RadoNorm
Managing risks from radon and NORM

Deliverable 4.4

Molecular characterization of lung cancer in never smoker patients and miners exposed to radon. Comparison to lung cancers in animal model. Discussion on biological consequences of homogeneous and heterogeneous exposures using organotypic models of human

Work Package 4

This project has received funding from the Euratom research and training programme 2019-2020 under grant agreement No 900009.

Document information

Project Acronym	**RadoNorm**
Project Title	**Towards effective radiation protection based on improved scientific evidence and social considerations - focus on radon and NORM**
Project Type	**RIA**
EC grant agreement No.	**900009**
Project starting / end date	**1st September 2020 – 31 August 2025**
Work Package No.	**4**
Work Package Title	**Effects and Risk Assessment**
Deliverable No.	**4.4**
Deliverable Title	**Molecular characterization of lung cancer in never smoker patients and miners exposed to radon. Comparison to lung cancers in animal model. Discussion on biological consequences of homogeneous and heterogeneous exposures using organotypic models of human.**
Lead Beneficiary	**IDIBAPS** (Spain) – **Gustave Roussy** (France)
Contractual Delivery Date	**31/08/2025**
Actual Delivery Date	**22/08/2025**
Type	**Report**
Dissemination level	**PU**
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To be cited as:

Mezquita L, Castellano G, Boei J, Jiménez M, Bernabeu M, Madas B. and Besse B. (2025): Molecular characterization of lung cancer in never smoker patients and miners exposed to radon. Comparison to lung cancers in animal model. Discussion on biological consequences of homogeneous and heterogeneous exposures using organotypic models of human. Final version as of 25.08.2025 of deliverable D4.4 of project RadoNorm.

Disclaimer

Acknowledgement

This document is a deliverable of the RadoNorm project. This project has received funding from the Euratom research and training programme 2019-2020 under grant agreement No 900009.

Status of deliverable	By	Date
Delivered (Lead Beneficiary)	Dr. Laura Mezquita Prof. Benjamin Besse	11/08/2025
Verified (WP Leader)	Dr. Valtteri Nieminen, UEF Prof. Jonne Naarala, UEF	22/08/2025
Reviewed (Reviewers)	WPL, PC	25/08/2025
Approved (PC)	PC	25/08/2025
Submitted to EC (PC)	PC	25/08/2025

Executive Summary

Deliverable D4.4 summarizes the outcomes of a multi-model investigation aimed at characterizing the molecular features of lung cancer associated with radon exposure. The study integrates findings from four complementary models: a controlled preclinical exposure system, an occupational cohort of miners with historical exposure data, a clinical cohort of never-smoker patients with non-small cell lung cancer (NSCLC) and measured residential radon levels, and human lung organotypic models designed to simulate heterogeneous exposure scenarios.

Key achievements include the identification of recurrent molecular alterations observed in both human and animal tumours, evidence of DNA repair pathway involvement and genomic instability, and recognition of exposure-specific profiles influenced by environmental co-exposures. Additionally, a human lung organoid model was developed, demonstrating persistent DNA damage responses following heterogeneous alpha-particle irradiation.

Detailed findings from the preclinical and occupational studies are documented in previous project milestones. The clinical cohort study is ongoing, with data integration and analysis planned to align with the completion of recruitment. An original research article synthesizing results across models is under preparation, and further in-depth molecular characterization of clinical samples is planned.

Preliminary data from this integrated work have been presented at the final meeting of the RadoNorm Consortium in Budapest (June 2025) and in international meetings. The emerging findings support radon's role in lung carcinogenesis through genotoxic mechanisms and may contribute to the future identification of biomarkers of exposure, as well as inform prevention, molecular surveillance, and risk stratification strategies for populations at elevated risk of radon-related lung cancer.

Table of content

1. Results

1.1 Molecular alterations in radon-induced lung tumours in rats

This subtask aimed to define the molecular characteristics of lung tumours induced by controlled radon exposure in a preclinical rat model. Lung primary tumours from the French Alternative Energies and Atomic Energy Commission (CEA) lifespan experiments were analysed, integrating clinical, pathological, and molecular data obtained through whole exome sequencing (WES) and RNA sequencing (RNA-seq). A total of 36 lung tumours were evaluated across different radon exposure groups and cumulative dose categories, comparing low and high cumulative exposures.

Controlled radon exposure in this model induced primary lung tumours with molecular features involving key oncogenic and DNA repair pathways. Histological patterns varied with cumulative exposure, and transcriptomic analyses revealed differential expression signatures associated with higher-dose groups. Structural genomic alterations and changes in mutational patterns were observed, reflecting both direct DNA damage and downstream cellular responses. These results, detailed in Milestone 46, represent the most comprehensive molecular description of radon-induced tumours in rats to date.

1.2 Molecular profile of lung adenocarcinoma in uranium miners

This subtask examined lung adenocarcinomas from a cohort of uranium miners with well-documented historical radon exposure, using WES analysis. Missense mutations predominated, with substitution patterns consistent with DNA damage typically associated with ionising radiation. Several recurrent alterations were identified in key oncogenic pathways, including those involved in cell proliferation, stress response, and DNA repair. Structural variants affecting relevant cancer driver genes were also detected.

High-exposure groups displayed a greater prevalence of DNA repair pathway alterations, and combined radon and tobacco exposure was associated with distinct mutational patterns, suggesting synergistic carcinogenic effects.

