FY2016 Plans of Activities

Introduction

Research activities are undergoing a transition in 2016. The Department of Radiobiology and Molecular Epidemiology and the Department of Genetics have merged and have become the Department of Molecular Biosciences. More significantly, research activities are transitioning from individual projects within departments to a more integrated mission oriented approach with a focus on projects encompassing interactions of multiple department approaching key question from different directions with each component building on each other to provide more information with respect to risks and mechanisms than would be possible by conducting separate individual projects. These integrated programs will be developed by research clusters (Cancer, Genetics, and Cardiovascular/non-cancer diseases) comprised of members from all departments meeting as groups to develop such integrated programs. Another important direction is the expansion of national and international collaborations to take advantage of the valuable biosamples using state-of-the-art technologies that are not possible at RERF. These two initiatives should significantly enhance our ability to fulfill our missions

I. Major Activity Plans

1. Research Projects Examining A-Bomb Survivors Health

1) Radiation and Cancer Risks

- *Liver Cancer:* The risk of liver cancer (HCC) following radiation exposure has been shown to significantly increase over that of controls. To understand mechanisms we will investigate the joint effect of radiation and hepatitis virus infection or the association with time-dependent BMI on HCC risk. Additional studies will investigate the contribution of chronic inflammation, insulin resistance, and liver fibrosis, indexed by CRP, TNF-α, IL-6, adiponectin, leptin, and type4 collagen, to HCC risk and to assess their possible mediation effects, along with HBV/HCV, in the etiology of radiation-related HCC.
- *Thyroid Cancer:* The risks of thyroid cancer are a major concern not only for A-bomb survivors but also other exposed or potentially exposed populations. Biosamples at RERF provide a unique opportunity to critically evaluate risks and mechanisms. Toward that end we are approaching this problem in several ways. As part of these ongoing as well as future mechanistic studies using biosamples we will continue to preserve fresh thyroid samples removed from thyroid tumor cases detected among AHS subjects for future mechanistic studies. Such studies include a new initiative examining the whole genome of thyroid cancers with the US NCI and RIKEN. With respect to gene analysis two additional projects will be continued: 1) the biological significance of rearranged *ALK* gene (*EML4-ALK),* that has been frequently detected in radiation-exposed PTC cases, will be evaluated in a conditional transgenic mouse model, in terms of radiation dose and the time required for tumor formation, histological changes of thyroid tissue prior to tumor development, and histological features of the developed PTC; 2) For fifteen PTC cases with no known PTC related gene alterations, we will examine *FGFR2-KIAA1967*, *FGFR2-CIT* and *FGFR3-TACC3* which are rearrangements of MAPK-related genes and whose partner gene has coiled coil domain.
- *Updated cancer incidence:* A comprehensive analysis of the risk of radiation for all solid cancer incidences in the LSS during 1958–2009 will be published in early 2016 in collaboration with the US National Cancer Institute. Papers on analyses of individual sites will be published in 2016 and beyond. Annual reports of cancer incidence in the local populations will also be released.

- *Site-specific cancer studies with histological reviews in the LSS cohort:* Active studies will be continued in collaboration with the US National Cancer Institute. Data analyses of radiation risks will be conducted and papers will be submitted for bone and soft tissue tumors, malignant lymphoma, and breast cancer. Histological reviews by pathologists will be continued for uterine (corpus) cancer. A collaborative pooled study of breast cancer and serum hormone biomarkers will be completed.
- *Radiation risk at low dose levels:* A collaborative study within RERF to investigate low-dose risks will be initiated. Analyses limited to low-doses need to consider heterogeneity of background rates of cancer incidence and cancer and non-cancer mortality due to lifestyle and other factors. Dose errors as well as potential impact of exposure to residual radiation and medical radiation will also be considered.
- *Radiation-induced mutations:* The role of mutations, both somatic and heritable, in radiation induced cancer continues to be of major interest and importance. To facilitate quantification and characterization of such mutations RERF investigators are developing a new *in vivo* mutation detection system using newly created p53-GFP mice. We are actively seeking outside investigators for collaborative studies for radiation-induced cancer.
- *In utero exposure:* Previous studies in 2015 on chromosomal effects in hematopoietic stem cells and thyroid cells as a function of time post conception and after birth have suggested mechanisms associated with stem cell development within the specific organ system. In 2016 we will conduct a series of studies directly testing this hypothesis.

