DISCOVER22

"DNA Damage and Immune System COoperation in VEry low Radiation environment"

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(Università di Roma 3 – INFN-RM 3)

Responsabile Locale: G. Baiocco
(Università di Pavia – INFN-PV)

Durata del progetto: 3 anni (2023 – 2025)

La collaborazione

INFN Sezione di Roma
INFN Roma 1


INFN Roma 3

INFN Istituto Nazionale di Fisica Nucleare SEZIONE DI PAVIA

INFN LNGS **INFN-LNGS**

Istituto Superiore di Sanità

ROMA TRE UNIVERSITÀ DEGLI STUDI **Università degli Studi Roma 3**

UNIVERSITÀ DI PAVIA

INFN LNL Istituto Nazionale di Fisica Nucleare Laboratori Nazionali di Legnaro

TIFPA Trento Institute for Fundamental Physics and Applications

Università degli Studi dell'Aquila

CdS Pavia – July 7, 2022

The background

Low-dose radiation (LDR)
modulates a variety of processes related to immune response

In vitro and *in vivo* studies have confirmed that **the regulatory effect of Low Dose Radiation (LDR) on innate and adaptive immunity** depends on **many factors**: status of immune cells, microenvironment of the immune system, immune cell-cell interaction

Radiobiological data suggest that certain **cytokines** modulating immunological responses are differentially up- or downregulated with **doses around 0.5 Gy** and that the **anti-inflammatory vs pro-inflammatory responses at doses as low as 10 and 50 mGy** are not clear-cut but rather the result of the balance between the two types of effect.

Investigation of the **immune system response to low radiation doses/dose rates** is also identified as **a priority** by the Multidisciplinary European Low Dose Initiative **(MELODI) European Strategic Research Agenda** (SRA) (http://www.melodi-online.eu/sra. html).

Further studies are needed in this field, in particular **systematic studies** regarding the **modulation of the immune response**

Question:

Does low radiation background influence the response of innate immune system ?

GB1 GB [2]1

The availability of **cell culture's facilities** outside and inside the **Gran Sasso National Laboratory (LNGS)** of the Italian Institute of Nuclear Physics (**INFN**) represents a great opportunity to **investigate both the influence of low doses as the environmental ones and even lower in triggering immune response(s).**

The instrumentation present in both laboratories is currently being implemented as part of the INFN funding provided for in the "**Operating Convention for research and development in the field of Radiobiology**" stipulated between INFN and ISS with reference to the Framework Agreement between Institutes.

Diapositiva 3

GB1	fotoni 20 nGy/h; muoni 40 nGy/h; neutroni (<20 MeV)= 1.4 nGy/h Giorgio Baiocco; 07/07/2022
GB [2]1	fotoni 20 nGy/h; muoni 0 nGy/h; neutroni (<20 MeV)= 0 nGy/h Giorgio Baiocco; 07/07/2022

A detailed characterisation of the radiation field outside and inside the LNGS is crucial for understanding Low Dose Radiation effects on the immune system.

Microdosimetry becomes very important. This is a well-established theoretical and experimental methodology and it **is focused in the stochastic aspects of energy deposition** by single events at low doses.

Simulations are also important because they allow to **identify of the microscopic features of the track structures,** that are predominantly responsible for any observed radio-induced effect.

Biophysical radiation models development can help to **understand response mechanisms** and **identify even subtle changes** in the behaviour of biological systems, as can be expected following exposure to LDR.
Biophysical models represent the appropriate **link between microscopic physical quantities and the corresponding biological response** to radiation.

The proposal

Discover22 is organised in three work packages (WP), declined in the following tasks:

WP 1: Radioimmunobiology: *in vitro* studies

- **Task 1.1 - Immune system pathway activation**
- **Task 1.2 - Modulation of immune system's differentiation**

WP 2: Radioimmunobiology: *in vivo* studies

- **Task 2.1 – *In vivo* gene of the immune system**

WP 3: Physic studies

- **Task 3.1 - Radiation field characterization and MC simulations**
- **Task 3.2 - Biophysical modelling**

