LILT
LEGA ITALIANA PER LA LOTTA CONTRO I TUMORI
prevenire è vivere

ALLEGATO B

Bando 2020-21 - Programma 5 per mille anno 2018-2019
Investigator Grant (IG)

TRANSLATIONAL RESEARCH

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

1. Principal investigator's full name and qualification:
Aldo Venuti, MD PhD, Head HPV-Unit UOSD Tumor Immunology and Immunotherapy

2. Proposal title. New prognostic and predictive biomarkers for HPV-associated oropharyngeal cancer

3. Primary area of Relevance: Improving cancer diagnosis and treatment

4. Relevance for the National Health System. The validation of new biomarkers in a multicentric study involving LILT Provincial Committees might have short-medium term effects in clinical practice allowing a more accurate identification of HPV-associated oropharyngeal cancer patients with a better prognosis and response to therapy, compared to patient stratification currently based on HPV-DNA and/or p16 positivity.

5. Institution: IRCCS Regina Elena National Cancer Institute – via Elio Chianesi 53 00144 Rome Italy phone +390652662520 e-mail aldo.venuti@ifo.gov.it

6. Authorized Administrative Official…Dr. Francesco Ripa di Meana Istituti Fisioterapici Ospitalieri via Elio Chianesi 53 001444 Rome Italy; phone +390652662702 e-mail dirgen@ifo.gov.it

7. Proponent's signature… Dott. VENUTI ALDO RESPONSABILE HPV - UNIT

8. Authorized Administrative Official's signature ISTITUTI FISIOTERAPICI OSPITALIERI Direttore Generale Dott. Francesco Ripa di Meana

9. Place and date… Roma 14/01/2021

SELF EVALUATION FORM

1. Investigator's full name: (PI) Aldo Venuti
2. Total papers…124… IF 445.397…….
3. Total papers (last 10 years) 58 IF 223.179……
4. Total Papers as first/last author or corresponding author 83……
5. Total H-index …31

PROPOSAL MAIN BODY

Proposal title: New prognostic and predictive biomarkers for HPV-associated oropharyngeal cancer

Abstract

Globocan 2018 estimates for 2018 18.1 million new cancer cases (17.0 million excluding NMSC) and 9.6 million cancer deaths (9.5 million excluding NMSC) worldwide. Of these cancers, approximately 15% is associated with infective agents such as bacteria, viruses, parasites and protozoa. High-risk Human Papillomaviruses (HPVs) are considered the causal agent of ano-genital cancers, and a subset of head-neck cancers. Persistent oral HPV infection, in particular HPV16 infection, is associated with possible development of oropharyngeal carcinoma (OPC). In the last decades, a significant increase in the incidence of HPV-associated OPC has been observed in several western countries. Noteworthy, the presence of HPV in OPC is associated with a better response to therapy and a better prognosis. Nevertheless, it should be noted that around 20% of HPV-positive tumors do not show these advantageous characteristics, indicating the need to improve patient stratification, currently based on the presence of HPV DNA and/or its surrogate marker p16.E5 is the smallest oncoprotein of high-risk HPVs. Previously, we demonstrated that E5 is more consistently expressed in the early stage of viral infection and in cervical precancerous lesions. Therefore, E5 could represent a target to prevent progression of precancerous lesions into invasive cancers. So far, only few studies have assessed the expression of E5 in OPC, with conflicting results regarding its significance as a prognostic marker. In collaboration with other groups, we demonstrated that E5 levels may affect HLA expression and that OPC patients with high expression of E5 and low expression of HLA had significantly worse disease-free survival and tended towards decreased overall survival. Recently, we developed an assay to detect specifically E5 transcripts in cervix scrapes and preliminary data showed that this transcript can be detected in oral clinical samples from HPV-positive healthy individuals. However, none of the available studied conducted on OPC investigated the presence of the E5 specific transcript. This multicentric Project is focused on evaluating for the first time the expression of HPV16 E5 specific transcript in a retrospective cohort of HPV-associated OPC patients, for which follow-up data are available. Therapies targeting the Epidermal Growth Factor Receptor (EGFR) have been proposed as de-escalation strategies in HPV-related OPC patients. E5 expression in tumor specimens will be thus correlated with EGFR and HLA expression, and with the clinical outcome, in order to explore the prognostic significance of these biomarkers, alone or in combination, for HPV-related OPC. Previously, we have identified HPV16-positive healthy subjects expressing mRNA for E6/E7, the main viral oncoproteins, suggesting a possible transforming infection. In order to better clarify the role of HPV16-E5 in the identification of potentially transforming infections, HPV16-E5 mRNA as well as E6/E7 mRNA expression will be investigated in oral rinses of HPV16-positive healthy individuals from ENT outpatient of several LILT Provincial Committees. This study will provide valuable information on the presence of E5 mRNA in HPV-associated OPC samples. We expect to identify subsets of HPV-related OPC with expression of E5, EGFR and HLA, associated with diverse response to therapy and prognosis. This is of critical importance because patients eligible for therapy de-intensification could be identified based on the presence of these biomarkers. A precise patient stratification would allow us to reduce side-

effects associated with current therapeutic approaches. Thus, the present Project could provide new low-cost tools useful to ameliorate management and therapy of HPV-associated OPC patients.

