# 1 - General information

**Project code:** PNRR-MCNT1-2023-12377359

**Project topic:** E1) Malattie croniche non trasmissibili (MCnT2) ad alto impatto sui sistemi sanitari e socio-assistenziali: innovazioni in campo diagnostico

**PI / Coordinator:** Licitra Lisa Francesca Linda

**Applicant Institution:** Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano

**Call section:** Malattie Croniche non Trasmissibili (MCnT1) ad alto impatto sui sistemi sanitari e socio-assistenziali

**Proposal title:** Matching primary tumor and blood gene expression analysis of HPV-negative squamous head and neck cancers (BIOMATCH - Head and Neck)

**Duration in months:** 24

**MDC primary:** Oncologia

**MDC secondary:** Otorinolaringoiatria e Odontoiatria

**Project Classification IRG:** Oncology 2 - Translational Clinical

**Project Classification SS:** Cancer Biomarkers - CBSS

**Project Keyword 1:** The use of specific assays or global molecular profiling to identify novel biomarkers based on DNA, RNA, protein, lipids, or metabolites obtained from tumor tissue or bodily fluids

**Project Request:** **Animals:** ☐ **Humans:** ☒ **Clinical trial:** ☐

**Project total financing request to the MOH: €** 1.000.000

**Free keywords:** Prognostic factors; Transcriptomics; Circulating biomarkers; Head and neck cancer

## *Declarations*

In case of a Synergy grant application 'Principal Investigator'(PI) means 'corresponding Principal Investigator on behalf of all Principal Investigators', and 'Host Institution' means 'corresponding Host Institution'.

| | |
|---|---|
| 1) The Principal Investigator declares to have the written consent of all participants on their participation and on the content of this proposal, as well as of any researcher mentioned in the proposal as participating in the project (either as other PI, team member or collaborator). | ☒ |
| 2) The Principal Investigator declares that the information contained in this proposal is correct and complete. | ☒ |
| 3) The Principal Investigator declares that all parts of this proposal comply with ethical principles (including the highest standards of research integrity — as set out, for instance, in the European Code of Conduct for Research Integrity — and including, in particular, avoiding fabrication, falsification, plagiarism or other research misconduct). | ☒ |
| 4) The Principal Investigator is only responsible for the correctness of the information relating to his/her own organisation. Each applicant remains responsible for the correctness of the information related to him and declared above. | ☒ |

**Personal data protection**

| | |
|---|---|
| **Project Code:** PNRR-MCNT1-2023-12377359 | **Call section:** Malattie Croniche non Trasmissibili (MCnT1) ad alto impatto sui sistemi sanitari |
| **Applicant Institution:** Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano | **Applicant/PI Coordinator:** Licitra Lisa Francesca Linda |

The assessment of your grant application will involve the collection and processing of personal data (such as your name, address and CV), which will be performed pursuant to Regulation (EC) No 45/2001 on the protection of individuals with regard to the processing of personal data by the Community institutions and bodies and on the free movement of such data. Unless indicated otherwise, your replies to the questions in this form and any personal data requested are required to assess your grant application in accordance with the specifications of the call for proposals and will be processed solely for that purpose. Details concerning the purposes and means of the processing of your personal data as well as information on how to exercise your rights are available in the privacy statement. Applicants may lodge a complaint about the processing of their personal data with the European Data Protection Supervisor at any time.

### *Abstract*

Head and Neck cancers (HNC) are the 7th most frequent cancer types worldwide, prevalently constituted by squamous cell carcinomas (HNSCC) which can arise from different subsites, such as oral cavity, oropharynx, hypopharynx and larynx. Almost 70% of cases are diagnosed at locoregionally-advanced stage, but only 50% of patients (pts) achieve long-term survival with surgery and/or concomitant platinum-based chemoradiation (cCTRT) with curative intent. Currently, the choice of treatments is based on two macroscopic factors (stage of disease and primary tumor subsite) with a ¿one-fits-all¿ approach, without taking into account the molecular heterogeneity of HNSCC subtypes. Human Papillomavirus (HPV) infection-related oropharyngeal cancers have better prognosis compared with HPV-negative ones, but the prognostic impact of HPV infection is irrelevant in non-oropharyngeal subsites. Therefore, the molecular characterization of HPV-negative HNSCC in the clinical practice is an unmet clinical need.
In previously published works, our group at Fondazione IRCCS Istituto Nazionale dei Tumori (INT - Milan, Italy) demonstrated that the differential gene expression (GE) signatures on solid tumor specimens allow to stratify HNSCCs, independently from the subsites, into 6 groups with predictive and prognostic value. However, GE analysis is not an easily applicable tool in the clinical practice. Our central hypothesis is that a machine learning model can match for each patient the GE obtained from primary cancer specimens with the corresponding GE found on liquid biopsies at baseline (before treatment) and with digital pathology images of the corresponding tumor at diagnosis, discovering the pathological features associated with each prognostic HNSCC cluster.
Here we present the research project BIOMATCH that will be coordinated by two referral academic cancer centers in Northern Italy (INT - affiliated with the University of Milan) and Southern Italy (Azienda Ospedaliera Universitaria di Sassari, AOUSS) with an expertise in clinical and translational research of patients with HNSCC. Both institutions will be actively involved in the collection of data and materials (specimens/liquid biopsies/digital pathology images) from 15 Italian centers. Overall, 300 patients will be included, and the project will last 2 years.
With the capillary diffusion of digital pathology in the next years, the results of the BIOMATCH study will increase the accessibility of clinicians to the prognostic information correlated with the heterogeneity of HNSCC, and also it will open to its applicability in clinical trials for precision oncology.

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In order to best review your application, do you agree that the above non-confidential proposal title and abstract can be used, without disclosing your identity, when contacting potential reviewers? Yes

# 2 - Participants & contacts

| | |
|---|---|
| **Project Code:** PNRR-MCNT1-2023-12377359 | **Call section:** Malattie Croniche non Trasmissibili (MCnT1) ad alto impatto sui sistemi sanitari |
| **Applicant Institution:** Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano | **Applicant/PI Coordinator:** Licitra Lisa Francesca Linda |

## Operative Units

| Institution that perform as UO | CF Institution | Department / Division / Laboratory | Role in the project | Southern Italy | SSN |
|---|---|---|---|---|---|
| 1 - Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano | 80018230153 | Medical Oncology | Coordinator; "omics" analyses; model development and data integration | | X |
| 2 - Azienda Ospedaliero Universitaria Sassari | 02268260904 | Unità Operativa di Oncologia Medica | Responsible of biobanking for Southern Italy; digital pathology | X | X |

## Principal Research Collaborators

| Key Personnel Name | Operative Unit | Role in the project |
|---|---|---|
| 1 - De Cecco Loris | Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano | coPI |
| 2 - Bergamini Cristiana | Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano | Ulteriore collaboratore UO1 |
| 3 - DEGANELLO ALBERTO | Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano | Ulteriore collaboratore UO1 |
| 4 - Bussu Francesco | Azienda Ospedaliero Universitaria Sassari | Responsabile UO2 |
| 5 - FADDA GIOVANNI MARIA | Azienda Ospedaliero Universitaria Sassari | Ulteriore collaboratore UO2 |
| 6 Under 40 - Cavalieri Stefano | Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano | Ulteriore collaboratore UO1 (under40) |
| 7 Under 40 - Colombo Elena | Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano | Ulteriore collaboratore UO1 (under40) |

| Key Personnel Name | Co-PI | Resp. CE | Resp. Animal | Birth Date | Gender |
|---|---|---|---|---|---|
| 1 - De Cecco Loris | X | | | 03/04/1973 | M |
| 2 - Bergamini Cristiana | | | | 08/02/1976 | F |
| 3 - DEGANELLO ALBERTO | | | | 10/01/1973 | M |
| 4 - Bussu Francesco | | | | 04/04/1974 | M |
| 5 - FADDA GIOVANNI MARIA | | | | 22/08/1973 | M |
| 6 Under 40 - Cavalieri Stefano | | | | 06/01/1988 | M |
| 7 Under 40 - Colombo Elena | | | | 26/05/1985 | F |

## Additional research collaborators under 40 to hire

| Key Personnel Name | Operative Unit | Birth Date | Gender | Role in the project | Degree | Actual Pos. and Inst. |
|---|---|---|---|---|---|---|
| 0 - Bacciu Andrea | Azienda Ospedaliero Universitaria Sassari | 02/07/1990 | M | Additional research collaborator | Master Degree in Biotechnology | Specializzando in Patologia Clinica e Biochimica Clinica - University of Sassary (UNISS) |
| 1 - VARRUCCIU SIMONA | Azienda Ospedaliero Universitaria Sassari | 28/03/1992 | F | Additional research collaborator | Master Degree in Biological Sciences | Specializzanda in Patologia Clinica e Biochimica Clinica - University of Sassary (UNISS) |

# 2.1 Administrative data of participating

## Operative Unit Number 1:

**Address:** ¿ Fondazione IRCCS Istituto Nazionale dei Tumori
¿ Dipartimento di Oncologia ed Ematologia; SC Oncologia Medica 3 - Tumori testa-collo
¿ Via Venezian 1
¿ 20133 Milan
¿ Italy

**PEC:** direzione.scientifica@pec.istitutotumori.mi.it

## Operative Unit Number 2:

**Address:** ¿ Azienda Ospedaliero Universitaria di Sassari
¿ Dipartimento di Medicina, Chirurgia e Farmacologia, DS Otorinolaringoiatria
¿ Viale San Pietro 43
¿ 07100 Sassari
¿ Italy

**PEC:** protocollo@pec.aou.ss.it

## Operative Unit Number 3:

**Address:** NA

**PEC:** NA

## Operative Unit Number 4:

**Address:** NA

**PEC:** NA

## Operative Unit Number 5 (self financing):

**Address:** NA

**PEC:** NA

## 2.2 Principal Investigator (PI) Profile

**Last Name:** Licitra
**First Name:** Lisa Francesca Linda
**Last name at birth:**
**Gender:** F

**Title:** Principal investigator
**Country of residence:** ITALY
**Nationality:** italiana
**Country of Birth:** ITALY
**Date of birth:** 24/09/1959
**Place of Birth:** Milano

**Official H index (Scopus or Web of Science):** 68.0

**Scopus Author Id:**7004094069 **ORCID ID:**0000-0003-0623-4118 **RESEARCH ID:**C-6271-2017

*Contact address*

**Current organisation name:** Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano
**Current Department / Faculty / Institute / Laboratory name:** Medical Oncology
**Street:** via Venezian 1
**Postcode / Cedex:** 20133 **Town:** Milano
**Phone:**+393482625482 **Phone 2:**

**Education / training**

| Educational institution and location | Degree | Field of study | From year | To year |
|---|---|---|---|---|
| University of Parma, Italy | Specialization / Specializzazione | Postgraduate board in Clinical Oncology, cum laude | 1984 | 1987 |
| Università degli Studi di Milano¿, Milan, Italy | Single-cycle master's degree / Laurea magistrale a ciclo unico | Degree in Medicine, cum laude | 1978 | 1984 |

**Personal Statement:**

Lisa Licitra, MD, is a globally recognized Medical Oncologist and Professor of the University of Milan dedicated since three decades to both clinical and translational research on cancers of the head and neck district (HNC). In this context, she leads a group of highly committed professionals focused on the multidisciplinary management of these complex patients. In this project she will supervise the Team activities and will provide her expertise to ensure the full development of the project as expected.

### Positions and honors

**Positions**

| Institution | Division / Research group | Location | Position | From year | To year |
|---|---|---|---|---|---|
| Fondazione IRCCS ¿Istituto Nazionale dei Tumori, Milan, Italy<br>University of Milan | Division of Medical Oncology Department of Oncology and Hemato-oncology | Via Venezian 1, 20133, Milano, Italy | Director of Head and Neck Cancer Medical Oncology Department 3 & Associated Professor | 2016 | 2023 |
| Fondazione IRCCS ¿Istituto Nazionale dei Tumori, Milan, Italy. | Division of Medical Oncology | Via Venezian 1, 20133, Milan, Italy | Director ad interim of Head and Neck Cancer Medical Oncology Department 3 | 2015 | 2016 |
| Fondazione IRCCS ¿Istituto Nazionale dei Tumori¿, Milan, Italy. | Division of Medical Oncology | Via Venezian 1, 20133, Milan, Italy | Director of the departimental medical oncology unit devoted to patients with Head and neck Cancer | 2011 | 2015 |
| Fondazione IRCCS ¿Istituto Nazionale dei Tumori¿, Milan, Italy. | Division of Medical Oncology | Via Venezian 1, 20133, Milan, Italy | Director of Head and Neck medical oncology unit | 2007 | 2011 |
| Fondazione IRCCS ¿Istituto Nazionale dei Tumori¿, Milan, Italy. | Division of Medical Oncology | Via Venezian 1, 20133, Milan, Italy | Medical Oncologist involved in head and neck cancer patients (with functional responsability from 2002) | 1997 | 2007 |
| Fondazione IRCCS ¿Istituto Nazionale dei Tumori¿, Milan, Italy. | Division of Medical Oncology ¿A¿ | Via Venezian 1, 20133, Milan, Italy | Full staff position as a Medical Oncologist | 1994 | 1996 |
| Fondazione IRCCS ¿Istituto Nazionale dei Tumori¿, Milan, Italy | Division of Medical Oncology ¿A¿ | Via Venezian 1, 20133 Milan, Italy | Non full staff position as a Medical Oncologist | 1992 | 1994 |
| Fondazione IRCCS ¿Istituto Nazionale dei Tumori¿, Milan, Italy | Division of Medical Oncology ¿C¿ | Via Venezian 1, 20133 Milan, Italy | Non full staff position as a Medical Oncologist | 1991 | 1992 |
| Fondazione IRCCS ¿Istituto Nazionale dei Tumori¿, Milan, Italy | Division of Medical Oncology ¿A¿ | Via Venezian 1, 20133 Milan, Italy | Non full staff position as a Medical Oncologist | 1989 | 1991 |

**Other awards and honors**

¿ 2008 Honorary Membership Award ESTRO

¿ 2010 Award ¿Guido Venosta 2010¿, for important head and neck cancer studies that, with a multidisciplinary approach, brought to new personalized treatments

¿ 2010 Honorary Membership Award ¿Israeli Society of Head and Neck Surgery and Oncology¿

¿ 2015 Hubertus Wald Award University of Hamburg ¿in recognition of her outstanding achievements in the field of cancer research and cancer therapy¿

¿ 2021: ESMO Award 2021

**Other CV informations**

Lisa Licitra is Board-certified in medical oncology, with special expertise in the treatment of head and neck cancers. She is currently Chief of the Head and Neck Cancer Medical Oncology Unit and Head & Neck research program at the Istituto Nazionale Tumori in Milan, Italy.

Since more than 30 years she is involved in clinical and translational research in the field of head and neck cancer. She is

conducting as PI several clinical both sponsored studies but more importantly academic studies with original translational research.

She is actively involved in international education and research programs for head and neck cancer through EORTC, ESMO; ESTRO as well as national societies like AIOM, AIOCC.

