

Editorial

Here is SEPARATE, a project dedicated to partial body exposure. With this project we close the series of special issues dedicated to the presentation of the results obtained by the 9 projects selected by CONCERT. Now AIR2, edited in the framework of the CONCERT project, is going to stop. I found the coordination of its publication throughout its 40 issues plus 15 special issues very exciting. I would like to take this opportunity to thank all those who have worked on its publication, during these 5 years, the numerous authors who presented their infrastructures, their projects, gave their point of view in "the floor to" but also all those who worked in the shadow at the conception of each issue, Jean-Michel Dolo, William Hempel, Elisabeth May, Maria Panagiotopoulou, Laure Piqueret-Stephan. But that does not mean that we will never again publish this newsletter, which has been very much appreciated by its readers. We are taking a break for a while in order to reinvent it better and I hope that you will be able to find this appointment with research infrastructures in a not so distant future. I let you enjoy this last issue and wish you a great summer.

Dr Laure Sabatier, CEA

The floor to...

Data on out-of-target responses obtained in vivo can be very powerful, since physiological cellular connections within a tissue, or cross-talk among tissues, allow living organisms to withstand injury through coordinated cell/tissue response. Nevertheless, there is a general lack of in vivo data for the radiation out-of-target effects relevant for human exposures and only limited data are available that allow understanding of their generality in tissues/systems in vivo and underlying mechanistic bases. These effects have not been adequately investigated in

the context of brain (i.e. cancer and cognitive risks), heart (i.e. cardiovascular risks), or liver (i.e. cancer and metabolic alteration risks) exposure. SEPARATE (Systemic Effects of Partial-body Exposure to Low Radiation Doses) is an interdisciplinary project, combining in vivo irradiation, molecular/cellular biology, omics and bioinformatics to investigate how partial body irradiation may have significant implications regarding systemic consequences and human health at low and intermediate doses of ionizing

radiation. The project, organized in 5 WPs, had a duration of 30 months (01/10/2017-31/03/2020) with an EC contribution of 1.201,742 €.

Changes in the transcriptome (coding and non-coding), proteome and metabolome have been explored in control, out-of-target and irradiated tissues and analyzed by an integrative bioinformatics analysis. We also examined the role of exosomes as possible mediators of radio-induced damage signals and extensively characterized their size, concentration and cargo after extraction from tissues and blood of animals from

all experimental conditions.

Results clearly indicate that the out-of-field organs, especially hippocampus and heart, exhibit molecular changes nearly identical to those found in directly irradiated organs, improving the current understanding of the contribution of systemic "out of target" effects caused by radiation signaling which potentially have important implications in the clinic and impact the risks of long-term health detriment by radiation.

SEPARATE
Systemic Effects of Partial-body Exposure to Low Radiation Doses

SEPARATE Coordinator

Dr. Mariateresa Mancuso

Head of Biomedical Technologies Laboratory

ENEA (Italy)



Photo: ENEA

SEPARATE

Partners:

- Agenzia Nazionale per le Nuove Tecnologie, L'Energia e lo Sviluppo Economico Sostenibile (ENEA, Italy)
- Helmholtz Zentrum München, German Research Center for Environmental Health (HMGU, Germany)
- Oxford Brookes University (OBU, United Kingdom)
- Technological University of Dublin (TU Dublin, Ireland)

Duration of the project:
30 months

Total project budget:
1 741 655 €

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SEPARATE WP1

Partial-body irradiation and dosimetry

This WP was built to optimize and verify dosimetrically the irradiation procedures and to perform mouse partial-body exposure (PBI) using shield leads, sparing as much as possible tissues outside the volume from direct energy deposition. The WP 1 is articulated in three tasks: Task 1 concerning dosimetry; Task 2 relative to animal breeding, irradiations and organ collection; Task 3 dealing with microvesicle isolation.

respectively (Fig. 1A). In addition, dose measurements were done using an EBT2 gafchromic film that become green upon irradiation