WP1 Radioimmunology - *in vitro* studies

Task 1.1 - Immune system pathway activation

INFN Roma 3

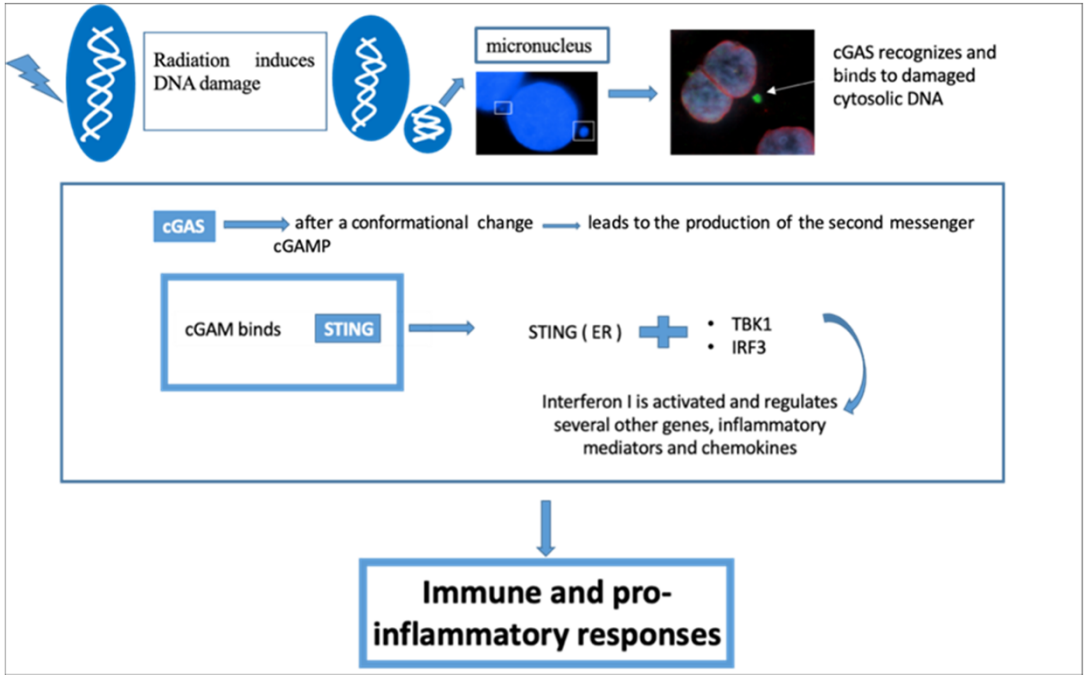
Istituto Nazionale di Fisica Nucleare
SEZIONE DI PAVIA

Trento Institute for Fundamental Physics and Applications

To investigate whether low radiation background influences the innate response of the immune system following radiation-induced DNA damage in human fibroblasts.

immortalized human fibroblasts will be grown in LRE and RRE for the 0.5, 1 and 2 months. Afterwards, the cells will be brought to Rome, irradiated at the dose of 2 Gy of X-ray (*challenging dose*) and the number of induced micronuclei (MN) analyzed in terms of cGAS-positive vs -negative.

cGAS-STING pathway activation is directly linking to DNA damage (MN) and to innate immune system activation: **quantifying the number of cGAS-positive MN will then allow quantification of the first step of the cGAS-STING pathway**.



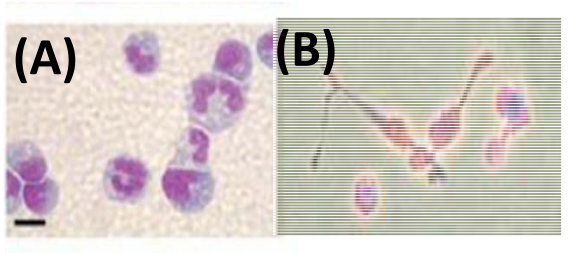
WP1 Radioimmunology - *in vitro* studies

Task 1.2 - Modulation of immune system's differentiation

To investigate whether low radiation background influences the ability of immature immune cells (HL60) to differentiate into macrophages and neutrophiles and to maintain their biological function.

