Bando 2019 - Programma 5 per mille anno 2019

Investigator Grant (IG)

TRANSLATIONAL RESEARCH

1. **Principal investigator's full name and qualification:** CAPALBO CARLO MD PhD Oncologist (Please include: CV in European format with list of publications; IF end Hi-index)

2. **Proposal title:** Galectins as target and predictive biomarkers in Immune checkpoint inhibitors therapy: a multicentric study

3. **Primary area of Relevance:** Predictive biomarkers for checkpoint Inhibitor-Based Immunotherapy

4. **Relevance for the National Health System:** Checkpoint inhibitor-based immunotherapy is going to alter the approach to cancer treatment. A meaningful improvement in survival outcomes versus standard of care has been reported both in monotherapy as well as in combined therapeutic approaches. However, to date in most indications, the majority of patients fail to respond to ICI tharapies. These therapies present a substantial economic challenge, especially considering the lack of accurate predictive biomarkers and undefined optimal treatment duration. The cost-effectiveness of ICIs could be mainly improved by better patient selection and by determination of the optimal treatment length. In our recent pilot study we discovered that tumor resistance to Immune checkpoint inhibitor (pembrolizumab) strongly correlated with high expression of galectin-3 in cancer cells, whereas an objective clinical response was obtained in the vast majority of tumors with a negative–low or intermediate galectin-3 phenotype after 12 and 24 weeks of treatment. This clinical observation is supported by a strong biological rationale. If the results of this pilot study will confirmed in multicentric validation study, a cheap and easy immunohistochemical method could be used to evaluate the expression of galectins in tumor biopsies, allowing a better selection of patient candidates for immunotherapy. This approach will avoid exposure to ineffective treatments as well as social costs

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IL RESP. AMM.VO DELEGATO
Dott. Carlo Appetecchia

Proponent's signature.....

Place and date...../..../....

Rome, 14/02/20

SELF EVALUATION FORM

1. Investigator's full name: (PI) CAPALBO CARLO

2. Total papers: 46 IF: 273,1

3. Total papers (last 10 years) 37 IF: 182,5

4. Total Papers as first/last author or corresponding author 11

5. Total H-index: 13 (scopus) IF: 273,1

PROPOSAL MAIN BODY

1. Proposal title: Galectins as predictive biomarkers and target in Immune checkpoint inhibitors (ICIs) therapy

2. Abstract

Rationale: Checkpoint inhibitor-based immunotherapy is opening a promising scenario in oncology with relevant outcomes registered in several tumors. However reliable predictive markers of tumor responsiveness are still lacking. These markers need to be urgently identified for a better selection of patients candidate to immunotherapy. In solid tumors, the pleiotropic molecules Galectins 1, 3 and 9 regulate apoptosis and tumor immune-escape and are in the right position to play as predictive marker in anti-PD1/PDL1 based immunotherapy. To investigate this hypothesis, the expression profile of galectins 1,3 and 9 will be assessed in a panel of PD-L1-positive (tumor proportion score > 50%) non-oncogene-addicted tumors from treatment-naive metastatic NSCLCs and melanoma patients treated with checkpoint inhibitor-based immunotherapy. The aim of the present multicenter study is to validate, in a large sample, the predictive value of specific galectin signatures in this setting. This retrospective observational, non-interventional study will include patients aged ≥18 years with stage IV NSCLCs or melanoma candidate to checkpoint inhibitor-based immunotherapy treatment in routine clinical practice across 4 centers (academic and hospital settings) since June 2017.

preliminary results: The primary research objective of the the present proposal is to validate the predictive and/or prognostic value of galectins 1,3,9 in melanoma and NSCLC stage IV treatment-naive patients treated with checkpoint inhibitor-based immunotherapy. For the later a pilot study of a cohort of 34 consecutive patients bearing programmed death-ligand 1 (PD-L1)-positive non-small cell lung carcinoma (NSCLC), treated with pembrolizumab, was considered; in particular, this study provided exciting preliminary results which deserve to be validate in a large multicentric trial. In this recent published pilot study we observed that the large majority of patients (about 90%) with high galectin-3 tumor expression (score 3+) showed an early and dramatic progression of the disease after three cycles of treatments. In contrast, all patients with negative or low/intermediate expression of galectin-3 in tumor cells showed an early and durable objective response to pembrolizumab, indicating galectin-3 as an interesting predictive marker of tumor responsiveness; as secondary objective we will test the effects of galectin-3 inhibition using several pharmacologic approaches in combination with ICIs on syngeneic mouse lung adenocarcinoma and human lung adenocarcinoma xenografts in order to explore the possibility of combined therapeutic strategies that target galectins.