Photo: ENEA

Dr Simonetta Pazzaglia

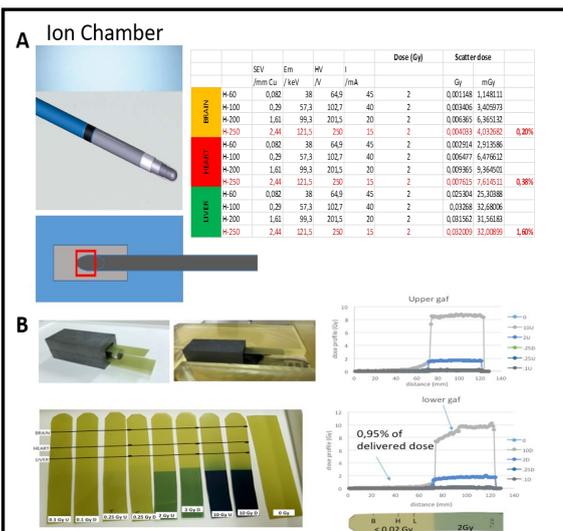


Photo and Graph: ENEA

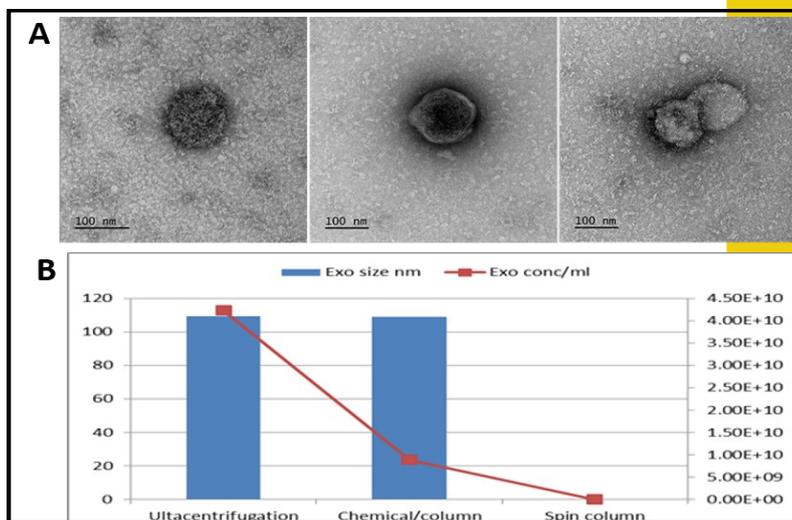
because of the undergoing polymerization in the active layer. Film calibration was done with increasing X-rays doses from 1 cGy to 10 Gy, followed by film digitalization at 48h after exposure. The calibration curve was extrapolated by an interpolation (3 degree) using the red channel. Dose verification in a plan under the phantom indicated for the three shielded organs that the scatter dose was 0.95% of the delivered dose (2 Gy), i.e., 19.0 mGy (lower gaf) (Fig. 1B). Overall, both dosimetric methods did not detect significant doses to the shielded organs.

Irradiation of 80 days old C57Bl/6 mice (n=500) with 0.1 or 2 Gy of X-rays was carried out with lead shield to protect the anterior two-thirds of the body, with the hindmost part directly exposed to radiation for PBI or with no shielding for whole-body irradiation (WBI). At 24 hours, 15 days or 6 months after irradiation animals were sacrificed and blood or hippocampus, cortex, heart and liver were snap frozen in liquid nitrogen and stored at -80°C for distribution to partners.

Pilot experiments with three different methods of exosome's isolation were also conducted and the ultracentrifugation method resulted in the highest yield (Fig. 2). Exosome size and concentration were assessed through TRPS system via qNano.

