TRANSCAN-3

ERA-NET: Sustained collaboration of national and regional programmes in cancer research

Joint Transnational Call for Proposals 2023 (JTC 2023)

" Translational research on cancer epigenetics "

Pre-proposal Application Form

All fields must be filled in using Arial font, size 11, single-spaced.
Applications should be submitted as a PDF file, formatted in DIN-A4.

Please note that proposals either incomplete, using a different format or exceeding length limitations of any sections will be rejected without further review.

1a. Project title (maximum 150 characters, including spaces)**:**

Sarcoma subtyping and stratification using genome-wide epigenetic profiling

1b. Project acronym (maximum 10 characters)**:**

EpiNanoSarc

2. Project duration (months)**:**

36

3. Project coordinator (research partner 1 in the consortium):

Name	Silvestro Conticello
Country	Italy
Position	Primo Ricercatore CNR
Institution/Department	Istituto per lo Studio, la Prevenzione e la Rete Oncologica (ISPRO)
Address	Via Cosimo il Vecchio 2, 50139 Firenze
Phone + Fax	+39 055 7944565
E-mail address	s.conticello@ispro.toscana.it
Type of entity (tick as appropriate)	☐ Academia (universities or other higher education or research institutions) **x** Clinical or Public Health Sector (hospitals/public health and/or other health care settings and health organisations) ☐ For-profit Private Organisation ☐ Nonprofit PItalyrivate Organisation

4. Other research partners

No.	Country	Name of research partner (principal investigator)	Institution, department & full address	Phone & Fax	Email address	Type of entity			
						Academia	Clinical or Public Health	For-profit Private	Nonprofit Private
2	Italy	Alessandro De Vita	Preclinic and Osteoncology Unit, Biosciences Laboratory, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori"	0543 739239/9935 0543 739221	alessandro.devita@irst.emr.it		X		
3	Israel	Aviad Zick	Hebrew University, Hadassah Medical Center, Sharett institute	+972 52 709 7940	aviadz@hadassah.org.il	X	X		
4	Spain	Atocha Romero	Hospital Universitario Puerta del Hierro Majadahond, Madrid, ES	+3463 4524396	atocha10@hotmail.com	X	X		
5	Romania	Anca Zgura	Department of Oncology-Radiotherapy, Prof. Dr. Alexandru Trestioreanu Institute of Oncology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania	+4075 1110005	medicanca@gmail.com	X	X		
6	Turkiye	Kazım Yalçın Arga	Department of Bioengineering, Faculty of Engineering, Marmara University, 34854 Maltepe İstanbul, Turkiye	0090 216 7773570	kazim.arga@marmara.edu.tr	X			

5. Total requested funding: € 1,268,706

6. Keywords

Please indicate three to seven keywords by using the MeSH vocabulary representing: the scientific content (type of cancer; specific aim(s) and topic(s), see *Call Text, Chapter 2: Aim of the Call*); the methodological and technological approach(es).

Sarcoma, Epigenetics, Genome Profiling, Patient-derived Primary Culture, Nanopore Sequencing, Pharmacologic Profiling, Precision Medicine

7. Project abstract (max 3,000 characters including spaces, equivalent to about ¾ of an A4 page)

Background, rationale
Sarcoma subtyping is crucial for determining the prognosis of the disease, as it helps predict how rapidly the cancer will grow and spread. While several diagnostic tools exist to support subtyping and patient classification, a high inter-observer variability makes their classification harder, with 15-20% of cases remaining unclassified.
Molecular analyses greatly improve classification by detecting typical genetic alterations and, recently, a methylation-based classifier has been shown to be capable of reliably classifying most sarcoma subtypes. Yet, this approach cannot be easily translated into routine diagnostics and comprehensive molecular analysis requires access to several analytical tools - not always available, especially in smaller hospitals.
Oxford Nanopore Technologies (ONT) sequencing is a competitive platform in terms of sample handling and instrumental costs. We have shown that ONT sequencing can detect multiple genome-wide genetic/epigenetic tumor DNA features from a single assay.

Hypothesis
Our hypothesis is that comprehensive genome-wide characterization of sarcomas using ONT sequencing will result in enhanced patient stratification.

Aims
Main aim of the project is to validate methylation-profiling by ONT sequencing as a viable alternative to more complex approaches. Additionally, we will develop multi-modal analysis to correlate epigenetic (methylation levels, specific alterations), genetic (CNAs, structural variants, signatures of CNAs, mutational signatures), and clinical features with treatment outcomes and evolution of the disease.

Methods
A retrospective and a prospective study will be carried out on tumor tissues (WP1), tumor-derived cell lines (WP2), and liquid biopsy (WP3). ONT sequencing will be performed on the various samples to extract genome-wide methylation profiles. Additional epigenetic/genetic features will be extracted as well. A multi-modal Machine Learning model will be trained based on all these features for sarcoma subtype classification and patient stratification.

Expected results and potential impact
We expect that our methylation profiling via ONT sequencing will match or improve other subtyping approaches, and that this novel approach will facilitate more effective and streamlined diagnostic management for sarcoma patients, ultimately leading to improved outcomes.

8. Adherence of the proposal to the scope, aims and specific topics of the call. Proposals will have to cover at least one of the undermentioned aims. Please select as appropriate.

Aim 1. The role of epigenetics in cancer initiation and progression

- ☐ To understand cancer initiation and progression by characterisation of the epigenetic landscape.
- ☐ To define epigenetic features of cells in the tumour microenvironment that may promote tumour progression (e.g., immune cells, vascular cells, microbiota).
- ☐ To study the role of epigenetic modifications as predictors of cell persistence or treatment resistance.
- X To validate epigenetic markers useful to improve early detection and diagnosis by exploring the correlation between epigenetics and clinical cancer manifestation.

Aim 2. Validation of new epigenetics-based therapeutic strategies to limit cancer progression, prevent relapse/recurrence or increase the efficiency or reduce toxicity of existing anti-cancer therapies

- ☐ To validate novel therapeutic targets.
- ☐ To study the potential use of epigenetic modulators to overcome resistance to anti-cancer therapies.
- ☐ To develop novel epidrugs/therapeutic approaches, through phase I and II clinical trials to improve safety and efficacy of treatments.
- ☐ To develop novel theranostic approaches involving epigenetics of cancer.

Has the project been submitted elsewhere?

- X Yes
- ☐ No

9. Project description (maximum 20,000 characters including spaces, equivalent to about five A4 pages)

Background and Rationale

Sarcomas, a diverse group of cancers arising from mesenchymal cells in various tissues, pose a complex challenge in classification due to their different origins and this, in turn, affects patient management and therapeutic strategies. They are broadly categorized into bone and soft-tissue sarcomas, but their diversity extends beyond these distinctions (*1*).

The prognosis of sarcomas varies significantly depending on several factors, such as tumor type, stage, location, and size. Five-year survival rates for sarcomas range from 50% to 80%, emphasizing the importance of early detection and classification for a more favorable outlook (*2*). While surgical removal can be curative for low-grade and slow-growing sarcomas, challenges arise when dealing with tumors situated in critical locations, leading to a higher risk of recurrence. Moreover, many sarcomas demonstrate aggressive behavior and are prone to metastasize, especially when diagnosed at an advanced stage.

Surgery remains the elective choice of treatment, and chemotherapy complements the approach either as adjuvant or neoadjuvant therapy in selected cases (*1*). Radiation therapy is sometimes used, especially in higher grade sarcomas. Due to the risk of recurrence and metastasis, multiple lines of chemotherapy, typically starting with anthracycline-based regimens are often necessary in advanced and metastatic disease (*3*).

