RadioTranNet Strategic Research Agenda

1) The first taskforce is dedicated to "Target volume definition".

This taskforce is led by Vincent Grégoire, Charlotte Robert and Benjamin Lemasson.

- General background and scientific needs:

The last two decades have seen the development of imaging modalities and RT treatment techniques. 3D and 4D imaging modalities make it possible to describe tumor and normal tissues and their motions. However, radiotherapy's anti-tumor efficacy must increase since about one third of cancer deaths involve local-regional failure. That fact demonstrates the need to better define the target.

- Main scientific objectives:

With modern imaging modalities and registration software, spatial definition of the macroscopic target can be achieved. From a biological point of view, different phenomena should be included in definition of the target. Hypoxia, vascularization and other processes related to the microenvironment should be known at least at the voxel scale. From an anatomical point of view, delineation is still a time-consuming operator-dependent action. Automation should be added to produce more consistent volumes irrespective of the clinician and to pave the way for adaptive radiotherapy.

Functional imaging with PET and MRI should provide specific sequences or radiopharmaceuticals to derive biological sub-volumes representative of a biological phenomenon able to help define the target volume or prescribed dose. Additionally, biological sub-volumes should be spatially registered to the planning images to define planning volumes. Research should be conducted to better understand the biological mechanisms and pathways needed to improve the sensibility and specificity of imaging exams that can derive accurate and quantitative hypoxia, tumor vascularization or other biomarker maps defining criteria for radioresistance. As biological mechanisms occur at cellular scale and imaging modalities use comparatively large voxels, studies should also consider this multiscale problem to fully exploit biological findings. In addition, quantitative imaging should make it possible to convert the detected signal into a required dose to prescribe for these sub-volumes. Such quantitative imaging is a prerequisite for developing treatments using dose painting by contours or by numbers.

These steps are fundamental to pave the way for adaptive radiotherapy. Indeed, adaptive radiotherapy is limited due to the necessary interventions of different operators. Fully adaptive radiotherapy could be developed only if progress is made in automation. Many algorithms already exist and prove satisfactory for some organs, especially when contrast is high. However, they still fail in some situations and always require expert validation. Research should continue, including by exploring neural networks and machine learning.

- Program areas and tasks:

The RADIOTRANSNET consortium identifies as a collective goal the progressive implementation of a strategic research agenda for optimizing radiation exposure and standardizing practices in radiotherapy. The "Target Volume Definition" research topics considered necessary and most urgent for effective medical care and efficient radiation protection are outlined below:

A. BIOLOGY-DRIVEN IMAGE ACQUISITION

To develop and validate new *in vivo* imaging strategies reflecting tumor biology and microenvironments in order to personalize radiotherapy planning. The long-term goal of the methods developed will drive future clinical trials validating multiparametric imaging's contribution to improving tumor control and/or reducing the toxicities of treatments. Therefore, research should focus on the following issues:

– Preclinical and/or clinical development and validation of new tracers (PET, SPECT) or NMR sequences to assess the tumor's heterogeneity and microenvironment (e.g., inflammatory infiltrate and its characterization).

– Preclinical validation of imaging modalities (already used in clinical practice or in development) on adapted animal models (e.g., collaboration with veterinary schools for studies on large animals) with suitable imaging systems (e.g., micro-CT, micro-PET, micro-NMR) is mandatory to better characterize the sensitivity and specificity of the imaging modalities, define biological significance (comparison with anatomopathological data) and assess the relationship with tumor heterogeneity.

– Evaluation of the interdependence of these multiparametric imaging modalities using AI, e.g., to transfer the result of one imaging modality to another.

B. EXTRACTION METHODS FOR MULTIPARAMETRIC IMAGING-BASED TARGET VOLUME(S) DELINEATION

– Development and validation of segmentation methods to extract relevant imaging information for target volume delineation; various approaches could be used, e.g., comparison with a ground truth (prostate, larynx) or pattern of local recurrences, to validate the use of these imaging modalities.

– Assessment of the segmentation methods' influence on dose distribution using both photons and proton beams.

– To evaluate the segmentation methods' robustness with images acquired during radiotherapy treatment in the framework of adaptive planning.

– Development of models for the automatic selection and delineation of the microscopic infiltration around the gross tumor volume (GTV) and in the drainage lymph nodes, e.g., Bayesian models of lymph node infiltration, AI-based delineation of clinical target volume (CTV).

– Development of OAR segmentation methods to account for the difference in function between various subareas of a given organ, e.g., lung, parotid glands.

C. VALIDATION OF THE CONCEPTS OF PERSONALIZED IMAGE-GUIDED RADIATION THERAPY

In the era of personalized medicine, new radiation therapy concepts have been proposed, including dose painting, i.e., modulation of the prescribed dose on a subregion or voxel scale, and adaptive radiation therapy, which tailors the treatment plan to the anatomy of the day and could also benefit from daily dose adjustment. Today, the clinical impact of these concepts has yet to be validated. To do this, the following research is proposed:

– Definition of the optimal scale of tumor heterogeneity characterization affecting the variation of dose distribution in photon and hadron beams and on patients' clinical response based on preclinical and clinical models.

– Characterization of the variation in tumor heterogeneity during treatment for different tumor locations (preclinical and clinical) to provide recommendations on which criteria a decision for treatment adaptation should be based.

– Development of preclinical models on large animals (e.g., in collaboration with veterinary schools) to validate the concept of adaptive radiotherapy and dose painting.

