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: Elena Barbieri, Associate Professor of Applied Biology, SSD BIO-13 (F1/05). Department of Biomolecular Sciences (DISB), University of Urbino Carlo Bo. Professor of 'Human Biology' in the Sport, Health and Physical Exercise Degree program (L-22) School of Health and Physical Exercise, University of Urbino Carlo Bo (P.I. 's Curriculum Vitae is attached as Supplementary documents, Addendum n.1).

2. Proposal title: Oncoprotective effect of exercise in breast cancer survivors: breast cancer cell proliferation and systemic adaptations in response to single exercise sessions performed at different intensities.

3. Primary area of Relevance: Exercise Oncology.

4. Relevance for the National Health System: Investigation in the context of tumor dormancy, recurrences and control of cell proliferation induced by exercise after primary BC surgery, in patients with high risk of recurrence, will improve the knowledge about exercise prescriptions and will ameliorate the quality of life, prognosis and survival of these patients.

5. Institution: University of Urbino Carlo Bo; address: Palazzo Bonaventura Via Saffi, 2 61029 Urbino PU; e-mail: rettore@uniurb.it; phone: +39-0722-3305343.

6. Authorized Administrative Official: Department of Biomolecular Sciences; address: Via Sant'Andrea, 34 61029 Urbino PU; segreteria.disb@uniurb.it; phone: +39-0722-304582.
Dr Mara Mancini (Responsabile Plesso Scientifico DISB - DISPEA)

7. Proponent's signature.....

8. Authorized Administrative Official's signature: Rettore Prof. Giorgio Calcagnini

.....

Place and date: Urbino, 15/01/2021

SELF EVALUATION FORM

1. Investigator's full name: **Elena Barbieri**
2. Total papers (Scopus) **60**;
3. Total papers (last 10 years) Scopus **33**;
4. Total Papers as first/last author or corresponding author **45**;
5. Total H-index **21**.

PROPOSAL MAIN BODY

1. Proposal title

Oncoprotective effect of exercise in breast cancer survivors: breast cancer cell proliferation and systemic adaptations in response to single exercise sessions performed at different intensities.

2. Abstract

Rationale. Physical inactivity is associated with an increased risk of breast cancer (BC). The cellular and molecular mechanisms mediating this effect are only partially known. Exercise is associated with improved survival among women with BC and recent studies show benefits in their quality of life.
Objective. We aim to examine if systemic responses to acute exercise in BC survivors could modulate BC cell proliferation and/or intracellular IGF-1/PI3K/AKT/mTOR signalling pathway activation using three-dimensional (3D) BC cell culture models.
Particular attention will be focused on the anti-proliferative effect of serum, circulating exosomes and specific antitumor miRNA released pre- and post- exercise on BC cell lines. We also aim to assess if exercise changes the pathological associated systemic factors in BC survivors.
Study Design. Cross-over, open-label, and randomized trial. Subjects will perform two steady-state exercise sessions in randomized order, with randomized permutation blocks, in order to ensure the balance.
Study population and Intervention. Twenty BC survivors will be included. *Inclusion criteria*: 40-65 years; non-physically active; non-metastatic; treated for early-stage BC (stage 0-III); between 6-18 months post-surgery; post chemo- or radio-therapy; with medical clearance to perform maximal exercises. E*xclusion criteria:* uncontrolled hypertension, cardiac or psychiatric illness, other relevant clinical contraindications provided by the oncologists. BC patients will perform 3 sessions of exercise, one preliminary session, and two experimental steady state exercise sessions at a different intensity. Blood samples will be collected before and after each aerobic exercise session. The BC cell lines will be exposed to both pre- and post-exercise serum and isolated exosomes. Changes in patients' functional parameters such as cardiorespiratory fitness, body composition, and pathological associated systemic factors (metabolic, hormonal, and inflammatory) will be evaluated as well.
Preliminary results. We obtained preliminary data from a pilot study in which we evaluated the anti-cancer potential of single aerobic exercise bouts with an *in vitro* 3D cell growth assay, using a triple-negative BC cell line cultured with exercise-conditioned serum. We also provide evidence that the transient serological responses to acute exercise reduces cancer cell growth. Furthermore, we are carrying out a clinical trial with the Unit of Oncology of Urbino Hospital named "Movement and Health Beyond Care" (Movis), under a partnership agreement signed on 4 December 2019. The analyses performed within the

