



Sigla: MIRO (Minibeam Radiotherapy)

Durata proposta: 3 years

Area di ricerca: Interdisciplinare

Responsabili nazionali: Fabio Di Martino (PI), Francesco Romano (CT)

Unità partecipanti: CT, LNS, PI, RM1, TIFPA, TO

WP3 CATANIA (LNS)					
Cost category	Item	I YEAR	II YEAR	III YEAR	Totale
Consumables	Reagents for inflammatory cytokines study and Luminex assay			7.500,00 €	7.500,00 €
Travels	travel for in-vitro experimental activities at CPFR in PISA with the minibeam	2.500,00 €	2.500,00 €	2.500,00 €	7.500,00 €



T3.1 In Vitro characterization of the overall response to minibeam RT

Task 3.1 focuses on spatially integrated observation of different radiobiological endpoints to elucidate the overall minibeam RT effects on in vitro systems as a function of the average irradiation equivalent dose. The different irradiation conditions and the possible differential effects on different cell lines will be tested.



T3.2 In vitro characterization of the local response to minibeam RT

In Task 3.2 a multiscale approach will characterize the pattern-dependent effects both at the cellular and subcellular level. Spatially resolved measurements and advanced fluorescence imaging will locally correlate the cellular position with respect to the irradiation pattern and a quantitative evaluation of molecular determinants associated to cellular damage, repair and functions will be performed.

Task	Deliverable	Description	When	Division
Task 3.1	D 3.1.3	Investigation of minibeam effect in breast tumorigenic and nontumorigenic cells	6-12	LNS, TIFPA
Task 3.2	D 3.2.3	DNA damage high-content analysis induced by minibeam irradiation in breast tumorigenic and non-tumorigenic cells	12-36	LNS, TIFPA



MIRO Working Package 3 (WP3) - Radiobiology



D 3.1.3 Investigation of minibeam effect in breast tumorigenic and non tumorigenic cells (LNS-TIFPA) (6-12 months)

D 3.2.3 DNA damage high-content analysis induced by minibeam irradiation in breast tumorigenic and non-tumorigenic cells (12-36 months)

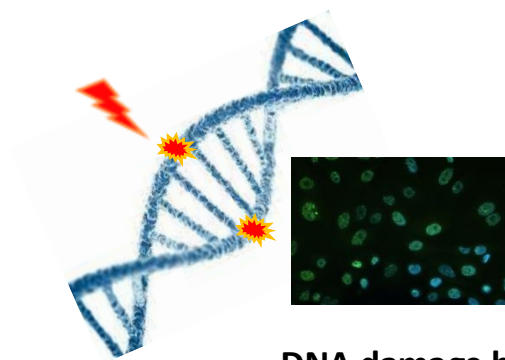
Cell models and Experimental Conditions

Cell Lines
MCF10A
MDA-MB-231

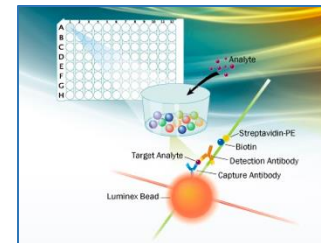
Irradiation modalities

- CONV
- FLASH
- Minibeam CONV
- Minibeam FLASH

Biological endpoints



DNA damage by
immunofluorescence
assay for γ H2AX and 53BP1



Immunological profile
(cytokines expression
by Luminex assay)

WP3
Radiobiology & Modeling
LNS, TIFPA, PI, CT
WP Leaders: Emanuele Scifoni-
TIFPA, Francesca Cella-PI
FTE: 5