2) Radiation and Risks of Circulatory Disease:

- *Cardiovascular disease:* We will continue collecting data cross-sectionally to investigate the relationship between radiation dose and heart function or manifestations among the AHS participants using echocardiography and pertinent preclinical biomarkers. A CVD incidence study will also be initiated using consistent criteria over the study period since AHS had started, especially for ischemic heart disease (IHD) and stroke, in order to examine the association between radiation dose and long-term IHD incidence in the AHS. Plans to examine relationships between radiation dose and blood biomarkers such as inflammatory cytokines (IL-6, TNF-α, etc.), adipocytokines (adiponectin, etc) and IGF-1, and ensuing CVD risk are also underway.
- *Chronic kidney disease (CDK):* As part of a continuing interdepartmental collaboration among a team of investigators at RERF we will continue to review the association between radiation and CKD and the joint role of CKD in the radiation-CVD association based on diagnoses and detailed categorizations of CKD using e-GFR and microalbuminuria among Hiroshima and Nagasaki AHS subjects.
- *Animal models of cardiovascular disease:* SHRSP animal models will continue to focus on mechanisms. Such studies will analyze the blood cell constituents (e.g., platelets), biochemical molecules (e.g., HDL), cytokines (e.g., IL-10) and metabolites in this model system. These studies will help to clarify the mechanisms of radiation-associated CVD in rats and try to infer potential mechanisms underlying radiation effect on CVD in A-bomb survivors.

3) Other Non-Cancer Risks:

- *Cataractogenesis:* We will continue collaborations with ophthalmologic specialists and internal investigators to conduct a new ophthalmologic study regarding radiation effects on cataract. We will start full-scale ophthalmologic examinations in both Hiroshima and Nagasaki after revising the research study plan based on results from the preliminary survey.
- *Diabetes:* There are inconsistent data regarding the association between the development of diabetes and radiation exposure in the A-bomb survivors. We will continue these studies to examine the dose response for the development of diabetes in Hiroshima and Nagasaki and to evaluate whether the radiation dose response for diabetes is modified by city and age at the timing of A-bomb.
- *Neurocognitive functions:* Regarding late-life neurocognitive function among subjects exposed in utero and at ages <13 years at the time of the bombings, we will conduct an exploratory factor analysis using all self-reports collected from non-exposed subjects of neurocognitive-deficit symptoms on the 25 item Neurocognitive Questionnaire (NCQ) survey instrument in cooperation with Statistics Department. We will then, analyze radiation effects on neurocognitive-deficit symptoms based on NCQ and/or cognitive function based on Cognitive Abilities Screening Instrument (CASI).
- *Dose response relationships:* Interdepartmental collaborative studies to estimate radiation risks and shapes of the dose-response curves for non-cancer diseases in the AHS, including the expanded younger AHS participants exposed before age 10 will continue. Such studies will consider any potential survivor or participation biases in the newly added young subcohort, and to determine how to analyze the resultant incidence and prevalence data appropriately.
- *Co-morbidity and non-cancer diseases:* In collaboration with Kurume University we will continue studies on the influence of co-morbidities on the radiation risk estimates of death due to non-cancer diseases.

4) Activities to Enhance Ongoing and Future Analysis

- *Mortality surveillance:* Mortality follow-up for all cohorts will continue and the data will be completed through the 2012 timeperiod. Archiving early-time materials will be continued.
- *LSS mail survey:* The results of the new LSS mail survey will be summarized. A paper about whether medical radiation exposures are a confounding variable for LSS risk estimates will be published.
- *Hiroshima and Nagasaki tumor/tissue registries:* Case collection on population-based cancer registries will be completed through 2014 in both Hiroshima and Nagasaki. The dataset of cancer incidence through 2011 will be biennially updated in 2016 on the LSS, *in utero*, and F_1 cohorts. The data will regularly be reported to the Monitoring of Cancer Incidence in Japan (MCIJ) and cooperative studies with the National Cancer Center of Japan are also being conducted. Analyses of the prefectural data of Hiroshima and Nagasaki will be conducted. RERF needs to adapt to the new national cancer registry system.
- *Pathology studies:* The indexing of specimens of formalin-fixed paraffin-embedded tissues within a new database is continuing. The system to preserve surgically resected materials from the A-bomb survivors will be organized with community hospitals in Hiroshima and

Nagasaki. These activities will be performed in cooperation with the RERF Biosample Center of RERF.

