Active Research Protocols by Study Program

1 April 2014–31 March 2015

The 103 research protocols (RPs) including smaller-scale Type A protocols that were active during the fiscal year are listed below by study program with brief progress reports prepared by primary investigating departments, and listings of publications and oral presentations emanating from related studies follow. In addition, the title and researcher names for any smaller-scale, preliminary or pilot Type B and Type P protocols are listed at the end of each section of the Active Research Protocols by Study Program.

Investigating departments are identified by the following codes:

Clinical Studies, Hiroshima **(CH)**
Clinical Studies, Nagasaki **(CN)**
Epidemiology, Hiroshima **(EH)**
Epidemiology, Nagasaki **(EN)**
Genetics **(G)**
Radiobiology/Molecular Epidemiology **(R)**
Statistics **(S)**
Information Technology **(IT)**
Biosample Center **(BC)**
RERF Director **(D)**
RERF Associate Chief of Research **(ACR)**
RERF Chief Scientist **(CS)**

Outside researchers are not listed with their affiliations here.

The following marks are used for publications and oral presentations based on RPs:

♦ Publications
⌘ Manuscripts in Press
❖ Oral Presentations

Protocols are presented by study program in reverse chronological order, and entries include the protocol title, investigators, and a brief description of the RP.

Each study program is followed by a listing of any publications that emanated from these studies as well as manuscripts accepted for publication during the fiscal year. These are presented in alphabetical order by first author. (Major publications are listed with a "Summary Explanation.")

Most of the outside authors are RERF consultants, expert advisors, or part-time professionals, and their listings with affiliations appear in a separate section of the annual report.

(Japanese) indicates that the original publication is in Japanese.

Oral presentations are included by study program after publications and manuscripts in press, and listed chronologically by meeting date.

Research Protocols 2-08, 1-75 (Platform Protocol), 2-61, A2-13, A1-13, A2-11, A2-10, A1-09, A1-08

Life Span Study (LSS)

RP 2-08 Mail survey 2008 on epidemiological factors in the Extended Life Span Study population

Sakata R (EH), Nagano J, Grant EJ (EH), Sugiyama H (EH), Sadakane A (EH), Ohishi W (CH), Hida (CN), Moriwaki H (EH), Mabuchi K, Ozasa K (EH), Kodama K (CS)

Objectives: To update the information on epidemiological factors that may confound or modify radiation effects among LSS subjects, and to obtain information on subsequent medical radiation exposures.

Background and Significance: Increased rates of malignant tumors and circulatory diseases indicate that important late effects of A-bomb radiation on health include common multifactorial diseases. The questionnaire information will permit more insights about the joint effects of or possible confounding by various environmental, lifestyle, and endogenous factors, upon the health effects of A-bomb radiation. ABCC-RERF has conducted several mail surveys for this purpose in the past. Since more than 15 years had passed since the last mail survey, another survey was conducted to update information on factors that might have changed longitudinally and to obtain new information on factors that need clarification from previous studies. For the first time, the survey collected information on exposures from radiotherapy and relatively high-dose diagnostic radiation procedures (e.g., CT scans).

Study Methods: Although the original targets of the survey were all 47,000 subjects who were alive as of 1 July 2007 in the Extended Life Span Study cohort (LSS-E85), the number was reduced to about 25,000 because of a new legal restriction regarding access to the resident registries, which limited our ability to obtain addresses. The content, validity, reproducibility, and feasibility of the questionnaire were evaluated through a pilot study (B45-06) as well as outside review. Multiple mailings were conducted for nonrespondents.

Study Progress: The survey was sent to 24,640 subjects. A supplemental interview, which was performed by the Department of Clinical Studies to collect information on smoking and alcohol drinking during clinic visits from AHS subjects who had not responded to the mail survey, was completed at the end of March 2014. Entry and cleaning of questionnaire response data have been completed. Cleaned data for general use were uploaded to the RERF database in November.

Results and Conclusions: The number of returned questionnaires with answers was 14,091. Information was also obtained from 338 AHS subjects who had not filled out a questionnaire but later visited RERF for a health examination. A preliminary examination of the medical irradiation data gave little indication that cumulative doses from medical x-ray were higher among those exposed to high doses of A-bomb radiation, though more detailed analyses are not yet completed.