Sarcoma classification is crucial for determining the prognosis of the disease, as it helps predict how rapidly the cancer will grow and spread, and helps determine the most effective therapeutical options (*1*, *4*). While sarcomas can be classified according to their histology and probable cell-of-origin, et a high inter-

observer variability makes their classification harder, **with 15-20% of cases remaining unclassified** (*5*). Molecular analyses greatly improve classification by detecting point mutations, translocations, copy number alterations (*5*, *6*). In particular, most sarcomas can be reliably identified based on a classifier trained on a dataset of 1077 methylation profiles comprising 62 methylation classes (*7*). Yet, bisulfite-based protocols are difficult to translate into routine diagnostics as biases can be introduced during the handling (e.g. *8*).

Nonetheless, comprehensive molecular analysis requires access to a diverse set of analytical tools, ranging from Sanger and high-throughput sequencing to methylation based arrays.

Oxford Nanopore technology (ONT) is a third-generation sequencing platform based on an array of nanoscale proteic holes through which single DNA filaments are pushed by motor proteins. The passage of DNA through the pore produces an electrical signal that depends on its sequence (*9*). Unlike other technologies, where sequencing is obtained through synthesis of a complementary strand, ONT sequencing allows analysis of the original DNA without amplification steps. It has two unique features, namely the capacity to obtain direct information on long DNA fragments and on base modifications. The former simplifies genome reconstruction, including allele phasing and identification of structural variants (*10*); the latter provides information on the epigenetic layer with performances similar -or better- to that of bisulfite-based methods (*11*). Considering all these elements, ONT sequencing represents a unique platform to investigate whole genomes.

Project aims

Main goal of the project is to use ONT sequencing to extract epigenetic as well as other features and establish correlations between sarcoma subtype and evolution of the disease. This goal will be articulated through three work packages:

WP1: Sarcoma subtyping and stratification from bioptic/postoperative samples

WP2: Impact of epigenetics on drug screening in patient-derived cell lines

WP3: Potential use of cell-free DNA for patient management

Statement of the research hypothesis(es)

The central hypothesis of this project is that comprehensive genome-wide characterization of sarcomas will result in enhanced patient stratification. In contrast to the current requirement for multiple single-feature platforms, ONT sequencing offers the advantage of providing access, with a single-run, to multiple epigenetic and genetic features. This novel approach will facilitate more effective and streamlined diagnostic management for sarcoma patients, ultimately leading to improved outcomes.

Preliminary data

WP1: Methylation-based profiling - Based on the reference set from Koelsche et al (*7*), Partner 3 has implemented a classifier using Random Forest and t-SNE to classify 23 suspected sarcomas based on the epigenetic profiles and the genetic alterations obtained through ONT sequencing (Fig. A-B).

WP2: Preliminary results - Partner 2 has more than 10 years of experience in patient-derived sarcoma cells isolation and characterization. In this regard, they have developed a 3D culture model that allows for extensive sarcoma genomic profiling and pharmacological characterization (Fig. C-E)

WP3: cfDNA analysis - Partner 1 has demonstrated the use of ONT sequencing for cell-free DNA (cfDNA) analysis (*12*). More importantly, together with Partner 3, we have demonstrated that methylation profiling by ONT sequencing can be used to deconvolute the cell-of-origin of cfDNA (*13*).

Methods/Workplan

WP1: Sarcoma subtyping and stratification from bioptic/postoperative samples

Task 1.1 - We will set-up methylation-based profiling of sarcomas using ONT sequencing with the aim of confirming its capability to classify the tumors based on Koelsche et al (*7*). We will use primary tumor tissue either from biobanks or freshly collected bioptic/postoperative samples.

The retrospective study will center on a case series of almost 250 soft tissue sarcoma patients from Partners 2 and 4 Biobanks (including ~40 liposarcomas, ~30 undifferentiated pleomorphic sarcoma (UPS), and ~30 Mixofibrosarcoma). Additional samples to cover subtypes not available will be obtained from other BBMRI-affiliated Biobanks.

In parallel we will set up a prospective observational study, which will allow greater versatility with regard to follow up and type of sample (WP2, WP3). The study, which will be carried forward in Israel, Italy, Spain, and Romania, aims at recruiting ~200 sarcoma patients. A balanced sex distribution will be maintained. Samples obtained through the observational study will be assayed both via ONT sequencing and via approaches that are routinely used for sarcoma stratification (immunohistochemistry, FISH, H&E staining, CINSARC - *14*). End result of this task will be understanding whether methylation-based analysis via ONT sequencing provides a more robust, cheaper, and user-friendly alternative to other diagnostic approaches.

Task 1.2 - While CpG methylation is the most characterized epigenetic feature in cancer, other DNA modifications such as 5-hydroxymethylcytosine (5hmC) and N6-methyladenine (6mA) have been recently shown to be valuable markers.

5hmC is another important epigenetic marker and its levels in cancer are increased at genomic regulatory regions (*15*, *16*). 6mA has only recently been detected in the human genome and its levels have been associated to tumorigenesis (*17–19*). We will thus use available pipelines to quantify these modifications (*19–21*) and we will investigate the association of their levels and profiles with cancer subtypes.

Task 1.3 - Tumor subtyping is crucial for better patient stratification as it allows for personalized treatment approaches. Indeed, methylation profiling has a prognostic and predictive value (*22*). We will thus compare genome-wide methylation profiles from Task 1.1 with treatment outcomes to assess their prognostic and predictive value in soft tissue sarcomas.

Additionally, methylation-based deconvolution approaches are able to determine the tissue of origin of the analyzed DNA. We will use deconvolution to quantify the proportion of immune cell-derived DNA that, as we previously demonstrated (*23*), is predictive/prognostic with regard to Trabectedin treatment.

As ONT sequencing provides much more than methylation information, we will extract additional features (CNAs, structural variants, signatures of CNAs, mutational signatures). This allows to combine the aforementioned features into a multi-modal single assay for sarcoma subtyping and stratification, potentially increasing the informativeness compared to mono-modal analyses. To this aim we will develop and test a machine learning (ML) classifier that combines all genetic/epigenetic/clinical tumor characteristics and treatment outcomes.

WP 2: Impact of epigenetics on drug screening in patient-derived cell lines

Task 2.1 - We have previously obtained patient-derived primary cell lines as 2D cultures and 3D scaffolds for, respectively, 60 and 40 patients, and exploited them to screen 4-8 first/second line chemotherapy treatments. From 12 patients we have also obtained immortalized cell lines available for further drug testing.

We will use ML to identify tissue-based epigenetic profiles (from WP1) correlated to in-vitro drug responsiveness, with the aim of: 1) investigate the use of ONT analysis on tissues as an alternative to cell culture to predict drug screening results, 2) assess the impact of genetic/epigenetic features alone on treatment response, reducing the effect of other clinicopathological variables (i.e. clean resection margins, lesion site).

Task 2.2 - Patient-derived cell lines are a powerful tool for pre-clinical analyses allowing extensive drug screening. However they may not fully retain the characteristics of the original tumor due to

epigenetic/genetic drift during in-vitro culturing (*24*), with potential implications on their reliability as pre-clinical model. To highlight epigenetic drift due to culture, a multilevel mixed linear model will be constructed using R package lme4 (previously exploited to quantify age-related drift, *25*) comparing methylation profiles of the cell lines (both primary and immortalized) and primary tissues. Methylation rates will be compared using both overall methylation profiles and hypo/hypermethylated regions, considering that alterations in specific regions are more likely to happen (*24*). Since DNA from previous primary lines is not available, new primary lines will be produced from the prospective case series.

Task 2.3 - We will sequence cells from Task 2.2 after treatment, we will compare results from both drug sensitive and insensitive cell lines with their respective pre-treatment sequencing, with the aim of detecting acquired alterations potentially responsible for resistance. Candidate alterations will be also investigated in tissue-based profiles from WP1 and correlated to treatment failure in-vivo to validate their biological and clinical meaning.

