Istituto Superiore di Sanità

MARIE CURIE FELLOWSHIP HOSTING AGREEMENT

BETWEEN:

(1) ISTITUTO SUPERIORE DI SANITA' - whose registered office and place is at Viale Regina Elena, 299, 00161 Roma, Italy hereby represented by Professor Enrico Garaci, legal representative (**"Beneficiary"**); and

(2) IOR Istituto Oncologico di Ricerca - Fondazione per la Ricerca e la Cura dei Linfomi, Ticino whose registered office and place is at Via Vincenzo Vela 6, 6500 Bellinzona, Switzerland, hereby represented by Prof. Dr. med. Franco Cavalli, (**"Host Institution"**).

WHEREAS:

ISS have signed with the REA, in response to Call FP7-PEOPLE-COFUND 2008" a Grant Agreement No 246549, hereinafter referred as the "Grant Agreement", for the implementation of the Programme called "Training through Research Application Italian iNitiative - TRAIN"

In the context of the mentioned programme, Dr Andrea Alimonti submitted the project entitled **"Relevance of PTEN loss induced cellular senescence on tumor-induced immunotolerance in prostate cancer"** to be implemented at **IOR Istituto Oncologico di Ricerca - Fondazione per la Cura e la Ricerca dei Linfomi, Ticino** (Host Institution) that has been selected for funding.

NOW, THEREFORE, IT IS HEREBY AGREED AS FOLLOWS:

Section 1: Definitions

1.1.Definitions

"Access Rights"	means licences and user rights to Foreground or Background;
"Agreement"	this strategic partnership agreement including its annexes between the Beneficiary and the Host Institution for the purpose of the Research Training Activities to be provided to the Researcher during the Secondment Period of the Project.
"Background"	means IPR or information or know-how other than Foreground which belongs to or is accessible to a Party and is Needed for carrying out the

Project or for using Foreground;

"Confidential Information" any data, documents or other material that is identified as 'confidential' by the disclosing Party;

"Effective Date" the date on which this Agreement comes into force, which is the date of the Marie Curie Fellowship Hosting Agreement signature.

"Foreground" the results, including information, whether or not they can be protected by means of IPR, which are generated under the Project;

"Grant Agreement" the grant awarded by the European Commission to the Beneficiary for carrying out the Project, pursuant to the Seventh Framework Marie Curie Programme of the European Community - COFUND, project called "Training trough Research Application Italian iNitiative TRAIN2009 – grant agreement n. 246549 and all the terms and conditions of said grant agreement;

"IPR" any intellectual property rights of any description including but not limited to patents, copyrights, design rights (registered or unregistered), trademarks, know-how and database rights, trade secrets, and any applications for the same anywhere in the world;

"Jointly Generated Foreground" Foreground developed jointly by at least one employee from each of the Parties in circumstances where the respective contributions of the Parties are bound together such that under applicable law it is not possible to separate them for the purpose of applying for, obtaining and/or maintaining and/or owning the relevant IPR protecting or available to protect such Foreground for purposes of IPR protection;

"Needed" in the case of Access Rights for implementation of the Project that without the grant of such Access Rights, carrying out the tasks assigned to the recipient Party would be impossible, significantly delayed, or require significant additional financial or human resources; and in the case of Access Rights for *use*, that without the grant of such Access Rights the *use* would be technically or legally impossible;

"Party" Beneficiary or Host Institution, and **"Parties"** shall be construed accordingly;

"Personal Career Development Plan" a plan established by the Researcher, together with the Scientist in Charge, indicating his training needs (including complementary skills) and scientific objectives as well as the foreseen measures to meet these objectives and a description of his Research Training Activities;

"Project" the 12 month project entitled **"Relevance of PTEN loss induced cellular senescence on tumor-induced immunotolerance in prostate cancer"** as further specified in Schedule 1 of this Agreement, including

the Research Training Activities;

"Research Activities" Training the activities related to the research training and career development to be provided to the Researcher under the Project;

"Researcher" Dr.ssa Diletta Di Mitri

"Scientist in Charge" The scientist in overall charge of supervision of the Researcher during the Project, namely Dr Andrea Alimonti of the Host Institution.

"Secondment Period" means the months of the Project, which is the period spent by the Researcher at the Host Institution's premises for the purposes of the Research Training Activities.

Section 2: Purpose

The purpose of this Agreement is to specify with respect to the Project the relationship between the Parties concerning the organisation of the Research Training Activities during the Secondment Period.

Section 3: Entry into force and term

This Agreement shall have effect from the Effective Date and shall continue in full force and effect until the completion of the Project, unless terminated earlier in accordance with clause 12.

Section 4: Responsibilities of Parties

4.1 Each Party undertakes to take part in the efficient implementation of the Project, the Research Training Activities, and to cooperate, perform and fulfil, promptly and on time, all of its obligations under this Agreement.

4.2 Each Party undertakes to notify the other promptly of any significant information, fact, problem or delay affecting or likely to affect the Project or delay its implementation.

4.3 Each Party shall use reasonable endeavours to ensure that the Researcher devotes himself full time and continuously to the pursuit of the Project;

4.4 Each Party shall abide by all applicable local laws and regulations in relation to the carrying out of the Project and to abide by fundamental ethical principles.

Section 5: Liability towards each other

5.1 No warranties

Each Party shall take reasonable measures to ensure the accuracy of any information or materials it supplies to the other Party, and to correct any errors of which it is notified.

The recipient Party shall in all cases be entirely and solely liable for the use to which it puts such information and materials.

5.2 Limitations of liability

The liability of either Party to the other for any breach of this Agreement, any negligence or arising in any other way out of the subject matter of this Agreement and the Project will not extend to any indirect damages or losses, or any loss of profits, loss of revenue, loss of data, loss of contracts or opportunity, whether direct or indirect, even if the Party bringing the claim has advised the other of the possibility of those losses, or if they were within the other Party's contemplation.

5.3 Damage caused to third parties

Each Party shall be solely liable for any loss, damage or injury to third parties resulting from the performance of the said Party's obligations under this Agreement, except to the extent that such loss, damage or injury results from the negligence of the other Party.

Section 6: Secondment provisions

6.1 During the Secondment Period the Host Institution shall conclude a fellowship agreement for a duration of the project and covering the duration of the research training activities in accordance to the provision of the Grant Agreement including its Annexes in particular Annex III of the Grant Agreement. For this period, the Researcher shall be a Marie Curie Fellow with an employment contract with the Host Institution. Compatible with Host Institution's national legislation. In all cases, the Host Institution must ensure that the Researcher is covered under the social security scheme which is applied to Host Institution workers. The Researcher employment contract terms and conditions will remain unchanged with the exception of those matters set out below:

6.1.1 The Researcher will not be required to undertake any work for the Beneficiary during the Secondment Period.

6.1.2 The hours of work during the Secondment Period will be the normal working days and hours of the Host Institution. To determinate the actual hours worked in the project the Researcher should fill out every month the timesheet duly signed by Scientist in charge of the Host Institution.

6.1.3 The Researcher will be expected to adhere to the Host Institution's policies and procedures during the Secondment Period.

6.2 The Beneficiary shall reimburse to Host Institution only the expenses that are directly connected with the execution of the Secondment Period in accordance to the following budget that shall be considered as a maximum amount reimbursable, and the financial provision indicated in the Grant Agreement. Payments shall be execute only if the Host Institution has fulfilled all his contractual obligations.

Table 1 - Cost Breakdown per fellowship

Cost categories	
Living allowance (EUR/year)	€ 36.000,00
Travel/Mobility (EUR/year)	€ 1.200,00
Research cost contribution (EUR/year)	€ 4.800,00

At the end of each six month of the Fellowship Agreement, the Hosting Institution shall submit to the Beneficiary a formal request of payment accompanied by those of the following documents:
⇨ a copy of the six month technical report relative to the Researcher Training Activities;
⇨ a six month detailed statements of reimbursable expenses, included all supporting documents as timesheets, invoices, receipts and used tickets for travel necessary to perform duties related to the Researcher's research activities.

