FY2022 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:

- *AHS liver Cancer mechanistic study (RP1-09, Ohishi W):* We will continue current collaborative studies with Statistics and Epidemiology Departments to investigate: 1) the contribution of the latent factors (chronic inflammation, insulin resistance, and liver fibrosis), indexed by CRP, TNF-α, IL-6, adiponectin, leptin, and type 4 collagen, etc. to HCC risk; 2) mediation of radiation risk for HCC by the latent factors; and, 3) determine to what extent viral hepatitis is involved in that risk by separating HCC with chronic viral infection from non-B/non-C HCC.

- *Pathogenesis of MDS (RP1-17, Miyazaki Y and Imaizumi M):* We will conduct whole genome sequencing of blood samples collected before and after MDS diagnosis for analyses 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, Yoshida N)*: We will assess whether DNA and RNA from FFPE samples can be analyzed by high-throughput sequencing. We are also going to 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).

- *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).

- As part of our goal to examine genetic susceptibility and gene-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 21,000 A-bomb survivors, using old samples preserved since 1958. We will analyze genetic polymorphisms that may be involved in radiation-related cancer development in A-bomb survivors using the SNP Array developed for analyzing genetic polymorphisms that may be involved in cancer development in a Japanese population. DNA samples extracted from either old

Wright-stained smears, blood-infiltrated paper discs, or Giemsa-stained chromosome slides 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 SNP array is possible by amplifying a whole genome, using a very small amount of DNA obtained from a blood specimens stored for many years. In FY2022, DNA extraction from preserved, old blood specimens 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 SNP array. (Hayashi, RP-P1-19, and a new RP in preparation).

- Most solid cancer tissue samples of the A-bomb survivors have been preserved as formalin-fixed paraffin-embedded (FFPE) tissues in the pathology laboratory. 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. Future research will analyze the survivors' FFPE samples to identify radiation injury-related biomarkers using the protocols established in these studies. Tsuruyama (new RP)

2) Radiation and Non-Cancer Effects:

- *Atherosclerosis study [RP2-11 (Part 2 of RP7-09), Nakamizo T]*: We will finish assessing the availability of the values of multi-functional cytokines, the results of which will be hopefully published. Subsequently, we will start analyzing the data on cytokines representing disturbed tissue repair and differentiation potentially involved in the pathogenesis of radiation-induced atherosclerosis. We will also examine associations among these cytokines, radiation, and clinical physiological indicators related to atherosclerosis using integrated omics and image analysis. These analyses will be conducted jointly with the clonal hematopoiesis program project because those pathological process are interrelated to inflammation.

- In the Project 1) Detection of clonal hematopoiesis in AHS samples, 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 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 Biological Samples, and applied for the IRB review. 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. (K. Yoshida and Kusunoki, Clonal Hematopoiesis Program, Project 1).

- In the project 2) Evaluation of inflammation markers, 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 start analyzing the data on inflammatory cytokines, T cell subsets representing T cell aging, and hematological profiles potentially representing clonal hematopoiesis.

- In the project 3) Mouse model of clonal hematopoiesis, in another mouse model using B6 mice, we will first validate our current findings of a high prevalence of radiation-induced CH (a manuscript in preparation) and then investigate longitudinal trajectories of clonal mutations, using blood cell phenotypes already analyzed and blood samples already completed for cell collections at various time points before and after irradiation. In addition, we will preliminarily analyze CH-related atherosclerosis development in LDLR-KO mice fed a high fat diet following whole-body irradiation, to design a full-scale study investigating characteristics of radiation-induced CH and the involvement of CH in atherosclerosis formation. (Kusunoki, K. Yoshida, Taga, Hamasaki, Satoh, Uchimura, Misumi and Noda, Clonal Hematopoiesis Program, Project 3).