WP 3: Potential use of cell-free DNA for patient management

Analysis and quantification of circulating tumoral DNA (ctDNA) in plasma samples has been proposed as both prognostic and monitoring tool for sarcoma patients (*26*), with the advantage of being highly non-invasive. In this contest, the design of patient-specific assays (ddPCR, or targeted sequencing) is typically used in order to retrieve patient-specific variants used as a proxy of ctDNA presence (*26*). In contrast, low-coverage sequencing represents a genome-wide alternative, which has proven useful in different types of sarcomas (*26*).

We will collect and analyze both pre- and post-operative plasma samples from the prospective case series, to answer different biological questions:

Task 3.1 - It has been previously demonstrated that patient-specific single-read methylation status can be used to quantify ctDNA with high sensitivity (*27*) without the need of developing patient-specific targeted assays. Also, it has been demonstrated that fragmentomic features (i.e. fragment length) can be used to furtherly discriminate ctDNA from healthy cell-free DNA (cfDNA) fragments, increasing the sensitivity of downstream analyses (*28*). We have previously demonstrated that ONT-seq can detect both methylation and fragmentomics in plasma cfDNA (*13*), so we propose a combined multimodal approach to maximize the sensitivity of ctDNA quantification.

Task 3.2 - The aim is to assess the potential of liquid biopsy as a preoperative diagnostic tool instead of tissue-based analysis. Unfortunately, plasma cfDNA is a mixture of ctDNA and healthy-derived DNA (mainly from blood cells, *13*), which acts as a contaminant. We aim at using the methylation-based classifier from WP1; however, low ctDNA fraction possibly prevents its use (as a tumor purity >60% is required). We will compare preoperative cfDNA with matched tissue results, assessing the concordance of the two sample sources. To increase tumor purity, we'll develop an approach to deplete blood-derived signals: we'll create a reference methylation atlas of blood-cell lines via ONT-seq to be able to subtract blood-specific signals from cfDNA mixture. We'll initially focus on advanced/metastatic patients since such cancers shed more ctDNA in the circulation. Depending on the results/feasibility we'll extend the analysis to less advanced diseases.

Task 3.3 - When possible, the primary tumor is treated with surgery, but metastases usually are not removed surgically (*29*). Since the primary tumor is removed, the post-treatment cfDNA should be enriched in metastasis-derived ctDNA allowing a characterization of the metastasis. ONT-seq profiles (including candidate alterations from Task 2.3) will be correlated with survival/response to treatment.

WP4: Project Coordination and Management

This WP will handle all organizational aspects of the project.

4.1 - Study coordination - Partners will design together the observational study and submit it to local Ethical Committees. A shared eCRF system will be set up for the recording of patients' information.

4.2 - Capacity Building - Training courses on ONT-sWGS, bioinformatic analysis, and primary culture establishment will be organized both at the coordinator's lab and locally.
4.3 - Dissemination and exploitation - Early results will be disseminated through conferences and consolidated ones will be published. Sequencing data will be shared through EGA and portable bioinformatic workflows through the NextFlow platform.

Novelty and originality of the project;

As we were the first to develop ONT sequencing for cancer diagnostics, the proposed project is the first attempt to bring genome-wide analyses to the bedside. Despite growing evidence of the value of "omics" approaches, genome-wide sequencing analyses are rarely performed in clinical contexts. The technical and analytical complexity of the approaches and poor access to expensive sequencers are two of the main obstacles to their use in clinical settings. Our project has the potential to direct towards a decentralization of epigenetic/genetic analysis and, consequently, a widespread increase in the quality of cancer management.

Feasibility of the project

The feasibility of the project relies on the transdisciplinary expertises present in the research consortium. These expertises will determine the role in the WPs: Silvo Conticello and Amir Eden's experience in ONT sequencing will be central for the development of new analyses. Aviad Zick and Anca Zgura are clinical oncologists with a long clinical experience. Alessandro De Vita's research has focused on molecular characterization of Sarcomas. The great experience of Atocha Romero in liquid biopsy analysis and clinical studies will be crucial. Finally, Kazim Arga is an expert in bioinformatics and multi-omics research, and will be central for the statistical and mathematical modeling of the analyses.
With regard to the management structure, a steering committee including all PIs will drive the implementation of the project. Each partner will be responsible for individual elements of the implementation plan (e.g., study design, protocols harmonization, compliance with ethical rules, dissemination strategy, stakeholder involvement, career development of early stage researchers, and resolution of the problems). The scientific WPs will be developed throughout the entire project: the retrospective study will begin immediately, for the prospective one we envision a 6 months delay in order obtain the authorization.

Impact

Identification of novel classes of biomarkers obtained from a single assay may allow for patient stratification well beyond the reach of existing methods, with a direct effect on patient's management.
ONT sequencing is still in a steep growth phase and it now represents an extremely competitive alternative to other sequencing technologies. Minimal instrumentation costs make ONT sequencing accessible to most hospitals. Its simplicity, as all data are produced within a single experiment, via a streamlined pipeline, reducing overall hands-on time and user required expertise, could be the key to make it a ubiquitous technology for rapid and decentralized diagnostics.
Methylation profiling by ONT sequencing could have applications well beyond cancer subtyping. For example, it could be used to monitor collateral damage from immunotherapy in non-cancer organs.
Development of an optimized and simple workflow, for the integration of multi-modal data using ONT sequencing, would improve the characterization and the management of cancer patients, with an eventual effect on treatment strategy choice. Likewise, setting-up automated and portable bioinformatics pipelines will benefit all potential stakeholders (clinicians, scientists, healthcare administrators) to provide a simple turn-key solution to facilitate adoption by smaller groups worldwide.
Dissemination and exploitation are described in Task 4.3.

References

1. Gómez, Tsagozis, *World J Clin Oncol* **11**, 180-189 (2020).
2. Siegel *et al.*, *CA Cancer J Clin* **70**, 7-30 (2020).
3. Hua *et al.*, *J Chemother* **33**, 319-327 (2021).
4. Callegaro *et al.*, *EClinicalMedicine* **17**, 100215 (2019).
5. Gounder *et al.*, *Nat Commun* **13**, 3406 (2022).
6. Nacev *et al.*, *Nat Commun* **13**, 3405 (2022).
7. Koelsche *et al.*, *Nat Commun* **12**, 498 (2021).
8. Olova *et al.*, *Genome Biol* **19**, 33 (2018).
9. Kono, Arakawa, *Dev Growth Differ* **61**, 316-326 (2019).
10. Cretu Stancu *et al.*, *Nat Commun* **8**, 1326 (2017).
11. Yuen *et al.*, *Nat Commun* **12**, 3438 (2021).
12. Martignano *et al.*, *Mol Cancer* **20**, 32 (2021).
13. Katsman *et al.*, *Genome Biol* **23**, 158 (2022).
14. Chibon *et al.*, *Genes Chromosomes Cancer* **58**, 124-129 (2019).
15. Pfeifer *et al.*, *Cell Tissue Res* **356**, 631-641 (2014).
16. Tian *et al.*, *Cell Res* **28**, 597-600 (2018).
17. Xie *et al.*, *Cell* **175**, 1228-1243.e20 (2018).
18. Xiao *et al.*, *Mol Cell* **71**, 306-318.e7 (2018).
19. Cui *et al.*, *Gene* **822**, 146353 (2022).
20. Zhang *et al.*, *Front Cell Dev Biol* **10**, 827391 (2022).
21. Liu *et al.*, *Genome Biol* **22**, 295 (2021).
22. Starzer *et al.*, *J Immunother Cancer* **9**, e001458 (2021).
23. Fausti *et al.*, *Cancers (Basel)* **15**, 1080 (2023).
24. Franzen *et al.*, *Commun Biol* **4**, 598 (2021).
25. Bates *et al.*, *Journal of Statistical Software* **67**, (2015).
26. Namløs *et al.*, *Mol Aspects Med* **72**, 100827 (2020).
27. Lau *et al.*, *Genome Med* **15**, 33 (2023).
28. Peneder *et al.*, *Nat Commun* **12**, 3230 (2021).
29. von Mehren *et al.*, *J Natl Compr Canc Netw* **16**, 536-563 (2018).