MoviS include preliminary data available for 32 non-physically active BC survivors. This partnership will allow the BC patients enrolment for the present study.
Detailed description of the translational value of the research and the expected impact on the NHS. This study will influence research in the field of exercise-oncology, and it will also provide insight into the effects of the exercise-induced systemic changes in relevant molecular pathways related to tumor dormancy and control of BC cell proliferation. Since recent studies have shown that exercise during cancer care reduces the overall healthcare costs per patient, progress in this area would potentially not only yield health benefits but also prove to be a cost-effective health promotion strategy that policymakers could embrace.

3. Introduction

Breast cancer (BC) is the most common invasive cancer in the world in women (Jemal et al., 2011). BC should not be considered as a single disease but includes a set of pathologies with biologically distinct entities and characteristics that require different therapeutic strategies. In addition to the biological-molecular aspects associated with the prognosis and development of BC, growing evidence highlights the role of lifestyle in influencing disease-related outcomes (Agostini et al., 2018). Unhealthy nutritional habits and low levels of physical activity, in fact, are associated with overweight and obesity that seems to have a negative impact on BC (Duggan et al., 2011), with an increased risk of recurrences and death in all subtypes (Ademuyiwa et al., 2012). Conversely, the correction of diet, hydration levels, overweight, and increased physical activity are associated with better outcomes in the short and long term (Ibrahim et al., 2011, Brenner et al., 2016). Recent epidemiological studies have shown that adequate levels of physical activity in the first 6 months post-diagnosis are positively correlated with survival rates, both overall and associated with recurrences (Fang et al., 2019; Bao et al., 2015).

4. Background and rationale

Recently, *in vitro* studies have demonstrated the ability of a single exercise session in reducing cancer cell proliferation, showing that 2 hours of combined aerobic and resistance exercise or high-intensity aerobic exercise are able to induce serum modifications that inhibit the proliferation of cancer cells (Devin et al. 2019; Dethlefsen et al., 2016) and reduce their tumorigenic potential (De Santi et al., 2019; Baldelli et al., 2020).
Our studies also highlighted, although preliminarily, a significant association between the intensity of exercise achieved by the subjects and a lower tumorigenic capacity of cancer cells (Baldelli et al., 2020). This allows us to hypothesize a different efficacy of the single exercise session in controlling cell proliferation as a function of exercise intensity.
In this study, two sessions of exercise at different intensities, moderate and vigorous, will be conducted, and serum samples taken before and after the single exercise session will be used to evaluate their effectiveness in slowing down the *in vitro* tumorigenic potential of BC cells and the induced systemic biochemical changes.

5. Experimental design (organized in tasks)

5.1 Objectives

The study arises from the hypothesis that exercise can exert an oncoprotective effect by inducing systemic biochemical-metabolic modifications capable of reducing / controlling the proliferation of BC cells.

Primary outcome:
Modulation of BC cell proliferation induced by testing pre- and post-exercise serum from BC patients.
Outcome Measures:
Cell proliferation and tumorigenic potential in three-dimensional (3D) cultures, IGF-1 receptor phosphorylation (IGF1-R), ERK1 / 2, AKT and Hippo / YAP pathways activation in BC cell lines (i.e. MCF-7 and MDA-MB-231).
Secondary outcomes:
1. Modification of biomolecular markers such as pro and anti-inflammatory myokines in BC patients before and after exercise.
2. Isolation and biochemical characterization of microvesicles from BC patients' pre- and post-exercise serum.
Outcome Measures:
Biomolecular markers: Creatine kinase, pro and anti-inflammatory myokines such as: IL-6, IL-8, IL-10, and TNF-alpha. Assessment of the state of hydration: change in plasma volume, haematocrit, and haemoglobin levels. Proteomic and miRNA profile of isolated microvesicles.

5.2 Study Design

Cross-over, open-label, randomized trial. The order in which the subjects will perform the two steady-state exercise sessions will be randomized, with randomized permutation blocks.

