

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: 31 March 2012

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: Cytogenetics and a-CGH as tools to find and monitor chromosome changes acquired in the course of Shwachman-Diamond syndrome (SDS): molecular mechanisms primed and risk of myelodysplastic syndrome (MDS) / acute myeloid leukaemia (AML)

2. Applicant Information:

Name: Emanuela MASERATI

Title and Degree(s): Associate Professor of Medical Genetics, Università dell'Insubria

Work Address: Dipartimento di Medicina Clinica e Sperimentale
Università dell'Insubria
Via J. H. Dunant, 5
21100 Varese

Telephone: +39 0332 217181

FAX: +39 0332 217119

Email: emanuela.maserati@uninsubria.it

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

CV

- Born on February 7th, 1956 in Piacenza
- Graduated in Scienze Biologiche in 1980, University of Pavia
- In 1984 she obtained the specialization in Human Cytogenetics
- In September 1990 she obtained the title of Dottore di ricerca (PhD) at the Dipartimento di Patologia Umana ed Ereditaria, University of Pavia
- She collaborates with the Pediatric Onco-haematology Division of Policlinico San Matteo, Pavia, since 1983
- In 1984 she was at the Department of Cell Biology and Genetics, Erasmus University of Rotterdam with a scholarship of the Foundation "Villa Anna Rusconi" of Varese
- In 1986 she was "visiting scientist" at the Department of Clinical Genetics of the Karolinska Hospital in Stockholm as fellow of the "Wenner-Gren" Foundation
- In February 1990 she won a competition for a position of Assistant Professor in the faculty of Medicine of the University of Sassari, and held this capacity 1990-2002
- She is involved in the Progetto Nazionale sulla Biologia delle Leucemie Infantili (ACRO) as cytogeneticist for pediatric leukaemias since 1992
- She is Associate Professor since 1/10/2002 in the Faculty of Medicine of the University of Insubria, Varese

Research interests

The principal research lines in the field of Medical Genetics were and still are:

- studies on karyotype/phenotype correlation in constitutional chromosome anomalies;
- studies on the chromosome variability in leukaemia, and, in particular, in myelodysplasia and myeloproliferative disorders at the diagnosis, during the course of the disease, and after bone marrow transplantation;
- studies to identify the parental origin of gained and lost chromosomes preferentially involved in myeloproliferative disorders and to investigate on the related mechanisms of origin;
- studies on families with Mendelian diseases associated to myeloproliferative disorders;
- studies on Shwachman Diamond Syndrome, and other inherited bone marrow failure syndromes predisposing to myelodysplastic and myeloproliferative disorders;
- studies on chromosome anomalies with gene effects leading to peripheral cytopenias (uni-, bi-, and three-linear) and/or bone marrow aplasia/hypoplasia.

All research work is carried out by means of conventional and molecular cytogenetic methods, and of molecular techniques, besides basic cell biology methods, as different cell culture techniques: the most relevant and informative methods used in relation to projects goals include Fluorescent In Situ Hybridization (FISH) and Multipainting, and by microarray-based comparative genomic hybridization (a-CGH); quantitative PCR for expression studies.

Teaching experience

The teaching experience includes:

- Courses of General Biology and Human Genetics in the Course for the degree in Medicine and Surgery, since 1986, at the Universities of Sassari, and Insubria;
- Courses of General Biology and Human Genetics in the Course for the degree in Dentistry, since 2008, at the University of Insubria;
- Courses of General Biology, of Human and Medical Genetics, of Cytogenetics in the Courses for the degree in Medical Laboratory Techniques, in Nursing, in Dental Hygiene, in Physiotherapy, since 2000, at the University of Insubria;
- Courses of Human and Medical Genetics in many different Postgraduate Schools of Specialization (such as Ophthalmology, Orthognatodonty, Pediatrics, Obstetrics, Pediatric Neuropsychiatry, Psychiatry, etc.), and in Masters Courses, since 1995, at the Universities of Sassari, and Insubria, and in several other institutions.

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 or of the Ethical Committees of the institutions where the clinical work is carried on.