**Selected peer-reviewed publications of the PI valid for minimum expertise level**

| Title | Type | Pag | Vol | Year | DOI | PMID | Cit.** | P.* |
|---|---|---|---|---|---|---|---|---|
| The EORTC module for quality of life in patients with thyroid cancer: Phase III | Article | 197-207 | 24 | 2017 | 10.1530/ERC-16-0530 | 28223365 | 23 | L |
| Big Data in Head and Neck Cancer | Article | NOT_FOUND | 19 | 2018 | 10.1007/s11864-018-0585-2 | 30361937 | 25 | L |
| Individualised quality of life as a measure to guide treatment choices in squamous cell carcinoma of the head and neck | Review | 18-23 | 52 | 2016 | 10.1016/j.oraloncology.2015.10.020 | 26578389 | 25 | F |
| Activity and safety of afatinib in a window preoperative EORTC study in patients with squamous cell carcinoma of the head and neck (SCCHN) | Article | 985-991 | 29 | 2018 | 10.1093/annonc/mdy013 | 29346507 | 25 | L |
| Quality-of-life priorities in patients with thyroid cancer: A multinational european organisation for research and treatment of cancer phase I study | Article | 1605-1613 | 26 | 2016 | 10.1089/thy.2015.0640 | 27605136 | 29 | L |
| Therapeutic strategies in the management of patients with metastatic anaplastic thyroid cancer: Review of the current literature | Review | 224-228 | 25 | 2013 | 10.1097/CCO.0b013e32835ff44b | 23493194 | 29 | L |
| Treatment-related outcome of oropharyngeal cancer patients differentiated by HPV dictated risk profile: A Tertiary Cancer Centre series analysis | Article | 694-699 | 25 | 2014 | 10.1093/annonc/mdu004 | 24510315 | 30 | L |
| Patients with adenoid cystic carcinomas of the salivary glands treated with lenvatinib: Activity and quality of life | Article | 1888-1894 | 126 | 2020 | 10.1002/cncr.32754 | 32031693 | 36 | L |
| International validation of the revised European Organisation for Research and Treatment of Cancer Head and Neck Cancer Module, the EORTC QLQ-HN43: Phase IV | Article | 1725-1737 | 41 | 2019 | 10.1002/hed.25609 | 30636188 | 42 | L |
| Does a multidisciplinary team approach in a tertiary referral centre impact on the initial management of head and neck cancer? | Article | 54-57 | 54 | 2016 | 10.1016/j.oraloncology.2016.01.001 | 26774920 | 44 | L |
| A randomized, phase 2 study of cetuximab plus cisplatin with or without paclitaxel for the first-line treatment of patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck | Article | 2820-2826 | 28 | 2017 | 10.1093/annonc/mdx439 | 28950305 | 48 | L |
| A phase II study of sorafenib in recurrent and/or metastatic salivary gland carcinomas: Translational analyses and clinical impact | Article | 158-165 | 69 | 2016 | 10.1016/j.ejca.2016.09.022 | 27821319 | 52 | L |

| Title | Type | Pag | Vol | Year | DOI | PMID | Cit.** | P.* |
|---|---|---|---|---|---|---|---|---|
| Preoperative chemotherapy in advanced resectable OCSCC: Long-term results of a randomized phase III trial | Article | 462-466 | 25 | 2014 | 10.1093/annonc/mdt555 | 24401930 | 64 | L |
| Evaluation of the benefit and use of multidisciplinary teams in the treatment of head and neck cancer | Review | 73-79 | 59 | 2016 | 10.1016/j.oraloncology.2016.06.002 | 27424185 | 73 | F |
| Clinical activity of androgen deprivation therapy in patients with metastatic/relapsed androgen receptor-positive salivary gland cancers | Article | 724-731 | 38 | 2016 | 10.1002/hed.23940 | 25522335 | 85 | L |
| Evidence-based treatment options in recurrent and/or metastatic squamous cell carcinoma of the head and neck | Review | NOT_FOUND | 7 | 2017 | 10.3389/fonc.2017.00072 | 28536670 | 100 | L |
| Buparlisib and paclitaxel in patients with platinum-pretreated recurrent or metastatic squamous cell carcinoma of the head and neck (BERIL-1): a randomised, double-blind, placebo-controlled phase 2 trial | Article | 323-335 | 18 | 2017 | 10.1016/S1470-2045(17)30064-5 | 28131786 | 146 | L |
| Durvalumab with or without tremelimumab in patients with recurrent or metastatic head and neck squamous cell carcinoma: EAGLE, a randomized, open-label phase III study | Article | 942-950 | 31 | 2020 | 10.1016/j.annonc.2020.04.001 | 32294530 | 161 | L |
| Impact of tumor HPV status on outcome in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck receiving chemotherapy with or without cetuximab: Retrospective analysis of the phase iii extreme trial | Article | 801-807 | 25 | 2014 | 10.1093/annonc/mdt574 | 24577117 | 171 | L |
| Predictive value of epidermal growth factor receptor expression for first-line chemotherapy plus cetuximab in patients with head and neck and colorectal cancer: Analysis of data from the EXTREME and CRYSTAL studies | Article | 1161-1168 | 49 | 2013 | 10.1016/j.ejca.2012.11.018 | 23265711 | 131 | F |

* Position: F=First L=Last C=Correspondent O=Other N=Not applicable

** Autocertificated

## Selected peer-reviewed publications of the PI for the evaluation CV

| Title | Type | Pag | Vol | Year | DOI | PMID | Cit.** |
|---|---|---|---|---|---|---|---|
| Afatinib versus methotrexate as second-line treatment in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck progressing on or after platinum-based therapy (LUX-Head & Neck 1): An open-label, randomised phase 3 trial | Article | 583-594 | 16 | 2015 | 10.1016/S1470-2045(15)70124-5 | 25892145 | 308 |
| Cisplatin and fluorouracil with or without panitumumab in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck (SPECTRUM): An open-label phase 3 randomised trial | Article | 697-710 | 14 | 2013 | 10.1016/S1470-2045(13)70181-5 | 23746666 | 372 |

| Title | Type | Pag | Vol | Year | DOI | PMID | Cit.** |
|---|---|---|---|---|---|---|---|
| Descriptive epidemiology of sarcomas in Europe: Report from the RARECARE project | Article | 684-695 | 49 | 2013 | 10.1016/j.ejca.2012.09.011 | 23079473 | 394 |
| Nivolumab vs investigator's choice in recurrent or metastatic squamous cell carcinoma of the head and neck: 2-year long-term survival update of CheckMate 141 with analyses by tumor PD-L1 expression | Article | 45-51 | 81 | 2018 | 10.1016/j.oraloncology.2018.04.008 | 29884413 | 457 |
| Cabozantinib in progressive medullary thyroid cancer | Article | 3639-3646 | 31 | 2013 | 10.1200/JCO.2012.48.4659 | 24002501 | 812 |
| Pembrolizumab versus methotrexate, docetaxel, or cetuximab for recurrent or metastatic head-and-neck squamous cell carcinoma (KEYNOTE-040): a randomised, open-label, phase 3 study | Article | 156-167 | 393 | 2019 | 10.1016/S0140-6736(18)31999-8 | 30509740 | 846 |
| Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study | Article | 1915-1928 | 394 | 2019 | 10.1016/S0140-6736(19)32591-7 | 31679945 | 1241 |
| Nivolumab for recurrent squamous-cell carcinoma of the head and neck | Article | 1856-1867 | 375 | 2016 | 10.1056/NEJMoa1602252 | 27718784 | 3037 |
| Durvalumab with or without tremelimumab in patients with recurrent or metastatic head and neck squamous cell carcinoma: EAGLE, a randomized, open-label phase III study | Article | 942-950 | 31 | 2020 | 10.1016/j.annonc.2020.04.001 | 32294530 | 161 |
| Impact of tumor HPV status on outcome in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck receiving chemotherapy with or without cetuximab: Retrospective analysis of the phase iii extreme trial | Article | 801-807 | 25 | 2014 | 10.1093/annonc/mdt574 | 24577117 | 171 |

** Autocertificated

**Grant**

| Funded by Institution | Researcher inst. where grant is/was performed | Year | Title | Position in Projects | Fund (euro) | Source website grant listed |
|---|---|---|---|---|---|---|
| Ministery of Health | Fondazione IRCCS Istituto Nazionale Tumori | 2017 | Det 374 DG 21/12/16 ¿ ¿Identification of head and neck cancer and HPV-related infections by profiling volatile organic compounds in exhaled breath¿ | Coordinator | 225.570,00 | - |
| AIRC | Fondazione Istituto Nazionale tumori | 2014 | Translational research to a practice changing global prospective study of androgen deprivation in salivary gland cancers | Coordinator | 424.000,00 | - |

| Funded by Institution | Researcher inst. where grant is/was performed | Year | Title | Position in Projects | Fund (euro) | Source website grant listed |
|---|---|---|---|---|---|---|
| Italian Ministry of Health ¿ Alleanza contro il cancro | Fondazione IRCCS Istituto Nazionale dei Tumori | 2021 | WP 17 - Studio multidimensionale sui meccanismi di risposta e di tossicità alla radioterapia in neoplasie primariamente (chemio)radiotrattate ¿ RADECISION Programma nazionale di oncologia personalizzata per gli IRCCS della rete ACC (codice progetto RCR-2021-23671213) | Coordinator | 37.800,00 | - |
| ERAPERMED2019 | Fondazione IRCCS Istituto Nazionale Tumori | 2020 | Supporting Personalized Treatment Decisions in Head and Neck Cancer through Big Data¿ Cofund within the Horizon H2020 European programme | Coordinator | 1.518.790,00 | https://erapermed.isciii.es/funded-projects/ |
| Horizon 2020 Framework Programme | Fondazione IRCCS Istituto Nazionale Tumori | 2020 | BD4QoL - Big Data Models and Intelligent tools for Quality of Life monitoring and participatory empowerment of head and neck cancer survivors | Coordinator | 4.985.975,00 | - |
| European Commission ID 724161 | Fondazione IRCCS Istituto Nazionale Tumori | 2016 | Joint Action on rare cancers | Collaborator | 1.499.848,00 | - |
| H2020-PHC-30-689715 | Fondazione IRCCS Istituto Nazionale tumori | 2015 | Big data and models for personalized head and neck cancer decision support-BD2 Decide | Collaborator | 835.000,00 | - |
| Translational Cancer Research (TRANSCAN) Joint Transnational Call for Proposals 2013 (JTC 2013) | Fondaziobe IRCCS Istituto Nazionale Tumori | 2015 | ¿DietINT Project - A randomized phase II study for tertiary prevention of squamocellular cancer of head and neck (SCCHN) with a dietary intervention¿ | Coordinator | 26.000,00 | - |
| SWISS BRIDGE Award | Fondazione IRCCS Istitutot Nazionale tumori | 2014 | Health and economic outcomes of two different follow up strategies in effectively cured advanced head and neck cancer | Coordinator | 102.680,00 | - |
| AIRC | Fondazione IRCCS Istituto Nazionale tumori | 2010 | Innovative Approach for Discovery and Development of Promising Targeted Agents in Head & Neck Cancer | Coordinator | 130.000,00 | - |

## 2.3 CO-PI Profile

**Last Name:** De Cecco
**First Name:** Loris
**Last name at birth:**
**Gender:** M

**Title:** coPI
**Nationality:** Italiana
**Date of birth:** 03/04/1973
**Country of residence:** ITALY
**Country of Birth:** SWITZERLAND
**Place of Birth:** Svizzera

**Official H index (Scopus or Web of Science):** 33.0

**Scopus Author Id:**8948006200 **ORCID ID:**0000-0002-7066-473X **RESEARCH ID:**K-7036-2016

*Contact address*

**Current organisation name:** Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano
**Current Department / Faculty / Institute / Laboratory name:** Medical Oncology
**Street:** via Amadeo
**Postcode / Cedex:** 20133
**Town:** Milano
**Phone:**+393337658753
**Phone 2:**

**Education / training**

| Educational institution and location | Degree | Field of study | From year | To year |
|---|---|---|---|---|
| Open University - London UK | PhD | Gene-expression profiling on breast cancer to identify pathways associated to response to targets therapies (tamoxifen and trastuzumab); experimental and bioinformatics activities for the project | 2004 | 2009 |
| University of Trieste | Single-cycle master's degree / Laurea magistrale a ciclo unico | Degree in Biological Sciences. Molecular Biology. | 1992 | 1998 |

**Personal Statement:**
He will be responsible of the experimental activities on tissue specimens and liquid biopses; he will design and plan the experimental activities; he will oversee the bioinformatics analyses including the validation of the signatures. As co-PI, he will maintain the relationships between experimental/bioinformatics collaborators and clinicians.

## Positions and honors

**Positions**

| Institution | Division / Research group | Location | Position | From year | To year |
|---|---|---|---|---|---|
| Fondazione IRCCS Istituto Nazionale Tumori, Milan | Department of Experimental Oncology | Via amadeo 42, 20133, Milan | Staff scientist | 2023 | 2023 |
| Fondazione IRCCS Istituto Nazionale Tumori, Milan | Department of Applied Research and Technology Development / Platform of Integrated Biology | via Amadeo 42, 20133, Milan, Italy | Reasearcher | 2009 | 2022 |
| Fondazione IRCCS Istituto Nazionale dei Tumori | Department of Experimental Oncology and Molecular Medicine | Via Venezian 1, 20133, Milan | PhD Student | 2008 | 2009 |
| IFOM (Fondazione FIRC di Oncologia Molecolare) | Molecular genetics of cancer unit | Via Adamello 16, Milan, Italy | PhD Student | 2005 | 2007 |
| IFOM (Fondazione FIRC di Oncologia Molecolare), | Molecular genetics of cancer unit | Via Amadello 16, Milan, Italy | FIRC and AIRC fellow | 2000 | 2004 |

**Other awards and honors**

2006 Award from Associazione Amici di Milano for original work in De Cecco L, et al Gene expression profiling of advanced ovarian cancer.

**Other CV informations**

Dr DeCecco, Senior scientist at the Integrated Biology of Rare Tumors. ( Fond. IRCCS Istituto Nazionale Tumori). Since 2000 Dr. De Cecco¿s scientific activity has been focused on the analysis of ¿multi-omics¿ NGS data and in bioinformatics underlying the progression and the clinical outcome in tumors. He awarded his PhD in 2009. From then on, he has focused his scientific interest on Head and Neck cancer with the aim at improving patient stratification by capturing heterogeneity in the response to treatment and reflecting factors related to the host and the tumor. His commitment and scienti¿c independence is proven by the publication of more than 140 peer reviewed papers.

**Selected peer-reviewed publications of the Co-PI valid for minimum expertise level**

| Title | Type | Pag | Vol | Year | DOI | PMID | Cit.** | P.* |
|---|---|---|---|---|---|---|---|---|
| An Inflammatory Signature to Predict the Clinical Benefit of First-Line Cetuximab Plus Platinum-Based Chemotherapy in Recurrent/Metastatic Head and Neck Cancer | Article | NOT_FOUND | 11 | 2022 | 10.3390/cells11193176 | 36231138 | 0 | L |
| A microRNA Prognostic Signature in Patients with Diffuse Intrinsic Pontine Gliomas through Non-Invasive Liquid Biopsy | Article | NOT_FOUND | 14 | 2022 | 10.3390/cancers14174307 | 36077842 | 1 | L |
| hacksig: A unified and tidy R framework to easily compute gene expression signature scores | Article | 2940-2942 | 38 | 2022 | 10.1093/bioinformatics/btac161 | 35561166 | 1 | L |
| Complete Response to Nivolumab in Recurrent/Metastatic HPV-Positive Head and Neck Squamous Cell Carcinoma Patient After Progressive Multifocal Leukoencephalopathy: A Case Report | Article | NOT_FOUND | 11 | 2022 | 10.3389/fonc.2021.799453 | 35083153 | 3 | L |

| Title | Type | Pag | Vol | Year | DOI | PMID | Cit.** | P.* |
|---|---|---|---|---|---|---|---|---|
| Prognostic evidence of the mirna-based ovarian cancer signature mirovar in independent datasets | Article | NOT_FOUND | 13 | 2021 | 10.3390/cancers13071544 | 33801595 | 2 | F |
| Clinical Validity of a Prognostic Gene Expression Cluster-Based Model in Human Papillomavirus¿Positive Oropharyngeal Carcinoma | Article | 1666-1676 | 5 | 2021 | 10.1200/PO.21.00094 | 34738049 | 3 | L |
| Tumor biomarkers for the prediction of distant metastasis in head and neck squamous cell carcinoma | Article | NOT_FOUND | 12 | 2020 | 10.3390/cancers12040922 | 32283719 | 10 | L |
| Mining of self-organizing map gene-expression portraits reveals prognostic stratification of HPV-positive head and neck squamous cell carcinoma | Article | NOT_FOUND | 11 | 2019 | 10.3390/cancers11081057 | 31357501 | 15 | L |
| A functional gene expression analysis in epithelial sinonasal cancer: Biology and clinical relevance behind three histological subtypes | Article | 94-101 | 90 | 2019 | 10.1016/j.oraloncology.2019.02.003 | 30846184 | 9 | F |
| Gene signatures and expression of miRNAs associated with efficacy of panitumumab in a head and neck cancer phase II trial | Article | 144-151 | 82 | 2018 | 10.1016/j.oraloncology.2018.05.013 | 29909889 | 11 | L |
| Gene Expression Signatures for Head and Neck Cancer Patient Stratification: Are Results Ready for Clinical Application? | Review | NOT_FOUND | 18 | 2017 | 10.1007/s11864-017-0472-2 | 28474265 | 34 | L |
| Integrative miRNA-Gene expression analysis enables refinement of associated biology and prediction of response to cetuximab in head and neck squamous cell cancer | Article | NOT_FOUND | 8 | 2017 | 10.3390/genes8010035 | 28098823 | 22 | F |
| Are Fusion Transcripts in Relapsed/Metastatic Head and Neck Cancer Patients Predictive of Response to Anti-EGFR Therapies? | Article | NOT_FOUND | 2017 | 2017 | 10.1155/2017/6870614 | 29259349 | 3 | L |
| Functional genomics uncover the biology behind the responsiveness of head and neck squamous cell cancer patients to cetuximab | Article | 3961-3970 | 22 | 2016 | 10.1158/1078-0432.CCR-15-2547 | 26920888 | 54 | L |
| Whole exome sequencing and single nucleotide polymorphism array analyses to identify germline alterations in genes associated with testosterone metabolism in a patient with androgen insensitivity syndrome and early-onset colorectal cancer | Article | 51 | 35 | 2016 | 10.1186/s40880-016-0115-1 | 27267075 | 3 | L |
| Interleukin 21 controls mRNAand MicroRNA expression in CD40-activated chronic lymphocytic leukemia cells | Article | NOT_FOUND | 10 | 2015 | 10.1371/journal.pone.0134706 | 26305332 | 12 | F |
| Head and neck cancer subtypes with biological and clinical relevance: Meta-analysis of gene-expression data | Article | 9627-9642 | 6 | 2015 | 10.18632/oncotarget.3301 | 25821127 | 86 | F |
| Comprehensive gene expression meta-analysis of head and neck squamous cell carcinoma microarray data defines a robust survival predictor | Article | 1628-1635 | 25 | 2014 | 10.1093/annonc/mdu173 | 24827125 | 43 | F |

| Title | Type | Pag | Vol | Year | DOI | PMID | Cit.** | P.* |
|---|---|---|---|---|---|---|---|---|
| Identification of a gene expression driven progression pathway in Myxoid liposarcoma | Article | 5965-5977 | 5 | 2014 | 10.18632/oncotarget.2023 | 25115389 | 15 | F |
| Measuring microRNA expression levels in oncology: From samples to data analysis | Review | 273-287 | 18 | 2013 | 10.1615/CritRevOncog.2013007207 | 23614615 | 23 | F |