Introduction

Globocan 2018 estimates for 2018 18.1 million new cancer cases (17.0 million excluding NMSC) and 9.6 million cancer deaths (9.5 million excluding NMSC) worldwide. Among these cancers, approximately 15% is associated with infective agents such as bacteria, viruses, parasites and protozoa. Head and neck cancers represent the fifth most common malignancy worldwide. Among these cancers, oral, laryngeal and particularly oropharyngeal carcinoma (OPC) may be associated with HPV infection. Globally, HPV is responsible for around 38,000 head and neck cancer cases, with a dominating role for HPV16 (de Martel et al, Int J Cancer 2017). Notably, the largest burden of HPV-associated OPC is mainly observed in developed countries, among which Europe and North America. A significant increase in the incidence of this neoplasia has been observed in recent years. Notably, HPV-associated OPCs represent a different biological and clinical entity compared to the HPV-negative counterparts. The presence of HPV is associated with a better response to therapy and a better prognosis. This has prompted several clinical trials for the de-intensification of therapy in patients with HPV-associated OPC. Nevertheless, it should be noted that about 20% of HPV-positive tumors do not show these favorable prognostic characteristics, indicating the need to improve patient stratification currently based on the presence of HPV-DNA and/or its surrogate marker p16.

Background and rationale

Persistent oral HPV infection is responsible for the development of a significant subset of OPC. An HPV-attributable fraction (i.e., the proportion of tumors with HPV-related aetiology) of 30% has been estimated worldwide (de Martel et al, Int J Cancer 2017). We showed that mucosal (AlphaHPV) as well as skin (BetaHPV) HPVs can be detected in this region but only alpha HPVs seem to be linked to tumor development. In previous studies, we assessed the HPV-attributable fraction in a series of cases diagnosed at IRE. We found that 39.8% of the OPC were HPV-associated, with HPV16 representing the most frequently detected genotype (Donà et al, Future Microbiol 2015). We also investigated HPV prevalence in oral samples from 310 healthy individuals at increased risk for HPV infection. We found that 19.7% of these subjects harboured at least one HPV type, with 10.6% of the participants being infected with high-risk genotypes. Notably, 12.6% of the individuals with high-risk HPV infection also expressed the mRNA for E6/E7, the main viral oncoproteins (Rollo et al, Cancer 2019). However, natural history and carcinogenesis seem to be quite different from that of genital area. Indeed, we observed that incidence of oral HPV infection is low, whereas its clearance is much higher (Giuliani et al, STI 2020). Nevertheless, there is need to further study this aspect.

E5 is the smallest viral oncoprotein and is considered the third oncogene of high-risk HPVs. E5 is extremely hydrophobic and is a trans membrane protein with many different functions spanning from anti-apoptotic activity to EGFR recycling and immune evasion (for review see Venuti et al, Mol Cancer 2011). It was reported that E5 can play an active role only in the early phases of transformation because during viral integration that takes part in most genital tumors, E5 ORF is lost. Indeed, the region of the viral genome containing E5 is 180 degrees from the viral replication origin, so it would be the point in theta replication where the replication forks would meet, and may be more prone to damage or deletion than other regions of the genome. However, a subset of HPV16-positive invasive cervical carcinomas, however, maintains viral DNA only as episomes indicating that integration-associated and episome-associated pathways of HPV16-induced cervical carcinogenesis might exist (Gray et al, Cancer Res 2010). Besides the integrated monomeric forms, head-to-tail concatemers of full-length HPV genomes flanked by truncated copies also exist, exemplified by the cervical cancer cell line CaSki. (Bo Xu et al, PLoS One 2013). It is interesting to note that E5 protein was identified by mass Spectrometry in CaSki cells, a cervical carcinoma cell line harboring around 600 copies of integrated HPV16 genome, giving the definitive proof that HPV-16 E5 does exist and may contribute to the malignant phenotype of some cervical cancers, even in cells containing exclusively an

integrated HPV genome, like CaSki cells. (Sahab et al, J Virol 2012). In previous works we demonstrated that E5 is more consistently expressed in the early stage of viral infection and in precancerous lesions, and therefore E5 altered pathways or E5 itself could provide new markers of progressive precancerous lesions (Lorenzon et al, J Clin Virol 2011). Fourteen species of HPV16 mRNA transcripts with various coding potential exist, but only one transcript with coding capacity for E5 was identified in clinical samples (Chen et al, Virology 2014). We derived specific primers spanning E5 intron region to detect only this transcript (Paolini et al, Hum Vaccin Immunother 2017). A Real Time PCR was developed and used to detect E5 mRNA of HPV16 (HPV16-E5) in clinical samples showing that this transcript is overexpressed in early stage of evolving cervical lesions.