RP 1-75 Research plan for RERF study of life span of A bomb survivors, Hiroshima and Nagasaki

Ozasa K (EH), Shimizu Y, Grant EJ (EH), Sugiyama H (EH), Sakata R (EH), Sadakane (EH), Utada M (EH), Soda M (EN), Cologne JB (S), Kodama K (CS)

Objectives: To ascertain the long-term health effects of exposure to ionizing radiation in the Life Span Study (LSS) cohort of A-bomb survivors.

Background and Significance: Because the LSS is a large cohort of all ages with a wide range of radiation doses and a long-term follow-up for mortality and cancer incidence, it provides key information for radiation risk assessment. Evaluation of the late health effects of radiation from the atomic bombs is the central goal of RERF research.

Study Methods: A cohort study. The LSS cohort of about 94,000 A-bomb survivors and 26,000 subjects who were not in city at the time of the bombing has been followed up since 1950 for their vital status and cause of death and since 1958 for cancer incidence. Individual A-bomb radiation doses have been estimated. Mail surveys have been conducted to obtain information on factors that might confound or modify the radiation effects.

Study Progress: Survivors' locations at the time of the bombings and corresponding individual dose estimates of LSS subjects were updated in collaboration with the RERF Dosimetry Committee. A paper of the dose response for mortality of various heart disease subtypes over separate observation periods from 1950 to 2008 is being drafted. Analyses of the updated cancer incidence data (through 2009) is being conducted using the updated doses and information on lifestyle factors in collaboration with the U.S. NCI. These include detailed analyses of total solid cancers and of a variety of individual tumor sites focusing on the shape of the dose-response curves, low-dose risk, and risks among those young at exposure. A paper on the analyses of the effects of co-morbidities from other chronic diseases on the mortality of major noncancer diseases is being drafted under RP-A2-11.

Results and Conclusions: The risk of rheumatic heart diseases, hypertensive organ damage, and heart failure increased while the risk of ischemic heart disease did not increase except for the most recent period. Those findings were thought to reflect not only the pathogenesis of radiation effects on different subtypes of heart diseases but also secondary associations through socioeconomic situations and misclassification of diagnosis on death certificates. The risk estimates of solid cancer incidence in the new analyses were mostly similar to those previously reported, but relative risks were slightly increased after adjusting for lifestyle factors such as smoking due the estimates now being relative to nonsmokers. Since a majority of the subjects who were exposed when young are still alive and risk estimates for them are uncertain at this stage, a number of years of further follow-up will provide a much more informative characterization of radiation risks. The public continues to be concerned about the effects of early entry into the city and further work is required in this area.

RP 2-61 Study of mortality and cancer incidence in people exposed *in utero*

Sugiyama H (EH), Shimizu Y, Preston DL, Suyama A, Cologne JB (S), Misumi M (S), Ozasa K (EH), Kodama K (CS)

Objectives: To investigate the nature of radiation effects on mortality and cancer incidence among people exposed *in utero* to atomic-bomb radiation.

Background and Significance: Prenatal medical diagnostic x-ray exposure has been associated with increased risk of childhood cancer in several case-control studies. The RERF *in utero* cohort, although small in size, has a large collective dose, with a subgroup with substantial radiation doses, and so can provide much information on the sensitivity of the embryo and fetus to late radiation effects. It is the only available cohort in the world with exclusively *in utero* radiation exposure and adulthood data on health risks.

Study Methods: Follow-up of mortality and cancer incidence in a cohort of 3,600 people who were *in utero* at the time of the bombings has now continued to age 68.

Study Progress: Analysis of *in utero* data between 1950 and 2008 for cancer and noncancer death has been completed and a paper is being drafted.

Results and Conclusions: Significant associations between radiation exposure and solid cancer mortality among females and noncancer disease mortality in both childhood and at ages of 40 years and older were detected. The adult noncancer mortality risk was detected among *in utero* exposed individuals of low birth weight. The possibility that there is mediation between radiation and noncancer mortality by birth weight is being considered.

RP-A2-13 A proposal to join the biliary tract cancers pooling project

Grant EJ (EH), Hida A (CN), Ohishi W (CH), Ozasa K (EH)

Objectives: This is a proposal to join the Biliary Tract Cancers Pooling Project (BTCPP) being organized by Dr. Jill Koshiol of the U.S. National Cancer Institute (NCI). The BTCPP is a pooled analysis of individual-level data collected in cohort settings to study the etiology of biliary tract cancers, specifically studying possible associations with body mass index (BMI), smoking, and diabetes mellitus.