All expenses shall always be reported in EUR with accounts in currencies other than EUR on the basis of the exchange rate that would have applied either:

* on the date that the actual costs were incurred or
* on the basis of the rate applicable on the first day of the month following the end of the reporting period.

For both options, the daily exchange rates are fixed by the European Central Bank (ECB) and may be obtained at the following internet address: http://www.ecb.int/stats/eurofxref/ or, for the rate of the first day of the month following the reporting period, in the relevant OJ of the European Union.

6.3 The payments will be made by Beneficiary to Hosting Institution within 30 days after the Beneficiary receives the request for payment of the instalment, together with the documents referred to in the point 6.2 and upon acceptance of the documents. The payments will be made in EURO.

6.4 The Host Institution will provide reasonable assistance to the Researcher in obtaining visas or other work permissions from the relevant authorities for the purposes of the secondment.

6.5 The Host Institution shall have throughout the duration of the Secondment Period the necessary means, including the infrastructure, equipment and products to enable the Researcher to carry out the Project and the Host Institution shall make available to the Researcher access to its facilities, an adequate work station and other means necessary to enable the Researcher to carry out the Project during the Secondment Period.

6.6 The Host Institution shall ensure the supervision of the Scientific in charge, and in a way that enables the achievement of the Personal Career Development Plan.

6.7 The Host Institution shall notify the Beneficiary of any leave of absence taken by the Researcher, such as through annual leave or sickness.

6.8 Should the Researcher be charged with any criminal offence, whether connected with the secondment or not, to the knowledge of the Host Institution, or should the Researcher

be the subject of any disciplinary proceedings with the Host Institution, these must be reported immediately to the Beneficiary.

Section 7: Reporting and audits

7.1 The European Commission may require an audit and review may be carried out remotely at the Researcher's place of work or involve sessions with Project representatives either at the Commission premises or at the premises of Host Institution. The Host Institution shall provide reasonable access by prior arrangement to the European Commission or its external scientific or technological expert to the locations and premises where the work is being carried out, and to any document concerning the work.

7.2 The Host Institution shall make available directly to the Commission all detailed information and data that may be requested by it or the external scientific or technological expert with a view to verifying that the Project is being/has been properly implemented and performed in accordance with this Agreement.

Section 8: IPR

8.1 This Agreement does not affect the ownership of any IPR in any Background. Such IPR will remain the property of the Party that contributes them to the Project (or its licensors). No licence to use any IPR is granted or implied by this Agreement except the rights expressly granted in this Agreement.

8.2 For the avoidance of doubt, Foreground shall be owned by the Party who carried out the work generating the Foreground, or on whose behalf such work was carried out. Foreground generated by the Researcher during the Secondment Period shall belong to the Beneficiary.

8.3 Unless agreed otherwise by the Parties in writing, the Parties shall jointly own Jointly Generated Foreground. In such case the Parties shall agree at their sole discretion in a separate written agreement on all protection measures and the division of related costs in advance of protection of Jointly Generated Foreground. Within a reasonable period following creation of any Jointly Generated Foreground, the Parties shall enter into good faith discussions in order to agree on an appropriate course of action for filing applications for patent protection or other protection, including the decision as to which Party is to be entrusted with the preparation, filing and prosecution of such applications and in which countries or territories such applications are to be filed. Except for any priority applications, the filing of any applications for patents or other IPR and the decision as to which territories to file Jointly Generated Foreground shall require mutual agreement between the Parties. Each Party shall have the perpetual and irrevocable right, without territorial or other restriction, to use the Jointly Generated Foreground and resulting patents, patent applications and other IPR protecting such Foreground, and to grant non-exclusive licences to third parties under the Jointly Generated Foreground and under any IPR protecting such Foreground providing that:

8.3.1 at least 45 days prior notice must be given to the other joint owner, within which time the other joint owner shall notify the licensor whether its legitimate interests are or may be unduly prejudiced by such license; and

8.3.2 fair and reasonable compensation as well as appropriate indemnification must be provided to the other joint owner.

8.4 Where free and reasonably able to do so each Party grants the other a royalty-free, non-exclusive licence to use its Background where Needed for the purpose of carrying out the Project, but for no other purpose. Neither Party may grant any sub-licence to use the other's Background for the purposes of this licence.

8.5 Each Party shall act in good faith to consider granting the other a non-exclusive licence to use its Background or Foreground where Needed for the purpose of using or commercially exploiting the grantee Party's Foreground but for no other purpose. Neither Party may grant any sub-licence to use the other's Background. Such licence shall be on fair and reasonable commercial conditions to be agreed in writing and in good faith.

Section 9: Dissemination and publication of *foreground*

9.1 Each Party shall ensure that its Foreground is disseminated as swiftly as possible in a way that is compatible with the protection of IPR and its confidentiality obligations and the legitimate interests of the owner(s) of the relevant Foreground.

9.2 The Beneficiary must under the Grant Agreement compile a report on dissemination activities and to this end the Host Institution agrees to report promptly on request to the Beneficiary any and all dissemination activities. With regard to scientific publications relating to Foreground such details/references and an abstract of the publication must be provided by the Host Institution within thirty (30) days following publication, including an electronic copy of the published version or the final manuscript accepted for publication

9.3 For the avoidance of doubt, a Party may not publish Foreground or Background of another Party, even if such Foreground or Background is amalgamated with the Party's Foreground, without the other Party's prior written approval. However, in no case shall publication of a Party's own Foreground be delayed for a period of longer than sixty (60) days from date of first submission of the proposed publication to the other Party.

9.4 Nothing in this Agreement shall be construed as conferring rights to use in advertising, publicity or otherwise the name of the Parties or any of their logos or trademarks without their prior written approval.

Section 10 Non-disclosure of information

10.1 During the Project and for a period of three years after its completion, each Party undertakes to preserve the confidentiality of any Confidential Information, which must be disclosed as confidential at the time of disclosure, or, where Confidential Information was communicated orally, its confidential character must be disclosed at the time of initial disclosure and confirmed by the disclosing Party in writing within 15 days after disclosure as "Confidential Information".

10.2 Clause 10.1 shall not apply to any information which:

- is or becomes publicly available by means other than a breach of confidentiality obligations or is independently developed by the receiving Party;
- the disclosing Party subsequently informs the recipient that the Confidential Information is no longer confidential;
- the Confidential Information is subsequently communicated to the recipient without any obligation of confidence by a third party who is in lawful possession thereof and under no obligation of confidentiality;
- the disclosure or communication of confidential information is required by the national law of the recipient or required under the Grant Agreement.

10.3 A recipient of Confidential Information undertakes to use such confidential information only in relation to the execution of the Project unless otherwise agreed with the disclosing Party.

Section 11: Termination

11.1 In event of termination of the Grant Agreement this Agreement shall be deemed to have terminated and the Parties shall work together in good faith to bring about an orderly termination of arrangements already in place. In the event that this Agreement terminates for whatever reason, the Researcher will return to their employment with the Beneficiary.

11.2 In the event of termination of this Agreement the Host Institution will return any unspent monies from the Research Expenses Fund to the Beneficiary without delay.

11.3 Without prejudice to its other rights or remedies a Party may terminate this Agreement with immediate effect by giving notice to the other Party if:

11.3.1 the other Party is in breach of any provision of this Agreement and (if it is capable of remedy) the breach has not been remedied within 30 days after receipt of written notice specifying the breach and requiring its remedy; or

11.3.2 the other Party becomes insolvent, or if an order is made or a resolution is passed for its winding up (except voluntarily for the purpose of solvent amalgamation or reconstruction), or if an administrator, administrative receiver or receiver is appointed over the whole or any part of the other Party's assets, or if the other Party makes any arrangement with its creditors.

11.4 The provisions of Sections 5, 7, 8, 9, 10, 11 and 12 shall survive the expiration or termination of this Agreement. Termination shall not affect any rights or obligations of a Party incurred prior to the date of termination.

