Associazione Giovanna Tosi per la lotta contro i tumori
Young Researchers Mobility Programme
Application Form

PART A – ADMINISTRATIVE DATA

A1. APPLICANT

Name, Surname: Gloria Delfanti

Date of Birth: 25.05.1989

Nationality: Italian

Address: *(street, postal code, city)* via Clitumno 9, 20131, Milano

Phone number: 3381245732 02 26434735

Email: delfanti.gloria@hsr.it

Researcher status:

☑ PhD student

Planned graduation date: January 2019

☐ Postdoctoral fellow (☐ PhD or ☐ MD)
Graduation date:
PhD/MD school:

Title of PhD or MD thesis:

Title of current research project: Harnessing invariant NKT cells for cancer immunotherapy

A2. HOME INSTITUTE*

Institute Name: Università Vita-Salute San Raffaele / San Raffaele Scientific Institute

Department/Research Unit: Division of Immunology, Transplantation and Infectious Diseases; Experimental Immunology Unit

Address: *(street, postal code, city)* via Olgettina 58, 20132 Milano, Italy

Supervisor name: Paolo Dellabona

Supervisor e-mail: dellabona.paolo@hsr.it

Supervisor phone number: 02 2643 4727

*only Italian Institutes are eligible

A3. HOSTING INSTITUTE

Institute Name: Houston Methodist Research Institute

Department/Research Unit: Department of Nanomedicine

Address: *(street, postal code, city)* 6670 Bertner Street Houston, Texas 77030

Supervisor's name: Haifa Shen

Supervisor's e-mail: hshen@houstonmethodist.org

Supervisor's phone number: 713-441-7321

A4. MOBILITY PERIOD

Starting date: 1st August 2018

Ending date: 30th October 2018

Duration: *(number of days-including travelling days) 91*

PART B – PLANNED VISIT

B1. PROPOSAL

B1.1 TITLE and ACRONIM

Employing nanotechnologies to enhance iNKT cell-based cancer immunotherapy (Nano-K-Therapy)

B1.2 PRIORITY AREAS

Tick up to three most pertinent

☐ Human oncogenic viruses
☐ Microbiome and cancer
☑ Molecular targets of the immune response against tumors
☐ -OMICS approaches
☑ pre-clinical research

Implementation :

☑ development of an experimental part of a research project
☑ use of facilities/instruments not available at the home institute
☑ acquisition of a new technique/skills development
☐ development of clinical research activities as part of a research project

☐ development of a translational research activity as part of a research project

B1.3 TOPIC. *Describe the topic chosen for the visit and its connection with cancer research and the priority areas. Maximum 2,500 characters, including spaces.*

Nanoparticles have been defined as the "magic bullets" for targeting the immune system and for treating cancer. One limitation of cancer therapy is the delivery of the therapeutic compounds into the tumor for a convenient period of time. Application of nanotechnologies to package drugs, small molecules, or immunomodulatory compounds into nanometer- or micrometer- size particles facilitate the transport of therapeutics into the tumor, overcoming physical and biological barriers. The released therapeutics can affect not only cancer cells but also surrounding immune cells, modifying the tumor microenvironment.
Nanoparticles have already been employed in cancer immunotherapy strategies, with the aim of ameliorating anti-tumor responses. Examples include cancer vaccines, immune checkpoint blockade, expansion of adoptively transferred TCR-engineered T cells and tumor extracellular matrix remodelling (Ferrari et al.2017; Xia et al. 2015). During my visit I will learn how to customize and handle an injectable nanoparticle generator platform, namely the multistage vector (MSV), and employ it to sustain the anti-tumor activity of invariant natural killer T cells (iNKTs). iNKTs are CD1d-restricted innate-like T lymphocytes expressing a conserved semi-invariant T cell receptor that recognizes self- and non-self- lipid antigens. These MHC-independent cells are highly effective in the tumor immune-surveillance process, either by directly killing CD1d+ tumors or indirectly, by reprogramming the tumor microenvironment towards a tumor-opposing state (Cortesi et al. 2018). The MSV is designed to sequentially overcome biological barriers and consists of three components. First stage component, is a porous silicon microparticle that preferentially binds to diseased vasculature and protects the cargo from degradation. As the silicon material gradually degrades, second stage nanoparticles are released into pathological tissue: these nanoparticles further protect the therapeutic payload from degradation and promote intracellular uptake in cancer/immune cells where the active molecule is eventually deployed. I will generate microparticles, with pre-incorporated liposomes delivering the powerful iNKT cell agonist αGalactosylCeramide (αGalCer), alone or in combination with Stat3 inhibitors and/or tumor antigens, in order to enhance and prolong iNKT cells' natural anti-tumor functionality.