* Position: F=First L=Last C=Correspondent O=Other N=Not applicable

** Autocertificated

**Grant**

| Funded by Institution | Researcher inst. where grant is/was performed | Year | Title | Position in Projects | Fund (euro) | Source website grant listed |
|---|---|---|---|---|---|---|
| Fondazione Celeghin | Fondazione IRCCS Istituto Nazionale Tumori | 2015 | Characterization of a circulating miRNA signature in response to targeted therapy in Diffuse Intrinsic Pontine Glioma (DIPG) | Coordinator | 121.000,00 | https://www.fondazioneceleghin.it/progetti-2/ |
| Associazione Bianca Garavaglia | Fondazione IRCCS Istituto Nazionale Tumori | 2018 | circulating microRNA in DIPG patients | Coordinator | 93.000,00 | - |
| ERAPERMED2019 | Fondazione IRCCS Istituto Nazionale Tumori | 2020 | Supporting Personalized Treatment Decisions in Head and Neck Cancer through Big Data | Collaborator | 1.518.790,00 | https://erapermed.isciii.es/funded-projects/ |
| AIRC | Fondazione IRCCS istituto Nazionale Tumori | 2020 | Optimizing biomarkers of immunotherapy response in clinical studies summarizing head and neck cancer natural history | Coordinator | 1.253.000,00 | https://www.airc.it/ |
| AIRC | Fondazione IRCCS Istituto Nazionale Tumori | 2016 | Radiogenomics Framework for Non-Invasive Personalized Medicine in Head and Neck Cancer | Coordinator | 450.000,00 | - |

## 2.3 Research Collaborators n. 2

**Last Name:** Bergamini
**First Name:** Cristiana
**Last name at birth:**
**Gender:** F

**Title:** Ulteriore collaboratore UO1
**Country of residence:** ITALY
**Nationality:** Italiana
**Country of Birth:** ITALY
**Date of birth:** 08/02/1976
**Place of Birth:** Seriate

**Official H index (Scopus or Web of Science):** 23.0

**Scopus Author Id:**8905689200 **ORCID ID:**0000-0001-7616-5125 **RESEARCH ID:**H-9431-2017

*Contact address*

**Current organisation name:** Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano
**Current Department / Faculty / Institute / Laboratory name:** Medical Oncology
**Street:** Fondazione IRCCS Istituto Nazionale dei Tumori di Milano
**Postcode / Cedex:** 20133 **Town:** Milano
**Phone:**+393479690827 **Phone 2:**

**Education / training**

| Educational institution and location | Degree | Field of study | From year | To year |
|---|---|---|---|---|
| Università degli Studi, Milan, Italy | Specialization / Specializzazione | Clinical Oncology | 2005 | 2007 |
| Università degli Studi, Milan, Italy | Single-cycle master's degree / Laurea magistrale a ciclo unico | Medicine | 1996 | 2004 |

**Personal Statement:**

Cristiana Bergamini is a Medical Oncologist and Senior Consultant working at the Head and Neck Medical Oncology Unit (INT) focused on the clinical management of head and neck patients and research. She has been working with Prof. Licitra since 15 years, covering the role of sub-investigator in several Italian and international trials on head and neck cancers. She has also a prominent role in the weekly multidisciplinary discussions with ENT surgeons and radiotherapists. In this study she will ensure the timely and accurate enrollment of cases from the outpatient setting of the Medical Oncology visits.

## Positions and honors

**Positions**

| Institution | Division / Research group | Location | Position | From year | To year |
|---|---|---|---|---|---|
| Fondazione IRCCS Istituto Nazionale tumori | Division of Medical Oncology / Head and Neck Cancer Unit | Via Venezian 1, 20133, Milan, Italy | Associate Physician in Oncology | 2007 | 2023 |

**Other awards and honors**

-

**Grant**

| Funded by Institution | Researcher inst. where grant is/was performed | Year | Title | Position in Projects | Fund (euro) | Source website grant listed |
|---|---|---|---|---|---|---|
| - | - | - | - | Collaborator | 0,00 | - |

## 2.4 Research Collaborators n. 3

**Last Name:** DEGANELLO
**First Name:** ALBERTO
**Last name at birth:**
**Gender:** M

**Title:** Ulteriore collaboratore UO1
**Country of residence:** ITALY
**Nationality:** Italiana
**Country of Birth:** ITALY
**Date of birth:** 10/01/1973
**Place of Birth:** Conselve

**Official H index (Scopus or Web of Science):** 20.0

**Scopus Author Id:**20734160200 **ORCID ID:**0000-0003-1008-7333 **RESEARCH ID:**DTY-2291-2022

*Contact address*

**Current organisation name:** Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano
**Current Department / Faculty / Institute / Laboratory name:** Medical Oncology
**Street:** Viale Giacomo Venezian n.1
**Postcode / Cedex:** 20133
**Town:** Milano
**Phone:**+393203075573
**Phone 2:**

**Education / training**

| Educational institution and location | Degree | Field of study | From year | To year |
|---|---|---|---|---|
| Vrije Universiteit Amsterdam, The Netherlands | PhD | Head and Neck Surgery and Skull Base Surgery | 2013 | 2017 |
| University of Florence | PhD | Head and Neck Surgery | 2008 | 2011 |
| University of Padua | Specialization / Specializzazione | Otolaryngology | 1997 | 2001 |
| University of Padua | Single-cycle master's degree / Laurea magistrale a ciclo unico | Medicine | 1992 | 1997 |

**Personal Statement:**
Alberto Deganello, MD, PhD, is the Director of the ENT Surgery Division of the Fondazione IRCCS Istituto Nazionale dei Tumori and Professor of ENT Surgery at the University of Milan. He took part in more than 20 research projects and has a strong international background. He supports the cooperation in multidisciplinary teams. In this study he will ensure the timely and accurate enrollment of cases from the inpatient and outpatient setting of the ENT Division (INT).

### Positions and honors

## Positions

| Institution | Division / Research group | Location | Position | From year | To year |
|---|---|---|---|---|---|
| Fondazione IRCCS Istituto Nazionale Tumori | Otolaryngology Head and Neck Surgery Department | Via Venezian 1, 20133, Milan, Italy | Director of Otolaryngology Head and Neck Surgery Department | 2022 | 2023 |
| University of Brescia | Otolaryngology Head and Neck Surgery Department | Brescia, Italy | Associate Professor in Otolaryngology | 2017 | 2023 |
| University of Florence | Otolaryngology | Florence, Italy | Associate Professor in Otolaryngology | 2015 | 2017 |
| University of Florence | Medicine | Florence, Italy | Assistant Professor in Otolaryngology | 2010 | 2015 |
| University of Florence | ENT Department | Florence, Italy | Otolaryngologist / Head and Neck Surgeon | 2006 | 2010 |
| National Cancer Institute ¿Regina Elena¿ | Otolaryngology Head and Neck Surgery Department | Rome, Italy | Otolaryngologist / Head and Neck Surgeon | 2005 | 2006 |
| Castelfranco Veneto Hospital | Otolaryngology Department | Castelfranco Veneto, Italy | Otolaryngologist / Head and Neck Surgeon | 2004 | 2005 |
| VU Medical Center Amsterdam | Otolaryngology Head and Neck Surgery Department | Amsterdam, The Netherlands | Otolaryngologist / Head and Neck Surgeon | 2002 | 2004 |

### Other awards and honors

-

## Grant

| Funded by Institution | Researcher inst. where grant is/was performed | Year | Title | Position in Projects | Fund (euro) | Source website grant listed |
|---|---|---|---|---|---|---|
| none | none | none | none | Coordinator | 0,00 | - |

## 2.5 Research Collaborators n. 4

**Last Name:** Bussu
**First Name:** Francesco
**Last name at birth:**
**Gender:** M

**Title:** Responsabile UO2
**Nationality:** Italiana
**Date of birth:** 04/04/1974
**Country of residence:** ITALY
**Country of Birth:** ITALY
**Place of Birth:** Cagliari

**Official H index (Scopus or Web of Science):** 31.0

**Scopus Author Id:**57215521657 **ORCID ID:**0000-0001-6261-2772 **RESEARCH ID:**AAA-7610-2019

*Contact address*

**Current organisation name:** Azienda Ospedaliero Universitaria Sassari
**Current Department / Faculty / Institute / Laboratory name:** Unità Operativa di Oncologia Medica
**Street:** via san Pietro 10
**Postcode / Cedex:** 07100
**Town:** Sassari
**Phone:**+393296024900
**Phone 2:** 079/228509

**Education / training**

| Educational institution and location | Degree | Field of study | From year | To year |
|---|---|---|---|---|
| Università di Roma Tor Vergata | Specialization / Specializzazione | Otorhinolaryngology | 2006 | 2010 |
| Università Cattolica del Sacro Cuore, Facoltà di Medicina e Chirurgia, Roma | PhD | Phisiopathology of the rhinopharyngo-tubaric distric | 2002 | 2006 |
| Università Cattolica del Sacro Cuore, Facoltà di Medicina e Chirurgia, Roma | Master's Degree / Laurea Magistrale | Medicine and Surgery | 1993 | 1999 |

**Personal Statement:**

Francesco Bussu, is the project coordinator of OU2. He will be in charge for the clinical aspects of the project along with the PI of the project, and for the clinical validation of technological tools developed through digital pathology. He will also participate to the validation studies to be submitted to the ethical committee(s).

## Positions and honors

## Positions

| Institution | Division / Research group | Location | Position | From year | To year |
|---|---|---|---|---|---|
| Università degli Studi di Sassari | Dipartimento di Medicina, Chirurgia e Farmacia | Sassari, Italy | Professore Ordinario (full Professor) | 2019 | 2023 |
| Azienda Ospedaliera Universitaria di Sassari | Otolaryngology | Sassari; Italy | Head of Division (Primario) | 2017 | 2023 |
| Policlinico Agostino Gemelli | Rome | Otorhinolaryngology | Dirigente Medico di Primo Livello | 2004 | 2017 |
| Università Cattolica del Sacro Cuore | Facoltà di Medicina e Chirurgia | Rome | Assistant Professor | 2004 | 2009 |

## Other awards and honors

¿ 1999 - Cash prize for the best oncologic degree theses of the year; 1999 - `Award Agostino Gemelli
¿ 1999', to the best Medical graduated of the year in Università Cattolica del Sacro Cuore;
¿ 2013 - `Award Italo Serafini 2013', by the Italian Society of Head and Neck Oncology (AIOCC) for the best free paper in the national congress;
¿ 2018 Cash prize for 'High quality publication' from Catholic University of the Sacred Heart

## Grant

| Funded by Institution | Researcher inst. where grant is/was performed | Year | Title | Position in Projects | Fund (euro) | Source website grant listed |
|---|---|---|---|---|---|---|
| Università Cattolica del Sacro Cuore | Università Cattolica del Sacro Cuore | 2016 | Definizione del ruolo complessivo dei virus oncogeni nel distretto testa e collo | Coordinator | 7.200,00 | www.unicatt.it |
| Università Cattolica del Sacro Cuore | Università Cattolica del Sacro Cuore | 2017 | Ruolo epidemiologico e prognostico dei Papillomavlrus tunani nella carcinogenesi della testa e del collo. Approcci diagnostici ed implicazioni per la pratica clinica | Coordinator | 7.200,00 | www.unicatt.it |
| Fondazione Banco di Sardegna | AOU Sassari | 2018 | Screening neonatale e sorveglianza audiologica infantile | Coordinator | 20.000,00 | https://www.fondazionedisardegna.it/ |
| Sardegna Ricerche | AOU Sassari | 2020 | Revealing - Studio osservazionale per la valutazione della storia clinica (pattern di presa in carico e di trattamento) e trattamento delle neoplasie del distretto cervico-facciale nel centro di riferimento per il Nord Sardegna | Coordinator | 50.000,00 | https://www.sardegnaricerche.it/ |

## 2.6 Research Collaborators n. 5

**Last Name:** FADDA
**First Name:** GIOVANNI MARIA
**Last name at birth:**
**Gender:** M

**Title:** Ulteriore collaboratore UO2
**Country of residence:** ITALY
**Nationality:** Italiana
**Country of Birth:** ITALY
**Date of birth:** 22/08/1973
**Place of Birth:** Sassari

**Official H index (Scopus or Web of Science):** 5.0

**Scopus Author Id:**57197246994 **ORCID ID:**0000-0003-4422-042X **RESEARCH ID:**-

*Contact address*

**Current organisation name:** Azienda Ospedaliero Universitaria Sassari
**Current Department / Faculty / Institute / Laboratory name:** Unità Operativa di Oncologia Medica
**Street:** Via Enrico De Nicola
**Postcode / Cedex:** 07100
**Town:** Sassari
**Phone:**+393396876707
**Phone 2:**

**Education / training**

| Educational institution and location | Degree | Field of study | From year | To year |
|---|---|---|---|---|
| University of Sassari | Specialization / Specializzazione | Residency in Medical Oncology | 2000 | 2004 |
| University of Sassari | Single-cycle master's degree / Laurea magistrale a ciclo unico | Medicine | 1992 | 1999 |

**Personal Statement:**

Giovanni Maria Fadda, MD, is a Medical Oncologist working as Senior Consultant at the Medical Oncology Unit of the University Hospital of Sassari. His research achievements are focused mainly on head and neck and lung cancers, including also studies on the molecular biology of these cancer types. In this study he will ensure the timely and accurate enrollment of cases from the inpatient and outpatient setting of the Medical Oncology Unit of Sassari.

## Positions and honors

**Positions**

| Institution | Division / Research group | Location | Position | From year | To year |
|---|---|---|---|---|---|
| Azienda Ospedaliera Universitaria AOU Sassari | Division of Medical Oncology | Via Enrico De Nicola, 07100 ¿ Sassari, Sardinia, Italy | Staff Physician, Medical Oncologist | 2016 | 2023 |
| Cesare Zonchello General Hospital, Azienda ASL 3 Nuoro | Division of Medical Oncolgy | Piazza Sardegna, 08100 ¿ Nuoro, Sardinia, Italy | Staff Physician, Medical Oncologist | 2006 | 2015 |

## Other awards and honors

-

**Grant**

| Funded by Institution | Researcher inst. where grant is/was performed | Year | Title | Position in Projects | Fund (euro) | Source website grant listed |
|---|---|---|---|---|---|---|
| none | none | none | none | Coordinator | 0,00 | - |

## 2.7 Research Collaborators n. 6 - Under 40

**Last Name:** Cavalieri
**First Name:** Stefano
**Last name at birth:**
**Gender:** M

**Title:** Ulteriore collaboratore UO1 (under40)
**Country of residence:** ITALY
**Nationality:** Italiana
**Country of Birth:** ITALY
**Date of birth:** 06/01/1988
**Place of Birth:** Aosta

**Official H index (Scopus or Web of Science):** 15.0

**Scopus Author Id:**57191971361 **ORCID ID:**0000-0003-1294-6859 **RESEARCH ID:**AAB-3879-2019

*Contact address*

**Current organisation name:** Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano
**Current Department / Faculty / Institute / Laboratory name:** Medical Oncology
**Street:** Via Venezian 1
**Postcode / Cedex:** 20133
**Town:** Milano
**Phone:**+393480625361
**Phone 2:**

**Education / training**

| Educational institution and location | Degree | Field of study | From year | To year |
|---|---|---|---|---|
| Università degli Studi di Milano | Specialization / Specializzazione | Medical Oncology | 2014 | 2019 |
| Università degli Studi di Milano | Single-cycle master's degree / Laurea magistrale a ciclo unico | Medicine | 2007 | 2013 |

**Personal Statement:**

Stefano Cavalieri, MD, is a Medical Oncologist and Assistant Professor at the University of Milan running both clinical and research activities at the Head and Neck Medical Oncology Unit (INT). He has achieved a broad expertise on the applications of Big Data and Artificial Intelligence in Head and Neck cancers. In this project he will ensure the timely and accurate enrollment from the outpatient setting of the multidisciplinary ENT-Medical Oncology visits and will take part in the interpretation of the results focusing on the potential applications in the clinical practice.

