FY2023 Plans of Activities

I. Plans of Major Activities

Because much of RERF's research involves longitudinal or large scale long-term studies that take a significant time to plan and conduct, so a number of ongoing plans for such studies have already been presented under research achievements. Here we provide highlights of new research plans or selected plans of note.

1. Research Projects Examining A-bomb Survivors Health

1) Radiation and Cancer:

- *Pathogenesis of MDS (RP1-17, Miyazaki Y and Imaizumi M):* We will continue analysis of whole genome sequencing of blood samples collected before and after MDS diagnosis for identification of mutational signature and structural variation and assess specific alterations in high-dose exposed patients compared with low-dose exposed patients.

- *CML study (RP-P2-19 and RP-P1-23, Yoshida N)*: We will assess whether DNA and RNA from FFPE samples can be analyzed by high-throughput sequencing. We will prepare a full-scale research project proposal to evaluate the effects of radiation exposure on molecular and pathological findings among leukemia cases that developed shortly after A-bombing. We will continue current collaboration with Epidemiology Department and external leading specialists to conduct the study.

- *Updated LSS mortality report (RP 1-75) on cancer and noncancer diseases:* Analyses and publication of the LSS Report 15 will be the highest prioritized project in the next several years and will be continued in collaboration with the Dept. of Statistics (Sakata R and all members).

- *Molecular epidemiological studies on radiation and cancer:* Number and quality of available samples from Adult Health Study (AHS) subjects will be assessed and possible study designs will begin to be developed and the current efforts on inventory of autopsy specimens at RERF for availability of molecular investigations in collaboration with Dept. of Molecular Biosciences will be continued (Brenner A, Sugiyama H, Sakata R).

- *Providing information on vital status, cause of death, cancer incidence, and risk factors on the subjects to all departments:* Information will be provided upon request of scientists in all departments for planning their research protocols.

- *Hiroshima and Nagasaki tumor/tissue registries (RPs 18-61, 29-60):* Regular activities in National Cancer Registry and Nagasaki tissue registry will be continued under the contract with each authority. The Dept. of Epidemiology will continue to crosscheck the subjects of RERF major cohorts against newly registered data with approval from each authority. By March 2024, resource database of LSS cancer incidence will be updated for LSS through 2019 as the data collection from registries to RERF cohorts is performed biennially. Alternative methods of the ceased Hiroshima tissue registry will be developed in collaboration with local pathologists in Hiroshima. Analyses of population-based information (RP-S2-17) will be continued (Sugiyama H).

- *Pathology studies (RPs 5-89, 1-12):* The indexing of specimens of formalin-fixed paraffin-

embedded tissues within a new database will be continued in collaboration with the Biosample Research Center of RERF. Efforts to preserve and utilize pathological materials from A-bomb survivors will be continued in collaboration with local hospitals in Hiroshima and Nagasaki (Sugiyama H, Sakata R). Last several years, there has been no research scientist in the Laboratory of Pathology who is a pathologist. Dr. Tsuruyama of the Dept. of Molecular Biosciences was assigned a research scientist, concurrently.

- As part of our goal to examine genetic susceptibility and gene-radiation/environment interaction, we are planning a genome analysis study as part of a potential large-scale multidisciplinary GWAS program for all AHS subjects, consisting of approximately 25,000 A-bomb survivors, using old samples preserved since 1958. We will analyze genetic polymorphisms that may be involved in radiation-associated cancer development in A-bomb survivors. Studies will use the SNP array developed for analyzing genetic polymorphisms in a Japanese population. DNA samples extracted from either old Wright-stained smears, blood-infiltrated paper discs, or Giemsa-stained chromosome slides can be used to conduct a genome study for all AHS subjects. For this reason, it is necessary to determine whether SNP analysis using this SNP array is possible by amplifying the whole genome using a very small amount of DNA obtained from blood specimens stored for many years. In FY2023, an appropriate whole genome amplification method using the REPLI-g amplification kit will be continued using DNAs extracted from smears stored 10, 30, and 50 years ago, paper discs stored 20 years ago, and Giemsa-stained specimens stored 30 years ago. The amounts of DNAs extracted and amplified, and the size and characteristics of the DNAs will be measured. The DNA derived from fresh blood specimens and amplified DNA derived from stored specimens will be genotyped for approximately 670,000 or more SNPs using SNP arrays. SNP assays of fresh DNA and amplified stored DNA will be compared, and the applicability of stored-derived DNA will be evaluated by RERF statisticians. (Hayashi, RP-P2-22).