Background and Significance: Biliary tract cancers are relatively rare and little is known about their etiology. Due to rarity, few prospective cohort studies have sufficient statistical power to study biliary tract cancer etiology; therefore a pooled study using prospective data is proposed.

Study Methods: Data will be pooled at the U.S. NCI and harmonized. Data will include height, weight, date of birth, age at baseline, BMI, smoking, diabetes, incident cancer (gallbladder) diagnosis date, and others if available (family history, NSAID drug use, physical activity, etc.). To avoid any concern of bias due to radiation exposure, only data from subjects exposed to less than 100 mGy were provided to NCI.

Study Progress: This RP was approved in July 2013. Data have been prepared and sent to NCI. Current efforts are underway at NCI to analyze the data.

Results and Conclusions: The consortium has recruited 25 cohorts representing 2,866 biliary tract cancers (BTC) and 2,635,854 non-cases. RERF has contributed 340 BTCs,

the second most of any of the collaborating cohorts. Women suffer the cancer more often than men (61% vs 39%). Smoking increases the risk of extrahepatic bile duct and ampulla of Vater cancers. There was little evidence of a risk due to alcohol consumption. A manuscript is in process.

RP-A1-13 A proposal to join the pooled analysis of radiation-related primary neoplasms of the brain and nervous system

Sakata R (EH), Sugiyama H (EH), Soda M (EN), Inskip P, Brenner A, Ozasa K (EH), Shore RE (CR)

Objectives: To characterize in more detail the association between radiation and tumors of the central nervous system (CNS), particularly considering: 1) the shape of radiation dose-response curve, 2) the effect modification by age at irradiation and sex, 3) temporal patterns of radiation-related risks, 4) the effect of dose fractionation, and 5) possible joint effects of radiotherapy and chemotherapy.

Background and Significance: It is well established that ionizing radiation can induce tumors of the CNS. Exposure during childhood appears to be more effective in tumor induction than adult exposure, but the data concerning adult exposure are sparse. Little is known about other factors that modify radiation-related risk. The association between radiation exposure and risk appears to be stronger for benign tumors (meningioma and schwannoma), than for malignant tumors (glioma).

Study Methods: Fifteen studies (11 cohort and 4 case-control studies) will join this pooled analysis. RERF will provide most of the data used in the previous study (Preston et al., JNCI 2002), but DS02 dose estimates will be used instead of the original DS86 doses. After the harmonizing of the pooled data, excess relative risks and excess absolute rate will be estimated at the National Cancer Institute (NCI). Using the best fitting dose-response model, multiplicative interactions of the ERR and EAR with sex, attained age, age at exposure, time since exposure, dose fractionation, and some study-specific characteristics (type of first cancer, chemotherapy, etc.) will be evaluated.

Study Progress: The project was approved by the National Institute of Health Office of Human Subjects Research Protections in January 2014. RERF data were provided to the NCI in April.

Results and Conclusions: Not yet. Results expected in 2015.

RP-A2-11 Mortality analysis of Life Span Study (LSS) cohort taking into account multiple causes of death indicated in death certificates

Takamori A, Kasagi F, Takahashi I (CH, EH), Ozasa K (EH), Yanagawa T

Objectives: To study associations between atomic bomb radiation exposure and mortality from combinations of underlying cause of death and comorbidities in LSS subjects. Circulatory diseases were targeted as underlying causes of death in this project.

Background and Significance: The LSS has mainly focused on relationships between underlying causes of death and radiation dose. However, diseases other than underlying causes of death (hereunder referred to as comorbidities) may modify the radiation risk estimates of death compared to the underlying cause of death and have rarely been studied.

Study Methods: LSS subjects (n = 86,611) were followed-up over the period 1950–2002. We investigated the relationship between dose and mortality for cases having different combinations of underlying cause of death and comorbidities.

Study Progress: The analysis has completed and a paper is being drafted.

Results and Conclusions: Among 49,603 deaths occurring during the follow-up period, circulatory disease was identified as the underlying cause of death in 9,670 deaths in 1977–2002 (the period in which death certificate accuracy was thought to be reliable). There was a significant relationship between dose and circulatory disease mortality in the 168 cases that had cancer as comorbidity while no significant relationship for other comorbidities was observed, such as pneumonia (908 cases), renal disease (447 cases), or diabetes mellitus (291 cases).