B1.4 AIMS. *Detail the general and specific aims of the visit. Maximum 1,500 characters, including spaces.*

The "Porous silicon microparticle-based cancer vaccines and methods for potentiating anti-tumoral immunity" has been patented by Dr.Haifa Shen in 2017. For this reason, the first aim of my visit to his laboratory, in the Department of Nanomedicine at Houston Methodist Research Institute, is to set up a collaboration in order to exploit this technology in my PhD research project, that aims to harness iNKT cells for cancer immunotherapy. More specifically I will learn how to prepare and handle the multistage vectors (MSVs) platform, encompassing the generation of the porous silicon microparticle according to United States Food and Drug Administration's good manufacturing practices (cGMP), and the formulation of the nanoparticles with αGalCer incorporated on their surface and immunomodulators inside them. When the system will be settled up, the second aim will be to assess the use of nanoporous silicon microparticles to activate CD1d-restricted iNKT cells at the tumor site, by this verifying the hypothesis that iNKT cell-dependent therapeutic modulation of the tumor microenvironment may be sustained via repeated stimulation with specific agonists and immunomodulators, locally delivered by next generation MSVs.

B1.5 ACTIVITIES. *Describe the activities to be implemented during the visit, including their schedule. Indicate any interdisciplinary activities. Maximum 10,000 characters, including spaces.*

We hypothesize that iNKT cells anti-tumor activity may be sustained over time using multistage vectors (MSVs) to combine repeated intra-tumor delivery of the potent iNKT cells agonist αGalactosylCeramide (αGalCer), together with drugs that inhibit immunosuppressive pathways and stimulate Th1 immunity. Based on results we generated in the lab, we will target the pro-angiogenic and immunosuppressive IL-6-JAK-STAT3 signaling pathway, frequently active in mouse and human cancers and highly upregulated in cancer-infiltrating immune cells, when tumor-bearing mice lack iNKT cells (Cortesi et al, 2018). MSVs carrying αGalCer, Stat3 inhibitors and tumor antigens-loaded liposomes are expected to be phagocytosed by myeloid cells in the tumor microenvironment (e.g. tumor-associated macrophages or dendritic cells) by this generating a pronounced pro-inflammatory milieu that sustains iNKT cell activation, prevents their exhaustion and possibly aids the restimulation of tumor-specific T cell responses.

1) Manufacturing MSVs. In collaboration with Dr. Haifa Shen, I will develop different MSVs for intra-tumor delivery of liposomes loaded with αGalCer, commercially-available Stat3 inhibitors and tumor antigen peptides (Ova, TRP-2, SV40, TagIV). MSVs are innovative nanoplatforms for in vivo delivery, composed by a "stage 1" discoid porous silicon microparticles (1-2,5 μm diameter, 400-700 nm thickness), whose pores are preload by "stage 2" nanoparticles (any currently available nanoparticle: liposomes, dendrimers, carbon structures, etc.), that in turn carry the active cargo up to albumin size (66.5 kDa).
First stage porous silicon microparticle will be generated according to United States Food and Drug Administration's good manufacturing practices (cGMP) and protocols. Concurrently, 100mm heavily doped silicon wafers (p++ type) will be exposed with resistivity of 0.005 Ω cm to electro-chemical etching in aqueous hydrofluoric acid to create pores in the silicon material. Then a layer of silicon oxide will be deposited on the film using low-pressure chemical vapor deposition, followed by photolithography and reactive-ion etching in fluorocarbon plasma, to form geometrical patterns outlining particle dimensions and enhance particle stability. Finally, the particles will be subjected to sonication in isopropanol to release them from the silicon substrate (Wolfram, Shen and Ferrari, 2015). Porous silicon microparticles can be produced with distinct dimension and porous structures, that will lead to particles with different performance attributes: nanoparticle loading capacity, drug release profile, degradation kinetics, and biodistribution. The extensive expertise of Dr. Shen's lab will guide me to choose the most suitable particle structure for our application. We expect this to be feasible in the short time as the group has recently optimized and patented a silicon microparticle-based platform for cancer nanovaccine (Xia et al., 2015; Xu et al., 2016; patent number: AU2016243027).
Second stage nanoparticles (NPs) will be generated according to Dr. Shen's lab protocols, and αGalCer will be directly incorporated on the liposome membrane. Following to this, αGalCer-liposomes will be loaded with different types of third stage therapeutics agents, including pharmaceutical compounds (Stat3 inhibitors) and synthetic antigenic peptides. To target the STAT3 pathways, I will use the commercially-available Stattic, a small molecule active in the low μM range to inhibit Stat3 phosphorylation, and Stat3-specific siRNA. Both strategies have already been used successfully to inhibit Stat3 in vivo; scrambled siRNA will generate control NPs. Additionally, I will generate NPs carrying tumor antigens synthetic peptides such as the immunodominant CTL epitopes of Ova (SIINFEKL), with the aim of eliciting or boosting cancer-specific immunity. The structure of the MSV ensures high grade of versatility and flexibility of use. For example, it is possible to simultaneously load multiple types of second stage NPs, thus allowing the deployment of tailored cocktail of therapeutic agents (Chiappini et al., 2010). I will take advantage of this feature during the testing phase, as I will test different combinations of NPs loaded on porous silicon microparticles to reach the best biological activity.

