAR TO OTHER MARROW FAILURE SYNDROMES, PATIENTS WITH SDS HAVE AN INCREASED RISK FOR MYELODYSPLASTIC SYNDROME (MDS) AND MALIGNANT TRANSFORMATION, IN PARTICULAR, DEVELOPMENT OF ACUTE MYELOID LEUKEMIA (AML). DESPITE IN RECENT YEARS, THE PARADIGM THAT TUMOR STROMAL COMPONENTS ARE JUST BYSTANDERS IN THE ONCOGENIC PROCESS HAS CHANGED, AND THE PREVAILING VIEW IS NOW THAT THE TUMOR MICROENVIRONMENT IS A DYNAMIC ENTITY PROMOTING LEUKEMIA EVOLUTION AND INVASION THROUGH MECHANISMS RELATED TO LEUKEMIC CELL PROLIFERATION, IMMUNOSUPPRESSION AND DRUG RESISTANCE, THE ROLE OF THE STROMAL COMPONENT HAS NOT BEEN FULLY ELUCIDATED. WE AIM TO STUDY THE ROLE OF THE BONE MARROW BM) MICROENVIROMENT IN SDS IN VIVO USING A MODEL OF MINIATURIZED HUMAN BONE MARROW NICHE IN IMMUNODEFICIENT MICE, ALREDY SET-UP IN OUR LABORATORY. THIS PROJECT PROVIDES A DIRECT PRECLINICAL FEASIBILITY STUDY OF A SCAFFOLD-FREE, CELL ONLY APPROACH TO STUDY THE ROLE OF STROMA IN BONE MARROW FAILURE AND LEUKEMIA EVOLUTION IN SDS PATIENTS. THE ELUCIDATION OF TUMOR-PROMOTING FUNCTIONS OF THE NICHE AND MSCS MAY IDENTIFY POTENTIAL THERAPEUTIC TARGETS TO PREVENT OR TREAT LEUKEMIA EVOLUTION IN SDS PATIENTS.

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): PI: Name: Giovanna D'Amico Co-I Name: Additional personnel (identify role): Name: Donatella Bardelli	0	0
Equipment:	0	0
Supplies:	40.000	10.000
Other Expenses:	0	0
Indirect Costs (not to exceed 10% of total)	0	0
TOTAL COSTS:	40.000	10.000

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.

	Euro
Culture medium, fetal calf serum Cell separation reagents	8000
Disposable material for cell Cultures, transwell	1000
Flow-cytometry reagents	7000
ELISA reagents	6000
Confocal Microscopy reagents	8000
Immunocompromised mice	10000

Giovanna D'Amico. Head of the Immunology and cell therapy Unit at the Centro Ricerca Tettamanti. She will have the function of scientific responsible for investigating the potential role of the human niche in inducing and supporting myelodysplstic syndrome and/or leukaemia evolution in Shwachman-Diamond Syndrome patients. She will supervise and coordinate Dr Claudia Cappuzzello.

Donatella Bardelli, Biotechnologist, PhD student at Milano-Bicocca University. She has a knowledge in molecular and cellular biology. Dr Bardelli has been already trained to autonomously generate semi cartilaginous pellets and transfer into SCID/bg mice to generate HPSC niche. She will work on the generation and characterization of the semi-cartilaginous pellet (SCP) derived from SDS-MSC. In addition she will work on the functional analysis of Human Pluripotent Stem Cells (HPSC) niche induced by SDS-MSC

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.

Part of reagent costs, indirect costs and overheads will be covered by Fondazione "Matilde Tettamanti Menotti De Marchi" Onlus.

Research Plane

Part A: Specific aims

Shwachman–Diamond syndrome (SDS) is an autosomal recessive multi-system disorder characterized by exocrine pancreatic dysfunction, bone marrow failure and skeletal abnormalities (1). Similar to other marrow failure syndromes, patients with SDS have an increased risk for myelodysplastic syndrome (MDS) and malignant transformation, in particular, development of acute myeloid leukemia (AML). To date, the mechanisms underlying the bone marrow failure in SDS patients and their predisposition to cancer is not fully understood. Despite in recent years, the paradigm that tumor stromal components are just bystanders in the oncogenic process has changed, and the prevailing view is now that the tumor microenvironment is a dynamic entity promoting leukemia evolution and invasion through mechanisms related to leukemic cell proliferation, immunosuppression and drug resistance, the role of the mesenchymal component has not been fully elucidated.