## Positions and honors

**Positions**

| Institution | Division / Research group | Location | Position | From year | To year |
|---|---|---|---|---|---|
| University of Milan | Department of Oncology and Hemato-Oncology | Milan, Italy | Researcher | 2021 | 2023 |
| Fondazione IRCCS Istituto Nazionale Tumori | S.C. Medical Oncology 3 ¿ Head and Neck Tumors | Via Venezian 1, 20133, Milan, Italy | Medical Oncologist | 2019 | 2021 |
| Fondazione IRCCS Istituto Nazionale Tumori University of Milan | Fondazione IRCCS Istituto Nazionale dei Tumori | Via Venezian 1, 20133, Milan | Resident in medical oncology | 2014 | 2019 |

**Other awards and honors**

-

**Grant**

| Funded by Institution | Researcher inst. where grant is/was performed | Year | Title | Position in Projects | Fund (euro) | Source website grant listed |
|---|---|---|---|---|---|---|
| Horizon 2020 Framework Programme | Fondazione IRCCS Istituto Nazionale Tumori | 2020 | BD4QoL - Big Data Models and Intelligent tools for Quality of Life monitoring and participatory empowerment of head and neck cancer survivors | Collaborator | 4.985.975,00 | - |

## 2.8 Research Collaborators n. 7 - Under 40

**Last Name:** Colombo
**First Name:** Elena
**Last name at birth:**
**Gender:** F

**Title:** Ulteriore collaboratore UO1 (under40)
**Nationality:** Italiana
**Date of birth:** 26/05/1985
**Country of residence:** ITALY
**Country of Birth:** ITALY
**Place of Birth:** Udine

**Official H index (Scopus or Web of Science):** 5.0

**Scopus Author Id:**57328785000 **ORCID ID:**0000-0003-3132-844X **RESEARCH ID:**FYR-3783-2022

*Contact address*

**Current organisation name:** Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano
**Current Department / Faculty / Institute / Laboratory name:** Medical Oncology
**Street:** via Venezian 1
**Postcode / Cedex:** 20133
**Town:** Milano
**Phone:**+393471294203
**Phone 2:**

**Education / training**

| Educational institution and location | Degree | Field of study | From year | To year |
|---|---|---|---|---|
| Fondazione IRCCS Istituto Nazionale tumori University of Milan | Specialization / Specializzazione | Medical Oncology | 2016 | 2021 |
| University of Milan | Single-cycle master's degree / Laurea magistrale a ciclo unico | Medicine and Surgery | 2009 | 2015 |
| Catholic University of Milan | Master's Degree / Laurea Magistrale | Philosophy | 2007 | 2009 |

**Personal Statement:**

Elena Colombo, MD, is a Medical Oncologist working as Associate Physician of the Head and Neck Medical Oncology Unit (INT). Since 2021, she is member of the institutional Molecular Tumor Board as referee for the head-and-neck cancer cases and she attends weekly multidisciplinary meetings with molecular biologists and pathologists, enriching her knowledge on translational research methods applied to the clinical practice across different cancer types. In this project she will ensure the timely and accurate enrollment of cases from both the inpatient and outpatient Medical Oncology settings.

### Positions and honors

**Positions**

| Institution | Division / Research group | Location | Position | From year | To year |
|---|---|---|---|---|---|
| Fondazione IRCCS Istituto Nazionale dei Tumori | Head and Neck Medical Oncology Unit | Via Venezian 1, 20133, Milan, Italy | Medical Oncologist | 2021 | 2023 |

**Other awards and honors**

-

**Grant**

| Funded by Institution | Researcher inst. where grant is/was performed | Year | Title | Position in Projects | Fund (euro) | Source website grant listed |
|---|---|---|---|---|---|---|
| Italian Ministry of Health ¿ Alleanza contro il cancro | Fondazione IRCCS Istituto Nazionale Tumori | 2021 | WP 17 - Studio multidimensionale sui meccanismi di risposta e di tossicità alla radioterapia in neoplasie primariamente (chemio)radiotrattate ¿ RADECISION | Collaborator | 37.800,00 | - |

## 2.9 Additional Research Collaborators n. 2 - Under 40 to hire

**Last Name:** Bacciu
**First Name:** Andrea
**Last name at birth:**
**Gender:** M

**Title:** Additional research collaborator
**Country of residence:** ITALY
**Nationality:** italiana
**Country of Birth:** ITALY
**Date of birth:** 02/07/1990
**Place of Birth:** Nuoro

**Official H index (Scopus or Web of Science):** 3.0

**Scopus Author Id:**57205235847 **ORCID ID:**0009-0004-7426-5279 **RESEARCH ID:**-

*Contact address*

**Current organisation name:** Azienda Ospedaliero Universitaria Sassari
**Current Department / Faculty / Institute / Laboratory name:** Unità Operativa di Oncologia Medica
**Street:** Via tempio 5
**Postcode / Cedex:** 07100
**Town:** sassari
**Phone:**+393482346318
**Phone 2:**

**Education / training**

| Educational institution and location | Degree | Field of study | From year | To year |
|---|---|---|---|---|
| Universita' degli studi di Sassari | Specialization / Specializzazione | Resident in clinical pathology and biochemistry | 2018 | 2023 |
| Università degli Studi di Sassari | PhD | Scienze biomediche | 2017 | 2021 |
| Universita' degli Studi di Sassari | Master's Degree / Laurea Magistrale | Biotecnologie Sanitarie Mediche Veterinarie | 2014 | 2016 |
| Università degli Studi di Sassari | Bachelor Degree / Laurea Triennale | Scienze Biologiche | 2009 | 2014 |

**Personal Statement:**

Andrea Bacciu, BSc, is a Biologist and Researcher working at the Department of Medical Sciences of the University of Sassari. His has achieved a relevant knowhow on molecular biomarkers, biotechnology and biosensors¿ design and applications. In this project he will be responsible of the accurate sample storage, sample processing and data annotation. Moreover, he will provide the timely delivery of results for the statistical analysis that will be held in INT.

## Positions and honors

**Positions**

| Institution | Division / Research group | Location | Position | From year | To year |
|---|---|---|---|---|---|
| Università degli studi di Sassari | Medicina, Chirurgia e Farmacia | Sassari, Italiy | Resident in clinical pathology and microbiology | 2021 | 2023 |

## Other awards and honors

-

**Grant**

| Funded by Institution | Researcher inst. where grant is/was performed | Year | Title | Position in Projects | Fund (euro) | Source website grant listed |
|---|---|---|---|---|---|---|
| none | none | none | none | Coordinator | 0,00 | none |

## 2.10 Additional Research Collaborators n. 3 - Under 40 to hire

**Last Name:** VARRUCCIU
**First Name:** SIMONA
**Last name at birth:** Sassari
**Gender:** F

**Title:** Additional research collaborator
**Country of residence:** ITALY
**Nationality:** Italiana
**Country of Birth:** ITALY
**Date of birth:** 28/03/1992
**Place of Birth:** Sassari

**Official H index (Scopus or Web of Science):** 0.0

**Scopus Author Id:**- **ORCID ID:**0009-0009-7210-0995 **RESEARCH ID:**IAR-9548-2023

*Contact address*

**Current organisation name:** Azienda Ospedaliero Universitaria Sassari
**Current Department / Faculty / Institute / Laboratory name:** Unità Operativa di Oncologia Medica
**Street:** Viale San Pietro 43
**Postcode / Cedex:** 07100 **Town:** Sassari
**Phone:**+393913815704 **Phone 2:**

**Education / training**

| Educational institution and location | Degree | Field of study | From year | To year |
|---|---|---|---|---|
| Università degli Studi di Sassari | Master's Degree / Laurea Magistrale | Biologia Sperimentale Applicata | 2017 | 2019 |
| Università degli Studi di Sassari | Bachelor Degree / Laurea Triennale | Scienze Biologiche | 2011 | 2016 |

**Personal Statement:**

Simona Varrucciu, BSc, is a Biologist focused on Clinical Pathology and Clinical Biochemistry at the University Hospital of Sassari. In this project she will ensure the accurate collection and storage of samples for the Digital Pathology analyses.

## Positions and honors

**Positions**

| Institution | Division / Research group | Location | Position | From year | To year |
|---|---|---|---|---|---|
| Università degli Studi di Sassari | Division of Pathology | Sassary; Italy | Resident in clinical pathology and biochemistry | 2021 | 2023 |
| Ospedale Mater Olbia | Laboratorio analisi | Olbia | Biologist | 2020 | 2021 |

**Other awards and honors**

-

| | |
|---|---|
| **Project Code:** PNRR-MCNT1-2023-12377359 | **Call section:** Malattie Croniche non Trasmissibili (MCnT1) ad alto impatto sui sistemi sanitari |
| **Applicant Institution:** Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano | **Applicant/PI Coordinator:** Licitra Lisa Francesca Linda |

**Grant**

| Funded by Institution | Researcher inst. where grant is/was performed | Year | Title | Position in Projects | Fund (euro) | Source website grant listed |
|---|---|---|---|---|---|---|
| none | none | none | none | Coordinator | 0,00 | - |

# 2.17 Expertise Research Collaborators

**Selected peer-reviewed publications of the Research Group / Collaborators**

| Collaborato | Title | Type | Pag | Vol | Year | DOI | PMID | Cit.** | P.* |
|---|---|---|---|---|---|---|---|---|---|
| Cavalieri Stefano | Efficacy and safety of single-agent pan-human epidermal growth factor receptor (HER) inhibitor dacomitinib in locally advanced unresectable or metastatic skin squamous cell cancer | Article | 7-15 | 97 | 2018 | 10.1016/j.ejca.2018.04.004 | 29734047 | 28 | F |
| Bacciu Andrea | Propylene glycol stabilizes the linear response of glutamate biosensor: Potential implications for in-vivo neurochemical monitoring | Article | NOT_FOUND | 6 | 2018 | 10.3390/chemosensors6040058 | NOT_FOUND | 6 | O |
| Bacciu Andrea | Low-temperature storage improves the over-time stability of implantable glucose and lactate biosensors | Article | NOT_FOUND | 19 | 2019 | 10.3390/s19020422 | 30669626 | 14 | O |
| Bacciu Andrea | The presence of polysaccharides, glycerol, and polyethyleneimine in hydrogel enhances the performance of the glucose biosensor | Article | NOT_FOUND | 9 | 2019 | 10.3390/bios9030095 | 31366026 | 6 | O |
| Bacciu Andrea | A new perspective on using glycols in glutamate biosensor design: From stabilizing agents to a new containment net | Article | NOT_FOUND | 8 | 2020 | 10.3390/CHEMOSENSORS8020023 | NOT_FOUND | 3 | F |
| Bacciu Andrea | A study on the combination of enzyme stabilizers and low temperatures in the long-term storage of glutamate biosensor | Article | NOT_FOUND | 9 | 2021 | 10.3390/chemosensors9060129 | NOT_FOUND | 1 | F |
| Colombo Elena | The association of cemiplimab plus sonidegib for synchronous cutaneous squamous cell carcinoma and basal cell carcinoma of the head and neck: Two case reports | Article | NOT_FOUND | 13 | 2023 | 10.3389/fonc.2023.1111146 | 36925925 | 0 | F |
| FADDA GIOVANNI MARIA | Liquid Biopsy in the Oncological Management of a Histologically Undiagnosed Lung Carcinoma: A Case Report | Article | NOT_FOUND | 12 | 2022 | 10.3390/jpm12111874 | 36579578 | 2 | F |
| Colombo Elena | Salivary gland cancers in elderly patients: challenges and therapeutic strategies | Review | NOT_FOUND | 12 | 2022 | 10.3389/fonc.2022.1032471 | 36505842 | 1 | F |

| Collaborato | Title | Type | Pag | Vol | Year | DOI | PMID | Cit.** | P.* |
|---|---|---|---|---|---|---|---|---|---|
| Colombo Elena | Abiraterone Acetate in Patients With Castration-Resistant, Androgen Receptor-Expressing Salivary Gland Cancer: A Phase II Trial | Article | 4061-4068 | 39 | 2021 | 10.1200/JCO.21.00468 | 34597119 | 11 | O |
| Bergamini Cristiana | Bleeding complications in patients with squamous cell carcinoma of the head and neck | Review | 2844-2858 | 43 | 2021 | 10.1002/hed.26772 | 34117666 | 7 | F |
| DEGANELLO ALBERTO | Prognostic significance of cd4+ and cd8+ tumor-infiltrating lymphocytes in head and neck squamous cell carcinoma: A meta-analysis | Review | 1-15 | 13 | 2021 | 10.3390/cancers13040781 | NOT_FOUND | 41 | O |
| Colombo Elena | Recent Advances in the Management of Typical and Atypical Lung Carcinoids | Review | 161-169 | 22 | 2021 | 10.1016/j.cllc.2020.12.004 | 33618994 | 14 | O |
| DEGANELLO ALBERTO | Genome-wide study of salivary miRNAs identifies miR-423-5p as promising diagnostic and prognostic biomarker in oral squamous cell carcinoma | Article | 2987-2999 | 11 | 2021 | 10.7150/THNO.45157 | 33456584 | 27 | O |
| Bergamini Cristiana | Local therapies for liver metastases of rare head and neck cancers: a monoinstitutional case series | Article | 188-195 | 107 | 2021 | 10.1177/0300891620952844 | 32924878 | 4 | F |
| Colombo Elena | Differential diagnosis and management of diarrhea in patients with neuroendocrine tumors | Review | 1-19 | 9 | 2020 | 10.3390/jcm9082468 | NOT_FOUND | 10 | O |
| Bergamini Cristiana | Real-world efficacy and safety of lenvatinib: data from a compassionate use in the treatment of radioactive iodine-refractory differentiated thyroid cancer patients in Italy | Article | 35-40 | 118 | 2019 | 10.1016/j.ejca.2019.05.031 | 31299580 | 58 | O |
| Cavalieri Stefano | Adjuvant androgen deprivation therapy for poor-risk, androgen receptor¿positive salivary duct carcinoma | Article | 62-70 | 110 | 2019 | 10.1016/j.ejca.2018.12.035 | 30771738 | 43 | O |
| Bussu Francesco | HPV as a marker for molecular characterization in head and neck oncology: Looking for a standardization of clinical use and of detection method(s) in clinical practice | Review | 1104-1111 | 41 | 2019 | 10.1002/hed.25591 | 30747478 | 38 | F |
| Cavalieri Stefano | Current status and perspectives in immunotherapy for metastatic melanoma | Article | 12452-12470 | 9 | 2018 | 10.18632/oncotarget.23746 | NOT_FOUND | 61 | O |

| Collaborato | Title | Type | Pag | Vol | Year | DOI | PMID | Cit.** | P.* |
|---|---|---|---|---|---|---|---|---|---|
| Cavalieri Stefano | Immuno-oncology in head and neck squamous cell cancers: News from clinical trials, emerging predictive factors and unmet needs | Review | 78-86 | 65 | 2018 | 10.1016/j.ctrv.2018.03.003 | 29574334 | 26 | F |
| Cavalieri Stefano | Management of tyrosine kinase inhibitors (TKI) side effects in differentiated and medullary thyroid cancer patients | Review | 349-361 | 31 | 2017 | 10.1016/j.beem.2017.04.012 | 28911730 | 57 | O |
| Bergamini Cristiana | Systemic therapy in metastatic salivary gland carcinomas: A pathology-driven paradigm? | Review | 58-63 | 66 | 2017 | 10.1016/j.oraloncology.2016.12.016 | 28249649 | 79 | O |
| De Cecco Loris | Development and validation of a microRNA-based signature (MiROvaR) to predict early relapse or progression of epithelial ovarian cancer: a cohort study | Article | 1137-1146 | 17 | 2016 | 10.1016/S1470-2045(16)30108-5 | 27402147 | 91 | O |
| FADDA GIOVANNI MARIA | Adjuvant hormonal therapy in women with early-stage breast cancer | Article | 261-267 | 12 | 2016 | 10.2174/1573406412031 60331211021 | 27056135 | 1 | O |
| DEGANELLO ALBERTO | Pharyngocutaneous fistula following total laryngectomy: Analysis of risk factors, prognosis and treatment modalities | Article | 400-405 | 35 | 2015 | 10.14639/0392-100X-626 | 26900245 | 48 | O |
| FADDA GIOVANNI MARIA | Advances in the treatment of triple-negative early breast cancer | Article | 268-272 | 12 | 2016 | 10.2174/1573406412666 151116144026 | 26567618 | 12 | O |
| FADDA GIOVANNI MARIA | Adjuvant treatment of early breast cancer in the elderly | Article | 280-284 | 12 | 2016 | 10.2174/1573406412666 151116144352 | 26567616 | 3 | F |
| De Cecco Loris | Augmentation of Response to Chemotherapy by microRNA-506 Through Regulation of RAD51 in Serous Ovarian Cancers | Article | NOT_FOUND | 107 | 2015 | 10.1093/jnci/djv108 | 25995442 | 98 | O |
| De Cecco Loris | Head and neck cancer subtypes with biological and clinical relevance: Meta-analysis of gene-expression data | Article | 9627-9642 | 6 | 2015 | 10.18632/oncotarget.3301 | 25821127 | 86 | F |
| FADDA GIOVANNI MARIA | Impact of tissue type and content of neoplastic cells of samples on the quality of epidermal growth factor receptor mutation analysis among patients with lung adenocarcinoma | Article | 187-191 | 12 | 2015 | 10.3892/mmr.2015.3347 | 25683726 | 16 | O |

| Collaborato | Title | Type | Pag | Vol | Year | DOI | PMID | Cit.** | P.* |
|---|---|---|---|---|---|---|---|---|---|
| Bergamini Cristiana | Clinical activity of androgen deprivation therapy in patients with metastatic/relapsed androgen receptor-positive salivary gland cancers | Article | 724-731 | 38 | 2016 | 10.1002/hed.23940 | 25522335 | 85 | O |
| DEGANELLO ALBERTO | Is elective neck dissection necessary in cases of laryngeal recurrence after previous radiotherapy for early glottic cancer? | Article | 1089-1094 | 128 | 2014 | 10.1017/S0022215114002709 | 25418930 | 28 | F |
| Bussu Francesco | Human papillomavirus (HPV) infection in squamous cell carcinomas arising from the oropharynx: Detection of HPV DNA and p16 immunohistochemistry as diagnostic and prognostic indicators - A pilot study | Article | 1115-1120 | 89 | 2014 | 10.1016/j.ijrobp.2014.04.044 | 25035216 | 35 | F |
| De Cecco Loris | miR-199a-3p displays tumor suppressor functions in papillary thyroid carcinoma | Article | 2513-2528 | 5 | 2014 | 10.18632/oncotarget.1830 | 24810336 | 97 | O |
| De Cecco Loris | Results of nimotuzumab and vinorelbine, radiation and re-irradiation for diffuse pontine glioma in childhood | Article | 305-312 | 118 | 2014 | 10.1007/s11060-014-1428-z | 24696052 | 77 | O |
| DEGANELLO ALBERTO | Cost analysis in oral cavity and oropharyngeal reconstructions with microvascular and pedicled flaps | Article | 380-387 | 33 | 2013 | NOT_FOUND | 24376293 | 30 | F |
| Bussu Francesco | New insights into human papillomavirus-associated head and neck squamous cell carcinoma | Review | 77-87 | 33 | 2013 | NOT_FOUND | 23853396 | 85 | O |
| Bussu Francesco | HPV infection in squamous cell carcinomas arising from different mucosal sites of the head and neck region. Is p16 immunohistochemistry a reliable surrogate marker? | Article | 1157-1162 | 108 | 2013 | 10.1038/bjc.2013.55 | 23403821 | 81 | F |
| Bussu Francesco | Comparison of total laryngectomy with surgical (cricohyoidopexy) and nonsurgical organ-preservation modalities in advanced laryngeal squamous cell carcinomas: A multicenter retrospective analysis | Review | 554-561 | 35 | 2013 | 10.1002/hed.22994 | 22495830 | 39 | F |

