Italian National Agency for New Technologies,
Energy and Sustainable Economic Development

Presentation of the projects proposed to GA for selection within the 1st Open Call

PIANOFORTE Event Budapest - Mercure Budapest Castle Hill, *5 and 6 December 2023*

Simonetta Pazzaglia

DISCOVER PIANOFORTE 2023-041

Dissecting radIation effectS into the Cerebellum micrOenVironmEnt driving tumour pRomotion

SCIENTIFIC AREA: **TOPIC 1** - Developing a knowledge base for a better understanding of disease pathogenesis of ionising radiation induced cancer to improve human health risk assessment.

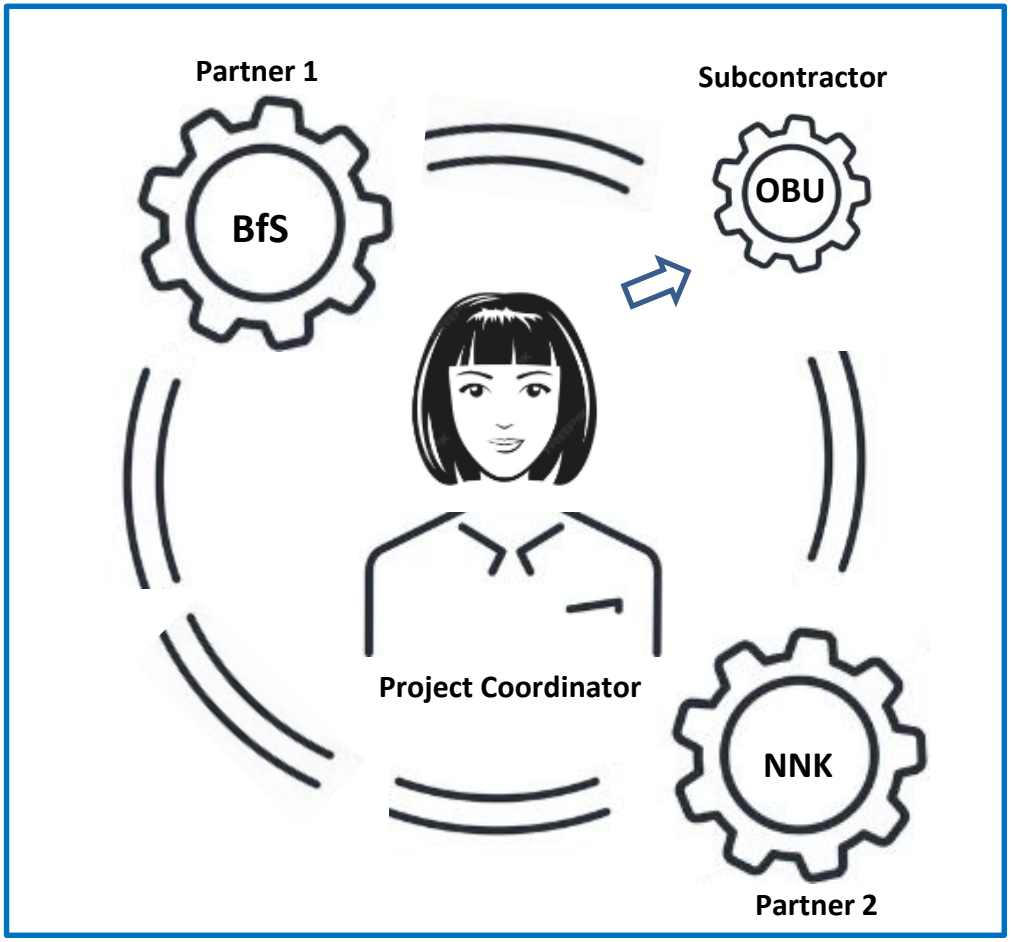
Project duration: 36 months

Total project costs/budget (€) 1,344,970

Total project requested funding (€) 847,350

Project participants

Simone MOERTL



Munira KADHIM

Katalin LUMNICZKY

Project Coordinator: ENEA - Italian National Agency for New Technologies, Energy and Sustainable Economic Development