- Most solid cancer tissue samples of the A-bomb survivors have been preserved as formalin-fixed paraffin-embedded (FFPE) tissues in the pathology laboratory of the Department of Epidemiology. We will aim to establish techniques enabling the molecular characterization of FFPE tissue samples. In particular, we will create a pipeline consisting of protein elution-liquid chromatography/mass spectrometry (LC/MS)-algorithm for biomarker identification. This study will include multi-processes, such as chemical pretreatment of FFPE, optimization of protein extraction efficiency, the establishment of the pipeline of LC/MS, artificial intelligence algorithm, and MALDI-TOF/ICP mass spectrometry imaging. A lung cancer case study has been planned in a new RP, and plans to analyze approximately 60 lung cancer samples. The plan will include the re-preparation of HE-stained specimens for reevaluation of the tissue subtypes. In addition, we will perform immunostaining to identify molecular profiles of the lung cancers (TTF1 for adenocarcinoma; p60, p63, and CK5/6 for squamous cell carcinoma; Napsin A, EGFR, ALK, ROS1, PD-L1, and BRAF). Neuroendocrine tumor markers, such as NSE and chromogranin A, will be used for diagnosing small-cell lung cancer. In addition, differentiation from mesothelioma (Calretinin, D2-40, WT-1) is necessary. This year, proteins related to stress response, such as RhoA and vigilin, were identified. They are available as immunostaining markers that distinguish between malignant mesothelioma and lung cancers. (Hiratuska T, Tsuruyama T et al, Scientific Rep., 2022). Evaluation will be performed according to the pTMN classification with the addition of progression data. The lymph node metastasis and distant organ metastasis will be checked using autopsy records. The diagnostic report will serve as basic data for future molecular epidemiological research. Presentations on 1) molecular pathology

associated with radiation exposure and lung cancer and 2) tissue profiling of lung cancer in atomic bomb survivors are expected. Interim follow-up reports may be submitted to pathology journals and lung cancer and respiratory disease journals. The research period is planned to be three years. (Tsuruyama, new RP).

2) Radiation and Non-Cancer Effects:

- Examine the relationships between the incidence of noncancer diseases and atomic bomb radiation dose using the updated longitudinal data with about 20 years of follow-up since the last report. Study population includes not only ME 200 (original AHS cohort) but also ME 200-1 expanded in 1977 (survivors with radiation dose of 1Gy or greater and their controls) and ME200-3 expanded in 2008 (younger survivors exposed at the age of less than 10 years).

- *Atherosclerosis study [RP2-11 (Part 2 of RP7-09), Nakamizo T]*: We will finish the assessment of the availability of the values of multi-functional cytokines and publish the results. Subsequently, we will start analyzing the data on cytokines representing disturbed tissue repair and differentiation potentially involved in the pathogenesis of radiation-induced atherosclerosis. The analysis will be conducted jointly with the clonal hematopoiesis program project because those pathological process are interrelated to inflammation.

- *Program project: Clonal hematopoiesis and inflammatory phenotypes potentially related to atherosclerosis risk in atomic-bomb survivors, project 2 (Nakamizo T):* We will finish examining the availability of the cytokine measurements. Subsequently, we will first evaluate the relationship between inflammatory biomarkers and atherosclerotic indicators gathered in RP7-09. The analysis will be extended to T cell subsets representing T cell aging, and hematological profiles potentially representing clonal hematopoiesis.

- In the Project 1), we have developed strategies for assessments of clonal expansion of HSCs (i.e., clonal hematopoiesis) and inflammatory changes in the hematopoietic system potentially contributing to the radiation-associated noncancer diseases, specifically atherosclerosis. Project 1 of this program aims to test the hypothesis that, in AHS subjects who were exposed to high-dose (> 1Gy) radiation several decades ago, clonal hematopoiesis is promoted with recurrent somatic mutations in epigenetic modifier genes (*TET2, DNMT3A, ASXL1,* etc) and/or DNA damage response genes (*TP53*, *PPM1D*, etc). Clonal hematopoiesis with somatic mutations will be evaluated by performing NGS using cryopreserved blood cells from about 100 subjects, in collaboration with Nagasaki University, Kyoto University, and the University of Tokyo. Plasma levels of endogenous danger signals (alarmins), which may promote clonal expansion of HSCs, will also be assessed in relation to the development of clonal hematopoiesis following radiation exposure. This project has been approved by the Non-cancer Research Cluster, outside scientific experts, and the RERF Committee on Biological Samples, and the IRB. This study is expected to be fully approved and initiated in 2023. (Yoshida and Kusunoki, Clonal Hematopoiesis Program, Project 1).