- *Biosample Center*: The center will continue development of infrastructure necessary for future use of biosamples. The major steps for 2016 are listed below:
  a. Create a manual on quality control: review methods of quality control (including storage temperatures and effects of thawing and refreezing) as necessary to improve the quality control of samples and examine their stability.
  b. Work on the establishment of a laboratory information management system (LIMS).
  c. Prepare more specific and detailed regulations on sample usage, including the limit of amount used, and a sample use request form, by incorporating the opinions of experts.
  d. Complete inventory of biosamples stored in -80°C deep freezers and transfer them to robotic biorepository system.
  e. Continue an effort to have understanding and acceptance on the use of biosample for research from A-bomb survivors and the local community.
  f. Establish internal/external advisory committees on the operation of Biosample Center and protocols for distribution of samples.
- *Research Resource Center (RRC):* The biosamples center is one component of a planned research resource center that will combine data management and distribution with biosamples. The initial steps in the development of this RRC will begin in 2016.

5) Mechanistic Studies to Provide a Better Understanding of Risks:

- *Immune response and radiation exposure:* Studies of radiation effects on immune function are important for understanding the mechanisms involved in both cancer and non-cancer diseases in A-bomb survivors. In this regard we are examining immune function using several different approaches. One set of studies has tested the hypotheses that the radiation-impaired immune system modifies the influenza vaccine response. Analyses include data on pre-existing human cytomegalovirus antibody titers and other immunological biomarkers, such as lymphocyte and profiles. Studies are also examining: 1) type-3 innate lymphoid cells generated from HSCs through Notch1-dependent and independent pathways: 2) radiation and aging effects on alterations of dendritic cells toward immune suppressor phenotypes in AHS subjects: and 3) alterations in thymic architecture and functional capacity in 205 LSS thymus autopsy cases.
- *Methylation Status:* RNA-seq will be used for investigation of genome-wide DNA methylation status and gene expression in naïve CD4 T cells among A-bomb survivors. Data analysis for T-cell receptor (TCR) diversity evaluated by deep sequencing will be completed, and a future *TCR* deep sequencing study in A-bomb survivors will be initiated. Aging- and radiation-related changes in the DNA methylation status will also be initiated using a genome-wide RRBS protocol with blood cell subsets from the AHS subjects comparing young and elderly non-AHS volunteers.
- *T cell immunity and inflammation:* We will analyze longitudinal study data on numerical changes in naïve CD4 T cells in 1,000 AHS participants to test the hypothesis that declining T-cell immunity may precede enhanced inflammation relating to elevated risks of arteriosclerotic diseases, such as myocardial and cerebral infarctions, and stroke, in A-bomb survivors.
- *SNP analysis:* We will select candidate SNPs, specifically those of immune-,

inflammation-, and DNA repair-related genes, that are potentially involved in the development of radiation-associated breast cancer. This will be accomplished using a targeted gene SNP array system (4,000 genes), as well fine mapping and validation of candidate genes with the use of MALDI-TOF mass spectrometry technology. On the basis of the targeted gene SNP array system, we will analyze the association of DNA-repair gene polymorphisms, particularly in the non-homologous end-joining pathway, with development of radiation-associated breast cancer as well as somatic genome mutability phenotype as an outcome variable. In addition we will continue to develop and adapt new methods to improve analyses of gene association studies and related studies, such as weighting schemes for gene-set and pathway analyses that take into consideration biological function and measures of effect (some preliminary work is being done in collaboration with several researchers at the University of Hawaii, Cancer Center).

