LILT

Bando 2019 - Programma 5 per mille anno 2019
Investigator Grant (IG)

TRANSLATIONAL RESEARCH

LILT will support research projects in the field of cancer aimed at improving cancer diagnosis and treatment. Particularly considered will be those translational research projects that promise short-medium term effects in clinical practice, concerning new diagnostic methodologies and new therapies. Multicentric studies with national coordination, aimed at validating new diagnostic methods, diagnostic, prognostic and predictive tumor markers, able to improve the clinical management of cancer patients are potentially eligible for funding. Specific research projects on new oncological therapeutic approaches are also eligible for LILT funding as IG. For this type of grants it is necessary to demonstrate solid preliminary experimental data supported by a rigorous biological rationale.

1. Principal investigator's full name and qualification:

(Please include: CV in European format with list of publications; IF end Hi-index)

2. **Proposal title:** BDNF/TrkB axis as a source for squamous cell carcinoma prognostic biomarkers and therapeutic targets

3. **Primary area of Relevance:** Translational research in the field of squamous cell carcinoma (SCC) prognostic biomarkers. Based on our strong preliminary results on BDNF/TrkB pathway, the proposal intends to identify new prognostic SCC biomarkers and therapeutic approaches able to improve the clinical management of cancer patients.

4. **Relevance for the National Health System:** We forecast a possible short-term effect on the National Health System. Indeed, obtained results will help to outline a suitable SCC management strategy and to contribute to reduce poor clinical outcomes.

5. **Institution** Fondazione Luigi Maria Monti (FLMM) IDI-IRCCS, via Monti di Creta 104, 00167 Roma **phone** 0666464321-2429, **e-mail** dirscient@idi.it

6. **Authorized Administrative Official** Prof. Avv. Antonio Maria Leozappa, via Monti di Creta 104, 00167 Roma **phone** 0666464485, **e-mail** presidenza@idi.it

7. **Proponent's signature.**

8. **Authorized Administrative Official's signature:**

FONDAZIONE LUIGI MARIA MONTI
Antonio Maria Leozappa
Presidente e R.L.

9. **Place and date Rome, February 13th 2020**

SELF EVALUATION FORM

1. Investigator's full name: (PI) **Elena Dellambra**
2. Total papers n°**38** IF **228,82**
3. Total papers (last 10 years) n°**22** IF **102,90**
4. Total Papers as first/last author or corresponding author n°**18** IF **106,76**
5. Total H-index **20** (Scopus) **22** (Google Scholar)

PROPOSAL MAIN BODY

1. **Proposal title:** BDNF/TrkB axis as a source for squamous cell carcinoma prognostic biomarkers and therapeutic targets
2. **Abstract** (1 page)

Cutaneous squamous cell carcinoma (SCC) is one of the most common cancers and an age associated malignancy. Although most of SCCs are treated by surgery, a subset of them displays a higher likelihood of recurrence and metastasis causing death. SCCs may arise from the field cancerization, a chronically photo-injured skin area in which clinical and subclinical lesions coexist. The presence of multiple lesions often reduces the efficacy of treatments allowing SCC recurrence with worse prognosis, risk of spread to regional lymph nodes and distant metastases. Given its increasing incidence and potential for poor outcomes, SCC is emerging as a public health problem.

Aging is a major risk factor for tumorigenesis as the accumulation of senescent cells in tissues may worsen the senescence response efficacy in tumor suppression and induce a chronic inflammatory status that may increase tumor incidence and progression. Therefore, the differential evolution of lesions might be due to age-related changes in dermal compartment secretion that fuel the uncontrolled proliferation and migration of some mutated keratinocytes.

Our preliminary results identified the neurotrophin Brain-Derived Neurotrophic Factor (BDNF) as paracrine factor able to promote proliferation, migration, epithelial-mesenchymal transition (EMT) and de-differentiation features in primary keratinocytes derived only from old donor skin through the binding to its high affinity receptor, the tropomyosin receptor kinase (Trk)B. Moreover, the age-related switch of BDNF/TrkB pathway expression in fibroblasts and keratinocytes affects cell plasticity and may make skin prone to initiate tumorigenesis in the elderly.

Nowadays, no satisfactory prognostic biomarkers for primary skin SCC or diagnostic biomarkers for relapse has been proposed. Based on our preliminary results on BDNF/TrkB pathway, the proposal intends to identify new prognostic SCC biomarkers and therapeutic approaches.

