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MARIE CURIE ACTIONS

Intra-European Fellowships (IEF)
Call: FP7-PEOPLE-2012-IEF

PART B

“WASHSCGENETHERAPY”

“Preclinical studies in mouse Hematopoietic Stem Cells for GENE THERAPY of Wiskott-Aldrich Syndrome”
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B1 Scientific and technological Quality

B1.1 Research and technological quality, including any interdisciplinary and multidisciplinary aspects of the proposal

**Wiskott-Aldrich Syndrome (WAS)** (MIM no.301000) is a rare X-linked hereditary immunodeficiency (1:250,000) characterized by eczema, microthrombocytopenia, proneness to infection, bloody diarrhoea and lymphoid malignancies (Hacein-Bey-Abina, Le Deist et al. 2002; Thrasher 2002; Badour, Zhang et al. 2003; Notarangelo and Ochs 2003). WAS is generally diagnosed early in the life and many severe patients do not survive past of 10 years without definitive treatment (Imai, Morio et al. 2004). *Was* gene (Derry, Ochs et al. 1994) (Gene map locus Xp11.23-p11.22) codify for a cytosolic protein of 502 amino acids, WASP protein (WASP), that is expressed exclusively in hematopoietic cells and is present in all types of leukocytes (Parolini, Berardelli et al. 1997) where it has roles in signalling (Snapper and Rosen 1999) and as a key regulator of actin cytoskeleton reorganization (Gallego, Santamaria et al. 1997; Ochs and Thrasher 2006). The clinical manifestations are: hemorrhage, eczema, autoimmune manifestations and tumours.

The first successful demonstration that allogenic hematopoietic stem cell transplantation (HSCT) could be used to cure inherited hematological diseases (Galy and Thrasher 2011) was obtained almost simultaneously in two patients with WAS and a Severe Combined Immunodeficiency (SCID) more than 40 years ago (Good 1987). Moratto et al (Moratto, Giliani et al. 2011) analyzed long-term outcome and donor cell engraftment in 194 patients with WAS who have been treated by HSCT in the period 1980-2009. Nowadays compatible HLA-matched-donors not always are available and presence of a low clinical score at the time of treatment. For more than 10 years now, gene-correction autologous HSCs have been used as an alternative to HLA mis-matched HSCT (see table):

<table>
<thead>
<tr>
<th>Disease</th>
<th>Type of vector</th>
<th>Centers</th>
<th>No. of patients</th>
<th>Efficacy</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA/SCID</td>
<td>Gamma retrovirial vector</td>
<td>Italy, UK, US, Japan</td>
<td>36</td>
<td>Yes for most patients receiving conditioning prior to gene therapy</td>
<td>No</td>
</tr>
<tr>
<td>SCID-X1</td>
<td>LTR-driven transgene expression</td>
<td>France, UK, US</td>
<td>22</td>
<td>Yes (patients &lt; 10 year old)</td>
<td>Yes, insertional mutagenesis in 5 patients</td>
</tr>
<tr>
<td>CID</td>
<td>Gamma retrovirial vector, LTR-driven transgene expression</td>
<td>Germany, UK, Switzerland, US, South Korea</td>
<td>17</td>
<td>Initial benefit only, 1 patient died of sepsis</td>
<td>Yes, insertional mutagenesis in 3 patients</td>
</tr>
<tr>
<td>WAS-Andre syndrome</td>
<td>Gamma retrovirial vector, LTR-driven transgene expression</td>
<td>Germany</td>
<td>10 included, 2 reported</td>
<td>Yes</td>
<td>Yes, insertional mutagenesis in 1 patient</td>
</tr>
</tbody>
</table>

In fact, WAS disease is very attractive for gene-cell-therapy because the expression of the therapeutic gene confers a selective advantage to the correct cells (Ariga, Kondoh et al. 2001; Martin, Toscano et al. 2005).

**State of the art**

Gammaretroviral vectors derived from Moloney leukaemia virus (MLV) and expressing a therapeutic transgene from the retroviral 5' long-terminal repeat were the first effective gene transfer vectors developed for these clinical applications. Gene therapy has been highly successful for restoration of T-cell homeostasis and T-cell function in SCID patients, follow-up in some patients now being more than 10 years (Galy and Thrasher 2011). The clinical trial performance in Hannover demonstrated engraftment of gene-correct cells in multiple lineages, sustained expression of WAS protein in HSC, lymphoid and myeloid cells, and platelets (Boztug, Schmidt et al. 2010). T and B cells, natural killer (NK) cells, and monocytes were also functionally corrected. However, the authors found vector insertion near the oncogene LMO2, the same that has been reported in SCID-X1 patients. The process of vector-induced insertional mutagenesis is still understood but several factors seem to be implicated. The presence of strong viral enhancers in the vector is thought to contribute by influencing the expression of genes in the host genome. Also, the LTR-driven gammaretroviral vectors are susceptible to epigenetic silencing, losing the therapeutic effect. Other inconvenient is that gammaretroviral vectors are produce in unpurified cell culture
supernatants harvested from packaging cell lines and several rounds of infections (in presence of cytokines to induce cell-division) are needed to achieve high levels of gene transfer.

Vectors derived from HIV-1 present advantages in terms of efficiency and their capacity of transduction of quiescent cells. The lentiviral vectors (LVs) are a powerful system of integration (Wiznerowicz and Trono 2005) and the last generation of self-inactivated-LVs express the transgene under internal promoters instead the 5´-LTR (long terminal repeat) (Zufferey, Dull et al. 1998; Kraunus, Schaumann et al. 2004) (Avedillo Diez, Zychlinski et al. 2010). This characteristic allows the use of physiologic or tissue-specific promoters to express the transgene (Toscano, Romero et al. 2011) (Bosticardo, Draghici et al. 2010).

To improve the biosafety of WAS GT, Martín et al. (Martin, Toscano et al. 2005) and Dupre et al. (Dupre, Trifari et al. 2004) developed hematopoietic-specific LVs expressing the human cDNA driven through different fragments of the WAS internal promoter. Their hypothesis was that the use of tissue-specific promoters should reduce genotoxicity by eliminating the enhancer activity of the commonly used viral long terminal repeat (LTR) and potential problems of ectopic expression of the transgene due to its physiological expression pattern.

Currently there are several clinical trials in phase I/II that use a HIV-derived LV to correct genetically autologous CD34+ HSCs from WAS patients. Of particular interest is the joint effort of UK, France and USA sponsored by Généthon (a non-profit company) to test safety and efficacy of LVs expressing was cDNA under the 1.6kb fragment of was proximal promoter. However, was gene can be expressed using two different promoters separated over 6kb; the proximal promoter and the alternative promoter. Dr. Martin’s group at GENYO, has developed an improved therapeutic LV expressing the was cDNA through a combination of the two was gene promoters (AWW) (Frecha, Toscano et al. 2008). This new vector backbone sustained high transgene levels along the whole lymphoid lineage in vivo and its performance was superior to proximal-promoter driven LVs. This data indicated that could be a good candidate for its use for WAS gene therapy.

There are two main reasons to hypothesize that chromatin insulators should be helpful for reducing retroviral vector-mediated genotoxicity. At the simplest level, the ability of barrier insulators to help reduce the rate of vector silencing can translate into the need for a lower vector “dose” necessary to achieve therapeutic (Emery, Yannaki et al. 2002). Although a direct relationship between vector dose and vector-mediated genotoxicity was not evident in clinical studies (Hacein-Bey-Abina, Von Kalle et al. 2003) several preclinical models have clearly demonstrated a dose-dependent component of vector-mediated genotoxicity (Modlich, Bohne et al. 2006) (Montini, Cesana et al. 2006). In addition, because many documented examples of vector-mediated genotoxicity involve cellular gene activation, it is likely that the enhancer-blocking capacity of chromatin insulators such as cHS4 hold the most promise for abrogating this genotoxicity.

We think that it is important to achieve physiological expression of was gene with a minimal vector dose. Previous studies demonstrated that the therapeutic WW and AWW LVs follow a physiological expression pattern but they also showed that one single integrant only achieved about half of the normal WAS protein levels. In addition, they could not study the behaviour of the therapeutic vectors in WAS-deficient HSCs (real target cells for WAS GT) due to the difficulties to obtain these samples.

Therefore, in order to improve the safety and efficiency of the LVs for GT of WAS, the aims of this project are:
1- To improve safety and efficiency of the gene transfer vectors AWW and WW for WAS GT by the introduction of insulator sequences (based on chicken HS4 and SAR sequences) and analysis of these new LVs (AWWINS, WWINS) in mouse Hematopoietic Stem Cells (HSCs).

2- Analysis of the capacity of AWWINS, WWINS (0.5kb) and the WW (1.6kb) LVs (used in the UK-France-USA clinical trial) to rescue mouse WAS Knockout (KO) phenotype.