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)"

Varese, March 31st 2012

Emanuela Maserati

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: Annamaria Grandi, professor

Title: Head

Address:

Dipartimento di Medicina Clinica e Sperimentale – Università dell'Insubria
Via J. H. Dunant, 5
21100 Varese

Phone: 0332 278830

Fax: 0332 217119

E-mail Address: <anna,grandi@uninsubria.it>

Varese, March 31st 2012

Anna Maria Grandi
Head Dept. Clinical and Experimental Medicine

ABSTRACT OF RESEARCH PLAN

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

TITLE: Cytogenetics and a-CGH as tools to find and monitor chromosome changes acquired in the course of Shwachman-Diamond syndrome (SDS): molecular mechanisms primed and risk of myelodysplastic syndrome (MDS) / acute myeloid leukaemia (AML)

Scientific aims

The project is intended to demonstrate the relevance of long-lasting cytogenetic monitoring, included the results of microarray-based Comparative Genomic Hybridization (a-CGH), in SDS, in relation with molecular mechanisms that may be primed by the chromosome anomalies acquired in the bone marrow (BM). The results may be relevant for the study of pathogenetic mechanisms leading to (or protecting from) MDS and AML, with obvious practical implications for the clinical management of the patients.

Methodology

We are performing cytogenetic investigations since 1999 in a cohort which is now of 68 SDS patients, with follow up lasting from few months to 12 years, and 27 of them showed clonal anomalies in the BM, whereas MDS/AML developed in three: several considerations concerning clonal chromosome anomalies, karyotype instability, and their role have been already reported.

The material for the present project includes BM cells and DNA of the patients of our cohort. The a-CGH system which will be used is the 244K genome-wide system (Agilent Technologies Inc., Santa Clara, CA, USA), applied and analyzed according to the manufacturer's instruction and software. The results will be confirmed by FISH with the probes indicated as informative by a-CGH. The possibility that chromosome unbalances detected are in fact benign "copy number variations" (CNV), not acquired in the BM and not linked with the SDS chromosome instability will be investigated by a-CGH on DNA from peripheral blood of the patient and/or on DNA from his/her parents. The molecular consequences of the chromosome changes will be investigated with expression and gene product analyses.

Long-term objectives

The demonstration, in recent years, of the appearance of chromosome changes in BM several years after the diagnosis of SDS, in parallel with higher risk of MDS/AML, and the fact that a-CGH provided a powerful tool to investigate unbalanced chromosome anomalies, lead the present project to investigate the molecular consequences of the clonal anomalies detected and to explore the relevance of using a-CGH in the clinical monitoring of SDS patients to prevent MDS/AML.

In particular:

1. The results of a-CGH will have a pivotal role in the precise definition of acquired unbalanced chromosome anomalies already detected by chromosome analysis and FISH;
2. The precise definition of clonal anomalies will be instrumental to infer and demonstrate their pathogenetic significance: this will be the case, in particular, of the *i(7)(q11)* in relation with the expression of the *SBDS* gene, and of the *del(20)(q11)* as to the expression of the *EIF6* gene;
3. Cryptic unbalanced chromosome anomalies, undetected by conventional cytogenetics, may be found by a-CGH (as was the case of two of the patients of our cohort already analysed), and should be discussed in relation to the SDS karyotype instability and to their significance;
4. The possibility and the clinical relevance to integrate the usual cytogenetic monitoring of the BM in SDS patients with a-CGH will be analyzed.

So, it will be possible to shed further light on the natural history of SDS, and to set up better weapons against MDS/AML development.

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 € 10,000)
Personnel (including fringe benefits): PI: Name: Co-I Name: Additional personnel (identify role): Name:	-	-
Equipment:	-	-
Supplies:	€ 27,840	€ 10,000
Other Expenses:	-	-
Indirect Costs (not to exceed 10% of total)	-	-
TOTAL COSTS:	€ 27,840	€ 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.

Personnel and equipment for this research project are already available.

The entire group of researchers is involved in all parts of the research work.

The costs concern only supplies, and are in part covered by other grants already available.

- Each a-CGH test costs approximately € 670, including:
 - o Array platform
 - o Labelling kit
 - o Gasket slides
 - o Washing solutions

No. a-CGH tests planned: 25 = cost € 15,340

- Costs for cell cultures, conventional cytogenetics, FISH, and expression studies: approximately € 500 per case

Total foreseeable cost: € 12,500

- Total supplies cost: € 27,840

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.

- Università dell'Insubria: FAR 2010 e 2011
- Erogazione straordinaria da parte della Presidenza di Facoltà di Medicina e Chirurgia dell'Università dell'Insubria per la valenza didattica delle ricerche perseguite

Research Plane

Part A – Specific aims

The project is intended to demonstrate the relevance of long-lasting cytogenetic monitoring in SDS, including the results of microarray-based Comparative Genomic Hybridization (a-CGH) and through several years, in relation with molecular mechanisms that may be primed by the chromosome anomalies acquired in the bone marrow (BM). The results may be relevant for the study of pathogenetic mechanisms leading to (or protecting from) MDS and AML, with obvious practical implications for the prevention of MDS/AML and for the optimal clinical management of the patients.