– Development of planning tools making it possible to calculate the "dose of the day" based on the consideration of positioning variations as well as intrinsic variations of target volumes (GTV and CTV) and at-risk organs.

– Adaptation of target volume selection/delineation in the framework of combined modality treatments, e.g., should we decrease target volumes in combined immunotherapy/radiation therapy strategies?

D. IMAGING DATABASES FOR TARGET VOLUME DEFINITION

Robust evaluations of any new personalized imaging-based radiation therapy are required prior to use in clinical practice. Such evaluations need large, structured databases (images and biological data) dedicated to radiation therapies. Moreover, the variability of images between centers is one of the major issues in multicenter studies.

– Creation of preclinical and/or clinical image databases to facilitate validation of the use of multimodal imaging. Proposals should not only aim to collect raw data, but also to propose the development of one or more automated data pre-processing modules in order to standardize the images, resample them, readjust, etc. and make them insofar as possible "ready to use" to answer various clinical questions. This initiative will build on existing initiatives and institutions such as ONCOshare (Rennes), Shanoir, HealthDatahub and France Life Imaging. The databases could be prospective or retrospective on preclinical or clinical data.

– Propose methods of standardizing clinical practices in image acquisition, reconstruction and segmentation and developing a "best practice guide". These proposals should be based on collaborations between experts (pathologists, radiologists, physicists, radiation oncologists) as well as on various ongoing international initiatives such as QIBA, EIBALL, etc.

- Description of collaborations and synergies between the teams and members of the network for each theme:

To ensure open and inclusive research, contributions from a large number of scientists, clinicians, physicists and patient associations are needed. Many RadioTransNet partners involved in such research topics are also members of the France Life Imaging Network (FLI, www.francelifeimaging.fr) coordinated by CEA.

Indeed, research progress, and thus cancer treatment improvement, may benefit from contributions from advanced dosimetry, radiobiology, systems biology, physics and mathematics developments. Scientific expertise is available in several research centers and collaboration will be encouraged. This could benefit from coordination, guidance and support. The development of a network of centers sharing tools, methods and outcome data would facilitate and accelerate implementation of findings.

- International relevance:

Synergy between advanced dosimetry, radiobiology, systems biology, physics and mathematics will enhance clinical practice and radiation protection in the medical field. International collaboration will be encouraged. Moreover, SFPM organizes annual international workshops for young scientists. This topic is consistent with the European Horizon program supporting “Scaling up multi-party computation, data anonymization techniques, and synthetic data generation” (HORIZON-HLTH-2022-IND-13-02) and the European Cancer Imaging initiative.

2) The second taskforce is dedicated to “ionizing radiation interaction with normal tissues”.

This taskforce is led by François Paris, Carmen Villagrassa and Renaud de Crevoisier. They were assisted by Fabien Millat and Thomas Lacornerie.

- General background and scientific needs:

Considerable progress towards reducing the toxicity of radiation therapy has been made by the introduction of so-called “dose-sculpting” treatment techniques. High-tech RT enables precise beam

delivery that closely follows the shape of tumors, yielding an improved efficacy/toxicity ratio. Identification of the molecular and cellular basis of radio-induced side effects could help define new therapeutic strategies to prevent them and improve the quality of life of patients who receive radiotherapy.

- Main scientific objectives:

Reducing the risk of sequelae and second cancer occurrence was identified as one of the seventeen objectives of the French “Plan Cancer 2014–2019” and was also a priority of the last ten-year national program. It will be a key point of the next Cancer Plan. Reducing adverse effects represents a major challenge for better quality of life for long-term cancer survivors. Preclinical research investigating the mechanistic processes of normal tissue response will pave the way for optimizing radiation exposure and reinforcing the emergence of new therapeutic approaches to cancer treatment.

- Program areas and tasks:

The RADIOTRANSNET consortium identifies as a collective goal the progressive implementation of a strategic research agenda for the optimization of radiation exposure and standardization of radiotherapy practices. The research topics on “normal tissue” considered necessary and most urgent for effective medical care and efficient radiation protection are summarized in six main areas:

A. CLINICAL OBSERVATION - CORRELATION TO DOSE

Patients’ individual tissue response may be considered in the choice of therapeutic strategies. This can be based on intrinsic factors (age, gender, genomics/epigenomics…) of the normal tissues, but also on concomitant diseases impacting the general or specific normal tissue tolerance. Patients with a high risk for certain severe normal tissue responses may require a change in dose distribution or treatment strategy. Follow-up protocols may need to be adjusted to the individual morbidity risk pattern based on early predictive molecular or functional marker expression. New predictive tests for individual susceptibility and response to normal tissue toxicity will contribute to developing personalized cancer treatment. The key points to explore are:

– Implementation of a robust methodology to standardize input dosimetric data and standardized registers allowing correlation to the deterministic effects observed
– Development of artificial intelligence models or deep-learning methods to utilize these data and find correlations
– Identification of biomarkers associated with late complications and related quantitative imaging biomarkers (e.g., sarcopenia, heart calcification)
– Evaluation of the effects of confounding factors and reirradiation when studying correlations
– Development of preclinical radiomics approaches.

Further development of NTCP models is required and needs standardized input data, delivered dose data and not only planned dose data.