Before moving to the testing phase, quality control of the generated NPs will be performed according to the expertise and resources available in Dr. Shen's lab. The chemo-physical properties of the NPs can be evaluated by z-potential, dynamic light scattering, electron microscopy imaging, and x-ray spectroscopy elemental mapping. Subsequently, the biological features that can be evaluated are (i) the number of molecules conjugated on the surface of the MSVs and NPs; (ii) the loading of different amounts and types of NPs; (iii) the release over time of second stage NPs in different media conditions and at different temperatures; (iv) the structural stability of the MSVs and NPs and their degradation kinetics and patterns. We will discuss with Dr.Shen which of these evaluation are required for our purpose.

2) Investigating MSVs use. After quality controls, the potency of the loaded MSVs will be first tested in vitro. In this testing phase, the iNKT hybridoma cell line DN3A4-1.2 will be co-cultured with bone marrow derived dendritic cells (DCs) and macrophages loaded with different types of generated NPs or soluble αGalCer. In this setting we will measure both the uptake of NPs by phagocytic cells, employing for example FITC-conjugated OVA peptide loaded NPs, and their ability to activate iNKT cells, by measuring IL-2 production through ELISA assay after 2, 6, 12 and 24 hours of co-culture. Following this first assessment with the hybridoma cell line I will also test the MSVs on primary iNKT cells. In the last year at San Raffaele, I set up and optimized a reliable protocol to expand high numbers of primary iNKT cells starting from the spleen of commercially-available Vα14-Jα18 transgenic mice (which harbor increased numbers of iNKT cells). These mice are also present in the animal facility of the hosting lab, ensuring a high feasibility of the experiments.
The biological activity and potential toxicity of MSVs will be also tested in vivo. During my stay in Houston, I will perform a preliminary experiment with B16-OVA tumor-bearing mice. B16-OVA cells, which are available in the lab, will be subcutaneously injected in the flank of C57BL/6 mice, and MSVs will be administered when a palpable tumor is established, first route of administration will be intra-tumor. The assessment of tumor growth (or rejection) will be the read out of the experiment, providing evidences of the direct effect of MSV in a subcutaneous tumor model. This experiment will unveil additional features such as MSV capacity of activating intra-tumor iNKT cells in vivo. This will be done by cytokine release in the serum of treated vs untreated animals. In a second experiment we will administer MSV intravenously, read out will be tumor growth, iNKT cells activation and MSVs distribution (systemic vs intra-tumor). This will be done by microscopy or flow cytometric analysis of iNKT cells in different locations (i.e. tumor, liver, spleen). If time allows, I will also perform two broader experiments: one to assess the most active NPs (or combination of NPs) and one to compare the effect of MSVs, MSVs plus iNKT cells adoptive transfer and iNKT cells only. Groups of at least 5 mice for each different treatment will be investigated and replicated until the appropriate statistics defines the significance. As in vivo experiments require longer time frames, the in-depth characterization of the MSV biological effects could also be performed once I will be back in Milano, when I will be supplied with the nanoparticles and all the know-how for any further developments that will be prompted by the results.