Traslational value: The *galectins signature*, at least in NSCLCs, promises a better selection of patient candidates for checkpoint inhibitor-based immunotherapy. In particular, it is urgent to optimize the clinical use of novel biomarkers by improving the selection of potentially responsive tumors. This will reduce patients' exposure to ineffective and potentially harmful treatments, as well as social costs. In order to examine the role of galectin-3 inhibition and to identify new treatment strategies associated with checkpoint inhibitor-based immunotherapy in NSCLC, as secondary objective we will test the effects of galectin-3

inhibition using several pharmacologic approaches on syngeneic mouse lung adenocarcinoma and human lung adenocarcinoma xenografts. Considering that phase II trial with gelectin 3 inhibitors are ongoing in several tumor types, we belive that this translational research projects promise short-medium term effects in clinical practice.

Introduction

Immune checkpoint therapies refers to drugs that target regulatory pathways on immune cells to promote antitumor immune responses. These innovative drugs have revolutionized cancer treatment across various tumor types. The immune system is crucial in cancer cell surveillance and tumoral immune-evasion by various mechanisms is considered one of the hallmarks of cancer. Understanding these immune evasive programs has been instrumental for the successful implementation of cancer immunotherapeutic modalities, particularly those targeting the programmed death-1 (PD-1)/programmed death ligand-1 (PD-L1) immune checkpoint pathways. The cancer immunity cycle described by Chen and Mellman describes the foundation for strategies involved in augmenting antitumour immune responses. These strategies include steps such as: cancer antigen release and presentation by dendritic cells, priming and activation of peripheral immune cells, trafficking and infiltration of T cells to the tumour compartment, and tumour-cell recognition and immune-mediated cell death. The steps after priming and activation of peripheral immune cells result in what has been described as the *T-cell inflamed phenotype*, which includes the local production of chemokines, interferon signalling, and expansion of CD8+ cytotoxic T cells. However, mechanisms of tolerance are common, which diminishes the ability for immune-mediated tumour eradication. In particular, the efficacy of anti-PD-L1 or anti-PD-1 immunotherapy is limited by several and complex immunosuppressive mechanisms, which are present at least in part, within the tumor microenvironment (TME). Several regulatory mechanisms inducing immunosuppression occur through the galectins.

background and rationale

Clinical use of anti-PD-1/PD-L1 antibodies as checkpoint inhibitors is rapidly becoming a promising therapeutic approach in treating tumors. However, not all patients show responses and adverse events have been reported, suggesting a better understanding of PD-1 pathway mediated immunosuppression is needed to predict patient response and improve treatment efficacy. Signals arising from the TME may also favor tumor growth by promoting several immune evasive pathways. Galectins are key players in this process by thwarting antitumor immunity through several mechanisms, including promotion of T cell apoptosis, inhibition of T cell activation, induction of anti-inflammatory T helper type 2 (Th2) responses, expansion of Foxp3+ regulatory T (T reg) cells, induction of tolerogenic dendritic cells (DCs), inhibition of natural killer (NK) cell function, and polarization of macrophages toward an M2 phenotype. In particular, Galectin-1 (Gal-1) is a member of the galectin family of β-galactoside-binding proteins. Previous studies have indicated that Galectin-1 (Gal-1) is highly expressed in many kinds of tumors and in the tumor stroma. Since 1995, Gal-1 has been found to induce the apoptosis of T cells. Gal-1 triggers T cell apoptosis by redistributing and segregating the clustering of CD3 and CD45 and the clustering of CD7 and CD43 into membrane microdomains. Gal-1 also acts as an antagonist in T cell receptor (TCR) signal transduction. The first in vivo evidence of Gal-1-mediated immune regulation and tumor immune escape was demonstrated in a study on melanoma. In lung cancer, moreover, cancer-derived Gal-1 activated lung cancer-associated fibroblasts and triggered the tryptophan 2,3-dioxygenase (TDO2)/kynurenine axis, which impaired T cell differentiation and function. In conclusion, several data are supporting the evidence that Gal-1 acts as an immune suppressor by directly promoting T cell apoptosis or indirectly impairing T cell differentiation in tumor cells and their microenvironment. Therefore, Gal-1 could be a good candidate as predictive marker and potential therapeutic target in cancer immunotherapy. Galectin-3 is the only chimera-type galectin. Human galectin-3 is a 35-kDa protein that is coded by a single gene, LGALS3, located on chromosome 14. The N-terminal domain of galectin-3 is essential