B. TISSUE RESPONSE RELATIONSHIP WITH HIGH DOSE, HIGH DOSE/FRACTION AND HIGH DOSE RATES

Radiation therapy treatments are mainly delivered by high-energy photon beams produced by linear accelerators according to conventional fractionation. The development of hypofractionated radiotherapy treatments requires a new determination of normal tissue complication probabilities. The biological mechanisms involved after high dose per fraction are controversial. Dose/volume constraints and tolerance doses of at-risk organs have been widely documented for "normo-fractionated" protocols. There is a very significant lack of knowledge on the biological effects associated with both high doses per fraction and high dose rates. The relative biological effectiveness

(RBE) concept of normal tissue response to new radiation modalities must be reconsidered. Preclinical research studies must be conducted to optimize new irradiation schemes.
In addition, the use of proton or carbon ion beams makes it possible to reduce the number of beams necessary to deliver highly conformal plans. The biological mechanisms involved after particle beam therapy differ from photon therapy. Consequently, radiobiological studies should consider the particle's impact on normal tissue side effects.

The identified goals are:

– Define a "safe" dose level: optimal and validated dose constraints to be used in the optimization process?
– Where can we get reliable evidence to spare one structure (or sub-structure) over another?
– How can we anticipate real clinical benefit from new advanced radiotherapy technology?
– Investigate the patient susceptibility effect
– Combine effects between organs
– Investigate severe hypofractionation and normal tissue responses
– Explore FLASH protection mechanisms
– Evaluate the irradiation of large volumes at low dose with respect to stochastic risks (IGRT)
– Move RBE concepts forward in line with the development of new techniques and practices in radiotherapy (FLASH electrons, FLASH protons, heavy ions, effects of high dose rate, therapeutic nuclear medicine)
– Initiate preclinical projects investigating reirradiation's consequences.

C. RESPONSES TO TARGETED RADIONUCLIDE THERAPY

Among the innovations developing targeted and personalized therapy, radionuclide/vectorized therapy is very promising. Methodologies like those previously described need to be adapted to radionuclide and vectorized therapy in order to investigate this field and develop new radioisotopes and vectors.

D. VALIDITY OF THE TRANSPOSITION OF THE "SMALL ANIMAL" MODEL TO HUMANS

Radiation-induced morbidity may be observed early (< three months) or late (several months or years) after cancer treatment. Emerging knowledge at the frontier between biology, chemistry and physics is required to better anticipate the risk of new treatment protocols such as hypofractionated schemes and stereotactic approaches. Exploring the role of tissue microenvironments is required to better characterize the normal tissue versus tumor response, with a system radiobiology approach making it possible to identify new molecular pathways of normal tissue response. Current morbidity risk models and normal tissue complication probability (NTCP) models are largely phenomenological in nature and aim to select a data-driven parsimonious correlation between the clinical, dosimetric and biological data with an observed treatment outcome with no assumed damage development processes or evidence of a mechanistic basis. Systems/computational radiobiology approaches and the use of up-to-date localized preclinical modeling as well as transgenic models able to elucidate the role of different cellular compartments will help address the problem. Preclinical models may help us implement NTCP models. The tasks to perform in priority are:

– Introduce relevant preclinical models using adapted irradiation tools
– Validate models for acute and mainly long-term effects
– Introduce preclinical models to study both healthy tissue and tumor cell effects, i.e., develop preclinical models of healthy tissue damage with implanted tumors
– Promote the standardization of preclinical models between different teams
– Introduce relevant preclinical models in large animals (pigs or others).

E. MOLECULAR AND CELLULAR MECHANISMS INVOLVED IN NORMAL TISSUE INJURY

Understanding the molecular and cellular mechanisms and pathways involved in normal tissue injury is a prerequisite to determining new irradiation schemes and therapeutic approaches to prevent and reduce irradiation side effects and improve patient wellbeing. Therefore, the following tasks have been deemed priorities:

– Preclinical research projects on at-risk organs such as the lung, heart, brain, digestive tract and bone
– Study the effects of high doses by fraction and decipher the molecular and cellular mechanisms by *in vitro* / *in vivo* approaches
– Develop systems biology projects applied to radiobiology: radiobiology–molecular biology–mathematics–bioinformatics interface, and share resources and skills on these integrated approaches
– Determine the role of different cell types in the initiation and progression of lesions in healthy tissues after irradiation: vascular compartments, immune cells, stem cell compartments, specialized cell types according to the organs
– Study the different cell fates of normal cells after radiation: type of cell death, senescence, differentiation, autophagy…
– Decipher senescence's role in the responses of tumors and healthy tissues: beneficial and deleterious effects
– Study dose–response relationships in line with modern molecular concepts (cGAS/STING activation, IFNg, activation…)
– Promote preclinical projects to better study combinations of radiotherapy / targeted therapies / immunotherapy / chemotherapy and their effects on toxicity in healthy tissue
– Develop *in vitro* 3D organoid/spheroid models applied to normal tissue radiobiological studies.

F. THERAPEUTIC STRATEGIES TO PREVENT, LIMIT AND TREAT NORMAL TISSUE DAMAGE

The future challenge in the biological response of normal tissues will be to identify 1) key compartments/processes and 2) sequences of action for temporal therapeutic intervention. Clarification of these mechanisms will help develop specific strategies for protection, mitigation or management of severe radiation side effects to the at-risk organ. The identified priority actions are:

– Develop relevant preclinical models to test therapeutic strategies to prevent and limit severe damage to healthy tissue after radiation therapy
– Develop original and modern therapeutic approaches (stem cell therapy, new pharmacological drugs) applied to normal tissue toxicity
– Study the role of microbiota and microbiota-based therapeutic approaches in RT-induced tumor response and normal tissue injury.