3) Genetic Effects of Radiation:

- The current plan for the trio study is to analyze a total of 580 children, 290 children born to parents in the high-dose group (>1 Gy) and 290 children born to parents in the control group (negligible exposure dose). Initially, genomic DNA will be extracted from stored blood lymphocyte samples of the family trios, including atomic bomb survivors. The DNA will be used for SNP-chip analysis and WGS analysis using short read NGS. SNP-chip analysis will

confirm parent-child relationships and detect newly occurring copy number mutations in the children. WGS analysis will be performed with an average coverage of 60x to detect newly occurring de novo mutations (base substitutions, small indels, structural variants) in the children. For mutation validation, some samples will be subjected to WGS analysis using long read NGS. We will then evaluate the transgenerational effects of radiation exposure at the whole genome level by examining the relationship between the number and characteristics of mutations occurring in each child and the parental exposure dose. Analysis of the genomic data will be conducted in collaboration with Dr. Chanock of NCI, USA, and Dr. Nakagawa of RIKEN, Japan. Access to the genome data for analysis will be through a cloud-based environment. (Uchimura, new RP in CR162 F_1 umbrella program project).

- Based on published paper this year (Uchimura et al 2022), we are planning to start several new collaborative studies, including a project to analyze radiation-induced mosaicism in clonal hematopoiesis (PI: Dr. K. Yoshida). This year, Dr. Uchimura has applied for a new MEXT research grant of 150 million yen (for a period of 3 years) in collaboration with external researchers: Associate Prof. Hashimoto (Osaka University), Prof. Honda (Okayama University), Prof. Matsuura (Hiroshima University) and Associate Prof. Matsumoto (Nagasaki University). If the application is accepted, we plan to begin detailed analysis of mutation history and cell lineages in early human embryos. (Uchimura, New RP to be developed as a part of F_1 umbrella program project).

- RERF is conducting genomics and omics studies of animal experiments and planning those utilizing atomic bomb survivors samples. Drs Liu and Misumi are working with a single cell sequence data of mice experiment conducted by Dr. Yoshida and applying a standard pipeline to investigate differential expression of genes associated with radiation exposure. Also, Dr. Liu and other members of the Department of Statistics are collaborating with MBS and Dr. Ono of the ITD to find out the needs of our future bioinformatics capability including the computational infrastructure. Moreover, massive multi-omics data are available in public domain. Drs Liu, Misumi, and other members of Department of Statistics are considering exploring the potential of integrating public omics data with ongoing genetics and omics studies at RERF.

2. Research Projects on the Health of A-bomb Survivors Children (F_1)

- *Mortality surveillance in LSS, in utero, and F_1 cohorts (RPs 1-75, 2-61, 4-75):* Mortality follow-up for all cohorts will continue and the data will be completed through 2019. Archiving of early-period materials will continue in collaboration with the Research Resource Center of RERF (Sakata R).

3. Research to Elucidate Individual Radiation Doses and the Effects of A-bombs

- With respect to dosimetry error specifically, there are a number of potential issues with the current dose error corrections used at RERF that we plan to address. First, the current dose error adjustments are based only on a classical dose error model – e.g., errors due to misspecification of the location of the subject – but do not consider additional so-called Berkson type error – e.g., that due to the fact that individuals at far distances, and therefore with lower doses, do not have shielding histories, so that average transmission factors for the corresponding shielding scenarios (inside, outside, etc.) are assigned. Second, the current classical regression calibration dose error corrections are only applied at higher doses (> ~500 mGy in Hiroshima, > ~ 700 mGy in Nagasaki, shielded kerma). In addition, the recent

analysis of chromosome aberrations points to a systematic overestimation of doses for Nagasaki factory workers. In light of these issues and also of the likely adoption of new organ dose estimates, a re-evaluation of the issue of dosimetry errors is warranted.