3- Analysis of the safety and efficiency of the different therapeutic vectors in a human cell model of WAS: HSCs (WAS-) derived from human Embryonic Stem Cells (hESCs) WAS KO.

With these results we are developing new strategies for WAS GT applications.
The best LVs will be present as candidates for clinical trials of WAS.

B1.2 Appropriateness of research methodology and approach

The studies of safety and efficiency of the LVs AWW, WW, AWWINS and WWINS vectors for WASP restauration will be carry out in mouse HSCs WAS KO and human HSCs derived from hESCs WAS KO. These “corrected” cells will be inject in mouse WAS KO model to analyze their repopulation capacity. In relation to the overall project, the methological approach to be employed in our specific aims will be as follows:

**Aim 1:** To improve safety and efficiency of the gene transfer vectors AWW and WW for WAS GT by the introduction of insulator sequences (based on chicken HS4 and SAR sequences) and analysis of these new LVs (AWWINS, WWINS) in mouse HSCs.

The objective is to obtained insulated AWW and WW LVs so they would not affected by enhancer sequences present in places near the integration site and that the expression profile of the target cells is not affected by the integration of the LVs. In colaboration with Dr. Francisco Martín, we will introduce insulators sequences in the therapeutic vectors (AWWINS, WWINS). The plasmid pNI-CD (Dr. Felsenfeld, NIH-Bethesda) contains two copies of the nucleous insulator of the chicken β-globin (cHS4) of 250 bp. It is described that the insertion of this element improve the expression pattern and the genotoxicity when is incorporated into gammaretroviral and lentiviral vectors (Hanawa, Yamamoto et al. 2009). The sequence will be insert in the 3´LTR of AWW and WW by standard tecniques of clonning.

**Vector production.** AWW, WW(0.5) and WW(1.6) LVs will be produced by standard procedure in 293T packaging cells using the pMVCΔ R8.9 and pMD.G plasmids to express packaging and VSVg proteins respectively. The LVs particles will be used immediately or frozen at -80º C.

**HSC (WAS-) gene modification.** Lin-Sca1+ cells obtained from six-eight weeks old WASp^-/- mice will be isolated by AutoMACS and transduced with the different therapeutic LVs (AWW, WW(0.5) and WW(1.6) +/-insulator) as previously described (Zanta-Boussif, Charrier et al. 2009). The gene-modified cells will be expanded for 1-2 days and placed on methylcellulose (StemCells) to allow myeloid differentiation. was gene expression will be analyze by FACS and Western-Blot (WAS protein). For each vector, was mRNA expression levels (analyzed by RT-Q-PCR) will be related to the number of integration per cell (obtained by Q-PCR of the genomic DNA).

**Aim 2:** Analysis of the capacity of AWWINS, WWINS (0.5kb) and the WW (1.6kb) LVs (last one used in the UK-France-USA clinical trial) to rescue mouse WAS KO phenotype.

**Phenotypic rescue of WASp^+/-mice.** The therapeutic vectors that behave best in the previous study will compared with the WW(1.6) in their ability to express was mRNA and protein at physiological levels in reconstituted WASp^+/- mice. For that, we will inject 2-3x10e5 corrected HSC(WAS-) cells in irradiated WASp^+/- mice (9.5Gy). Peripheral blood will be analyzed 5.5 weeks after transplantation to evaluate the repopulation capacity of these transduced cells. Six-ten weeks after transplantation, recipient mice will be killed and cells obtained from bone marrow, spleen, lymph nodes and liver. The different hematopoietic populations will be
analyzed for was expression by FACS and by sorting followed by Western-blots and/or RT-QPCR. Functional rescue of T cells and macrophages will be studied by analysis of CD3 response and podosome formation respectively. The therapeutic effects of the treatment will be evaluated by measuring development of colitis, bleeding, (Zanta-Boussif, Charrier et al. 2009)

**Aim 3:** Analysis of the safety and efficiency of the different therapeutic vectors in a human cell model of WAS: HSCs (WASp deficient) derived from human Embryonic Stem Cells (hESCs) WAS KO.

This third aim of the project will be performed in collaboration with Dr. Francisco Martín (GENyO, Spain). His group has developed **WAS-deficient human embryonic stem cells (hESC WAS KO) as human model** to study safety and efficacy of LVs for GT of WAS. In collaboration with his group, we will isolate CD45+CD34+ derived from the hESC (WASKO) cells (equivalent to the HSCs from WAS patients) and transduced them with the different therapeutic LVs. After transduction we will analyze:

1- Expression levels of was mRNA in the different population derived from this LVs-transduced-HSC (WAS-) (macrophages, granulocytes, NK, etc) compared to HSC derived from hESC wild-type. These experiments will tell us which vectors offer the expression pattern that best mimic the endogenous was gene.

2- Potential alteration in the expression profiles caused by the integrations of the different therapeutic vectors in the HSC (WAS-) cells.

![WAS expression](image)

**B1.3 Originality and innovative nature of the project, and relationship to the 'state of the art' of research in the field**

Recent trials of Gene Therapy (GT) of primary immunodeficiencies (PID) have demonstrated recovery of functional immunity in more than 60 patients, showing the therapeutic potential of this technology. However, up to 10% of the patients treated in clinic trials of SCID-X1, CGD and WAS developed leukaemia or myelodysplasia. These results are promising but also demonstrate the necessity of improvement of the strategies used for genetic modification and preclinical analysis. In addition, WAS gene therapy may benefit from near physiologic expression since was gene under expression may not be fully corrective of B cell immunity and thrombocytopenia (Meyer-Bahlburg, Becker-Herman et al. 2008). **This project aims to improve the current technologies for genetic modification of the HSC from WAS patients to avoid transformation and to achieve physiological expression. In this sense, several important points will be original and novel:**

- **The improvement of therapeutic vectors (WW (1.6))** already in used in clinical by: 1- including new regulatory regions from was gene own promoter and 2- including insulator sequences that could enhance expression and reduce genotoxicity.

- **The use of a human WAS cellular model (hESC WAS KO) to study efficacy and safety.** The clinical trials that are currently on going use WAS-patient derived HSCs. For this reason, the ideal cellular model for studies of
efficiency and biosecurity should allow the study of the vectors in this target cells. However, HSCs from WAS patients are difficult to obtain (it is a rare disease and predominantly children), and do not survive in culture for long time. To address this problem Dr. Francisco Martín has developed several WASP deficient hESC lines (hESC WAS KO) using targeted gene disruption by Zinc-Finger nucleases. These cell lines maintain their pluripotent capacity and they are capable of hematopoietic lineage differentiation (CD45$^+$). For this reason, they are the first human model that allows the study of WASP function in lineages difficult to obtain from patients samples. The use of this novel human model to test potential therapeutic vectors will greatly assist the analysis of vector performance in terms of correction of cellular phenotype and also biosafety.

B1.4 Timeliness and relevance of the project

The incidence of Wiskott-Aldrich syndrome in the European population has been reported to be 1 in 250,000 live male births (rare disease). A study from Switzerland reported that the prevalence is 4.1 cases per 1 million live births and found that this condition occurred in 2-8.8% of patients with primary immunodeficiencies. The life expectancy is 15 years in a patient lacking WASP expression and the only cure is Hematopoietic Stem Cell (HSC) transplant for different reasons: matched sibling most successful, unrelated donor much more risky and greatest success when done before 5 years of age. Despite the promising results in GT of PIDs, vector insertion was found near the oncogene LMO2, the same that has been reported in SCID-X1 patients. The presence of strong viral enhancers in the vector is thought to contribute to insertional mutagenesis by influencing the expression of genes in the host genome. Also, LTR-driven gammaretroviral vectors are susceptible to epigenetic silencing, losing the therapeutic effect. Also, because many documented examples of vector-mediated genotoxicity involve cellular gene activation, it is likely that the enhancer-blocking capacity of chromatin insulators such as cHS4 hold considerable promise for abrogating this genotoxicity.

For these reasons, this project has a strong relevance in order to improve the therapeutic strategies for Gene Therapy of WAS and for the scientific excellence of the European Research, contributing to the better understanding of the LVs for GT of WAS and the use of insulators in order to improve safety and to avoid genotoxicity as much as possible.

References


B1.5 Host scientific expertise in the field

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University College London (UCL) is ranked amongst the World’s top universities (ranked 7th by the QS World University Rankings 2011) and is amongst the World’s premier centres for biomedical research. In RAE 2008, the Institute of Child Health (ICH) was part of a UCL return to Unit of Application 4 in which 70% of staff were rated to be of international quality (4* 40%; 3* 30%); for both Environment and Esteem indicators the ICH's return was judged to be of world-leading quality (100% 4*).

ICH is a University College London Research Institute, within which the Molecular Immunology Unit has a longstanding interest in the understanding and treatment of primary immunodeficiency. It is affiliated with Great
The Molecular Immunology Unit has a highly active basic and translational research programme aimed at developing understanding of the molecular and cellular basis of primary immunodeficiencies and developing strategies to improve haematopoietic stem cell transplantation and somatic gene therapy techniques. The Unit comprises over 50 basic and clinical scientists and currently has 10 full-time PhD students and 3 clinical fellows. There are numerous international collaborations with research groups in Europe, the US, Australia and China and the unit attracts visiting researchers and students from around the world.