Partner 1: BfS - Federal Office for Radiation Protection, Oberschleissheim, Germany

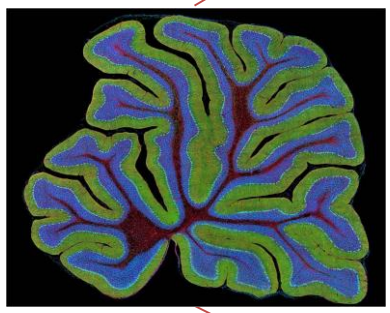
Partner 2: NNK - National Public Health Centre, Budapest, Hungary

Subcontractor: OBU - Oxford Brookes University, Oxford, UK

DISCOVER GOAL

There is increasing recognition that radiation can induce changes within the microenvironment and cause epigenetic modifications

Ionizing Radiation



Cerebellum

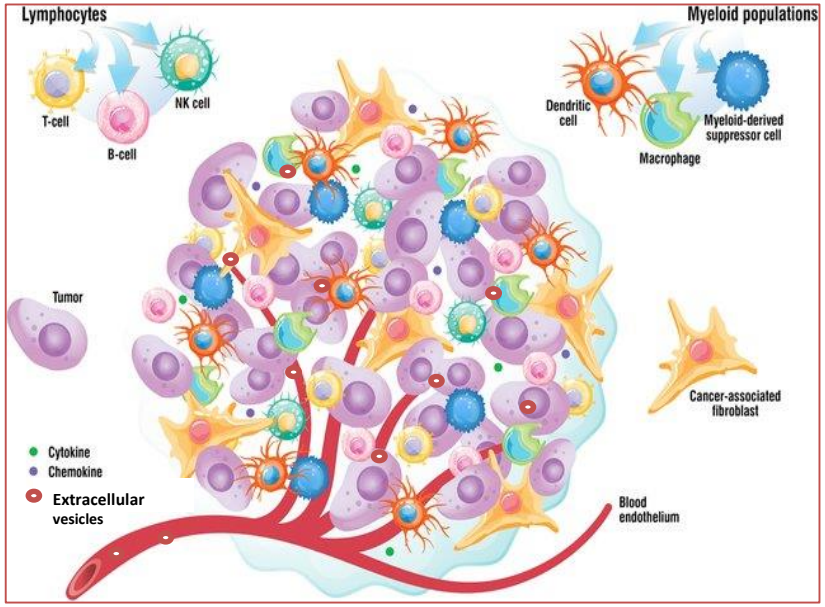
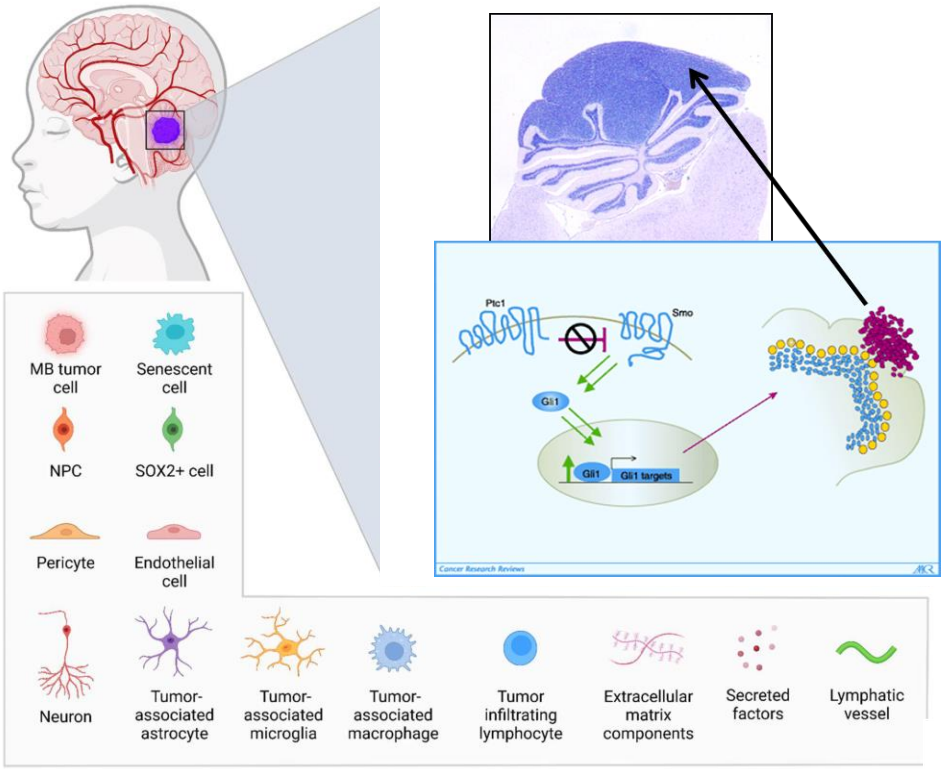