Section 12: Miscellaneous

12.1 Inconsistencies and severability

In case this Agreement is in conflict with the Grant Agreement, the terms of the latter shall prevail.

Should any provision of this Agreement become invalid, illegal or unenforceable, it shall not affect the validity of the remaining provisions of this Agreement. In such a case, the Parties shall negotiate a valid and practicable provision which fulfils the purpose of the original provision.

12.2 No representation, partnership or agency

No Party shall be entitled to act or to make legally binding declarations on behalf of any other Party, except that the Beneficiary shall be entitled where required under the Grant Agreement to make representations on behalf of the Parties. Nothing in this Agreement shall be deemed to constitute a joint venture, agency, partnership, interest grouping or any other kind of formal business grouping or entity between the Parties.

12.3 Notices

Any formal notice, consent or approval shall be signed by an authorised representative of a Party and shall either be served personally or sent by mail with recorded delivery notice to be given under this Agreement, and such notices shall be in writing to the addresses and recipients listed below:

12.4 Assignment and amendments

No rights or obligations of the Parties arising from this Agreement may be assigned or transferred, in whole or in part, to any third party without the other Party's prior formal approval.

Amendments and modifications to the terms and conditions of this Agreement require agreement in writing of the authorised signatories of the Parties.

12.5 Mandatory statutory law

Nothing in this Agreement shall be deemed to require a Party to breach any mandatory statutory law under which the Party is operating.

12.6 Force Majeure

If the performance by either Party of any of its obligations under this Agreement is delayed or prevented by circumstances beyond its reasonable control, that Party will not be in breach of this Agreement because of that delay in performance. However, if the delay in performance is more than 3 months, the other Party may terminate this Agreement with immediate effect by giving written notice.

12.7 Rights against the European Commission

The Host Institution agrees that it has no rights against the European Commission under the Grant Agreement, and expressly waives any such rights as it may have by law.

12.8 Applicable law and Compent Court.
This Partnership Agreement shall be governed and interpreted under the terms of the Grant Agreement, by the European Union Law, by and, on a subsidiary base, by the Italian law.
In case of dispute, the competent Court shall be exclusively the Court of Milan Italy

Signatures

AS WITNESS:

The Parties have caused this Agreement to be duly signed by the undersigned authorised representatives in two or more counterparts the day and year first above written.

SIGNED for and on behalf of the
ISTITUTO SUPERIORE DI SANITA'

Name: Prof. Enrico GARACI

Position: President

Signature

SIGNED for and on behalf of
IOR Istituto Oncologico di Ricerca - Fondazione per la Ricerca e la Cura dei Linfomi, Ticino

Name: Prof. Dr. med. Franco Cavalli

Position: President

Signature

SIGNED for and on behalf of
IOR Istituto Oncologico di Ricerca - Fondazione per la Ricerca e la Cura dei Linfomi, Ticino

Name: Andrea Alimonti, MD

Position: Group Leader Molecular Oncology

Signature

Schedule 1

Project

STARTPAGE

TRAIN

(Co-funded by Marie Curie Actions)

Outgoing Grants (OG)
Call: "TRAIN-001"

PART B

"Cancer senescence"

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Relevance of PTEN loss induced cellular senescence on tumor-induced immunotolerance in prostate cancer

Abstract

Cellular senescence is a terminal arrest of cell growth that is observed in response to various insults including an abnormally high activity of oncogenes (2, 5). Recent papers have demonstrated the relevance of cellular senescence in opposing tumorigenesis *in vivo*, opening up new potential opportunities for cancer treatment (1, 2, 3). *Pten*-loss Induced Cellular Senescence (PICS) is a novel type of cellular senescence response, which significantly differs from replicative senescence and oncogene-induced senescence (OIS) (4). These differences are characterized by lack of a DNA damage checkpoint response (DDR) and hyper-replication, breaking the current dogma for senescence induction (4). In addition, PICS can also be triggered in non-proliferating cells (4). The ability to induce senescence without a requirement for hyper-replication and DNA damage opens up the possibility of targeting, quiescent cells including quiescent cancer initiating cells (qCICS) for cancer therapy. A significant recent advance in the senescence field has been the demonstration that senescence cancer cells secrete growth factors and inflammatory cytokines that influence the tumor microenvironment, a process known as senescence-associated secretory phenotype (SASP). While in certain models, the SASP of a tumor is essential to sustain senescence (11) and promote tumor clearance (8) in others it drives tumor development and aggressiveness (10). Aim of the current application is to characterize the SASP of PICS and its role in the tumor immune response. Notably, we'll investigate whether the SASP *of Pten* null tumor, triggers premature senescence in the tumor infiltrating lymphocytes (TILs), quiescing the tumor immune response. Finally we will also assess *in vivo* whether compounds that enhance or block PICS can modify the PICS-associated secretory phenotype and whether combination of pro-senescence compounds with immunomodulators may be effective in restricting prostate cancer initiation and progression.

B1 SCIENTIFIC AND TECHNOLOGICAL QUALITY

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

Cellular senescence describes a terminal arrest of cell growth that is observed in response to various insults including an abnormally high activity of oncogenes (1, 5). Several papers have recently demonstrated the relevance of cellular senescence in opposing tumorigenesis *in vivo*, opening up new potential opportunities for cancer treatment (1, 2, 3). The hosting laboratory of this application has recently identified a novel type of cellular senescence response, which can be triggered by complete loss of the tumor suppressor *PTEN* (PICS) (4) and therefore offers a radical therapeutic approach to target cancer cells including quiescent cancer initiating cells (qCICs). This particular type of senescence response is mediated by mTOR- p53 up regulation, and is characterized by a lack of DNA damage response and hyper-replication.

Recent papers demonstrate that senescent tumor cells secrete growth factors and inflammatory cytokines in the tumour microenvironment, a process known as senescence-associated secretory phenotype (SASP) (9, 10). While in some models, the SASP of tumors is essential to reinforce cell cycle arrest and to help the immune system to restrict tumorigenesis (8) in other models, the release of pro-inflammatory cytokines can exert harmful effects on the tumour microenvironment, promoting tumour progression and invasion (10). Preliminary data from the host laboratory of this fellowship indicate that *PICS* drives the release of pro-inflammatory cytokines that regulate the immune response in the *Pten* null tumor microenvironment. PICS drives also the recruitment of both T and B lymphocytes in the tumor proximity, in both the *Pten* heterozygous and *Pten* null prostate conditional mouse models (Fig.1). Main objective of the present proposal is to characterize the SASP of *Pten* deficient cells and prostate tumors, before and after treatment with enhancers or inhibitors of PICS. We will characterize the immune mediators that can drive tumor clearance promoting a beneficial peri-tumoral immune response *in vivo*. In addition we aim at exploring the dark side of PICS, by focusing our attention on the immunomodulatory mechanisms that may create tolerance against the tumor itself. In particular we will focus on the tumour release of TGFβ and on the recruitment in the tumor microenvironment of T regulatory (Treg) lymphocytes and of CD4+Foxp3+CD103+ Treg cells (12, 13). As previously shown in animal models and prostate cancer patients, the recruitment of CD4+ Treg lymphocytes in the microenvironment suppress the effector function of tumor antigen-specific T lymphocytes and plays a prominent role in tumor-associated immunosuppression (14, 15). We will consequently investigate the release of TGFβ in *Pten* null MEFs and *Pten* null tumours before and after treatment that increases or block PICS (4) and we will try to interfere with the TGFβ release.

