

FY2021 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:

- *Updated cancer incidence (RPs 1-75, 18-61)*: Papers on comparison of cancer incidence and mortality (Brenner A, et al.) and a summary paper (Brenner A, et al.) will be published then this series of papers will be completed.
- *Updated LSS mortality report (RP 1-75) on cancer and noncancer diseases*: Periodical publication of mortality risk of cancer and noncancer disease due to atomic bomb radiation is the most essential work of the Dept. of Epidemiology and the LSS Report 15 will be the highest prioritized project in the next few years. Available data and the framework of analyses will be determined and preliminary analyses will begin in collaboration with the Dept. of Statistics. Dose-response shape and radiation risk at low dose levels will be investigated considering potential confounding by geospatial factors and variation in baseline rates. Those analyses will include lifestyle factors (smoking, alcohol drinking, body mass index), some indicators representing socioeconomic status, and so on. This is a complex project that will require several years to complete (Sakata R and all members).
- As part of our goal to examine genetic susceptibility and gene-environment interactions, we are planning a genome analysis study as part of a potential large-scale GWAS (genome-wide association study) program for all AHS subjects, consisting of approximately 20,000 A-bomb survivors, using old samples preserved after 1958. We will analyze genetic polymorphisms that may be involved in radiation-related cancer development in A-bomb survivors using the Japonica Array developed for analyzing genetic polymorphisms that may be involved in cancer development in a Japanese population. DNA samples extracted from old Wright-stained smears must be used to conduct a genome study for all AHS subjects. For this reason, it is necessary to determine whether an SNP analysis using the Japonica Array is possible by amplifying a whole genome, using a very small amount of DNA obtained from a blood smear stored for many years. In FY2021, DNA extraction from preserved, old blood smears of AHS subjects will be carried out to examine a suitable preparation method for REPLI-g amplified DNA. Then, we will evaluate DNA availability by analyzing those DNA samples with the Japonica Array. (Hayashi, RP-P1-19, and a new RP).
- *Pathogenesis of MDS (RP1-17, Miyazaki Y and Imaizumi M)*: We will continue to conduct phylogenetic analyses to clarify clonal dynamics and evaluate genome data with clinical course to find driver mutations and CNAs (copy number alterations), and assess specific alterations in high dose patients compared with low dose patients. We will submit a manuscript for publication in 2022.
- *CML study (RP-P2-19, Yoshida N)*: We will continue current collaboration with Epidemiology Department and external leading specialists to evaluate the availability of

DNA and RNA extracted from old formalin-fixed paraffin embedded (FFPE) samples in molecular analysis. We will confirm whether DNA and RNA from FFPE samples can be analyzed by droplet digital PCR and high-throughput sequencing. We will complete the series of current pilot studies in 2021 and prepare a full research project proposal based on the current results in 2021.

- Biologically-based mechanistic modeling will be one of the approaches to derive biological hypotheses from our epidemiological data. As a next project, we will analyze the LSS colon cancer data. We described a possibility of screening effects related to colonoscopy and different radiation risks among the sites of colorectal cancers in the latest update of LSS colon cancer incidence (Sugiyama, Misumi, Brenner, International Journal of Cancer, 2020). An application of a mechanistic model to the previous LSS colon cancer data showed an association between radiation and chromosome instability (CIN) pathway of colon carcinogenesis, but not between radiation and microsatellite instability pathway (Kaiser et al. PLoS One, 2014). We will investigate these associations between radiation and molecular pathways taking account of the possible screening effects utilizing the latest data in collaboration with Dr. Kaiser of Helmholtz Zentrum Munchen. The result will give us better understanding of the applicability and possibility of the mechanistic modeling. With the better understanding, we will consider extending the application of this approach to other cancer sites.

2) Radiation and Non-Cancer Effects:

- Conduct our Clonal Hematopoiesis program project that consists of 4 projects 1) Detection of clonal hematopoiesis in AHS samples, 2) Evaluation of inflammation markers, 3) Mouse model of clonal hematopoiesis and 4) Mathematical design. We are now working on 1) and 3).