- *Clonal hematopoiesis and inflammatory phenotypes in radiation-associated CVD:* To fully test the hypothesis that radiation promotes arteriosclerosis through clonal hematopoiesis and pro-inflammatory phenotypes, we will examine somatic mutations that cause clonal growth of hematopoietic cells, immune cell phenotypes and inflammatory markers. We expect that assessments of these molecular and cellular endpoints are feasible in both a large-scale (N=3,000) and a longitudinal (N=30-50, 30 years) studies, by using blood samples collected from AHS participants, and by analyzing these endpoints in relation to inflammation and arteriosclerosis indices evaluated in the AHS. The effects of radiation exposure on clonal hematopoiesis and chronic inflammatory phenotypes including atherosclerosis development will also be evaluated in mouse and mathematical simulation models. Investigators to be involved will include Drs. K. Yoshida, N. Yoshida, Nakamizo, Taga, Satoh, Hamasaki, Uchimura, Hayashi, Noda, Misumi, Cordova, Ohishi, Kusunoki, Cologne, Imaizumi, and Hida.

3) Genetic Effects of Radiation:

- For WGS studies of the human trios comprised of atomic bomb survivors and their offspring, we will respond to the suggestions from the Genetics Research Cluster and external reviewers and proceed to approve the research protocol. 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. After getting informed consent, we will start experiment using WGS (Uchimura, new RP in CR162 F1 umbrella program project).

- *GWAS on radiation-associated cancers in all AHS subjects:* One of the important future research goals of RERF is to study the association between the inherited genomes and radiation-associated risks of disease. Much remains to be learned about genetic susceptibility to radiation-associated cancers and other diseases. A future study that aims to detect genetic factors that confer higher sensitivity to radiation exposure should, therefore, provide useful information for informing radiation protection. Blood specimens from the RERF Adult Health Study (AHS), which have been preserved since 1958 and cover approximately 21,000 A-bomb survivors, will be used in a genome-wide analysis by SNP array using these unique resources. The results obtained by the GWAS will be further analyzed by large-scale phenotype-genotype association analysis by combining the

biomarker data of the AHS subjects. Thus it is expected that this study will help to clarify the susceptibility and mechanisms of radiation diseases, including radiation-related cancers. This study will become a typical case of DSU-collaboration where epidemiologists, clinical scientists and data scientists get together to make a large-scale association study. Investigators to be involved include Drs. Hayashi, Ohishi, K. Yoshida, Cologne, Brenner, Kato, Noda, newly hired data scientists, and a young scientist to be newly recruited.

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

- *F_1 offspring clinical study (FOCS) [RP4-10, Ohishi W, Tatsukawa Y, and Hida A]:* We continue further discussion to establish methods of analysis based on multi-state models and the datasets will be prepared in collaboration with Departments of Statistics and Epidemiology. We will start analyses to investigate the effects of parental radiation exposure on hypertension, dyslipidemia, and diabetes in offspring of A-bomb survivors.
- *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 2018. 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 light of these issues and also of the likely adoption of new organ dose estimates, Dr. Sposto and Dr. Misumi have initiated a working group within the Department to explore improvements in the method for correcting for dosimetry error, inspired in part by the success of the working group led by Drs. Cologne and Yamamura on multistate modelling. The dosimetry error working group will include discussion of the implications on dosimetry error adjustments of primary vs secondary responses to the shielding survey, timing of the shielding interview/multiple interviews, different shielding details dependent on distance from hypocenter and its implications for dose error corrections at low dose, and other characteristics of dose error estimation that have heretofore not formally been considered. We hope to identify specific areas of methodologic research to clarify these issues.

  Related to this, Ms. Cordova will continue her work utilizing a structural equation modeling approach to assess the magnitude of dose error in DS02 doses using various sources of data about physiological exposure (biodosimetry), with the aim to draft a manuscript describing the results in 2022.

- The primary focus of our work on dosimetry during FY2022 is continued preparation for the implementation of the revised organ dosimetry using updated computational phantoms, as described above. While the Department will become prepared to implement the new organ dosimetry, the ultimate decision of if 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). The planning is under way for a joint meeting in early 2022 between representatives of DOE, MHLW, the ODWG, and RERF, to present the work that has been done thus far and to discuss any issues that may exist regarding the adoption of this new dosimetry system at RERF. If there is agreement to proceed to utilize the new organ dosimetry, Ms. Funamoto and Mr. Shimizu will continue the work of integrating this new dosimetry into the current RERF dosimetry software and database.