- The primary focus of our work on dosimetry during FY2023 is continued preparation for the implementation of the revised organ dosimetry using updated computational phantoms, as described above. Implementation of the revised organ dosimetry is planned for 2023.

4. Project to Release of Research Results and to Collaborate with Other Scientific Organizations

Continuing collaborations: Long term collaborations are listed below and these are expected to continue in 2023:

a. Partnership with the University of Washington
b. Partnership with Kurume University
c. Collaborations with the US National Cancer Institute
d. Collaborations with the University of Florida
e. Collaborations with Outside Investigators:
   45 Japanese Institutions
   7 North American Institutions
   8 European Institutions

5. Training Programs for Domestic and Overseas Specialists

RERF will hold a training course for non-epidemiologist radiation researchers to learn the basics of epidemiological research and increase understanding of radiation health risks. In addition, RERF will train persons capable of working in the fields of radiation protection, radiation emergency medical care, and radiobiological research. Decisions on whether these activities could be held and how they would be held will be made considering the status of COVID-19 pandemic.

Activity plans for FY2023:

i) RERF intends to hold an online or in-person epidemiological training course for radiation biologists in Japan to enhance understanding of results from epidemiology research on A-bomb survivors.

ii) RERF will accept overseas research trainees to support the activities of such organizations like the International Council for Health Care of the Radiation-exposed (HICARE), the Nagasaki Association for Hibakusha's Medical Care (NASHIM), and the Japan International Cooperation Agency (JICA).

iii) If there are any invitations for the MHLW-sponsored FY2023 International Exchange and Research Program, RERF will consider accepting physicians and researchers from overseas to provide them with training.

iv) The Department of Statistics will apply to the International Fellowships for Research in Japan program, sponsored by the Japan Society for the Promotion of Science (JSPS), upon application from candidate trainees, and provide research opportunities to young post-doctoral researchers from various countries.

6. Public Information Programs

From the days of its establishment until the present, RERF has investigated medical effects of radiation in atomic bomb survivors and their children (second-generation survivors). Of utmost importance among RERF's public information programs are its efforts to communicate research results in simple and concise fashion to atomic bomb survivors and their children, both of whom have long cooperated in RERF's research, as well as to the public. In fiscal year 2023, RERF will work on the public information programs outlined below to ensure that such target audiences can gain further insight into RERF's work.

i) RERF Open House event

The Hiroshima and Nagasaki RERF laboratories will hold their 28th and 26th Open House events, respectively. Keeping in mind the downgrade of the coronavirus classification in Japan under the infectious disease law in May 2023, RERF is considering holding the 2023 Open House event with a focus on an exclusive online portal site. However, RERF is also looking into the creation of a 'hybrid' Open House event that includes not only video postings on the portal site, but also 'live events' and limited in-person activities.

ii) Public outreach related to RERF's genome sequencing analysis study

RERF will initiate public relations activities when consent for this study is obtained from participants. RERF will publicize information about the genome sequencing analysis study through social media—with a focus on the creation of videos introducing the research—and share information with the media when necessary. In both cases, the genome sequencing analysis research will be explained to the public using language that the layperson can readily understand.

iii) Promotion of public relations activities targeting media

RERF will respond to media requests for information in an effort to ensure that coverage is fair and unbiased. In FY2023, RERF will continue to hold guidance sessions for the media as necessary to ensure accurate understanding of RERF's activities. In particular, RERF will respond to requests for reporting on genome sequencing analysis research and relocation from the Hiroshima Laboratory, topics in which media interest is high, in consultation with relevant parties.

iv) Enhancement of social media-related activities

RERF will work to publicize its genome sequencing analysis research in FY2023 in concert with public relations activities on its Facebook and Twitter accounts. RERF will manage risk on its social media accounts with users always in mind, fully utilizing the unique characteristics of RERF and making an effort to consistently develop follower support not merely increase follower numbers.

v) Full utilization of video-production system

In place of its email magazine (E-News), which was terminated late last year, RERF will focus on the production of videos that involve RERF's planned genome sequencing analysis study and the foundation's latest research results. Such video production will also include interviews with scientists about specific research in which they are engaged as well as other related stories, as an enhancement of the RERF research-paper synopsis project.

vi) Enhancement of RERF website

In FY2023, RERF will continue aiming at enhancement of its homepage through further refinement of information delivery related to our research-paper synopsis project. Particular focus will be placed on conveying readily understandable research and other information to the public through utilization of more informational videos and other methods. RERF will renew the formatting and content of the homepage, which appears slightly dated at this point, five years after its 2018 inception.