for its multimerization, sensitive to proteolysis by matrix metalloproteinases and may participate in the interaction with other intracellular proteins. Galectin-3 is predominantly located in the cytoplasm and shuttles into the nucleus. In addition, it is secreted to the cell surface and into biological fluids. The different locations of galectin-3 contribute to its various functions. Galectin-3 is expressed in a consistent percentage of NSCLCs and melanoma, as well as in other malignancies. Soluble galectin-3 derived by tumor shedding, binds specific glycan residues in the tumor microenvironment, forming a complex lattice. This complex molecular structure reduces IFNγ diffusion through the tumor matrix and the chemokine gradient that is necessary to favor T lymphocytes migration into the tumor: The finding that experimental transfer of cytotoxic T lymphocytes in vivo reduces tumor growth only after galectin-3 inactivation further support the key role of galectin-3 in favoring tumor immune escape. According to these evidences we recently discovered that tumor resistance to pembrolizumab strongly correlated with high expression of galectin-3 in cancer cells, whereas an objective clinical response was obtained in the vast majority of tumors with a negative–low or intermediate galectin-3 phenotype after 12 and 24 weeks of treatment. In addition, Galectin-9 expression was found in all NSCLC pathological type and on tumor cells, galectin-9 level had correlation with TIM-3 level. Patients with low galectin-9 level on tumor cells or high galectin-9 level on TILs were more likely to have poor prognosis. In this complex scenario galectin-1,3 and 9 deserve consideration for the functional implications they can have on tumor immune-response. Expression of galectin-1,3 and 9 in tumor cells can block apoptosis; when shed in the tumor microenvironment both of them induce T-cell apoptosis via CD45 and CD7 binding on T-cell surface, favoring, indeed, tumor immune-escape. This scenario opens the possibility that a tumor-specific "*galectin signature*" could be a surrogate predictive marker of tumor responsiveness to checkpoint inhibitors-based immunotherapy.

experimental design (organized in tasks)

1st task

In the first part of project, the samples for analysis will be collected in a centralized laboratory in coordinator center, while the clinical information will remain in the referral center and will be disclosed only after the IHC analysis. In referral center, baseline and follow-up data must be available for each enrolled patient (minumun follow-up: 12 months); the patient should have measurable disease according to iRECIST criteria. This research will carried out following the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines. Present study is multicenter, retrospective observational cohort study of checkpoint inhibitor-based immunotherapy in treatment-naive in NSCLCs and melanoma stage IV patients. This non-interventional study includes patients aged ≥18 years with metastatic NSCLCs or melanoma candidates to checkpoint inhibitor-based immunotherapy treatment in routine clinical practice across 4 centers (Istituto Tumori Regina Elena IRCCS – Roma, Istituto Tumori Fondazione Pascale IRCCS-Napoli, Istituto Europeo Oncologia IRCCS-Milano, Casa Sollievo della Sofferenza IRCCS-Foggia) since June 2017. The study will include patients which already concluded the ICI and those for which ICI is still ongoing. Demographic, efficacy data are collected from patient medical charts at study entry and at routine care visits. Paraffin-embedded samples will be prepared from specimens obtained from primary tumors at least 369 (95% CI) patients diagnosed with metastatic lung (NSCLC) or melanoma. Two pathologists will review all tissue specimens. Inclusion Criteria: Histologically or cytologically confirmed metastatic NSCLC or metastatic melanoma; The participant has measurable disease per iRECIST; has provided an archival tumor tissue sample; Eastern Cooperative Oncology Group (ECOG) Performance status of 0 or 1; Adequate organ function. Exclusion Criteria :Prior therapy with an anti-PD-1, anti-PD-L1, CTLA-4 agents or other anticancer drugs; Radiotherapy within 2 weeks of start of ICIs treatment; Vaccination with a live vaccine within 30 days prior to the first dose of trial treatment. Has a diagnosis of immunodeficiency or is receiving chronic systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior the first dose of trial treatment; Has a known additional malignancy that is progressing or has required active treatment within the past 2 years.; Has known active