Time Frame of the study: 24 months
Phase I (0 to 6 months): Clinical assessment of patients' cardiorespiratory fitness, pre steady-state exercise (SSE) test and SSE test with related haematological samples.
Phase II (7 - 18 months): *in vitro* 3D culture analysis using sera collected in Phase I; analysis of the molecular mechanisms involved and biochemical analyses and biochemical and biomolecular characterization of the microvesicles in the most responsive samples, an exploratory cluster analysis will be performed to find any subgroups showing different patterns.
Phase III (18 - 24 months): data processing and statistics.

Task 1. Study Population and recruitment
We will take advantage of the large training intervention trial, 'Movement and Health Beyond Care (MoviS), in collaboration with the Oncology Unit of the Hospital of Urbino, that aims to educate BC survivors in follow-up on the benefits of personalized exercise and proper nutrition based on Mediterranean Diet.
In the present project, we will enrol a sub-group of participants from the trial.
Inclusion criteria. Diagnosis of BC (stage 0-II-III, without metastases or recurrences diagnosis at recruitment); after surgery and chemotherapy and/or radiotherapy treatments. Maximum 12-month from surgical treatment; minimum 6-month from the end of chemotherapy; risk of recurrence identified with meeting at least 1 of the following criteria: BMI at diagnosis ≥25; testosterone ≥ 0.4 ng/mL (for women); serum insulin ≥ 25 μU/mL (170 pmol/L); metabolic syndrome (at least 3 of the following 5 factors): a. glycemia ≥ 100 mg/dL (6.05 mmol/L); b. triglycerides ≥150 mg/dL (1.69 mmol L); c. HDL-C <50 mg/dL (1.29 mmol/L) (female), <40 mg/dL (1.04 mmol/L) (male); d. waist circumference ≥ 80 cm (woman), ≥ 90 cm (man); e. blood pressure ≥ 130/85 mmHg. Non-physically active: subjects who have not performed regular PA (assessed by IPAQ) for at least 6 months.
Exclusion criteria. Contraindication to moderate to vigorous aerobic exercise intensities after the cardiological medical examination. Disabling pneumological, cardiological,

neurological, orthopaedic comorbidities, and mental illness that prevent exercise performance. Treatment with drugs that alter the heart rate response to exercise. Treatment with antidepressant drugs.

Task 2. Physical exercise sessions

Participants will perform 3 sessions of exercise, one preliminary session, and two experimental SSE sessions at different intensities. During the preliminary session (pre-SSE test), the pre-exercise and the maximal heart rate and oxygen uptake will be measured. Then, two experimental SSE tests at two different conditions will be performed (in random order): 45 minutes at moderate (between 40 and 45% of heart rate reserve [HRR]) and vigorous (between 65 and 75% HRR) intensities according to the classifications proposed by the American College of Sports Medicine (ACSM, 2018).

Blood samples will be taken immediately before (T0) and after 1 (T1), 3 (T2), and 24 (T3) hours after the SSE test for the evaluation of primary and secondary outcomes.

The exercise sessions will be separated from each other for about a week and will be performed at approximately the same time of day to minimize the possible effect of the circadian rhythm on HR and $\dot{V}O2$ and outcome variables. The testing sessions will be performed under controlled room temperature and humidity.

Participants will be asked to avoid changes in their exercise program and eating habits and to avoid vigorous physical activity or drinking alcohol or caffeine both the day before the tests and on the testing days. They will also be asked to drink plenty of fluids the day before testing and to drink 3 to 5 mL of water per kg of body weight one hour before scheduled testing sessions (ACSM, 2007). Furthermore, participants will be required to arrive in the laboratory after a fasting period of at least 2 hours. Compliance with these instructions will be assessed on each testing day using a questionnaire specifically designed for this study.

The $\dot{V}O2$ and HR will be monitored and recorded, respectively, breath-by-breath using the COSMED portable metabolimeter (Cosmed, Rome, Italy) and in beat-to-beat intervals using a Polar heart rate monitor (Polar Electro Oy, Kempele, Finland).

Pre-SSE test (Day 1)

Pre-exercise assessments

Firstly, anthropometric and body composition assessments will be performed. Then, participants' HR and $\dot{V}O2$ pre-exercise values will be continuously recorded for 20 min with the subject in a position similar to the one assumed during the prescribed exercise mode. Both HR and $\dot{V}O2$ recordings will be divided into four 5-min intervals, and the interval with the lowest average will be assumed as the pre-exercise value of standing HR and $\dot{V}O2$.