RP-A2-10 A semi-parametric survival extrapolation method: Model validation using RERF cohort

Fang CT, Wang JD, Hwang JS, Furukawa K (S), Kasagi F, Soda M (EN), Suyama A, Ozasa K (EH), Cullings HM (S)

Objectives: To examine the robustness of a semi-parametric survival extrapolation method based on the constant excess hazard model (developed by Dr. Hwang) using LSS cohort data. The long follow-up data in the LSS cohort provide a good opportunity to evaluate temporal extrapolation using the model.

Background and Significance: The knowledge of how long patients can expect to live after the diagnosis of a disease is essential for cost-effectiveness evaluation of medical interventions. Researchers need to look beyond the follow-up limit of clinical trials and take a lifetime perspective when measuring the impact of a new medical therapy. Because there are usually only very limited follow-up data for newer medical interventions, a robust statistical method for survival extrapolation is particularly important. Previously used parametric survival models can make reasonably good short-term predictions. Problems may arise, however, when there is a high right-censoring rate or long-term projection.

Our group therefore conceived an innovative approach that borrows information from national vital statistics data, and incorporates this information in a semi-parametric survival extrapolation method based on the logit survival ratio between patients with a disease and an age- and gender-matched reference population (see details in the Study Methods section), assuming a constant excess hazard. In that case, the logit survival ratio curve will converge to a straight line over time, which allows linear extrapolation.

Study Methods: We propose to examine whether the logit survival ratio curves between atomic bomb survivors who (1) are with or without radiation exposure, or (2) had a specific cancer vs. an age- and gender-matched reference population generated from Japanese vital statistics by a Monte Carlo method, will converge to a straight line over time. In addition, we will test different strategies for estimating the slope in logit survival ratio plots in order to find the best strategy for choosing the sample size. A software program has been developed by Prof. J-S Hwang (Academia Sinica, Taiwan) to facilitate these calculations.

Study Progress: A manuscript has been submitted to a journal.

Results and Conclusions: For the period 1950–1998, the logit survival ratio between atomic bomb survivors who were exposed to the highest-dose radiation (HDR) (dose ≥ 1,000 mGy) (2,375 persons) and an age- and gender-matched reference group selected from those without radiation exposure (17,830 persons), the slope eventually randomly fluctuated between +0.005 and –0.020. For site-specific cancers (stomach, lung, liver, colon, breast, pancreas), the slopes of the logit survival ratio between patients with cancer and the matched references also show overt random variations over time as the sample size decreases, especially for cancers with a smaller sample size. To solve this problem, we derived two new mathematical theorems on the properties of the logit survival ratio, based on the constant excess hazard assumption. Using these, we developed a set of simple yet robust rules for selecting the best slope for extrapolating the survival curves. With the selected slope, we further evaluated the accuracy of long-term projection by comparing the difference between the predicted and actual survival curves. The semi-parametric model had excellent accuracy in extrapolation: the differences between the predicted and actual survival probabilities at the end of follow-up were all below 0.05 (in absolute value). We concluded that the semi-parametric method based on the constant excess hazard assumption is a robust statistical method for lifetime survival extrapolation.

RP-A1-09 Biologically based mechanistic modeling of leukemia in the Life Span Study

Dekkers F, Bijwaard H, Cullings HM (S), Soda M (EN), Sugiyama H (EH), Kasagi F, Suyama A

Objectives: Apply a biologically based two-mutation carcinogenesis model to individual leukemia incidence data in the LSS cohort.

Background and Significance: The Netherlands National Institute for Public Health and the Environment (RIVM) has a history of developing Moolgavkar-type two-mutation models for leukemia in laboratory animals and humans that attempt to incorporate biological concepts of the disease. The biologically based leukemia model results can be compared with our empirical-model results, and may be informative as to how to transfer risk estimates to other radiation-exposed groups, such as western populations with chronic or low-dose exposures.

Study Methods: RIVM has developed a maximum-likelihood, Two Mutation Carcinogenesis (TMC) model that takes relevant radiobiological information into account more than most other two-stage models have done by estimating mutation rates of interest. Risks will be estimated for Life Span Study (LSS) data for three main radiogenic leukemia subtypes (acute myeloid leukemia, chronic myeloid leukemia, and acute lymphocytic leukemia).