B1.6 EXPECTED RESULTS. *Describe the expected results and their impact. Maximum 2,500 characters, including spaces.*

Based on the extensive and widely recognized expertise acquired by Dr. Shen lab and Houston Methodist Institute in the nanomedicine field, the first expected result is the proper MSVs assembly, including appropriate incorporation of αGalCer on the liposome membrane, the generation of NPs carrying Stat3-inhibitors / tumor antigen and their assembly on MSVs.
Then we expect that functional MSVs carrying αGalCer, Stat3 inhibitors and tumor antigens will sustain iNKT cells anti-cancer response persistently, further controlling and delaying disease progression. The purpose of my PhD project is to harness iNKT cells for cancer immunotherapy, enhancing their capability of reprogramming the tumor microenvironment towards a tumor-opposing state and controlling cancer progression. Results obtained so far indicate that iNKT cells adoptive

transfer into tumor-bearing mice control tumor progression for up to two weeks. After this time a decrease in the number and/or function of iNKT cells at the tumor site causes tumor relapse. Exploiting MSVs we expect silicon microparticles to efficiently protect and transport the loaded NPs to the terminal capillaries of tumor lesions, where also in response to reactive oxygen species, present in high levels in tumor tissue, they will gradually degrade releasing NPs. As demonstrated by the hosting lab, NPs infiltrate cancer lesions following the tumor-mediated enhanced permeability and retention (EPR) effect and can be actively phagocytosed by stromal and cancer cells within the tumor microenvironment, thus achieving a high local cargo concentration. In our case, the use of αGalCer-coated NPs will create an enhanced pro-inflammatory milieu specifically sustaining iNKT cell activation at tumor site, preventing their exhaustion. In accordance to our recent findings (Cortesi et al., 2018), as well as other published data, we expect that this strategy will improve the therapeutic effectiveness following iNKT cell-based immunotherapy.

B1.7 DELIVERABLES. *Describe one or more tangible deliverable(s) resulting from the activityies performed during the mobility period (e.g. raw data and record of experiments, number of samples collected, scientific paper written, application for a grant). Do not indicate the "Mobility Report" as a deliverable. Maximum 2,000 characters, including spaces.*

The primary deliverable resulting from my visit at the Houston Methodist, will be the final MSVs. This includes MSVs encompassing all the combinations of NPs and third stage agents that I previously described, namely: (i) αGalCer plus small molecules Stattic (ii) αGalCer plus Stat3-specific siRNA (iii) αGalCer plus scrambled siRNA (iv) αGalCer plus OVA peptide antigen SIINFEKL (v) αGalCer alone. Moreover, we will also keep free liposome to be compared with MSVs. This generated library of particles will comprehend a detailed record with the chemical/physical characterization. I will acquire the know-how for this technology from production to handling and application and collects several results from in vitro characterization experiments and functional test/co-culture, as well as preliminary in vivo experiment with B16-Ova tumor bearing mice. All these results will be fundamental to further implement my PhD project based on iNKT cells immunotherapy and will be enclosed in my thesis. Additionally, if the hypothesis is confirmed, the generated data will be added to the scientific paper that we are currently structuring.

B1.8 HOSTING CAPACITIES AND ARRANGEMENTS. *Describe the competences and facilities of the hosting organisation, limited to those used during the mobility period. Describe the hosting arrangement and the complementarities between the home and hosting institutes in relation to the young researcher's project. Maximum 2,500 characters, including spaces.*

Houston Methodist is a very innovative research centre, committed to "develop and move the best". The Department of Nanomedicine is focused on interdisciplinary research by combining nanoengineering, mathematical modelling and biomedical sciences to develop nanotechnology-enabled therapeutics and diagnostic platforms for clinical applications. At Houston Methodist, the Nanoengineering Core facility develops and provides silicon-based nanotechnology for biomedical research, taking advantage of industrial silicon microfabrication techniques. They have developed a series of fabrication protocols through a combination of microfabrication and chemistry to make porous silicon particles for MSVs system. The Lab of Dr.Shen recently patented the "Porous silicon microparticle-based cancer vaccines and methods for potentiating anti-tumoral immunity". Thus, they have all the know-how and forefront instrumentation for MSVs fabrication, to be employed in cancer immunotherapy. This aspect is of great relevance for the purpose of my visit, as in my home institution we have a high knowledge in immunology and cancer, but we have really poor understanding and knowledge in the nanotherapeutics field. Spending some time in the lab of Dr. Shen will give me the possibility to learn how to prepare and handle the MSVs at the best, to be use in the iNKT cells adoptive transfer experiments, which are the main focus of my research project. I will also perform both in vitro and in vivo preliminary experiments with the newly produced MSVs. Once I will be back to San Raffaele

Research Institute, I will continue my collaboration with Dr.Shen and further implement the application of MSVs as adjuvant for cancer immunotherapy.