There are a wide range of interests within the Unit. These include a core of work understanding the molecular and cellular pathogenesis of immunodeficiency syndromes using relevant systems. These studies will enhance our basic understanding of immunological processes and aid the development of diagnostic and therapeutic strategies.

The techniques that are required for this project are already established in the host laboratory

B1.6 Quality of the group/scientist in charge

Prof. Adrian Thrasher is a leading researcher in the Molecular Immunology Unit at ICH and is Director of Research in Primary Immunodeficiency across the whole UCL Campus, which incorporates the Royal Free Hospital Hampstead Campus. He is well known for his contribution to the field of primary immunodeficiency (PID) and stem cell research, is a recognized expert in the clinical and pathophysiological features of PID and has published many papers in this area which are directly relevant to the proposal. He is also a leading researcher in the development of new forms of treatment for primary immunodeficiency disorders and is currently directing 3 Phase I/II clinical trials for the treatment of primary immunodeficiency disorders by retroviral-mediated somatic gene therapy. More relevant publications related to this proposal (of the last 6 years):


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14-Gene Therapy: SCID-X1 transgene leukemogenicity Thrasher A; Gaspar H; Baum C; Modlich U; Schambach A; Candotti F; Otsu M; Sorentino B; Scobie L; Cameron E; Blyth K; Neil J; Hacein-Bey Abina S; Cavazzana-Calvo M; Fischer A.. Nature, 443, E5-E6, 2006.

European Commission Grants:
Professor Thrasher has supervised two recipients of Marie-Curie Fellowships, one of which remains within his group. In addition, he has been the co-ordinator of a framework V EU grant and a partner in a further 8 EU FP6 or FP7 awards, two of which are current and one in the negotiation stage and these are detailed as follows:
2008-2012 Persisting transgenesis (PERSIST). FP7, Co-ordinator Dr Luigi Naldini (ICH portion Euros 777,600)

Prizes:
In 2005, Professor Thrasher was a recipient of the Descartes Laureate award for the European Initiative on Primary Immunodeficiency (EURO-PID), his collaborators on the project and fellow recipients being Prof Alain Fischer, Prof Jean-Laurent Casanova, Dr Lennart Hammarstrom, Prof Luigi Notarangelo, Dr Edvard Smith, Dr Anna Villa.

Collaborations:
Professor Thrasher’s group has collaborations with researchers across the UK, Europe and elsewhere in the world. In this particular project, there are strong associations with the following research groups:
Prof. G.E. Jones: Cell Motility & Cytoskeleton group, Randall Division, Kings College, London (UK) “Molecular mechanisms of WASp in DC migration”.
Dr. A. Galy: Généthon, Evry (France) Dept of Biochemistry, University of Bristol (UK) “Lentiviral vector-mediated gene therapy and RNAi
B2 Training

B2.1 Clarity and quality of the research training objectives for the researcher

I have chosen this host institution because it is the best place which combines the clinics with the research advances (full applicability of the great steps forwards). The Institute of Child Health-UCL, in partnership with Great Ormond Street Hospital, is the largest centre in Europe devoted to clinical and basic research and postgraduate teaching in children’s health. The focus of its research is to understand the causes and complications of primary immunodeficiencies, to translate the research into optimal diagnostics, state-of-the-art clinical trials in cell and gene therapy and to develop optimal hematopoietic stem cell transplantation approaches. They are conducting trials of somatic gene therapy for various forms of PID including WAS. Other research interests includes the pathophysiology of primary immunodeficiency syndromes especially WAS, the actin cytoskeleton in hematopoietic cells (with Dr Siobhan Burns), the development of somatic gene therapy (with Professor Bobby Gaspar, Professor Christine Kinnon, Dr Waseem Qasim, and for ocular disease with Professor Robin Ali), and thymus transplantation (with Dr Graham Davies). With the interaction and collaboration with these other researches, I will gain inter-disciplinary expertise from the large research community at the host institution. I will have the chance to discuss my project with expertise in different multidisciplinary areas and also it will be possible to collaborate with them during my stay or during future research projects. Another benefit of this project is my contribution to a better translation of the research into optimal diagnostics, state-of-the-art clinical trials in cell and gene therapy and to develop optimal hematopoietic stem cell transplantation approaches, thanks to the improvement of new therapeutic LVs.

For the introduction of the gene therapy of PID into clinic in an efficient and safe way, it is necessary the development of new therapeutic strategies for the genetic modification of HSCs of patients and the analysis of these strategies in mice models and human cell models, that mimic as far as possible the therapeutic target cells of the patients. With the project I will acquire the capacity of mice transplantation as a useful tool for the engraftment studies of HSCs. I will have the capacity of hematopoietic repopulation of AWW, WW and the LV used in clinical trial (with the promoter of 1.6 kb) of WASP rescue in HSCs of mice WAS KO. I will obtain new technical abilities in molecular biology with the performance of new therapeutic LVs vectors with the insertion of insulator sequences (based on chicken HS4 and SAR sequences) and subsequent analysis of these new LVs (AWWINS, WWINS) in mouse and human HSCs.

I already know different methods to differentiate hESCs towards hematopoiesis, but with my work in the host institution I am sure that I will acquire expertise in new experimental approaches and methods. With my participation in lab meetings, journal clubs and congresses I will enrich my knowledge, not only in terms concerning my project, but also in subjects that could give new ideas and new supports.

As my first language is not English, the continued interaction with the researches at the ICH as well as attending an English language course (UCL Organizational Staff Development) will be of huge benefit to me. I will feel more comfortable writing articles and it will give me the opportunity to apply for European or International grants and the capacity of a better understanding with other researchers.

B2.2 Relevance and quality of additional research training as well as of transferable skills offered with special attention to exposure to industry sector, where appropriate

This research training will give me the opportunity to acquire and improve other scientific and related skills essential for the development of my scientific career:

- The new results obtained during this project will be published and I expect that the acquisition of this knowledge and skills will me to increase the number of publications. The course that the UCL IT Training offers “Endnote: Bibliography Management and On-line Databases” and the course “Getting Published in the Sciences” of the UCL Online Learning Resources for Research Staff will be very useful for this purpose together with the performance of this project in the UCL Institute of Child Health, (GOSH), the largest centre in Europe devoted to clinical and basic research and postgraduate teaching in children’s health, will increase my CV and possibilities to get grants in the future. As I mentioned before, my first language is not English. Although I have publications in a wide range of important journals and I am familiar with commonly accepted standards of scientific writing, there is no doubt that during the time of this project I will develop listening skills and the
ability to communicate more fluently in English. I will feel more confidence in lab meetings, congress and
journal clubs where to express much better my queries or to share my ideas.

- Thanks to my confidence talking English, it will be easier for me to transfer my research knowledge
acquired with this project to the industry sector and also, to study the possibility to patent any results of my project
during my stay in the UCL (in fact, two patents resulted from Dr. Francisco Martín’s laboratory during my postdoc
in his lab). Also, UCL Advances gives a variety of courses, providing me with the opportunity to develop skills
for effective interaction with researchers.

- This project in the ICH-UCL gives me the opportunity to present this research at national and
international congresses, conferences, symposiums, department conferences or lab meetings. This will surely
result in an improvement of my oral presentations.

- During my career in research I have supervised undergraduate students, so I will improve my leadership
abilities for supervising my own PhD students in the future.

- I expect to make a link between European institutions thanks to the collaboration with Dr. Francisco
Martín (GENyO, Spain) and other researchers at the ICH-UCL (UK).

B2.3 Measures taken by the host for providing quantitative and qualitative mentoring/tutoring

The Molecular Immunology Unit has a strong record of supervision and training for both students and staff,
some of whom have remained within MIU establishing their own research groups, others moving on to research
establishments elsewhere as successful independent scientists. Support for Miss Muñoz will be provided in many
ways. She would receive informal training where applicable in generic skills such as literature appraisal,
experimental design, troubleshooting, data analysis and presentation of results on an on-going basis from her
supervisor, Prof Thrasher. As mentor and supervisor, he will provide immediate guidance of both academic and
professional development through these informal sessions but there is also a formal requirement for yearly
appraisal of all students and staff. The appraisal process provides a regular opportunity to assess progress and
development and also provides an opportunity for Miss Muñoz to raise any concerns regarding the project direction
and/or support provided. Additionally, our Head of Unit, Professor Christine Kinnon, has an ‘open door’ policy for
all researchers within the department. There are also frequent group meetings, more formal weekly unit meetings
and journal clubs where regular attendance is expected. Researchers are expected to present their data at Unit
meetings on a regular basis.