Few studies were performed on the expression of E5 in HPV-associated OPC, with conflicting results regarding its significance as a prognostic marker: Ramquist T et al. (Oral Oncol 2015), did not find any correlation. Variable levels of E5 expression in HPV-positive OPC were also detected by Uo Um SH et al (J Clin Virol 2014) whereas Taberna M et al. (Front Oncol 2018) showed that HPV16-E5 is highly expressed in HPV16-positive OPCs and has prognostic significance together with EGFR. In a cohort of patients of University of San Diego, we demonstrated, in collaboration with other groups, that E5 levels may affect HLA expression and patients with high expression of E5 and low expression of HLA had significantly worse disease-free survival and tended towards diminished overall survival. These findings demonstrated that expression levels of HPV E5 and HLA may impact on the clinical outcome (Miyauchi et al, Cancer Res 2019). However, none of these studies was conducted to detect the presence of the E5 specific transcript. For this reason, this Project is focused on evaluating viral HPV16-E5 oncogene expression (for the first time, E5 specific early transcript) as well as EGFR and HLA subtype as biomarkers for clinical outcome in a retrospective cohort of patients with HPV-associated OPC (1st aim). To better clarify the role of HPV16-E5 in the identification of potentially transforming infections, oral samples from normal individuals enrolled at ENT outpatient of several LILT Provincial Committees will be also evaluated for HPV infection, and those positive for HPV16 will be analyzed to assess the presence of E5 mRNA (2nd aim). In addition, current data on the differential response of OPCs to anti-EGFR therapies as a function of the HPV status are still inconsistent and, importantly, 20% of HPV-related OPSCC patients fail to treatment. Thus, in an era where anti-EGFR therapies are being studied on de-escalation clinical trials, this Project evaluating the prognostic and predictive values of these biomarkers and their differential association with clinical outcome, might have a profound and immediate impact on patient's health by indicating patients where therapy de-escalation can be conducted more safely.

Experimental design

The study will be conducted within three tasks.

Task 1. To assess E5 mRNA, pEGFR and HLA expression in archival samples of HPV16-positive OPC. DNA/RNA will be extracted from formalin-fixed, paraffin-embedded (FFPE) tissue samples of cancer patients, retrieved from the archives of the Department of Pathology of Regina Elena National Cancer Institute IRCCS and Radiation Medicine and Applied Sciences of University of California, San Diego (USA). HPV16-positive cases, already characterized for the expression of p16, will be selected. Nucleic acid extracts will be used to investigate the presence of HPV16-E5 mRNA viral expression. pEGFR expression as well as the expression of major and minor MHC class I subtypes will be assessed by immunohistochemistry.

Task 2. To correlate HPV16-E5 mRNA, pEGFR and HLA expression with the clinical outcome. Follow-up data of the patients selected for the study will be retrieved from a dedicated database. Patients will be stratified based on E5 mRNA, pEGFR and HLA expression, as single and combined biomarkers. Their expression will be correlated with the overall survival (OS) and recurrence-free survival (RFS). Kaplan-Meyer curves will be used. Log-rank test will be used to compare survival curves. Age groups, pathological staging, therapy, and smoking status will be also taken into account.

Task 3. To assess HPV infection and E5 mRNA expression in oral samples from healthy subjects. Oral rinse-and-gargles will be collected from consented healthy subjects enrolled at

the ENT outpatient clinic of the LILT Provincial Committees. Each center will provide at least 30 samples from individuals at increased risk for high-risk HPV infection, i.e., male subjects, current smokers, reporting at least 5 lifetime oral sex partners (D'Souza et al, Annals Oncol 2017). These samples will be tested for the presence of HPV-DNA and those positive for HPV16 will be also analyzed for E5 and E6/E7 mRNA.
Since the Project must be developed in two years, all tasks will start together and interim analysis of obtained results will be performed after 12 months.

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

Sample size calculation

To estimate the sample size for HPV16-positive OPC, the following assumptions were taken into account: E5 expression in 77% of the cases (Taberna et al, Front Oncol 2018); RFS at 30 months of 60% for E5-negative cases vs. 90% for E5-positive cases (Ho Um et al, J Clin Virol 2014). Considering an alpha error of 0.05, with a power of 90%, 84 HPV16-positive OPC were estimated to be necessary for this study.

HPV 16 E5 expression

RNA will be extracted from FFPE and fresh samples by RNeasy kit (QIAGEN), according to the manufacturer's instructions. Total RNA pre-treated with DNase I, (Invitrogen), will be retro-transcribed into cDNA for 1 h at 42C using a random hexamer primer kit as described by the manufacturer (GeneAmp RNA PCR kit Applied Biosystem). The synthesized cDNA underwent real time-PCR with 2X Kapa SYBR Fast qPCR Master Mix (KAPA). Briefly, the amplification of E5 and β-Actin sequences will be performed in a 20 µl final volume containing, for 40 cycles: 3 sec denaturation at 95C, annealing and extension at 60C for 30 sec, with the initial denaturation at 95°C for 3 min. The expression levels of E5 specific transcripts will be normalized with respect to those of housekeeping gene. The specific E5 primers were already designed by Beacon Design software (BioRad) encompassing splicing site (880–3358): forward primer (5'-GCGACGTGAGAGCAACG-3') and reverse primer (5'-AGGGGTTTCCGGTGTCTGG-3'). Relative quantity of genes will be determined by the ΔΔCt method normalized to housekeeping genes.