Study Progress: An earlier analysis under the assumption of a fixed lag time led to problems. A minimum lag time between the creation of the first malignant cell and leukemia diagnosis provides better model fitting. The value for lag time obtained in statistical data fitting is largely determined by the earliest cases. Since the LSS does not contain information on cases that occurred in the first years after the bombings, using the LSS data alone results in implausibly large values. In the past year, the limited information available on early cases was used to obtain a more realistic value for the lag time. Data fitting to determine the sensitivity of the other parameters in the model to the uncertainty in the lag time are in progress.

Results and Conclusions: Preliminary TMC analyses indicated that different background mutation rates were found for men and women. All other parameters are identical for the two sexes. This suggests that risk estimates for populations with different baseline leukemia incidence can be obtained from the model. Similarly, risks can be derived for chronic exposures. This work was presented at the 14th International Congress of Radiation Research (ICRR), Warsaw, Poland, 2011.

RP-A1-08 Bayesian MCMC applied to individual cancer incidence data in atomic bomb survivors

Cullings HM (S), Little MP, Furukawa K (S), Nishi N, Soda M (EN), Suyama A, Sakata R (EH), Kasagi F, Molitor J

Objectives: The uncertainty in survivor dose estimates and the resulting effect on RERF studies is a longstanding concern at RERF. It is currently addressed by adjusting doses using correction factors based on regression calibration and assumptions about the probability distributions of the errors. This study intends to examine the effect of assumptions about the error distributions on important types of RERF risk estimation studies by using Bayesian models for individual data with fully specified likelihoods rather than the standard approach of Poisson regression on grouped data. For a variety of radiosensitive cancer sites, estimates obtained using Bayesian Markov Chain Monte Carlo (MCMC) methods will be compared with those obtained using the regression calibration (substitution) method, where both methods are based on individual data.

Background and Significance: A major source of uncertainty concerns extrapolation of risks at high doses and high-dose rates to low doses and low-dose rates and the impact of both systematic and random dosimetric errors on those. It is well recognized that measurement error can alter substantially the shape of this relationship and hence the derived population risk estimates.

Pierce et al. (*Radiat Res* 1990; 123:275–84) carried out a dose adjustment prior to the model fitting to allow for random dosimetric errors by substituting the expectation of the "true dose" given the estimated dose. This leads to reasonable adjusted point estimates of the model parameters for linear dose-effect relationships but is only approximate for non-linear ones, and does not fully take account of all the variability induced by the measurement errors. In fact, the published work by Pierce and others to date has focused on the effect of random dose error with regard to bias in risk estimates but not in regard to the uncertainty of those estimates.

The proposed Bayesian approach to the measurement error problem links three basic sub-models: the disease model, the measurement model and the exposure model, and dosimetric uncertainty is reflected in the variability of the model parameters. In principle, this allows for more of the uncertainties to be taken into account and will produce

a wider, but more realistic, uncertainty envelope than a single error model with a specific form for the error distribution. However, it is possible that in practice this may not happen to any great extent.

Study Methods: The posterior distribution of the risk parameters for the various data sets of interest will be obtained by samples from MCMC algorithms. Using individual data will allow a fully Bayesian analysis to be carried out, in particular one that embraces the full range of uncertainties, for example relating to the form of the error distribution. However, given the complexities and iterative nature of the model and the large sample size, the computational requirements are a hurdle.

Study Progress: In April 2009, Dr. Furukawa presented some initial results from simulation studies to compare a Bayesian approach for the dose error adjustment to regression calibration in normal and logistic regression. The work initiated by Dr. Li (Imperial College London, UK) in 2008 (nested case-control data of leukemia and thyroid cancer) made little progress after that due to Dr. Li's relocation.

The computational burden was still a major issue for Cox regression on individual data, the primary goal of this project. We held discussions with Dr. Pierce about computational feasibility issues during his visit in October 2010. As he suggested, we started exploring some alternative approaches to Bayesian MCMC that may be more computationally tractable, such as multiple imputation. Some related results from a collaboration with Dr. Doi were presented at the International Biometrics Society meeting in Kobe in August 2012. This work made no progress in 2014, primarily due to lack of time because of other priorities related to dosimetry and dose-error, particularly the work described under the dosimetry RP 18-59 and the dose-error RPs A4-10 and (now terminated) A5-11.

Results and Conclusions: None yet.

Effects of rain falling shortly after the atomic bombings

Ozasa K (EH), Grant EJ (EH), Sakata R (EH), Cullings HM (S)

Objectives: To clarify the association of exposure to rain falling shortly after the atomic bombings (fallout rain) in Hiroshima and Nagasaki and the development of acute radiation syndromes and long-term health outcomes.