1) (A) Summary of the experimental measurements with the NE 2571 ionization chamber. Table: values of scatter doses to the brain, heart and liver at 60, 100, 200, and 250 kVp. (B) Assessment of the scattered dose using gafchromic film to brain (B), heart (H) and liver (L) at 250 kVp. (B)

Irradiation was delivered with a X-ray generator (Gilardoni CHF 320) operating at 60, 100, 250 kVp, 15 mA, with HVL = 1.6 mm Cu (additional filtration of 2.0 mm Al and 0.5 mm Cu). Dosimetry was carried out mimicking the experimental settings, i.e. mice irradiated with individual lead parallelepiped to protect the anterior two-thirds of the body, with the hindmost part directly exposed to radiation. Dose measurements were carried out with the support of the Italian National Metrological Institute using two different approaches. First, experimental measurements were carried out setting a ionization chamber in the same position as the shielded organs (brain, heart and liver) of the irradiated mice. Organ dose estimation at 2 Gy (from 10 measurements) are shown in Fig. 1A. There was a dose of 4 mGy to the shielded brain, 7.6 mGy to the shielded heart and 32.1 mGy to the shielded liver, corresponding to 0.2%, 0.38% and 1.6% of the total dose,



2) (A) TEM representative images of 1:10 diluted plasma exosome samples. (B) Data from the three different methods of exosome extraction

Photo and Graph : OBU

ID Card:

Work Package leader
Simonetta Pazzaglia
ENEA (Italy)

Partners involved in the WP:
OBU (UK)

Keywords:

Whole-body irradiation (WBI) Partial-body irradiation (PBI) Dosimetry Exosomes

Duration:

16 months
October 2017-February 2019

Infrastructures:

Exposure platforms: X-rays irradiation facility at ENEA;
Electron microscopy facility at OBU

Analytical platforms:

Esistive pulse sensing (tRPS) technology, using the qNano system, at OBU
Models and tools:
C57Bl/6 mouse strain

Project website:

<https://sites.google.com/view/separate-project/home>

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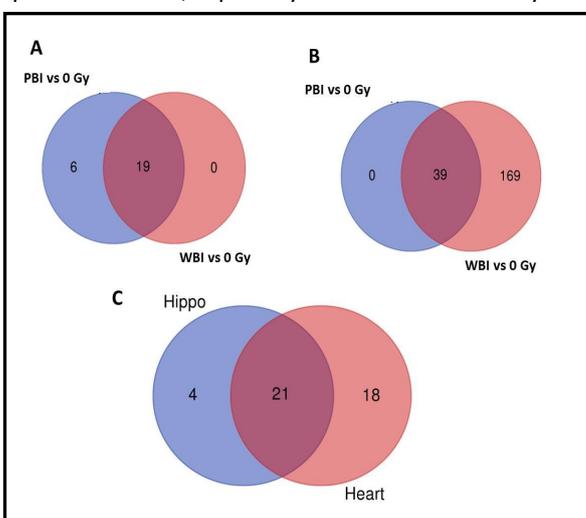
Related to:

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EURADOS



Omics analyses and biomarkers

The aim of this WP was to identify signal transduction pathways involved in long-range communication between exposed and out-of-field organs. For this purpose, we analysed the changes in the miRNome, proteome, and metabolome in directly irradiated and corresponding out-of-field organs. The analyses were performed in murine hippocampus and heart, 2 weeks and 6 months after whole- (WBI) or partial body irradiation (PBI) using X-ray doses of 0.1 Gy or 2.0 Gy. By means of these omics analyses we could demonstrate that in-field and out-of-field irradiation cause nearly identical alterations in non-coding RNAs, proteome, and metabolome 15 days post-irradiation, especially after the dose of 2 Gy.