CNS metastases and/or carcinomatous meningitis.; Has an active autoimmune disease that has required systemic treatment in past 2 years; Has a history of (non-infectious) pneumonitis that required steroids or has current pneumonitis; Has an active infection requiring systemic therapy; Has a known history of human immunodeficiency virus (HIV) infection; Known active Hepatitis B or Hepatitis C;Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the study, interfere with the patient's participation for the full duration of the trial, or is not in the best interest of the patient to participate, in the opinion of the treating investigator; Has known psychiatric or substance abuse disorders that would interfere with cooperating with the requirements of the trial. Monoclonal Antibodies and ImmunohistochemistryMouse monoclonal antibodies to PD-L1 (clone 22C3 DakoCytomation, Glostrup, Denmark) and a rat mAb to Galectin-3 (clone M3/38, Mabtech, Nacka, Sweden) and galectins 1 and 9 (R&D System) were commercially acquired and will be use according to the manufacturer's instructions. Briefly, antigen-retrieval microwave treatment (0.01M citrate buffer pH 6.0) will applied when required for three cycles of 5 min each, at 750 W. Endogenous peroxidase activity will quenched with methanol–hydrogen peroxide (3%) for 15 min. After blocking with unrelated antiserum, tissue slides will incubated with the primary monoclonal antibodies in a moist chamber at 4 °C. The immune reaction will visualized by using the Envision System (DakoCytomation, Glostrup, Denmark) for indirect immunoperoxidase, as required. The expression analysis of PD-L1 and galectins 1,3 and 9 will performed by IHC on NSCLC or melanoma tumor biopsies collected before treatment. Microscopic evaluation will performed independently by two experienced pathologists. Two discordant IHC evaluations were resolved after a consensus meeting with experts in the pathology laboratory. Most importantly, galectins expression analysis will performed in blind, meaning that no clinical information will available to pathologists at the time of IHC evaluation of the tumors. The expression analysis of galectins 1,3 and 9 in this study will allow to identify three groups of patients: (a) a high galectin-3 expression group (score 3+) with positive tumor cells >70%; (b) an intermediate galectin-3 expression group (scores 2+ and 1+) with >50–70% and 10–50% positive cells, respectively; (c) a low expression group (negative score and +/− score) including galectin-3-negative cases and cases showing scattered positive tumor cells <10% [23]. Tissues labelled without the primary antibody will use as negative controls. Statistical Analisis: Bivariate comparisons of clinicopathological features between patients will performed using the chi-squared test (Fisher's exact test). The association of multiple prognostic factors with cancer-specific survival will assessed using univariate and multivariate Cox proportional hazard model analyses. Survival curves will calculated using the Kaplan-Meier method, and the difference between survival curves was analyzed using the log-rank test. Regression analysis will use to assess the relationship between two variables. Differences will considered statistically significant at P<.05. Sample size required: 369 patients (95 % confidence interval).

2nd task

Recently, innovative and more promising treatments are designed on the combination of therapeutic agents able to hit gelectin-3. To examine the role of galectin-3 inhibition in NSCLC, in second phase we will test the effects of galectin-3 depletion using several pharmacologic approaches on syngeneic mouse lung adenocarcinoma and human lung adenocarcinoma xenografts. In this part of project, we proposed to investigate the efficacy improvement on tumor cell growth, cell death, and drug-resistance by combined treatments with Smo-Hedgehog (Hh)/Galectin-3 +/- PD1 inhibitors. These combinations will be investigated in vivo by using syngeneic mouse lung adenocarcinoma and human lung adenocarcinoma xenografts. To this end lung cancer cell lines will be isolated and equal volume of single-cell suspension were injected s.c. at the posterior flank of mice. Animals will be randomly divided into different groups and treated with solvent only, single agent (smo-HH inhibitors, Galectin 3 inhibitor, PD1 inhibitor) or combinations (Smo-HH/Galectin-3 inhibitors, Smo-HH/PD1 inhibitors, PD1/Galectin-3 inhibitors, PD1/Smo-HH/Galectin-3 inhibitors). Tumor growth will be monitored by measuring the size with caliper and tumors will be allowed to growth until a