Preclinical research requires dedicated radiation facilities with configurations representative of clinical use for cancer treatment. Specific action for the identification and networking of the whole national infrastructure will be necessary to facilitate joint efforts for the upgrading of and open access to dedicated platforms for preclinical radiotherapy research. Several radiation facilities specializing in preclinical research for radiation oncology, including image-guided small animal irradiators, medical linear accelerators, proton and carbon beams, are already installed and could be used. Numerous platforms have already been organized into different networks. Also, management of dose/volume constraints for preclinical modeling would be necessary (ablative doses, low doses on large volumes…) to develop standardized preclinical models with high clinical significance. These aspects are common to some WP4 and are also developed in the corresponding section.

- Description of collaborations and synergies between the teams and members of the network for each theme:

This topic relies on multidisciplinary collaboration: scientists specialized in radiobiology, immunology, functional genetics, systems biology, methodologists, radiation oncologists, imaging specialists, pathologists, etc. The scientific expertise is available from the RadioTransNet network as identified partners are already working on the topic, such as IRSN, Gustave Roussy or Institut Curie. This would benefit from coordination and funding support.

- International relevance:

Synergy between advanced dosimetry, radiobiology, systems biology, physics and mathematics will enhance clinical practice and radiation protection in the medical field. International collaboration will be encouraged. Moreover, SFPM organizes annual international workshops for young scientists. Moreover, these topics are major concerns for the EURAMED and MEDIRAD European consortia and are consistent with the 'Better Life for Cancer Patients Initiative' underpinning Europe's Beating Cancer Plan.

3) The third taskforce is dedicated to "Combined Treatments".

This taskforce is led by Stéphane Supiot, Jean-Noël Badel and Sophie Pinel.

- General background and scientific needs:

The large number of preclinical data concluding that *in vitro* or *in vivo* experiments suggest a potential for clinical benefit in radiotherapy contrasts with the fact that very few new drugs were approved for concurrent radiotherapy administration in the last fifteen years.

It seems obvious that there might have been several gaps between experimental models and clinical reality. Only a minority of various preclinical experiments with targeted therapies and radiotherapy studied these combinations' impact on normal tissue response. The transition to the targeted therapies era has profoundly challenged the value of preclinical models. We propose focusing on few combinatory strategies, which should contribute to producing significant advances and novelty in the field; key aspects will be outlined.

- Main scientific objectives:

One important aspect is to focus on very few key combined strategies among the broad spectrum of potential approaches, based on several selection criteria at both preclinical and clinical levels and the availability of industrial support. With that aim, we propose developing a strategy to enhance the anti-tumor effect of radiotherapy, focusing on various aspects of the combinatory approach such as targeting the tumor cells' intrinsic signals, DNA damage and response pathways involved in resistance to radiation, the interplay between the tumor and vascular network, the cellular dynamics, motility and plasticity involved in resistance to radiation, and the interplay of the cancer cell and immune host.

The choice of a preclinical focus is relevant **in defining how preclinical evaluation of novel combinations would be performed in a network**. We will focus on the major dose-limiting organs; in particular, we will develop and make available to the consortium models of lung, bowel and brain late toxicities.

In addition, the emerging concepts of radiotherapy's immune effects require the development of and access to relevant murine models to evaluate its systemic immune effects. Response to highly specific targeted agents often relies on pre-existing mutations. The molecular profiling of tumors plus data from the TCGA are underscoring the emerging needs for more relevant tumor models reflecting the diversity of molecular subtypes. In the same way, patient cellular models that are fully characterized in terms of genomic profile and clinical response to irradiation will be used to study the efficacy of

targeted drugs directed against the activated pathways involved in these models' radio-resistance and irradiation.

- Program areas and tasks:

The project greatly depends on the involvement of partners in imaging, radiotherapy and the pharmaceutical industry.

The potential benefits of the planned structure are the following:

− Faster and earlier testing in combination with RT of some drugs in the development pipeline based on scientific rationale (preclinical data showing that the drugs' target is of interest as well as clinical data showing that it has clinically significant relevance in terms of radiation response)

− Access to a preclinical and clinical platform and network of excellence in the field, incorporating the latest advances in the molecular classification of tumors

− Better identification of unmet clinical needs, which could lead to faster registration of the drugs in combination with RT, ultimately benefiting patients.

The network would select the drugs of interest and suggest the appropriate methodology, including access to technological platforms according to the potential selected targets. The role of each platform was defined during the dedicated workshop on this topic, under the three coordinators' supervision.

The selection of candidates to be transferred into the clinic will incorporate translational molecular, genomic and imaging data demonstrating target engagement to ensure the clinical relevance of the hypothesis and optimize patient population selection for clinical transfer. Candidate targets and agents will encompass various fields such as oncogene addiction, DNA damage repair and signaling, metabolism, tumor stroma and vasculature and immunotherapy.

Results will be presented during international meetings held by NCI-AACR-EORTC, ESTRO, ASTRO and AACR. Translational research and clinical research will be performed under the umbrella of collaborative groups such as EORTC and UNICANCER and organ-oriented groups such as GERCOR, FFCD, GORTEC, IFCT, etc. There will be a yearly meeting in the form of a specific session during the SFRO annual conference.