1- (A) Venn diagram of the significantly deregulated miRNAs ($|\log_2FC| > 3$ and p -value of < 0.1) shared in the hippocampus of PBI and WBI mice vs control mice. (B) Venn diagram of the significantly deregulated and shared miRNAs in the heart of PBI and WBI mice vs control mice. (C) Venn diagram of the significantly deregulated and shared miRNAs between hippocampus and heart. All analyses were performed 15 days after irradiation with 2 Gy

The miRNome analysis based on next generation sequencing showed similar changes in the miRNA content in the out-of-field hippocampus and out-of-field heart as in the directly irradiated organs (Fig 1A, 1B). This analysis also revealed that, compared to the heart, the hippocampus showed a higher number of deregulated miRNAs in common between in-field and out-of-field tissues. This suggests that the hippocampus, the brain region where memory formation and adult neurogenesis takes place, is also able to build an “abscopal memory”. Interestingly, many of the miRNAs that altered their expression both after direct or out-of-field irradiation in hippocampus or heart were involved in brain and heart pathologies, respectively. Noteworthy, over 54% of

the deregulated miRNAs were in common between out-of-field hippocampus and heart (Fig. 1C), suggesting that, in addition to organ-specific out-of-field responses, there could be a common miRNA-mediated systemic mechanism playing a role in abscopal effects. At the low dose of 0.1 Gy, only 3 miRNAs, miR-143-5p, miR-378a-3p, miR-378a-5p, were significantly differentially expressed in the WBI hippocampus but no significantly deregulated miRNAs were found after PBI. This suggests a dose-dependent deregulation of miRNA expression both in irradiated and out-of-field hippocampus. After 6 months, the expression of miR-1a-3p was still significantly upregulated in the WBI and PBI hippocampus and heart. This makes miR-1a-3p a good biomarker candidate and suggests a role in mediating systemic effects after out-of-field radiation exposure.

Similarly, proteomic profiles indicated that a high percentage of the significantly deregulated proteins in PBI hippocampi overlapped with those altered following WBI especially at 2.0 Gy (80%; 89/111) 15 days after the radiation exposure (Fig. 2A). Both direct and abscopal radiation effects altered biological pathways that are associated with learning and memory, suggesting that WBI and PBI caused similar alterations in the proteome. The metabolic analysis using Raman spectroscopy also indicated that the level of many metabolites was similarly altered in PBI and WBI especially at 2.0 Gy 15 days (Fig. 2B) and 6 months post-irradiation.



Soile Tapio

Photo: IHMGU

ID Card:

Work Package leader:
Soile Tapio, HMGU

Partners involved in the WP:
ENE, OBU, TU Dublin

Keywords:
miRNome, proteomics, metabolomics, radiation biomarkers

Duration:
24 months (February 2018-January 2020)

Infrastructures:
Exposure platforms: X ray irradiation facility at ENEA

Databases: Ingenuity Pathway Analysis (IPA), STRING (<http://string-db.org>), Gene Ontology (GO), DAVID (Database for Annotation, Visualization and Integrated Discovery) and PANTHER (<http://www.pantherdb.org/pathway>) at HMGU.

Analytical platforms: TU Dublin Spectroscopic platform for radiation biology and biodosimetry

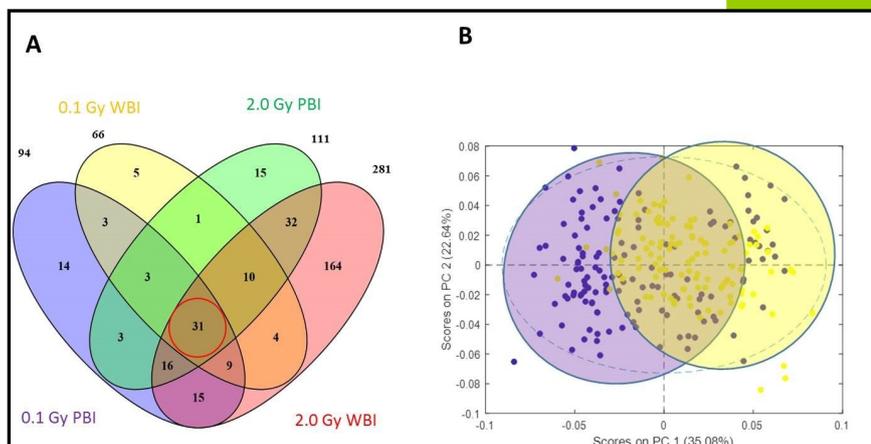
Models and tools: C57BL/6 mouse strain

Open Access of produced data:
STOREdb (www.rbstore.eu)