HPV 16 E6/E7 expression

Analysis of transcripts for E6/E7 will be performed with the above reported methodology but utilizing specific primers for E6 and E7 genes (Badaracco et al, Oncol Rep. 2010, Donà et al, PLoS One. 2013).

Immunohistochemistry for pEGFR and HLA

From each FFPE block the following sections will be obtained: a) 2x2µm outer sections, used for H&E staining and diagnosis confirmation; b) 1x2µm section for pEGFR and HLA staining. OPC diagnosis will be confirmed by an experienced pathologist. Immunostaining will be performed using commercially available antibodies, following the antibody instruction for staining procedure and the instructions given by the manufacturer for Ventana Benchmark XT System. Staining will be independently examined by two investigators.

Oral sample collection

Oral rinses will be collected in the ENT outpatients of the LILT Provincial Committees. Male attendees will be informed of the study and, once verified the eligibility, they will be asked to sign an informed consent. Participants will be subjected to a full otolaryngology examination to exclude the presence of lesions suspicious for head and neck cancer. The ENT outpatients will be furnished with a collection kit composed of 15 ml of Listerine® mouthwash (or similar) and containers with a solution for nucleic acid preservation (PreservCyt or similar) for the shipment. Participants will be asked to alternatively rinse and gargle for a total time of 30 seconds. Samples in preserving solution will be sent to the Regina Elena Laboratories for molecular analysis.

HPV-DNA detection in oral samples

The Xpert® HPV assay (Cepheid,Inc, Sunnyvale,CA), which detects 14 high-risk HPV types, will be used. This test is based on a real-time PCR that targets a sequence in the E6/E7 region. The test result is given with a concurrent partial genotyping for HPV16 as a single result, for HPV 18 and 45 as a pooled result, and collectively for the "other HR-HPVs". The assay incorporates an internal control which detects a human gene in single copy in order to monitor sample quality. One ml of the oral sample will be tested with this assay.

Work carried out and preliminary results

All IRE archival samples which will be used for this study have been already characterized for HPV presence together with p16 immuno-staining. The same data were already collected from patients in the cohort of University of San Diego. In addition, follow-up data for the patients included in the study have been already retrieved from the medical records and stored in a dedicated database.

The methodology for collection and HPV-DNA and mRNA testing of oral rinse-and-gargles from healthy individuals has been already set up and largely used in previous studies (see above). Methods used for collection and analysis of oral samples are routinely utilized. The transport medium used for this study can guarantee the stability of the samples at room temperature for at least a week, avoiding problem of shipment from ENT outpatient of different LILT Provincial Committees to the central laboratory.

The included picture shows preliminary results for the detection of HPV16-E5 transcripts in clinical samples of oral cavity, demonstrating that this method can be used to detect E5 specific transcripts, which, indeed, can be found in some oral samples. (Figure 1)

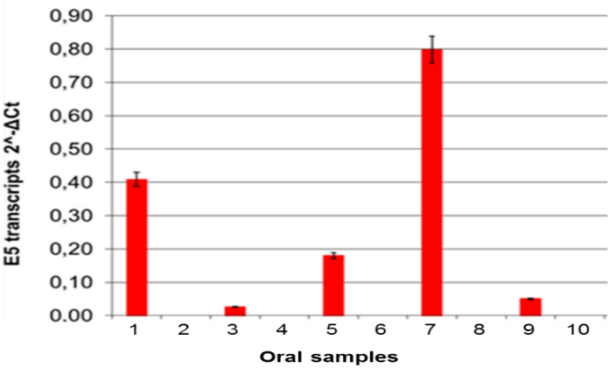

Figure 1 Specific E5 transcripts in clinical samples. The 2-ΔCT method was used as a relative quantification strategy for quantitative real-time polymerase chain reaction (qPCR) data analysis. The expression levels of E5 sequence and β-actin were analyzed in triplicate samples using qPCR as described in methods.
Error bars = ±SD.

During last year (2020) these preliminary data were further improved by starting a Pilot Study on 10 archival samples in which viral transcripts were detected. This work was conducted by a small grant of Fondazione Calabresi - Roma

Expected results and relevant corresponding milestones

This study will provide valuable information on the possible role of E5 as a prognostic marker in patients with HPV-associated OPC (1st aim) but also as a marker of transforming infection in healthy individuals with oral HPV16 infection (2nd aim).

Among the HPV-positive OPCs cases, we expect to identify subsets of cases with differential expression of E5, pEGFR and HLA, associated with diverse response to therapy and prognosis. This is of critical importance because patients eligible for therapy de-intensification could be identified based on the presence of these biomarkers. A precise patient stratification would allow us to reduce side-effects associated with current therapeutic approaches. Thus, the present Project could provide new low-cost tools useful to ameliorate management and therapy of HPV-associated OPC patients.