10. Capacity building activities (if eligible for the funding organisation/country), (maximum 2,000 characters including spaces, equivalent to about half of an A4 page)

Please specify whether the project will include capacity building activities. If so, please describe the nature and purpose of the planned activities taking into account information described in section 2.2 of the Call Text. The budget will have to be mentioned in the financial plan (sections 12 and 13) in the appropriate line.

ONT sequencing: Since only the Conticello and the Zick/Eden Labs have prior experience with ONT sequencing, it is essential to transfer this expertise to the other partners. To accomplish this, two types of activities have been planned:
The first round involves visits to the Conticello lab, where biologists from the Romero, Zgura, and De Vita labs will learn the experimental settings and technical details of ONT sequencing through hands-on training.
The second round entails bioinformaticians from the Conticello lab visiting the other labs to set up and load the pipelines for analyzing the sequencing data onto their servers. Again, hands-on activities will be performed to ensure understanding of the current procedures.

Primary cell lines establishment: biologists from the various labs will visit Alessandro De Vita's lab to learn how to establish cultures from tumor tissues.

Data management: The expertise of Amir Eden, associated with the Zick lab, in shared data management will be leveraged to train all partners in the use of NextFlow workflows (online courses).

Statistical and mathematical analysis: Kazim Yalçın Arga, professor of bioengineering and expert in bioinformatics and multi-omics research, will organize online sessions to teach the basics of mathematical modeling in the context of genome-wide analysis.

11. Brief CV for each partner in the research consortium (i.e. the project coordinator and each principal investigator) including a description of the main domain of research and a list of the five most relevant publications within the last five years regarding the proposal (once converted into PDF document: max 1 page for each partner).

Partner 1 – Silvestro Conticello
ORCID: 0000-0002-4244-1846

My area of expertise is the analysis of mutational events in physiology and pathology. In particular, I study the role of the AID/APOBECs, a family of deaminases that edit cytosines in uracils in DNA and RNA. These enzymes are involved in the immune response, through the antibody diversification processes and an innate pathway against viruses and mobile elements. Their aberrant activity in cancer cells leads to the onset of mutations and genetic alterations, which have been shown associated to the selection of therapy-resistant clones and to the response to immunotherapy. Starting from this experience, I have recently expanded my research towards the analysis of genetic alteration in cancer and we were the first group to report the use of ONT-seq to analyze cfDNA from liquid biopsies.

Education
1995-1999 PhD, University of Catania and University of Bary, Italy
1996 Italian Medical license
1989-1995 MD, Medical School - University of Catania, Italy

Positions
2019-current Primo Ricercatore, Secondo livello, CNR IFC, presso ISPRO
2007-current Group Leader at ISPRO, Firenze, Italy
2002-2007 Postdoctoral fellow at the MRC-LMB
1999-2002 Postdoctoral fellow at the Weizmann Institute of Science

Grants (selected)
2021 Ministero dell'Università (FISR COVID19 2020 - HackTheCoV). "Exploiting RNA editing against the SARS-CoV-2: hacking the virus to identify molecular targets and dampen the infection" (€70648). PI (€42388.80)
2021 Istituto Buddista Italiano Soka Gakkai. "Host genetics and pathogenetic mechanisms of COVID-19" (€640000). Unit Coordinator
2021 European Innovative Training Network (ROPES, 956810). "Roles of epitranscriptomic in diseases" (€3095828,86). ESR Coordinator
2020 Ministero della Salute Starting Grant 2019 (SG-2019-12370279). "Third-generation sequencing and liquid biopsy: a Nanopore-based approach for copy number variation analysis from cell-free DNA of cancer patients" (€130000) – Fellow Supervisor
2020 JPND 2019 (NMJ-on a -Chip, JPND2019-466-146). "Humanized high-throughput co-culture system for motor neuron diseases" (€1079915). Unit Coordinator
2016 Ministero della Salute – PE 2013 (PE-2013-02357669). "The oncogenic potential of the AID/APOBECs: involvement in tissue transformation and oncogenesis - new tools to better model cancer" (€382008). Project Coordinator (€ 305617.53)

Selected Publications (**#** Lead or corresponding author)
- Katsman et al. **#** "Detecting cell-of-origin and cancer-specific methylation features of cell-free DNA from Nanopore sequencing" **Genome Biol.** 2022 DOI:10.1186/s13059-022-02710-1
- Boccaletto et al. "MODOMICS: a database of RNA modification pathways. 2021 update" **Nucleic Acids Res**. 2021 doi: 10.1093/nar/gkab1083
- Rogier et al. "Fam72a enforces error-prone DNA repair during antibody diversification" **Nature** 2021 doi: 10.1038/s41587-020-00775-6
- Martignano et al. **#** "Nanopore sequencing from liquid biopsy: analysis of copy number variations from cell-free DNA of lung cancer patients" **Molecular Cancer** 2021 doi: 10.1186/s12943-021-01327-5
- Di Giorgio et al. **#** "Evidence for host-dependent RNA editing in the transcriptome of SARS-CoV-2" **Science Advances** 2020 doi: 10.1126/sciadv.abb5813

Partner 2 - Alessandro De Vita
ORCID:0000-0002-1677-5797

My area of expertise is oncology translational research focusing on sarcoma, pharmacology and nanotechnology. I'm currently holding the Assignment of High Specialization in Nanotechnology and Sarcoma in IRCCS IRST

Education
2004-2009 Master degree in Pharmacy, University of Modena and Reggio-Emilia, Italy
2009 Italian Pharmacist license
2010-2012 PhD in Nanomedicine, University of Modena and Reggio-Emilia, Italy

Positions
2012-2012 Teaching assistant, Traditional and Innovative Pharmaceutical Technology Research Center, University of Modena and Reggio-Emilia, Italy
2012-2013 Phase III clinical trial Coordinator, Health Research and Development, Spin Out
Bologna University, General Confederation of Italian Industry Emilia-Romagna and Saint Lucia Garden company
2013-2014 Research collaborator, Istituto scientifico romagnolo per lo studio e la cura dei tumori (IRST) IRCCS
2014-2015 Visiting Postdoctoral Fellow, Methodist Hospital Research Institute, Texas, USA
2017-2017 Visiting Researcher National Cancer Institute (CRO), Aviano, Italy
2015-2018 Researcher, Istituto scientifico romagnolo per lo studio e la cura dei tumori (IRST) IRCCS
2019-2019 Visiting Researcher, Centre Léon Bérard Lyon, Lyon, France
2019-2022 Senior Researcher, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori"
2022-current Adjunct Professor in General Pathology at the Department of Pharmacy and Biotechnology of the Alma Mater Studiorum Bologna University.
2023-current High Specialization Assignment in Nanotechnology and Sarcoma, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori"

Grants (selected)
2017 Italian Ministry of Health, An in vitro and ex vivo model of biomimetic regenerative devices to treat bone metastases and soft tissue tumors: (BIOBOS PROJECT) GR-2016- 02364704. Co-PI (€250.000,00)
2022 Italian Ministry of Health, Nanotechnology-based Platforms for the improvEment of therapeutic strateGies in soft tissue sArcoma and melanoma leSiOns (PEGASO). PI (€260.000,00)
2022 Italian Ministry of Health, Engineered lisyl oxidase lipid-based nanovesicles for the treatment of solid tumors: on the path to clinical application TRUST. Collaborator (€420.000,00)