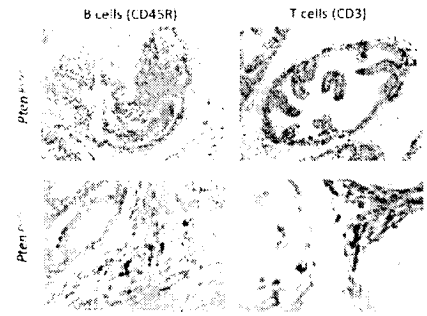

Fig1. TILs in *Pten*^{wt} and *Pten*$^{pc-/-}$ tumors

A Final and essential objective of this proposal is to evaluate whether stress signals that mediate p38 activation in TILs such as certain types of cytokines and reactive oxygen species (ROS) may induce premature senescence of the T lymphocytes, thus altering their effectors functions and survival (16). As the releases of cytokines and ROS are known to be closely related to senescence tumors, the demonstration that PICS drive premature senescence in TILs of *Pten* null tumors will be extremely relevant and provocative.

The present proposal will give us the opportunity to conjugate the most advanced findings in the field of cancer research with an immunological approach. Moreover it will allow us to identify potential entry points for the designs of pre-clinical trial assessing the efficacy of the combination of pro-senescence compounds with immunotherapy. We believe that this combinatorial approach may have a terrific effect on the inhibition of cancer initiation and progression and may represent a promising therapeutic strategy.

References

1. Chen, Z., *et al.* Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature* **436**, 725-730 (2005).
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B1.2 Research methodology

Aim of the current proposal is to explore the role of the senescence-associated secretary phenotype in PICS and to establish whether it is either beneficial or harmful for prostate tumorigenesis. Therefore we will focus on those immunomodulators that can promote or block the PICS immune response with the aim to identify possible entry points for the development of combination of pro-senescence compounds with immunomodulators.

AIM 1 To evaluate whether PICS triggers a tumour immune response. Our aim is to evaluate whether the SASP, in PICS, induces the recruitment, activation and differentiation of TILs *in vivo*. In vitro analysis of inflammatory cytokines release and adhesion molecules expression in both *Pten* null mouse embryonic fibroblasts (MEFs) and *Pten* null prostate tumors ($Pten^{pc-/-}$) will be performed both before and after treatments with compounds that enhance PICS (e.i. Nutlin-3 and VO-OHpic). Cytokines considered determinant for T lymphocytes activation and polarization, such as IL6, IL1ß, IL2 and TGFß will be analyzed. We will then monitor the expression of adhesion molecules that belong to the family of the integrins and selectins, known to be determinant for the migration of cells from both the innate and the adaptive immune response. The obtained data will be correlated to the analysis of markers considered specific of the senescence response in tumors and with the percentage of T lymphocyte subsets.

A) *Pten* Lox/Lox and Lox/+ MEFs will be infected with either a retroviral construct expressing pIRES-GFP- Cre or control. After infection, MEFs will be selected for two days in the presence

of puromycin and seeded in six well dishes. Eight hours after the seeding, compounds that potentiate cell senescence such as Nutlin-3 and VO-OHpic, will be administered. After 24 hours, supenatants will be collected and analyzed by ELISA in order to check the cytokines release. In a separate experiment, brefeldin will be added to the colture and the cells will be collected, stained for specific surface markers and then fixed and permeabilized in order to perform an intracellular staining for the cytokines of interest.
Also the expression of adhesion molecules on the MEFs cells before and after treatment will be assessed by multicolour flow cytometry.

B) At least three *Pten* null tumors from control mice and mice treated with PICS enhancers (e.i. Nutlin- 3) will be collected. Tumour and mononuclear cells will be isolated from cancer tissues in vivo. T lymphocytes subsets will be identified by flow cytometry by using αCD3, αCD4, αCD8, αCD56, αVd2/Vg9 MoAb. MoAb such as αCD69, αCD45RO, αCD45RA, αCD27, CD28 will be utilized to monitor T lymphocytes differentiation; the release of IFNγ and IL17 will be check by flow cytometry upon stimulation with PMA and ionomycin. Granzyme B and perforin expression will be analyzed in order to evaluate the cells effector functions ex vivo. The expression of adhesion molecules on the surface of isolated tumor cells will be assessed by multicolour flow cytometry.
Finally the lymphocytes subsets will be sorted basing on the expression of surface markers and plated with tumor cells isolated from the cancer tissue in order to perform a test of cytotoxicity by flow cytometry.

AIM 2 To assess whether the SASP of PICS triggers immunotolerance. We will evaluate whether PICS alters the release of immunomodulatory cytokines, such as TGFbeta and IL10 from Pten null cells in vitro and in vivo. Moreover we will assess *in vivo* the tumor recruitment of immunosuppressive cell subsets, such as CD4+CD25+Foxp3+ regulatory cells and myeloid suppressor cells, before and after treatment with Nutlin-3. The release of TGFβ from senescence cancer cells will be correlated with the frequency of regulatory T cells found in the proximity of the tumor microenvironment.

A) At least three *Pten* null tumors from control mice and mice treated with PICS enhancer (Nutlin-3) will be collected as mentioned before. Tumour cells will be isolated from cancer tissues, cells will be lysed and RNA will be extracted and converted in cDNA. Quantitative RealTime PCR will be performed to analyze the modulation of TGFβ expression following PICS induction. In a separate experiment tumor cells *will be* isolated and put in colture for 24 hours. The supernatants will be collected and the release of TGFβ will be investigated by ELISA. Mononuclear cells from the same mice will be isolated and the presence of T regulatory cells will be identify by using αCD3, αCD4, αCD25, αFoxp3 and αCD103 MoAb. Also the frequency of myeloid suppressor cells in the proximity of the tumor site will be evaluated by flow cytometry, by using αCD11 and αGr1 antibodies.

B) We will confirm the effect of pro-senescence compounds on TGFβ release on human prostate cancer cell lines. Human prostate cancer cells will be cultures in absence or presence of PICS enhancing treatments such as Nutlin-3 and VO-OHpic. After 24 hours the supernatants will be collected and the release of TGFβ will be investigated by ELISA. In a separate experiment the cells will be treated with brefeldin and then stained in order to check the expression of TGFβ by flow cytometry.

AIM 3 To evaluate whether PICS can induce premature senescence in TILs. Immunosenescence refers to the gradual deterioration of the immune system brought on by natural age advancement. T lymphocytes can also undergo premature senescence under stress conditions that activate the p38 MAP kinase signalling pathway (16). Our idea is that *Pten* null senescence tumor cells can induce premature immune senescence in the tumor microenvironment by secreting cytokines and enhancing the accumulation of ROS.

A) Three *Pten* null tumors from control mice and mice treated with PICS enhancer (e.i. Nutlin- 3)

will be collected as described before. Mononuclear cells will be isolated from cancer tissues in vivo and CD4+ and CD8+ T lymphocytes subsets will separated by cell sorting. Markers of cell senescence such as KLRG1 and CD57 will be analyzed in the sorted cell subsets. Moreover lymphocytes will be stimulated with αCD3 and αCD28 and the telomerase activity will be evaluated by TRAP assay. Also the expression of γH2AX will be investigated by flow cytometry

AIM 4 To evaluate whether immunotherapy can improve the efficacy of pro-senescence compounds in *Pten*$^{pc-/-}$ tumors. We will make use of a preclinical mouse model to combine pro-senescence compounds with immunomodulatory treatments in the attempt to promote the clearance of prostate oncogenic cells. A cohort of *Pten*$^{pc-/-}$ mice will be enrolled to set up a preclinical trial aimed to assess the therapeutic efficacy of Nutlin-3 (at a dose of 100 mg/kg/day) in combination with anti-TGFβ treatment, at a standard dose. Also the combination of Nutlin-3 and anti-CD103 monoclonal antibody will be investigated. Treatments will begin once the mice reach 4 weeks of age, prior to puberty and the onset of PIN, and it will be stopped after 4 weeks of treatment. Tumor response, senescence and apoptosis induction will be evaluated by histological analysis and immunoassaying as previously described (4)