Prof Thrasher has had extensive experience of supervising students and post-doctoral fellows, acting as
principal supervisor for 8 completed PhD students and as secondary supervisor for another 14 completed PhD
students within the Molecular Immunology Unit, and a further 8 students nationally and internationally. Currently,
within the MIU, he is primary supervisor to three PhD students, has 10 postdoctoral fellows within his group, and a
team of clinical trials researchers and co-ordinators under his supervision. The Head of Unit (Prof Christine
Kinnon) has hosted several Marie Curie Training Fellows previously and also held a Marie Curie PhD Training
Site Fellowship (2000-2004 Human haematopoietic stem cell gene therapy) at ICH under the European
Commission’s IVth Framework Programme.

It is a feature of the Institute that a large number of the current senior academic staff have been recruited at
early stages in their career and have developed their careers at the ICH, although it continues to recruit high-calibre
senior staff from elsewhere. In the past twenty years the size of the Institute, and the calibre of its research
programme, have increased enormously, reflecting this proactive policy. The Molecular Immunology Unit has a
long standing successful record in the development of the careers of both non-clinical and clinical academics. The
Unit presently has four Professors, two Readers, two clinical senior lecturers (plus honorary readers and senior
lecturers based at Great Ormond Street Hospital), and two clinical lecturers, the majority of these academics having
been promoted in successive stages throughout their career in the Molecular Immunology Unit.

UCL has an active programme of training and development for post-graduates, post-doctoral fellows and
lecturers in order for them to progress to senior independent research investigators. There are also PhD supervision
courses in order to learn how to supervise and provide help and guidance to PhD students. UCL provides formal
taught courses on presentation skills, experimental design, data handling, statistics, risk assessment, scientific paper
writing, project and general management and also IT courses in both general and research-relevant software
packages. The applicant would be expected to be able to develop the necessary skills to generate her own ideas and
experimental protocols in discussion with colleagues and more senior members of the Unit. As the host laboratory
is comprised of scientists from many different nationalities and cultures, the applicant will benefit from working in a multicultural international environment, which provides a platform for easy exchange of ideas and discussion.

**B3 Researcher**

**B3.1 Research experience**

After graduating in Biochemistry at the University of Granada (1999), I started working in Dr. Jaime Sancho’s laboratory (Parasitology and Immunology Institute “López-Neyra” (CSIC), Granada, Spain). During this time (2001-2003) I learnt different methodologies that were very useful for the PhD. In 2003, I obtained a predoctoral fellowship from the Spanish National Research Council (FPI Fellowship).

**2003-2007:** as a PhD student, I studied the CD38 protein in membrane microdomains of T lymphocytes. In this way, I analyzed the signaling mechanisms of CD38 and its function in immunological synapses. During this period, I optimized different experimental techniques including: Western-blot, FACS-Cytometry, cell cultures, and confocal microscopy. I also actively collaborated in other projects of the laboratory, particularly on the role of CD38 in T lymphocytes of Lupus Erythematosus patients. At that time, I attended and participated in the lab meetings and internal meetings of the institute, and my results were also presented at national scientific meetings. During my fellowship I had the opportunity to do two stays outside of my laboratory. The first one was in the laboratory of Dr. Francisco Sánchez-Madrid in “La Princesa Hospital” (Madrid). Thanks to that I learned different techniques that were essential for my thesis and for the collaboration, resulting in one paper as first author (Blood 2008). My second stay was in Boston, in Dr. Cox Terhorst’s laboratory (Beth Israel Deaconess Medical Center, Harvard Medical School), collaborating in the study of the SLAM family proteins. Thanks to this stay we initiated a collaboration which resulted in one paper (J.Exp.Med 2011).

**2007-present:** as a postdoctoral researcher in the laboratory of Dr. Francisco Martín’s (first at the Andalusian Stem Cells Bank and in the actuality at GENyO, Granada (Spain)). My project is the study of safe gene therapeutic strategies for Wiskott - Aldrich syndrome (WAS). This syndrome is caused by mutations in was gene and is only expressed by hematopoietic cells. At the moment, there is no human disease model where we can study and evaluate new gene therapeutic lentiviral vectors for WAS. Our approach to this problem has been to use human embryonic stem cells (hESCs) as source of hematopoietic cells and for the study of our lentiviral vectors during hematopoietic differentiation. With this hESCs we could demonstrate the specificity of the WAS promoter to express eGFP only in hemogenic precursors and hematopoietic cells (PLoS One 2012). For this work I established in the laboratory all the techniques for hESCs culturing, hematopoietic differentiation (embryoid bodies’ formation and OP9 system), and cytometry analysis for hematendothelial precursors (CD31, CD34, and CD45). The laboratory of Dr. Francisco Martín’s has a wide experience in gene therapy and lentiviral vectors production so it was very easy for me to learn the different methods and also improve the protocol for hESCs transduction. In the laboratory I am also participating in the development of hESCs WAS KO (human cellular model for WAS) and in the study of the insulators sequences under doxycycline-regulable vectors in hESCs.

**Present professional position:**

Postdoctoral researcher (“Sara Borrell” Fellowship from the Spanish National Research Council) at GENyO (Pfizer-University of Granada-Junta de Andalucía Center for Genomics and Oncology) (www.genyo.es). Avda. de La Ilustración nº 114 C.P. 18007 Granada (Spain)

**Education:**

Biochemistry graduate (1994-1999): Faculty of Sciences (University of Granada) Spain
PhD Degree (2003-2007): Institute of Parasitology and Biomedicine “López-Neyra” (CSIC), Granada (Spain)

**Professional experience**

<table>
<thead>
<tr>
<th>Date</th>
<th>Position</th>
<th>Place and Supervisor</th>
</tr>
</thead>
<tbody>
<tr>
<td>29/01/2010-Present</td>
<td>Postdoctoral researcher</td>
<td>GENyO (Andalusian Government) Spain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dr. Francisco Martín Molina</td>
</tr>
</tbody>
</table>
B3.2 Research results including patents, publications, teaching etc., taking into account the level of experience

-In 1999 I obtained the **Biochemistry Graduated** by the University of Granada.

-From 2001 to 2003 I have been working in the laboratory of Dr. Jaime Sancho at the Institute of Parasitology and Biomedicine “López-Neyra” (Spain) as postgraduate researcher. My research was focused on the role of CD38 in the immunological synapse of the T lymphocyte and its role as a marker of Lupus Erithematosus Disease. I obtained my **PhD degree with Honours in 2007**. The results of my work have been published in high impact journals and are taken as work of reference influencing current research. In total, I published 9 articles in peer-reviewed international journals (3 as first author) and 2 of them as result of the 2 stays that I did during my PhD. I have participated in 4 research projects financed by national funding and I have been a participant in 10 congresses.

-From 2007-present I am a postdoctoral researcher at the laboratory of Dr. Francisco Martín. My interest is focused on the study of safety gene therapeutic strategies for Wiskott-Aldrich syndrome and the use of human embryonic stem cells as source of hematopoietic precursors. All our work has been published in 6 international journals and 3 patents have been developed as result of our studies. I have participated in 5 projects and I have attended to 6 national and international congresses (one of the congress (Spanish Society for Gene and Cell Therapy 2009) was realized in our city (Granada) and I was the local secretary of the organizing committee and participant). Also, during this time I have been teaching in the European Master of Advance Therapies (IAVANTE) for two courses.

**In summary**, all the studies that I have performance during my career as a researcher, have given me a wide and solid experience in developmental biology, embryonic stem cells, lentiviral vectors, mouse models disease (EAE) and immunology, which are beneficial for the execution of my project at Prof Thrasher’s laboratory with a Marie Curie Fellowship.