Selected Publications (**#** Lead or corresponding author)
- Vanni S, et al. **#** “Unveiling the Genomic Basis of Chemosensitivity in Sarcomas of the Extremities: An Integrated Approach for an Unmet Clinical Need”. **Int J Mol Sci.** 2023. doi: 10.3390/ijms24086926.
- Fausti V, et al. **#** **“**Systemic Inflammatory Indices in Second-Line Soft Tissue Sarcoma Patients: Focus on Lymphocyte/Monocyte Ratio and Trabectedin”. **Cancers (Basel).** 2023 doi: 10.3390/cancers15041080.
- Mercatali L, et al. **#** “The emerging role of cancer nanotechnology in the panorama of sarcoma”. **Front Bioeng Biotechnol.** 2022 doi: 10.3389/fbioe.2022.953555.
- Vanni S, et al. **#** Myxofibrosarcoma landscape: diagnostic pitfalls, clinical management and future perspectives. **Ther Adv Med Oncol**. 2022 doi: 10.1177/17588359221093973.
- De Vita A, et al. **“**The potential role of the extracellular matrix in the activity of trabectedin in UPS and L-sarcoma: evidences from a patient-derived primary culture case series in tridimensional and zebrafish models”. **J Exp Clin Cancer Res**. 2021 doi: 10.1186/s13046-021-01963-1.

Partner 3 - Aviad Zick
ORCID: 0000-0002-5077-9249

Dr Aviad Zick is a clinical oncologist and an expert in the analysis of cfDNA methylation and copy number alteration in cancer.

Education
2010-2011 Medical Epidemiology, Hadassah School of Public Health, Israel
2004-2009 PhD, Hebrew University- Hadassah Medical School, Israel
1996-2005 MD, Hebrew University- Hadassah Medical School, Israel

Current Positions
2017-present Lecturer, Hebrew University- Hadassah Medical School
2015-present Member of Institutional Helsinki Committee, Hebrew University
2014-present Member of scientific-ethical committee of the Israeli National Biobank
2012-present Senior Medical Oncologist, Head of Cancer Genetics Laboratory, Sharett Institute of Oncology, Hadassah Hebrew University Hospital

Awards and Fellowships (selected)
2013 David S. Lando Memorial Fund
2011 Health Education fund in honor of Lou Sand
2010 Young researcher award of the Hadassah Medical Center

Grants (selected)
2020 JFC-UIA grant. “A cell free DNA (cfDNA) based blood test to detect breast cancer (PI). US $10,000
2018 Gross Cancer Research Fund of Sarita and Elmer Gross zl, “Identification of tissue specific sarcoma markers using single cell analysis” (PI). US $70,000
2016 Israel Cancer Research Fund, “Tissue-specific methylation patterns of circulating DNAs as biomarkers for neurotoxicity” (PI). US $100,000
2013 Israel Science Foundation, “NF-kappaB regulation of DNA double-strand break repair” (PI). US $180,000

Selected Publications (**#** Lead or corresponding author)
- Katsman et al. “Detecting cell-of-origin and cancer-specific methylation features of cell-free DNA from Nanopore sequencing” **Genome Biol**. 2022 DOI:10.1186/s13059-022-02710-1
- Sadeh et al. “ChIP-seq of plasma cell-free nucleosomes identifies cell-of-origin gene expression programs.” **Nat. Biotech**. 2021 DOI:10.1038/s41587-020-00775-6
- Moss et al.**#** “Circulating breast-derived DNA allows universal detection and monitoring of localized breast cancer.” **Ann. Oncol**. 2020. DOI:10.1016/j.annonc.2019.11.014
- Maoz et al.**#** “Clinical Implications of Sub-grouping HER2 Positive Tumors by Amplicon Structure and Co-amplified Genes”. **Sci. Rep**. 2019. DOI:10.1038/s41598-019-55455-6
- Moss et al. “Comprehensive human cell-type methylation atlas reveals origins of circulating cell-free DNA in health and disease.” **Nature Communications** 2018. DOI:10.1038/s41467-018-07466-6

Partner 4 - Atocha Romero
ORCID: 0000-0002-1634-7397

Dr. Romero is the head of the Liquid Biopsy Laboratory (LBL) at Puerta de Hierro Hospital, which provides diagnostic services and conducts research projects on the field of biomarkers. Being the author of more than 75 publications in international journals, her research focuses on the usefulness of liquid biopsies for the management of lung cancer patients in daily oncology as well as in the context of clinical trials, which has received uninterrupted funding from public and competitive grant calls. Atocha Romero has supervised several theses, and she is accredited by ANECA as an assistant professor. Atocha Romero has has participated in the European H2020 IASIS project (727658) and is currently involved in CLARIFY project (875160). She also participates in the Tentacles Research Network (Translational Network for the clinical application of Extracellular Vesicles (RED2018-102411-T).

Education
PROJECT PARTNER (FIB University Hospital Puerta de Hierro)
Chief Liquid Biopsy Lab, FIB University Hospital Puerta de Hierro (since 2018) PhD in Biochemistry and Molecular Biology Master in Clinical Genetics
Positions
2015-2018 Researcher Joan Rodés, Hospital Puerta de Hierro
2014-2015 Reseacher Post-Doc, FIB San Carlos Clinical Hospital
2012-2014 Researcher Río Hortega, FIB San Carlos Clinical Hospital
2010-2011 Researcher, FIB San Carlos Clinical Hospital
2005-2009 Laboratory Medicine Residency, San Carlos Clinical Hospital
2003-2004 PhD, National Centre of Biological Research & National Centre for Biotechnology
Most relevant R&D competitive projects obtained as PI:
2021-2023 Biomarkers4Cure: Predictive biomarkers in response to combinations of immunotherapy plus chemotherapy neoadjuvant in patients with operable lung cancer
2021- 2023 Clinical validation of a system to detect ALK translocations in extracellular vesicles in plasma III
2020-2023 BLI-O. Development of molecular markers in liquid biopsy for long survival in oncoimmunotherapy
2017-2021 Clinical utility of liquid biopsy in non small lung cancer patients with EML4-ALK translocation
2016-2019 Study of the mutational dynamics in cancer patients of advanced non microcytic lung with EGFR mutation in first treatment line
2016-2019 Correlation with radiographic imaging of tumors and survival
Grants:
2020-2023 CLARIFY Cancer Long Survivors Artificial Intelligence Follow Up (H2020-SC1-DTH-2018-2020; 875160).
2017-2020 IASIS: Integration and analysis of heterogeneous big data for precision medicine and suggested treatments for different types of patients (H2020-SC1-PM-18-2016; 727658).