medium size of ~ 100 mm3. The effects of pharmacological inhibition on the both Galectin 3 and Hh pathways activity will be evaluated by assessing the expression levels of the Galectin 3 and Hh target. Tumor volume (V) was expressed in mm3 using the following formula: V = 0.5 a × b2; where a and b were the long and short diameters of the tumor, respectively. As a measurement of efficacy, a T/C (%) value was calculated at a time point according to: T/C (%) = (TRTV/CRTV) × 100%; where TRTV was the relative tumor volume (RTV) of the treatment group and CRTV was the RTV of the control group. RTV = Vt/V1; where V1 and Vt were the average tumor volumes on the first day of treatment (day 1) and the average tumor volumes on a certain time point (day t), respectively. Additional measurements of response included stable disease (SD), partial tumor regression (PR), and complete regression (CR) were determined by comparing tumor volume change at day t to its baseline: tumor volume change (%) = (Vt-V1/V1). The criteria for response (mRECIST) were adapted from RECIST criteria [32, 33] and defined as follows: mCR, BestResponse < − 95% and BestAvgResponse < − 40%; mPR, BestResponse < − 50% and BestAvgResponse < − 20%; mSD, BestResponse < 35% and BestAvgResponse < 30%; mPD, not otherwise categorized. SD, PR, and CR were considered responders and used to calculate response rate (%). Body weight of animals were monitored simultaneously. The change in body weight was calculated based on the animal weight of the first day of dosing (day 1). Tumor volume and changes in body weight (%) were represented as the mean ± standard error of the mean (SEM).

work carried out and preliminary results

The research group involved in the present proposal has specific expertise in biology of galectins and evaluation of their potential clinical applications. In the field of this specific proposal a resent pilot study gas been alreay publishedwe observed that the large majority of patients (about 90%) with high galectin-3 tumor expression (score 3+) showed an early and dramatic progression of the disease after three cycles of treatments. In contrast, all patients with negative or low/intermediate expression of galectin-3 in tumor cells showed an early and durable objective response to pembrolizumab (table 1). Thus we belive that the galectins signature, at least in NSCLCs, promises a better selection of patient candidates for checkpoint inhibitor-based immunotherapy. This finding deserves to be confirmed in multicenter clinical study.

Table 1. Clinical features of the patients and non-small cell lung carcinoma (NSCLC) phenotypes.

Patients	Gender	Age	Performance Status (ECOG)	Tumor Subtype	Gal3 Tumor score	Response iRECIST
1	F	58	1	SCC	neg	Response (PR)
2	F	59	1	SCC	+/-	Response (PR)
3	M	67	0	Adc	+++	PD
4	M	71	0	Adc	+/-	Response (CR)
5	M	78	1	Adc	+++	PD
6	F	70	0	Adc	+++	PD
7	M	72	1	Adc	+++	PD
8	M	82	1	Adc	+++	PD
9	M	54	0	Adc	+	Response (PR)
10	M	58	0	Adc	++	Response (PR)
11	F	67	0	SCC	++	Response (PR)
12	F	70	1	Adc	+++	PD
13	F	70	1	SCC	++	Response (PR)
14	M	54	0	Adc	+++	PD
15	F	59	1	Adc	+++	PD
16	F	78	1	Adc	+++	PD
17	M	45	0	Adc	+++	PD
18	M	63	0	Adc	+++	Response (PR)
19	F	55	1	Adc	+++	Response (PR)
20	F	60	1	Adc	+++	PD
21	M	71	1	SCC	+/-	Response (PR)
22	M	50	0	Adc	++	Response (PR)
23	F	64	0	Adc	+++	PD
24	M	60	0	Adc	+/-	Response (PR)
25	M	69	1	Adc	+++	PD
26	M	55	0	Adc	+++	PD
27	M	71	1	SCC	++	Response (PR)
28	M	69	0	Adc	neg	Response (PR)
29	M	73	1	Adc	+++	PD
30	M	49	1	Adc	+++	PD
31	M	53	1	Adc	neg	Response (PR)
32	M	70	0	Adc	+++	PD
33	M	72	1	Adc	+++	PD
34	M	55	1	Adc	+++	PD

Legend: F: Female; M: Male; SCC: Squamous Cell Carcinoma; Adc: Adenocarcinoma; Gal3: Galectin-3 expression (neg: negative; +/−: < 10%; +: >10<50%; ++: >50<70%; +++: >70%). Immune-related response criteria (iRECIST): CR: Complete Response; PR: Partial Response; SD: Stable Disease; Progressive Disease: PD.