**Publications**

<table>
<thead>
<tr>
<th>Impact factor assessed by the ISI® Journal Citation Reports (JCR® 2011)</th>
</tr>
</thead>
</table>


**Patents**

" LENTIVIRAL VECTORS FOR THE IDENTIFICATION OF HEMATOPOIETIC LINEAGE" Francisco Martín-Molina, Pilar Muñoz-Fernández, Miguel García-Toscano, Karim Benabdellah-el-Khlanji

SOLICITUDE N°: P201230449 (2012). Andalusian Public Foundation “PROGRESO Y SALUD” (Andalusian Government), Spain

"HIGHLY INDUCIBLE TET-ON VECTOR SYSTEM" Francisco Martin-Molina, Karim Benabdellah-el-Khlanji, Marién Cobo-Pulido, Miguel García-Toscano, Pilar
Participation in research projects

   "Terapia génica del Síndrome de Wiskott-Aldrich: Desarrollo de un modelo celular humano para estudios preclínicos"
   FIS Project (ISCIII) (Instituto de Salud Carlos III/Spanish National Research Council) Spain (PS09/00340) 2010-2013

2. "Gene-cell therapy for Wiskott-Aldrich Syndrome by genetic surgery"
   "Terapia celular génica del Síndrome de Wiskott-Aldrich mediante cirugía genética”. Consejería de Salud/Regional council of Health (Junta de Andalucía-Andalusian Government) Spain (PI0001/2009) 2010-2013

3. "Gene-cell therapy for Multiple Sclerosis"
   "Terapia celular-génica para esclerosis múltiple” Consejería de Innovación, Ciencia y Empresa/Innovation, Science and Business Council (Junta de Andalucía-Andalusian Government) Spain (P09-CTS-04532) 2010-2014

4. "Support to the activities developed by the investigation group BIO"
   "Apoyo a las actividades desarrolladas por el grupo de investigación BIO” Grupos PAI/PAI groups. Consejería de Innovación, Ciencia y Empresa/Innovation, Science and Business Council (Junta de Andalucía-Andalusian Government) Spain 2010-2013

5. "Development of regulable-lentiviral vectors for application in gene and cellular therapy"

   "Compartimentación subcelular y enfermedad. Nuevas estrategias diagnósticas y terapéuticas”. Programa I+D, Proyectos de Excelencia de la Junta de Andalucía/ I+D program, Excellence Investigation Project of Andalusian Government, Spain (P05-CVI-00908) 2006-2009

7. "Role of CD38 in the regulation of inflammatory process in autoimmune diseases"
   "Papel de CD38 en la regulación de los procesos inflamatorios en enfermedades autoinmunes”. FIS Project (ISCIII) (Instituto de Salud Carlos III/Spanish National Research Council) Spain (PI030389) 2004-2006

8. "Discovery of biomarkers of autoimmune diseases"

9. "Identification by proteomic techniques of the signaling complexes used by T regulatory lymphocytes in the autoimmune diseases"
   "Identificación por técnicas de proteómica de los complejos señalizadores utilizados por los linfocitos T reguladores en las enfermedades autoinmunes”. Ministerio de Ciencia y Tecnología/Ministry of Science and Technology, Spain (SAF2002-00721) 2002-2005

Fellowships

<table>
<thead>
<tr>
<th>Date</th>
<th>Position</th>
<th>Fellowship</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2010-Present</td>
<td>Postdoctoral researcher</td>
<td>“Sara Borrell” (CD09-00200) awarded by the Spanish National Research Council (ISCIII)</td>
</tr>
</tbody>
</table>
6/2001-11/2003 Graduate Student - I3P Fellowship awarded by the Spanish National Research Council (CSIC)
- Predoctoral researcher contract associated to the projects: SAF-2000-00721, R01 AI48448-01, CV1 156.
- Superior graduated in Investigation and Laboratory Contract (INEM-CSIC/National Institute of Employment-Spanish National Research Council)

Stays at National and International R+D Centers
As predoctoral researcher:

<table>
<thead>
<tr>
<th>Name of the Center</th>
<th>Supervisor</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beth Israel Deaconess Medical Center (Harvard Medical School). Boston (Massachusetts) USA</td>
<td>Dr. Cox Terhorst</td>
<td>May-September 2005</td>
</tr>
<tr>
<td>Hospital de la Princesa (Princess Hospital) Immunology Department. Madrid, SPAIN</td>
<td>Dr. Francisco Sánchez-Madrid</td>
<td>February-April 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>January-April 2004</td>
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</tbody>
</table>

Teaching


Courses

<table>
<thead>
<tr>
<th>Name of the course</th>
<th>Organizer/Place</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular Reprogramming and Induced Pluripotent Stem Cells (iPSCs)”</td>
<td>Cell-Therapy Net and Spanish Society for Gene and Cell Therapy (SETGyC) Madrid, Spain</td>
<td>2010</td>
</tr>
<tr>
<td>“Technological advances in human reproduction”</td>
<td>University of Granada. Spain</td>
<td>2000</td>
</tr>
<tr>
<td>Pedagogic Aptituated Certificate</td>
<td>University of Granada. Spain</td>
<td>2000</td>
</tr>
<tr>
<td>“The biologic engineering of XXI century, science of fiction?”</td>
<td>University of Granada. Spain</td>
<td>1999</td>
</tr>
<tr>
<td>2nd course of Scientific Present “Comunication, Science and Technology in the XXI Century”</td>
<td>Sciences Park of Granada. Spain</td>
<td>1999</td>
</tr>
</tbody>
</table>

Scientific Excellence

1- Secretary of the Local Organizer Committee of the “V Congress of Spanish Society for Gene and Cell Therapy (SETGyC) celebrated in Granada (Spain) 30th September-2nd October 2009

2- Member of the group P.A.I.D.I BIO326 (Plan Andaluze Investigación y Desarrollo/ Andalusian Plan of Investigation and Development, Andalusian Government): “Molecular bases and therapeutic strategies of immune system diseases”. 30/01/2009-present

Participation in Conferences

5. “V Congress of the SETGyC”. Granada, Spain 30th September-2nd October 2009. 2 Posters presentation

B3.3 Independent thinking and leadership qualities

During my career as a researcher, my capacity for independent thinking has been demonstrated. Since the beginning I learnt to work in a team and to establish and improve new protocols in the laboratory. I always have been working with new students that I instructed them with the techniques of the laboratory. When I got the fellowship of the Spanish government for my PhD, I was allowed to carry out my project. For this purpose, the two stays that I carried out in the laboratory of Dr. Francisco Sánchez-Madrid in the Hospital “de La Princesa” were crucial. I learned different techniques for the study of the immunological synapse such as confocal microscopy, and migration assays…all of which I implemented in the laboratory of Dr. Jaime Sancho. Also the stay at Dr. Cox Terhorst’s laboratory in the Beth Israel Deaconess Medical Center, (Harvard Medical School, Boston, USA) was very important. After my PhD, I contacted with Dr. Francisco Martín and under his supervision I have been in charge of managing my research projects, proposing new experiments, analysing the data, participating in the lab meetings for new ideas and writing the publications. I collaborate in the projects of the people in the laboratory, with experiments, giving new perspectives and in the organization of the laboratory (congresses inscriptions and making orders of laboratory materials). Also I am the coordinator of the cell culture facility of Dr. Francisco Martín’s group and part of my function is to coordinate the correct use of the facilities. Dr. Francisco Martín has promoted my leadership qualities by allowing me to participate as a teacher during two courses of the “Master of Advances Therapies”, and by encouraging me to write grants to the Andalusian Government. Also he has
encouraged me to discuss my results in English making me feel very confident with my language skills. All these experiences have been reinforcing my leadership capabilities.

**B3.4 Match between the fellow’s profile and project**

During my PhD I received an excellent training in immunology and cell biology techniques. In 2007 I joined Dr. Francisco Martín’s laboratory, a pioneer group in gene therapy of Wiskott-Aldrich syndrome (WAS). In his laboratory I am currently widening my expertise in stem cell biology looking at the cellular and molecular aspects of hESC growth and early differentiation, and I now have the skills to maintain and differentiate hESCs towards hematopoietic lineage. Moreover, I have learned how to genetically manipulate hESC with lentiviral vectors. In Dr. Martín’s lab we work on the improvement of therapeutic lentiviral vectors (using insulators and new promoters) using hESCs as a cell model. Our next goal is to test these therapeutic vectors for WAS in animal models and cells obtained from WAS patients. However, WAS is a rare disease, so to obtain samples from patients is difficult, as well as our planned work with animal models that requires good animal facilities. Prof. Thrasher’s lab is the best place to perform my studies, as it is a strong and competitive group in the understanding of the causes and complications of primary immunodeficiencies, to translate the research into optimal diagnostics, state-of-the-art clinical trials in cell and gene therapy and to develop optimal hematopoietic stem cell transplantation approaches. The training that I will receive in his laboratory is a clear opportunity to perform basic research with translational goals and setting a platform for the study of new therapeutic lentiviral vectors for Wiskott-Aldrich syndrome that would be crucial to move forward in my career to become an independent researcher. Hence, I can receive all the support and facilities for the work with a mouse model for Wiskott-Aldrich and samples from patients. I feel confident that I can contribute to the host group with the human cellular model of WAS that we have developed in Dr. Martín’s laboratory and my expertise in hESCs culture and lentiviral vectors among other techniques.

**In conclusion, my background in lentiviral vectors for WAS will be very useful for the development of this project, improving new therapeutic lentiviral vectors for gene therapy of WAS.**

**B3.5 Potential for reaching a position of professional maturity**

During this project I expect to acquire complementary abilities in the field of the use of mouse model or WAS patients’ cells and also, in the proper environment and under good supervision, I will be able to initiate my own projects in close contact with the host group, his collaborators and of course the group of Dr. Martín (GENyO). At present I count on the full support of the department of Gene and Cell Therapy of GENyO where I currently am a postdoctoral researcher with a fellowship “Sara Borrell” (CD09-00200) awarded by the Spanish National Research Council (ISCI) and I feel confident that I will have the necessary experience to apply for new projects and also I consider other professional openings in any European country. In each laboratory that I have been, I learnt so many things, not only scientific techniques but I have also matured and gained a clearer perspective of what I want and what I can get. Dr. Martín encouraged me to acquire groundbreaking knowledge in the WAS mouse model and at this point I am certain that I will apply for other calls in Europe and also in Andalusia (Spain). Getting this Marie Curie fellowship would be important for my future career, firstly because it would allow me to carry out my studies in one of the most relevant places in gene therapy clinical trials, and secondly, to obtain this prestigious fellowship would certainly boost my curriculum. I strongly believe that after the end of the fellowship and with the support of the host institution, the Institute of Parasitology and Biomedicine “López-Neyra” (CSIC) and GENyO (Andalusian Government), I will have good chances to find national or European funding to carry out my research and of course establish myself as principal investigator of my own laboratory.