Background and Significance: Although some information on exposure to fallout rain was collected in the early interview surveys in 1950s–60s, it had not been used for the analysis of health effects because the data were crude and non-quantitative. However, as a result of the findings on the effects of "black rain" published and/or reported to news media by outside groups and the nuclear power plant accident in 2011, public concern about radioactive fallout has grown, and it was thought that an investigation of the association of exposure to fallout rain and health outcomes was needed in the light of such concerns.

Study Methods: Individual information on exposure status (yes/no/unknown) to fallout rain and development of acute symptoms was abstracted. Combined with follow-up information, the risk of exposure to fallout rain was estimated for overall death, cause of death, and cancer incidence. Cross-sectional analyses between rain exposure and acute symptoms were also conducted. The work has been conducted in coordination with the RERF Dosimetry Committee and the Department of Statistics.

Study Progress: A paper on the risk of fallout rain exposure for long-term health effects was published in *Radiation Research* and another paper on the association of rain exposure and development of acute symptoms is under internal review.

Results and Conclusions: No substantial increases in risks of cancer or other diseases were observed in association with reported exposure to fallout rain. Therefore, we concluded that the reported fallout rain was not likely to be homogeneously radioactive at a considerable level, which accords with area measurements made soon after the bombings.

Life Span Study Publications

RERF Reports (RR)

♦ Furukawa K, Preston DL, Misumi M, Cullings HM: Handling incomplete smoking history data in survival analysis. Stat Methods Med Res 2014 (October) doi:10.1177/0962280214556794 [Epub ahead of print]. **(RR 4-13)** (related to *Special Cancer Studies*)

[Study Findings] This study proposes a new method based on multiple imputation for analysis involving incomplete smoking history data, a situation that could be problematic when analyzing cancer incidence data in the Life Span Study (LSS) of atomic bomb survivors. Applying the proposed approach to the LSS lung cancer incidence analysis, we observed the radiation-associated lung cancer risk to be larger among moderate smokers than heavy smokers. The smoking-radiation interaction, which had been estimated to be unusually strong in the previous analysis using a naïve approach (relatively simple methodology to analyze missing data) with missing data indicators, was slightly weaker in the current analysis, which seems to be more interpretable. The proposed approach is expected to reduce estimation bias that might be unavoidable in naïve analyses while preserving efficiency by using all available information, which should lead to radiation risk estimation with improved precision.

[Explanation] In studying the radiation effects on, for example, cancer incidence among atomic bomb survivors, it is important to account for effects of other important risk factors, such as smoking habit. However, data for such risk factors are frequently missing or only available incompletely. Generally, the choice of a method to analyze data involving missing data may have large impacts on the results of risk estimation. In particular, it is well known that a naïve approach, such as the simple deletion of subjects with missing data, can lead to substantial bias in estimation. This study proposed a new approach using multiple imputation for analysis involving missing or incompletely observed smoking history data, applied it to analysis of the LSS lung cancer incidence, and compared the estimation results with those based on the simpler missing-data approaches. ***Study purpose*** To develop a new multiple imputation method to analyze data involving missing or incompletely observed time-dependent smoking history data and apply it to the LSS lung cancer

incidence analysis. ***Study methods*** In analyzing the LSS lung cancer incidence during the period 1958–1999, about 40% of the 105,401 eligible subjects had no smoking data, and many of the others had smoking history known for only part of the time period. As a new approach of multiple imputation for incompletely observed smoking history data, prediction models for the age of smoking initiation and, given initiation, smoking intensity and cessation age, were estimated; based on which missing smoking data were predicted and imputed. Complete data sets including the imputed data were analyzed by multiple imputation. ***Study results*** In an analysis of the LSS lung cancer incidence, we confirmed that when one had both radiation exposure and smoking habit, the radiation-associated excess relative risk of lung cancer would be higher if he/she were a moderate smoker than a heavy smoker. The smoking-radiation interaction, which had been found to be unusually strong by a naïve approach using indicators for missing data, was slightly weaker with the new approach, which seems to be more interpretable for the joint effect of radiation exposure and smoking habit. The proposed approach is particularly appealing in a large-scale cohort study such as the LSS, since it can reduce estimation bias that might be unavoidable in naïve analyses while maintaining efficiency by retaining known information, which is expected to lead to radiation risk estimation with improved precision.