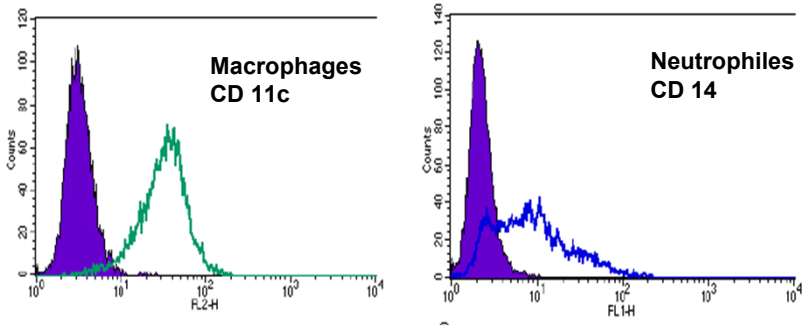
HL60
human promyeloblasts

HL60 will be grown in LRE and RRE for the 0.5, 1 and 2 months. Afterwards, the cells will induced to differentiate in: (A) **macrophages**; (B) **neutrophils.**



Flow cytometry will be used to study

- the **changes on expression of surface markers (i.e. CD) during differentiation.** Any CD ratio deviation in LRE and RRE will be indicative of immune system modulation.



- the **capability of the macrophages and the neutrophils to maintain their phagocytic function**: the ROS production and the uptake of FITC-labelled microsphere and/or of fluorescent viable bacteria.
- the **formation of neutrophil extracellular traps (NETs) in a process called NETosis**

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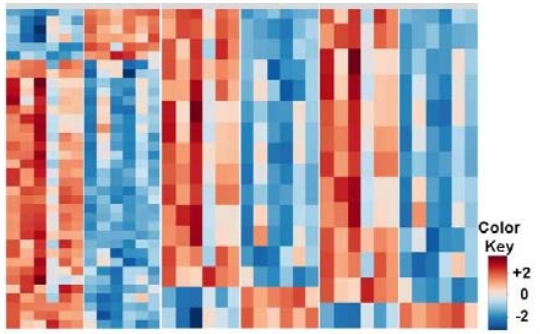
WP2 Radioimmunology - *in vivo* studies

Task 2.1 - *In vivo* gene of the immune system

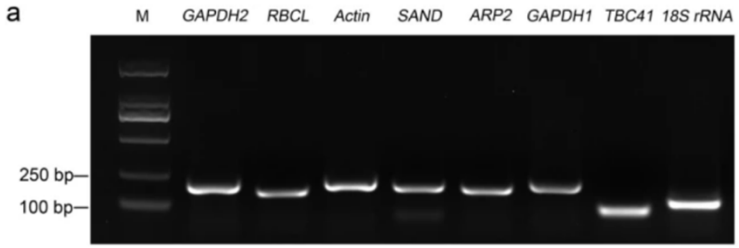
To get information on the expression of genes related to the immune response in *Drosophila melanogaster*, taking advantage from the transcriptomic analysis performed in RENOIR experiment.

Drosophila melanogaster

Analysis of a subset of the transcriptional profiling experiments from RENOIR to find a possible gene expression modulation specifically in the **expression of immune system genes related to low radiation enviroment**.



gene expression profiling



Validation by PCR of the modulated genes considered most significant and indicative of the modulation related to low radiation environment.

WP3 Physic studies

Task 3.1 - Radiation field characterization and MC simulations

Istituto Nazionale di Fisica Nucleare
Laboratori Nazionali di Legnaro

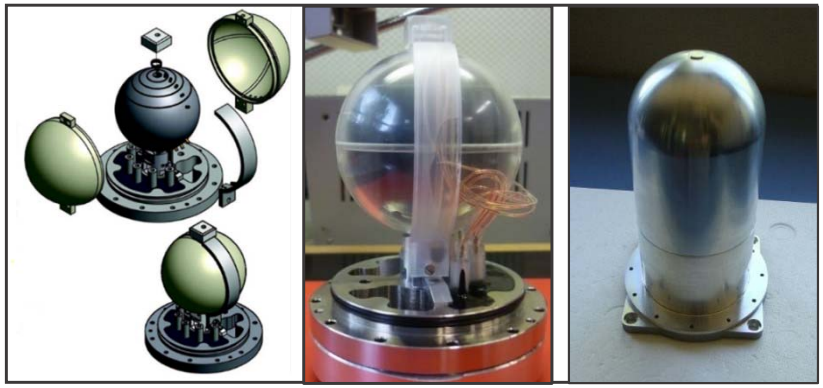
Trento Institute for Fundamental Physics and Applications

To supplement the absorbed dose measurements with a complete microdosimetric characterization of the radiation field in the outside reference environment and in the underground laboratory.