A specific SRA for this area is presented below:

A. IMPROVING PRECLINICAL TESTING

The relevance and predictive value of preclinical trials are often challenged because of various scientific, methodological, technical and ethical limitations and biases.

- Concerted guidelines for good practice are needed to standardize and coordinate preclinical studies. The future guidelines should address the following issues:
  - Defining the scope of preclinical research: Does the "preclinical field" include cognitive research in radiation physics and biology? Is the "preclinical field" limited to translational/clinical questions?
  - Prioritizing the radiation therapy fields affected by combined therapies: conventional radiotherapy versus new irradiation modalities (FLASH RT, hadrontherapy, etc.)
  - Referencing the *in silico*, *in vitro* and *in vivo* models currently used for combined treatment research (state of the art and critical analysis) + highlighting their respective advantages/interests and drawbacks/limitations, and positioning them in the development chain.
  - Validating the design of preclinical studies according to the scientific or clinical questions addressed: irradiation (radiation equipment, doses and fractionation), delivery of the

combined molecule (route of administration, doses, schedules), therapy scheme (neoadjuvant, concomitant or adjuvant drug…)

- Defining the most relevant methods and tools (imaging monitoring, biomarkers…) and setting the required criteria to evaluate combined treatments' efficacy in preclinical trials: the best balance will have to be found between the possibility of applying these tools and criteria in preclinical studies and the possibility of translating them into clinical practice.

Many technical and ethical limitations stem from the use of small rodents (mice and rats) for preclinical experiments in radiation therapy. Immunocompetent or immunodeficient rodents are easy to use and well known in preclinical oncology but (i) the tumor microenvironment is not fully representative (ii) significant adaptations are needed for targeted irradiation and imaging and for collecting blood samples (detection of biomarkers).

- Promoting research on new preclinical models dedicated to combined "drug and radiotherapy" treatments;
- Facilitating the development and/or access to technical tools adapted to small animals;
- It would be useful to consider the use of larger animals (such as minipigs or dogs) to overcome the limitations. Two questions need to be addressed: is it scientifically and clinically relevant (i.e., will results obtained from big animals have a better predictive value)? is it technically and ethically feasible in France (i.e., are there sufficient infrastructures and know-how to conduct combined therapy trials using big animals)?

Researchers working in the field of "preclinical studies for combined radiotherapy and drug regimens" regret that access to new molecules is difficult, uncertain and often delayed, probably because pharmaceutical and biotech industries are reluctant. Worse, in some cases, combination therapies are applied in clinical practice without an established preclinical rationale.

- Facilitating partnerships with industry by creating the conditions for trust is urgent.

B. DIRECT RADIOSENSITIZATION

Three priorities are identified: delivery of the treatment, prediction and effects of radiation therapy, and the transfer of preclinical results to clinical practice.

- Treatment delivery raises questions about dose per fraction, standardization of irradiation protocols and access to linear accelerators for preclinical research.
    - The dose per fraction, particularly the effect of ultrahigh dose rate or FLASH irradiation, combined with nanoparticles such as radio sensitizers should be investigated. Moreover, the impact of hypofractionated treatment must be considered and studied.
    - The lack of standardized irradiation protocols is still a real problem because irradiation techniques cannot be compared between them. It would be very useful to form a working group to develop a standard irradiation protocol.
    - Researchers have limited access to linear accelerators for preclinical research, making it difficult to organize experimental measurements and wasting time. Financing solutions must be found to improve the conditions for implementing experimental measurements. The creation of specific calls for tender would be a solution.
- Prediction and effects of radiation therapy are essential in the study of direct radiosensitization. These require the implementation of preclinical imaging, research on dose calculation methods at cellular level and the choice of evaluation criteria.
    - Preclinical imaging is an essential tool to evaluate and understand the biodistribution of nanoparticles, particularly small-animal PET/MR imaging, for which further research on sensitivity and specificity is needed. Scintigraphic imaging (PET, SPECT) is another modality

to be developed for the quantification of biodistribution and pharmacokinetics of radiolabeled nanoparticles.
  - Knowledge of the absorbed dose at cell level is essential to quantify the effect of interactions between radiation and nanoparticles. Research on calculation and simulation methods must be reinforced.
  - Consideration should be given to the selection of simple common endpoints to assess tumor control, preservation of healthy tissue and activation of the immune system.
- Transfer from the preclinical to clinical stage requires an evaluation of preclinical results.
  - Standard criteria for evaluating preclinical results should be developed. A working group could be formed to propose this standard.
  - The framework of partnerships to evaluate molecules with manufacturers must be reviewed.

C. RADIOSENSITIZATION BY TARGETING THE MICROENVIRONMENT

To carry out this work, the choice of tumor models respecting the tumor microenvironment, particularly the immune one, is essential.

– The use of sequential biopsies or samples after preoperative radiotherapy should be encouraged to better characterize radiotherapy's interactions with molecules targeting the microenvironment. Mathematical modeling of these interactions will make it possible to better anticipate the clinical protocols that will emerge from these preclinical results. Evaluation of the tumor response by specific imaging will make it possible to transpose preclinical experiments to clinical practice.