Obtained results will help to outline a suitable SCC management strategy and to contribute to reduce poor clinical outcomes. Therefore, the study will have relevant positive socio-economic effects for the National Health System.

3. **Introduction**

Cutaneous squamous cell carcinoma (SCC) is one of the most common cancers in Caucasian populations[1], accounts for 20–30% of skin malignancies and its prevalence is increasing[2,3]. The most significant risk factors are sun exposure, age, fair skin, smoke and immunosuppression. Over half of patients develop multiple tumors during life. Although most of SCCs can be treated by surgery, a subset of them displays a higher likelihood of recurrence and metastasis causing death[4]. Clinical studies show that 65% of SCCs arises

from an early lesion named actinic keratosis (AK). AK and SCC are stages of a continuous multistep tumorigenic process that derives by specific gene mutations in keratinocytes. AKs may arise from the field cancerization, a chronically photo-injured skin area in which clinical and subclinical lesions coexist. The presence of the field cancerization is a clinical condition of major morbidity and lethality as reduces the efficacy of treatments and allows AK persistence and SCC recurrence [3,5].

In Italy, around 80.000 new cutaneous SCC/year have been found and this number is increasing with the steadily growing elderly population. Given its increasing incidence and potential for poor outcomes, SCC is emerging as a public health problem.

4. Background and rationale

Once a SCC has recurred, it has a much worse prognosis with risk of spread to regional lymph nodes and distant metastases[3]. The risk of local recurrence depends on increased tumour dimension, thickness or on localization. However, specific prognostic markers are not available.

Interestingly, some AKs do not progress into SCC, even if keratinocytes harbor typical genetic or epigenetic changes. These alterations have been also found in clusters of keratinocytes of normal skin, especially in sun-exposed areas of aged individuals[5]. Thus, these changes may be permissive for cancer-spreading instead of being the driver mutations. Indeed, cancer may result from altered tissue homeostasis or immune surveillance inhibition rather than from deregulated control of single or groups of cells[6].

SCC is the most common age-associated malignancy[3]. Aging is a major risk factor for tumorigenesis as the accumulation of senescent cells in tissues may worsen the senescence response efficacy in tumor suppression and induce a chronic inflammatory status that may increase tumor incidence and progression[6–8]. Therefore, the differential evolution of field cancerization clinical and subclinical lesions might be due to age-related changes in dermal compartment secretion that fuel the uncontrolled proliferation and migration of some mutated keratinocytes.

Studying the effect of aged fibroblast secretome, we identified the neurotrophin Brain-Derived Neurotrophic Factor (BDNF) as paracrine factor able to promote proliferation, migration, epithelial-mesenchymal transition (EMT) and de-differentiation features in primary keratinocytes derived only from old donor skin (see preliminary results). The tropomyosin receptor kinase (Trk)B is a high affinity receptor tyrosine kinase activated by BDNF and acts on RAS/MAPK, the PI3K/Akt, and the PLCγ pathways[10]. We demonstrated that aged fibroblasts secrete an active BDNF and that aged keratinocytes express higher levels of TrkB receptor. Moreover, a functional BDNF/TrkB signaling is required for enhanced proliferation and migration, and induction of EMT and de-differentiation processes in keratinocytes from old subjects. Thus, the age-related switch of BDNF/TrkB expression in fibroblasts and keratinocytes may make skin prone to initiate tumorigenesis in the elderly.

Although BDNF/TrkB axis influences the evolution of some cancers, still little is known about cutaneous SCCs. Thus, the investigation of BDNF/TrkB axis dysregulation in SCC initiation, progression and/or relapse will allow the identification of new prognostic biomarkers and therapeutic approaches in SCC management strategy.

5. Experimental design

To achieve the broad objective of the project, the following specific aims will be addressed:

Task1. Identification of new prognostic SCC biomarkers (Months 0-12) - To investigate if the proteins of the BDNF/TrkB axis may be relevant as prognostic biomarkers, expression of BDNF, TrkB and selected down-stream targets (e.g Yap1, Notch1, Ki-67, p16, H-Ras, E-cadherin, β-catenin) will be evaluated by immunohistochemical assays in SCC specimens and

healthy skin samples, enrolled at IDI-IRCCS and Policlinico Tor Vergata (about 400 patients suffering from SCC are yearly examined in each centre). Specimens from patients will be selected on the basis of clinical outcomes to obtain groups of individuals prone to relapses and/or metastasis and others not predisposed. SCCs will be classified according to Tumor-Node-Metastasis (TNM) classification.