♦ Sakata R, Grant EJ, Furukawa K, Misumi M, Cullings HM, Ozasa K, Shore RE: Long-term effects of the rain exposure shortly after the atomic bombings in Hiroshima and Nagasaki. Radiat Res 2014 (December); 182(6):599–606. **(RR 7-14)**

[Study Findings] Using a population of individuals responding to surveys from 1949 to 1961, we compared mortality and cancer incidence between those reporting rain exposure shortly after the atomic bombing and those reporting no such rain exposure. No increase in mortality or cancer incidence was observed in those reporting rain exposure.

[Explanation] ***Objectives*** The objective of the study was to determine whether mortality or cancer incidence was higher among individuals reporting rain exposure shortly after the atomic bombing than individuals reporting no such rain exposure. ***Methods*** The study included 86,609 Life Span Study cohort members who had an estimated Dosimetry System 2002 (DS02) radiation dose. The Atomic Bomb Casualty Commission, the predecessor to the Radiation Effects Research Foundation, conducted surveys through personal interviews from 1949 to 1961. Based on these survey results, the respondents were categorized into three groups according to their response to the question on rain exposure (i.e., "Yes," "No," "Unknown"). The excess relative risks (ERRs) of mortality between 1950 and 2005 and cancer incidence between 1958 and 2005 were estimated among those reporting rain exposure relative to those reporting no rain exposure. The data were adjusted for city, sex, year of birth, attained age, and individual DS02 dose estimates from direct radiation exposure. ***Results*** (1) Of the individuals interviewed, 42,050 (72%) in Hiroshima and 25,064 (89%) in Nagasaki responded to the question on rain exposure. Of these, 11,661 (20%) in Hiroshima and 733 (2.6%) in Nagasaki reported rain exposure. (2) The ERR of all-cause mortality from 1950 to 2005 among those who reported rain exposure in Hiroshima was 0.01 (95% confidence interval (CI): 0.02, 0.04). The ERRs of mortality for solid cancer and leukemia for the same period were 0.02 (95% CI: 0.06, 0.04) and 0.06 (95% CI: 0.15, 0.32), respectively. No significant increase in risk was observed. (3) A weak association was observed between all-cause mortality and rain exposure in Nagasaki (ERR = 0.08, 95% CI: 0.00006, 0.17) for the entire period of observation (1950 to 2005). However, the association was not statistically significant for the period after all surveys had been completed (1962 to 2005). The ERRs of mortality for solid cancer and leukemia between 1950 and 2005 were 0.14 (95% CI: 0.01, 0.33) and 0.03 (95% CI: 0.07, 0.02), respectively, suggesting no significant increase in risk. (4) No increase in cancer incidence in those who reported rain exposure was observed in either city.

The individuals reporting rain exposure in Hiroshima were not at significantly higher risk of mortality from all cause, solid cancer, or leukemia or of developing solid cancer or leukemia. The findings, however, were inconsistent for those reporting rain exposure in Nagasaki; all-cause mortality risk was higher for certain observation periods and statistical models but not others. In addition, risk estimates for cancer mortality and incidence were inconsistent with each other. These inconsistencies could be attributable to the low number of individuals who reported rain exposure in Nagasaki (733), which is too small to provide reliable statistical inference. In addition, answers to the question on rain exposure would more likely be "unknown" when the 1949–1961 surveys, including the question on rain exposure, were conducted after individuals died and questions were answered by their proxies who had no knowledge regarding whether or not that individual had been caught in rain (outcome-dependent bias). It was also possible when the surveys were conducted after individuals had developed cancer or died that the respondents might have associated this health outcome with their rain-exposure experience (recall bias). For the observation period after completion of the surveys (i.e., starting in 1962), analysis results were probably less affected by these biases. For this observation period, therefore, no significant increase in risk was observed in Nagasaki. No increased risk was observed in either city for mortality from or the occurrence of leukemia, which is often taken to be a "sentinel" indicator of radiation effects. For these reasons, the association observed in Nagasaki is unlikely to be attributable to exposure to radiation from rain. In this study, a large percentage of individuals have missing data on rain exposure. In addition, we did not obtain any information on rain exposure from a considerable proportion of the subjects and no details were available on the circumstances surrounding the rainfall and how individuals experienced the rain from those providing the information on rain exposure. The results should thus be interpreted with caution.