Figure modified from Zhang and Veeramachaneni, Biomarker Research (2022) 10:5

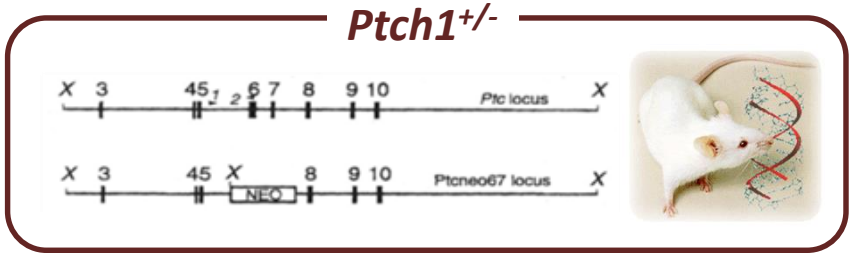
Do radiation-induced modifications in the microenvironment contribute to cancer development?

- **DISCOVER** is a multi-faceted research proposal focusing on the effects of ionizing radiation on the **cell microenvironment communication** and its role in tumour development
- The integration of **highly innovative** approaches and comparisons with **human data** aims to significantly advance our mechanistic understanding of radiation-induced cancer and its potential implications for risk assessment and personalized medicine

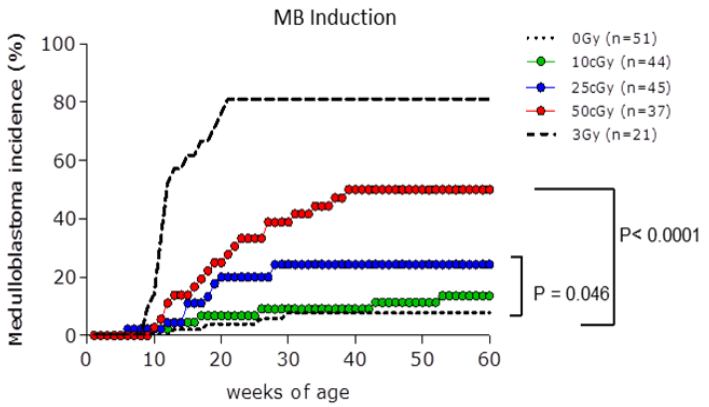
Experimental model: medulloblastoma in *Ptch1* heterozygous mice



Medulloblastoma (**MB**) is the most common malignant pediatric brain tumor. It arises from mutated granule cell precursors (**GCPs**), a transient population of progenitors that, in normal conditions gives rise to granule neurons of cerebellum