Maximal exercise tests

After the pre-exercise assessments, participants' HRmax and $\dot{V}O2max$ will be measured using a personalized graded exercise test (GXT) to exhaustion. The GXT will be created for each participant using the Excel spreadsheet provided by Ferri Marini et al., (2020).

When the GXT will end, participants will sit quietly for 20 min and then will perform the $\dot{V}O_2$ verification trial (VT) proposed by Nolan et al., 2014. During maximal tests (i.e., GXT and VT), participants will receive strong verbal encouragement to make their maximum effort.

The $\dot{V}O2$ and HR raw data will be smoothed as a 15-breath moving average (Robergs et al., 2010) and a 5-second stationary time average (Midgley et al., 2009), respectively. The highest values of $\dot{V}O2$ and HR that will be recorded during either GXT or VT will be assumed to be maximal values if at least one $\dot{V}O2$ plateau will be identified or if the highest HRs recorded during the GXT and VT will be within 4 bpm (Midgley et al., 2009).

SSE test (Day 2 and 3)

The target HRs corresponding to the desired HRR percentages, corresponding to moderate and vigorous exercise intensities, will be calculated, using maximal and pre-exercise values

resulting from pre-SSE test assessments, with the following formula: (maximal value – pre-exercise value) x desired percentage + pre-exercise value.
SSEs will start with a 5-min warm-up at an exercise intensity corresponding approximately to 30% of HRR, followed by 45 min at either a moderate or vigorous exercise intensity (random order), corresponding approximately to 40% or 70% of the HRR (random order).
Due to the high interindividual variability of the predicted %HRR at different exercise intensities (Ferri Marini et al., 2021), to determine the exercise intensities corresponding to the target HRs, individual linear regressions between HRs (independent variable) and exercise intensity expressed in W (dependent variable) will be performed using the data recorded during the GXT. Then, the exercise intensities (W) corresponding to the target HRs will be predicted, for each participant, using the slopes and intercepts of the individual linear regressions in the following formula: slope x target HR + intercept.
After the warm-up, the exercise intensity will be linearly increased every 30 seconds to reach the desired SSE intensity (which should yield approximately 40% or 70% of the HRR) in 2.5 min. If needed, an experienced exercise scientist will adjust the treadmill belt velocity according to the HR response to the exercise to reach and maintain the target HRs throughout the SSE sessions.

Task 3. *In vitro* study
The *in vitro* biological responses induced by conditioned serum derived from pre- and post-SSE blood samples will be evaluated in BC cells such as MCF-7 and MDA-MB-231. Cell proliferation will be evaluated in a 3D growth model. Cells will be cultured in a semisolid medium supplemented with 5% serum taken from patients for 2-3 weeks. After the incubation period, the number and dimension of microtumors will be analysed analysing the stimulation induced by post-exercise sera in comparison to the stimulation obtained with pre-exercise sera as previously described (De Santi et al., 2019; Baldelli et al., 2020).
The analysis of intra and extracellular responses will be evaluated as well. Cells will be cultured for 24 hours in a serum-free medium and stimulated with different percentages of the sera obtained from the patients for a time ranging from 10 minutes to 24 hours according to the cellular processes considered. The activation (phosphorylation) of the IGF-1 receptor (IGF-1R), MAP kinases (ERK1 / 2, p38, JNK), and AKT, the modulation of the proteins involved in the Hippo/YAP pathway, and the production of microvesicles by the treated tumor cells will be analysed. Molecular analyses will be performed by western blotting, immunofluorescence and real-time PCR.
Part of the blood samples from the study will be used for the isolation of the microvesicles and for subsequent in vitro tests. Microvesicles will be purified by differential ultracentrifugation and analysed to evaluate quantity and size as described in Guescini et al., 2015. Subsequently, human BC lines (MCF-7, MDA-MB-231) will be treated with serum at a concentration equal to 5% in the culture medium, and with different aliquots of microvesicles isolated from serum. The same biological and molecular responses described above will be evaluated in the treated cells, such as the microtumors formation and the activation of specific molecular mechanisms involved.