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 2022:

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:
   39 Japanese Institutions
   12 North American Institutions
   4 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 status of COVID-19 pandemic.

Activity plans for FY2022:

i) RERF will 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 FY2022 International Exchange and Research Program, RERF will consider accepting trainees from overseas.

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 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 FY2022, 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 27th and 25th Open House events at the Hiroshima and Nagasaki RERF Laboratories, respectively. In continuation from last year, in FY2022, RERF will once again consider holding the Open House events 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 limited contact with the public due to the COVID-19 pandemic, social media such as Facebook and Twitter have become the most effective method of communicating with the outside community. In FY2022, social media platforms will be used effectively to achieve the goal of obtaining understanding from atomic bomb survivors, the second-generation, local communities, and the media. Through a particular focus on visual information on Facebook and Twitter, a series of videos, including those related to the Open House, 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 FY2022, RERF's PR staff plans to hold lectures and study sessions for the media, in continuation from similar efforts made last year.

v) Public outreach related to RERF's biosample-related studies

Regarding the conduct of RERF's biosample-related research, information about stakeholder and other outside committee deliberations will continue to be actively conveyed to the public through the use of media and social media.

vi) Enhancement of RERF website

In FY2022, RERF will continue aiming at enhancement of its homepage through further refinement of information delivery related to our research synopsis project. 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.

vii) Enhancement of online news-delivery system

RERF's email magazine system, E-News, which replaced the printed *Update* newsletter, will be streamlined to increase work efficiency and ease with which the latest research results and information about RERF events and activities can be distributed, attracting readers to RERF as 'members' by offering a sense of 'buy-in' with respect to RERF as an organization.

viii) 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. In FY2022, RERF will keep this program in place in preparation for a time when interest in the activity recovers once the pandemic is contained. In the future, in addition to in-person teaching, RERF will focus on creation of videos with the same content as school visits to allow schools across Japan, and perhaps even the world, to access the information for learning about radiation health effects.

ix) 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.

x) 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 all of our research with simpler prose and smaller word counts than used previously in our "summary explanation" series for specialists. Through continuation of this series in FY2022, the aim is to improve understanding by the public and media of RERF's research results.
- A persistent goal again in FY2022 will be the creation of a system for 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 FY2022, 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

With the introduction of the Research Resource Center Operating Committee, personnel will be assigned to work on the RRC development as a primary work assignment for the first time. In addition, a senior contract programmer will be hired to oversee technical development. Major efforts on RERF's data infrastructure are needed. The identification of an appropriate underlying framework is required and will receive top priority.

The Content Management System (CMS) pilot will be completed and a full rollout of the CMS should be anticipated. Integration of the CMS with RERF's newly-started "Scanning Center" should also occur. 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.

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 and be developed in conjunction with the new Office of Research Support.

2. Review on the relocation of the Hiroshima Laboratory

We will continue deliberating the two candidate sites for the relocation of Hiroshima Laboratory: The Hiroshima Comprehensive Health Center and Hiroshima University's Kasumi Campus. At the same time, we will also carefully examine the facilities needed to achieve RERF's mission and implement our strategic plans.

3. Transition to a full Audit

RERF is aiming to obtain a full audit by an external auditing firm to supplement the audit by the Auditors. Deloitte Touch Tohmatsu LLC, with whom we have a contract for investigation services for the audit, will conduct an investigation of the opening balances for FY2022 from March to May 2022 and submit the investigation report in June of the same year. If it is determined in the investigation report that it is feasible to undertake a full audit, an audit contract will be signed in July 2022, and RERF will begin receiving full audits from FY2022.

4. Revision of the rules and regulations

RERF revises the current regulations and establishes new regulations by responding to the Deloitte Touche Tohmatsu's findings and results of regular reevaluation by the sections in charge. Along with continuing to respond to the Deloitte's findings quickly and suitably in FY2022, required revisions will be made to keep the foundation's management operations appropriate. Doing so will provide regulations befitting of a public interest incorporated foundation funded by the U.S. and Japanese government subsidies.