- General growth
- Radiosentivity
- Cataract
- Tumor predisposition

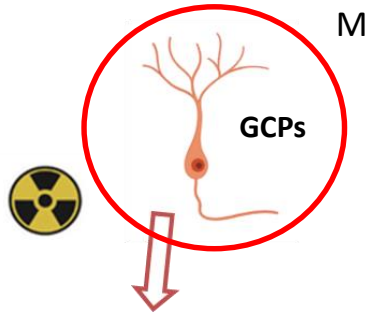


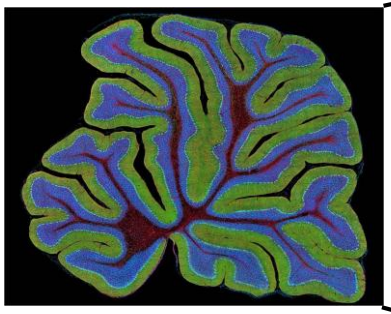
Pazzaglia et al. International journal of radiation oncology, biology, physics, 73; 1203–1210, (2009)

Dissecting rad**I**ation effect**S** into the **C**erebellum micr**O**en**V**ironm**E**nt driving tumour p**R**omotion

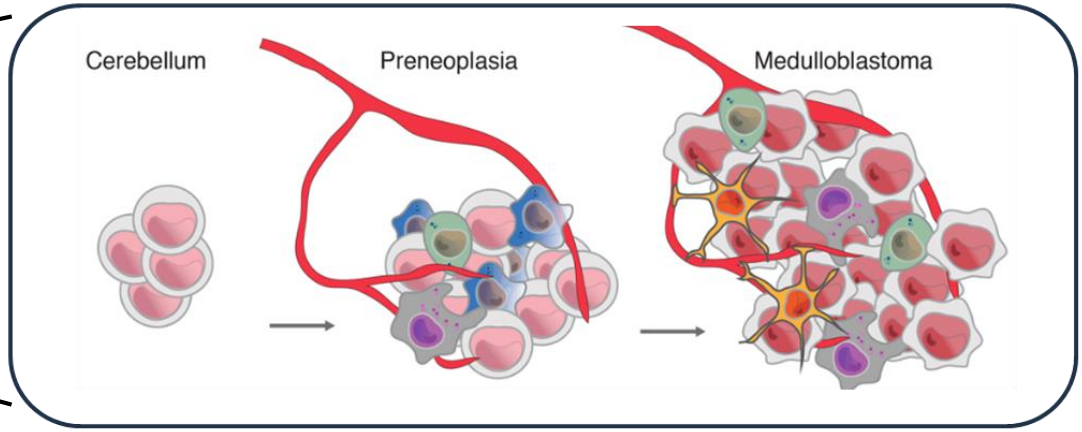
0.1 Gy or 2 Gy

MB cell of origine

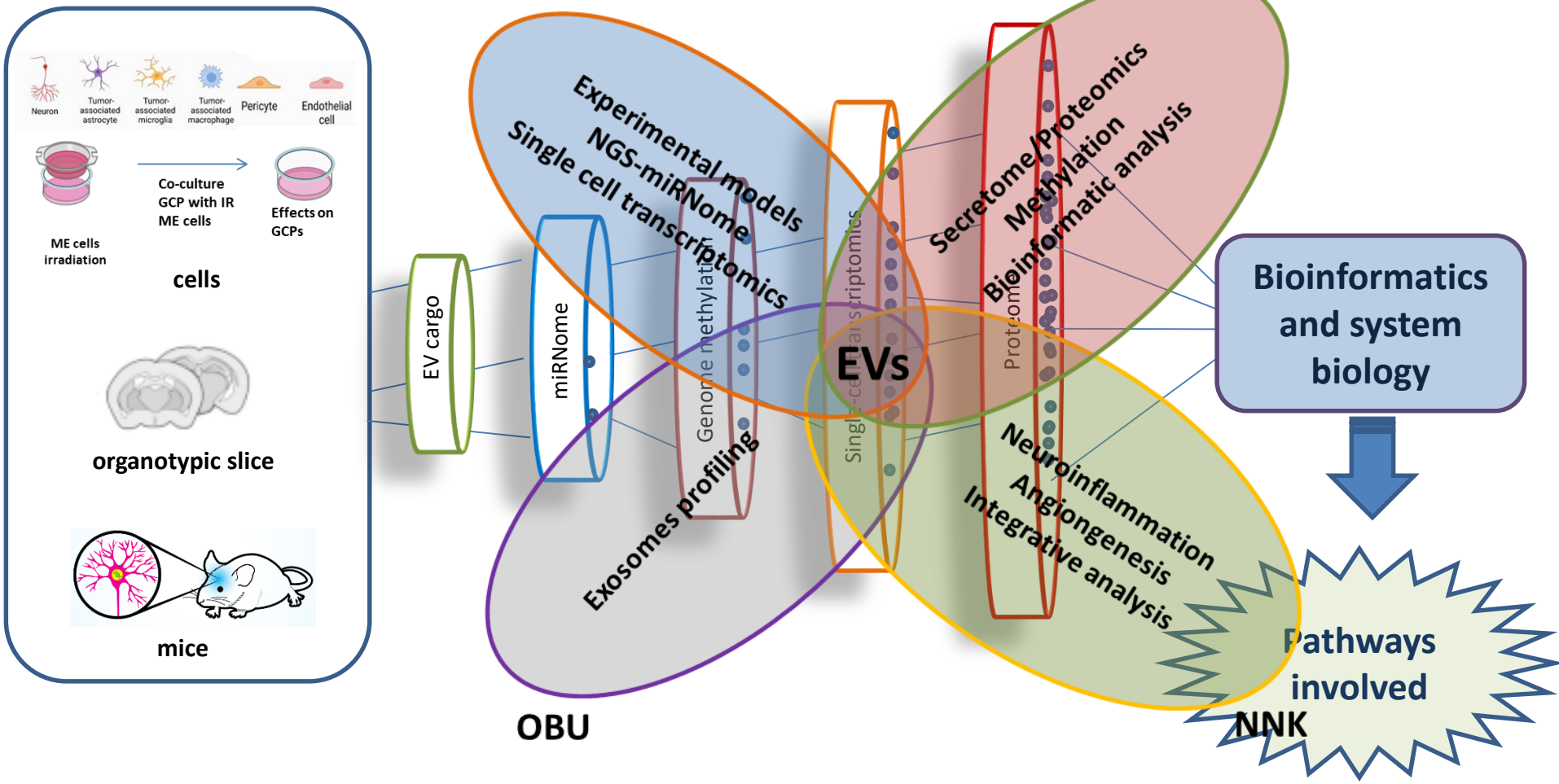