B2. MOTIVATION LETTER

Explain the scientific and personal reasons for the proposed mobility and how the mobility could contribute to your career development. Maximum 7,000 characters, including spaces.

Visiting Houston Methodist Research Institute during my PhD, will be a unique experience and opportunity, that will positively influence both my scientific career and personal growth.
Working in Dr. Shen lab, will give me the possibility to develop an important task of my PhD project, whose final aim is to ameliorate cancer immunotherapy by using innate-like T cell (iNKT cells) that are independent of the MHC restriction and, if harnessed correctly, could therefore constitute a universal therapeutic option. We will generate cutting-edge multistage vectors (MSVs) carrying a specific iNKT cells activating lipid and combined with immunomodulatory drugs. Thanks to the intrinsic characteristics of these nanoporous silicon microparticles, this innovative system is able to reach the tumor site at high specificity, and directly deliver herein the therapeutic drug combination of choice.
I strongly believe that the time I will spend in Houston will not only have a great impact on my PhD project, but will also set a major step forward in my career development that could not be achieved elsewhere. I am convinced that Dr. Shen's lab in Houston is the place to be to push my scientific knowledge forward: indeed, I will immerse myself into the field of bio-engineering and merge it with my immunology background to generate a new immunotherapeutic approach. Also, Dr. Shen's lab is the patent holder of the MSVs technology so suitable for our approach, therefore moving to Houston for this external period is the only possibility to develop this application.
The lab where I will set up this part of the project is in one of the most forefront American research institute. Houston Methodist is an excellence research centre, which foster innovations and multidisciplinary approach for the development of new platform for clinical applications. The entire Nanomedicine department, and in particular Dr. Shen and his team are worldwide recognized experts in the field of nanotherapeutics. Working there will give me the special opportunity to immerse myself in a completely new field, learning cutting-edge techniques and methodologies that are not available in our Institute. The scientific environment will be extremely multidisciplinary, with people coming from a variety of scientific backgrounds. Here clinicians, engineers, mathematicians, physicists, statisticians, biologists and veterinarians are all collaborating for the development and the implementation of new technologies. Because of this, I will be exposed to lot of different scientific stimuli that will surely influence my way of thinking and approaching science. This will also be a great opportunity for discussing my data with people outside my home lab, by this broadening my horizons and prompting new ideas and suggestions, and possibly raising new interesting questions or challenges. I am counting on the completely different perspective I will immerse myself into to help me facing and solving problems and issue in the project that I could not think of while being in my current reality. Joining such a leading research institute will also give me the opportunity to attend seminars, lectures and meetings from other laboratories, all key occasions to learn as much as possible from other scientific fields and to create a solid working network. Networking will be of fundamental relevance for my future scientific career, creating the basis for future collaboration and scientific exchange.
Apart from the scientific aspect, living in the US for some time will also influence the way I live and face things in everyday life. Compared to the reality I grew up in, this experience will put myself in a completely different setting. Houston is America's fourth largest city, there are vast number of educational, business, cultural, and recreational opportunities. It has a high cultural diversity, it homes 45 religions and it has over 80 foreign consulates. Being an international city, living in Houston will give mi the opportunity for cultural exchange. I will live by myself in a foreign country, with different habits, rules and lifestyle and this will further encourage me to interact with other people, to create a

new group of friends and get integrated into the multi-cultural Houston community. I believe that this aspect will be of pivotal importance to improve my interpersonal skills and strengthen my personality. At last, I will improve my English in both scientific communication and every day speaking.
In conclusion, I am convinced that all the aspects described above will have a major impact on my career development; I will work out an important part of my PhD project, merge my immunology background into the fields of bio-engineering and nanomedicine and acquire a broad range of interdisciplinary skills. This will definitely concur to make me a more complete scientist.

PART C– ADDITIONAL DOCUMENTS

Tick each item in the check-list

- ☑ **Applicant's *Curriculum Vitae***
- ☑ **Photocopy of applicant's valid identification card or passport**
- ☑ **Home Institute's endorsement letter, signed by both the home scientific supervisor and chief administrator**
- ☑ **Hosting Institute's acceptance letter signed by both the hosting scientific supervisor and the legal representative or chief administrator**

Date of submission 30.05.2018

Signature

Send completed forms by email to: associazionegiovannatosi@gmail.com