UNIVERSITÀ DEGLI STUDI DI MILANO

TO MAGNIFICO RETTORE OF UNIVERSITA' DEGLI STUDI DI MILANO ID CODE ____4488____

I the undersigned asks to participate in the public selection, for qualifications and examinations, for the awarding of a type B fellowship at **Dipartimento di Oncologia Ed Emato-Oncologia** _____

Scientist- in - charge: _ **Prof. Vincenzo Costanzo** _____

Vincenzo Sannino

CURRICULUM VITAE

PERSONAL INFORMATION

Surname	**Sannino**
Name	**Vincenzo**
Date of birth	**30, 07, 1982**

PRESENT OCCUPATION

Appointment	Structure
Post-doc	**IFOM – Istituto FIRC di Oncologia Molecolare**

EDUCATION AND TRAINING

Degree	Course of studies	University	year of achievement of the degree
Degree	**Biologia**	**Università Degli Studi Di Napoli Federico II**	**2009**
Specialization			
PhD	**Scienze Biotecnologiche**	**Università Degli Studi Di Napoli Federico II**	**2013**
Master			
Degree of medical specialization			
Degree of European specialization			
Other			

REGISTRATION IN PROFESSIONAL ASSOCIATIONS

Università degli Studi di Milano – Direzione Risorse Umane
Ufficio Contratti di formazione e Ricerca
Via Sant'Antonio 12 - 20122 Milano, Italia
Assegni.ricerca@unimi.it

Date of registration	Association	City

FOREIGN LANGUAGES

Languages	level of knowledge
English	**Very Fluent**

AWARDS, ACKNOWLEDGEMENTS, SCHOLARSHIPS

Year	Description of award
2009	**Borsa di Studio (awarded fellowship) from ASI (Agenzia Spaziale Italiana) for the program "From Molecules to Man: Space Research Applied to the Improvement of the Quality of Life of the Ageing Population"**
2016	**Borsa di Studio (awarded fellowship) for the program "FUV (Fondazione Umberto Veronesi) post-doc fellowships 2017"**
2017	**Borsa di Studio (awarded fellowship) for the program "FUV (Fondazione Umberto Veronesi) post-doc fellowships 2018"**
2018	**Borsa di Studio (awarded fellowship) for the program "FUV (Fondazione Umberto Veronesi) post-doc fellowships 2019"**

TRAINING OR RESEARCH ACTIVITY

description of activity

POST-DOCTORAL FELLOWSHIP (April 2018 - Current)

FIRC Institute of Molecular Oncology (*IFOM*) - Via Adamello 16, 20139, Milan, Italy

DNA Metabolism lab

Supervisor: Prof. Vincenzo Costanzo

Project Title: Identification of targetable DNA repair pathways activated in the absence of tumor suppressor BRCA2

Funding: Fondazione Umberto Veronesi

I am currently working on a project aimed at the identification of uncharacterized DNA repair pathways activated in the absence of the tumor suppressor BRCA2. Cancer cells mutated in the BRCA1/2 genes lose their ability to proper repair DNA lesions and rely on alternative DNA repair pathways for their survival. Thus, the knowledge of such alternative repair pathways would provide us informations about new druggable targets that can be taken into account for alternative or support cancer therapies.

My approach is based on a starting proteomic screening carried out with the *Xenopus* system and a second validation phase carried out with both *Xenopus* extracts and mammalian cells. Mass spectrometry analyses

are carried out on the protein pool enriched on *Xenopus* chromatin isolated after replication of genomic DNA in the absence of the BRCA2 or Rad51 factors. Any eventual hits are then evaluated by following the DNA replication dynamics of the isolated factor in *Xenopus* extracts by western blot analyses. Validated protein targets are eventually investigated in the mammalian DLD-1 BRCA2-KO cell line. Some interesting targetable enzymatic factors have been already identified. I am currently working to determine whether the chemical inhibition of these enzymes has an effect on BRCA2-KO cells viability.