Part B – Significance and background

The proportion of SDS patients who develop myelodysplastic syndromes (MDS) and/or acute myeloid leukaemia (AML) is approximately 30% (Dror, 2005), and evaluations which take into account the age of the patients estimate a risk of 19% at 20 years and of 36% at 30 years (Shimamura, 2006).

Clonal chromosome changes, mainly involving the chromosomes 7 and 20, are often found in the bone marrow (BM) of SDS patients, the most frequent being an isochromosome for the long arms of chromosome 7, i(7)(q10), and a deletion of the long arms of chromosome 20, del(20)(q11) (Maserati et al, 2006). The relationship between these and other chromosome changes in the BM and the risk of MDS/AML is object of discussion (Dror et al, 2002; Dror, 2005). The cytogenetic follow-up lasting several years in the cohort of SDS patients monitored by our group since 1999 showed that the acquisition of BM clonal chromosome anomalies is age-related, in parallel with the risk of MDS/AML (Maserati et al, 2009).

We have already ruled out the hypothesis that the karyotype variability in the BM of SDS patients is linked to an increase in the frequency of spontaneous breaks, as in customary breakage syndromes (Maserati et al, 2006), but a specific kind of karyotype instability is undoubtedly present in SDS. So, we have suggested a mutator effect of *SBDS* mutations leading to the karyotype instability which leads, in turn, to clonal anomalies in the BM (Maserati et al, 2006). This view was supported by evidence that *SBDS* protein promotes spindle stability and normal chromosome segregation (Austin et al, 2008): the defect in SDS cells might so explain the karyotype instability, possibly through cytokinesis failure and tetraploidy, with subsequent chromosome changes. These changes may be structural, as often is the case in SDS, according to a model which has been proposed for cancer, via defective telomere function (Storchova and Pellman, 2004). The MDS/AML risk in SDS is likely to be due to the clonal chromosome variability so arising.

Comparative Genomic Hybridization on microarray (a-CGH) provides a powerful tool to investigate unbalanced chromosome anomalies, and some results in SDS have been already reported (Maserati et al, 2009), with a better definition of clonal BM anomalies and with the definition of other possible cryptic chromosome anomalies not detected by conventional cytogenetics and linked to the karyotype instability.

Some examples of the pathogenetic mechanism which may be primed by the clonal chromosomal anomalies and may promote a positive or negative effect in the disease course, concerning in particular the risk of MDS/AML, have been shown or postulated: this was the case of the mutations of the *SBDS* gene present on the i(7)(q10) (Minelli et al. 2009), and of the loss of the *EIF6* gene in the deletion del(20)(q11) (Pressato et al, 2012).

Part C – Preliminary studies

Cytogenetic investigations were performed in a cohort of 68 SDS patients, with follow up lasting from few months to 12 years, and 27 of them showed clonal anomalies in the BM, whereas MDS/AML

developed in three: a number of the considerations concerning clonal chromosome anomalies, karyotype instability, and their role have been reported (Maserati et al, 2000; Maserati et al, 2006; Maserati et al, 2009).

We obtained preliminary results by means of a-CGH in 12 SDS cases, with informations regarding the exact nature of the i(7)(q10) (Pressato et al, 2010), and with cryptic unbalanced anomalies undetected by chromosome analyses. Evidence of the positive prognostic relevance, as to MDS/AML risk, of the mutations present on the i(7)(q10) was obtained and reported (Minelli et al, 2009). A benign prognosis in the same sense in the patients with the del(20)(q11) and loss of the *EIF6* gene has been postulated (Pressato et al, 2012).

Part D – Experimental design and methods

The material for the project includes cells from BM and peripheral blood, as well as DNA from BM of the patients of our cohort. All informative techniques of cytogenetics and of molecular cytogenetics will be used, and in particular Fluorescent in Situ Hybridization (FISH) with informative libraries and probes and with the multipainting technique. The a-CGH system which will be used is the 244K genome-wide system (Agilent Technologies Inc., Santa Clara, CA, USA), applied and analyzed according to the manufacturer's instruction and software. All a-CGH results will be confirmed, when necessary, by FISH with the probes indicated as informative by a-CGH. The possibility that chromosome unbalances detected are in fact benign "copy number variations" (CNV), not acquired in the BM and not linked with the SDS chromosome instability will be investigated by a-CGH on DNA from peripheral blood of the patient and/or on DNA from his/her parents.

The molecular consequences of the chromosome changes will be investigated with expression and gene product analyses. The preliminary results on the molecular effects primed by the chromosome anomalies mentioned above will be confirmed and analyzed in relation with the course of the disease at clinical and haematological levels. All other possible molecular mechanisms primed will be investigated in particular as to their possible prognostic relevance.