(1) For Project 1, we have developed strategies for assessments of clonal expansion of hematopoietic stem cells (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 next-generation sequencing (NGS) using cryopreserved blood cells from about 100 subjects, in collaboration with Nagasaki University and Kyoto University. 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 Biosamples, and applied for the IRB review (Yoshida and Kusunoki, a new RP of the Clonal Hematopoiesis Project 1). This study as yet to be fully approved even next year, until RERF develops institutional policy concerning the use of cryopreserved samples for obtaining NGS data in A-bomb survivors. With application of an established policy to this study, we will start the study as soon as possible. (Yoshida, a new RP in the Clonal Hematopoiesis Program Project, Project 1).

(2) Project 3 involves the use of mouse models. Current mouse CH studies rely on very

limited preliminary data that can support the feasibility of the study and that enable power calculations to determine the sample size. Thus, we are analyzing preliminary whole-exome sequencing (WES) data in 12 and 7 mice irradiated with 3 and 0 Gy, respectively, and are detecting recurrent mutations in bone marrow (BM) cells with their frequencies >0.02 . By targeted amplicon sequencing, we will analyze the frequencies of the mutations in hematopoietic cell populations including myeloid, lymphoid, and stem and progenitor cells, as well as in non-hematopoietic tissue cells including brain and tail tissue cells, which will determine whether the mutations are embryonic mosaic or hematopoietic specific and whether they are the hematopoietic stem or lymphoid (i.e., mature T or B cell) origins. We will also develop experimental protocols that analyze LDL-receptor knockout (LDLR-KO) mice fed a high fat diet following whole-body irradiation, for 1) identification and phylogenetic tree construction of early embryonic spontaneous somatic mutations (SPMs), 2) CH identification in BM through WES, and 3) SPM-based evaluation of clonal monocyte accumulation in atherosclerotic plaques. Preliminary experiments using these experimental protocols will enable power calculation to adequately determine the sample size and a detailed design of a full-scale study investigating the frequency of radiation-induced CH and the involvement of CH in atherosclerosis formation. (Kusunoki, Yoshida, Taga, Hamasaki, Satoh, Uchimura, Misumi and Noda, a new RP in the Clonal Hematopoiesis Program Project, Project 3).

- *Cataract study*: We will write a manuscript regarding the relationships between several types of cataracts and radiation among subjects who were <15 years of age at the time of bombings (excluding *in utero* exposed subjects). This will be submitted for publication during FY2021. Analysis of the relationships between radiation and cataracts among *in-utero* exposed subjects will be started in collaboration with the Dept. of Statistics.
- *Atherosclerosis study (RP2-11, part 2 of RP7-09, Nakamizo T)*: We will start analyzing data on cytokines representing disturbed tissue repair and differentiation potentially involved in the pathogenesis of radiation-induced atherosclerosis. This analysis will be conducted jointly with the clonal hematopoiesis program project because those pathological processes are interrelated to inflammation, a key player involved in the pathogenesis of radiation-induced atherosclerosis.

3) Genetic Effects of Radiation:

- Previous animal model studies identified more small deletions and multisite mutations in F1 mice of 4 Gy-irradiated spermatogonia and mature oocyte than in those from unexposed parents. However, larger size mutations including structural variants are not fully understood. Currently, we are developing a new pipeline to detect larger size mutations using long-read NGS techniques such as PacBio, Oxford nanopore and Bionano Saphyr. Our new pipeline is meant to give us the ability to uncover spontaneous de novo mutation rate and characteristics of such variants (especially in transposon mutations) in mice. (Satoh, RP 2-13 addition).
- For our germline stem (GS) cell studies described in the achievements section, mutations in irradiated GS cell clones will be characterized by array CGH (comparative genomic hybridization) and whole-genome sequencing (WGS) analyses, and then the cell clones will be transplanted into Busulfan-treated testes of adult male mice. We also plan to make artificial large-scale deletions or inversions that simulate radiation-induced chromosome alterations in GS cells via newly developed gene editing systems. After the transplantation, we will examine the offspring derived from the GS cells for heritable genetic changes.

(Noda, RP-P3-17).

2. Research Projects on the Health of A-bomb Survivors Children (F₁)

- Continue to conduct health examinations among the cohort members of the FOCS. This will continue at least until approximately 2050 to evaluate lifetime risk.
- Continue data cleaning to verify development of multifactorial diseases among about 10,000 FOCS participants.
- Establish methods of analysis based on multi-state models to investigate the effects of parental radiation exposure on hypertension, dyslipidemia, and diabetes in F₁ offspring of A-bomb survivors in collaboration with Department of Statistics.
- *F1 cohort study (RPs 4-75, 18-61)*: Regular collection of follow-up information will be continued.
- For WGS studies of the trios comprised of atomic bomb survivors and their offspring, we will develop a research protocol to conduct these studies. In addition to the scientific proposal, a key element in the initiation of these studies are ELSI and obtaining informed consent since many of the parents in the trio sets are deceased.