Selected Publications (**#** Lead or corresponding author)
- Provencio et al. **#** **"**Perioperative Nivolumab and Chemotherapy in Stage III Non-Small-Cell Lung Cancer**"** **N Engl J Med.** 2023 DOI: 10.1056/NEJMoa2215530
-Sanchez-Herrero et al. **#** "ALK-Fusion Transcripts Can Be Detected in Extracellular Vesicles (EVs) from Nonsmall Cell Lung Cancer Cell Lines and Patient Plasma: Toward EV-Based Noninvasive Testing" **Clin Chem**. 2022 DOI: 10.1093/clinchem/hvac021
-Provencio et al. **#** "Overall survival and biomarker analysis of neoadjuvant nivolumab plus chemotherapy in operable stage IIIA non-small-cell lung cancer (NADIM phase II trial)" **J Clin Oncol**. 2022 DOI: 10.1200/JCO.21.02660
-Sanchez-Herrero et al. **#** "NGS-based liquid-biopsy profiling identifies mechanisms of resistance to ALK inhibitors: a step towards personalized NSCLC treatment" **Mol Oncol**. 2021 DOI: 10.1002/1878-0261.13033
-Provencio et al. **#** "Analysis of circulating tumour DNA to identify patients with epidermal growth factor receptor-positive non-small cell lung cancer who might benefit from sequential tyrosine kinase inhibitor treatment" **Eur J Cancer** 2021 DOI: 10.1016/j.ejca.2021.02.031

Partner 5 - Anca Zgura
ORCID: 0000-0002-2370-5762

Dr. Anca Zgura is a specialized medical oncologist who focuses on treating various types of tumors. Her research activities primarily revolve around this field and are carried out at the University of Medicine and Pharmacy Carol Davila in Bucharest. Her major interests lie in studying predictive and prognostic factors in cancers, as well as exploring the sequencing of therapies and novel combinations of treatments for tumors.Throughout her career, Dr. Anca Zgura has been actively involved in numerous clinical trials, both as a Principal Investigator (PI) and a sub-investigator, sponsored by various organizations and academic institutions. She has also made substantial contributions to the scientific community by publishing over 40 papers in international peer-reviewed journals listed on Pubmed. Additionally, she has presented several abstracts at international conferences, focusing on the topics of her expertise.

Education
2012-2018 PhD , University of Medicine and Pharmacy Carol Davila Bucharest
Title of the paper: DETERMINATION OF IMMUNOLOGICAL FACTORS WITH IMPACT ON PROGNOSIS AND TREATMENT RESISTANCE IN BREAST CANCER
2004-2010 Graduated in general medicine, University of Medicine and Pharmacy Carol Davila Bucharest
Positions
2023 Associate Professor, University of Medicine and Pharmacy Carol Davila Bucharest
2019-2023 Lecturer, University of Medicine and Pharmacy Carol Davila Bucharest
2017-2019 Assistant Professor, University of Medicine and Pharmacy Carol Davila Bucharest

Grants
1. Excellence Award for outstanding achievements in research work carried out - Foundation Awards Ceremony "Acad. Marin Voiculescu"
2. CERO – Career profile: Romanian Researcher", grant number POSDRU/159/1.5/S/135760, cofinanced by the European Social Fund for Sectoral Operational Programme Human Resources Development 2007-2013"
3. Project „Skills development in transplant "POSDRU/186/3.2/S/155295,

Selected Publications (**#** Lead or corresponding author)
- Badiu DC, Zgura A, Mehedintu C, Haineala B, Anghel R, Bacinschi X **#** "The Role of Programmed Cell Death Receptor 1 in Lung Cancer" **In Vivo**. 2022 doi: 10.21873/invivo.12794.
- Bacinschi X, Popescu GC, Zgura A, Gales L, Rodica A, Mercan A, Serban D, Haineala B, Toma L, Iliescu L **#** "A Real-World Study to Compare the Safety and Efficacy of Paritaprevir/Ombitasvir/Ritonavir and Dasabuvir, with or without Ribavirin, in 587 Patients with Chronic Hepatitis C at the Fundeni Clinical Institute, Bucharest, Romania". **Med Sci Monit**. 2022 doi: 10.12659/MSM.936706.
- Bacinschi XE, Zgura A, Safta I, Anghel R. **#** "Biomolecular Factors Represented by Bcl-2, p53, and Tumor-Infiltrating Lymphocytes Predict Response for Adjuvant Anthracycline Chemotherapy in Patients with Early Triple-Negative Breast Cancer". **Cancer Manag Res**. 2020 doi: 10.2147/CMAR.S274104.

Partner 6 - Kazım Yalçın Arga
ORCID: 0000-0002-6036-1348

K. Yalçın Arga is a professor of bioengineering and senior expert on bioinformatics and multi-omics research. His research centers on translation of big data to applications in systems biology, pharmaceutical innovation, drug repositioning, health care, and clinical practice. His works are interdisciplinary and integrative, spanning the continuum of metabolic systems engineering in bacteria to drug repurposing and diagnostics innovation for common complex human diseases. His research currently focuses on developing pipeline to analyze, integrate and translate multi-omics data (i.e., genomics, epigenomics, transcriptomics and proteomics) to clinical and health care innovation.

Education
2007 PhD, Boğaziçi University, Istanbul, Turkiye
2002 M.Sc. Chemical Engineering, Boğaziçi University, Istanbul, Turkiye
2001 B.Sc. Chemical Engineering, Boğaziçi University, Istanbul, Turkiye
Positions
2007-current, Professor, Department of Bioengineering, Marmara University, Istanbul, Turkiye
2022-current, Principle Investigator, Health Biotechnology Joint Research and Application Center of Excellence, Istanbul, Turkiye
2019-2021, Director of R&D, Health Institutes of Turkey, Istanbul, Turkey
2005, Visiting Researcher, Technical University of Denmark, Copenhagen, Denmark
2001-2007, Research Assistant, Boğaziçi University, Istanbul, Turkiye

Grants (selected)
2023, TUBİTAK-International, Circulating Tumor Microenvironment Components as Urothelial Cancer Immunotherapy Response Markers (UCIPREDICT), Researcher (€36000).
2021, TÜBİTAK-1001, Identification of Biomarkers and Drug Candidates for Early Diagnosis and Treatment of Abdominal Aortic Aneurysm, Researcher (€36000).
2022, TÜBİTAK-1001, Performing In-Vivo Tests of Drug Formulation of Axololt Blastema in Wound Model to be Created in Healthy and Diabetic Rats and Investigation of Wound Healing Mechanism of Blastema Content, Researcher (€36000).
2017, TÜBİTAK-1001, Drug repositioning applications for prostate cancer through biological molecules at different levels with a systems biology perspective, Project manager (€24000).

Selected Publications (**#** Lead or corresponding author)
- Aydin et al. “Epigenomic and transcriptomic landscaping unraveled candidate repositioned therapeutics for non-functioning pituitary neuroendocrine tumors”, **Journal of Endocrinological Investigation** 2023. doi:10.1007/s40618-022-01923-2
- Kori et al. **#** “Drug repositioning via host-pathogen protein-protein interactions for the treatment of cervical cancer”, **Front Oncol**. 2023. doi:10.3389/fonc.2023.1096081
- Kori et al. **#** “A Pan-Cancer Atlas of Differentially Interacting Hallmarks of Cancer Proteins”, **Journal of Personalized Medicine** 2022. doi:10.3390/jpm12111919
- Gulfidan et al. **#** “Pan-cancer mapping of diffferential protein-protein interactions”, **Scientific Reports** 2020. doi:10.1038/s41598-020-60127-x
- Turanli et al. “Systems biology based drug repositioning for development of cancer therapy”, **Seminars in Cancer Biology** 2021. doi:10.1016/j.semcancer.2019.09.020
- Caliskan et al. **#** “Novel Molecular Signatures and Potential Therapeutics in Renal Cell Carcinomas: Insights from a Comparative Analysis of Subtypes”, **Genomics** 2020. doi:10.1016/j.ygeno.2020.06.003

12. Global financial plan: sum of year 1-3. Please describe the requested budget (grant to be covered by the funding organisation) only.
(Please note that eligibility of costs is subject to national rules and regulations: refer to Annex 1 of the Guidelines for Applicants).