We aim to study whether Mesenchymal Stem Cells (MSCs) from patients with SDS are able to sustain *in vivo* the evolution to MDS and/or AML. In our study we will use an innovative *in vivo* system, already set-up in our lab, based on the ex vivo generation of semi-cartilaginous pellets from human mesenchymal stromal stem cells transplanted in immunocompromised mouse. This project provides a direct preclinical feasibility study of a scaffold-free, cell only approach to study the role of MSC in leukemia evolution in SDS as a model of bone marrow failure and predisposition to hematological tumors. The elucidation of tumor-promoting functions of the niche and MSCs may identify potential therapeutic targets to prevent or treat leukemia evolution in SDS patients.

Part B: Significance and background

The Shwachman diamond sindrome and leukemia evolution. SDS is a hereditary disorder characterized by pancreatic insufficiency, bone marrow failure and skeletal dysplasia (1). Approximately 90% of patients meeting clinical criteria for the diagnosis of SDS harbour mutations in the *SBDS* gene (Shwachman-Bodian-Diamond syndrome) (2). Similar to other marrow failure syndromes, patients with SDS have an increased risk for MDS and AML. The risk of leukemic and dysplastic transformation in SDS patients enhance with age varying from 14% to 30% (3). Rujkijyanont and coworkers (4) demonstrated that SDS marrow mononuclear cells of nine SDS patients exhibit abnormal gene expression patterns. They showed that among 154 known leukemia-related genes, several oncogenes were found to be up-regulated including TAL1 and MLL, and several tumor suppressor genes were down-regulated including DLEU1, RUNX1, FANCD2 and DKC1. Recent

studies show that specific changes in MSC of the haematopoietic microenvironment may be sufficient to initiate a complex phenotype of disordered homeostasis with similarities to myelodysplasia (5). Furthermore, Raaijmakers demonstrates the ability of this abnormality to result in the emergence of a clonal neoplasm in a cell type of clearly distinct lineage with distinct secondary genetic changes. The data indicate that individual, well-defined, mesenchymal microenvironment constituents can be primary enablers of neoplastic changes in a heterologous cell type.

Part C: Preliminary studies

It has been demonstrated that patients with SDS had significantly lower number of CD34**+** cells on BM aspirates (6). In addition, SDS CD34**+** cells showed markedly impaired colony production potential when plated in methylcellulose for clonogenic assays. The ability of marrow stromal cells from SDS patients to support normal CD34+ cells in long-term colony assays was also diminished (6). In addition, patients have a generalized marrow dysfunction with abnormal bone marrow stroma in terms of its ability to produce fat cluster and to support and maintain hematopoiesis. Patients with SDS have an increased risk to develop MDS and/or AML. Rujkijyanont and coworkers demonstrated that SDS marrow mononuclear cells of nine SDS patients exhibit abnormal gene expression patterns. They used oligonucleotide microarray to identify gene expression patterns, which were shown to be associated with leukemogenesis, without overt transformation compared to healthy controls. Recently, Scaddens' group demonstrated that targeted deletion of the miRNA processing endonuclease Dicer1 osteoprogenitors-mesenchymal cells (MSC) could induce complex secondary changes in the organization of the hematopoietic (parenchymal) lineage, including the development of independent genetic mutations and frank leukemia. This model supports the hypothesis that MSC comprising tissue stroma may serve as the initiating "hit" in the multi-hit process of oncogenesis.

Several studies (7-9) demonstrated that Mesenchymal Stromal Cells (MSCs) represent the pivotal organizers for the generation, maintenance and plasticity of hematopoietic stem cell (HSC) niche thorough supporting the proliferation and differentiation of HSCs and their progenies. MSC generate a number of stromal cells which have been shown to impact HSC behavior, including adipocytes, pre-osteoblasts, osteoblasts and chondrocytes. The hematopoietic microenvironment controls the formation of blood cells through the production and secretion of cytokines and extracellular matrix molecules.