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

1) Clinical

- *Health exams:* We will continue to conduct health examinations among the cohort members of F_1 follow-up clinical studies.
- *Data analysis:* We will conduct data cleaning and final tabulation of multifactorial disease outcomes among participants of the 2nd round FOCS examination. We will also develop the statistical analysis plan with the aim of full analyses in collaboration with Statistics and Epidemiology Departments and internal investigators.

2) Studies to Investigate Potential Transgenerational Effects

- *Hereditary mutations in a murine model:* Radiation induced hereditary mutation can differ based on gender. We have recently completed analysis of effects in females following radiation exposure. New studies will evaluate radiation-induced mutations and analyze parental origins of *de novo* base substitutions in male F1 mice born before and after irradiation to male germ cells by whole genome sequencing.
- *Hereditary mutations in the F1 population:* Previous studies examining hereditary mutation in survivors and their offspring (F1) have found no evidence of increased risks in the F1 population after radiation exposure to the parents (A-bomb survivors). However, these studies used methods that weren't sensitive enough to exclude effects. The development of new techniques that involve the sequencing of the whole genome (WGS) allows us to examine the effects of radiation on hereditary effects in detail in a quantitative and qualitative manner. Such studies will allow us to critically examine potential effects. Toward that end we are developing strategies for collaborative studies for WGS of the trios and initiate the analysis. These studies will be linked with the F1 clinical studies and epidemiological studies to allow us to not only identify mutations but also evaluate their potential significance with respect to disease outcome. We are currently exploring a collaborative study with the NCI.
- *Bioinformatics:* Proper analysis of WGS data is essential to interpret any results obtained. RERF investigators in the statistics department will collaborate with other RERF investigators involved in these WGS studies to initiate analyses of the F1 population. Analysis also requires collaborations with other bioinformatics research teams outside of RERF.

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

- *Statistical uncertainties:* Individual-data analytic methods will be used to characterize sources and types of uncertainties in atomic-bomb radiation dosimetry and their impact (covariate error) on risk estimates and develop ways to correct for this, including simulations and use of biodosimetric information (chromosome aberration analysis and ESR studies on tooth enamel) obtained by RERF investigators. We are working with several groups of external collaborators and expect continued production of statistical papers and useful results for practical application at RERF.
- *Survivor location:* We will begin studies to develop ways to apply geospatial methods to RERF data to take full advantage of existing two-dimensional information on survivor locations at the time of the bombings, testing assumptions such as circular symmetry in the dosimetry and spatial homogeneity of background rates over large areas of the cities. This involves modern methods such as hierarchical Bayesian or empirical Bayesian methods. RERF data present challenges for spatial analysis, as they are strongly influenced by direct radiation dose from the bombs and other covariates, the effects of which must be estimated from the same data as part of a simultaneous analysis. Specific projects may ultimately include spatial analyses of acute epilation, chromosomal aberrations, and cancer incidence.
- *Measurement errors:* Studies will be initiated to develop general and basic theoretical considerations related to regression calibration for mitigating the effect of measurement error in problems that involve additive measurement error, for possible application to general measurement-error problems other than the radiation dose-response problem.
- *Organ doses:* We have recently begun discussions aimed at evaluating, in collaboration with a binational scientific working group, the potential costs and benefits of a plan to provide improved computational phantoms for use with DS02 to calculate organ doses, fetal doses and related quantities. Studies will be initiated in 2016.
- *Indirect radiation exposure:* Because of potential confounding factors in risk estimates we are continuing to investigate the possible doses that survivors may have received from indirect sources, i.e., residual radioactivity, as we have done for DOE-sponsored workshops in 2012 and 2014 and in support of two recent Epidemiology papers on exposure to rain shortly after the bombings.

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

1) Release of research results:

An important activity with respect to release of research results is a special symposium to be held at the Radiation Research Society in collaboration with the Committee on Radiation Health in October. This symposium is devoted solely to ongoing studies being conducted at RERF. It is expected that approximately 800 radiation scientist will attend.