Comparative analysis with the preclinical model revealed both overlapping and divergent molecular features, with shared pathway disruptions across models but model-specific differences likely attributable to co-exposure to tobacco and variations in exposure context.

1.3 Indoor radon exposure and molecular profile in patients with NSCLC (1920-EORTC LCG BioRadon study)

The BioRadon study is an ongoing, multicentre, prospective cross-sectional investigation within the EORTC SPECTA framework, enrolling patients with non-small cell lung cancer (NSCLC) of all stages who have both molecular tumour profiles and measured residential radon exposure. Recruitment is active across several European countries, with all participating sites fully operational.

Biological samples, including tumour tissue and blood, are centrally stored at a dedicated biobank for germline and somatic genomic analyses. Patients are categorised into distinct molecular subgroups according to their tumour profiles, with recruitment ongoing in some cohorts and complete in others.

Once enrolment concludes, the study will perform data harmonisation and conduct integrative analyses to evaluate associations between molecular tumour characteristics, measured indoor radon exposure, and potential germline susceptibility. The aim is to validate molecular patterns identified in preclinical and occupational models and translate these into a clinical context.

Comprehensive genomic and transcriptomic characterisation is planned for both tumour and matched blood samples. Initial results will be compared with those from animal and occupational cohorts to assess cross-model concordance. Findings are expected to inform biomarker development and risk stratification strategies for radon-related lung cancer.

1.4 Comparative cross-model analysis

The integrated assessment of radon-associated lung cancers in preclinical, occupational, and *in vitro* human organotypic systems identified both shared and model-specific molecular features, underscoring alpha-particle radiation as a driver of lung carcinogenesis. Common patterns included disruption of key oncogenic signalling cascades, evidence of high genomic instability in high-exposure contexts, and consistent mutational trends across models.

Differences between models reflected variations in exposure conditions and co-exposures. The preclinical model showed alterations predominantly affecting certain growth and repair pathways, while the occupational cohort exhibited enrichment of DNA damage response and repair pathway alterations, likely influenced by concurrent tobacco exposure.

These findings suggest the presence of a conserved molecular footprint of radon exposure, modulated by environmental and biological context. Validation in the prospective BioRadon study will be essential to confirm these patterns in patients and determine their clinical utility as biomarkers of radon-related lung cancer.

1.5 Biological consequences of homogeneous vs. heterogeneous exposures using human organotypic models

1.5.1 Consequences of inhomogeneous exposure at the tissue (model) level

In reconstituted human bronchial epithelium models, alpha-particle exposure did not result in marked cell death or loss of epithelial barrier integrity. However, persistent DNA damage markers remained detectable long after exposure, accompanied by reduced proliferation of basal cells, a critical process for epithelial repair and homeostasis. When proliferation was experimentally stimulated, genomic instability features were observed, suggesting that accumulated damage in progenitor cells could compromise tissue regeneration.

Under conditions of heterogeneous exposure, increased proliferation was observed in unexposed regions, together with evidence of cell migration from unexposed to exposed areas. This behaviour is consistent with epithelial unjamming processes and was associated with activation of signalling pathways implicated in tissue remodelling. These observations point to complex spatial interactions within tissue following localised alpha-particle exposure, which could be further modulated by other environmental insults, such as tobacco smoke.

1.5.2 Consequences of inhomogeneous exposure at the cellular level

Efforts were made to detect potential mutational fingerprints of alpha-particle exposure using whole genome sequencing. While initial attempts using the human epithelial model faced challenges in achieving sufficient clonal expansion, an alternative approach employing a cell type amenable to stable clonal growth allowed the generation of material for genomic analysis.

Preliminary results indicate the feasibility of identifying distinct genomic alteration patterns following alpha-particle exposure, including complex structural rearrangements do not present in unexposed controls. However, the number of samples analysed to date is insufficient to confirm the existence of a definitive alpha-particle-specific mutational signature. Establishing such a fingerprint could provide a direct molecular link between radon exposure and its health effects, with potential application in human tissue studies from high-radon environments.

2. Final note & next steps

This deliverable provides a synthesis of findings from preclinical, occupational, and in vitro models investigating the molecular effects of radon exposure in lung cancer. The results strengthen the evidence for radon as a carcinogenic driver, revealing both conserved molecular features across models and patterns that are specific to particular exposure contexts.

In the controlled experimental model, frequent alterations in signalling and DNA repair pathways were detected, together with structural changes and transcriptional patterns associated with high cumulative exposure. The occupational cohort demonstrated pathway alterations consistent with the preclinical findings, alongside features likely influenced by additional environmental co-exposures. While no definitive radon-specific molecular signature was established, recurrent patterns across datasets suggest the existence of a conserved molecular footprint.

An integrated peer-reviewed publication combining results from the preclinical, occupational, and clinical subcohorts is in preparation. Upon completion of BioRadon recruitment, data will be curated and harmonised for presentation in 2026. Planned analyses include comprehensive genomic and transcriptomic profiling, enabling correlation of indoor radon exposure with tumour and molecular characteristics in patients with NSCLC. This will test the translational relevance of candidate biomarkers and alterations identified in earlier models.

The integration of BioRadon with existing datasets will support improved molecular risk stratification for radon-related lung cancer and inform the development of targeted prevention, surveillance, and therapeutic strategies.