Task 4. Biochemical analysis
The blood samples obtained pre-post-SSE will be analysed for the outcome measures: cytokines (such as: IL-6, IL-8, IL-10, and TNF-alpha); differentially expressed serum proteins (2D-PAGE Proteomics); isolation of microvesicles from serum. These analyses will be carried out following the work protocols described in Guescini et al., 2015 and Annibalini et al., 2017 at the Department of Biomolecular Sciences of the University of Urbino Carlo Bo. The determination of haematocrit and haemoglobin will be carried out, in addition to the blood chemistry analysis provided by the MoviS project at the Clinical Chemistry Laboratory of the U.O.C. of Clinical Pathology of the Urbino Hospital.

Task 5. Statistical analyses

The analysis of the sample size was carried out with the aim of verifying the differences in the microtumors formation induced by exercise-conditioned serum from BC patients subjected to a single session of aerobic exercise on BC cells.

Considering the significance level $\alpha = 0.05$ (two-tailed), standard deviation of the difference = 19.6 (difference in the number of colonies between pre and 24 hours after exercise sessions), minimum difference between treatments equal to 15 number of colonies (with power = 0.9), a total of at least 20 patients will be involved in this two-treatment cross-over study. This means that the probability that the study detects a treatment difference at a bilateral 0.05 significance level, if the actual difference between treatments is 15 colony numbers, is 90% (1-β). The standard deviation of the difference in response was obtained considering the results reported in Baldelli et al. (2020).

Continuous variables will be subjected to normal or Gaussian distribution testing; if outcome measures appear not normally distributed, pre-post SSE comparative statistics will be performed using the Friedman test and Mann-Whitney for group comparison. The two-way ANOVA test for repeated measures will be applied in order to measure the post-treatment variations of the outcome measures. Categorical variables will be compared using the Chi-square test. The post-hoc analysis will be carried out using the Bonferroni test. Factors predicting the serum-induced effects will be analysed by multiple linear regression. Statistical analysis will be carried out using SAS software version 9.1.3.

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

Prior to every visit to the University campus, all participants involved in human research must be informed on the 'University pandemic management model'. All procedures will take place after inspection and under the supervision of the University prevention office and in strict compliance with the government provisions and the ministerial and university safety protocols in force.

7. Work carried out and preliminary results

We obtained preliminary data from a pilot study showing how to evaluate the anti-cancer potential of single exercise bouts with an *in vitro* 3D cell growth assay, using a triple-negative BC cell line cultured with exercise-conditioned serum (De Santi M, 2019). Other Authors also highlight how cancer protection is driven by accumulative effects of repeated acute exercise responses (Dethlefsen C, 2016; Orange ST, 2020).

We also provide evidence that the transient serological responses to acute exercises reduce cancer cell growth (Baldelli G. 2020). In this study, healthy sedentary subjects were recruited and performed two high-intensity endurance cycling (HIEC) sessions before and after a nine-week period of high-intensity interval training (HIIT). We revealed that the microtumor formation of both breast and prostate cancer cells cultured with HIEC-conditioned HS was significantly lower in comparison to cells cultured with HS taken at rest. In addition, multiple linear regression analysis showed relationships between the effects of HIEC-conditioned HS in PC cells, lactate threshold, and VO2max. These results highlight the potential of HIEC bouts in tumor progression control and the importance of optimizing an approach to identify physiological predictors of the effects of acute exercise in tertiary cancer prevention.

Furthermore, the Department of Biomolecular Sciences at the University of Urbino and the Unit of Oncology of Urbino Hospital have been working together on the "Movement and Health Beyond Care" research project (MoviS) for about one year, under a partnership agreement signed on 4 December 2019. The analyses performed within the MoviS include

preliminary data are available for 32 non-physically active BC Survivors. Since this study will adopt the same enrolment criteria used in the MoviS, these preliminary data will be considered as the outcome of a pilot study. This study will also have advantages thanks to the above-mentioned partnership agreement.