To our knowledge no study has examined the functional properties of MSCs obtained from patients with Shwachman-Diamond syndrome (SDS-MSCs). We have already obtained MSCs from 27 out of 80 SDS patients included in the Italian registry (10 and *Paper in preparation)*. These cells displayed typical

fibroblastoid morphology; they were consistently devoid of contaminating hematopoietic cells and expressed common MSC markers. Similarly to MSCs obtained from healthy donors (HD-MSCs), these cells were able to differentiate into adipocytes and osteoblasts. In addition, SDS-MSCs drastically decreased the mitogen-induced lymphocyte proliferation, in a dose dependent manner. We also cultured CD34+ cells obtained from HD in presence or absence of MSCs at different time points. We demonstrated that SDS-MSCs were comparable to HD-MSCs in supporting the viability and clonogenic potential of CD34+ cells. In addition, the genome wide gene expression analysis carried out using HG-U133 Plus 2.0 Arrays showed that SDS-MSCs had a specific profile, significantly different from HD-MSCs.

Recently we have set up, a new and innovative experimental model in which a perfect architecture of a miniaturized human bone marrow niche has been developed in mice by ex vivo generation of semicartilaginous pellets from human bone marrow-derived MSC (Serafini et al. Stem Cell Research. *In press*). Preliminary results demonstrated that human CD34+ hematopoietic cells injected into immunocompromised mice previously implanted with cartilage pellets could stably engraft into the generated human niche, showing inside the ossicles the presence of engrafted human hematopoiesis (Serafini et al., European Bone Marrow Transplantation meeting, Oral communication). The possibility of generating *in vivo* the human niche, gives us the unique opportunity to identify the stromal-derived signals driving the maintenance of pre-leukemic cells and their evolution to overt leukemia, in order to discover the molecular mechanisms that might be the targets for preventive and therapeutic intervention.

Part D: Experimental design and methods

1) Generation and characterization of the semi-cartilaginous pellet (SCP) derived from SDS-MSC:

In preliminary experiments we also obtained SCP from 7 SDS-MSCs. Ematossilina-eosina and Safranina analysis showed that cartilaginous tissues was formed correctly. In this task we will further characterize the SCP by assessing chondroid differentiation markers (upregulation molecules of cartilage and regulators - collagen type II, collagen type X, aggrecan and Sox-9), and abrogation of undifferentiated MSCs markers (CD146, CD105, NG2) through histology, immunohistochemistry (IHC) and qPCR analyses

2) Optimization of the protocol for the generation of HPSC niche by SDS-MSC in an vivo model:

In preliminary experiments we will test the formation of Human Pluripotent Stem Cells (HPSC) niche at different time points (20, 40, 60 day) after SCP-derived SDS-MSC transplanted into subcutaneous tissue of

immunosuppressed mice. The extent and the distribution of newly formed bone and BM will be detected by histomorphometry. Donor origin of formed tissues will be assessed using IHC for human Lamin A, or in situ hybridization for human Alu sequences. Reconstitution of an MSC compartment will be detected by CFU-F assays and cytofluorimetric analysis.