A tissue equivalent proportional counter (TEPC) will be used where 1 μm of tissue is simulated with a larger volume filled with low density gas. With a site diameter of 10 cm, the expected number of events increases to 5000/h, for a total of 1.2×10^5 events detected in one day. This **new TEPC** will be constructed at LNL and installed in the underground laboratory of LNGS and it will allow **to monitor** both **the dose** and **the microdosimetric spectrum** on a daily base with significant statistics. The detector and data acquisition will be controllable both locally and remotely.

First microdosimetric measurements will be performed with the **EUTEPC**, a spherical TEPC of 5 cm diameter with segmented cathode, which is **already available at LNL**.

Two more detectors might be used to further monitor the natural radiation: a **Radon detector** (MR1-MIAM) and a **muon detector**, which are **already available at LNL**.



The EUTEPC of LNL. A schematic view (left); internal sensor (centre); external case (right).

WP3 Physic studies

Task 3.2 - Biophysical modelling

Istituto Nazionale di Fisica Nucleare SEZIONE DI PAVIA

INFN Roma 3

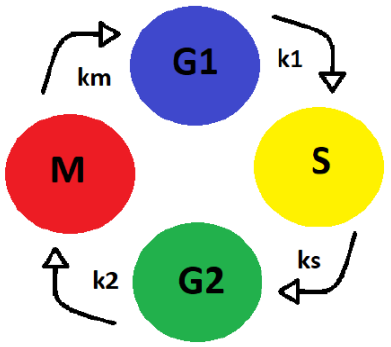
Trento Institute for Fundamental Physics and Applications

To develop a biophysical model of a proliferating human cell population subject to radiation-induced DNA damage (-> WP1, Task 1.1)

The **compartmental model** will use as input flow-cytometry data measured at INFN-Pavia (samples from Roma3) and allow extraction of parameters to identify differences:

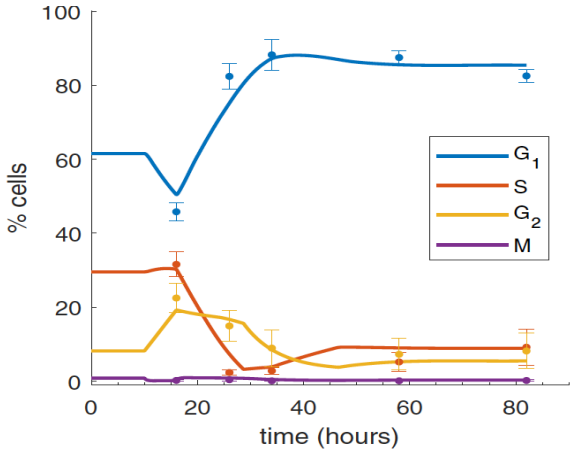
- in cell-cycle progression
- in innate-immune system response through micronuclei induction and and cGAS-STING pathway activation after a dose of 2Gy of X rays

for cells grown in LRE and RRE conditions



scheme of the cell-cycle compartmental model with transition rates as parameters

example of model reproduction (lines) of cell-cycle perturbation (experimental changes in % of phases) after irradiation