Cerebellum

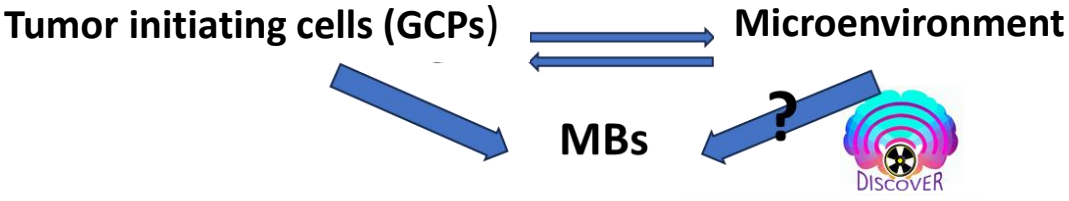


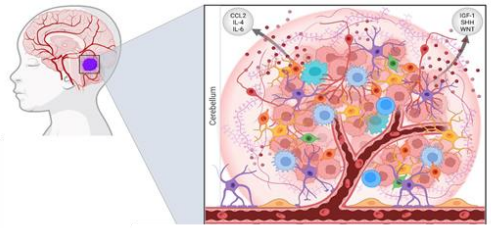
Technological approaches



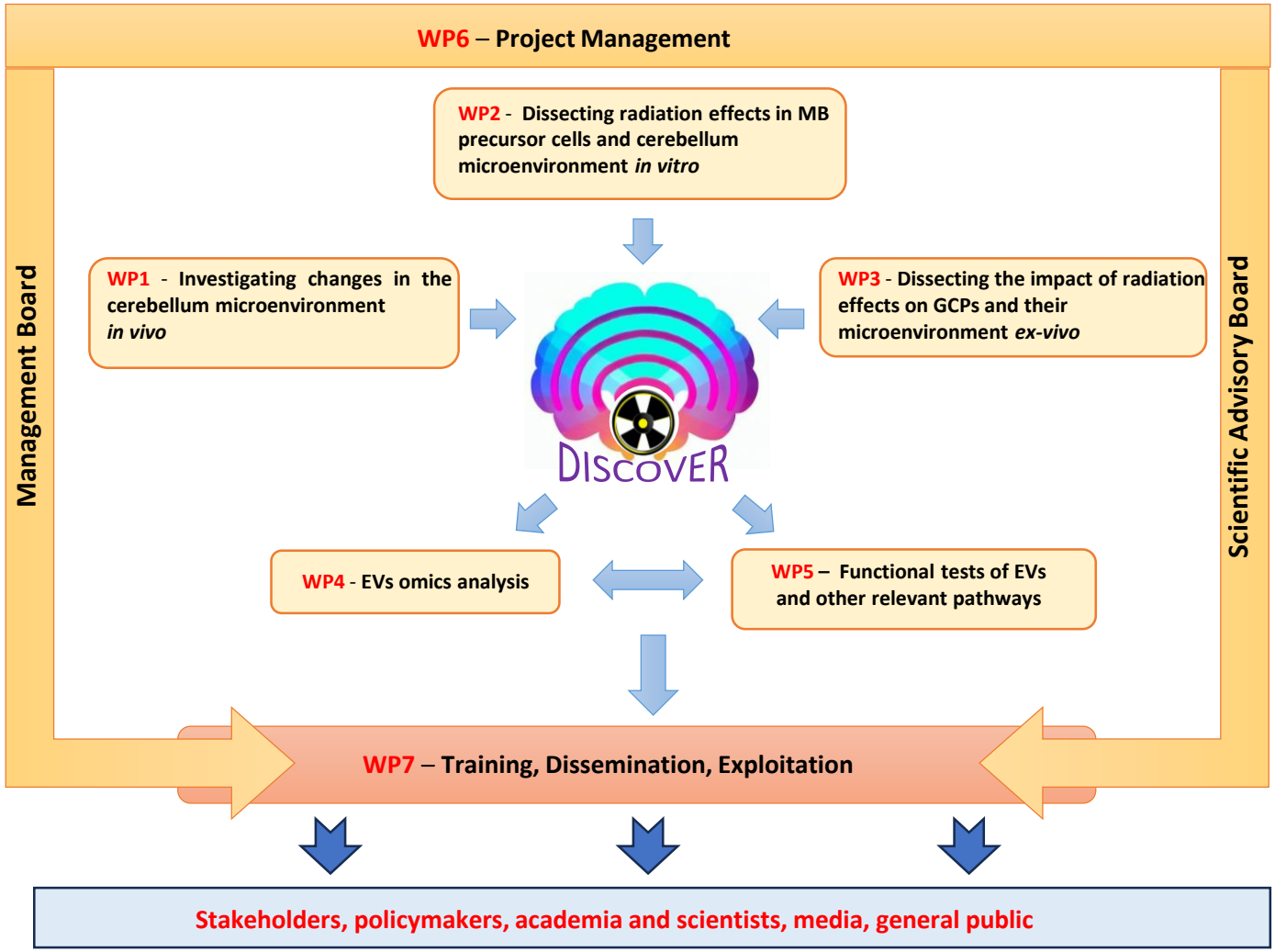
The expertise of the partners complements each other and aligns with the proposed project