**B3.6 Potential to acquire new knowledge**

Throughout the course of my PhD and my postdoctoral research I have demonstrated a potential to acquire knowledge as well as the capacity to learn and optimize specific techniques, experimental design and analytical skills. Also I took special care to attend internal and external seminars and courses because I always had considered their special importance. It is essential to now the state-of-the-art, not only for your project, but also the understanding and knowledge of other disciplines that can be beneficial for the development of my project.
At this point, I feel particularly confident that I have the full capacity to acquire the knowledge and skills required for successfully conduct this project, with as much success as I acquired new knowledge and defeated complex challenges during my PhD and postdoc. My stay at the host institution will be very profitable in terms of publications and acquisition of knowledge for the study of new gene therapeutic lentiviral vectors for Wiskott-Aldrich syndrome. Also the establishment of collaborations with other research groups at the ICH-UCL will be beneficial for my current and future projects.

B4 Implementation

B4.1 Quality of infrastructures/facilities and international collaborations of host

The UCL Institute of Child Health is fully equipped with all relevant infrastructures. Of particular significance for this project are the microscopy facilities, including confocal and fluorescence microscopes, fully equipped FACS facility and animal facilities, which are equipped with a fully functional transgenic unit and have excellent breeding facilities. The Molecular Immunology Unit is sited within newly refurbished and fully equipped dedicated laboratory space that contains specially designed and commissioned tissue culture suits for cell culture techniques, and a specialised facility for lentiviral work. The Unit’s fully equipped molecular biology laboratories include real-time PCR machines, a Megabase sequencing facility as well as biochemical and protein labs.

The applicant will have her own dedicated laboratory bench, as well as office space, and will be assisted and trained where necessary on all necessary equipment. Comprehensive training is provided in all relevant laboratory techniques and courses are offered in a wide variety of relevant research skills, both organisational and practical, including flow citometry, and confocal microscopy. Where applicable, the applicant will be encouraged to attend formal in-house training sessions and external specialist training courses, for example, in microscope techniques.

The Unit is internationally renowned for its contribution to the WAS research field and has many collaborators on national and international level. For this proposal the more relevant collaborators are:

**Prof G.E. Jones**  
Cell Motility & Cytoskeleton group, Randall Division, Kings College, London, UK  
Molecular mechanisms of WASp in DC migration

**Dr A. Galy**  
Généthon, Evry, France  
Lentiviral vector-mediated RNAi to specifically inhibit WASp expression, lentiviral production.

**Dr D. Becker**  
Dept of Anatomy & Developmental Biology, University College London, UK  
Muti-photon laser microscopy to study real time in vivo migration

B4.2 Practical arrangements for the implementation and management of the research project

The administrative staff of both the Molecular Immunology Unit and the UCL Institute of Child Health will assist Miss Muñoz in the aspects of administrative procedures relating to the project, for example, creating risk assessment documentation, registering with UCL financial systems, applying for core facility access and procurement operations. The MIU has an in-house expert for advice with animal licensing and procedures as well as dedicated staff for this purpose at ICH and UCL.

Professor Thrasher will supervise and mentor Miss Muñoz at regular meetings and will designate postdoctoral fellows / PhD students to support her in everyday experimental work.
B4.3 Feasibility and credibility of the project, including work plan

The duration of this project is 24 months. During this time I will develop experiments, data analysis and discussion, write manuscripts and other complementary skills, such as oral and posters communications to congress, lab meetings, supervision of students, etc. The main aim of this project is to improve the safety and efficiency of the lentiviral vectors (LVs) for gene therapy of Wiskott - Aldrich syndrome (WAS). For this purpose I will develop new LVs by the introduction of insulator sequences in the gene transfer vectors AWW, WW (0.5kb) and WW (1.6kb). These new lentiviral vectors will be analyzed in HSCs from mouse WAS KO model and HSCs obtained from hESCs WAS KO (aims 1, 2 and 3 of the proposal).

We envision these studies will provide biological tools for the study of new insulated-LVs for gene therapy of WAS by providing key information about the safety and efficiency of these therapeutic LVs. The following table depicts the work plan in terms of specific tasks and the objectives of each part.

**Aim 1:** To improve safety and efficiency of the gene transfer vectors AWW and WW for WAS GT by the introduction of insulator sequences (based on chicken HS4 and SAR sequences) and analysis of these new LVs (AWWINS, WWINS) in mouse HSCs

<table>
<thead>
<tr>
<th>Months</th>
<th>Specific Tasks</th>
<th>Objectives</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>1.1- Training courses (animal facility) and paperwork for the UCL.</td>
<td>-To initial contact with the lab to start the project</td>
</tr>
<tr>
<td></td>
<td>1.2- Learning about the organization of the lab: rules, people, machines,</td>
<td>-To interact with researches of the lab and gain basic technical background.</td>
</tr>
<tr>
<td></td>
<td>materials…</td>
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<td></td>
<td>1.3- Initial scientific presentation and discussions</td>
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</tr>
<tr>
<td>2-5</td>
<td>1.4- Cloning of insulator sequences into 3’-LTR of AWW, WW (0.5kb) by</td>
<td>-To obtain the insulated-lentiviral constructs.</td>
</tr>
<tr>
<td></td>
<td>standard techniques.</td>
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<td></td>
<td>1.5- Analysis of the constructs by digestion and sequencing</td>
<td></td>
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<tr>
<td>6</td>
<td>1.6- Lentiviral production by standard procedure in 293T packaging cells using</td>
<td>-To obtain the lentiviral particles for the experiments.</td>
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<td></td>
<td>pMCMVΔ R8.9 and pMD.G plasmids. The LVs particles will be used immediately or</td>
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<tr>
<td></td>
<td>frozen at -80º C</td>
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<tr>
<td>7-10</td>
<td>1.7- Isolation of LinSca1+ cells of WASp+/− mice by AutoMACS.</td>
<td>-To analyze the restoration of WASp+/− cells with the new LVs</td>
</tr>
<tr>
<td></td>
<td>1.8- Isolation of HSCs from WAS patients (when it is possible)</td>
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<tr>
<td></td>
<td>1.9- Transduction of those cells with AWWINS and WWINS (0.5kb) for methylcellulose assay (myeloid differentiation)</td>
<td></td>
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<td></td>
<td>1.10- Analysis of WAS protein expression: FACS and Western-blot</td>
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<td></td>
<td>1.11- Analysis of was gene expression by RT-QPCR.</td>
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<tr>
<td></td>
<td>1.12- Data collection and writing/analysis of the results obtained.</td>
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</tbody>
</table>
**Aim 2:** Analysis of the capacity of AWWINS, WWINS (0.5kb) and the WW (1.6kb) LV (used in the UK-France-USA clinical trial) to rescue mouse WAS KO phenotype.

<table>
<thead>
<tr>
<th>Months</th>
<th>Specific Tasks</th>
<th>Objectives</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-16</td>
<td><strong>2.1</strong>-Injection of corrected HSCs (as described in the previously table) into irradiated WASp°-mice (9.5Gy).</td>
<td>-To analyze the phenotypic and functional rescue of WASp°- mice in terms of WASp expression and functionality of T cells and macrophages.</td>
</tr>
<tr>
<td></td>
<td><strong>2.2</strong>-Analysis of peripheral blood 5.5 weeks after transplantation.</td>
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<tr>
<td></td>
<td><strong>2.3</strong>-Sacrifice of the mice 6-10 weeks after transplantation to obtain bone marrow, spleen, lymph nodes and liver.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>2.4</strong>-Analysis of the different hematopoietic populations for WAS protein (WASp) expression by FACS followed by Western-Blot and/or RT-QPCR.</td>
<td></td>
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<tr>
<td></td>
<td><strong>2.5</strong>-T cells and macrophages study by analysis of CD3 response and podosome formation respectively.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>2.6</strong>-Data collection and writing/analysis of the results obtained.</td>
<td></td>
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<tr>
<td></td>
<td><strong>2.7</strong>-Writing to publish the results obtained.</td>
<td></td>
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</tbody>
</table>

**Aim 3:** Analysis of the safety and efficiency of the therapeutic vectors in a human cell model of WAS: HSCs derived from human Embryonic Stem Cells (hESCs) WAS KO