- Ongoing collaborations: Long term collaborations are listed below and these are expected to continue in 2016:
  a. Partnership with Kurume University
  b. Collaborations with the US National Cancer Institute
  c. Collaborations with the US National Institute of Allergy and Infectious Diseases
  d. Collaborations with Outside Investigators:
     45 Japanese Institutions
     22 North American Institutions

12 European Institutions
6 Asian Institutions
Nuclear Emergency Workers Study (NEWS): 10 Japanese Institutions

While the National Institute of Allergy and Infectious Disease (NIAID) project is coming to a close as of 9/1/2016 (we are currently in a no cost extension), we are exploring several other collaborative opportunities both within and outside Japan. These potential projects are all directly applicable to RERF and its mission.

There are currently opportunities to expand our collaborations with the US NCI with respect to genomic sequencing of thyroid tumors in the survivors to examine potential radiation specific mutations, deletions, etc. We are also planning with NCI a study on the trios (parents and children of survivors) with respect to genomic and other changes such as epigenetic changes associated with radiation exposure.

Beyond the expansion of collaborations with NCI we are initiating overtures to Japan Science and Technology Agency (JST) and to the European Communities integrated program on radiation risks and mechanisms (MELODI). This initiative would serve to facilitate closer collaborations with the radiation program conducted in inside and outside of Japan. The MELODI collaboration would involve direct collaborations between RERF and the European Communities radiation program on specific collaborative projects. Both of these initiatives reflect our desire to explore new collaborative opportunities.

5. Training programs for domestic and overseas specialists

RERF will hold a training seminar 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.

Activity plans for this fiscal year

i) RERF will hold an epidemiological training seminar for radiation biologists in Japan again this year for enhanced understanding of results from epidemiology research on A-bomb survivors.

ii) RERF will accept overseas research trainees to support the activities of such organizations as the Hiroshima 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) Besides the above activities, RERF will accept students from domestic and overseas schools/universities for facility tours, and will provide training sessions on the foundation's research activities.

iv) RERF will continue to review directions as to its training activities and publicly invite overseas trainees in the International Exchange Research Program for this fiscal year also.

6. Public information programs

RERF will provide radiation-related information to the public by explaining radiation and its effects in an understandable and easily accessible manner through educational support to classes for the general public and students, public seminars, the Open House event, and the provision of relevant information on the Internet and in the form of pamphlets. We will also respond to questions and inquiries submitted from within Japan and from overseas.

Activity plans for this fiscal year

i) RERF Open House event

RERF will hold its 22nd and 20th Open House events at the Hiroshima and Nagasaki RERF Laboratories, respectively. The event features various programs, including exhibitions, and lectures, and is scheduled to be held in Hiroshima and Nagasaki on August 5–6 and August 8–9, respectively.

ii) RERF Public Lecture

RERF will hold public lectures to provide the general public with an opportunity to enhance their understanding of the foundation's research and to learn more about radiation's health effects.

Dates: Hiroshima (undecided); Nagasaki (undecided)

iii) Permanent exhibits

Our permanent exhibits at the Hiroshima and Nagasaki Laboratories introduce the history of ABCC-RERF, the organization's study/research activities, its domestic and overseas collaborative activities, and contributions RERF has made to society.

iv) Updating of public relations materials

RERF will update its various public relations materials.

v) Enhanced RERF website

RERF will provide information on its research activities in a more prompt and readily understandable manner using its public website, by posting short summaries of new papers and other such efforts. The existing Q&A section will be updated appropriately and systematized. Educational web seminars (slides with explanatory audio) for the general public will be added. RERF will continue to post information, with a focus on video format, on its Facebook page.

vi) Other public relations activities

- RERF will actively promote the foundation's important papers to the domestic and overseas media via press releases.
- RERF will organize annual forums for media representatives in Hiroshima and Nagasaki.
- RERF will develop a proactive public relations system collaborating with all its professional and general staff in a unified manner.
- RERF will seek an efficient approach with respect to its facility tours.

II. Activities necessary for the above projects

1. Implementation of Secretariat reorganization due to continued personnel reduction

To cope with downsizing of the number of general employees, we have reviewed the reorganization of Secretariat, etc., while continuing negotiations with the RERF Labor Union for a labor-management agreement over revisions of a portion of the allowances.