Acronym:						
Partner	Coordinator	Partner 2	Partner 3	Partner 4	Partner 5	Partner 6
Name (principal investigator)	Silvestro Conticello	Alessandro De Vita	Aviad Zick	Atocha Romero	Anca Zgura	Kazim Yalçın Arga
Country	Italy	Italy	Israel	Spain	Romania	Turkiye
Funding organisation	TuscReg	MOH	MOH	ISCIII - FAECC	UEFISCDI	TÜBİTAK
Personnel (€) - Scientist	90,000				132,300	16,000
- PhD-Student		100,000	55,000			12,000
- Technician			27,750	62,000		
- Other		100,000	15,000			
Person months - Scientist	36		20%		36	100%
- PhD-Student		100%	100%			100%
- Technician			20%	24		
- Other		100%	Programmer 10%			
Consumables (€)	164,720	148,000	27,023	80,000	42,840	
Equipment (€)						
Study/Clinical trial (€)[1]						
Travel (€)[2]	6,000	12,000	2,500	3,000	11,900	5,350
Capacity building (€)[3]	6,000					
Other direct costs (€)[4]	6,000				12,959	
(national) Overheads (€)	27,272	40,000	12,727	29,820		20,545
Total requested budget (€)	299,992	400,000	140,000	174,820	199,999	53,895

[1] If applicable: incl. clinical trial drugs/compounds, clinical trial fees and insurance.
[2] Travel expenses should include the participation of the coordinators and/or principal investigators in an intermediate and/or a final status symposium to present the results of their projects (organised by the Joint Call Secretariat).
[3] Separate budget for capacity building activities (if eligible for the funding organisation/country).
[4] e.g. subcontracting, provisions, licensing fees.

13. Individual financial plans: sum of year 1-3.

(Please note that eligibility of costs is subject to national/regional rules and regulations: refer to Annex 1 of the Guidelines for Applicants)

13.1 Partner 1

Project Coordinator Partner (n.1) name:	Silvestro Conticello	
Funding organisation	Tuscany Region (TuscReg)	
Country	Italy – Tuscany (TuscReg)	
	Requested budget	Justification
Personnel (€)	90,000	A Bioinformatician who will be involved for the entirety of the project (36PM) in all Tasks
Consumables (€)	164,720	Nanopore MinION and PromethION flow cells, library preparation kits, and related reagents
Equipment (€)		*Please indicate and justify the equipment to be acquired in accordance to project tasks and objectives. Applicants should also check if equipment is eligible in accordance to their national regulations.*
Study/Clinical trial (€)		*Please indicate the concrete participation/work package(s) in the study/clinical trial*
Travel (€)	6,000	Two international meetings in the 2nd and 3rd years
Capacity building (€)	6,000	Biologists and Bioinformaticians will travel to the partners labs to teach ONT sequencing techniques and data analysis (1PM, travel expenses)
Other direct costs (€)	6,000	Cost of publications
Overheads (€)	27,272	10% overhead
Total budget (€)	299,992	

13.2 Partner 2

Partner (n.2) name:	Alessandro De Vita	
Funding organisation	MOH	
Country	Italy	
	Requested budget	Justification
Personnel (€)	200,000	PhD student, (100%) genomic profiling analysis Master Degree(100%) pharmacological profiling analysis
Consumables (€)	148,000	Sequencing flowcells & kits, Plasticware, patient-derived primary culture establishment and pharmaoclogical profiling, nanopore analysis
Equipment (€)		*Please indicate and justify the equipment to be acquired in accordance to project tasks and objectives. Applicants should also check if equipment is eligible in accordance to their national regulations.*
Study/Clinical trial (€)		*Please indicate the concrete participation/work package(s) in the study/clinical trial*
Travel (€)	12,000	Participation fees for scientific congresses and workshops to present study data (project dissemination activity), travels and accommodation costs for congresses to present data of the project
Capacity building (€)		PhD student/MSc students will visit Conticello's lab to learn implementation of nanopore system
Other direct costs (€)		*May include subcontracting, fees, insurances, etc. Please justify each predicted expenditure with relation to project tasks and objectives*
Overheads (€)	40,000	10% management and general costs
Total budget (€)	400,000	

13.3 Partner 3

Partner (n.3) name:	Aviad Zick	
Funding organisation	MOH	
Country	Israel	
	Requested budget	Justification
Personnel (€)	97,750	PhD student, (100%) Data analysis, pipeline and tool development Lab technician (20%) Sample collection $ prep, sequencing run Programmer, (10%) Data production, integration, QC and pipeline implementation
Consumables (€)	27,023	Sequencing flowcells & kits, Plasticware, ddPCR
Equipment (€)		*Please indicate and justify the equipment to be acquired in accordance to project tasks and objectives. Applicants should also check if equipment is eligible in accordance to their national regulations.*
Study/Clinical trial (€)		*Please indicate the concrete participation/work package(s) in the study/clinical trial*
Travel (€)	2,500	Student/Technician visit to De Vita lab to learn implementation of 3D patient-derived primary culture systems
Capacity building (€)		*Please indicate the type of capacity building and necessary efforts (PMs, travel etc.).*
Other direct costs (€)		*May include subcontracting, fees, insurances, etc. Please justify each predicted expenditure with relation to project tasks and objectives*
Overheads (€)	12,727	10%
Total budget (€)	140,000	

13.4 Partner 4

13.4 a

Partner (n.4) name:	Atocha Romero	
Funding organisation	ISCIII	
Country	Spain	
	Requested budget	Justification
Personnel (€)	62,000	24 person/month (2.583,33€ x 24 months) of a Graduate student to implement the following tasks: -Genomic Studies´ tasks: •DNA, RNA, cfDNA extraction. Nucleid acid quantification and quality evaluation. •Raw data analysis. -Scientific contribution to the Consortium´s tasks under de supervision of Dr Atocha Romero: •support that the S&T objectives of the project are met with quality and in time. •collaborate in the assessment of the project´s progress •support the implementation of the quality procedures and the verification of the project results
Consumables (€)	80,000	Lab consumables for the Genomic Studies for 3 years: •ctDNA: Price per sample: 400 x nº of samples: 100. Total: 40.000 EUR
Equipment (€)		*Please indicate and justify the equipment to be acquired in accordance to project tasks and objectives. Applicants should also check if equipment is eligible in accordance to their national regulations.*
Study/Clinical trial (€)		*Please indicate the concrete participation/work package(s) in the study/clinical trial*
Travel (€)		*Please give an estimate on the number and main reasons for the travels within the project*
Capacity building (€)		*Please indicate the type of capacity building and necessary efforts (PMs, travel etc.).*
Other direct costs (€)		*May include subcontracting, fees, insurances, etc. Please justify each predicted expenditure with relation to project tasks and objectives*
Overheads (€)	29,820	21% of direct costs according to national regulations (ISCIII)
Total budget (€)	171,820	

13.4 b

Partner (n.4) name:	Atocha Romero	
Funding organisation	FCAECC	
Country	Spain	
	Requested budget	Justification
Personnel (€)		*Please indicate the number of PMs per type of personnel, indicating the project tasks that justify the inclusion of that number of PMs*
Consumables (€)		*Please identify the consumables to be included, and their importance within your projects' tasks and objectives*
Equipment (€)		*Please indicate and justify the equipment to be acquired in accordance to project tasks and objectives. Applicants should also check if equipment is eligible in accordance to their national regulations.*
Study/Clinical trial (€)		*Please indicate the concrete participation/work package(s) in the study/clinical trial*
Travel (€)	3,000	Travel expenses of at least 3 travels for 1/2 persons each including: -participation in an intermediate and/or a final status symposium to present the results of their projects (organized by the Joint Call Secretariat). -at least 2 international meetings of the Consortium during the implementation of the project. -other necessary meetings between project partners
Capacity building (€)		*Please indicate the type of capacity building and necessary efforts (PMs, travel etc.).*
Other direct costs (€)		*May include subcontracting, fees, insurances, etc. Please justify each predicted expenditure with relation to project tasks and objectives*
Overheads (€)		*Please refer to your national regulations before calculating overheads*
Total budget (€)	3,000	