<table>
<thead>
<tr>
<th>Months</th>
<th>Specific Tasks</th>
<th>Objectives</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-24</td>
<td><strong>3.1</strong>-Culture and expansion of human embryonic stem cells (hESCs) WAS KO</td>
<td>-To study hESCs WAS KO as human cell model for Wiskott-Aldrich-syndrome.</td>
</tr>
<tr>
<td></td>
<td><strong>3.2</strong>-Transduction of hESCs WAS KO with the insulated-LVs</td>
<td>-Analysis the safety and efficiency of the LVs in HSCs derived from hESCs WAS KO</td>
</tr>
<tr>
<td></td>
<td><strong>3.3</strong>-Analysis of the pluripotency capacity of these transduced-hESCs WAS KO: FACS analysis of SSEA-3, SSEA-4, TRA-1-60, TRA-1-81 and OCT3/4 and teratoma formation capacity.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>3.4</strong>-Hematopoietic differentiation of transduced-hESCs WAS KO (Embryoid bodies formation and/or OP9 cocultive) to obtain CD34+CD45+ cells.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>3.5</strong>-Expression levels of was mRNA in the different population derived from this LVs-transduced-HSC.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>3.6</strong>-Study of the potential alteration in the expression profiles caused by the integrations of the different therapeutic vectors in the HSC (WAS-).</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>3.7</strong>-Data collection and writing/analysis of the results obtained.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>3.8</strong>-Writing to publish the results obtained.</td>
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</tbody>
</table>
B4.4 Practical and administrative arrangements and support for the hosting of the fellow

Miss Muñoz will be appointed according to human resources policy of UCL Institute of Child Health, which covers a wide variety of supportive arrangements for new staff. The Human Resources Team is able to facilitate work permit applications and offer comprehensive guidance on UK tax, national insurance and pension procedures, supplying necessary documentation and forms and arranging appointments where necessary. The Human Resources Team have nurtured relationships with local banks so that they understand the dimensions of our overseas appointments and ICH/GOSH also have a highly efficient and effective accommodation bureau. This has a comprehensive and informative database of accommodation available to our staff, and can also supply advice on travel, costs, localities, and other practical help on living in London. In addition, the European Research and Development Office (ERDO) at UCL are specifically constituted to provide support to academic staff working on European funded projects. The Office has been managing European Commission funding since Framework Programme I and is currently active in 370 Framework 7 projects.

UCL Institute of Child Health staff has access to crèche facilities and application for a place can be discussed with our Human Resources Manager. Language courses can be applied for at the UCL Language Centre, and the following link http://www.ucl.ac.uk/language-centre/ provides further details on courses and application procedures. There is a comprehensive staff induction and staff development training programme at UCL, in which all new (and current) staff are encouraged to participate in order to enhance personal and career development as well as offering practical training in organisational and practical skills.

The Molecular Immunology Unit has one full time and one part time unit administrator and a full time lab manager, and the applicant will benefit from in-house induction and advice from these three personnel. On arrival, the Lab Manager will offer fire safety training and a local laboratory safety induction course and the Unit Administrator will initiate or assist in the completion of the paperwork necessary to facilitate a smooth introduction to the unit. Additionally, these personnel will perform introductions and provide timetables to include the fellow as quickly as possible in both the scientific and social networks of a very multidisciplinary and multicultural environment.

Apart from the extensive facilities provided centrally by UCL, the Institute of Child Health also shares an R&D Department with Great Ormond Street Hospital and has other administrative sections in-house, enabling the benefit of R&D, finance, funding, ethical and teaching advice from onsite.
B5 Impact

B5.1 Impact of competencies acquired during the fellowship on the future career prospects of the researcher, in particular through exposure to transferrable skills training with special attention to exposure to the industry sector, where appropriate

This Marie Curie Action represents an excellent opportunity for me to boost my future career as a researcher. The opportunity to work at the renowned ICH-UCL in London will allow me the acquisition of a wide repertoire of methodological techniques and invaluable contacts with new collaborators. Importantly, a stay at the UCL will surely broaden my perspective, improve individual thinking and boost my confidence. At the host group, under Prof. Thrasher’s supervision, I will acquire a wide knowledge in the manipulation and analysis of the mouse model of WAS and in the improvement of new lentiviral vectors for gene therapy of Wiskott-Aldrich syndrome. It is especially relevant to mention Prof. Thrasher’s relations with Great Ormond Street Hospital for Children (ICH/GOSH) NHS Trust, which is one of the major paediatric centres in Europe. The academic and clinical immunology and bone marrow transplantation services at ICH/GOSH form one of the largest European tertiary referral centres for research, diagnosis and treatment of inherited immunological disorders in children. This is an excellent opportunity to establish contact with Wiskott-Aldrich patients for future career prospects for the development of the most optimum gene therapy strategy for WAS. Furthermore, the realisation of this project, together with the collaboration established with Dr. Francisco Martín, will give me a huge opportunity to gain expertise from these research communities, to get visibility amongst researchers and to promote research collaborations during this stay and in the future. As leading fellow of this project, I will acquire experience and complementary skills as the best background to obtain an independent position in a relevant European institution. I would improve my skills at managing a research project being directly involved in the timeliness procedures and ethical approvals. Importantly, improving my knowledge in English, both spoken and written, will bring me the necessary skills to write good grant-proposals and to improve the quality of the research articles. I will have the opportunity to improve my general communication skills in English because of the interaction with native speakers, helping PhD students and undergraduates and by attending courses provided by the UCL. Also, the direct contact with students and undergraduates will be very beneficial for me in order to gain leadership skills for fellows in the future. The constant interactions with professionals in gene therapy through the presentation of my results in lab meetings, international congresses and collaborations will let me to improve the professional maturity in this field of research.

B5.2 Contribution to career development or re-establishment where relevant

This is an extraordinary opportunity to push my research career by adding complementary research competences at an advanced level. Firstly, the ICH and Great Ormond Hospital are top class institutions in clinical trials of primary immunodeficiencies (PID) and are the best places for the development of new strategies for gene therapy of WAS. Secondly, Prof. Thrasher has many years of internationally-competitive track record in PID as WAS. Therefore, the performance of this project at the ICH will give me a huge opportunity to acquire expertise on these topics. Also, I will have the opportunity to publish the results in international journals, which will have an exceptional impact in my curriculum vitae, allowing me to gain visibility in the research community and increasing my prospects of acquiring an independent position in the near future.

On the other hand, the proposed research has great potential in generating new hypotheses and future projects that can be pursue after the fellowship has ended. The use of insulators to improve the biosafety of lentiviral vectors for gene therapy is a wide field of study. Thus, this opportunity can open new doors in the design of new safety lentiviral vectors and, also I can establish international collaborations, acting as a bridge between the institutions, by promoting the development of shared innovative projects.
B5.3 Benefit of the mobility to the European Research Area

Firstly, I strongly believe that, with the execution of this project, the European Research Area will benefit enormously from the potential knowledge that I can acquire in the area of gene therapy/clinical trials for WAS and basic disease mechanisms using the WAS mouse model and hESCs WAS KO.

Secondly, for researchers, the option of mobility across countries constitutes one of the most efficient ways for the transmission and acquisition of knowledge and is very beneficial for establishing collaborations. The collaboration between UK and Spain could be consolidated by means of the development of the proposed project. In this sense, I can offer my experience to the host institution because this Marie Curie Action is an excellent opportunity to attract a Spanish trained scientist to UK and vice versa.

Thirdly, this IEF grant will give me new knowledge in gene therapy of WAS and increase my proficiency in English, both of which are crucial for my career as a future independent researcher. The formation of prepared scientists are of great importance for the competitiveness of the European Research Area in the years to come. In case of granting, this fellowship will be very fit for me because I can moved from a Spanish Research institution to a Research centre of Public Healthcare (Institute of Child Health) intimately connect with the Great Ormond Hospital. This will be a huge opportunity to develop better communication skills through full contact with a foreign scientific culture and the experience of learning new techniques basic for my future career. During my PhD I did a stay of 4 months in the Beth Israel Deaconess Medical Center (Harvard Medical School, Boston, USA) and it was an excellent opportunity to come in contact with its scientific community, the exchange of ideas and the collaboration with professionals in immunology (collaboration in J. Exp. Med). Now, funding this project is an excellent opportunity to increase my knowledge inside the Europe Research Area.

B5.4 Development of lasting cooperation and collaboration with other countries

The likelihood of creating collaboration between the ICH-UCL (UK) and other countries after the end of the fellowship is absolutely sure. In fact, the Molecular Immunology Unit of ICH (the host group) has numerous international collaborations with research groups in Europe, USA, Australia and China and the unit attracts visiting researchers and students from around the world. Prof Thrasher has European Commission Grants with Italy (Dr. Luigi Naldini and Prof. Alessandro Aiuti), Spain (Dr. Juan Bueren) among others, also his group has collaborations with researchers across the UK, Europe and elsewhere in the world: Prof. G.E. Jones (Kings College, London, UK), Dr. A. Galy (Généthon, Evry, France), Dr. D. Becker (University College London, UK).