Expression levels of BDNF, TrkB or both, will be correlated to the expression their down-stream targets, patient age, sex, clinical outcomes (number of relapses, metastasis) and well-established prognostic histologic features, including tumor size and depth, histologic subtype, grade of differentiation, level of dermal invasion (Clark's level), presence or not of perineural, lymphatic or vascular invasion[3,4]. Notably, BDNF promotes VEGF-A-dependent tumour angiogenesis and VEGF-C-dependent lymph angiogenesis in chondrosarcoma[11]. The expression of lymphangiogenic factors, such as vascular endothelial growth factor-A and -C (VEGF-A, VEGF-C), and their receptors (VEGFR-2, VEGFR-3) or co-receptors (neuropilin-1 and -2) will be evaluated in the same specimens and correlated to BDNF/TrkB axis expression.

Task2. Evaluation of the relevance of BDNF/TrkB in SCC initiation (Months 0-6) - Because EMT and migration are mandatory for tumor invasion and metastasis development, the project aims at verifying if age-related modifications are sufficient to induce matrix invasion or if probably additional modifications are necessary. To investigate if the BDNF/TrkB axis may have a role in SCC initiation in aged subjects, a 3D SCC organotypic model will be used (see preliminary results). This model allows testing both the capacity of fibroblasts to remodel the matrix and the potential of keratinocytes to invade it. Organotypic cultures will be composed of young, aged or tumor fibroblasts embedded in a matrix and young, aged or tumor keratinocytes seeded on the gel. The invasion ability of different conditions will be assayed in the absence or presence of recombinant BDNF in the medium. Quantification of the "invasive index" will be assessed using paraffin-embedded sections of the fixed organotypic cultures stained with hematoxylin and eosin. Expression modulation of selected genes, previously identified in BDNF-treated keratinocytes from aged subjects, will be evaluated by immunohistochemistry.

Task3. Identification of new therapeutic targets (Months 6-12) - To investigate if TrkB pathway may be relevant as potential therapeutic target for cutaneous SCC management strategy, two skin SCC cell lines derived from aged subjects will be used to study the effect of a TrkB specific inhibitor. Cells will be treated with ANA-12 (a selective TrkB antagonist that do not alter TrkA and TrkC functions) to analyze the recovery of specific BDNF-mediated features. To this aim, EMT markers and invasion ability will be evaluated on 3D SCC organotypic models by immunohistochemical assays. Expression modulation of selected selected down-stream targets will be evaluated on ANA-12 treated SCC cultures by qRT-PCR and western blot assays, and on ANA-12 treated SCC organotypic models by immunohistochemical assays. Two HNSCC cell lines derived from aged subjects will be used in parallel for comparison.

Based on the results obtained in Task1, the effect of ANA-12 will be also evaluated in combination with anti-angiogenic drugs such as the anti-VEGF-A antibody bevacizumab or additional pharmacological compounds targeting proteins downstream of TrkB.

6. Further details on the overall methods that will be used in this project

This project had the approval of the IDI-IRCCS Ethics Committee (Prot. 552, 14-12-18). Patients with SCC diagnosis to be subjected to surgical removal will be eligible for the study. To shortly increase the number of SCC cases, we also plan to analyze Formalin-Fixed Paraffin-Embedded (FFPE) specimens already collected at IDI-IRCCS and classified by

pathologists according to TNM classification.

The expression of BDNF, TrkB, selected down-stream targets and proteins of VEGF pathways will be evaluated by immunohistochemical assays in FFPE specimens (including lesional and nonlesional zones). Data will be described in terms of sex and age, level of identified markers, clinical outcomes (number of relapses, metastasis) and well-established prognostic histological features, as absolute numbers and percentages for categorical variables, as means and standard deviations, median for continuous variables. To test differences between the groups defined on the basis of clinical and/or histological data, one-way analysis of variance (ANOVA), or when the ANOVA assumptions are not met, non-parametric Kruskal-Wallis test will be used for continuous variables. Chi-squared test, or Fisher's exact test will be used to test differences for categorical variables. Multivariate logistic regression model will be used to examine variables potentially associated with the presence of clinical and/or histological data, and ORs and 95% confidence intervals (CI) will be estimated. All statistical analyses will be conducted using STATA, release 15 (StataCorp LLC, College Station, TX.).