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

Prostate cancer is the most common cancer in men and the second cause of mortality in men in the develop countries. While androgen ablation therapy has gained a discrete success in the treatment of patients affected by the early stage of disease, there are not effective treatments for hormone-refractory prostate cancer patients. Therefore, it is fundamental to develop novel therapeutic strategies for this type of cancer.
Several evidences have established that induction of cellular senescence in cancer cells mediates growth arrest and propose that senescence induction can be utilized to oppose tumorigenesis. In addition, senescence cells can trigger a tumor immune response and even promote tumor clearance in certain tumor models. In this context the possibility to develop novel *senescence-inducing* therapies for cancer treatment has become concrete. Recently the host laboratory of this application has characterize a novel type of cellular senescence identified in response to the complete loss of the tumor suppressor *PTEN,* termed *Pten*-loss Induced Cellular Senescence (PICS). This particular type of senescence came out to be uniquely distinctive from oncogenic induced senescence and replicative senescence. Due to the lack of DNA damage and hyper-replication, in fact, PICS appears to be less "harmful" than other form of OIS and can be used for cancer therapy. The efficacy of this approach has already been proven by several preclinical trials aimed to test the efficacy of compounds that can potentiate PICS either at advance stage of disease. The results of those trials show that novel pro-senescent compounds are effective in reducing tumor development and invasiveness, but haven't clarified whether the immune response associated to PICS induction plays a role in limiting the cancer progression *in vivo* or whether, on the other hand, the activation of the immune system may be harmful.
Several innovative immunotherapeutic approaches have been recently developed for the treatment of advanced prostate cancer. Indeed prostate cancer more than other types of tumors triggers a strong immune response and engages immunosuppressive mechanisms that can impair the reaction of tumor-reactive lymphocytes. The recruitment of regulatory cell subsets such as CD4+CD25+Foxp3+ T lymphocytes, mesenchymal stromal cells and macrophages is often observed in prostate cancers. Moreover TGFβ in the prostate cancer microenvironment has been shown to be determinant in the disease progression and invasiveness. A preclinical model of prostate tumor has recently revealed that the blockade of TGFβ signalling impairs cancer development. While those and other immunotherapeutic approaches are promising, none of these strategies have led to major clinical activity yet, thus suggesting that different approaches need to be used at the same time in the attempt to win the fight against prostate tumor progression.
The novelty of the present project relies on the idea to combine different therapeutic strategies in order to inhibit cancer progression and to promote tumour clearance. Preliminary data from the host institution suggest that PICS promotes the recruitment of the immune system to the tumour site. Therefore main objective of this application is to study the contribution of this immune response in PICS and identify possible mechanisms that can blow up the tumor immunotolerance with the aim to improve the efficacy of

pro-senescence treatments. We strongly believe that combination of pro-senescence therapy with immunomodulators may have a tremendous effect on cancer inhibition.

B1.4 Timeliness and relevance of the project

Prostate cancer is the most common cancer in America, affecting 1 in 6 men. The majority of all prostate cancers are diagnosed in men over the age of 65, and after that age, the chance of developing prostate cancer becomes more common than any other cancer in men. The high incidence of this disease all over the world makes the development of efficient therapeutic strategies a social and economic necessity. The prior target is represented by the hormone-refractory metastatic form of prostate cancer, which causes extensive morbidity and cannot be treated with either chemotherapy or hormonal therapy. New treatments, among them vaccines and immunomodulating agents, are currently under investigation and development, but the gained survival improvements are still modest, and the limited efficacy of drugs in limiting the overall survival of patients affected by the advanced form of prostate cancer has a significant impact on the disease burden. Hence, there is a need for novel approaches directed at the identification of compounds to be used either alone or in combination with the existing therapies. In this context the present project represents a completely novel approach that deserves a go. Recent evidences have established the importance of cellular senescence in opposing tumorigenesis both *in vitro* and *in vivo* and the possibility to develop new *pro-senescence* therapies to limit cancer development and progression has become concrete. The host institution contributed to the characterization of a novel type of cell senescence, called *Pten*-loss Induced Cellular Senescence (PICS) that has already been shown to be effective in limiting tumor growth in a preclinical model of prostate cancer. The competences of Dr. Di Mitri, acquired during her PhD and the post-doc experience in London, will be critical for the development of this project in the host institution. In fact, her knowledge in basic immunology, particularly focused on immunotolerance and T regulatory cells characterization, will perfectly fit with the qualification in the field of cancer research of the host institution. Such complementary collaboration will permit to develop the present project that aims to combine an already promising pro-senescence therapy with immunomodulators, in the treatment of prostate cancer. At the same time Dr. Di Mitri will make use of the high technical and scientific qualification of the host institution, in order to improve her skills and to extend her scientific background to the cancer research. The experience in the host institution will let her to acquire new high-level knowledge that may reveals to be determinant for a future reintegration in the European country of origin.

B1.5 Host scientific expertise in the field and quality of the group/researchers in charge/supervisors

The Molecular Oncology lab at oncology Institute of Southern Switzerland (IOSI) is directed by Dr. A. Alimonti that has recently started his laboratory with the contribution of the prestigious ERC starting grant. Dr Alimonti moved to IOSI from Harvard Medical School (HMS) in 2009 where he was working in the laboratory of Prof. PP Pandolfi. Dr. Alimonti, has recently identified PICS as novel type of cellular senescence and has an extensive expertise in mouse modelling, especially in models of *Pten* driven tumorigenesis.
IOSI is the main cancer Institute of the Italian region of Switzerland and is conceived as a comprehensive cancer canter with both clinical and research facilities. Among the clinical research activities phase I/II/III trials in solid tumors and haematological malignancies are ongoing, with large national and international collaborations (SAKK and IELSG, SENDO, IBCSG, EORTC respectively). Importantly the laboratories of IOSI are located in the Institute for Research in Biomedicine, directed by Prof. A. Lanzavecchia which is a recognized authority in the field of immunology. The Institute therefore offers the best background for the successful realization of this proposal thanks to the many competences, facilities and interactions available to each scientist (cancer/ immunology). Beside an extensive knowledge in the cancer research and immunology field, a widespread expertise that ranges from cell biology to molecular biology, pharmacology, functional genomics and bioinformatics is available.
Dr. Andrea Alimonti, the supervisor of this project, began working in clinic as clinical researcher in 2002, when the therapeutic approach to patients was shifting from the clinic to the laboratory where therapies were being developed to antagonize specific genetic alterations in tumors. After his clinical experience he joined one of the most prestigious cancer labs in the U.S. (Prof. PP Pandolfi laboratory) and once there he was able to learn the principles of basic cancer genetics and how to generate mouse models. Over the following years, he was able to adapt these mouse models to directly study the effect of several novel drugs on tumors

generated in specific genetic backgrounds. During his post-doc in Pandolfi lab at HMS, Dr. Alimonti intensively studied the mechanisms that drive *Pten* induce cellular senescence (PICS) and characterized the mains downstream regulator of this pathway, identifying the possible "targetable" effectors of this response. Finally, he has proposed the concept of "pro-senescence" therapy for cancer and his results on pro-senescence therapy and on the relevance of Pten dosage for cancer development, appeared in several papers published in The Journal of Clinic investigation and Nature Genetics. Dr. Alimonti has also published several papers in Nature and Cell as contributing author in the field of Pten breast and prostate tumorigenesis. Dr. Alimonti has been recently awarded with the prestigious ERC starting grant and the Swiss Bridge Award. His extensive expertise in the field of cancer senescence will be a key point for the success of the present project and for the training of the participant.