PROJECT ACTIVITY

Year	Project
2017 - 2018	FIRC Institute of Molecular Oncology (*IFOM*) - Via Adamello 16, 20139, Milan, Italy DNA Metabolism lab Supervisor: Prof. Vincenzo Costanzo Project Title: BRCA2 and the protection of the nascent DNA Funding: Fondazione Umberto Veronesi Aim of the project was to study the role of BRCA2 and Rad51 in the DNA replication process and the stabilization of replication intermediates of large eukaryotic genomes. We were able to show that BRCA2 and Rad51 are needed to protect newly synthesized DNA from degradation in the context of the replication fork during challenged DNA replication conditions. I personally carried out the electron microscopy analyses showing that lesions accumulate on the genomic DNA replicated in the absence of BRCA2. We also showed that this depends on the destabilization of Rad51 chromatin binding in absence of BRCA2. We also demonstrated that BRCA2 and Rad51 have an essential role in protecting replication forks from DNA degradation by the Mre11 nuclease. These degradation events are particularly extensive in the context of the reverse forks. These latter are peculiar DNA replication forks which have inverted their direction following DNA replication stressing conditions. The annealing helicase Smarcal1 can trigger the formation of reverse forks when ssDNA gaps are accumulated at the replication fork junction. I personally carried out the biochemical assays demonstrating an unbalance for Polymerase α in the absence of BRCA2/Rad51. This polymerase activity unbalance triggers the formation of ssDNA lesions which are then exploited by Smarcal1 to invert the direction of the fork, thus creating the structures for Mre11 nucleolytic attack. These findings were discussed in *Kolinjivadi et al. Mol Cell. 2017.* In the same period I was involved in a project with Dr. Alberto Ciccia (Columbia University - NY - USA) aimed to study replication fork stability dynamics in mammalian BRCA-mutated cells. For this project I personally carried out sample preparation and all the electron microscopy analyses aimed to evaluate the presence of lesions on the genomic DNA and the levels of reverse forks accumulation in both BRCA1 and BRCA2 mutated cell lines. We could demonstrate that BRCA-mutated cells accumulated reverse forks and that the formation of these structures was mediated by several fork remodelers such as Smarcal1, ZRANB3 and HLTF. In the absence of functional BRCA1 or BRCA2 reverse forks are actively degraded by the Mre11 nuclease during the synthesis phase. This causes the accumulation of DNA lesions and chromosomal aberrations visible during the following phases of the cell cycle. These findings have been discussed in *Taglialatela et al. Mol Cell. 2017.* Always in the same period a parallel collaboration was carried out with Prof. Lumír Krejčí (Masaryk University - Brno - Czech Republic) in order to study the effect of some specific mutants of the Rad51 protein which were recently categorized as Fanconia Anemia-associated mutants. Fanconi Anemia is a genetic disorder characterized by a defect in DNA inter-strand crosslink repair, chromosomal instability and predisposition to cancer. We were

	able to demonstrate that these Rad51 mutants fail to form a stable protein nucleofilament which is needed to protect the DNA replication forks under DNA perturbed conditions. For this project I personally carried out the biochemical analyses with the Xenopus system and all the electron microscopy analyses to investigate about the presence of lesions at the level of the genomic DNA. These findings have been discussed in *Zadorozhny et al. Cell Rep. 2017.*
2013 - 2017	FIRC Institute of Molecular Oncology (*IFOM*) - Via Adamello 16, 20139, Milan, Italy DNA Metabolism lab Supervisor: Prof. Vincenzo Costanzo Project Title: The role of DNA damage response factors in vertebrate DNA replication Funding: Armenise Foundation The aim of the project was the functional characterization of the alternative role played by the BRCA2 and Rad51 recombination factors during unperturbed DNA replication and upon stressing conditions. The *Xenopus laevis* egg extract experimental system was exploited in order to reproduce DNA replication stressing conditions in presence or absence of those specific factors. Electron microscopy analyses of the DNA replication intermediates were also exploited to evaluate the effect of such experimental conditions on the DNA replication process. Interesting preliminary results were obtained in this period which represented the starting point for the data published in 4 following articles: *Kolinjivadi et al. FEBS Lett. 2017, Kolinjivadi et al. Mol Cell. 2017, Taglialatela et al. Mol Cell. 2017, Zadorozhny et al. Cell Rep. 2017.* In the meanwhile, a parallel project was carried out aimed to describe DNA replication at some specific chromosomal regions such as centromeres. Using bacterial artificial chromosomes and the *Xenopus laevis* egg extract system we were able to characterize the chromatin assembly and replication dynamics of centromeric alpha-satellite DNA. By using mass spectrometry together with electron microscopy analyses we managed to describe the assembly of a specific protein matrix and the formation of Topoisomerase I-dependent DNA loops. This peculiar conformation has been shown to switch off the ATR-dependent checkpoint by preventing RPA hyper-loading thus facilitating the replication of centromeric DNA. These findings have been described in *Aze et al. Nat Cell Biol. 2016* and in *Sannino & Costanzo Cell Cycle 2016.* I personally carried out the electron microscopy analyses of the DNA molecules for this paper. I succeeded in setting up a method to directly visualize the centromeric DNA chromatin structure for the first time. Moreover, a successful collaboration has been carried out with Dr. Luca Pellegrini (Cambridge University, Cambridge, UK). This allowed me to continue the work previously done with Dr. Francesca Pisani at IBP-CNR to describe the Cdc45 DNA replication factor structure and function. By exploiting the structural data obtained by X-ray crystallography we managed to finely map regions of Cdc45 involved in the interaction with the other components of the CMG (Cdc45-Mcm2-7-GINS) complex, the eukaryotic replicative DNA helicase. Specific mutants of Cdc45 in those regions were functionally analyzed using the *Xenopus* egg extract system and DNA replication dynamics were described in the absence of a properly-assembled CMG complex. I personally carried out all the functional analyses with the *Xenopus* system. These findings have been reported in *Simon et al. Nat Commun. 2016.*
2009 - 2013	Università Degli Studi di Napoli Federico II - Naples, Italy The work has been carried out at the Institute of Protein Biochemistry (IBP) - National Research Council (CNR), Naples, Italy Supervisor: Dr Francesca M. Pisani Project title: "The Cdc45 DNA replication factor: biochemical studies and biotechnological perspectives" Funding: AIRC The aim of the project was to carry out a structural and functional characterization of the