The development of this project in the host institution involves the participation in meetings and congress with the collaborators of the group, so I will have the opportunity to interact with them and establish collaborations with specialists in gene therapy of primary immunodeficiencies.

B5.5 Contribution to European excellence and European competitiveness regarding the expected research results

The main aim of this proposed project is to develop safety strategies for gene therapy of Wiskott-Aldrich syndrome. This disease affects mainly children and this is one of the points of Health Research Envisages by FP7 (Child Health) and contributes completely to the excellence of European Research.

This proposal is totally in line with the 7 Framework Programme priorities:

-Pillar 1: Biotechnology, generic tools and medical technologies for human health: Innovative therapeutic approaches and interventions.

-Pillar 2: Translating research for human health: Rare diseases.
-**Pillar 3: Optimizing the delivery of health care to European citizens: Translating clinical research into clinical practice.**

The fellow has been trained in Dr. Francisco Martín’s lab (GENyO, Spain) since 2007. This is a leader group in gene therapy of Wiskott-Aldrich syndrome, and her stay in the hosting institution will clearly contribute to increase Europe’s competitiveness in the emerging field of new strategies for gene and cell therapy. Furthermore, the fellow will provide her expertise in basic cellular and molecular biology, which will help bridging the gap between basic research and clinical applications. Thanks to this fellowship, Miss Muñoz will become an expert on basic and translational research on development of new lentiviral vectors for gene therapy of PID. This study will provide new tools that facilitate disease research and the improvement of the lentiviral vectors existing in terms of safety and efficiency.

This project contributes to European excellence in order to the new strategies developed for gene therapy. Indeed gene therapy and advanced cell-based therapy for treatment of primary immunodeficiencies research receives continuous support through several EU Programmes, given its immediate relevance for research in health. Developing and consolidating the proposed research in Europe will certainly increase the European Research Area, enhancing the investment in research and innovation in the study of new lentiviral vectors for gene therapy.

The results of this innovative research will be disseminated through participation in National, European and International events and also through publications in international peer review journals, raising visibility of European expertise in this field. So, the integration of the candidate in the hosting institution will clearly contribute to increase Europe’s competitiveness in the emerging field of gene and cell therapy. Therefore, this fellowship will contribute to consolidating and raise the visibility of European expertise on this area amongst the global scientific community.

**B5.6 Impact of the proposed outreach activities**

The final objective of this proposal is the improvement of new safety strategies for gene therapy of patients with Wiskott - Aldrich syndrome, so is an essential factor, in the light of the principles of the “European Charter for Researchers” and “Code of Conduct” for the recruitment of researchers, that the research activities are made known to society at large in such a way that can be understood by public in general. This direct engagement will help us to better understand public interest in priorities for science and technology and also the public’s concerns. Scientific outreach, which promotes awareness and an appreciation of current research, has become an essential task for the research community and for many scientists.

The candidate will communicate the research outcomes through the UCL press office to see if any published outputs may generate media interest in outreach journals and local newspapers. Also the direct contact with students is an excellent form of learning from undergraduates, and might motivate them to embrace research careers in the future. The fellow will actively participate in the activities proposed by the UCL Outreach Office to link the research community to the public at large. In addition, the Marie Curie project runs several outreach activities which the fellow wishes to participate on the Marie Curie Ambassador, where Marie Curie fellows visit schools, universities and related institutions to promote research. As ‘Ambassador’ the fellow will assist teachers in preparing and delivering teaching materials. Finally the fellow will participate in the Marie Curie Workshop Day and the Marie Curie Open Day to raise scientific awareness within school and university students and the general public.
B6 Ethical Issues

The current project will be carried out in Spain, in compliance with fundamental ethical principals laid down in the European Carter of Fundamental Rights in the Seven Framework Programme (Decision 1982/2006/EC).

DISCLOSURE: This proposal has not any Conflict of Interest.

USE OF ANIMALS

To date, unfortunately, there is no human model for the study of Wiskott-Aldrich syndrome, so WAS KO mice are very useful in order to evaluate the safety and efficacy of the lentiviral vectors used for gene therapy of this disease.

The proposed research application involves the use of knockout transgenic mice (Wiskott-Aldrich mouse model disease) for scientific procedures. They are necessary to analyze in vitro and in vivo the capacity of restoration of WAS protein expression by the new lentiviral vectors. We will use 6 to 8 mice per experiment, no more than 80 in total for the project. Throughout the project, mice will be monitored by an expert veterinary and the “three Rs” policy of Refinement, Reduction and Replacement towards the use of animals for scientific procedures (99/167/EC: Council Decision of 25/1/99) will be adopted.

All the animal procedures will be approved by the corresponding ethical committees and will adhere to the national and international laws and provisions regarding the protection of animals. In particular, all animal experiments will be performed by authorized personnel under the rules of each given country according to EC Directive 86/609. The animal studies will be carried out under strict containment, in facilities that meet legal requirements and by qualified personnel in order to minimize the potential pain and/or distress in the animals. The Molecular Immunology Unit of UCL Institute of Child Health MIU has an in-house expert for advice with animal licensing and procedures as well as dedicated staff for this purpose. In addition, the proposed research will be continuously supervised in order to follow properly any ethical issues. All partners performing work which envisages the use of animals hold animal licences and adhere to the national regulations on animal experiments. After the research experiments the animals will be euthanized.

USE OF HUMAN EMBRYONIC STEM CELLS (hESCs)

THE PROPOSAL DOES NOT INCLUDE RESEARCH ACTIVITIES WHICH DESTROY EMBRYOS INCLUDING FOR THE PROCUREMENT OF STEM CELLS

As I described before, there is no human model available to analyze the safety of the therapeutic lentiviral vectors for gene therapy of WAS before going into clinic. Ongoing clinical trials apply genetically modified HSCs obtained from WAS patient. For this reason, HSCs would be the ideal cellular model for the study of the efficiency and biosecurity of the LVs. However, HSCs from WAS patients are difficult to obtain (it is a rare disease and the patients are predominantly children), and do not survive for a long time in culture.

The use of a human WAS cellular model (hESC WAS KO) to study efficacy and safety:

To address this problem we propose to use WASP deficient hESC lines (hESC WAS KO) obtained by Dr. Francisco Martín’s group. They represent the first human model that allows the study of WASP function in lineages difficult to obtain from patients samples. The use of this novel human model to test potential therapeutic vectors will greatly assist the analysis of vector performance in terms of correction of cellular phenotype and also biosafety.

The project will involve hESC WAS KO cell lines obtained by was gene editing with Zinc-Finger nuclease of H9 (WiCell, Wisconsin, USA) and AND-1 (derived in Spain and deposited in the National Bank of Embryonic Stem Cells) hESCs lines.
For all these cell lines we have the approval from the Autonomic and Central Government and this line of research is within the Spanish Biomedical Research Portfolio and constitutes a clear strategy being promoted by the Government.

The relevant authorizations by Regional and National Ethical Committees required for the development of the project will be provided at the time of contract signature if this project is approved.

**Related legislation and regulation (EU)**

- **COUNCIL DIRECTIVE** of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the **protection of animals used for experimental and other scientific purposes**.
- **COMMISSION RECOMMENDATION** of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes.
- **COUNCIL DECISION** of 23 March 1998 concerning the conclusion by the Community of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes.
- **COUNCIL DECISION** of 22 July 2003 concerning the conclusion of the Protocol of Amendment to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes.
### B6.1 ETHICAL ISSUES TABLE

(Note: Research involving activities marked with an asterisk * in the left column in the table below will be referred automatically to Ethical Review)

#### Research on Human Embryo/ Foetus

<table>
<thead>
<tr>
<th>Activity</th>
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<tr>
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<tr>
<td>* Does the proposed research involve human Foetal Tissues/ Cells?</td>
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#### Research on Humans

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<td>* Does the proposed research involve patients?</td>
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<td>* Does the proposed research involve persons not able to give consent?</td>
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<td>* Does the proposed research involve adult healthy volunteers?</td>
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#### Privacy

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<tr>
<td>Does the proposed research involve processing of genetic information or personal data (e.g. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?</td>
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<td>Does the proposed research involve tracking the location or observation of people?</td>
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#### Research on Animals

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<tr>
<td>Are those animals transgenic small laboratory animals?</td>
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<tr>
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<td>Are those animals cloned farm animals?</td>
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#### Research Involving Developing Countries

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<tr>
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<td>Is the proposed research of benefit to local communities (e.g. capacity building, access to healthcare, education, etc)?</td>
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#### Dual Use

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<td>Research having the potential for terrorist abuse</td>
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“WASHSCGENETHERAPY”

“Preclinical studies in mouse Hematopoietic Stem Cells for GENE THERAPY of Wiskott-Aldrich Syndrome”