B1.6 Potential of transferring knowledge to the host and/or bring knowledge to Europe

The participant was trained in Italy and then moved to UK for a post-doctoral experience. Her experience in a foreign country made her understand the importance of mobility between laboratories and geographical areas in order to improve a researcher's abilities and at the same time to transfer his/her knowledge to the host institution. During her training, the participant mainly focused on the field of immunology and immunotolerance. The participant's technical skills and her qualified preparation in the immunological field will be determinant for the development of this project. Dr. Diletta Di Mitri will in fact have the opportunity to transfer her experience and abilities to other researchers in the host institution. We believe that the combination of the participant's qualification in immunology and the wide expertise of the host in the cancer field will be the key of the success of the study.
In addition to this, we strongly believe that the combination of pro-senescence therapy with immunomodulators may potentiate the already promising results of senescence-inducing drugs, and may have a tremendous effect on cancer defeat. In case of positive results, the present project, led by Dr. Di Mitri, will raise the interest of the scientific community and will increase the level of scientific research in Europe.
Several evidences have recently established the importance of cellular senescence in opposing tumorigenesis both *in vitro* and *in vivo* and the possibility to develop new *senescence-inducing* therapies in order to combat cancer initiation and progression has now become concrete. Many U.S. institutes are in the process of starting large projects that aim to identify new compounds with "pro-senescence" properties. Therefore the development of a "pro-senescence" therapy approach in Europe offers great potential for improving EU competitiveness in the field of cancer treatment. Preclinical trials aimed to test the efficacy of pro-senescence compounds against cancer progression have revealed to be promising, thus giving to this field of research the ability to attract funding. This will surely help to increase the level of EU competitiveness in the field of cancer therapy and drug development.

B2 RESEARCHER

B2.1 Research experience

Dr. Di Mitri graduated in Bologna in the Ematology unit of The Sant'Orsola hospital, and got her degree working of hematopoietic stem cells. She was then trained for a PhD course in Neuroimmunology in the laboratory directed by Luca Battistini at the Santa Lucia Foundation (Rome). She was highly interested by the study of the regulation of immune responses and during her PhD she studied the involvement of human regulatory T cells in the pathogenesis of multiple sclerosis. In that period she got skilled in using the most advanced technologies and techniques, ranging from multicolour cytofluorimetric analysis to confocal microscopy and from molecular to cellular biology techniques. In March 2009 she started a post-doc experience at the University College London (UCL, London) in the Department of Pathology and Immunology directed by Arne Akbar. During these years she had the opportunity to study the role of senescence in human T lymphocytes. She was particularly interested to the study of p38 MAPkinase signalling and its implication in cell differentiation and senescence. She defined conditions modulating T cell aging and investigated a telomere-independent form of senescence in human T cells. Her findings contribute to new immunological knowledge that could open new perspectives in understanding lymphocytes functions in human inflammatory diseases and aging. Once back in Rome, Diletta focused her attention on the

phenotypical and functional characterization of terminally differentiated/senescent human T lymphocytes in patients affected by multiple sclerosis. At this stage of the carrier, Dr. Di Mitri wants to transfer all acquired knowledge to a different field of research, to develop a project focused on the combination of pro-senescence therapy with immunomodulators in the treatment of prostate cancer.

B2.2 Research result and scientific and technological quality of previous research

In 2005 Dr. Di Mitri won a fellowship at the Santa Lucia Foundation in Rome, and here she obtained her PhD degree in Neuroscience in 2008 working on human natural T regulatory cell. The result of this period is published in two papers that appeared in Blood (2007, 2009) and one paper as first author accepted by PlosOne (2011). These two publications clarify the role played by CD4+ regulatory cells in the pathogenesis of Multiple Sclerosis.
Soon after she moved to the University College London (UCL) to join the group of prof. Arne Akbar as a post-doctoral fellow. For 18 months she led a project on the study of senescence in T lymphocytes. The ensemble of this work is the subject of an article as first author that appeared in the Journal of Immunology (2011). This paper focuses on telomere-dependent and independent senescence in T lymphocytes, and opens new perspectives in understanding the mechanisms that lead to T cell aging and contribute to the senescence of the immune system in human.

Publications:

1 "Reversible Senescence in Human CD4+ CD45RA+CD27- Memory T Cell". J Immunology accepted (June 2011)
D. Di Mitri, R.I. Azevedo, S.M. Henson, V. Libri, N. Ridel , R. Macaulay, D. Kipling, M. V.D. Soares, L. Battistini, A. N. Akbar

2 "T regulatory cells are markers of disease activity in MS patients". Plos One, accepted (May 2011)
D. Di Mitri*, D. Dalla Libera*, A. Bergami, D. Centonze, C. Gasperini, M. G. Grasso, S. Galgani, V. Martinelli, G. Comi, G.Martino, G. Borsellino, F. Sallusto, L. Battistini, R. Furlan
*co-authorship

3 "Cytomegalovirus infection induces the accumulation of short-lived, multifunctional CD4(+) CD45RA(+) CD27(-) T cells: the potential involvement of interleukin-7 in this process." Immunology 2011.
Libri V, Azevedo RI, Jackson SE, Di Mitri D, Lachmann R, Fuhrmann S, Vukmanovic-Stejic M, Yong K, Battistini L, Kern F, Soares MV, Akbar AN.

4 "HIF1-positive and HIF1-negative glioblastoma cells compete in vitro but cooperate in tumor growth in vivo." Int J Oncol 2010.
Fiorenzo P, Mongiardi MP, Di Mitri D, Cozzolino M, Ferri A, Montano N, Trevisi G, Maira G, Battistini L, Falchetti ML, Levi A, Pallini R.

5 "Characterization of regulatory T cells identified as CD4(+)CD25(high)CD39(+) in patients with active tuberculosis." Clin Exp Immunol 2009.
Chiacchio T, Casetti R, Butera O, Vanini V, Carrara S, Girardi E, Di Mitri D, Battistini L, Martini F, Borsellino G, Goletti D.

6 "CD49d provides access to "untouched" human Foxp3+ Treg free of contaminating effector cells." Blood 2009.
Kleinewietfeld M, Starke M, Di Mitri D, Borsellino G, Battistini L, Rötzschke O, Falk K.

7 "Efficacy of a nanocochleate-encapsulated 3,5-diaryl-s-triazole derivative in a murine model of graft-versus-host disease." Trasplantation 2008.
Campo S, Arseni B, Rossi S, D'Alessio V, Lu R, Ngoje J, Ahl PL, Bonitz S, Mannino R, Di Mitri D, Battistini L, Carminati P, De Santis R, Ruggiero V.

8 "Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression." Blood 2007.
Borsellino G, Kleinewietfeld M, Di Mitri D, Sternjak A, Diamantini A, Giometto R, Höpner S, Centonze D, Bernardi G, Dell'Acqua ML, Rossini PM, Battistini L, Rötzschke O, Falk K.

Curriculum vitae

- Education and training

• DATES: From 11-2005 to 12-2007
• TITLE OF QUALIFICATION AWARDED: PhD in Neuroscience (Neuroimmunology)

• DATES: From 09-1999 to 07-2004
•TITLE OF QUALIFICATION AWARDED: Bachelor Degree cum Laude in Medical Biotecnology (University of Bologna)

- Languages

• MOTHER TONGUE: Italian
• OTHER LANGUAGES: English (fluent): very good writing and speaking skills, German: scholastic

- Courses

• IFCS (International Flow Cytometry School) Berlin, sept 27 – Oct 1 2005
• ESNI (European School for Neuroimmunology) Thessaloniki, Greece. September 12-15 2005

-Participation to conferences

ISNI (International Society of Immunology): ottobre 2008, Fort Worth (Texas); oral presentation
AINI XVIII (Italian association Neuroimmunology): ottobre 2008, Napoli (Italy); oral presentation
WIRM II (World Immune Regulation Meeting): aprile 2008, Davos (Switzerland); poster
AINI XVII (Italian association Neuroimmunology): Settembre 2007, Verona (Italy); oral presentation
5th SIICA (Italian Society of Immunology, Clinical Immunology and Allergology): Giugno 2007, Trieste (Italy); oral presentation
WIRM I (World Immune Regulation Meeting):: aprile 2007, Davos (Switzerland); poster
ISNI (International Society of Immunology): ottobre 2006, Nagoya (Japan); poster
AINI XVI (Italian association Neuroimmunology): ottobre 2006, Ostuni (Italy); oral presentation