By reaching the 2nd aim, we expect to better clarify the role of HPV16-E5 in the identification of potentially transforming infections. Based on HPV-DNA detection it is not possible to establish whether the infection is transient and clinically irrelevant or active and potentially transforming. Conversely, detection of mRNA for viral oncoproteins may provide information regarding HPV

activity. Presence of E5 mRNA together with mRNA for the established oncoproteins E6/E7 may suggest its potential role in transforming infections. In addition, important information about circulation of HPV in the healthy population from different Italian areas will be provided.

Milestones.
M0. Upgrading of clearance from the Ethics Committee n. 1443720 of 22-12-2020
A. 1st objective:
M1a. Retrieval of archival samples of HPV-associated OPC
M2a. Assessment of HPV16-E5 mRNA expression in tumor samples
M3a. Evaluation of pEGFR and HLA expression in tumor samples
M4a. Patient stratification according to the expression of the selected biomarkers
M5a. Survival analysis
B. 2nd objective:
M1b. Enrollment of the healthy study population by the Provincial LILT committees
M2b. Collection of oral samples and shipment to the central laboratory
M3b. HPV-DNA testing of the oral samples
M4b. HPV-mRNA testing of the oral samples positive for HPV16
M5b. Estimation of oral HPV DNA and mRNA prevalence in the healthy population
Overall Project
M6. Manuscript preparation

References and relevant publications by the research group, already available

References

Badaracco et al, Oncol Rep. 2010
Bo Xu et al, PLoS One 2013
Chen et al, Virology 2014
D'Souza et al, Annals Oncol 2017
de Martel et al, Int J Cancer 2017
Donà et al, Future Microbiol 2015
Donà et al, PLoS One. 2013
Giuliani et al, STI 2020
Gray et al, Cancer Res 2010
Ho Um et al, J Clin Virol 2014
Lorenzon et al, J Clin Virol. 2011
Miyauchi et al, Cancer Res 2019
Paolini et al, Hum Vaccin Immunother 2017
Ramquist et al, Oral Oncol 2015
Rollo et al, Cancer 2019
Sahab et al, J Virol. 2012
Taberna et al, Front Oncol 2018
Venuti et al, Mol Cancer 2011

Relevant publications by the research group

Donà et al, PLoSOne. 2013
Paolini et al, J Exp Clin Cancer Res. 2011
Paolini et al, Int J Immunopathol Pharmacol. 2011
Cercato et al, J Med Virol. 2010
Paradiso et al, Int J Biol Markers. 2009
Miyauchi et al, Cancer Res. 2019
Wu et al, Oral Oncol. 2018
Paolini et al, Hum Vaccin Immunother. 2017
Paolini et al, J Clin Virol. 2013
Venuti et al, Head Neck Pathol. 2012
Cercato et al, J Med Virol. 2010
Venuti et al, Mol Cancer.2011
Badaracco et al, Oncol Rep. 2010
Rollo et al, Cancer. 2019
Donà et al, Oral Oncol 2019.
Giuliani et al, Sex Transm Infect. 2020
Donà et al, Future Microbiol. 2015

PERSONNEL INVOLVED IN THE RESEARCH

Name and date of birth	Role on Project	Fellowship required	Effort on project (%)	Present position
Regina Elena National Cancer Institute - Rome				
1)Aldo Venuti 18-11-1954	PI	none	50	Head HPV-Unit
2)Francesca Paolini 05-04-1979	Co-PI	none	50	Health researcher
3)Claudia Bonomo 17-08-1992	Sample analysis	none	30	Health research technician
4)Francesca Rollo 01-08-1982	Sample analysis	none	30	Health researcher
5) Renato Covello 23-02-1964	Confirming histology	none	30	Executive Doctor
6)Maria Benevolo 01-07-1963	Supervising analysis results	none	30	Research executive
7) Flaminia Campo 04-05-1989	Healthy sample collection	none	20	Executive Doctor
San Gallicano Dermatologic Institute IRCCS - Rome				
8)Maria Gabriella Donà 08-05-1975	Co-PI	none	50	Health researcher
9)Eugenia Giuliani 05-04-1989	Sample analysis	none	40	Fellow
University of California at San Diego USA				
10)Andrew Sharabi 21-11-1979	Collection archival samples	none	20	Assistant Professor
Provincial LILT Palermo				
11)Letizia Davì 23-11-1983	Coordination of sample collection	none	20	General Director
Provincial LILT Livorno				
12)Fabio Cecconi 15-05-1969	Coordination of sample collection	none	20	Vice-President LILT Livorno
Provincial LILT Catania				
13)Aurora Scalisi 13-03-1957	Coordination of sample collection	none	20	President LILT Catania
Provincial LILT Roma				
14) Giuseppe D'Ermo 08-10-1956	Coordination of sample collection	none	20	Director Centro Prevenzione LILT Roma
Provincial LILT Parma				
15)Ilaria Gambardella 01-08-1980	Coordination of sample collection	none	20	Coordinator ENT outpatients