3D SCC organotypic model will be generated as described in preliminary results.

Methodologies for functional and pharmacologic in vitro studies will carried out as described[21,23].

7. Work carried out and preliminary results

7.1 Age-related switch of BDNF-TrkB expression in fibroblasts and keratinocytes is involved in inducing cell plasticity and may make skin prone to carcinogenesis.

Senescent cells accumulate in premalignant lesions but are not detectable after progression to malignancy. As cellular senescence is a highly dynamic and progressive process in which the properties of senescent cells continuously evolve and diversify, some senescent cells might become premalignant[13].

Primary keratinocyte cultures form elderly donors are characterized by cells with a heterogeneous proliferative potential and enriched in paraclones, composed of cells with senescence features[14]. We demonstrated that the secretome of aged human fibroblasts modifies the morphology of primary human keratinocytes derived from elderly subjects. Keratinocyte colonies are composed of elongated and irregular cells displaying enlarged intercellular spaces and resembling cells of SCC. The presence of continuous extracellular signals is required for maintaining these changes. On the contrary, the secretome does not induce any morphological changes in keratinocytes derived from young donors. SPN-treated aged keratinocytes are characterized by features associated to phenotypic plasticity that are relevant for tumor initiation. Indeed, they display increased activity of PI3K/Akt, STAT3 and Ras/MAPK pathways and increased expression of their effectors such as EMT master genes (Zeb1, Zeb2, Slug, Snail, Twist1). These transcription factors activate the EMT program resulting in the disjunction of cellular adhesions, loss of epithelial cell polarity, ECM remodeling and manifestation of a mesenchymal and motile phenotype[15]. Accordingly, SPN-treated cultures are characterized by decreased expression of the cell-cell adhesion proteins (E-cadherin, beta-catenin), increased expression of mesenchymal markers (vimentin, thy-1) and cell-matrix adhesion proteins (fibronectin, collagen 1, collagen 3), and, in turn, enhanced migration. Interestingly, aged primary keratinocytes recover proliferation, clonogenic ability and dedifferentiation features following SPN treatments. Moreover, the expression of the senescence marker p16 decreases, suggesting that these cells could have escaped senescence. Mechanical signals exerted by ECM rigidity, cell density and shape modifications regulate epidermal cell fate by YAP modulation. Indeed, the loss of E-cadherin and β-catenin, and the spreading of the cell on stiffen ECM activate YAP. The mechano-activation of YAP

promotes an undifferentiated state by DLL1-mediated inhibition of Notch1 signaling[16,17]. Notably, aging, inflammation or tissue damage alter mechanical homeostasis or induce ECM stiffening lowering the threshold for YAP activation[18,19]. Those pathways are accordingly modulated in EMT-like colonies, suggesting that SPN-treated aged keratinocytes may acquire some stem-like properties. Notably, most of the SPN-modulated genes are mutated in AK and SCC [20].

To our knowledge, this is the first demonstration that aged human fibroblast secretome promotes EMT and dedifferentiation programs on primary aged keratinocytes.

The integration of data from fibroblast secretome analysis, protein association database and literature evidences allowed us to identify BDNF as potential paracrine factor able to promote these features in aged human keratinocytes. BDNF is the ligand of TrkB, a receptor tyrosine kinase, that is a regulator of tumor progression in some human cancers[10]. Indeed, activated TrkB acts on RAS/ MAPK, the PI3K/PDK1/AKT, and the PLCγ pathways and induces proliferation and EMT through the induction of Twist and Snail in cancer cells[10].

We demonstrated that the BDNF-TrkB axis is a cross-talk signaling involved in the induction of aged keratinocyte plasticity. We found that both the BDNF secretion from fibroblasts and TrkB expression in keratinocytes increase with aging. The treatment of aged keratinocytes with recombinant BDNF recapitulates morphological and molecular modifications promoted by aged SPN treatment. Furthermore, aged fibroblasts released an active BDNF as the treatment of aged keratinocytes with a specific antibody against BDNF inhibits the paracrine signaling preventing SPN-mediated morphological changes, E-cadherin decrease and proliferation. What is more, blocking the receptor activity by a selective TrkB inhibitor prevents SPN-mediated changes.