Project website:
<https://sites.google.com/view/separator-project/home>

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2- (A) Venn diagram showing the number of significantly deregulated and shared proteins in irradiated hippocampus 15 days after exposure to 0.1 WBI (yellow), 0.1 Gy PBI (purple), 2.0 Gy WBI (red) or 2.0 Gy PBI (green). (B) Scatter plot showing principal component analysis of the metabolic changes in the 2 Gy WBI (purple) and PBI (yellow) groups using Raman spectroscopy

Graph: MSLTU Dublin



SEPARATE WP4&WP5

WP4: Project Management

WP5: Dissemination, Training, Exploitation

Project management is performed under WP4, composed by 4 tasks: Task 4.1 covering the Overall Project Management; Task 4.2 dealing with WP Management; Task 4.3 for Project Meetings; Task 4.4, for Risk Management. ENEA successfully managed the coordination of the project, including scientific management, project leadership and communication between the European

Commission and the partners. The ENEA Project Management was assisted by the Management Board, made up of work package leaders (ENEA, HMGU and OBU), and by the Scientific Advisory Board composed of two leaders in radiobiology field: Prof. C. Limoli (University of

California, Irvine, CA, USA) and Dr. K. Butterworth (Queen's University, Belfast, UK). As the Coordinator, ENEA also provided financial support to a Third Party represented by the Technological University of Dublin (Prof. Fiona Lyng).

By focusing on the mechanisms of risks posed by partial-body irradiation, SEPARATE specifically addressed the CONCERT research call Topic 1 "Understanding human health effects from ionizing radiation and improving dosimetry", fulfilling several of CONCERT challenges and long-term goals.

The consortium has met in person at the Kick-off meeting (Rome, March 2018), at the 1st Annual meeting (Dublin, December 2018), at the Member Board Meeting (Rome, May 2019) and at the Final Meeting (Rome, February 2020). In occasion of international meetings [i.e. International Congress on Radiation Research (ICRR) 2019 and European Radiation Protection Week (ERPW) 2018 and 2019], the SEPARATE partners discussed results and future planning. Being SEPARATE a small-scale consortium, it was easily

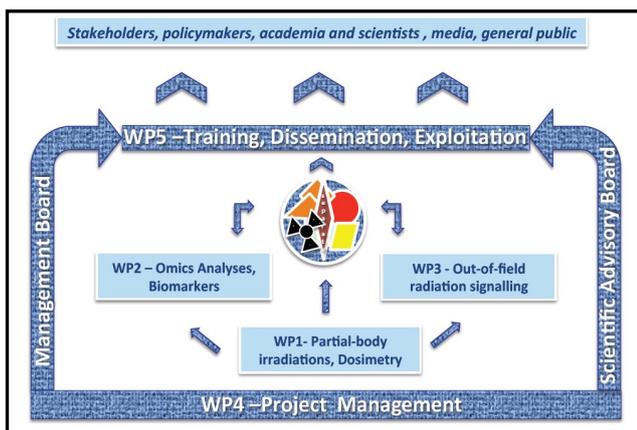
coordinated due to a prompt flow of information, and quick sharing of programming and results, through teleconferences frequently requested by partners.

WP5, dedicated to the dissemination activities of the SEPARATE Consortium, is articulated in 3 tasks: Task 5.1 devoted to Dissemination; Task 5.2 to Training; Task 5.3 to Protection of Intellectual Property. In total, 16 conference contributions,

including posters and oral presentations, were presented at international meetings. Up to now, two papers acknowledging SEPARATE financial contribution were published on peer-reviewed journals and other two are currently in preparation. Dissemination

activities also included the organization of a Satellite meeting focusing on "Extracellular vesicles in mediating bystander and systemic RT effects" during the ICRR in Manchester, UK, 25-29 August 2019 in conjunction with the CONCERT project LEU-TRACK.