Part E – References

- Austin KM, Gupta ML Jr, Coats SA, Tulpule A, Mostoslavsky G, Balazs AB, Mulligan RC, Daley G, Pellman D, Shimamura A. 2008. Mitotic spindle destabilization and genomic instability in Shwachman-Diamond syndrome. *J Clin Invest* 118:1511-1518.
- Dror Y. 2005. Shwachman-Diamond syndrome. *Pediatr Blood Cancer* 45:892-901.
- Dror Y, Durie P, Ginzberg H, Herman R, Banerjee A, Champagne M, Shannon K, Malkin D, Freedman MH. 2002. Clonal evolution in marrows of patients with Shwachman-Diamond syndrome: a prospective 5-year follow-up study. *Exp Hematol* 30:659-669.
- Maserati E., Minelli A., Olivieri C., Bonvini L., Marchi A., Bozzola M., Danesino C., Scappaticci S., Pasquali F. 2000. Isochromosome i(7q) in Shwachman Syndrome without MDS/AML and role of chromosome 7 anomalies in myeloproliferative disorders. *Cancer Genet Cytogenet* 121:167-171.
- Maserati E, Minelli A, Pressato B, Valli R, Crescenzi B, Stefanelli M, Menna G, Sainati L, Poli F, Panarello C, Zecca M, Lo Curto F, Mecucci C, Danesino C, Pasquali F. 2006. Shwachman syndrome as mutator phenotype responsible for myeloid displasia/neoplasia through karyotype instability and chromosome 7 and 20 anomalies. *Genes chromosome Cancer* 45:375-382.
- Maserati E, Pressato B, Valli R, Minelli A, Sainati L, Patitucci F, Marletta C, Mastronuzzi A, Poli F, Lo Curto F, Locatelli F, Danesino C, Pasquali F: The route to development of myelodysplastic syndrome/acute myeloid leukaemia in Shwachman-Diamond syndrome: the role of ageing, karyotype instability, and acquired chromosome anomalies. *Br J Haematol*, 145:190-197, 2009.
- Minelli A, Maserati E, Nicolis E, Zecca M, Sainati L, Longoni D, Lo Curto F, Menna G, Poli F, De Paoli E,

Cipolli M, Locatelli F, Pasquali F, Danesino C: The isochromosome i(7)(q10) carrying c.258+2t>c mutation of the *SBDS* gene does not promote development of myeloid malignancies in patients with Shwachman syndrome. *Leukemia*, 23:708-11, 2009.

- Pressato B, Marletta C, Montalbano G, Valli R, Maserati E: Improving the definition of the structure of the isochromosome i(7)(q10) in Shwachman-Diamond Syndrome. *Br J Haematol*, 150:632-633, 2010.
- Pressato B, Valli R, Marletta C, Mare L, Montalbano G, Lo Curto F, Pasquali F, Maserati E: Deletion of chromosome 20 in bone marrow of patients with Shwachman-Diamond syndrome, loss of the *EIF6* gene and benign prognosis. *Br J Haematol*, doi:10.1111/j.1365-2141.2012.09033.x, 2012
- Storchova Z, Pellman D. 2004. From polyploidy to aneuploidy, genome instability and cancer. *Nat Rev Mol Cell Biol* 5:45-54.
- Shimamura A. 2006. Shwachman-Diamond syndrome. *Semin Hematol* 43:178-188.

Part F – Relevance of the research to SDS

The results of the research will be both of theoretical and practical relevance. In particular:

1. The results of a-CGH will have a pivotal role in the precise definition of acquired unbalanced chromosome anomalies detected by chromosome analysis and FISH;
2. Cryptic unbalanced chromosome anomalies, undetected by conventional cytogenetics, may be found by a-CGH (as was the case of two of the patients of our cohort already analyzed), and should be discussed in relation to the SDS karyotype instability and to their significance;
3. The possibility and the clinical relevance to integrate the routine cytogenetic monitoring of the BM in SDS patients with a-CGH will be analyzed;
4. The precise definition of clonal anomalies in BM will be instrumental in all possible discussion on their pathogenetic significance;
5. The prognostic relevance of the mutations of the *SBDS* gene present on the i(7)(q10) will be confirmed and analyzed in relation with the course of the disease at clinical and haematological levels;
6. The possibility that the del(20)(q11) plays a benign role as to the risk of developing MDS/AML due to the loss of the *EIF6* gene will be analyzed, with preventive issues;
7. All other molecular effects which might be primed by the clonal chromosome anomalies in BM will be taken into account and studied properly.

Parts G, K

Not applicable.