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

- We plan to further dose error work related to biodosimetry. A joint collaborative project involving the Departments of Statistics and Molecular biosciences, will develop and execute a new analysis project which seeks to combine all currently available physiological information about radiation exposure (e.g., electron spin resonance of tooth enamel, chromosome aberration frequency scored using Giemsa and FISH methods, and the presence of acute symptoms) using structural equating modeling in order to characterize and quantify the extent and nature of dose error in the DS02 system.
- The primary focus of our work on dosimetry during FY2021 is preparation for the implementation of the revised organ dosimetry using updated computational phantoms. This includes completing the conversion of existing FORTRAN code and the development of additional computational modules. While the Department of Statistics will become prepared to implement the new organ dosimetry, the ultimate decision whether and when to implement this new dosimetry will be made by the RERF leadership in conjunction with US Department of Energy (DOE) and the Japanese Ministry of Health Labor and Welfare (MHLW).

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 2021:

- a. Partnership with the University of Washington
- b. Partnership with Kurume University
- c. Partnership with Osaka University
- d. Collaborations with the US National Cancer Institute
- e. Collaborations with the University of Florida
- f. Collaborations with Outside Investigators:

- 44 Japanese Institutions
- 9 North American Institutions
- 8 European Institutions
- 1 Asian, Oceanian Institution

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. Furthermore, decisions on whether these activities could be held and how they would be held will be made considering the rate of COVID-19.

Activity plans for FY2021:

- i) RERF will hold an online 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 FY2021 International Exchange and Research Program, RERF will consider accepting trainees from overseas.
- iv) The Department of Statistics will apply to the FY2021 International Fellowships for Research-in-Japan program, sponsored by the Japan Society for the Promotion of Science (JSPS), 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 the medical effects of radiation, in atomic bomb survivors and their children (the second-generation). In terms of RERF's public information programs of utmost importance are our efforts to communicate in simple fashion our research results to atomic bomb survivors and the children of A-bomb survivors, both of whom have long understood and cooperated in RERF's research, as well as members of the public. To succeed at communicating with such audiences, good relations with national and international media, starting with members of the media in Hiroshima and Nagasaki, are crucial to reaching a wide audience with our messaging. In FY2021, RERF will work on the public information programs, outlined below, to ensure that such target audiences can gain further understanding about RERF.

- i) RERF public lecture series

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. Based on such efforts, RERF will be able to provide an opportunity to enhance understanding

of the foundation's research and learn more about radiation's health effects to even greater numbers of atomic bomb survivors, second-generation survivors, and the public.

ii) RERF Open House event

RERF will hold its 26th and 24th Open House events at the Hiroshima and Nagasaki RERF Laboratories, respectively. In FY2021, RERF has decided to take a different stance and hold the Open House event in a virtual format, out of consideration of the effects of the continued spread of the coronavirus.

iii) Strengthening of social media-related activities

Given the limited contact with the public due to the COVID-19 pandemic, social media such as Facebook and Twitter has become the most effective method of communicating with the outside world. In FY2021, because the most important task facing RERF is obtaining understanding from atomic bomb survivors, the second-generation, local communities, and the media, social media will be used effectively to achieve that goal. Through a particular focus on visual information on Facebook and Twitter, a series of videos will be created to give outside people access to our facilities, research, explanations of research policies, and so on, through a virtual format. RERF will also continue refining our messaging on the platforms, with the aim of both increasing our follower numbers and expanding our reach and engagement through increased sharing of our posts by our followers.

iv) Promotion of public relations activities targeting media

RERF will respond to the extent possible to media requests for coverage of RERF related to various issues surrounding our research and policies this year, with the aim of allowing the media to accurately understand ABCC-RERF's research achievements and to report based on that clear understanding. In FY2021, RERF's PR staff plans to hold lectures and study sessions for the media, in continuation from similar efforts made last year.

v) Enhancement of RERF website

In FY2021, RERF will continue aiming at enhancing the homepage. Particular focus will be placed on conveying readily understandable research-related and other information to the public, through the utilization of more video and other methods.