Training activities involved 3 PhD students and 2 Postdocs that have been partially financed by the project, and actively contributing to project dissemination. In particular during the ERPW 2018 in Rovinj, Croatia, a session dedicated to young scientists was hosted, where young investigators were invited to present the results of their research.



SEPARATE Pert chart showing interdependency



SEPARATE meeting in Dublin (December 2018)



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Work Package leader:
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Partners

HMGU, Germany
OBU, United Kingdom
TU Dublin, Ireland

Keywords:

In vivo abscopal effects; omics, exosomes, inhomogeneous exposure

Duration:

30 months

Total budget:

1,741,655 €

Project website:

<https://sites.google.com/view/separate-project/home>

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Future events:

[ERRS 2020: European Radiation Research Society](#),
Lund, Sweden
13-17th September 2020
Digital meeting

[IRPA 15: International Congress of Radiation Protection Association](#)
Seoul, South Korea
17-22th January 2021

[1st ISORED meeting: International Society of Radiation Epidemiology and Dosimetry](#)
Sitges, Spain
Postponed to 2021

[ERPW2020: European Radiation Protection Week 2020](#)
Estoril, Portugal
Postponed to 2021

[EPRBiodose 2020:](#)
Okayama, Japan
Postponed to 2021

[See also CONCERT website](#)

Issue

Exposure platforms

Databases, Sample banks, Cohorts

Analytical platforms, Models & Tools

Published to date:

Oct 2015, #1	FIGARO	FREDERICA	RENEB
Nov 2015, #2	B3, Animal Contamination Facility	The Wismut Cohort and Biobank	The Hungarian Genomics Research Network
Dec 2015, #3	Pulex Cosmic Silence	STORE	METABOHUB
Feb 2016, #4	SNAKE	French Haemangioma Cohort and Biobank	Dose Estimate, CABAS, NETA
Mar 2016, #5	Radon exposure chamber	3-Generations exposure study	PROFI
Apr 2016, #6	Biological Irradiation Facility	Wildlife TransferDatabase	Radiobiology and immunology platform (CTU-FBME)
May 2016, #7	CIRIL	Portuguese Tinea Capitis Cohort	LDRadStatsNet
Jun 2016, #8	Mixed alpha and X-ray exposure facility	Elfe Cohort	ERICA Tool
Jul 2016, #9	SCRS-GIG	RES³T	CROM-8
Sep 2016, #10	Facility radionuclides availability, transfer and migration	INWORKS cohort	France Génomique
Oct 2016 #11	LIBIS gamma low dose rate facility ISS	JANUS	Transcriptomics platform SCKCEN
Nov 2016, #12	Microtron laboratory	EPI-CT Scan cohort	CATI
Dec 2016, #13	Nanoparticle Inhalation Facility	UEF Biobanking	The Analytical Platform of the PREPARE project
Feb 2017, #14	Infrastructure for retrospective radon & thoron dosimetry	Chernobyl Tissue Bank	HZDR Radioanalytical Laboratories
Special Issue 1	1st CONCERT Call: CONFIDENCE, LDLensRad, TERRITORIES	1st CONCERT Call: CONFIDENCE, LDLensRad, TERRITORIES	1st CONCERT Call: CONFIDENCE, LDLensRad, TERRITORIES
Mar 2017, #15	Alpha Particles Irradiator Calibration Laboratory at KIT		SYMBIOSE
Apr 2017, #16	Changing Dose rate (SU) Low dose rate (SU)		Advanced Technologies Network Center
May 2017, #17	Chernobyl Exclusion Zone	Chernobyl clean-up workers from Latvia	BfS whole and partial body Counting
Jun 2017, #18	MELAF	Belgian Soil Collection	INFRAFONTIER
Jul 2017, #19	MICADO'LAB	Estchern Cohort	ECORITME
Sep 2017, #20	DOS NDS		CERES
Oct 2017, #21	CALLAB Radon Calibration Laboratory		CORIF
Nov 2017, #22	Calibration and Dosimetry Laboratory (INTE-UPC)	German airline crew cohort	Centre for Omic Sciences (COS)
Dec 2017, #23	NMG	Techa River Cohort (TRC)	iGE3
Special Issue 2	MEDIRAD	MEDIRAD	MEDIRAD
Feb 2018, #24	UNIPI-AmBe	Greek interventional cardiologists cohort	SNAP