B2.3 Independent thinking and leadership qualities

Since her degree in Medical Biothecnology, Dr. Di Mitri demonstrated initiative and independent thinking in the development of her research projects. During the first years of research she was involved in a project aimed to investigate the role of T regulatory cells in the pathogenesis of Multiple Sclerosis. During her PhD she contributed to the discovery of new marker for the characterization of this cell subset and she then made use of it to follow the frequency of the CD4+ regulatory T lymphocytes in patients affected by the Multiple Sclerosis. In order to perform this study she collaborated with Dr. Olaf Rotsche in Germany and many Italian groups working on the same field.
After her PhD, she moved to work in London where she covered a post-doctoral position. She managed her project on human T cell differentiation and senescence by using a multiple techniques. She obtained interesting results, collaborating with colleagues of the Pathology and Immunology Department at the UCL. She also established connections with scientific experts research teams. In particular she collaborated with

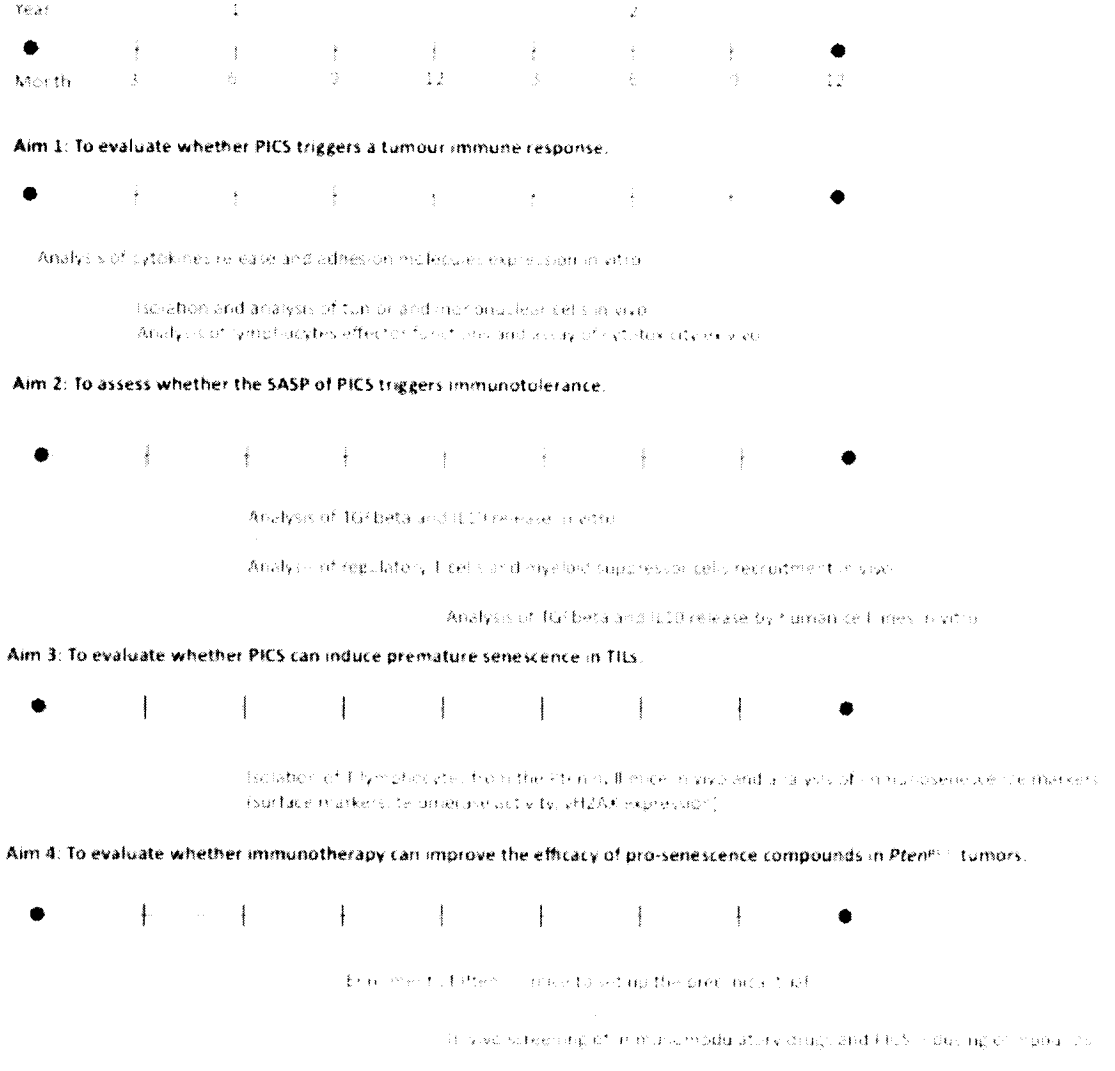
David Kipling. In light of this experience, the international and high-level environment of the UCL allowed Diletta to establish interesting collaborations with several high-specialized groups in immunology increasing the level of independence and educational training. Furthermore, she was involved in the teaching and training of a PhD student and in the collaboration with technicians. During all these years she always demonstrated a great interest in improving her immunological knowledge attending international conferences and participating in scientific meetings.

B2.4 Match between the fellow's profile and project

The most important results that Dr. Di Mitri obtained during her PhD training concern the study of human CD4+CD25highFoxp3+ regulatory T cells. In that period she contributed to the characterization of the phenotype of this cell subset and to the discovery of a new surface marker that revealed to be critical for Treg individuation in the peripheral blood. Moreover she defined a new suppressor mechanism utilized by human Treg in order to perform their modulatory activity. Diletta also investigated the frequency of human T regulatory cells in patients affected by Multiple Sclerosis thus contributing to a better understanding of the role played by this subset in the pathogenesis and progression of the disease. During her post-doctoral experience in London, Diletta led a project focused on T lymphocytes senescence, and acquired deep knowledge on T cells subpopulations and their effector functions.
Her interest and her results on T regulatory cells and the immune response in general are suitable for the project proposed, where she will study the recruitment of regulatory cell populations in the context of a pro-senescence therapy applied to prostate cancer. During her training, the applicant Dr. Di Mitri also acquired important skills that will be very important for the proposed project. In particular, the purification of lymphocytes subsets, the characterization of these subsets by multicolour flow cytometry, in vitro assays aimed to assess the cells cytotoxic activity and activation state, the release of cytokines by ELISA and Quantitative Real Time PCR.
The competences of Dr. Di Mitri, acquired during her PhD and the post-doc experience in London, her knowledge in basic immunology, particularly focused on immunotolerance and T regulatory cells characterization, will be critical for the development of this project in the host institution.

B3. IMPLEMENTATION AND THE HOST INSTITUTION

B3.1 Feasibility and credibility of the project, including work plan



1) The first 6-9 months of the present project will be dedicated to the evaluation of the impact of cancer cell senescence on the immune response in vivo and to characterization of the components of the immune system recruited to the tumor site.

A) The applicant will carry out cytometric analysis and ELISA assays to investigate the release of cytokines by MEFs cells in response to compounds that potentiate cell senescence such as Nutlin-3 and VO-OHpic; the expression of adhesion molecules on the MEFs cells before and after treatment will also be assessed by multicolour flow cytometry.

B) Mononuclear cells will be isolated from cancer tissues in vivo and T lymphocytes subsets will be identified by flow cytometry and their effector functions and activation state will be investigated. Both tumor cells and T lymphocytes will be isolated and purified by cell sorting. In vitro assays of cytotoxicity will be performed by flow cytometry.

C) The participant will present the progress of the project at one National or International meeting.

2) During the second half of the first year and the beginning of the second year, the participant will investigate the impact of cancer cell senescence on tumor-induced immunotolerance.

A) *Pten* null tumors from control mice and mice treated with PICS enhancer (Nutlin- 3) will be collected as mentioned before. Quantitative RealTime PCR will be performed on isolated tumor cells to analyze the modulation of TGFβ expression following PICS induction. The release of TGFβ will be also investigated by ELISA. We will confirm the effect of pro-senescence compounds on TGFβ release also on human prostate cancer cell lines.

B) Mononuclear cells from the mice will be isolated and the presence of T regulatory cells will be identified *ex vivo* by flow-cytometry. Also the frequency of myeloid suppressor cells in the proximity of the tumor site will be evaluated.