http://radbiophys.unipv.eu/

Lonati et al, Sci Rep 2021

WP3 Physic studies

Task 3.2 - Biophysical modelling

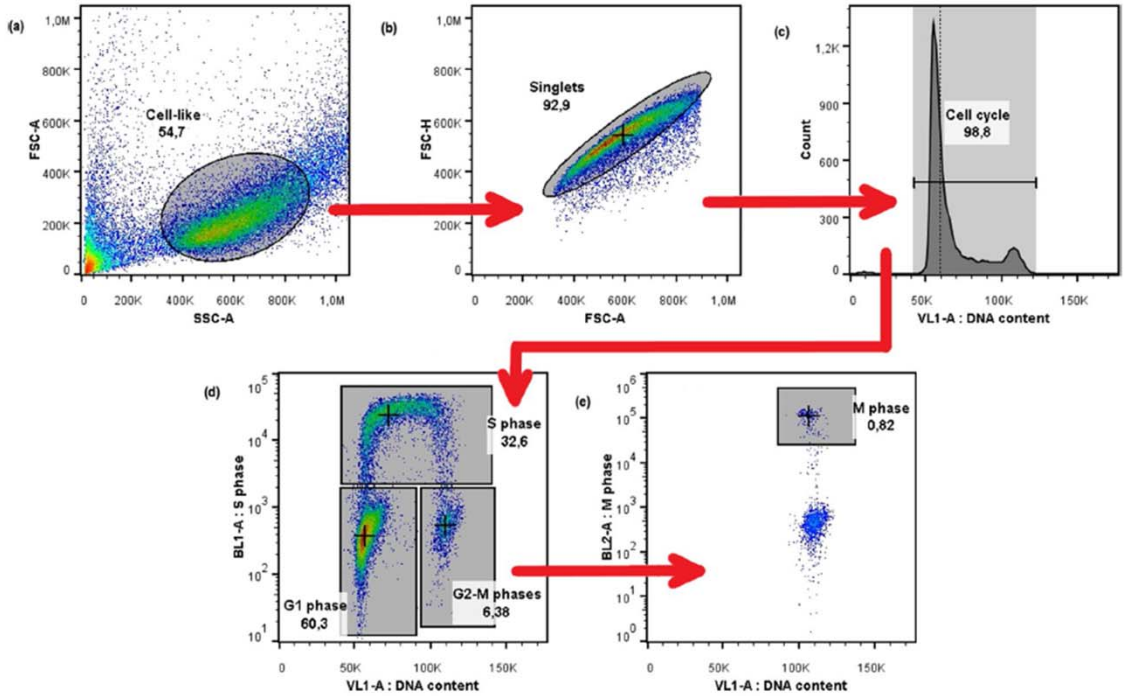
Istituto Nazionale di Fisica Nucleare
SEZIONE DI PAVIA

INFN Roma 3

Trento Institute for Fundamental Physics and Applications

To develop a biophysical model of a proliferating human cell population subject to radiation-induced DNA damage (-> WP1, Task 1.1)

Cell cycle data measured at the flow-cytometry facility of the RadBioPhys LAB



Hierarchical gating procedure for cell-cycle data acquisition by means of flow-cytometry

http://radbiophys.unipv.eu/

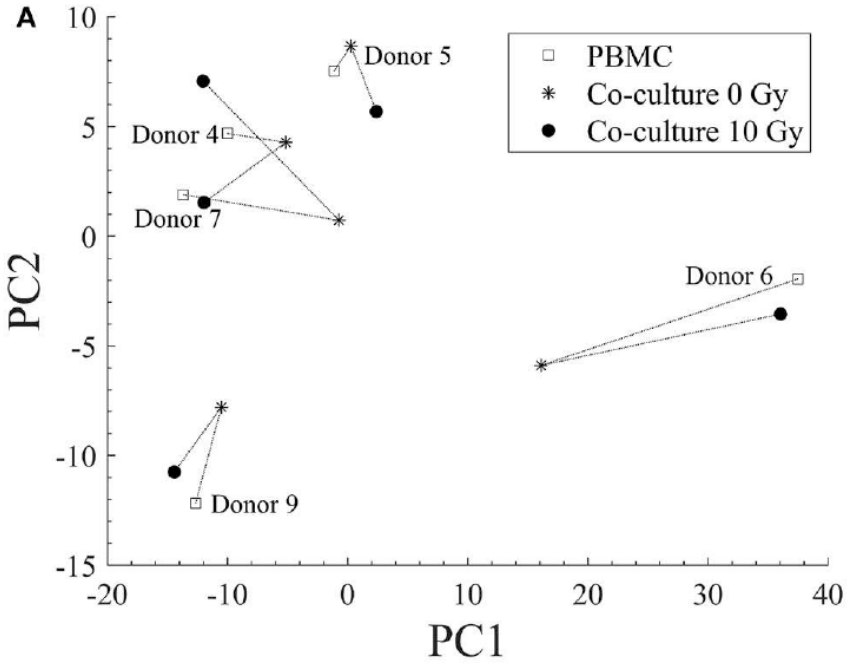
Lonati et al, Sci Rep 2021

WP3 Physic studies

Task 3.2 - Biophysical modelling

To apply advanced data analiysis techniques to identify changes in the ability of immature immune cells to differentate in a low-radiation background
(-> WP1, Task 1.2)

Data integration from all measured endpoints (from Roma1) and analysis based on machine learning algorithms and data dimensionality reduction (e.g. *Principal Component Analysis*, or *t-SNE, t-distributed Stochastic Neighbor Embedding*) will allow to identify subtle changes as generally expected for Low-Dose-Rate - related phenomena.