Actions to be taken in FY2016 include preparatory activities for obtaining BOD approval for revisions to RERF regulations based on the labor-management agreement; obtaining MHLW permission; and undertaking personnel assignment, review-related work transfer, and space reallocation. We hope to implement the reorganization in the first half of FY2016.

2. Review of personnel planning in preparation for the aging of the general staff

By the end of FY2016, the number of budgeted personnel, including research scientists, will be reduced to 209, down from 588 at the time RERF was established in 1975, as the result of implementing repeated personnel reduction plans.

In addition, we expect that a total of 53 general employees will retire mandatorily over the next five years: ten in FY2016, eight in FY2017, 13 in FY2018, 16 in FY2019, and six in FY2020. Prospective mandatory retirements in the coming five years will account for as much as approximately 32% of the current number of general employees (168).

Because it is predicted that loss of such a mass of personnel through mandatory retirement will affect stable, systematic operations, we need to promptly develop and implement mid-term personnel planning (including new hiring of mid-career and young employees) to cope with the aging of our personnel and the impact of mandatory retirement.

3. Improvement of the audit system

With regard to RERF's audit system, agreed-upon procedures established through a contract with an external auditing firm have been employed as a guide for the Foundation's financial reporting. However, the contract with the auditing firm expired in FY2015, and we need to establish a new internal audit system in FY2016.

To maintain RERF's activities as a public interest incorporated foundation, we will create an internal audit system to ensure the reliability of our financial reporting with regard to daily accounting work and accounting management systems; we will also implement systems that promote adherence to laws/regulations and conservation of assets.

4. Facility upgrades

(1) Upgrades to Hiroshima Laboratory

1) Following completion of the installation of the robotic biorepository system in October 2015, it is expected that existing generators, No.1 – No. 4, will not be able to produce sufficient backup power; therefore, an additional generator, No. 5, will be installed at an estimated cost of 50 million yen.

2) As the current total operating capacity of the emergency generators is only three days, underground tanks (20,000 liter of light oil) and an automatic lubrication system will be installed to increase the total operating capacity to seven days. The estimated cost is 40 million yen.

3) The three-year plan for facility upgrades includes the following projects for this year: convert to LED lighting in Buildings D and H for energy savings (estimated cost: 20 million yen) and replace old air conditioners, installed 17 years ago, in Buildings F and H (estimated cost of 24 million yen).

(2) The following two projects will be carried out on a continuing basis using the U.S. subsidy for FY2015:

1) Replacement of the electric cables of low-voltage distribution boards in each building (excluding Buildings A and Da) and the Hijiyama Hall will cost approximately 177 million yen.

2) An automatic gas fire extinguishing system will be installed to protect RERF's valuable research materials and data from fire at the Hiroshima and Nagasaki Laboratories. The estimated cost is 138 million yen.

Hiroshima Laboratory		**Nagasaki Laboratory**	
Storage sites	Floor area (m^2)	Storage sites	Floor area (m^2)
1) Bldg. C 131 Chart storeroom	123	1) 1F Chart storeroom	62
2) Bldg. H 129 Work station room	80	2) 2F X-ray film storeroom	29
3) Bldg. E 105 Epidemiology Dept. storeroom	135	3) 4F Master File Section storeroom	52
4) Bldg. I 205 Master File Section storeroom	83	4) 4F Tumor & Tissue Reg. Office and archives room	48
5) Bldg. C 212 Tumor & Tissue Reg. Office storeroom	10	5) 4F Server room	18
6) Bldg. J 207 Historical Archives Room	120		
Total for Hiroshima	551 m^2	Total for Nagasaki	209 m^2

(3) Waterproofing of the roof of the Nagasaki Laboratory will be fully redone at a cost of approximately 4 million yen. It was last carried out in full scale in 1997, and it has only been partially repaired since at times when rain leaked. Because the expense will be equally shared with the Nagasaki Prefectural Education Association, RERF expects to bear approximately 2 million yen of the cost.