13.5

Partner (n.5) name:	Anca Zgura	
Funding organisation	UMFCD	
Country	Romania	
	Requested budget	Justification
Personnel (€)	132,300	1 principal investigator, 2 senior researchers, 1 technician and 1 research assistant will be involved for the entirely project – 36 months
Consumables (€)	42,840	ctDNA extraction Kit
Equipment (€)		*Please indicate and justify the equipment to be acquired in accordance to project tasks and objectives. Applicants should also check if equipment is eligible in accordance to their national regulations.*
Study/Clinical trial (€)		*Please indicate the concrete participation/work package(s) in the study/clinical trial*
Travel (€)	11,900	Travel to partners labs to learn nanopore sequencing technique, other necessary meetings between project partners.
Capacity building (€)		*Please indicate the type of capacity building and necessary efforts (PMs, travel etc.).*
Other direct costs (€)	12,959	Project management consultancy , costs related to the 'open access' dissemination of project results
Overheads (€)		*Please refer to your national regulations before calculating overheads*
Total budget (€)	199,999	

13.6

Partner (n.6) name:	Kazim Yalçın Arga	
Funding organisation	TÜBİTAK	
Country	Turkiye	
	Requested budget	Justification
Personnel (€)	28,000	Scientist (Post-doc researcher): (100%), Data analysis, pipeline development and implementation PhD Student: (100%), Data analysis and integration, pipeline and tool development
Consumables (€)		*Please identify the consumables to be included, and their importance within your projects' tasks and objectives*
Equipment (€)		*Please indicate and justify the equipment to be acquired in accordance to project tasks and objectives. Applicants should also check if equipment is eligible in accordance to their national regulations.*
Study/Clinical trial (€)		*Please indicate the concrete participation/work package(s) in the study/clinical trial*
Travel (€)	5,350	2x Project meetings (international) 1x International scientific conference/symposium
Capacity building (€)		*Please indicate the type of capacity building and necessary efforts (PMs, travel etc.).*
Other direct costs (€)		*May include subcontracting, fees, insurances, etc. Please justify each predicted expenditure with relation to project tasks and objectives*
Overheads (€)	20,545	Overhead
Total budget (€)	53,895	

14. Reviewers

Please note that providing the information below is optional. The Call Steering Committee (CSC) will consider these suggestions provided that they do not interfere with the objective and thorough evaluation of the proposal.

Suggested reviewers for reviewing this proposal (up to five), without any conflict of interest (i.e. not working in the same institute, no co-publication in the past 5 years).

Name	Institute	Email address

Reviewers to be excluded from reviewing this proposal (up to five).

Name	Institute	Email address

15. Annexes (to be included in this document)

- Diagrams and figures (one page maximum)
- **IF APPLICABLE:** A signed written confirmation that the project partner with own funding (also from a country/region not participating in the JTC 2023) has secured his/her funding.

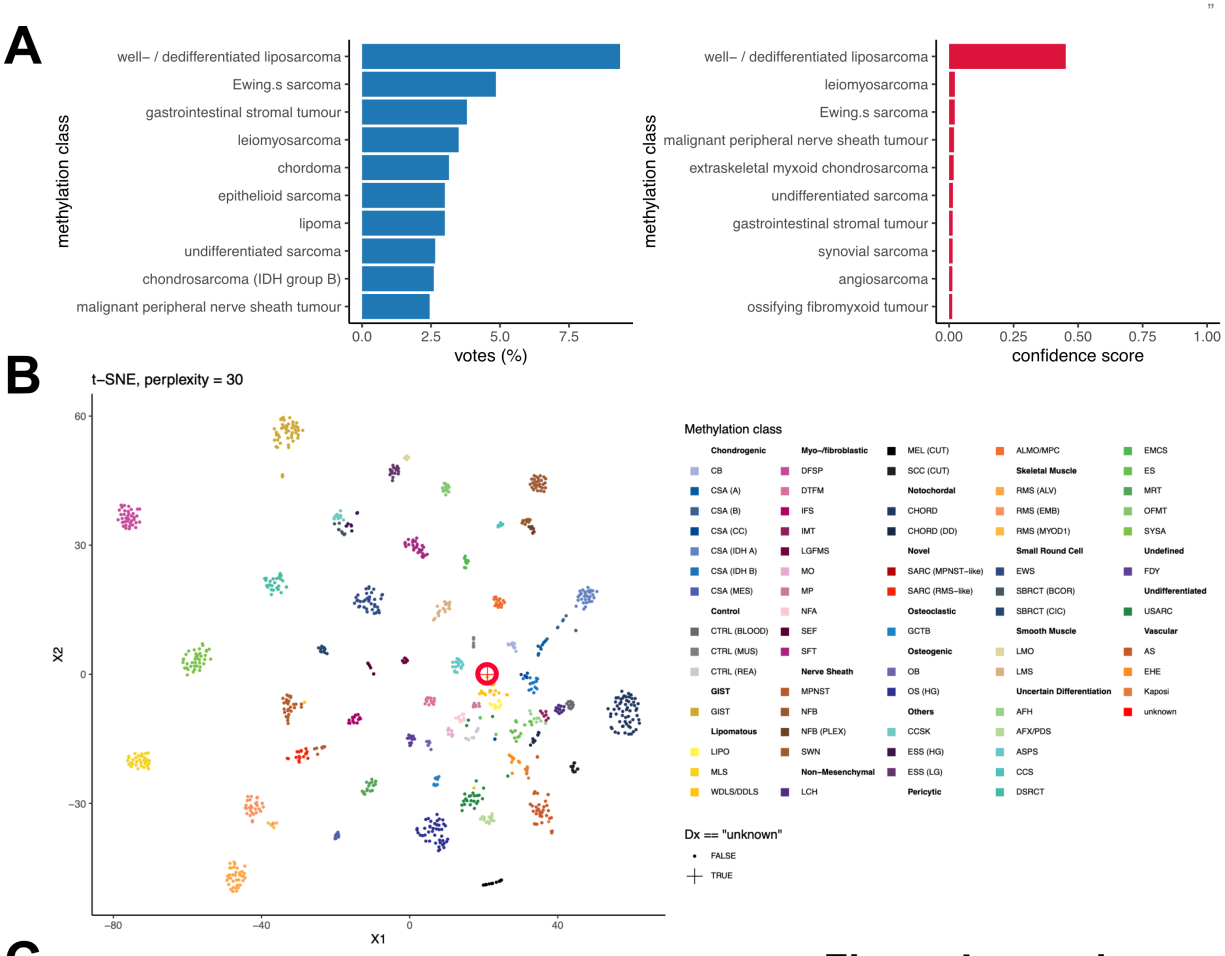

Figure Legend

(A) Methylation-based classification of a representative tumor based on 16427 CpG sites (overlap of sites covered in this sample and the training set). Raw (left) and calibrated (right) score distribution for the Top 10 entities is shown.

(B) The t-SNE plot shows the clustering of the methylation pattern of the specific sample (circled cross sign) among the other methylation patterns of the sarcomas in the reference set.

(C) 3D (a) scaffold and 3D culture models (b-d) from sarcoma tissues.

(D) 2D and 3D cultures (left) allow extensive sarcoma genomic profiling (right)

(E) Pharmacological characterization of sarcoma-derived cells

”

Signature of the Project Coordinator

Silvestro Conticello

PLEASE NOTE

- **Proposals that do not meet the national eligibility criteria and requirements will be declined without further review.**
- **Proposals must be sent in one single PDF document.**