Altogether, our results show that the age-related switch of BDNF-TrkB expression in fibroblasts and keratinocytes may make skin prone to carcinogenesis in the elderly (***Tinaburri L. et al., submitted to J Invest. Dermatology***).

7.2 Standardization of a 3D SCC organotypic model.

We have a long-lasting experience in isolation and sub-cultivation of primary keratinocyte stem cells and fibroblasts from skin biopsies[9,14,21–23] and in the generation of skin equivalents using primary keratinocytes and fibroblasts also using genetically-modified cells[23]. Recently, we standardized a 3D SCC organotypic skin model (***Dellambra E. et al., submitted to Journal of the European Academy of Dermatology and Venereology***). SCC cell lines and CAFs, isolated from patient specimens, have been used. We demonstrated that SCC cells were able to invade the matrix in the presence of CAFs, resembling SCC behaviour in vivo. This model has been generated by seeding primary keratinocytes or SCC cell lines on a biodegradable polymer substrate incorporating human dermal fibroblasts or CAFs. The embedded CAFs led to 72.29%±1.7 of gel contraction whereas primary fibroblasts to 13.05%±1.6, demonstrating that CAFs maintained their functional properties in the biopolymer. Organotypic cultures were exposed at the air-liquid interface to promote full epidermal differentiation and stratification. In 3D SCC organotypic cultures, SCC keratinocytes invaded into the matrix and displayed an invasion index (I.D.) of 0.71±0.06. where I.D. = 1 – (non-invading area/total area)

8. Expected results and relevant corresponding milestones

The proposal is based on our preliminary data and intends to define if the BDNF/TrkB axis dysregulation is involved in cutaneous cancerogenesis in aged people to identify new prognostic biomarkers and therapeutic approaches in SCC management strategy.

BDNF/TrkB dysregulation confers aggressive phenotypes underpinned by their resistance to chemotherapeutic agents[24] and is implicated in tumor relapse in breast cancer and HNSCC[25–

[27]. Up to date, no satisfactory prognostic biomarkers for primary cutaneous SCC or diagnostic biomarkers for relapse has been proposed[3,4]. The correlation between BDNF/TrkB axis protein expression in SCC specimens and histopathological characteristics associated with local recurrence and metastasis, and clinical data allow verifying if these proteins may be relevant as prognostic biomarkers.

Achieving this goal is of outmost clinical importance for SCC diagnosis and therapy. Identifying biomarkers associated with SCC relapse or poor prognosis will help to outline a suitable SCC work-up and management strategy.

Performing experiments using the organotypic models with different cell type and media compositions will allow to define the relevance of BDNF/TrkB in SCC initiation. Moreover, studying the effect of a TrkB specific inhibitor in cell culture and 3D organotypic systems will verify if the BDNF/TrkB axis may be a pharmacologic target for SCC therapy.

Identifying potential therapeutic targets will represent the basis for further clinical therapeutic studies of safety and efficacy.

Therefore, the study will contribute to reduce poor clinical outcomes and may have relevant positive socio-economic effects for the National Health System.

Milestones:

- Identification of biomarkers associated with SCC relapse or poor prognosis will help to outline a suitable SCC work-up and management strategy **(Month 12)**
- Assessment of therapeutic effects of pharmacological compound in pre-clinical 3D SCC organotypic models **(Month 12)**

9. References and relevant publications by the research group, already available

References (publications by research group are underlined)