8. Expected results and relevant corresponding milestones

The expected result is a minor proliferation and reduced tumorigenic potential (i.e., microtumor formation) of cancer cells induced by the serum extracted post-SSE, compared to the serum extracted before. In addition, on the basis of our previous evidence, we would expect a greater difference between the serum collected after the session carried out at vigorous intensity, compared to that collected after moderate intensity session.
From the treatments of cancer cells with post-SSE extracted serums, at vigorous intensity, we would expect to observe a greater involvement of molecular mechanisms such as HIPPO and/or AKT/ERK pathways and a greater release of microvesicles. The increase in knowledge regarding the mechanisms of action that involve potential microvesicles and or miRNA independently of the receptors of the surface of these cell lines or BC represents a new expected result.
This study does not include highly invasive measures and does not require too long an intervention time. It just requires a recruitment phase as the same patients have already been enlisted in the MoviS project.
The principal innovation of this study is represented by the comparison between two different exercise sessions, carried out at different intensities, with the aim of identifying which one is the more efficient in slowing the progress of cancer. In fact, although the evidence on the efficiency of an exercise session is well supported by scientific literature, the level of optimal intensity in order to have a protective effect against cancer has not yet been noted. This study will obtain a more solid scientific base regarding physical exercise as a prevention and protection in cancer patients. The planned exercise sessions in this study will help participants to get used to an activity supervised by exercise experts, which is useful for the course planned within the MoviS project.
This approach can be particularly useful for those patients who are reluctant to begin a traditional physical activity program as they fear that intense activity could worsen the symptoms related to BC, as well as undertake a dedicated nutritional program. The success of this study will also help to supply solid support to general practitioners and oncologists in the integrated management of patients in overcoming the barriers concerning the management of exercise with a correct lifestyle.

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

9.1 References of the research proposal

ACSM's position stands (2007) Exercise and fluid replacement. Med Sci Sports Exerc.;39(2):377-390.
ACSM's guidelines for exercise testing and prescription (2018) Tenth edition. edn. Philadelphia: Wolters Kluwer.
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Bao P et al. (2015) Epidemiology. 26(6): 909–916.
Brenner DR et al. (2016) Cancer Causes Control. Apr;27(4):459-72.
De Santi M et al. (2019) Data in Brief. 104704.
Dethlefsen C et al. (2016) Breast Cancer Res Treat. 159(3):469-79.

Devin JL, et al. (2019) J Physiol. Apr;597(8):2177-2184.
Duggan C et al. (2011) J Clin Oncol. 29(1):32-9.
Fang Q, et al. (2019) J Breast Cancer. Sep; 22(3):399-411.
Ferri Marini C et al. (2020) Strength and Conditioning Journal, e-Pub Jun 11.
Ferri Marini C et al. (2021) Med Sci Sports Exerc. 53(1):174–182.
Guescini M et al. (2015) PLoS One. 10(5):e0125094.
Ibrahim EM et al. (2011) Med Oncol. Sep;28(3):753-65.
Jemal A et al. (2011) Cancer J Clin 61(2):68-90.
Jordan AR, et al. (2020) Physiol Rep;8(22):e14635.
Lillelund C, et al. (2016) P. Breast Cancer Res Treat. 159(3):469-79.
Midgley AW et al. (2009) Applied physiology, nutrition, and metabolism, 34(2):115-123.
Orange ST, et al. (2020) Physiological Reports. e14635.
Robergs RA et al. (2010) Sports medicine, 40(2):95-111.

9.2 Relevant publications by the research group, already available

Agostini D et al. (2018) Oxid Med Cell Longev. 2018 Sep 30; 2018:5896786.
Annibalini G et al. (2017) Oxidative Medicine and Cellular Longevity. ID 3937842 Baldelli G et al. (2020) Clinical and Translational Oncology. e-Pub May 23.
Barbieri E et al. (2018) J Funct Morphol Kinesiol 3:23.
De Santi M et al. (2019) Data in Brief. 104704.
Emili R et al. (2020) Annals of Oncology 31, Suppl 4, S335-S336, Sept 01, 2020
Ferri Marini C et al. (2020) Strength and Conditioning Journal, e-Pub Jun 11.
Ferri Marini C et al. (2021) Med Sci Sports Exerc. 53(1):174–182.
Guescini M et al. (2015) PLoS One. 10(5): e0125094.