T3.1: *In Vitro*
characterization of the
overall response to
minibeam RT

T3.2: *In vitro*
characterization of the
local response to
minibeam RT

T3.3: *In vivo* studies of
radiobiological
Minibeam effects

T3.4: Analysis and
Modelling

24-Multiwell



Pisa, 12th November 2024

MCF10A Non tumorigenic Breast cell line

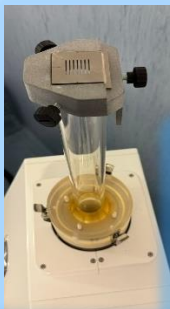
MDA-MB-231 Breast cancer cell line



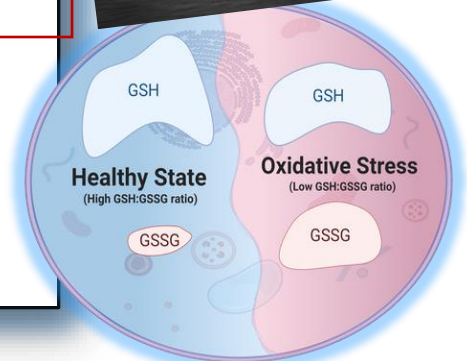
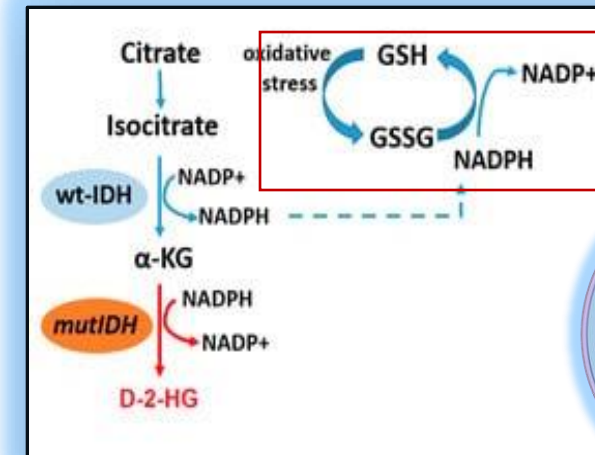
Dose-rate

Conv	5 Gy/min
Flash	291 Gy/sec
Minibeam-Conv	5,3 Gy/min
Minibeam-Flash	296 Gy/sec