* Position: F=First L=Last C=Correspondent O=Other N=Not applicable

** Autocertificated

# 3 - Ethics

| | |
|---|---|
| 1. HUMAN EMBRYOS/FOETUSES | |
| Does your research involve Human Embryonic Stem Cells (hESCs)? | No |
| Does your research involve the use of human embryos? | No |
| Does your research involve the use of human foetal tissues / cells? | No |
| 2. HUMANS | |
| Does your research involve human participants? | Yes |
| Does your research involve physical interventions on the study participants? | No |
| 3. HUMAN CELLS / TISSUES | |
| Does your research involve human cells or tissues (other than from Human Embryos/ Foetuses? | No |
| 4. PERSONAL DATA | |
| Does your research involve personal data collection and/or processing? | Yes |
| Does your research involve further processing of previously collected personal data (secondary use)? | No |
| 5. ANIMALS | |
| Does your research involve animals? | No |
| 6. ENVIRONMENT & HEALTH and SAFETY | |
| Does your research involve the use of elements that may cause harm to the environment, to animals or plants? | No |
| Does your research deal with endangered fauna and/or flora and/or protected areas? | No |
| Does your research involve the use of elements that may cause harm to humans, including research staff? | No |
| 7. DUAL USE | |
| Does your research involve dual-use items in the sense of Regulation 428/2009, or other items for which an | No |
| 8. EXCLUSIVE FOCUS ON CIVIL APPLICATIONS | |
| Could your research raise concerns regarding the exclusive focus on civil applications? | No |
| 9. MISUSE | |
| Does your research have the potential for misuse of research results? | No |
| 10. OTHER ETHICS ISSUES | |
| Are there any other ethics issues that should be taken into consideration? Please specify | No |

I confirm that I have taken into account all ethics issues described above and that, if any ethics issues apply, I will complete the ethics self-assessment and attach the required documents. [X]

# 4 - Call-specific questions

| Eligibility | |
|---|---|
| I acknowledge that I am aware of the eligibility requirements for applying as specified in the Call-PNRRXXXX_M6/C2, and certify that, to the best of my knowledge my application is in compliance with all these requirements. I understand that my proposal may be declared ineligible at any point during the evaluation or granting process if it is found not to be compliant with these eligibility criteria. | [X] |
| I confirm that the proposal that I am about to submit draws substantially don't repeat on an existing or recently finished GRANT funded. | [X] |
| Data-Related Questions and Data Protection<br>(Consent to any question below is entirely voluntary. A positive or negative answer will not affect the evaluation of your project proposal in any form and will not be communicated to the evaluators of your project.) | |
| For communication purposes only, the MoH asks for your permission to publish,in whatever form and medium, your name, the proposal title, the proposal acronym, the panel, and host institution, should your proposal be retained for funding. | [X] |
| Some national and regional public research funding authorities run schemes to fund MoH applicants that score highly in the MoH's evaluation but which can not be funded by the MoH due to its limited budget. In case your proposal could not be selected for funding by the MoH do you consent to allow the MoH to disclose the results of your evaluation (score and ranking range) together with your name, non- confidential proposal title and abstract, proposal acronym, host institution and your contact details to such authorities? | [X] |
| The MoH is sometimes contacted for lists of MoH funded researchers by institutions that are awarding prizes to excellent researchers. Do you consent to allow the MoH to disclose your name, non-confidential proposal title and abstract, proposal acronym, host institution and your contact details to such institutions? | [X] |
| The Ministry of Health occasionally could contacts Principal Investigators of funded proposals for various purposes such as communication campaigns, pitching events, presentation of their project's evolution or outcomes to the public, invitations to represent the Ministry of Health in national and international forums, studies etc. Should your proposal be funded, do you consent to the Ministry of Health staff contacting you for such purposes? | [X] |
| For purposes related to monitoring, study and evaluating implementation of MoH actions, the MoH may need that submitted proposals and their respective evaluation data be processed by external parties. Any processing will be conducted in compliance with the requirements of Regulation 45/2001. | |

# 5 – Description Project

**Summary description**

Our group at Fondazione IRCCS Istituto Nazionale Tumori previously developed and validated a molecular stratification in patients with curable head-and-neck squamous cell carcinomas (HNSCC) with prognostic and predictive values based on

tumor tissue gene expression (GE). The present project aims at translating the subtype stratification on liquid biopsies exploiting a cohort of patients with curable HPV-negative HNSCC to forecast and easily monitor the risk of relapse after treatment. 300 newly-diagnosed patients with matched primary tumor pathological slides and longitudinal blood series will be collected prospectively for biological analyses including: i) multi-omics integration at bulk, single-cell, spatial levels for subtype stratification; ii) profiling of circulating biomarkers; iii) AI model development to implement longitudinal monitoring on liquid biopsies; iv) model development on tissue tumor slides to optimize classification for future applications in the clinical practice.

**Background / State of the art and Preliminary data (if available)**

HNSCC are the 7th most frequent cancer types and in 70% of cases they are diagnosed at curable stage. Nevertheless, only 50% of patients with HPV-unrelated HNSCC receiving concomitant platinum-based chemoradiation (cCTRT) with curative intent will achieve long-term benefit from this approach (PMID 28542679). Currently, we lack reliable predictive and prognostic biomarkers associated with patients¿ outcomes. INT developed a cluster-based GE model able to stratify HNSCC pts in 6 groups with different biological, prognostic and predictive significance [PMID 25821127], paving the way to potential therapeutic implications to better inform treatment outcomes. In this scenario, the next steps for personalized treatment include the prediction of tumor radio-/chemotherapy sensitivity through the match of solid and liquid biomarkers. Digital pathology and Artificial Intelligence (AI) are expected to increase the capability of finding predictive features on digital tumor slides (PMID 31044723). Overall, literature data and preliminary evidences support the aim of finding an AI model able to predict HNSCC response to CTRT by combining features of tumor molecular subtypes with a corresponding profile found in liquid biopsies and the corresponding tumor images by digital pathology. In addition, the disease evolution after CTRT can be studied by analyzing subtype-specific circulating biomarkers monitored with longitudinal liquid biopsies.

**Description and distribution of activities of each operating unit**

The BIOMATCH research project will be conducted with the collaboration of 2 clinical institutions ¿ one in Northern and one in Southern Italy ¿ which are reference centers entirely dedicated to the clinical management and research for cancer patients.
1) Fondazione IRCCS Istituto Nazionale Tumori, hereafter INT (PI Licitra, coPI De Cecco; collaborators Deganello and Bergamini, U40 collaborators Cavalieri and Colombo)
2) Azienda Ospedaliero Universitaria di Sassari, hereafter AOUSS (collaborators Bussu and Fadda; U40 collaborators Bacciu and Varrucciu)
The consortium will be coordinated by INT, site of the PI/co-PI.
A prospective multicenter study will be designed to collect a cohort of 300 subjects affected by HPV-unrelated HNSCC candidate to curative standard treatment including radiation and chemotherapy. Both centers will collect specimens from primary tumors, hematossilin & eosin (H&E) slides of the primary tumor and blood at baseline and after 3 months from cCTRT treatment completion. The study and trial-related Standard Operating Procedures (SOPs) will be designed with the contribution of both centers.
The study will be conducted in a timeframe of 14 months and will involve 15 participating Italian clinical centers whose research activities will be harmonized and delivered to INT and AOUSS under the guidance of a Contract Research Organization (CRO) funded by the BIOMATCH project. Solid specimens and liquid samples centralisation are foreseen at both centers. Multi-omics analysis will be performed both on tissue and blood at INT; digital pathology activities will be performed at AOUSS, where dedicated tools and specific expertise are present. Tissue omics analysis will inform pathological H&E digital imaging to develop an image-based subtype classifier. Machine learning training and model validation will be performed at INT.
To manage the multidisciplinarity of the project, hierarchical organization scheme will be structured assigning responsibilities at the different levels of decision and operational intervention. The PI and coPI will ensure the proper functioning of the project to achieve the objectives. In month 1, the PI will schedule a kick-off meeting. Further consortium meetings will be

organized every four months and the interim progress will be monitored scheduling phone calls/skype meeting to support efficient cooperation.

## 5.4 Specific Aims and Experimental Design

**Specific aim 1**

Specific Aim 1:To classify the 6-cluster GE model of primary tumors in a prospective cohort of 300 HPV-negative HNSCC pts. To achieve this aim, the project foresees 3 tasks (T).
T1.1 Clinical study design and setup.
A specific study protocol compliant with Good Clinical Practice and the relevant ethical requirements will be designed by the Consortium through an ad hoc selected writing committee. The protocol will be integrated by the informed consent, electronic case report forms (eCRFs), material collection and storage SOPs. The finalized protocol will submitted to the independent Ethical Committees of all the participating Institutions recruiting pts. Data- and material-transfer agreements (DTA and MTA, respectively) will be set up before starting the study. We foresee to include 15 Centers with high volumes of HNSCC patients.
Inclusion criteria will be stage III and stage IV histologically proven HPV negative HNSCC.
INT and AOUSS centers are involved in recruiting patients and collecting clinical and biological data. A subcontract (e.g., Contract Research Organization, CRO) and specific DTA and MTA will be signed between beneficiaries. Prior to the start of the research activities, a study initiation visit (SIV) will be performed to secure staff information and training. During the whole project execution, the partners¿ activities, recruiting rates and the development of the scientific activities will be closely monitored. During the study conduction, to ensure the proper progressing of the research tasks, periodic monitoring activities will be conducted. After the completion of the study, a site closure visit (SCV) will be performed at each site.

T1.2 SOPs definition [months 1-6]. Prior to the study start, standard operating procedures (SOPs) will be provided for tumor tissue and blood sample collection. During the main protocol elaboration, case report forms (CRFs) will be developed to define the clinical variables that will be collected to comply with the study eligibility criteria and to ensure adequate data quality for the analyses foreseen in the project. The consortium plans to disseminate the project and its results in order to maximize the spread of knowledge that this project will generate. The study protocol will be submitted for publication on international registries (e.g., ClinicalTrials.gov) and impacted journals accepting trials-in-progress as full papers. The project consortium derives from a network of clinical institutions in close collaboration, with particular expertise on head-and-neck cancer (HNC) management. This research project will be presented and promoted at national meetings to foster patients recruitment. Articles will be prepared for publication in impacted scientific journals to disseminate project results.

T1.3. Clinical study execution. This will include the prospective collection of newly diagnosed pts data, including paired tumor tissue, H&E slides, and blood samples from 15 Italian centers. Eligibility criteria include: any age and sex; histological diagnosis of HPV-negative HNSCC (in case of primary oropharyngeal carcinoma, HPV status should be assessed either through negative p16 immunohistochemistry and/or with confirmation of absence of HPV infection); stage I-IVb non-metastatic disease; treatment with curative intent. Exclusion criteria include: non-squamous head and neck cancers (e.g., lymphoma, sarcoma, melanoma, salivary gland cancers); skin squamous cell carcinoma from the skin of the head and neck. Following the SOPs elaborated within T1.2, tumor specimens and blood samples will be collected at the participating institutions and shipped to Fond. IRCCS Istituto Nazionale Tumori for centralized at INT biological analyses, while H&E slides will be centralized and stored at AOUSS.

**Specific aim 2**

Specific Aim 2: Omics analyses

To assess the biological phenotype of circulating blood with matched tumor subtype stratification in a prospective cohort of 300 HPV-negative HNSCC pts from whom a primary tumor specimen is available along with two blood draws. The analysis will be conducted when the first 50 primary tumor specimens for which a paired baseline blood sample is available. The same numbers will be needed to progressively conduct all the following determinations till the last evaluable subject will be accrued. The analyses will be completed within 14 months.

T2.1: Omics data at bulk, single cell, spatial levels for biological implementation and subtype-based stratification. These analyses will be conducted on primary tumor specimens and will enable a more comprehensive understanding of the HNSCC biology and facilitate personalized medicine approaches. First, by bulk RNAseq we will gain the baseline six subtype stratification as defined in the original paper [PMID 25821127] representing our banchmark; this analysis will be done on all cases of our cohort (i.e. sample size calculated in T.3.3). Data will be implemented at single-cell omics level in selected cases allowing the identification of distinct cell types, characterization of cell states and transitions, and the discovery of rare cell populations that will explain the inherent heterogeneity of the disease. Spatially resolved information in selected cases will uncover the architectures and interactions of each cellular component. Single-cell and spatial analyses will be evaluated to cover representative cases based on subtype stratification and will be instrumental for digital pathology assays (T3.2). Eventually, an integrative approach will be performed using computational methods including dimensionality reduction techniques, clustering algorithms, network-based approaches, and machine learning models to summarize disease trajectories; these data will be associated to findings in T3.1 and T3.2.

T2.2: Fragmentomics. We will exploit emerging approaches in cfDNA fragmentomics implemented by methylation and topology assessment, in details: i) the fragmentation of plasma cfDNA is related the disease tumor state and linked to the nucleosomal organization, chromatin structure, gene expression, and nuclease content of the cell of origin, resulting in characteristic signatures in the form of fragment size, nucleotide motifs at the fragment ends, single-stranded jagged ends, and the genomic locations of the fragmentation endpoints; ii) DNA methylation profiles differ between cells reflecting the heterogeneous nature in tumors; we hypothesized that CpG methylation patterns of cfDNA offer invaluable information regarding the cancer specific patterns associated to the molecular subtypes; iii) besides linear double stranded filaments, cfDNA exist in other topological forms including single stranded DNA, circular/linear mitochondrial DNA, extrachromosomal circular DNA. Profiling of these cfDNA states will disclose circulating biomarkers and tumor biological features.

T2.3: circulating miRNA. BIOMATCH project supported by our preliminary data will investigate the profiles in blood circulating miRNA expression able to mirror the HNC subtypes. Accumulating knowledge in the field of miRNA research suggests that circulating miRNAs hold significant potential for unraveling subtle biological and clinical features of various disease subtypes, since they are released into the bloodstream by tumor or microenvironment cell types. Thus, they can reflect the pathological changes and molecular alterations, offering insights into the disease processes. Circulating miRNAs can be readily detected and analyzed in biofluids by small RNAseq. Their stability in these body fluids, coupled with advancements in high-throughput profiling technologies, makes them attractive candidates as non-invasive biomarkers for disease diagnosis, prognosis, and therapeutic response prediction.

**Specific aim 3**

Specific Aim 3: To correlate the biological phenotype defined in Aim 2 with the 6-cluster GE model obtained in Aim 1.

T3.1: Artificial Intelligence model development, and integration by Transfer Learning with Convolutional Neural Networks to implement longitudinal monitoring. We will implement Artificial Intelligence (AI) models to associate the six subtypes to liquid biopsies features. AI development, particularly using transfer learning with Convolutional Neural Networks (CNNs), will be empowered for implementing baseline data and longitudinal monitoring. In particular we will use transfer learning, a

technique that involves using pre-trained models developed on large-scale datasets, such as our molecular subtypes. A fine-tuning is required for this task and we will exploit CNN architectures, such as ResNet or VGGNet, which have proven effective for high dimensional-based tasks. The CNN model will be initialize with pre-trained weights from the chosen architecture and fine-tuned to train the model on the annotated data to learn the patterns and relevant features. The performance of the model will be evaluated using appropriate metrics, such as accuracy, precision, recall, or area under the curve (AUC). The model's performance will be validated on separate test datasets to ensure its generalizability and robustness.

T3.2 Development of actionable assays. Digital pathology will offer the frame for the acquisition, management, and interpretation of images of pathological specimens in a clinical setting to exploit our findings from T2.1 where our subtype stratification will be implemented at single-cell tissue level. This task will encompasse the digitization of glass slides and the use of computational techniques to analyze and interpret these digital images. Similarly, specific assay will be designed based on liquid biopsies results (T.2).