PERSONNEL INVOLVED IN THE RESEARCH

Name and date of birth	Role on Project	Fellowship required	Effort on project (%)	Present position
Elena Barbieri	Principal Investigator	No	10	Associate Professor in Applied Biology Department of Biomolecular Sciences University of Urbino Carlo Bo
Vincenzo Catalano	Subinvestigator	No	5	Staff oncologist Oncology Operative Unit of the Urbino Hospital ASUR Marche-AV1
Rita Emili	Subinvestigator	No	10	Staff oncologist Oncology Operative Unit of the Urbino Hospital ASUR Marche-AV1
Mauro De Santi	Subinvestigator	No	15	Department of Biomolecular Sciences

				University of Urbino Carlo Bo
Francesco Lucertini	Subinvestigator	No	10	Department of Biomolecular Sciences University of Urbino Carlo Bo
Michele Guescini	Subinvestigator	No	10	Department of Biomolecular Sciences University of Urbino Carlo Bo
Luciana Vallorani	Subinvestigator	No	10	Department of Biomolecular Sciences University of Urbino Carlo Bo
Annibalini Giosuè	Subinvestigator	No	5	Department of Biomolecular Sciences University of Urbino Carlo Bo
Davide Sisti	Subinvestigator	No	5	Department of Biomolecular Sciences University of Urbino Carlo Bo
Post Doc	Subinvestigator	Si	20	Department of Biomolecular Sciences University of Urbino Carlo Bo

DESCRIPTION OF THE WORK FOR EVERY UNIT OF PERSONNEL

1. Elena Barbieri: conception of outcomes and presentation of the data collected and of preliminary results to the research team; presentation of preliminary results in international meetings.
2. Vincenzo Catalano: patients' recruitment.
3. Rita Emili: patients' recruitment.
4. Mauro De Santi: three-dimensional cell culture models.
5. Francesco Lucertini: exercise protocol and fitness assessments.
6. Luciana Vallorani: proteomic analysis of biological samples.
7. Michele Guescini: microvesicle isolation and analysis.
8. Giosuè Annibalini: cytokines analysis.
9. Davide Sisti: statistical analysis.
10. Post Doc fellowship/s: exercise protocol/*in vitro* studies.

Scientific production of the research group as Supplementary documents (Addendum n. 2)

Budget Form /year

1.	Research costs	20.000 €
2.	Instruments	5.000 €
3.	Indirect costs	(*)
	Sub-total.....	**25.000** €
4.	Overheads	5.000 €
5.	Fellowships	10.000 €
	Total.....	**40.000** €

DETAILS OF BUDGET COSTS

Research Costs

Consumable 18.000

Cardiorespiratory fitness assessment and training intervention [consumables to use with the metabolimeter and the heart rate monitors].
ELISA: [molecular biomarkers such as IL-6, IL-8, IL-10, TNF-alpha].
Cell biology [cell lines; cell culture supplies for 3D cell proliferation; cell growth media; flask, tips, test tubes; Western blotting and (antibodies; membranes; photographic plates)].
Molecular biology and Proteomic [reagents for quantitative PCR (qPCR) and gene expression assay, kit for DNA, RNA extraction, purification; cDNA synthesis; DNA cloning; PCR Real Time; 2D-Page gel electrophoresis; Master mix, kit FLUO probe (PCR)].

Dissemination of the results obtained 2.000
(i.e. publications)

Instruments

Cardiorespiratory fitness assessment [treadmill] 5.000

(*) Indirect costs

According to an ongoing agreement between the Department of Biomolecular Sciences and the Oncology Operative Unit of the Urbino Hospital, the costs of several haematological analyses (such as IGF-1, glucose, insulin, c-peptide, LDL, HDL, triglyceride, free fatty acid, estradiol, progesterone, and testosteron, etc.) will be included in a specific budget item from the hospital. Therefore, the budget of the study will comprise the analyses not covered by the Urbino Hospital's budget. Furthermore, training facilities will be made available with no additional costs by the School of Sport, Health and Physical Exercise of the University of Urbino).

Overheads 5.000
Fellowship 10.000

EXISTING/PENDING SUPPORT

None

SUGGESTED REVIEWERS (MAX 3)

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Cancérologue médical
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BIOETHICAL REQUIREMENT

1. Human experimentation
YES, approval of the competent ethical committee is attached as Supplementary Documents (Addendum n. 3 and n. 4).

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 the 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 purposes.

Date: 15/01/2021. Name of PI: Elena Barbieri. Signature: .....

Principal investigator's signature .....

Authorized Administrative Official's signature .....

Date: Urbino, 15/01/2021

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