– The microenvironment is composed of cells of multiple types and origins, such as vascular cells (endothelial, lymphatic, pericytes, etc.), immune cells (lymphocytes, macrophages, MDSC, DC, etc.), or stromal cells (CAF, adipocytes, etc.). It is necessary to better characterize the cell subtypes forming this microenvironment. There are few data on the plasticity of the tumor microenvironment subjected to irradiation. One population of the microenvironment cannot be isolated from others and interaction between all the cell types that make up the microenvironment makes multiparametric analysis of these interactions essential.

– Many communication routes need to be explored in the specific context of irradiation, whether the role of substances released into the microenvironment (proinflammatory cytokines, extracellular vesicles, microRNA, pro-angiogenic / antiangiogenic substances, paracrine hormonal signaling, particularly in the bone microenvironment, etc.) or direct communications by intercellular junctions / desmosomes. In particular, the neoangiogenesis or neovascularization mechanisms and inflammatory responses occurring after irradiation require particular analysis.

– The physical parameters of this microenvironment (degree of oxygenation, pressure and acidity) require precise analysis. Studying radiotherapy's effects on the microenvironment's metastatic permissiveness would make it possible to determine new therapeutic targets. Questions relating specifically to the radiotherapy technique's influence on the tumor microenvironment can be asked: influence of fractionation, dose rate and type of particles. The sequence of administration of molecules targeting the microenvironment with radiotherapy is worth investigating. Joint analysis of the tumor's molecular characteristics and relationship to those of the microenvironment will be an essential step in implementing treatment personalization approaches.

- <u>Description of collaborations and synergies between the teams and members of the network for each theme:</u>

This topic relies on multidisciplinary collaboration: small animal modeling, immunology, pharmacology, biomathematics, experimental therapeutics, medical physics for local dose enhancement, etc. Many actors from the Nanotherad initiative are working on these topics and considering combined treatments with drugs or nanoparticles. Partners like Gustave Roussy have

developed strong industrial partnerships to investigate drug combinations with radiotherapy. Startups like NhTheraguix have fostered powerful collaboration throughout the country to evaluate the AguIX nanoparticles' potential.

- International relevance:

In accordance with European Directive 2010/63/EU on the protection of animals used for scientific purposes, the topics aim at improving the relevance and predictive value of preclinical trials based on good practice guidelines and improving animal models and dedicated tools for experiments. This topic also fits with the European horizon program supporting the preclinical development of next-generation immunotherapies for diseases with unmet medical needs (HORIZON-HLTH-2022-DISEASE-06-02).

4) The fourth taskforce is dedicated to "Dose Modelling"

This taskforce is led by Ludovic De Marzi, David Pasquier and Etienne Testa.

- General background and scientific needs:

Radiotherapy treatments are mainly based on single *in silico* tridimensional dose calculation using patient-specific CT images and contours. Indeed, dose modelling by advanced statistical methods is of great interest in some circumstances. Monte Carlo (MC) simulations seem to meet the expressed needs well: they accurately compute organ doses for every patient using computational 3D models based on their CT images.

However, dose distributions are usually calculated on a snapshot of the living and breathing patient. It is necessary to move from planned dose maps to delivered dose maps. This issue is of major interest in the context of stereotactic ablative radiotherapy, reirradiation, adaptive radiotherapy and clinical outcomes in order to derive dose–response relationships. However, scientific problems are still unsolved, especially validation of deformable registration algorithms, measurement or computation of the daily dose and dose accumulation.

Because reducing the risk of sequelae and cancer in irradiated areas is an objective of RadioTransNet, the use of MC simulations could help to address various issues related to the radiotherapy field and associated imaging techniques (kV- and MV-imaging, radiology), as well as out-of-field dose estimation or the determination of patient dosimetry caused by imaging CT examinations for treatment preparation, delivery and follow-up. Dose calculation is important to better understand the dose–carcinogenic effect based on CT images because a simple relationship between material density and Hounsfield Units is taking into account patient heterogeneities. However, the perspectives of an increased role of MRI in radiotherapy encourage to led researches on dose calculation on MR images instead of CT images.

Dose modelling will make it possible to estimate accurately for each patient and each treatment, the out-of-field doses delivered by the therapeutic beam and the imaging positioning procedure used for IGRT.

Finally, dose modelling using MC calculation will be used to answer current questions such as simulation of innovative treatments like:

- ultra-high dose rate (Flash) or mini-beam radiotherapy (MBRT) for which accurate dosimetry is currently limited using standard methods and dose incertitude will have more impact on patients compared to current radiotherapy protocols,
- ions beams, as an example, in the case of protons the appearance of necrosis just behind Bragg Peak deposition has to be elucidated to prevent associated sequelae,
- nanoparticles, radionuclide or vectorized: by, for example, determining the local dose enhancement factor is crucial for the optimization of protocol.

- Main scientific objectives:

There are no internationally recognized recommendations/protocols on how to plan radiotherapy to minimize the risk of cancer in irradiated tissue. Indeed, during the entire treatment, dose to patient is not always recorded and calculated due to different diagnostic procedures. Moreover, the addition of physical doses from orthovoltage and megavoltage energy beams is controversial. Thus, new models of biologically effective doses are required to accumulate doses and consider previously absorbed doses in cases of reirradiation.

The developments of innovative techniques such as nanoparticles/vectorized, flash therapy or MBRT stress the need for the development of new tools for dose measurement and calculation. Furthermore, it also exists a gap between dose deposition and biologically effective dose which is currently unsolved. This gap has expanded considering these new irradiating techniques for which the linear-quadratic model is far from sufficient.