T3.3. Ethics and Statistics. To reach the primary aim of the project, we will prospectively enroll non-metastatic HPV-unrelated HNSCC patients eligible for treatments with curative intent. To calculate the sample size, we considered the data published in our article on the six GE HNSCC subtypes [PMID: 25821127].
In the dataset used for the meta-analysis, 44% of patients had a GE cluster known to be related with a poor prognosis. In particular, in the database made of 527 cases, 77 belonged to cluster 2 (mesenchymal) + 154 to cluster 3 (hypoxia). To estimate the requested sample size, the following parameters are given:
- type I error (alpha, two-sided) = 0.01;
- power (1-ß) = 0.95;
- expected ratio of sample sizes in negative (GE clusters not related to poor prognosis) / positive (GE clusters related to poor prognosis) groups = 1.3 (i.e., 296 with clusters 1-4-5-6 vs. 231 cases with clusters 2-3);
- the null hypothesis (H0) is an AUROC (area under receiver operating characteristics) curve = 0.5 (i.e., the circulating biomarkers assessed at diagnosis have no accuracy in identifying the GE clusters detected at primary tumor);
- the alternative hypothesis (H1) is an AUROC curve = 0.66 (i.e., the newly developed circulating biomarker leads to the correct identification of the GE clusters associated with poor prognosis in two thirds of the tested cases).
In this case, 228 patients (99 with clusters associated with poor prognosis + 129 with GE clusters without poor prognosis) would be needed to reject the null hypothesis [PMID: 7063747].
Calculations were performed using MedCalc version 19 (MedCalc Software Ltd). Considering a dropout of 32% (72 cases), mostly due to technical reasons (i.e., insufficient amount of tumor cells in FFPE, low quality after nucleic acids extraction, etc.), a final cohort of 300 patients is required.
Ethical Committees¿ approvals, DTA and MTA from all participating centers will be obtained within 6 months from the BIOMATCH project start.

**Experimental design aim 1**

BIOMATCH goes beyond disciplinary silos by bridging the gap between molecular determinants in the tumor tissue and their corresponding features in liquid biopsies (i.e. plasma). We will exploit different computational disciplines through combining multidisciplinary state-of-the-art artificial intelligence / machine learning (AI/ML) (which seeks to identify correlations among big data to infer the behaviour of biological systems) and mechanistic models (which seek to identify causal relations between data in order to predict the behaviour of biological systems). Our original hypothesis is that molecular cluster subtpyes detected on tumor tissues are also detected by liquid biopsies by AI/ML and mechanistic models that will complement each other to create robust virtual twins that consider both underlying physics and mechanistic aspects. BIOMATCH will leverage the potential of both approaches to provide new insights into disease mechanisms, help

identify new targets and treatment strategies, and inform decision making. This paradigm shift is one substantial step towards the Virtual Twin to help the clinicians take into account individual characteristics and medical history of patients. Sharing of the patient health data across the partners will require the review by the Institutional Research Boards and Ethics Committees. They will make sure that all legal, regulatory, ethical, and patient permission criteria are met. More specifically, we shall adhere to all applicable laws and the General Data Protection Regulation (Regulation (EU) 2016/679). All of our cohorts will be analyzed in accordance with the main and secondary clinical goals for which the data were gathered and submitted to ethical committees and the authorities. In accordance with the data governance policies put in place within each center that should utilize these data, Data Protection & Chief Data Officers and Principal Investigators will complete and sign the various applications to the relevant Data Access and Ethics Committees.
To assess patients¿ eligibility for their inclusion in the project reach and to reach the project aims, we will design an electronic case report form (eCRF). For each recruited patient, the investigators will be requested to enter the following clinical data in the eCRF:
-age at diagnosis
-sex
-smoking (current, former, never)
-smoking exposure (pack-years)
-alcohol consumption (current, former, never)
-performance status (ECOG)
-tumor site (oral cavity, oropharynx, hypopharynx, larynx, other)
-tumor subsite (ICD-O)
-histology at diagnosis
-date of diagnosis
-p16 status
-HPV status
-clinical stage (AJCC/UICC eighth edition)
-pathologic stage (AJCC/UICC eighth edition) for cases receiving surgery
-comorbidities (ACE27)
-curative treatment (yes vs no)
-modalities (single modality vs multimodality)
-surgery (yes vs no)
-radiotherapy (yes vs no)
if yes radiotherapy:
- setting (post-operative vs definitive)
- energy (photons vs protons vs other)
- technique (IMRT vs VMAT vs other)
- total dose (in Gy)
- number of fractions
-systemic therapy (yes vs no)
if yes systemic therapy:
- setting (induction vs concomitant with radiotherapy vs induction and concomitant with radiotherapy)
- drugs for induction
- drugs for concomitant chemo-radiation
- acute G3 toxicity (yes vs no)
- if yes, specify
- late G3 toxicity (yes vs no)
- if yes, specify

-status at last follow-up (alive and disease-free; alive with disease recurrence; dead without evidence of disease; dead with disease recurrence)
-date of last follow-up for alive patients
-date of death
-cause of death (primary HNSCC under study; second primary tumor; treatment-related toxicity; other causes)
-date of recurrence
-site of recurrence (any combination of local, regional, distant)
-date of second primary tumor
-site of second primary tumor (head and neck, other site, combinations)

**Experimental design aim 2**

Data will be collected following FAIR (Findable, Accessible, Interoperable and Reusable) rules. The data will be curated, quality control will make it possible to discard potential outliers due to technical bias, and normalised to allow the comparison between them.
Our experimental activities on tissue specimens include: i) bulk RNAseq of the entire cohort (n=300) that will be stratified based on the 6 molecular subtypes; ii) selection of representative cases of each cluster for single cell RNAseq with Evercode Whole Transcriptome (Parse Biosciences) and spatial-omics with Visium Spatial Gene Expression (10x Genomics).
Our experimental activities on blood specimens include: i) fragmentomics analysis (PMID: 36675018) on ct-DNA by Oxford Nanopore Technologies (ONT) including detection mitochondrial DNA and extrachromosal circular DNA; ii) methylation calling on ct-DNA by ONT; iii) ct-miRNA profiles by smallRNAseq by QIAseq smallRNAseq (Qiagen).
Non-random fragmentation pattern of cfDNA reflects epigenetic regulation and is a new area of interest in liquid biopsies. We hypothesize that fragmentomics patters are linked to the cluster's biology. Nanopore sequencing can improve fragmentomics detection by: i) CNV calling in nanopore sequence data by NanoGLADIATOR tool; ii) methylation calling for CpG 5-methyl and 5-hydroxymethyl residues by Nanopolish. Based on plasma ct-DNA methylation, through deconvolution and machine learning techniques, we¿ll merge the CNV and methylation data, plus fragmentomics data, from the same ONT sequencing .
The issues of merging single cells and spatial omics will be addressed by using complementary methodologies including "Representations by Multiple Instance Learning" (MIL), "Self-supervised Learning" (SSL), and "Prediction by Human Interpretable Features" (HIF). MIL and SSL may be integrated extremely well, creating competitive and adaptable solutions that can be trained to predict a number of output variables, including treatment response (PMID: 33903725; PMID: 36516847). We will further extend the SSL paradigm to discover how to combine representations in order to cope with limited amount of data, a situation that could happen when small clusters of cells are identified. Using a method previously applied in breast cancer (PMID: 36516847) to discover topological molecular features and their spatial associations, domain adaptation (PMID: 26782180) will be put to the test. Based on the categorization of tissue and cell types, and cellular phenotypes (PMID: 33712588), HIF approaches will create picture representations from molecular interpretable characteristics. HIFs may represent the cellular and tissue-scale features, such as the proportion of different cell types in a given area or the contact surfaces between cell populations.
Although a huge amount of data is available, only a fraction is being integrated, understood, and analyzed. The challenge lies in harnessing volumes of data, integrating the data from hundreds of sources, and proving their value in answering clinical endpoints. To properly evaluate the capability of our findings to improve clinical decisions, bio-statistical model performance estimates will be assessed in terms of: i) prediction error based on Brier scores; ii) calibration; iii) discrimination; and iv) decision curve analysis. The Brier score gives an estimate of the mean squared error of a prediction. Regarding calibration, a graphical method will be used to gauge the extent to which the predicted risks matched the observed events (relative frequency). For a perfect fit, the plot should have a diagonal (45°) line, through the origin, whose

slope is 1. Discrimination will be examined using the receiver operating characteristic (ROC) curves with censored data. Decision curve analysis (DCA) (PMID: 30241973): This was used to examine the net benefit of the model for predicting outcome. The method establishes the ability of a prognostic model to be clinically useful.

**Experimental design aim 3**

Cutting edge AI/ML methodologies will be applied to learn patient data representations in order to build pragmatic prognostic or predictive statistical models that will be tested for their ability to support clinical decisions. It will evaluate the predictive performance of the different data types and their combinations with respect to the standard clinical data. In this frame we will also test the added value of digital pathology features. Graph-inspired neural networks will be used to incorporate a priori biological information. We will use AI/ML techniques to augment the data collected for real patient sets and generate synthetic but realistic datasets conditioned on the desired patient characteristics (such as clinical data, state of the disease), capturing the statistical dependencies within and between data layers. This is done in light of the intrinsic heterogeneity at molecular levels and to disclose the biology even in small clusters of patients. The creation of virtual patient cohorts with the required features for clinical trial simulation and models to explain disease trajectories are both made possible by generative models of the patient cohorts.
By combining multi-omics with a priori biological knowledge, biological driven representations of multi-omics datasets will be developed. Modern AI/ML techniques based on transfer learning, representation learning, and generative data modeling will be used to accomplish these objectives. To pre-train and fine-tune the ML models, transfer learning will be used. Our models will understand the intricate statistical relationships between biological and clinical variables using techniques like VAEs, autoencoders with structured latent space, and GANs. We will rely on the prior work on dimensionality reduction and explainable omics data-preprocessing (PMID: 35462395). We'll look at how generative models trained on single cell datasets made accessible by cutting-edge DL techniques like scVI or DCA (PMID: 33491336) might be used to liquid biopsies to provide useful representations of bulk data. We will apply knowledge graph embedding techniques based on the combined analysis of the multi-omics data, utilizing GNN or other comparable algorithms, to integrate multi-omics with a priori knowledge. To produce as many datasets that are close to realistic states (as much as feasible) for each individual modality, generative modeling will be used. We will create omics-to-omics translation models (doi: 10.1109/JBHI.2023.3284794) to recapitulate the dependencies among data layers by learning the connections among the different omics data, based on either coupled VAEs or GANs, in order to capture the relatioships across omics layers.
We will generate virtual cohorts of patients (i.e. virtual twins) using generative methods such as conditional GANs. These realistic populations of virtual patients will be generated (PMID: 31919373) with the desired clinical characteristics in terms of clinical stage, grade, molecular alterations. This will make it possible to equilibrate and augment the presence of rare but important phenotypes for the in silico experiments which will be performed in the in silico clinical trials. Virtual (based on simulations) trials will consist in predicting the effect of a drug or combination of drugs on a patient population together with uncertainty of the trial outcome. In this way we expect to foresee the most useful treatment modalities for each molecular subtype.
We also aim at going beyond traditional genomics approaches by incorporating multi-scale approaches including digital pathology in integrative analyses to train virtual twins for personalised disease management. We will build molecular representations of histopathological images which map complex cellular and tissue phenotypes to a latent space predictive of treatment response and we will use the data from digital pathology along with single cell and spatial omics.

**Picture to support preliminary data**

Preliminary_PNRR.pdf

**Hypothesis and significance**

Our central hypothesis is that matching the previously found 6 GE-based HNSCC molecular subtypes with corresponding

digital pathological imaging-based features (pathomics) implemented by insights from at single-cell and spatial resolution and their corresponding circulating profile at baseline will facilitate clinical uptake of prognostic and predictive tools in individual clinical decision-making. In addition, the development of specific circulating biological subtypes will enable to follow the disease trajectory at the subclinical level after treatment completion, serving as a signal of minimal residual disease (MRD). We expect 3 alternative scenarios:
1. at 3 months after curative cCTRT treatment no blood-based alterations will be found (absence of MRD = MRD-);
2. the blood-based profile, mirroring its specific HNSCC subtype, will be found at 3 months after curative treatment conclusion (presence of MRD = MRD+);
3. the blood-based profile identified at 3 months do not reflect the baseline characteristics (different MRD).
In scenario 1 and 2 we will test the sensitivity and specificity of the developed biomarker to predict pts outcome. We will assess if the blood results (MRD+ or MRD-) improve outcome prediction already enabled by tumor tissue-based subtype stratification. In scenario 3 we will correlate the new findings with pts outcome and compare it with the expected outcome based on primary tissue analysis.
We expect to foster clinical uptake and research in the field a personalized approach by translating the GE profile of patients with locally advanced curable HPV negative head-and-neck cancer in circulating biomarker and/or at the level of pathological images. This would lead to a significant improvement in the understanding of molecular mechanisms at the basis of an unfavorable outcome (almost 50% of patients) with standard of care including radiation and chemotherapy. We expect to find a wide range of resistance alterations possibly in relation to the cluster subtype to which a given tumor belongs. By dissecting these mechanisms, we will be able to envision more effective treatment approaches by correctly anticipating the individual disease trajectory, sparing futile treatments and designing new ones based on specific tumor evolutions after unsuccessful primary curative treatment.

## 5.5 Methodologies and statistical analyses

**Methods of data collection**

Both partners have the technological background, the expertise, and the capabilities to conduct the present project. For instance, the Head and Neck (HN) group of INT has established a strong networking with all the other groups devoted to research activities and clinical management in this setting at both national and international levels. The HN INT group has coordinated several studies, some of which [e.g. PMID: 28950305] within Gruppo Oncologico Nord-Ovest (GONO) network, which will be also involved in the current project proposal.
Data from WP2 will represent a few dozens of terabytes which require sufficient storage capacity and computing power to process them, as well as to train and evaluate the different models proposed in our computational framework. The dedicated infrastructure is available at INT and it will provide high performance computing resources, storage capacity and heterogeneous computing power (with both CPUs and GPUs). Estimated raw data size is 30TB that should be available for data pre-processing. Around 8,000 CPU-hours (with multi-threading using at most 16 cores over 64GB of RAM) will be needed by the bioinformatics pipelines (most of them implemented with nextflow and singularity). The preprocessing requires around 10TB of temporary scratch as the preprocessing will produce this amount of relevant output data which will be used to inform Machine Learning (ML)/Artificial Intelligence (AI) and mechanistic models (WP3). This relevant output data should be kept during the timeframe of the project. ML/AI and mechanistic models should be trained, tested on a regular basis on these data. Data will pseudo-anonymised or anonymised encrypted (e.g. SHA-256). Achieving the right level of parallelisation will allow the workloads to be run reducing costs and time; thus, the needed computational requirements are estimated and are in place to allow to accomplish the goals of the project.
The creation of reusable, reproducible, shareable analysis that can also be tailored to the characteristics of a specific computing environment will be made much simpler by workflow management systems like nextflow and container technologies like Docker or Singularity/Apptainer (a lesser known but more HPC-friendly container technology). These methods will guarantee the software's usability, maintainability, interoperability, sustainability, portability, reproducibility,

scalability, and effectiveness for increased acceptance and dissemination in the scientific community.

**Statistic plan**

Statistic plan
Objectives and endpoints
- Primary objective: to assess the accuracy of a blood profile (b-OMICS) in identifying the tumor tissue subtypes (t-OMICS) in loco-regionally advanced HPV-negative HNSCC patients
- Primary endpoint: sensitivity and specificity of b-OMICS (newly identified and developed in the current project) in diagnosing Cl2-Cl3 on t-OMICS (based on the transcriptomic model developed by De Cecco et al. Oncotarget 2015) in stage III-IVa/b (AJCC/UICC eighth edition) HPV-negative HNSCC patients treated with curative intent
- Secondary objective: to assess the survival outcomes of HPV-negative HNSCC patients based on their t-OMICS and b-OMICS
- Secondary endpoint: disease-free survival (DFS) and overall survival (OS) stratified according to t-OMICS (Cl2-Cl3 vs. others; presence or absence of b-OMICS signature)
- Exploratory objectives:
i. to assess the accuracy of a digital pathology model in identifying the t-OMICS in loco-regionally advanced HPV-negative HNSCC patients
ii. to assess the accuracy of a blood GE profile (b-OMICS) in identifying the digital pathology model in loco-regionally advanced HPV-negative HNSCC patients
iii. to assess the survival outcomes of HPV-negative HNSCC patients based on digital pathology model
- Exploratory endpoints:
i. sensitivity and specificity of digital pathology model (newly identified and developed in the current project) in diagnosing Cl2-Cl3 on t-OMICS (based on the transcriptomic model developed by De Cecco et al. Oncotarget 2015) in stage III-IVa/b (AJCC/UICC eighth edition) HPV-negative HNSCC patients treated with curative intent
ii. sensitivity and specificity of b-OMICS (newly identified and developed in the current project) in diagnosing the digital pathology model (newly identified and developed in the current project) in stage III-IVa/b (AJCC/UICC eighth edition) HPV-negative HNSCC patients treated with curative intent
iii. DFS and OS stratified according to the newly developed digital pathology model.
Analysis methods will include classical biostatistical analyses for diagnostic tests and time-to-event statistics (details are reported in the ¿Statistical analysis¿ section).
The associations between t-OMICS and b-OMICS will be assessed after the completion of the biological analyses of the last-patient-in samples and specimens. The associations between GE models and digital pathology will be analyzed after the completion of the digital pathology acquisition of the last-patient-in HNSCC specimen.
Data monitoring activities will include periodic remote visits to ensure clinical data quality. To this end, a monitor will assess the concordance between source data and the eCRFs once every 3 months for the first year and once every 6 month for the subsequent years. All the clinical partners will receive the data monitoring plan during the study setup phases. They will accept to agree with it to ensure the most timely data entry and the highest quality of the data.
Bioinformatics plan
Our bioinformatics plan will include the assembly and organization of data for querying and developing tools for its querying, computation, and modeling. Bioinformatics pipelines will be developed to process each data type to ensure harmonisation of molecular entities (gene ID, protein ID, ...), sample IDs, and detect potential errors introduced during data collections. Metadata collection will be formalised and validated as part of the bioinformatics pipeline. Pipeline development will also aim at integrating the data into the UNCAN European research data hub (https://uncan.eu/a-european-research-data-hub/).