GSH:GSSG ratio quantification as a marker for cellular oxidative stress

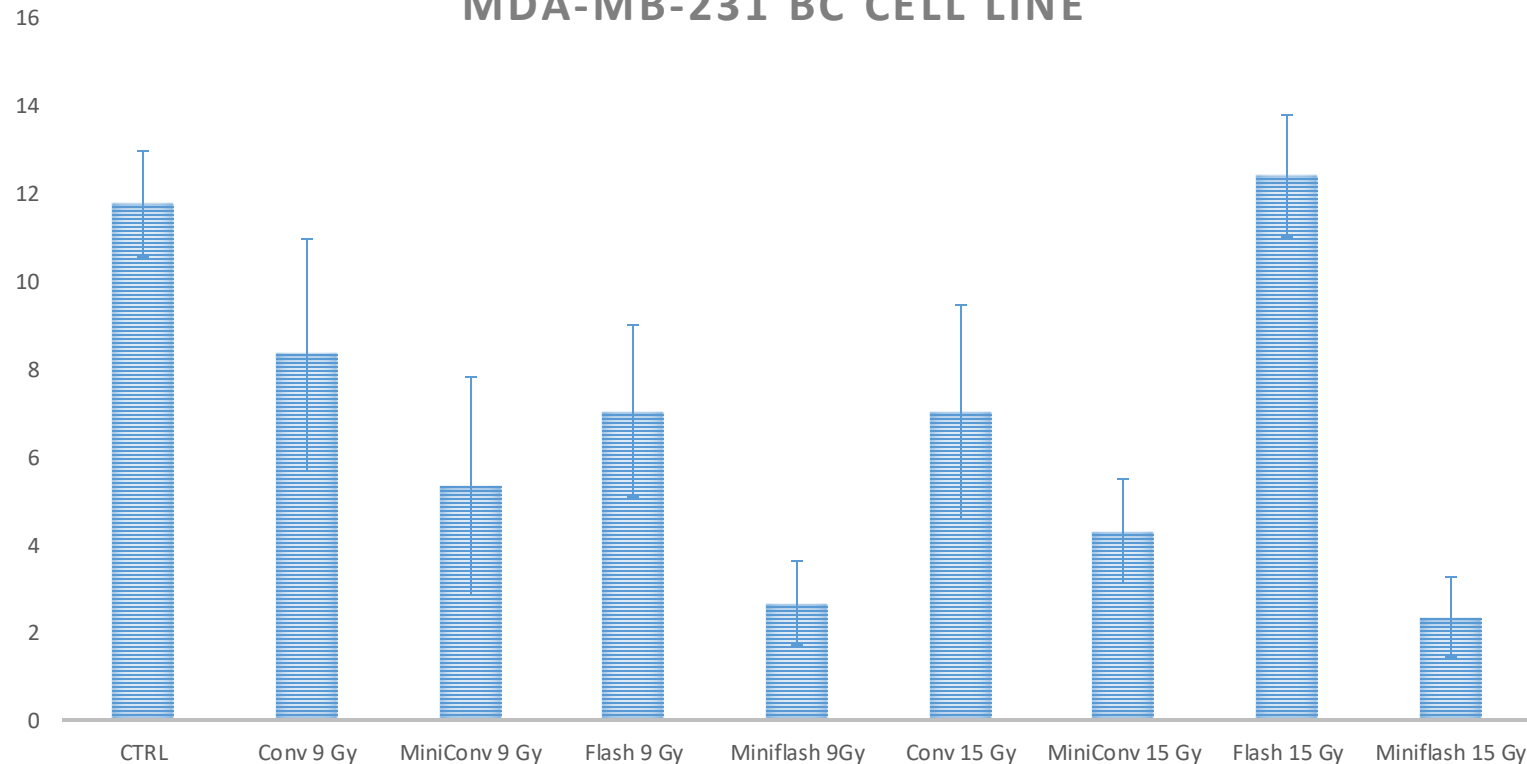


Pellets were collected 24h post RT waiting to be transferred to the Metabolomic laboratory of IBSBC-CNR of Segrate for the GSH/GSSG quantification by LC-QQQ (Liquid Chromatography-Triple Quadrupole Mass Spectrometry).



D 3.1.3 Investigation of minibeam effect in breast tumorigenic and non-tumorigenic cells (LNS-TIFPA) (6-12 months) *November 2024*

**GSH/GSSG RATIO
MDA-MB-231 BC CELL LINE**



The redox state of glutathione, and therefore the alternation of the oxidized form (**GSSG**) and the reduced form (**GSH**), is a fundamental indicator for defining the redox state of the cell itself.

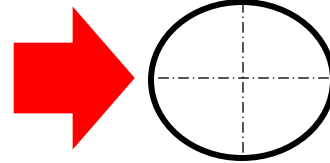
As shown in the graph, mini-beam irradiation enhances the intracellular oxidative stress, as supported by the decrease in **GSH/GSSG** ratio values in all the configurations tested and, notably, it seems not to be strictly linked to the dose (in terms of Gy) as well as to the irradiation beam schedules (conventional or flash) performed.



Ongoing: irradiation schedules programmed in Autumn 2025

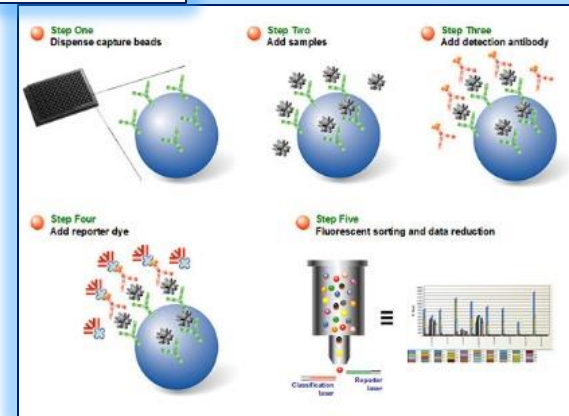
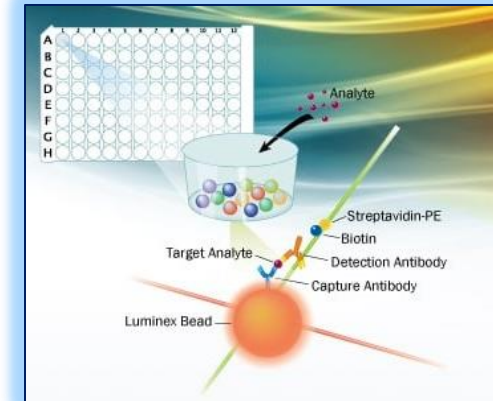


Soon



4,4 cm Diameter
higher amount of sample

Luminex Multiplex Assay



FTE

- Giorgio Russo – 0,1
- Giusi Irma Forte – 0,2
- Luigi Minafra – 0,6
- Valentina Bravatà – 1
- Gaia Pucci – 0,5