DESCRIPTION OF THE WORK FOR EVERY UNIT OF PERSONNEL

1 The PI will coordinate all the activities of the Project.
2 FP, as Co-PI, will help the PI in coordinating the project and will participate in sample and statistical analysis
3 FR will perform nucleic acid extractions and sample analysis
4 CB will work on sample retrieval from archival specimens, tissue block cutting and IHC
5 RC will check and confirm histology in archival sample.
6 MB will supervise all experimental results including IHC
7 FC will be in charge to coordinate healthy sample collection at ENT outpatients of Regina Elena National Cancer Institute
8 MGD, as Co-Pi, will help the PI in coordinating the project and will participate in sample and statistical analysis
9 EG will perform bio-molecular analysis in collected samples from Provincial LILT Committees
10 AS will be in charge to collect and send sample from archival repository together with relevant clinical information
11-15 They will be in charge to coordinate the activities of Provincial LILT Committees to collect samples from healthy individuals as well as clinical information.

Budget Form /year	
1. research costs:	70.000 euro
2. Instruments:	none
3. Indirect costs:	7.000 euro
4. Sub-total:	**77.000 euro**
5. Overheads:	7.700 euro
6. Fellowships :	none
7. Total:	**84.700 euro**

Justifications. To keep at minimum research costs, no equipment is required. The predicted number of samples to be analyzed requires that large part of financial support will be used for reagents and sample collection. Financial support is also required for dissemination and transfer of results.
Indirect costs are referred to secretary expenses, researcher PC and disposable material not project related. Overheads are for Utilities (gas, power, water consumption), for ordinary maintenance od Laboratories and for grant management fees.
Itemized research costs:
Reagents (enzymes, buffers, DNA/RNA extraction kit, antibodies, IHC reagents and buffers, sequencing and primers, oral sample collection and preservation media): 35.000€
Disposable material (plastic wares) 10.000€
Sample shipment and disposable material for ENT outpatients: 20.000€
Dissemination and transfer of study results (Congress Participation, Meetings with collaborating LILT, editorial cost for scientific publications): 5.000€

EXISTING/PENDING SUPPORT

Part of this research (see preliminary data) received a small financial support (5000 €) by a Grant of Fondazione Calabresi –Roma

SUGGESTED REVIEWERS (MAX 3))
-Prof. Giuseppe Spriano (Humanitas University – Milan): giuseppe.spriano@humanitas.it bspriano@email.it
-Prof. Debora French (Facoltà di Medicina e Psicologia "Sapienza" Università di Roma Azienda Ospedaliero-Universitaria Sant'Andrea): deborah.french@uniroma1.it
-Prof. Maria Teresa Sandri (Humanitas University – Milan): maria.sandri@humanitas.it

BIOETHICAL REQUIREMENT

1. Human experimentation ……(YES) – As stated in the declaration, before beginning of research, I will up-grade the clearance n. 1443/20 (22-12-2020) of the competent ethical committee.

2. Animal experimentation …..(/NOT)

Declaration
I shall confirm to the Declaration of Helsinki in its latest version.
I shall also apply the Bioethics Convention of the Council of Europe.
In implementing the proposed research, I shall adhere most strictly to all existing ethical and safety provisions applicable.
Before start of the research, I shall up-grade the clearance n. 1443/20 (22-12-2020) of the competent ethical committee.
I shall conform with all regulations protecting the animals used for research purpose.

Date: 14-01-2021…… Name of PI…Aldo Venuti

Principal investigator's signature …….

Dott. VENUTI ALDO
RESPONSABILE
HPV - UNIT

Authorized Administrative Official's signature……

ISTITUTI FISIOTERAPICI OSPITALIERI
Direttore Generale
Dott. Francesco Ripa di Meana

Date 14-01-2021

Si autorizza al trattamento dei dati ai sensi dell'articolo 5 del Regolamento (UE) 2016/679

CURRICULUM VITAE

PERSONAL INFORMATION

First name(s)/Surname(s)	VENUTI ALDO
Address	IRE-VIA ELIO CHIANESI 53 00144 ROME ITALY
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E-mail	aldo.venuti@ifo.gov.it
Nazionality	Italian
Date of birth	18-11-1954
Gender	male

WORK EXPERIENCE

• Dates	06-2012/to date
• Name and address of employer	IRCCS Regina Elena National Cancer Institute – Via Chianesi Rome Italy
• Main activity and responsability	Supervising all the activity of HPV-UNIT
Occupation or position held	Chief HPV UNIT
•	
• Dates)	11-2003/03-2013
• Name and address of employer	IRCCS Regina Elena National Cancer Institute – Via Chianesi Rome Italy
• Main activity and responsability	Supervising all the activity of Viroloy Lab.
Occupation or position held	Director of Laboratory of Virology
• Dates)	11-1991/10-2003
• Name and address of employer	IRCCS Regina Elena National Cancer Institute – Via Chianesi Rome Italy
• Main activity and responsability	Supervising HPV research group.
Occupation or position held	Medical/research officer
• Dates)	01-1988/10-1991
• Name and address of employer	IRCCS Regina Elena National Cancer Institute – Via Chianesi Rome Italy
• Main activity and responsability	Starting a new group of research on HPV
Occupation or position held	Senior research associate
à	
• Dates)	01-1984/12-1987
• Name and address of employer	Università of Rome "La Sapienza" Institute of Virology Rome Italy
• Main activity and responsability	Working on the sequencing of Hepatitis A virus