**Statistical analysis**

We hypothesize that the molecular profiling of tumor tissue at bulk level (t-OMICS) can be assessed and translated in blood determinants (b-OMICS)
The following definitions are given:
- Cl2-Cl3 on t-OMICS with the presence of the newly detected b- OMICS: true positive (TP)
- Cl2-Cl3 on t- OMICS with the absence of the newly detected b- OMICS: false negative (FN)
- t-OMICS other than Cl2-Cl3 with the presence of the newly detected b-OMICS: false positive (FP)
- t-OMICS other than Cl2-Cl3 with the absence of the newly detected b-OMICS: true negative (TN)

Given these definitions, after patient recruitment and translational analyses, we will measure sensitivity (true positive rate), specificity (true negative rate), positive and negative predictive values, false negative and false positive rates, false discovery and false omission rates, positive and negative likelihood ratios, errors of types I and II.
As secondary aims, we will assess the prognostic significance of the t-OMICS and b-OMICS considering the disease-free and the overall survivals (DFS and OS, respectively). In particular, we will assess these time-to-event outcomes with the Kaplan-Meier method and compare patient groups with log-rank tests. Stratification will be conducted based on the following categories: age, stage, HNSCC subsite, treatment (unimodal vs. multimodal; including surgery, radiotherapy, chemotherapy, combinations), t-OMICS (i.e., survival of patients with Cl2-Cl3 vs. non-Cl2-Cl3 t-OMICS clusters; each cluster one against the others), b-OMICS (i.e., survival of patients with the newly b-OMICS profile vs. those without), digital pathology models.
As exploratory objectives, we will evaluate the association of the newly developed digital pathology model to b-OMICS and t-OMICS, and assess any difference in survivals based on the digital pathology model.
Based on the follow-up and the global number of events, the Kaplan-Meier curves will be used; between curves comparison will be performed by using the log rank test. Every effort will be made to ensure an adequate follow-up history for the cohort entering in our study. Multivariable Cox regression model analysis will also be performed in order to evaluate whether the classifier will provide more accurate and independent predictions than that associated to known clinical covariates. Cox model results will be presented in terms of hazard ratios and their 95% confidence intervals, together with p values at two sided Wald test. The performance of the Cox model including the classifier together with the clinical covariates and, for the sake of comparison, the performance of the model including the clinical covariates only, will be quantified with the boostrap-corrected Harrell C statistic. In all cases, statistical significance will be set at 0.05.
Bioionformatics analyses
Raw data will be processed and normalized so that multiple datasets may be compared, preventing any bias in the downstream analysis. Dedicated bioinformatics pipeline (https://github.com/bioinfo-pf-curie/RNA-seq) will be used to analyze the RNA-seq/miRNA-seq data. Briefly, raw sequencing reads will be trimmed, matched to the Human reference genome, and evaluated for their quality. The samples' quality will be verified using cutting-edge quality criteria like library complexity, alignment statistics, or duplication level. On the Gencode annotations, the salmon tool will be used to quantify gene expression. Using the ComBat approach or by modifying the data prior to feature computation using cycled Generative Adversarial Networks, the consistency of the statistical distribution of the data will be examined and realigned as necessary. Dimension reduction (PCA, NMF, UMAP, tSNE, etc.) or hierarchical clustering will then be used to the data after normalization to ensure that any unfavorable technical biases have been eliminated.

**Timing of analysis data**

The research project aims at matching primary tumor gene expression based clusters and blood molecular features in HPV-negative squamous head and neck cancers. To this end, 300 patients will be recruited (details reported in the section dedicated to sample size calculation).
The first 4 months will be dedicated to establish a consortium agreement among partners and DTA/MTA will be signed among the centers that will be involved in patient recruitment.

Eligible patients will be recruited in 15 Italian centers over 14 months. Tissue specimens and blood samples will be collected at baseline (i.e., before starting curative treatments), and once after at least 3 months from treatment conclusion. For cases receiving multimodal strategies, tumor specimens and blood samples will be collected before the first therapy, whichever is delivered first. The average duration of a curative treatment usually ranges from 2 to 3 months: i.e., for patients candidate to surgery, approximately 1 month for surgery + 7 weeks for post-operative (chemo)radiation; for those candidate to definitive (chemo)radiation, approximately 6-7 weeks of treatment. After treatment completion, patients will be followed up as per clinical practice following the scheduled visits recommended by international clinical practice guidelines for managing head and neck squamous cell carcinoma patients, i.e., once every 3 months [PMID: 33239190]. Patients¿ status (alive or dead, with or without disease recurrence) and toxicities will be recorded once every follow-up visit. Given these timings and the fact that the project lasts 2 years, the last-patient-in will be recruited within the 18th month to ensure at least 3 months of post-treatment follow-up. In addition, every effort will be done in order to update the follow-up event after the end of the project and ensure reliability in time-to-event analyses.
As soon as the specimens will be collected we will proceed in nucleic acids extraction and quality checks from month 4. At first, tumor subtype stratification will be assigned by bulk RNAseq. The molecular subtype stratification will be transposed on liquid biopsies and these activities will start from month 6. We will test a number of determinants through ctDNA/ctmiRNAs exploring fragmentomics , methylation and miRNAs patterns (month 6) since they are closely associated to biological events as those found in tissue cluster. Further analyses on tissue specimens will complement the biological cluster membership at single cell/spatial omics levels (month 12) disclosing the cellular landscapes inherent in these tumors. Single cell/spatial omics will also be the foreground for the digital pathology workflow (month 12) . The details about the bioinformatics and AI analyses both on tissue and liquid biopsies are already addressed in the experimental design aim2 and aim3 as well as in the GANTT chart.
Our study is design to test a number of signatures (month 18) with the assessment of predictive models capable of providing new treatment modalities. Since our cohort of patient is treated with conventional radiation, we will apply the Radio Sensitivity Index (RSI), a pan-cancer signature based on 10 genes that was already successfully tested in head and neck tumors. We have previously demonstrated that RSI is able to reveal underlying heterogeneity in radiation treatment effect within groups presumed to have been treated uniformly (with approximately equivalent physical dose). We also already proved that RSI is associated to the subtype cluster stratification with CI1 showing high radiosensitivity while CI2/CI3 are radioresistent. Our project is expected to disclose features in blood that will help in defining the patients that will benefit from radiotherapy and in monitoring the trajectories of the disease over time explaining the wide heterogeneity in predicted RT effects in spite of relatively uniform RT dose prescribed.

## 5.6 Expected outcomes

In relation of our specific aims we expect the following:
1. We are confident to be able to set up within a short period of time a study that will collect material and clinical data of a selected head and neck cancer population. We base this assumption on our prior experiences on setting up clinical studies on head and neck cancer. As a matter of fact, we have already demonstrated that our team is able to act as a collection hub for translational projects involving tumor specimens. In this project we rely on a consolidated network of clinical centers used to collaborate within clinical trials. The study activation, conduction and monitoring will be supported by a CRO specifically appointed for the study. Among the expected outcome we envisage to count on a motivated group of investigators. This will consolidate the research network based on clinicians working in hospitals scattered over the Italian territory. In our project the two Units will act as hub respectively for the southern and for the northern located centers. This will give the opportunity also to reach out to hospitals that are generally not involved in clinical research, although they regularly treat a significant number of patients with head and neck cancer. This will allow to strengthen the existing network and it will contribute to distribute and support a collaborative research culture.
2. We aim at dissecting the HNSCC biology according to previous work that has significantly contributed to the knowledge

of its heterogeneity. Indeed identified gene expression clusters have a significant role in term of prognostic impact. In HNSCC, likely to other cancers, tumor subtypes will soon gain prime interest for treatment profiling (e.g. escalating treatment for high risk clusters, deescalating it in low risk populations); thus, it is becoming imperative to look for solutions that simplifies this analysis. Our project will significantly contribute to the acquisition of blood biomarkers that mirrors the primary tumor cluster characteristics to allow for an extended use of the individual biological features for optimal prognostication and treatment decision. In addition it is conceivable that the availability of such blood markers will allow for an easy, non-invasive, timely detection and monitoring of the treatment effect offering a unique opportunity to follow the real time tumor kinetic. In addition, it will potentially enable to track tumor evolution and escaping mechanisms in the unfortunate scenario of treatment resistance that in locally advanced HNSCC is reaching 50%. In this scenario we are essentially aiming at constructing the scientific, clinical and methodological basis for the development of a lab on chip device.
3. In line with the concept of simplifying thus potentially enlarge the feasibility and accessibility of biological subtype identification at the level of the primary tumor, we aim at exploiting the image based pathology information generated from digitalized glass slides. The development of a digital pathology based approach for quantitative pathological assessment that include artificial intelligence solutions allows for extraction information beyond the human visual perception. By recapitulating the gene expression clusters through the use digital pathology we will be able to empower histopathological diagnosis by ¿deep¿ biological significance thus adding a new layer to the standard diagnostic procedures.
4. A new and significant dataset including specimens and clinical data of highly selected subjects with HNSCC will be generated and it will be added to the existing database today kept by INT.
In summary, we expect to conduct a prospective collection of high quality clinically annotated tumor/blood specimen of a large cohort of patients. This will enable to simplify and to accelerate the uptake of a new diagnostic procedure that will be essential for personalising curative treatment of locally advanced HNSCC patients.

## 5.7 Risk analysis, possible problems and solutions

The strategy for Risk Management will be managed at key checkpoints (Month 8, Month 16, Month 24,). We will implement risk prevention strategies in critical areas (WPs and Tasks).
The project will include intermediate steps in order to monitor the completion of each steps and support actions to correct issues. The key step of BIOMATCH is the capability to integrate data coming from different sources obtaining models and graphical visualization tools. Although the feasibility of this approach is documented in literature, we have been dealing with a full genomics characterization at single-cell/spatial levels for the tissue and matched determinants in liquid biopsies. For this reason, we will indentify intermediate research endpoints to fully exploit the potentiality of the data. The project¿s development will start from the identification of the biological entities/features of the tumor assessed in WP2. As first, the assessment by single-cell sequencing will elucidate the cellular content of tumor microenvironment and this data will be matched to fragmentomics data including also methylation ctDNA data. At first genomics data will be analyzed individually, and then integrated using different frameworks. A preliminary assessment has been made as part of this proposal and some potential risks are estimated in advance, as follows.

Risk: delays in approval of clinical study from ethical review boards centers; reasons include lack of anticipation of ethical issues raised, insufficiently robust defense of research methods and different review boards raising different issues.
Mitigation: the study description has been extensively revised by the clinical collaborators to minimize this risk.

Risk: patient drop out; we expect a patient dropout of about 15-20% for technical reasons;
Mitigation: the risk was already evaluated and takes into account.

Risk: failure to detect meaningful molecular patterns in genomic analysis; we expect that the planned genomics activities

will allow detecting the expected alterations at single-cell level.
Mitigation: extensive literature supports this statement.

Risk: Issues in cognitive analytics tools
Mitigation: The architecture of the BIOMATCH platform will be developed on the basis of experience of the partners that ensures the success.

Risk: Issues Unforeseen complexity of developing and integrating data, models and algorithms; it is expected that in the near future new algorithms will be developed especially in the frame of spatial applications and liquid biopsies into biological field.
Mitigation: We will continuously update our bioinformatics and statistical plans to improve our analyses.

Risk: The discovery phase is not sufficient to fulfill the project aims; this risk is typical of a research projects in challenging clinical domains, especially in innovative approaches.
Mitigation: The PI, clinical and technical partners have acquired sufficient background experience in clinical projects, making the occurrence of this risk very low.

Risk: Project planning not precise.
Mitigation: Permanent coordination among PI, project management, and scientific collaborators will be sufficient to mitigate this risk.

## 5.8 Significance and Innovation

CtDNA has emerged as a diagnostic tool to detect the presence of cancer or quantify the tumor burden. The available commercial assays fail to detect ctDNA in more than half of the tested patients, thus, posing a significant uncertainty in relation to its clinical usefulness. In this context, new approaches such as detecting methylated DNA and ct-miRNAs may represent an added value in HNSCC. In this scenario, the liquid biopsy field is moving in the context of HNSCC surveillance after curative treatment. Given this background we believe that our project will be able to move the field ahead by exploring and establishing circulating biomarkers in the context of HPV negative tumors. These biomarkers will mirror the primary tumor clusters which are prognostically relevant. In this regard the project will advance the field by combining in the same diagnostic tool two properties: 1. early detection of tumor recurrence, 2. provide prognostic and predictive information.

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## 5.10 Timeline / Deliverables / Payable Milestones

The BIOMATCH project implementation plan is organised over 24 months with 3 work packages corresponding to the specific aims of the project, which are further structured in tasks. The overall strategy of the work plan is based on a division of the workload according to the target technologies of the project objectives and the achievement of specific goals in the project. The structure is worked out very detailed, to allow progress monitoring. While each tasks has clearly distinguishable activity fields and interfaces, all tasks consist of tight collaborative interactive efforts between the partners which are described later in the work plan.

**Milestones 12 month**

a) study approval by the local independent ethics committee; b) controlled collection of fresh frozen tissue specimens, plasma samples at baseline and during follow-up, banking of nucleic acids; c) collection of baseline clinical data, database update, and check on data quality focused on the project needs;

**Milestones 24 month**

a) gene-expression assessment and subtype stratification; b) single-cell RNAseq ; c) methylation and miRNA profiling in blood specimens; d) data processing; e) data analysis and association to clinical endpoints; f) data integration;

**Gantt chart**

gantt.pdf

## 5.11 Equipment and resources available

**Facilities Available**

The project is a multidisciplinary effort that involves biologists, bioinformaticians, biostatisticians, oncologists, surgeons, pathologists, covering the required needs and expertise for the project¿s completion.
INT is a comprehensive cancer center which - for almost a century - has been managing clinical and pre-clinical research, with national and international, sponsored or investigator-initiated trials. Moreover, INT is a referral center for treatments with Advanced Therapy Medicinal Products (ATMP). At INT, the level of global care for rare tumors is highly qualified as that provided for tumors with higher incidence. Throughout 2022, INT has carried out 818 clinical studies, of which 469 clinical trials, 333 observational studies and 16 registries. For information on all resources and technological facilities available at INT visit: https://www.istitutotumori.mi.it/web/guest/research-core-facilities.
The Azienda Ospedaliera Universitaria (AOU) of Sassari is the largest hospital in Sardinia. A multidisciplinary head and neck tumor board manages cancer patients in all phases from diagnostic work up and staging to primary treatment (all 3 modalities, chemo/radiotherapy and surgery), to salvage treatments to follow up. The ENT division is a referral center for head and neck oncology receiving patients from all the country. Day hospital, ward and surgical activities are carried out.

**Subcontract**

The prospective study will be multi-institutional to reach the desired sample size in the planned project timeline. It is essential that the complex involvement of the several centers with high volumes of HNSCC patients does not hamper the feasibility of the research project. Therefore, to ensure the timely conduction of the project tasks related to the clinical study design, setup, and conduction (detailed in tasks 1-3), a contract research organization (CRO) will be involved. In particular, during the clinical study setup, the CRO will provide substantial support in coordinating the agreements (e.g., DTA, MTA, contracts) between the legal and administrative offices of each institution recruiting patients, as well as the submission to the relevant ethical committees. The CRO will coordinate the smooth sharing of biological samples and data with the relevant institutions responsible for the analyses foreseen in the project. During the study execution, the CRO will substantially contribute to data monitoring activities.

## 5.12 Desc. of the complementarity and sinergy of secondary collab. researchers

The choice of secondary collaborator researchers was attentively carried by taking into account the criteria of complementary expertise and synergy of research roles. Secondary collaborator researchers are highly committed biologists, bioinformaticians, and clinicians. The INT collaborators have a relevant knowhow on molecular biomarkers, biotechnology and high throughput assay design. They in this project will be participate in the development of the project and overesee for the accurate sample storage, sample processing and data annotation. Also, they will provide the timely delivery of results for the statistical analyses held at INT. The AOUSS second collaborator are currently focused on clinical pathology and will ensure the standardized collection and storage of samples for the digital pathology analyses that will be performed at AOUSS under the supervision of Professor of General Pathology Maria Maddalena Simile and Dr. Rosa Maria Pascale. In addition at INT, material collection, data storage, bioinformatics and omics analyses will be run by a proactive group of researchers with an long-lasting expertise in this research area, including also covering computational, bioinformatics, and biostatistics areas. The 2 partners offer a unique complementarity in knowledge and expertise to cover the specific tasks of the project.

## 5.13 Translational relevance and impact for the national health system (SSN)

**What is already know about this topic?**

The majority of HNSCC pts are diagnosed with a loco-regionally advanced disease [https://seer.cancer.gov/statfacts/html/all.html], which recur in approximately 60 % within the first 2 years despite aggressive approaches. The standard of care includes radiation and chemotherapy, sometimes in combination with surgery. Apart from cancer (stage, HPV status) and pts-related characteristics we lack predictive biomarker so that at present HNSCC pts are treated with a ¿one-size-fits-all¿ approach. INT identified a GE model classifying HNSCC pts in 6 groups, with distinct biological and clinical characteristics. INT found that 44% of pts belonged to clusters with poor prognosis while one out of ten pts may potentially benefit from immune-based therapeutic approaches. At present, GE subtypes are not used to stratify patients for individual treatment approaches. There is ample literature in oncology where a biologically driven approach has led to significant outcome improvements.