Principal component analysis to identify changes in the response of individual healthy donors of PBMCs (donor clustering), when immune cells are co-cultured with irradiated cancer cells

Borsci et al, Front. Immunology 2020

http://radbiophys.unipv.eu/

Milestones - 1° year

M1.1 and M1.2: report on the data obtained to choose the optimal adaptation time for *in vitro* studies (***12*** *months*))

M1.3: report on data elaboration and analysis of *in vivo* effect on immune system response following Low Radiation Exposure (***12*** *months*)

M1.4: report on microdosimetric measurements with the **EUTEPC already available at LNL** (***12*** *months*)

M1.5: executive project of the new TEPC (10 cm diameter) **for low count rate** (***12*** *months*)

Milestones - 2° year

M2.1: cell-cycle model validation following underground and outside LNGS radiation exposure (***16*** *months*)

M2.2 – M2.3: preliminary results of *in vitro* effect on immune system response following Low Radiation Exposure (***23*** *months*)

M2.4: report on PCR validation of *in vivo* effect on immune system response following Low Radiation Exposure (***24*** *months*)

M2.5: report on measurements with the new TEPC and the remote control and DAQ system (***24*** *months*)

Milestones - 3° year

M3.1: MN induction and innate immune response model for underground and outside LNGS radiation exposure(***33*** *months*)

M3.2-M3.3: final report on results of *in vitro* effect on immune system response following Low Radiation Exposure (***34*** *months*)

M3.4: report on evaluation of changes in differentiation of immune cells in underground and outside LNGS radiation exposure (***34*** *months*)

M3.5: final report on microdosimetry under and above ground with the new TEPC (***36****months*)

M3.6: final report on data integration from radioimmunobiology, microdosimetry and biophysical modelling results (***36*** *months*)

Roma 1		FTE 1.2
Valentina Dini	Ric.	70
Massimo Sanchez	I Ric.	20
Valentina Tirelli	Ric.	20
Giuseppe Esposito	Ric.	10
Maria Antonella Tabocchini	*Ass. senior*	-
Pasquale Anello	*Tecnico TD*	-

Pavia		FTE 2.0
Giorgio Baiocco	Ric.TDB	100
Isabella Guardamagna	AdR	50
Leonardo Lonati	dottorando	50

LNL	FTE 1.8
Valeria Conte	50
Anna Selva	70
Stefania Canella	50
Massimo Rossignoli	30
Emanuele Scifoni (TIFPA)	10

Roma 3		FTE 2.3
Antonella Sgura	Prof. ass.	70
Francesco Berardinelli	Ric.	30
Ion Udroiu	AdR Univ. Roma3	50
Antonio Antoccia	Prof. ass.	10
Federica Barbato	dottoranda	60
Jessica Marinaccio	AdR	10

LNGS		FTE 0.8
Marco Balata	I° Tecnologo	10
Francesco Ferella	AdR	10
Angelo Galante	Prof ass	10
Mauro Maccarone	Prof ass	30
Daniela Grifoni	Prof ass	20
Patrizia Morciano		*40**

* In previsione appena parte l'assegno di ricerca

PAVIA – richiesta finanziaria sui tre anni di progetto

2023

Consumo: 2 kEuro

- Specific reagents, antibodies for biological tests
- Focusing fluid, wash, shutdown, tracking beads, for flow-cytometry

Missioni: 0.5 kEuro

- From Pavia to LNGS (2/anno per 1 persone)

Other costs: 0.5 kEuro

- Shipping fees and dry ice (shipping samples/reagents Roma3 – Pavia)

2024

Consumo: 1.3 kEuro

- Specific reagents, antibodies for biological tests
- Focusing fluid, wash, shutdown, tracking beads, for flow-cytometry

Missioni: 0.6 kEuro

- From Pavia to LNGS (2/anno per 1 persone)

Other costs: 0.4 kEuro

- Shipping fees and dry ice (shipping samples/reagents Roma3 – Pavia)

2025

Consumo: 1.3 kEuro

- Specific reagents, antibodies for biological tests
- Focusing fluid, wash, shutdown, tracking beads, for flow-cytometry

Missioni: 0.6 kEuro

- From Pavia to LNGS (2/anno per 1 persone)

Other costs: 0.4 kEuro

- Shipping fees and dry ice (shipping samples/reagents Roma3 – Pavia)