Occupation or position held	Research associate
•	
• Dates)	01-1982/12-1984
• Name and address of employer	BioLab Company Rome via Trionfale Italy
• Main activity and responsability	Supervising all activity of diagnostic Laboratory
Occupation or position held	Director of Laboratory
• Dates	12-1979/12-1982
• Name and address of employer	Università di Roma "La Sapienza" Institute of Virology Rome Italy
• Main activity and responsability	Working on picornavirus molecular biology
Occupation or position held	Internal Researcher

EDUCATION AND TRAINING

• Dates	2014-2018
• Name and type of oranization providing education and training	MIUR Italy
• Principalsubjects / occupational skills covered	Virology
• Type of qualification awarded	Full Professor Microbiology [National Habilitation (06/A3) 2014-2018]
• Dates)	06-1998/01-1999
• Name and type of oranization providing education and training	Laboratory of Immunology Queensland University- - Brisbane Australia
• Principalsubjects / occupational skills covered	HPV vaccine
• Type of qualification awarded	FIRC fellow
• Dates)	01-1988/12-1989
• Name and type of oranization providing education and training	Beatson Institute - Glasgow - Scotland
• Principalsubjects / occupational skills covered	HPV cancerogensis and diagnosis
• Type of qualification awarded	AIRC fellow
• Dates)	01-1984/05-1985
• Name and type of oranization providing education and training	INDIANA University - Bloomington, USA
• Principalsubjects / occupational skills covered	cloning of enterovirus
• Type of qualification awarded	Research Associate
• Dates)	01-1983/12-1985
• Name and type of oranization providing	Università di Roma "La Sapienza" - Institute of Virology

education and training	
• Principal subjects / occupational skills covered	molecular biology and virology
• Type of qualification awarded	PhD
• Dates)	01-1980/12-1983
• Name and type of oranization providing education and training	Università di Roma "La Sapienza" – Clinical Pathology School
• Principal subjects / occupational skills covered	Clinical Pathology
• Type of qualification awarded	clinical pthology Specialist
• Dates)	09-1973/07-1979
• Name and type of oranization providing education and training	Università di Roma "La Sapienza" - Institute of Virology
• Principal subjects / occupational skills covered	medical degree, virology
• Type of qualification awarded	MD

PERSONAL SKILL AND COMPETENCES

Acquisite nel corso della vita e della carriera ma non necessariamente riconosciute da certificati e diplomi ufficiali.

MOTHER TONGUE	**Italian**
OTHER LANGUAGES	
	English
Understanding	excellent.
• Writing	excellent.
• Speaking	excellent.
	Francais
Understanding	EXCELLENT.
• Writing	GOOD.
• Speaking	GOOD.

TECHNICAL SKILLS AND COMPETENCE

As Chief of HPV-Unit the mission was to formalize an organizational model of a "unified and coordinated space" in which originate jointly initiatives related to the topic of HPV: the clinical management (diagnosis and treatment guidelines, facilitated routes and more), and the scientific matching (creation of ad hoc database, sharing of researches and more). This organizational model is a tool to inform, train and network both patients and health workers involved in HPV-related pathologies, from gynecological area to the skin, comprising ENT, urological and proctologic diseases. Regarding tumor prevention, HPV vaccination, that is a program already started in 2014 for female, up to 45 years old, and later on extended to male, Dr. Venuti is in charge to render fully operating this preventive tool with a constant increase of vaccinated people both women and men. Dr. Venuti is actively involved in scientific activities focused on translational researches of virus–associated cancers, on cancer prevention and on the development of permanent professional training facilities and information addressed to the citizens
The training activity is scheduled with courses for HCWs while the activity of user's information is constantly carried out by telephone, by email and through the continuous up-grading the dedicated internet site. In addition, dedicated course and Meetings were organized by Dr. Venuti, the last three Meeting on cancer prevention were: "OPEN DAY. Difendiamoci dai Tumor: *Prevenzione & Informazione*" (Roma 10-12-2016); "10 Anni di Vaccinazione HPV" (Rome - 03-25-2017) HPV Related Diseases: Diagnostic and terapeutic strategies (28-11-2019
He has been and is scientific responsible of many Projects on HPV granted by CNR-ACRO, AIRC, Ministry of Health, Abbott Co. USA, Compagnia di San Paolo Torino, Fraunhofer Institute (USA), MSD (Europe), Roche (Europe), Lega Italiana Tumori (LILT).