Issue	Exposure platforms	Databases, Sample banks, Cohorts	Analytical platforms, Models & Tools
Published to date:			
Special Issue 3	2nd CONCERT Call: LEU-TRACK, PODIUM, SEPARATE, VERIDIC, ENGAGE, SHAMISEN-SINGS	2nd CONCERT Call: LEU-TRACK, PODIUM, SEPARATE, VERIDIC, ENGAGE, SHAMISEN-SINGS	2nd CONCERT Call: LEU-TRACK, PODIUM, SEPARATE, VERIDIC, ENGAGE, SHAMISEN-SINGS
Mar 2018, #25	IRRAD	MARiS	BIANCA
Apr 2018, #26	Forest observatory site in Yamakiya	BBM	OEDIPE
May 2018, #27	Belgian NORM Observatory Site	The German Thorotrast Cohort Study	VIB Proteomics Core
Jun 2018, #28	CERF	Mayak PA worker cohort	Geant4-DNA
Jul 2018, #29	TIFPA	RHRTR	D-DAT
Sep 2018, #30	HIT	The TRACY cohort	COOLER
Oct 2018, #31	PTB Microbeam	The BRIDE platform	BRENDA
Nov 2018, #32	AGOR Facility at KVI-CART LNK		MARS beamline at SOLEIL
Dec 2018, #33	PARISII	The ISIBELa cohort	CIEMAT WBC
Feb 2019, #34	The MIRCOM microbeam	The ISE cohort	EFFTRAN
Special Issue 4	NSRL	LSAH & LSDA	GeneLab
Mar 2019, #35	IRSE Experimental Farm	The MWF database	DSA Environmental Laboratory
Apr 2019, #36	PG stack at Barreiro, Portugal	CONSTANCES	The MCDA Tool
May 2019, #37	LERF	IMMO-LDRT01 cohort	Radiochemical and Radioactive Analysis Laboratory (INTE-UPC)
Jun 2019, #38	FAIR	The BACCARAT study	CIEMAT In Vitro Internal Dosimetry Laboratories
Jul 2019, #39	AMBIC	LSS	LRM
Sep 2019, #40	FRM II	REQUITE	TU Dublin Analytical Platform
Special Issue 5	CONFIDENCE	CONFIDENCE	CONFIDENCE
Special Issue 6	PODIUM	PODIUM	PODIUM
Special Issue 7	LDLensRad	LDLensRad	LDLensRad
Special Issue 8	ENGAGE	ENGAGE	ENGAGE
Special Issue 9	LEU-TRACK	LEU-TRACK	LEU-TRACK
Special Issue 10	CIEMAT External Dosimetry Service and Retrospective Luminescence Dosimetry Lab, AIFIRA Microbeam, The Calliope Facility, ZATU	The 'hematopoietic system' database for Mayak nuclear workers chronically exposed to ionizing radiation	
Special Issue 11	TERRITORIES	TERRITORIES	TERRITORIES
Special Issue 12	VERIDIC	VERIDIC	VERIDIC
Special Issue 13	HARMONIC	HARMONIC	HARMONIC
Special Issue 14	SHAMISEN-SINGS	SHAMISEN-SINGS	SHAMISEN-SINGS
Special Issue 15	SEPARATE	SEPARATE	SEPARATE