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2. Madan, V., Lear, J. T. & Szeimies, R. Non-melanoma skin cancer. *Lancet (London, England)* **375,** 673–85 (2010).
3. Stratigos, A. *et al.* Diagnosis and treatment of invasive squamous cell carcinoma of the skin: European consensus-based interdisciplinary guideline. *Eur. J. Cancer* **51,** 1989–2007 (2015).
4. Que, S. K. T., Zwald, F. O. & Schmults, C. D. Cutaneous squamous cell carcinoma: Incidence, risk factors, diagnosis, and staging. *J. Am. Acad. Dermatol.* **78,** 237–247 (2018).
5. Christensen, S. R. Recent advances in field cancerization and management of multiple cutaneous squamous cell carcinomas. *F1000Research* **7,** 690 (2018).
6. Dotto, G. P. Multifocal epithelial tumors and field cancerization: Stroma as a primary determinant. *J. Clin. Invest.* **124,** 1446–1453 (2014).
7. Loaiza, N. & Demaria, M. Cellular senescence and tumor promotion: Is aging the key? *Biochim. Biophys. Acta* **1865,** 155–67 (2016).
8. van Deursen, J. M. The role of senescent cells in ageing. *Nature* **509,** 439–46 (2014).
9. Tinaburri, L. *et al.* miR-200a Modulates the Expression of the DNA Repair Protein OGG1 Playing a Role in Aging of Primary Human Keratinocytes. *Oxid. Med. Cell. Longev.* **2018,** 1–17 (2018).
10. Radin, D. P. & Patel, P. BDNF: An Oncogene or Tumor Suppressor? *Anticancer Res.* **37,** 3983–3990 (2017).
11. Lin, C.-Y. *et al.* Brain-derived neurotrophic factor promotes VEGF-C-dependent lymphangiogenesis by suppressing miR-624-3p in human chondrosarcoma cells. *Cell Death Dis.* **8,** e2964 (2017).

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14. Cordisco, S. *et al.* Bmi-1 reduction plays a key role in physiological and premature aging of primary human keratinocytes. *J. Invest. Dermatol.* **130,** 1048–1062 (2010).
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16. Totaro, A. *et al.* YAP/TAZ link cell mechanics to Notch signalling to control epidermal stem cell fate. *Nat. Commun.* **8,** 15206 (2017).
17. Negri, V. A. *et al.* Delta-like 1-mediated cis-inhibition of Jagged1/2 signalling inhibits differentiation of human epidermal cells in culture. *Sci. Rep.* **9,** 1–11 (2019).
18. Zanconato, F., Cordenonsi, M. & Piccolo, S. YAP and TAZ: a signalling hub of the tumour microenvironment. *Nat. Rev. Cancer* **19,** 454–464 (2019).
19. Kim, N. G., Koh, E., Chen, X. & Gumbiner, B. M. E-cadherin mediates contact inhibition of proliferation through Hippo signaling-pathway components. *Proc. Natl. Acad. Sci. U. S. A.* **108,** 11930–11935 (2011).
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24. Lee, J., Jiffar, T. & Kupferman, M. E. A novel role for BDNF-TrkB in the regulation of chemotherapy resistance in head and neck squamous cell carcinoma. *PLoS One* **7,** 1–11 (2012).
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Our unpublished manuscripts:

Dellambra E., Cordisco S., Proto V., Nicodemi E.M., Didona B., Cesario C., Pisaneschi E., Teson M., Castiglia D., Guerra L. Fibroblasts isolated from nontumoral areas of palmoplantar keratoderma in patients carrying *RSPO1* mutation display a cancer-associated phenotype. submitted to Journal of the European Academy of Dermatology and Venereology

Tinaburri L., Valente C., Teson M., Cordisco S., Guerra L., Dellambra E. The secretome of aged fibroblasts promotes EMT-like phenotype in primary keratinocytes from elderly donors through BDNF-TrkB axis. submitted to Journal of Investigative Dermatology

(max 8 pages)

PERSONNEL INVOLVED IN THE RESEARCH

Name and date of birth	Role on Project	Fellowship required	Effort on project (%)	Present position
Elena Dellambra 29/04/1968	Coordination of the project and monitoring of the activities of internal and external collaborators	-	30%	Senior scientist
Cristina Failla 11/07/1964	Collection of clinical and histological data, supervisoring of Task 1 activities	-	10%	Senior scientist
Massimo Teson 28/03/1969	Performing cell culture activities	-	30%	Technician
Ylenia Minafò 07/01/1989	Performing activities of Tasks1-3	25.000,00€	100%	Post-doc
Maurizio Nudo 26/12/1963	Patient management	-	5%	Clinician
Luca Fania 10/09/1982	Patient management and collection of clinical data	-	5%	Clinician
Enzo Palese 07/04/1973	Patient management	-	5%	Clinician
Damiano Abeni 09/08/1956	Statistical study	-	5%	Epidemiologist
Simona Mastroeni 25/03/1973	Statistical study	-	10%	Statistician
Francesca Ricci 06/04/1976	PerformingTNM classification	-	5%	Anatomopathologist

The study involves also the participation of Policlinico Tor Vergata (Dr. **Elena Campione**) for patient enrollment. The clinical team will also work in concert with clinicians of the section of Rome of the "Lega Italiana per la Lotta contro i Tumori" (LILT) (Dr. **Elena Mari** and **Sara Tambone**).