Teaching activity:

1984 He was lecturer in a course for graduate student at the Dept. of Biology INDIANA University. 1999-2007 he was teaching at the Corso di Laurea in Infermiere Generico e Pediatrico" University "La Sapienza" – Rome and 2003-2008 he was giving integrate teaching activity at the Faculty of Veterinary, University "Federico II" Naples. 2007-2012 he was giving integrate teaching activity at the Faculty of Medicine and Psychology University "La Sapienza" –Rome. Since 2012 he was teaching HPV molecular biology at Course Master in Virology University "La Sapienza" –Rome

Editorial Board Membership:

Since 2006 is Board member of the Journal of Exp. and Clin. Cancer Research

Patents:

- National (Italy) on Vaccini a subunità e procedimenti per la loro produzione RM2001A000332, International (USA and EUROPE) on Subunit vaccines and processes for the production thereof. #PCT/IT02/00354; National (Italy) on Vaccini basati su chimere genetiche tra antigeni virali/tumorali (es. HPV) e proteine vegetali (es. RIPs). Submitted C-08/05. He is author of **124 publications on peer reviewed Journals with Total IF 435.507 and h index 31**.

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1) Franconi R, Massa S, Paolini F, Vici P, Venuti A. Plant-Derived Natural Compounds in Genetic Vaccination and Therapy for HPV-Associated Cancers. Cancers (Basel). 2020 Oct 23;12(11):3101. doi: 10.3390/cancers12113101.
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3) Miyauchi S, Sanders PD, Guram K, Kim SS, Paolini F, Venuti A, Cohen EEW, Gutkind JS, Califano JA, Sharabi AB. HPV16 E5 Mediates Resistance to PD-L1 Blockade and can be targeted with Rimantadine in Head and Neck Cancer. Cancer Res. 2019 Dec 17. pii: canres.1771.2019. doi: 10.1158/0008-5472.CAN-19-1771.
4) Pizzuti L, Krasniqi E, Barchiesi G, Mazzotta M, Barba M, Amodio A, Massimiani G, Pelle F, Kayal R, Vizza E, Grassadonia A, Tomao S, Venuti A, Gamucci T, Marchetti P, Natoli C, Sanguineti G, Ciliberto G, Vici P. Eribulin in Triple Negative Metastatic Breast Cancer: Critic Interpretation of Current Evidence and Projection for Future Scenarios. J Cancer. 2019 Oct 12;10(24):5903-5914. doi:10.7150/jca.35109. eCollection 2019. Review
5) Krasniqi E, Barchiesi G, Pizzuti L, Mazzotta M, Venuti A, Maugeri-Saccà M, Sanguineti G, Massimiani G, Sergi D, Carpano S, Marchetti P, Tomao S, Gamucci T, De Maria R, Tomao F, Natoli C, Tinari N, Ciliberto G, Barba M, Vici P. Immunotherapy in HER2-positive breast cancer: state of the art and future perspectives. J Hematol Oncol. 2019 Oct 29;12(1):111. doi:10.1186/s13045-019-0798-2. Review.
6) Conforti C, Paolini F, Venuti A, Dianzani C, Zalaudek I. Comment on `The detection rate of human papillomavirus in well-differentiated squamous cell carcinoma and keratoacanthoma: is there new evidence for a viral pathogenesis of keratoacanthoma?' - reply from authors. Br J Dermatol. 2019 Dec;181(6):1345-1346.doi: 10.1111/bjd.18385. Epub 2019 Oct 15
7) Conforti C, Paolini F, Venuti A, Dianzani C, Zalaudek I. The detection rate of human papillomavirus in well-differentiated squamous cell carcinoma and keratoacanthoma: is there new evidence for a viral pathogenesis of keratoacanthoma? Br J Dermatol. 2019 Dec;181(6):1309-1311. doi: 10.1111/bjd.18212. Epub 2019 Aug 13.
8) Massa S, Paolini F, Marino C, Franconi R, Venuti A. Bioproduction of a Therapeutic Vaccine Against Human Papillomavirus in Tomato Hairy Root Cultures. Front Plant Sci. 2019 Apr 11;10:452. doi: 10.3389/fpls.2019.00452. eCollection 2019.
9) Mora Román JJ, Del Campo M, Villar J, Paolini F, Curzio G, Venuti A, Jara L, Ferreira J, Murgas P, Lladser A, Manubens A, Becker MI. Immunotherapeutic Potential of Mollusk Hemocyanins in Combination with Human Vaccine Adjuvants in Murine Models of Oral Cancer. J Immunol Res. 2019 Jan 20;2019:7076942. doi: 10.1155/2019/7076942. eCollection 2019.
10) Venuti A. Review of DNA tumour viruses. Hum Vaccin Immunother. 2019;15(5):1133-1134. doi: 10.1080/21645515.2019.1577677. Epub 2019 Mar 20.
11) Dotta L, Notarangelo LD, Moratto D, Kumar R, Porta F, Soresina A, Lougaris V, Plebani A, Smith CIE, Norlin AC, Gòmez Raccio AC, Bubanska E, Bertolini P, Amendola G, Visentini M, Fiorilli M, Venuti A, Badolato R. Long-Term Outcome of WHIM Syndrome in 18 Patients: High Risk of Lung Disease and HPV-Related Malignancies. J Allergy Clin Immunol Pract. 2019 May - Jun;7(5):1568-1577. doi: 10.1016/j.jaip.2019.01.045. Epub 2019 Feb 2.
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Signature
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