DISCOVER


Tumor initiating cells (GCPs)
Microenvironment
MBs
?
DISCOVER


Cerebellum

DISCOVER PERT chart



Summary of the specific DISCOVER aims:

- To dissect microenvironment's contribution driving cerebellum tumourigenesis through use of model systems of different complexity from *in vitro* (**WP2**), ex vivo (**WP3**) and *in vivo* models (**WP1** and **WP5**) to understand the intricate interactions in radiation-induced tumourigenesis.

- To undertake a comparative analysis of transcriptomic profiles at the individual cell level to examine radiation effects on gene expression in the cerebellum *in vivo* (**WP1**).

- To investigate secretome, EVs and epigenetic changes as possible mediators of microenvironment signalling by integrating the information coming from different model systems (**WP1, WP3, WP4**).

- To perform omics on EVs cargo and integrative analysis to identify microenvironment signalling pathways (**WP4**).

- To use integrative bioinformatic analysis to identify the pathways affected in exposed cells/brains and potential biological mechanisms of exposures (**WP1 and WP4**).

- To perform functional tests *in vivo* to verify the involvement of EVs and identified deregulated pathways (**WP5**).

Understanding the mechanisms of the complex processes involved in radiation-induced carcinogenesis is a key element of risk assessment in radiation protection

Italian National Agency for New Technologies, Energy and Sustainable Economic Development

Bundesamt für Strahlenschutz

NATIONAL PUBLIC HEALTH CENTER

OXFORD BROOKES UNIVERSITY

WP1: to investigate the mechanisms underlying the transmission of oncogenic signals from the irradiated brain microenvironment (ME) *in vivo*

WP1

Investigating changes in the cerebellum microenvironment following cranial irradiation

IR
X-rays: 0.1, 2 Gy

P2 *Ptch*$^{+/-}$ mice
(ENEA)

Task 1.1

MB Development (ENEA)

1 or 6 weeks post-irradiation

- SCGE (ENEA)
- Proteomics (BfS)
- Genome methylation (BfS)

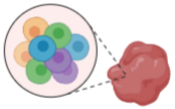
Task 1.2

- Neuroinflammaon
- Angiogenesis
- Gene expression (ENEA)
- ELISA (NKK)

WP1 - [M2-M20] - WP Leader ENEA; Participation by BfS and NNK

WP2: to dissect the interactions between GCPs, the main cell type of origin for MB, and the surrounding ME in radiation-induced carcinogenesis *in vitro*

WP2
Dissecting radiation effects in MB target cells and ME in cerebellum *in vitro*



GCPs/ME cells isolation

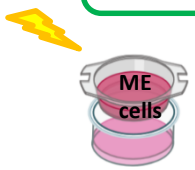
Task 2.1

IR
X-rays: 0.1, 2 Gy

3D Systems

Characterization of radiation effects on GCPs (ENEA, NNK)
- Carcinogenesis-related endpoints
- Neurosphere assay
- Loss of wild-type Ptch1 allele
- GCPs migratory capacity