	human DNA replication factor Cdc45. Protein sequence analyses revealed for the first time an evolutionary link for human Cdc45 with the DHH family of phosphoesterases. Structural data were obtained by SAXS analysis and the DNA binding activity of the protein was characterized. These findings were discussed in *Krastanova et al. J Biol Chem 2012*. As this paper reports the first structural and functional analysis of Cdc45, it is considered a seminal work in the field. In fact, it has been already cited by 50 different scientific reports (among these 25 PubMed Central articles). I have also collaborated in a study where the physical and functional interaction between Cdc45 and Mcm10 was analysed. Results of this work were described in *Di Perna et al. Biochem J. 2013*. During the same period a strategy for the production of the CMG complex in recombinant form was set up with the aim of finely analysing the role of the Cdc45 factor within the CMG complex. Exploiting the *MultiBacTurbo™* system for polycistronic viruses production, we were able to co-express the 11 subunits composing the CMG complex by co-infection of insect cells with only 3 baculoviruses, which could provide coordinate and balanced expression of each single factor. Moreover, a collaboration was started with Dr. Carlo Raia (IBP-CNR) to support cloning and over-expression of a NAD(H)-dependent dehydrogenase/reductase from *Sulfolobus acidocaldarius* in bacterial cells. The results of this study were published in *Pennacchio et al. Appl Microbiol Biotechnol 2013*.
March 2012 - May 2012	London Research Institute - Cancer Research - London, UK Host: Prof. Vincenzo Costanzo During this time I had the opportunity of learning the use of the *Xenopus laevis* egg extract experimental system. I had the possibility of familiarize with a very powerful experimental system for the study of protein factors involved in pivotal biological processes such as cell cycle, DNA replication, DNA repair. Acquiring this expertise has been fundamental for the next steps of my experimental researcher career. At that time the final goal was to exploit the Xenopus system to functionally characterize the human Cdc45 factor and derivative mutants of this protein. The study of this specific DNA replication factor has been started during the PhD studentship and continued during the first years of post-doc fellowship. Thanks to the results obtained I am co-author for 2 of the pivotal publications describing the function of this protein in the context of the CMG complex for the first time (*Krastanova et al. J Biol Chem 2012, Simon et al. Nat Commun. 2016*).
2008 - 2009	Università Degli Studi di Napoli Federico II - Naples, Italy The work has been carried out at the Institute of Protein Biochemistry (IBP) - National Research Council (CNR), Naples, Italy Supervisor: Dr Francesca M. Pisani Project title: "Site-specific phosphorylation of the human DNA primase by the cyclin-dependent kinase CDK2" The project aimed to identify some specific phosphorylation sites on the human DNA primase which are target of the Cyclin-Dependent Kinase 2 (CDK2). To this aim the p48/p58 complex (human DNA primase), CycE/CDK2 and CycA/CDK2 protein complexes were produced in bacterial cells and purified in order to carry out *in vitro* functional assays. The specific phosphorylation sites were identified by means of classical kinase assays, phospho-peptide mapping and mass spectrometry and validated by site-specific mutagenesis.

PATENTS

Patent

Università degli Studi di Milano – Direzione Risorse Umane
Ufficio Contratti di formazione e Ricerca
Via Sant'Antonio 12 - 20122 Milano, Italia
Assegni.ricerca@unimi.it

CONGRESSES AND SEMINARS

Date	Title	Place

PUBLICATIONS

Books
[title, place, publishing house, year ...]
[title, place, publishing house, year ...]
[title, place, publishing house, year ...]