**Details on what is already know about this topic**

In head and neck cancer, full tumor control remains an elusive goal and its response to curative treatment is still unpredictable. Currently patient¿s risk stratification and treatment decision are determined based on the consensus of a multidisciplinary team using international guidelines, which are based on the TNM staging system. Treatment at late stages needs a multimodal approach that includes radiation therapy either alone or in combination with chemotherapies. Despite improvements in diagnosis and therapy, as well as pre-clinical and translational research efforts, the 5-years mortality rate has not changed in the past 20 years and remained roughly 50%.
At present, little forward progress has been made to translate as much of basic biological and science knowledge as possible into strategies that may significantly alter patient outcomes in terms of local and loco-regional control, and eventually overall survival. A switch in the approaches of studying HNSCC is deeply required.

**What this reasearch adds?**

Given the dismal prognosis and the poor quality of life resulting from curative approaches of locally advanced HPV-negative HNSCC, the recently issued European Cancer Plan has indicated among others head-and-neck cancer a priority for efforts directed to improvement in treatment and research. Our ambition is to translate the biological information of primary tumor into the peripheral blood and also to be able to capture tumor biology heterogeneity through single-cell, spatial, and pathomic analyses. If we find circulating based biomarker profiles able to predict the molecular patterns of primary HNSCC specimens, we will deliver a trustable non-invasive tool which will easily assess the individual biological print. The results of our project is expected to guide and support the evolution of the head-and-neck cancer care towards biologically-driven approaches in the curative settings, which are still dominated by standard ¿one-size-fits-all¿ radiation and chemotherapy.

**Details on what this reasearch adds**

This study addresses an unmet clinical need in HNSCC patients care. Recently, the European Cancer Plan indicated Head and Neck Cancers as one of the priority subjects for efforts directed to improve standard-of-care therapies and research. In the current practice, no reliable tools are available to detect patients¿ risk groups. Previous studies validated 6 prognostic clusters based on genomic signatures and their application is eagerly needed. We aim to detect the biological information of HNSCC from peripheral blood and capture tumor biology heterogeneity through single-cell, spatial and pathomic analyses. By crossing data from genome profiling/liquid biopsies/digital pathology, this study will realize a pivotal step towards finding cost-effective and accurate prognostic methods potentially applicable on large scale. The results will guide HNSCC patients¿ care towards biology-driven approaches in the curative settings, still dominated by standard one-size-fits-all radio-chemotherapy.

**What are the implications for public health, clinical practice, patient care?**

The highest burden of disease recurrences and cancer-related mortality in HNSCC pts is owed to the HPV-negative disease. This is still a disease with high mortality, poor quality of life and a significant financial burden to patients and their families. Furthermore, HPV-negative HNSCC is non-preventable chronic conditions which have a high impact on health and health systems. Therefore, we deem that this pts group is the one with the strongest unmet need for innovative research approaches that has already proved to be successful in other solid cancers. We may anticipated that through a more biological driven treatment approach we may significantly add to generate new knowledge that will turn to be instrumental to support the change in the present "one fits all" treatment paradigm. By pursuing this approach we anticipate a deep impact on patient care and clinical practice resulting in a significant benefit for the public health especially in avoiding futile and toxic treatments.

**Details on what are the implications for public health, clinical practice, patient care**

HPV-negative HNSCC patients are burdened by high risk of recurrences, cancer-related mortality, poor quality of life and significant financial burden. HPV-negative HNSCC are non-preventable chronic diseases with a high impact also on sociality and workforce, as several patients struggle with impaired social interactions (for dysphonia, dysphagia) and depression. Patients¿ care is complex due to complications possibly occurring before/during/after oncological treatments. This study aims at informing the clinicians on HNSCC aggressivity by using accessible tools such as liquid biopsies and digital pathology. Also, it will help shaping clinical trials design and improving the eligibility of patients to precision-oncology treatments. We anticipate a deep impact on patients¿ care, resulting in a significant benefit for the public health, i.e. by de-escalating toxic treatments for patients with favorable prognostic signatures and intensifying the follow-up for those with unfavorable traits.

# 6 - Budget

**Total proposed budget ( Euro )**

| Costs | TOTAL BUDGET | Co-Funding | List of costs proposed for funding to the MOH | Percentage of total proposed to the MOH |
|---|---|---|---|---|
| 1 Staff Salary | 168.906,00 | 168.906,00 | not permitted | 0,00 |
| 2 Researchers' Contracts | 584.000,00 | 0,00 | 584.000,00 | 58,40 |
| 3a.1 Equipment (Leasing - | 16.000,00 | 16.000,00 | 0,00 | 0,00 |
| 3a.2 Equipment (buying) | 0,00 | 0,00 | 0,00 | 0,00 |
| 3b Supplies | 168.500,00 | 0,00 | 168.500,00 | 16,85 |
| 3c Model Costs | 0,00 | 0,00 | 0,00 | 0,00 |
| 4 Subcontracts * | 70.000,00 | 0,00 | 70.000,00 | 7,00 |
| 5 Patient Costs | 44.500,00 | 0,00 | 44.500,00 | 4,45 |
| 6 IT Services and Data Bases | 4.000,00 | 0,00 | 4.000,00 | 0,40 |
| 7 Travels | 7.000,00 | 0,00 | 7.000,00 | 0,70 |
| 8 Publication Costs | 10.000,00 | 0,00 | 10.000,00 | 1,00 |
| 9 Dissemination | 6.500,00 | 0,00 | 6.500,00 | 0,65 |
| 10 Overheads * | 60.500,00 | 0,00 | 60.500,00 | 6,05 |
| 11 Coordination Costs | 45.000,00 | 0,00 | 45.000,00 | 4,50 |
| **Total** | 1.184.906,00 | 184.906,00 | 1.000.000,00 | 100,00 |

* percentage calculated as average value between all the Operating Units.

Report the Co-Funding Contributor:

co-funding: PI effort 10%, 8574/yr; coPI effort 10%, 7713/yr; Bergamini effort 30%, 19595/yr; Cavalieri effort 20%, 10314/yr; Deganello effort 20%, 17148/yr; Bussu effort 10%, 13375/yr; Fadda effort 10%, 7734/yr
co-funding: Tapestation (Agilent) 8000 euro/yr

**Budget Justification**

| | |
|---|---|
| 1 Staff Salary | Salaries of the permanent staff (only co-fund) |
| 2 Researchers' Contracts | The project will support the research contract of young clinical and transnational researchers |
| 3a.1 Equipment (Leasing - Rent) | Platform for quality check of nucleic acids (Tapestation, Agilent) only co-fund |
| 3a.2 Equipment (buying) | n/a |

| | |
|---|---|
| 3b Supplies | reagents, kits, plasticware, buffers, enzymes, and consumables for experimental analyses on tissue specimens (transcriptomics, single cell, spatialmocis, digital pathology) and liquid biopsies (methlyation, fragmentomics, ct-miRNAs) |
| 3c Model Costs | n/a |
| 4 Subcontracts | Subcontract to a company providing support to study submission to ECs, study cunduction, and sample retrieval. |
| 5 Patient Costs | Cost for shipments and fees for in-site sample collection. |
| 6 IT Services and Data Bases | Fee to access platforms for data analyses. |
| 7 Travels | Costs for attending scientific conferences. |
| 8 Publication Costs | Fees for english editing and publication |
| 9 Dissemination | Attendance to conferences and meetings to present the work and results done in the project |
| 10 Overheads | Istitutional costs |
| 11 Coordination Costs | cost for the organization of two meetings among partners |

**Project Code:** PNRR-MCNT1-2023-12377359 **Call section:** Malattie Croniche non Trasmissibili (MCnT1) ad alto impatto sui sistemi sanitari

**Applicant Institution:** Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano **Applicant/PI Coordinator:** Licitra Lisa Francesca Linda

## Proposed total budget UO1 Institution: Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano (Euro)

| Costs | TOTAL BUDGET | Co-Funding | List of costs proposed for funding to the MOH | Percentage of total proposed to the MOH |
|---|---|---|---|---|
| 1 Staff Salary | 126.688,00 | 126.688,00 | not permitted | 0,00 |
| 2 Researchers' Contracts | 350.000,00 | 0,00 | 350.000,00 | 58,33 |
| 3a.1 Equipment (Leasing - Rent) | 16.000,00 | 16.000,00 | 0,00 | 0,00 |
| 3a.2 Equipment (buying) | 0,00 | 0,00 | 0,00 | 0,00 |
| 3b Supplies | 84.500,00 | 0,00 | 84.500,00 | 14,08 |
| 3c Model Costs | 0,00 | 0,00 | 0,00 | 0,00 |
| 4 Subcontracts | 40.000,00 | 0,00 | 40.000,00 | 6,67 |
| 5 Patient Costs | 22.000,00 | 0,00 | 22.000,00 | 3,67 |
| 6 IT Services and Data Bases | 4.000,00 | 0,00 | 4.000,00 | 0,67 |
| 7 Travels | 4.000,00 | 0,00 | 4.000,00 | 0,67 |
| 8 Publication Costs | 10.000,00 | 0,00 | 10.000,00 | 1,67 |
| 9 Dissemination | 4.000,00 | 0,00 | 4.000,00 | 0,67 |
| 10 Overheads | 36.500,00 | 0,00 | 36.500,00 | 6,08 |
| 11 Coordination Costs | 45.000,00 | 0,00 | 45.000,00 | 7,50 |
| **Total** | 742.688,00 | 142.688,00 | 600.000,00 | 100,00 |

| | |
|---|---|
| **Project Code:** PNRR-MCNT1-2023-12377359 | **Call section:** Malattie Croniche non Trasmissibili (MCnT1) ad alto impatto sui sistemi sanitari |
| **Applicant Institution:** Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano | **Applicant/PI Coordinator:** Licitra Lisa Francesca Linda |

## Budget Justification

| | |
|---|---|
| 1 Staff Salary | co-funding: PI effort 10%, 8574/yr; coPI effort 10%, 7713/yr; Bergamini effort 30%, 19595/yr; Cavalieri effort 20%, 10314/yr; Deganello effort 20%, 17148/yr |
| 2 Researchers' Contracts | Bioinformatician effort 100%, 30000/yr (tot 60000 euro) ; PhD student effort 100% 30000/yr (tot 60000 euro); Medical oncologist effort 100% 40000/yr (tot 80000 euro); three junior researcher effort 100% 25000/yr (tot 150000 euro) |
| 3a.1 Equipment (Leasing - Rent) | co-funding: Tapestation (Agilent) |
| 3a.2 Equipment (buying) | n/a |
| 3b Supplies | RNA/DNA extraction quality control (7000);<br>RNAseq: library prep(16000 euro); sequencing Illumina (16000)<br>fragmentomics/methylation: library prep (10000 euro); sequencing Nanopore (14000)<br>ct-miRNASeq: library prep (12000 euro); sequencing Illumina (9500) |
| 3c Model Costs | n/a |
| 4 Subcontracts | Subcontract for the management of clinical trials according to Good Clinical Practice standards according to its SOPs (constantly updated and monitored).<br>Site Monitoring Visits (on-site - 3/site), Close-out Visits (on-site) eCRF and data management |
| 5 Patient Costs | Costs of ancillary medical services for patients enrolled in the clinical study (i.e. Tubes/vials for blood and tissues, slides, shipments). Insurance and Safety management |
| 6 IT Services and Data Bases | Fees for clinical database/data analyses |
| 7 Travels | Costs for travels to attend international conferences |
| 8 Publication Costs | Fees and cost for english editing and publication |
| 9 Dissemination | Fee to attend conferences aiming at disseminating the results and findings of the project |
| 10 Overheads | Istitutional cost |
| 11 Coordination Costs | Cost for shipments to recover biological material from recruiting centers e between INT and AOUSS (42000 euro). Organization of two consortium meeting (tot 3000euro); i) kickoff meeting; ii) second year meeting |

## Proposed total budget UO2 Institution: Azienda Ospedaliero Universitaria Sassari (Euro)

| Costs | TOTAL BUDGET | Co-Funding | List of costs proposed for funding to the MOH | Percentage of total proposed to the MOH |
|---|---|---|---|---|
| 1 Staff Salary | 42.218,00 | 42.218,00 | not permitted | 0,00 |
| 2 Researchers' Contracts | 234.000,00 | 0,00 | 234.000,00 | 58,50 |
| 3a.1 Equipment (Leasing - Rent) | 0,00 | 0,00 | 0,00 | 0,00 |
| 3a.2 Equipment (buying) | 0,00 | 0,00 | 0,00 | 0,00 |
| 3b Supplies | 84.000,00 | 0,00 | 84.000,00 | 21,00 |
| 3c Model Costs | 0,00 | 0,00 | 0,00 | 0,00 |
| 4 Subcontracts | 30.000,00 | 0,00 | 30.000,00 | 7,50 |
| 5 Patient Costs | 22.500,00 | 0,00 | 22.500,00 | 5,62 |
| 6 IT Services and Data Bases | 0,00 | 0,00 | 0,00 | 0,00 |
| 7 Travels | 3.000,00 | 0,00 | 3.000,00 | 0,75 |
| 8 Publication Costs | 0,00 | 0,00 | 0,00 | 0,00 |
| 9 Dissemination | 2.500,00 | 0,00 | 2.500,00 | 0,62 |
| 10 Overheads | 24.000,00 | 0,00 | 24.000,00 | 6,00 |
| 11 Coordination Costs | not permitted | not permitted | not permitted | 0,00 |
| **Total** | 442.218,00 | 42.218,00 | 400.000,00 | 100,00 |

| | |
|---|---|
| **Project Code:** PNRR-MCNT1-2023-12377359 | **Call section:** Malattie Croniche non Trasmissibili (MCnT1) ad alto impatto sui sistemi sanitari |
| **Applicant Institution:** Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano | **Applicant/PI Coordinator:** Licitra Lisa Francesca Linda |

## Budget Justification

| | |
|---|---|
| 1 Staff Salary | co-funding: Bussu effort 10%, 13375/yr; Fadda effort 10%, 7734/yr |
| 2 Researchers' Contracts | Biologist effort 100%, 34000/yr (tot 68000 euro) ; Biologist effort 100% 34000/yr (tot 68000 euro); two junior researcher biologist/biothecnologist effort 100% 24500/yr (tot 98000 euro) |
| 3a.1 Equipment (Leasing - Rent) | n/a |
| 3a.2 Equipment (buying) | n/a |
| 3b Supplies | single cell isolation with Miltenyi gentleMACS (4000); library prep Parse Bioscience Evercode kits (16000); sequencing with Illumina (18000); kits for spatial omics: library pred (24000)<br>digital pathology: assays and reagents (19000); plasticware (3000) |
| 3c Model Costs | n/a |
| 4 Subcontracts | Subcontract for the management of clinical trials according to Good Clinical Practice standards according to its SOPs (constantly updated and monitored).<br>Site Monitoring Visits (on-site - 3/site), Close-out Visits (on-site) eCRF and data management |
| 5 Patient Costs | Costs of ancillary medical services for patients enrolled in the clinical study (i.e. Tubes/vials for blood and tissues, slides, shipments). Insurance and<br>Safety management |
| 6 IT Services and Data Bases | n/a |
| 7 Travels | Costs for travels to attend international conferences |
| 8 Publication Costs | n/a |
| 9 Dissemination | Fee to attend conferences aiming at disseminating the results and findings of the project |
| 10 Overheads | Istitutional costs |
| 11 Coordination Costs | n/a |

## Principal Investigator Data

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Struttura: Dipartimento di Oncologia ed Emato-Oncologia (UNIMI) - S.C. Oncologia Medica 3 (INT)
Istituzione: Università degli Studi di Milano - Fondazione IRCCS Istituto Nazionale dei Tumori di Milano
Datore/ente di lavoro? Yes
Datore/ente di lavoro SSN? No
Nome datore/ente di lavoro non SSN: Università degli Studi di Milano
Nome istituzione SSN: Fondazione IRCCS Istituto Nazionale dei Tumori di Milano
Tipo contratto: Professore Associato distaccato presso IRCCS/IZS/ISS/Ente SSN (convenzione di clinicizzazione e/o ricerca)

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**Con l'invio della presente proposta si dichiara che la stessa o parti significative di essa non sono oggetto di altri finanziamenti pubblici o privati e che di conseguenza vi è assenza del c.d. doppio finanziamento ai sensi dell'art. 9 del Regolamento (UE) 2021/241, ossia che non ci sia una duplicazione del finanziamento degli stessi costi da parte di altri programmi dell'Unione, nonché con risorse ordinarie da Bilancio statale.**

**By submitting this proposal, I declare that no significant part or parts of it are recipient of any other public or private funding and that consequently there isn't any so-called double financing pursuant to art. 9 of Regulation (EU) 2021/241, i.e. that there is no duplication in the financing of the same costs by other Euopean Union programs or any other ordinary resources from the State budget.**

| | |
|---|---|
| **Project Code:** PNRR-MCNT1-2023-12377359 | **Call section:** Malattie Croniche non Trasmissibili (MCnT1) ad alto impatto sui sistemi sanitari |
| **Applicant Institution:** Fondazione Istituto Nazionale per lo studio e la cura dei tumori - Milano | **Applicant/PI Coordinator:** Licitra Lisa Francesca Linda |

## Project validation result

Message: Success