The RadioTransNet consortium's main ambitions for this purpose are: to calculate delivered dose to patients instead of planned dose to derive dose–response relationships; to develop new and reliable built-in software tools to estimate accurately and rapidly imaging and out-of-field doses; to develop tools and methods for accurate dose measurement and calculation dedicated to innovative beams; to develop mathematical models to sum biological doses or calculate biologically effective doses delivered by various beams.

- Program areas and tasks:

The RadioTransNet consortium identifies as a collective goal the progressive implementation of a Strategic Research Agenda (SRA) for the optimization of radiation exposure and standardization of radiotherapy practices. The research topics on "Dose Modelling" could be summarized as follows:

The primary goal of RT is to reach tumor control by minimizing healthy tissue complications. During the last decade, intensive research was performed to quantify the out-of-field doses produced by scattered radiations, which are known to enhance the risk of inducing a secondary cancer and complications of the cardiovascular and central nervous systems, fertility problems and other toxicities, for pediatric and young adult populations. Research should also include proton and carbon ions beams.

In addition to out-of-field doses, we should also examine additional doses delivered by X-ray imaging systems used in image-guided RT for patient positioning, known as imaging doses. The RadioTransNet consortium will focus on assessing out-of-field doses and concomitant doses due to positioning imaging procedures during radiotherapy.

The quantity used at present for clinical prescriptions in radiotherapy deliveries is absorbed dose to water. Therefore, for several radiotherapy modalities, such as ion beams, treatment planning is based on the product of the absorbed dose and a weighting factor accounting for the relative biological effectiveness of the respective radiation type. The question is how characteristics of the track structure can be used to predict the biological outcome expected for a given irradiation. It has been shown that nanodosimetric quantities derived from ionizing radiation track structures in mixed radiation fields are directly related to the biological outcome of the mixed field. They can be the basis for future treatment planning systems with biological optimization based on nanometric characteristics of particle track structures.

Only 3% of adult patients are included in clinical studies, which take a long time to initiate, and clinical results are difficult to register. Finally, the results are often irrelevant considering the development of practice and knowledge. Therefore, it is necessary to use treatment data from all patients to determine feedback and improve clinical practice. The main barrier to creating a machine-learning database is the poor quality of data, which are not standardized. To build an efficient clinical decision support system and radiotherapy that can correlate toxicities and tumor control probability to treatment data, actually delivered doses are required. Functional imaging after or during treatment will improve disease response assessment and registering these images with dose distribution is an active area of

research. Such registrations are necessary to enable quantitative evaluation of the tumor or normal tissue response to radiation.

The dose accumulation during overall treatment is of major importance to derive dose–response relationships, tumor control probabilities and normal tissue complication probabilities. However, this concept only considers physical dose. Modified fractionations or modified treatment modalities make it necessary to consider biologically effective doses. In clinical practice, the linear-quadratic model and the equivalent dose to 2 Gy fractions (EQD2) are used to compare different fractionations. There is a strong need for mathematical models that can accommodate modern radiotherapy cancer care. Models aiming to derive dose/volume constraints in a context of reirradiation are essential. These investigations should also consider relative biological effectiveness according to the linear energy transfer of particles.

The taskforce's SRA is presented below:

A. DOSE CALCULATION

Dose calculations are the key component of treatment planning systems (TPS) that ensure proper tumor irradiation while sparing insofar as possible healthy tissues and at-risk organs. Such calculations must, therefore, be able to estimate doses in both tumor volumes and healthy tissues (out-of-field doses). Moreover, doses induced by imaging, especially during image-guided radiotherapy (IGRT), must be accurately considered, which is not the case currently. Finally, the impact of treatment uncertainties must be minimized with improvements in each treatment step (imaging, planning and delivery). Monte Carlo (MC) simulations are the gold standard in terms of precision and are extensively used in research to improve estimates of any kind of dose (transversal research focus). The main research areas identified are the following:

- Monte Carlo (MC) simulations
  - Key role in the connection between physical dose calculation and biological endpoint predictions (e.g., Tumor Control Probability and Normal Tissue Complication Probability) using biophysical models
  - Use of AI for computing time acceleration. If direct predictions of dose distributions can be considered as science fiction, AI might replace MC methods to predict some probability distributions, boosting the computing time efficiency
  - Modeling of MRI-guided linear accelerators (MRI-LINAC) for precise physical (and biological) dose predictions
- Modeling of out-of-field doses
  - Need for improved experimental data (for model validation) and beam models
  - Need for patient data standardization
- Modeling of doses induced by imaging, especially during image-guided radiotherapy (IGRT)
  - Need for patient data standardization
- Robust treatment planning to consider treatment uncertainties and organ motion. Main areas:
  - Active motion management (e.g., breath control)
  - Probabilistic treatment planning methods
- Improved tissue characterization (dual-energy computed tomography, MRI, proton tomography...)