Articles in reviews
Zadorozhny K., Sannino V., Belan O., Mlcouskova J., Spirek M., Costanzo V., Krejci L. **Fanconi Anemia-Associated Rad51 Mutations Impair Replication Fork Protection Due To Destabilization Of Rad51 Filament.** *Cell Rep. 2017 Oct 10;21(2):333-340*
Taglialatela A., Nanez S.A.*, Leuzzi G.**, Sannino V.**, Ranjha L.**, Huang J.W.**, Madubata C., Levy B., Rabadan R., Cejka P., Costanzo V., Ciccia A.* **Restoration of replication fork stability and genome integrity in BRCA1- and BRCA2-deficient cells by inactivation of SNF2-family fork remodelers.** *Mol Cell. 2017 Oct 19;68(2):414-430* ** These authors contributed equally, ** These authors contributed equally*
Kolinjivadi A.M., Sannino V.*, De Antoni A.*, Zadorozhny K., Kilkenny M., Técher H., Baldi G., Shen R., Ciccia A., Pellegrini L., Krejci L., Costanzo V.* **Smarcal1-Mediated Fork Reversal Triggers Mre11-Dependent Degradation of Nascent DNA in the Absence of Brca2 and Stable Rad51 Nucleofilaments.** *Mol Cell. 2017; 67:867-881* ** These authors contributed equally*
Sannino V., Pezzimenti F., Bertora S., Costanzo V. **Xenopus laevis as Model System to Study DNA Damage Response and Replication Fork Stability.** *Methods Enzymol. 2017; 591:211-232*
Kolinjivadi A.M., Sannino V., De Antoni A., Técher H., Baldi G., Costanzo V. **Moonlighting at replication forks - a new life for homologous recombination proteins BRCA1, BRCA2 and RAD51.** *FEBS Lett. 2017; 591:1083-1100*
Sannino V., Kolinjivadi A.M., Baldi G., Costanzo V. **Studying essential DNA metabolism proteins in Xenopus egg extract.** *Int J Dev Biol. 2016; 60:221-227*
Sannino V. & Costanzo V. **ATR checkpoint suppression by repetitive DNA.** *Cell Cycle 2016; 15:2993-2994*
Simon A.C., Sannino V., Costanzo V., Pellegrini L. **Structure of human Cdc45 and implications for CMG helicase function.** *Nat Commun. 2016; 7:11638*
Aze A., Sannino V., Soffientini P., Bachi A., Costanzo V. **Centromeric DNA replication reconstitution reveals DNA loops and ATR checkpoint suppression.** *Nat Cell Biol. 2016; 18:684-91*
Di Perna R., Aria V., De Falco M., Sannino V., Okorokov A., Pisani F.M., De Felice M. **The physical interaction of Mcm10 with Cdc45 modulates their DNA-binding properties.** *Biochem J. 2013; 454:333-43*

Università degli Studi di Milano – Direzione Risorse Umane
Ufficio Contratti di formazione e Ricerca
Via Sant'Antonio 12 - 20122 Milano, Italia
Assegni.ricerca@unimi.it

Pennacchio A., Sannino V., Sorrentino G., Rossi M., Raia C.A., Esposito L. **Biochemical and structural characterization of recombinant short-chain NAD(H)-dependent dehydrogenase/reductase from Sulfolobus acidocaldarius highly enantioselective on diaryl diketone benzyl.** *Appl Microbiol Biotechnol 2013; 97:3949-64*
Krastanova I., Sannino V.*, Amenitsch H., Gileadi O., Pisani F.M., Onesti S.* **Structural and functional insights into the DNA replication factor Cdc45 reveal an evolutionary relationship to the DHH family of phosphoesterases.** *J Biol Chem 2012; 287:4121-4128* ** These authors contributed equally*

Congress proceedings
[title, structure, place, year]
[title, structure, place, year]
[title, structure, place, year]

OTHER INFORMATION

Declarations given in the present curriculum must be considered released according to art. 46 and 47 of DPR n. 445/2000.

The present curriculum does not contain confidential and legal information according to art. 4, paragraph 1, points d) and e) of D.Lgs. 30.06.2003 n. 196.

Place and date: MILANO, 7/1/2020

SIGNATURE

Vincenzo Sannino

Università degli Studi di Milano – Direzione Risorse Umane
Ufficio Contratti di formazione e Ricerca
Via Sant'Antonio 12 - 20122 Milano, Italia
Assegni.ricerca@unimi.it