3) Functional analysis of HPSC niche induced by SDS-MSC

In order to study whether SDS-MSC are able to recreate *in vivo* the bone marrow failure typical of SDS patients, we will analyzed the reconstitution of a HPSC niche at the time point identified in the previous task. Analysis of niche cells will be performed by CXCL12/CD146/CD34 confocal IF, combined with transmitted light assessment of position of sinusoids with respect to endosteal surfaces, and with multicolor localization of HPSCs. Distribution of human stem cell (HSC) with respect to canonical landmarks of the HSC niche (sinusoidal walls, endosteal surfaces) will be obtained by histomorphometry on IF images. In addition, ossicles will be digested with collagenase and lineage (Lin)- stem-cell antigen 1 (SCA1)+ KIT+ (LSK) cells are separated by FACS or magnetic columns. Expression of hematopoietic TF (Lmo2, Scl, Gata2, Ikaros, or Pu.1) will be performed by qPCR. Moreover, mielo-monocytic, megacariocitic (CD41) and KLS antigen expressions will be detected by FACS analysis. In addition, the cytokine/chemokine profile will be analyzed by qRT-PCR arrays. The functional characterization of HPSCs will be assessed by CFC assays and hematopoietic reconstitution assays through trasplantation into lethally irradiated C57Bl6 mice. All these studies will be done in comparison with HPSC populations obtained from the long bones of the same mice carrying ossicles in close collaboration with Bianco's group.

2) Study the ability of SDS-MSC to induce AML or MDS in an vivo model:

In order to investigate the potential role of SDS-MSC in promoting malignant tranformation, mice implanted with SCP-derived SDS-MSC will be transplanted with purified human CD34+ cells obtained from BM of healthy donors or CD34+ cells derived from SDS patients, already collected in our laboratory. At different time points ossicles will be digested with collagenase and human hematopoietic cells are separated by FACS or magnetic columns. The ability of microenviroment to induce AML or MDS will be evaluated by FACS and cytogenetic analysis and by qPCR. Mutagenic treatment can be performed to damage DNA and simulate secondary hits to accelerate leukaemia.

Part E: References

1. Cipolli M. Shwachman-Diamond syndrome: clinical phenotypes. Pancreatology. 2001;1:543-8.
2. Boocock GR, et al. Mutations in SBDS are associated with Shwachman-Diamond syndrome. Nat Genet. 2003 33:97-101.
3. Vardiman JW, et al. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood. 2002;100:2292-302.
4. Rujkijyanont et al. Leukaemia-related gene expression in bone marrow cells from patients with the preleukaemic disorder Shwachman-Diamond syndrome. Br J Haematol. 2007 137:537-44
5. Raaijmakers MH, et al. Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. Nature. 2010;464:852-7.
6. Dror Y, Freedman MH. Shwachman-Diamond syndrome marrow cells show abnormally increased apoptosis mediated through the Fas pathway. Blood. 2001;97:3011-6.
7. Xie Y, et al. Detection of functional haematopoietic stem cell niche using real-time imaging. Nature. 2009;457:97-101.
8. Bianco P. Bone and the hematopoietic niche: a tale of two stem cells. Blood.;19;117:5281-8.
9. Sacchetti B, et al..Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell. 2007;131:324-36.
10. J M. Liu, et al. Sixth International Congress on Shwachman-Diamond syndrome: from patients to genes and back. Ann. N.Y. Acad. Sci. 2011;1242:26-39.

Part F: Relevance of the research to Shwachman-Diamond Syndrome

Patients with SDS evolve with high frequency into MDS and/or AML. The risk of leukemic and dysplastic transformation in SDS patients enhance with age varying from 14% to 30%. The model suggested by the Scaddens'group candidates, for the first time, MSCs as a new actor in the multi-hit process of oncogenesis. MSCs are thought to generate a number of non-hematopoietic cells including adipocytes, chondrocytes and osteoblasts and to support proliferation and differentiation of HSCs and their progenies. To our knowledge no study has examined the functional properties of MSCs obtained from patients with Shwachman-Diamond syndrome (SDS-MSCs). In this study we will analyze the potential role of SDS-MSCs in affecting hematopoiesis and in promoting malignant transformation by using an innovative *in vivo* system. This study will offer the great opportunity of integrating clinical and preclinical data obtained from the *in vivo* analysis of MSC isolated from SDS patients, allowing a deeper understanding of MSC biology and their role in leukemia evolution.The identification of signals from the microenvironment inducing transforming events could pave the way to new highly targeted strategies for the prevention an treatment of this syndrome.

Part K:

I herewith declare that the proposed project requires experimentation on specimens of human subjects and

animals.

However, the approval of the ethical committee is not available at the moment of submission.

I herewith declare that I am pledging to obtain the required approval before the start of the research.