B. ADAPTIVE RADIOTHERAPY

The dose accumulation during overall treatment is of major importance to specify dose–response relationships, tumor control probabilities and normal tissue complication probabilities. Nevertheless, the clinical benefit and benefit/cost ratio have yet to be demonstrated. Many biological parameters related to the biological effect (fractionation, treatment duration) must be taken into account. There is no consensus on the appropriate manner of accumulating the dose. We lack robust validation of

dose accumulation algorithms based on elastic registration and also robust parameters to decide on new planning. In addition, more and more patients benefit from reirradiations and clinicians need to know how to consider previously absorbed doses.
– Adaptive radiotherapy implies research in the following areas: auto-segmentation, deformable registration, automated planning, advances in workflows (off and online), anatomical and functional imaging, and evidence of benefit in clinical sites.
– All of these main adaptive radiotherapy components: delineation, deformable registration, planning and decision-making may benefit from AI-based algorithms.
– MR-LINAC machines are particularly suitable for adaptive radiotherapy due to high contrast for soft tissues. Adapting the treatment (volume, dose) to the changing signal (diffusion, perfusion...) during radiotherapy courses should be areas of preclinical and clinical investigation. Adaptive radiotherapy according to the changing PET signal could also be studied.
– The development of an image and dosimetric database is suggested. This large-scale database would make it possible to link outputs (tumor control, toxicity) to the accumulated dose.

C. INNOVATIVE RADIATION THERAPY TECHNIQUES

A main limitation of RT remains that the dose delivered to a tumor is conditioned by how the surrounding normal tissues will tolerate it. Consequently, the treatment of radioresistant tumors (e.g., gliomas), tumors close to a sensitive structure (e.g., the spinal cord) and pediatric cancers is limited. Finding new approaches or innovative techniques to shift the normal tissue complication probability (NTCP) curve towards higher doses is therefore a priority in RT today.
Spatial fractionation (micro, grid or minibeam radiation therapy) is a RT technique derived from synchrotrons (x-rays) that combines the use of spatial fractionation of the dose with submillimetric field sizes. This approach can, in particular, be developed with protons or electrons to partner the inherent physical advantages of charged particles for radiotherapy with the gain in normal tissue preservation observed when irradiated with narrow spatially fractionated beams. Several studies evidence a very significant increase of mean survival time in preclinical investigations with MBRT, this significant increase of the therapeutic window could provide real hope may open new treatment strategies for high-grade glioma patients.
FLASH radiotherapy, a new treatment modality and irradiation technology currently being developed by several groups, involves the ultrafast delivery of radiation treatment at dose rates several orders of magnitude greater than routine clinical practice. It has recently been shown that FLASH with electrons, photons or protons is as effective as conventional irradiation for tumor inhibition while dramatically less damaging for healthy tissue. While the mechanisms underlying the biological effects remain to be elucidated, the FLASH effect has been very recently demonstrated in the first human patient with promising results, supporting further studies.
Recently, several studies showed significantly enhanced biological effects when human tissues were treated with nanoparticles before being irradiated. The use of boron-containing compound is an example with protons or neutrons: thermal neutron interactions can result in the production of energetic charged particles with high relative biological effectiveness (called boron neutron capture therapy, BNCT) as well as in proton boron capture therapy (pBCT), where low-energy alpha particles with high RBE can be generated. These kinds of techniques could be used during the treatment of radioresistant cancers. Although the increased effectiveness was proven experimentally, the exact mechanisms behind this effect remain a source of debate and have still not been elucidated.

D. NEW TOOLS DEVELOPMENT FOR RADIOBIOLOGICAL DOSE MODELING

Despite multiple simulations, some radio-induced responses still do not fit with models, such as dose enhancement with nanoparticles. The biological mechanisms of chemo-radio-induced responses in tumors and neighboring tissues are very complex and vary between different signs (age, gender,

genomics, proteomics) and symptoms of morbidity (e.g., reduced lung/liver tolerance due to smoking and alcohol consumption) or previous/parallel treatments. A contemporary view of radiation-induced responses involves multiple cell compartments that interact in a complex sequence of events following the radiation insult. Adaptive and innate immune systems, vascular networks, mesenchymal and epithelial cells and even microbiomes can contribute to the radio-induced response. Moreover, radio-induced response results from various biological cascades at numerous levels (molecules, cells, organs…). All these parameters should be considered for accurate *in silico* modeling.

The following research is proposed:
- Database creation to collect preclinical (chemicals, *in vitro* and *in vivo* models) and clinical data and improved access to Biological Resource Centers (BRC) to facilitate the use of these data by computational tools.
- Structured analyses: creation of facilities dedicated to complex analyses, recruitment of qualified staff (biostatisticians, bioinformaticians and bioanalysts) to perform such deep analyses; creation of a working group on preclinical radiotherapy: definition of clear and common annotations, definition of minimal endpoints and analyses, definition of processes for improving data quality control and traceability.
- Generation of radiobiological data following the working group's recommendations for data standardization and using the various beams available in the RadioTransNet network (i.e., beams from ResPlaNdIr) to evaluate radiobiological responses depending on beam parameters (particles, energy, dose rate, fractionation…), in combination or not with nanoparticles.
- Identification and improvement of existing mathematic models as well as development of new computational tools for radiation biology modelling. Absorbed dose is a macroscopic average quantity, while the biological effects of ionizing radiation are known to be related to the pattern of radiation interactions on the micro- and nanoscopic subcellular scales.

- <u>Description of collaborations and synergies between the teams and members of the network for each theme:</u>

This work package relies on multidisciplinary collaboration: engineering, physics, dosimetry, medical physics, biomathematics and data processing. As mentioned above, identified RadioTransNet partners are already working on this topic, i.e., members of the ResPlaNdIr network, IRSN and teams from the Nanotherad–Paris Saclay consortium. The topics will also benefit from the Nanox project emerging from M. Beuve's team (Lyon).