DESCRIPTION OF THE WORK FOR EVERY UNIT OF PERSONNEL

Dr. **Elena Dellambra** will coordinate the project and monitor the activities of internal and external collaborators. Having long-lasting experience in keratinocyte biology and expertise in gene transfer in primary keratinocyte stem cells, she will be responsible for functional and pharmacological studies.

Dr. **Cristina M. Failla** will collaborate at this project due to her studies in inflammation and tumor angiogenesis. She will be mainly involved in the activity of Task 1.

Massimo Teson is a technician with 20 years' experience in skin cell and 3D organotypic cultures and will follow the cell culture activities and the standardization of methods described in Tasks 2 and 3.

Dr. **Ylenia Minafò** is a post-doc with specific proven expertise in molecular and cell biology. She will be in charge of collecting clinical and histological data and performing immunohistochemical analysis (Task 1) and functional and pharmacological studies (Tasks 2 and 3).

Patients with SCC will be visited in "Dermatologic surgery" by Dr. **Maurizio Nudo**, head of the Unit, and "Non melanoma skin cancer" clinic by Dr. **Luca Fania** and **Enzo Palese** at IDI-IRCCS. They are dermatologists with strong know-how in skin cancer patient management.

The Histopathology Department at IDI-IRCCS (Prof. G. Annessi and Dr. **Francesca Ricci**) has a collection of more than 2000 specimens of SCCs. Dr. Ricci will be in charge for TNM classification of SCC (Task 1) and for provide fresh samples for cellular and molecular assays (Tasks 2 and 3).

To perform the project the team will work in concert with the Epidemiology Laboratory at IDI-IRCCS (Dr. **Damiano Abeni** and Dr. **Simona Mastroeni**) for clinical data analysis and elaboration (Task 1).

A collection of 150 samples of nitrogen-frozen primary human keratinocytes and fibroblasts is available in the PI laboratory. Four cell lines from SCC (SCC4, SCC12, SCC13, SCC15) and two CAF samples are also available.

Budget Form /year

1. research costs	€ 45.125,00
2. instruments	€ 0,00
3. indirect costs (5%)	€ 2.375,00
4. **Subtotal**	**€ 47.500,00**
5. overheads (5%)	€ 2.500,00
6. Fellowships	€ 25.000,00
7. **Total**	**€ 75.000,00**

Justifications

Itemized research costs

1. research costs	Cell culture media, serum, growth factors	€ 12.000,00
	Collagen/Matrigel (3D models)	€ 8.000,00
	Disposable plasticware	€ 3.000,00
	Antibodies	€ 10.000,00
	Chemicals/Solvents	€ 3.000,00
	Revelation kits	€ 2.000,00
	Publication costs	€ 4.000,00
	Registration fees and travels for meeting	€ 3.125,00
6. Fellowships	A post-doctoral fellowship for Dr. Y. Minafò	€ 25.000,00

EXISTING/PENDING SUPPORT

Ministry of Health - Ricerca corrente € 10.000,00

SUGGESTED REVIEWERS (MAX 3)

Dr. Barbara Bellei
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Istituto Dermopatico dell'Immacolata-IRCCS, Rome, Italy.
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BIOETHICAL REQUIREMENT

1. Human experimentation **YES** – please provide clearance from the competent ethical committee as addendum A

2. Animal experimentation **NOT** – please include a statement as_addendum B specifying which regulations the proposed research meets

Declaration

I shall confirm to the Declaration of Helsinki in its latest version.
I shall also apply the Bioethics Convention of the Council of Europe.
In implementing the proposed research, I shall adhere most strictly to all existing ethical and safety provisions applicable.
Before start of the research, I shall obtain clearance from the competent ethical committee in case of involvement of human subjects in the research and /or in case of other ethical implications.
I shall conform with all regulations protecting the animals used for research purpose.

Date: February 13th 2020 Name of PI **Elena Dellambra** signature Elena Dell

Principal investigator's signature Elena Dell

Authorized Administrative Official's signature

FONDAZIONE LUIGI MARIA MONTI
Antonio Maria Leozappa
Presidente e R.L.

Date 17 FEB. 2020

Si autorizza al trattamento dei dati ai sensi del d.lgs. 196/2003

OK
GB