vi) Enhancement of online news-delivery system

RERF's email magazine system, E-News, which replaced the printed *Update* newsletter, will be enhanced in terms of content and used to not only distribute the latest research results and information about RERF events and activities but also to attract readers to RERF as "members," by offering a sense of "buy-in" with respect to RERF as an organization.

vii) School Visit Program

This program was first established in FY2016 to convey to elementary, junior-high, and high-school students the reality of radiation health effects. Based on the program's popularity, each year, numbers of requests for such classes increase and a now stable group of available teachers has provided RERF the opportunity to teach an increased number of classes. In FY2021, RERF will work to expand its public relations activities by, in addition to conventional on-site lessons, conveying to students how to teach the basics of radiation to their peers in a student-led education drive.

viii) Internship (work experience) project

RERF has been accepting interns, mostly those with scientific backgrounds, for some time, but RERF plans to invite trainees such as students to learn about how to perform RERF's facility tours and other public relations work.

ix) Other public relations activities

- RERF will actively promote the foundation's important scientific papers to the domestic and overseas media via press releases and press conferences.
- With the aim of improving public understanding of our research, RERF initiated in FY2018 a new series of paper synopses that explain research with simpler prose and smaller word counts than used previously in our "summary explanation" series for specialists. Through continuation of this series in FY2021, the aim is to improve understanding by the public of RERF's research results.
- A persistent goal again in FY2021 will be the training of more RERF personnel to handle facility tours in English for overseas visitors, in preparation for when the tours are restarted in the future after the coronavirus pandemic is contained.
- By increasing our effort at communicating RERF's research with the aim of improved transparency related to RERF research 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 FY2021, 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, who will be invited to come to RERF and speak with directors and staff about ABCC/RERF history and research results, with the aim of achieving greater understanding about RERF.

II. Operation and Management of RERF

1. Research Resource Center

The highest priority activity in 2021 is the formalization of the Research Resource Center (RRC) by the RERF ad hoc committees in charge of establishing the RRC. Decisions regarding physical location within the institute, internal structure (Sections), leadership, reporting lines, personnel and budget are all needed. After those decisions are made, obtaining personnel and initiating the Office of Collaborative Research would provide immediate benefits to the institute.

The RRC Technical Team needs to decide if Gen3 is a suitable Data Commons for RERF's needs. If yes, then a full-scale installation and support structure needs to be put in place. If Gen3 is abandoned, then a pilot project using different software will need to be initiated.

The Content Management System (CMS) should see significant progress in 2021 with a recently hired external consultant expert in CMS development. The CMS will store and categorize scans of paper documents and digital publications. It will allow full search, a feature that is currently not available at RERF.

RERF's multifunction copiers will be programmed so that scanning can be performed locally and deposited directly into the CMS.

A digital presence (web site) with rudimentary functions (data search/assembly, manuscript/RP search, streamlined procedures to initiate data sharing, etc.) will be put in place.

2. Review on the relocation of the Hiroshima Laboratory

We will proceed with deliberations on the proposals for candidate relocation sites for achieving RERF's strategic plans, revolving around the Hiroshima Comprehensive Health Center and Hiroshima University's Kasumi Campus.

3. Transition to a full Audit

To supplement the Auditors' audit, RERF aims to receive a full audit (financial audit) from an external corporation. From now, RERF will prepare to transition to a full audit from FY2022 because there are good prospects that the conditions for the transition will be met, recording the retirement allowance liability and evidence of US subsidy income. Furthermore, RERF plans to select a contractor for the financial audit, in keeping with the opinions of the Auditors and internal regulations, by the regular BOC meeting in June 2022 and sign an audit contract by August of that year when administrative procedures for the financial audit are set to begin.

4. Introduction of an attendance and work management system

Following the enforcement of the work-style reform law enacted in June 2018, RERF will introduce an attendance and work management system with the primary goal of monitoring employee work hours objectively. RERF will analyze the cause of past unsuccessful procurements, remove the causes of the failed bids from the specifications, and attempt to procure a system with these specifications. RERF plans to launch the system fully during FY2021.

5. Revision of the rules and regulations

We are revising current regulations and establishing new regulations to resolve the findings of the Auditors' management letter and Deloitte Touche Tohmatsu's internal audit. Along with continuing to resolve these findings quickly and suitably in FY2021, required revisions will be made to keep this institution's management operations appropriate. Doing so will provide regulations befitting of a public interest incorporated foundation funded by Japanese and U.S. government subsidies.