8. expected results and relevant corresponding milestones

The aim of this project is identify a specific galectin signature, which can predict tumor responsiveness to checkpoint inhibitor-based immunotherapy, contributing to optimize the clinical use of these innovative drugs (better patients' selection candidate to immunotherapy) avoiding unnecessary social costs. Furthermore, this study has the potential to provide the biological rationale for using a combined therapeutic approach for cancer treatment, which combine checkpoint inhibitors-based immunotherapy and targeting of specific galectins.

- to confirm in large multicentric study the predictive role of galectins in melanoma and NSCLC stage IV treatment-naive patients treated with checkpoint inhibitor-based immunotherapy (short-medium term effects in clinical practice).

- to calculate the NHS cost reductions for exposure to ineffective and potentially harmful treatments (short-medium term effects in clinical practice).

- to study the effects of galectin-3 inhibitions using several pharmacologic approaches in combination with checkpoint inhibitor-based immunotherapy on preclinical mice models (medium term effects in clinical practice).

References and relevant publications by the research group (bold), already available

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BUDGET FORM/YEAR

Research cost 53.000,00 euro

Instruments /

Indirect cost 2.000,00 euro

Sub-total 55.000,00

Overheads 5.500,00

Tolal: 60.500,00 euro

IUSTIFICATIONS/ITEMIZED RESEARCH COSTS

Research costs:

Consumables: Laboratory consumable and plastic disposables; Reagents and disposables for IHC analysis

Mice, animals model facility

Meeting and travel cost:

study coordination, scientific meeting and partecipation to national/international meeting.

Publication costs:

Publication fees on international journals

Indirect cost:

Stationery and office supplies

Overheads:

Administrative cost of the hosting institution amount to the 10% of the 10% of the sum of direct and indirect costs.

SUGGESTED REVIEWERS (MAX 3)

Michele Milella

Paolo Ascierto

Francesco Cecconi

PERSONNEL INVOLVED IN THE RESEARCH

Name and date of birth	Role on Project	Fellowship required	Effort on project (%)	Present position
Carlo Capalbo (04/11/1977)	PI	-	50	MD PhD
Salvatore Sciacchitano (07/12/57)	Medical Doctor	-	30	MD PhD researcher
Paolo Graziano	Medical Doctor		30	MD PhD researcher
Chiara Diletta Marini (16/04/1990)	Medical Doctor	-	50	MD
Belardinilli Francesca (29/06/1987)	Biologist	-	50	Early stage researcher
Fabrizio del Prete	Tech	-	30	Technician

DESCRIPTION OF THE WORK FOR EVERY UNIT OF PERSONNEL

Dr. Carlo Capalbo (PI, MD,) as PI will be involved in all Tasks of the proposal, providing overall supervision, coordination of the work, evaluation of results, writing of scientific reports and publications. (Task 1 and 2)

Dr. Chiara Diletta Marini (MD) will be primarily involved in histomorphological / immunohistochemical revision, surgical procedures in animals model; collection and analysis of clinical data. (Task 1 and 2)

Dr. Salvatore Sciacchitano (MD) will be primarily involved in collection and analysis of clinical data. (Task 1)

Dr. Paolo Graziano Marini (MD) will be primarily involved in histomorphological / immunohistochemical revision, surgical procedures in animals model; collection and analysis of clinical data. (Task 1 and 2)

Fabrizio del Prete will be primarily involved in collection of biological samples and pretaration of paraffin-embedded sections (FFPE) (Task 1)

Belardinilli Francesca will be primarily involved in collection of biological samples and clinico-pathologic data, preparation of animal models, tumor cell lines and paraffin-embedded tumor tissues (Task 1 and 2)

Declaration

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

Date: 14/02/20. Name of PI. CAPALBO CARLO signature

Principal investigator's signature.....

Authorized Administrative Official's signature.....

IL RESP. AMM.VO DELEGATO
Dott. Carlo Appetecchia

Date 14/02/20

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