C) The expression of markers of T lymphocytes senescence such as KLRG-1, CD57 and γH2AX and the up-regulation of the telomerase activity upon stimulation will be assessed on the isolated cell subsets.

3) During the second year preclinical studies will be set up to combine the administration of pro-senescence compounds with anti-TGFbeta treatment in a mouse model of prostate cancer.

A) A cohort of Pten null prostate mice will be enrolled to set up a preclinical trial; the therapeutic efficacy of Nutlin-3 in combination with antiTGFβ treatment will be assessed. Treatments will begin once the mice reach 4 weeks of age and it will be stopped after 4 weeks of treatment.
B) The applicant will carry out histological analysis and immunostaining to check tumor response, senescence and apoptosis induction

4) Finally the participant will present the results of the project at an annual international meeting.

The participant will work in the IOSI institute that is the main Oncology Institute of the Italian region of Switzerland. In this structure the participant will have the possibility to work in a fully equipped laboratory, which is already available. The institute offers several services to its employees including financial advice or basic services, such as supplying real estate agencies for researcher who needs accommodation or to open a new bank account. A childcare facility is also provided by the IOSI institute, and all employees who decide to use this service can take advantages of special rates or discounts. IOSI employers can also take advantage of several benefits such as specific private health care plans.
Dr. Di Mitri is mother tongue Italian and speaks fluent English so very few possible barriers exist that may interrupt or inhibit her integration into the international research community of the IOSI.

B3.2 Dissemination and exploitation of results

The key aspect of this study is the possibility to use and optimise an already promising therapeutic approach against prostate cancer, based on the induction of cancer senescence through pro-senescence compounds already used in clinic. We strongly believe that pro-senescence compounds may represent the future in the treatment of human cancer and that a great effort has to be done in order to improve pro-senescence therapies already tested in preclinical trials. In this context the combination of pro-senescence compounds with immunomodulators may have a tremendous effect on cancer defeat. We plan to expose our results to the public as soon as possible; to achieve this purpose we will submit one or more papers to scientific journals and we'll also give communication of the obtained results to newspapers and scientific magazines.
In addition to this, during the development of the project the participant will present her data in the context of national and international meetings. Furthermore, students will be invited to visit the research institutions and the laboratory and to participate to a one-day meeting focus on the present project.

B4 IMPACT

B4.1 Capacity to develop lasting cooperation and collaboration with the other countries

The participant was trained in Italy and then moved to UK for a post-doctoral experience. Her experience in a foreign country made her understand the importance of mobility between laboratories in order to improve a researcher's skills and knowledge and to create connections between groups that work in different field or different geographical areas. The development of the present project will involve several groups, also thanks to the numerous national and international collaborations of the IOSI institute in the field of basic and clinical oncology. The participant will also keep in contact with laboratories from her country of origin, where she spent most of her training period. Furthermore, the international background of the supervisor, Dr. Andrea Alimonti, will award the possibility to maintain and create new lasting collaborations with other countries that will surely reveal to be determinant for the success of the project.

B4.2 Contribution to career development of the researcher

The proposed grant will surely benefit the carrier development of Dr. Di Mitri. The participant will work in a fully equipped laboratory and the host institution the host institution will give her complete independence in leading her project. The participant will have the opportunity to learn from high-qualified researchers in the field of cancer research, thus extending her scientific background. At the IOSI Dr. Di Mitri will also learn many new methodologies associated with this area of research; she will be able to improve her knowledge regarding the design of pre-clinical and clinical trials including the legal processes and management required for their implementation. Such trials are critical for the future development of this project. The potential for Diletta to improve her communication skills through lab meetings, research presentations and tight collaboration with groups from multiple disciplines will also exist, thanks to the open scientific environment present at IOSI.
The experience in Bellinzona will benefit both the technical skills and the intellectual knowledge of the participant and such improvement may become determinant for her future career in the research field.
At the IOSI the participant will have the exciting prospect of working in a new and expanding research environment where she will have the opportunity to translate her ideas and research findings to a preclinical setting. The Experimental Oncology Laboratory, where Dr. Di Mitri will have the opportunity to carry out her work, represents an internationally renowned immunology institute. Such an institute will let the participant to benefit of the cooperation with high-qualified researchers and will give her the opportunity to acquire new knowledge and technical skills that may be determinant in the prospect to find a position of more responsibility in the future. Furthermore, in Bellinzona the participant will lead an independent project and will train and follow graduate and PhD students, thus improving her management and communication skills. This will surely have a positive impact on her future career.

B5 ETHICAl ISSUES

Informed consent:
No human subjects are used in this proposal

Date protection issues:
No personal date will be collected

Human embryonic stem cells:
No human embryonic stem cells are used in this proposal

Use of animals:
Animal care and use is authorized and audited by the Veterinary Officer of the Canton TICINO in

observance of the Swiss Federal Act on Animal Protection, the Swiss Animal Protection Ordinance and the Guidelines of the Swiss Veterinary Office. All necessary measures are taken, in accordance with current veterinary practices, to ensure minimum discomfort, distress and pain, using appropriate methods for sedation, analgesia or anaesthesia. The Animal Facility provides the materials and advice necessary for FMI researchers to conduct animal experiments in full accordance with ethical principles and national and EU statutes. Research work is only carried out by individuals trained in the proper care, handling and use of animals. Regular training sessions on animal welfare are provided as well as information on biosafety, zoonoses, personal hygiene and occupational health and safety.

The Ethical Issue Table

RESEARCH ON HUMAN EMBRYO/FOETUS	**YES**	**PAGE**
* Does the proposed research involve human Embryos?		
* Does the proposed research involve human Foetal Tissues/ Cells?		
* Does the proposed research involve human Embryonic Stem Cells (hESCs)?		
* Does the proposed research on human Embryonic Stem Cells involve cells in culture?		
* Does the proposed research on Human Embryonic Stem Cells involve the derivation of		
cells from Embryos?		
I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	X	
RESEARCH ON HUMANS		
* Does the proposed research involve children?		
* Does the proposed research involve patients?		
* Does the proposed research involve persons not able to give consent?		
* Does the proposed research involve adult healthy volunteers?		
Does the proposed research involve Human genetic material?		
Does the proposed research involve Human biological samples?		
Does the proposed research involve Human data collection?		
I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	X	
PRIVACY		
Does the proposed research involve processing of genetic information or personal data (e.g. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?		
Does the proposed research involve tracking the location or observation of people?		
I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	X	
RESEARCH ON ANIMALS		
Does the proposed research involve research on animals?	X	5-7
Are those animals transgenic small laboratory animals?	X	5-7
Are those animals transgenic farm animals?		
* Are those animals non-human primates?		
Are those animals cloned farm animals?		
I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL		
RESEARCH INVOLVING DEVELOPING COUNTRIES		
Does the proposed research involve the use of local resources (genetic, animal, plant, etc)?		
Is the proposed research of benefit to local communities (capacity building i.e.		

access to		
healthcare, education etc.)?		
I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	X	
DUAL USE		
Research having direct military use		
Research having the potential for terrorist abuse		
I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY PROPOSAL	X	

ENDPAGE

TRAIN

(Co-funded by Marie Curie Actions)

Outgoing Grants (OG)
Call: "TRAIN-001"

PART B

"Cancer senescence"

Schedule 2

Time sheet

TIMESHEET

Title of the Project:
Grant Agreement number:
Name of Host Institution:
Researcher Name:
Month, Year:

	Hours spent per day on quoted project to nearest 0.5 of an hour																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total
RTD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00.00
DEMO																																
OTHER																																
																																0.00.00
																				0.00												

Signed as a correct record (Project staff member) Date

Full name *and* signature

I hereby certify that the above represents a true and accurate record of the time spent in accordance to the hours registered in the TIMEWORK System

Countersigned (Hierarchical supervisor) Date

Full name *and* signature

Countersigned (Project manager) Date

Full name *and* signature