Task 2.2



GCPs

ME cells irradiation

Co-culture GCP with IR ME cells

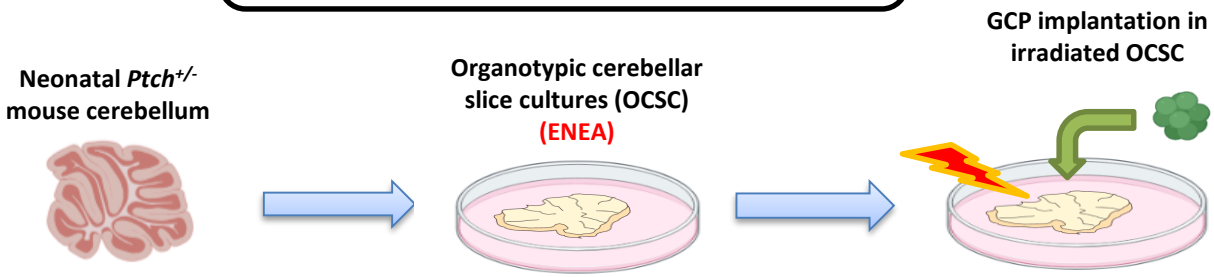
Effects on GCPs

Characterization of ME cells-mediated radiation effects on GCPs (ENEA, NKK)
- Carcinogenesis-related endpoints
- Migration
- Senescence

WP2 - [M2-M30] - WP Leader NNK; Participation by ENEA and BfS

WP3: to assess the impact of irradiated ME on neoplastic progression of GCPs through *ex-vivo* analysis

WP3
Dissecting the impact of radiation effects on GCPs and their ME ex-vivo



Task 3.1

- Proliferation, apoptosis, stemness/differentiation status and senescence in GCP spheroids (ENEA)
- LOH wild-type Ptch1+/− allele
- Methylation state of DNA

Task 3.2

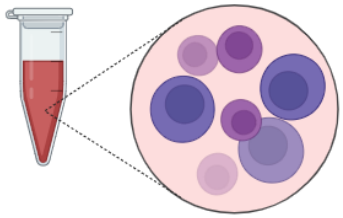
Collection of cell-free secretome and extracellular vesicles from OCSCs (ENEA, NKK)

WP3 - [M3-M30] –WP Leader ENEA; Participation by BfS

WP4: to characterize the secretome/EV mediated cellular effects induced by radiation

WP4

Role of secreted factors and EVs in radiation-induced signalling



Task 4.1

Secretome from WP2 and WP3 (BfS)

Multiplex immunoassay system

↓

Multiple secreted factors analysis

Task 4.2

EV isolation from WP1, WP2 and WP3 and their characterization (OBU)

Task 4.3

Characterization of EV cargo (ENEA, OBU, NNK, BfS)

- NGS – based miRNome analysis
- Proteomics
- Bioinformatic analysis

Task 4.4

Characterization of Cell-free EV-derived DNA from ME cells (NNK)

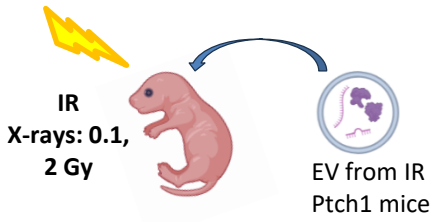
WP4 - [**M12-M32**] – **WP Leader BfS**; Participation by NNK, ENEA and OBU

WP5: to fully characterize potential EV transmitted radiation-induced oncogenic effects by adopting an *in vivo* experimental strategy

WP5

Functional tests of EV and other relevant pathways

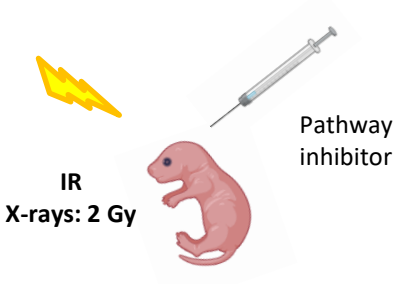
Task 5.1



Somministration of EV from WP1 to newborn *Ptch1*$^{+/-}$ mice cerebellum (ENEA, OBU)

- Apoptosis
- Tumorigenesis

Task 5.2



Validating the role of radiation-modulated pathways in MB promotion through their inhibition (ENEA, BfS)

- Tumorigenesis
- Preneoplastic lesions
- Proliferation

Task 5.3

Integrative analysis and system biology (NNK, ENEA)

WP5 - [**M16-M35**] –**WP Leader NNK**; Participation by BfS, ENEA, OBU