vii) Changes to facility tour program

This program has been used effectively for years to introduce RERF facilities and research results to large numbers of visitors, both domestic and overseas. However, with reduced staff numbers and the continued coronavirus pandemic, a more efficient system is necessary. In FY2023, RERF is considering offering limited periods and numbers of times during which people can make reservations to listen to presentations at Hijiyama Hall, a system designed to reduce coronavirus risk.

viii) Other public relations activities

- RERF will actively promote the foundation's important scientific papers to domestic and overseas media by sending out press releases and holding press conferences.
- RERF's new public lecture series, initiated in 2019, targets peace volunteer guides, among other such individuals, in partnership with external organizations such as the Hiroshima Peace Memorial Museum. This series was temporarily shelved due to the coronavirus pandemic but will be reinitiated once the coronavirus is contained.
- RERF has been accepting interns, mostly those with scientific backgrounds, for some time, but in 2023 RERF hopes to work on inviting students and trainees to learn about how to perform RERF's facility tours and other public relations work.
- With the aim of improving public understanding of its research, RERF initiated in FY2018 a new series of paper synopses that explains our research with simpler prose and smaller word counts than used previously in our "summary explanation" series for specialists. Through continuation of the new, shorter series in FY2023, the aim is to improve understanding by the public and media of RERF's research.
- A continued goal in FY2023 will be the creation of a system for RERF personnel to be able to handle facility tours/presentations in English for overseas visitors.
- To enhance our effort at communicating RERF's research with the aim of improved transparency related to RERF studies and establishment of good communication with the public, in particular A-bomb survivors, their children, and the media, the Public-Awareness Campaign working group was formed in January 2019. In FY2023, the PAC working group will meet and discuss about how to provide information to the public on important topics such as the human genome, genetics studies, and other such topics.
- When it is possible again after the coronavirus pandemic is contained, RERF will continue to target small public groups of A-bomb survivors and their children inviting them to visit RERF and speak with directors and staff about ABCC/RERF history and research results, with the aim of achieving greater understanding about RERF.
- Centered on the Public Relations and Publications Office, and in cooperation with other relevant departments and offices, RERF will take care to respond to daily inquiries that come from outside the institute. At the same time, the PR office will quickly manage matters that only it can handle and, in that way, work to improve operational efficiency.

II. Operation and Management of RERF

1. Research Resource Center

As its endeavor to preserve RERF's research assets and historical materials, the Research Resource Section will continue organizing and digitizing the inventories of pathological samples kept by the Department of Epidemiology, digitizing the negative films of chromosome images, and compiling an electronic database of their ledgers, which are kept by the Department of Molecular Biosciences. Furthermore, the preparation for introducing the Content Management System to centrally manage paper-based materials including paper manuscripts and research protocols will continue to be moved forward. The preparation to introduce the Data Management System to enhance the availability of RERF's research assets will be continued by evaluating the results of the pilot project involving the foundation's actual data. Finally, personnel will be assigned to the Office of Research Support to start the support for administrative research procedures, the management and use of intellectual property, and the support for external grant application, while the preparation to introduce the electronic workflow system to digitize the foundation's decision-making processes will continue to be advanced.

2. Relocation of the Hiroshima Laboratory

We will go ahead with the plan to relocate the laboratory to Hiroshima University's Kasumi Campus and, together with the demolition of the existing building on the campus, will aim to start construction work within the fiscal year by preparing execution design for the new building.

3. Full Audit

The audit report on the voluntary audit (full audit), which is a new process to be taken from FY2022, will be submitted by the auditing firm to the Auditors in May 2023. In addition, at the annual BOC meeting in FY2023, the Directors will report on the progress made in establishing an audit system, and the Auditors will present their audit report. It is planned to enter into another audit contract with an external auditing firm to undergo another voluntary audit in FY2023.

4. Revision of the rules and regulations

RERF revises the current regulations and establishes new regulations by responding to its auditing firm's findings and results of regular reevaluation by the sections in charge. Along with continuing to respond to the auditing firm's findings quickly and suitably in FY2023, required revisions will be made to keep the foundation's management operations appropriate. Doing so will provide RERF with regulations befitting of a public interest incorporated foundation funded by the U.S. and Japanese government subsidies.