Other Journal Publications

♦ Nakamura N: Hiroshima and Nagasaki, low dose risks (Radiation risk at low doses: Series 3). Nagase Landauer Newsletter 2014 (April); No.436:2. (Japanese)

♦ Nakamura N: How to explain low dose risks to the public. Hiroshima Igaku [J Hiroshima Med Assoc] 2014 (April); 67(4):294–7. (Proceedings of the 54th Late A-bomb Effects Research Meeting, 2013) (Japanese)

♦ Nakamura N: How to think about low-dose risks of radiation? Nihon Hoshasen Koushu Anzen Gakkaishi [JRPS: Japanese Society of Radiation Public Safety] 2014 (September); No.11:13–21. (Japanese)

♦ Nakamura N: Thoughts on Fukushima (Radiation risk at low doses: Series 4). Nagase Landauer Newsletter 2014 (May); No.437:2. (Japanese)

♦ Ozasa K: Epidemiology and pathogenesis. Byori to Rinsho [Pathol Clin Med] 2015 (January); 33(1):55–9. (Japanese)

♦ Ozasa K: Late health effects of atomic bomb radiation. Tajima K et al., eds. Advances and Future Directions of Cancer Epidemiology and Prevention. Extended Abstracts for the 44th International Symposium of the Princess Takamatsu Cancer Research Fund, Tokyo, Japan, 13–15 November 2013. Tokyo: Princess Takamatsu Cancer Research Fund, 2014, pp 49–53.

Manuscript in Press

⌘ Grant EJ, Ozasa K, Ban N, de Gonzalez AB, Cologne JB, Cullings HM, Doi K, Furukawa K, Imaoka T, Kodama K, Nakamura N, Niwa O, Preston DL, Rajaraman P, Sadakane A, Saigusa S, Sakata R, Sobue T, Sugiyama H, Ullrich RL, Wakeford R, Yasumura S, Milder C, Shore RE: A report from the 2013 International Symposium: The evaluation of the effects of low-dose radiation exposure in the Life Span Study of atomic bomb survivors and other similar studies. Health Phys.

Life Span Study Oral Presentations

❖ Shore RE, Grant EJ, Milder C, Sadakane A. The role of other exposures in radiogenic breast cancer risk. 105th Annual Meeting of the American Association for Cancer Research (AACR), 5–9 April 2014, San Diego, California, USA (related to *Special Cancer Studies*)

❖ Shore RE. Human radiation biology studies: issues and RERF biosamples. 5th EpiRadBio Contractors Meeting, 28–30 April 2014, Lisbon, Portugal (related to *Adult Health Study*)

❖ Shore RE. Health outcomes of those exposed to the A-bombing as fetuses or in childhood. IAEA Meeting on Low-dose Radiation, 2–4 July 2014, Vienna, Austria

❖ Grant EJ, Sakata R, Cullings HM, Ozasa K. Effects of fallout rain on mortality and cancer incidence among the Life Span Study of atomic bomb survivors. 59th Annual Meeting of the Health Physics Society, 13–17 July 2014, Baltimore, Maryland, USA

❖ Shore RE, Sadakane A. How much risk from CT exams of children? 56th Annual Meeting of the American Association of Physics in Medicine, 21 July 2014, Austin, Texas, USA

❖ Ozasa K, Shimizu Y, Pham TM, Grant EJ, Sakata R, Sugiyama H, Sadakane A, Soda M, Kodama K. Radiation risk of mortality of cancer and noncancer disease in the atomic-bomb survivors. 20th International Epidemiological Association World Congress of Epidemiology, 17–21 August 2014, Anchorage, Alaska, USA

❖ Brenner AV, Sakata R, Preston DL, RERF-NCI Cancer Risk Analysis Group. Incidence of female breast cancer in atomic bomb survivors in Hiroshima and Nagasaki; 1958–2009. 21st International Meeting Conference on Radiation and Health, 21–24 September 2014, Las Vegas, Nevada, USA

❖ Cahoon E, Furukawa K, RERF-NCI Cancer Risk Analysis Group. Lung and other respiratory cancers, smoking, and radiation risks in the LSS. 21st International Meeting Conference on Radiation and Health, 21–24 September 2014, Las Vegas, Nevada, USA

❖ Furukawa K. Non-parametric smoothing for radiation dose-response estimation. 21st International Meeting Conference on Radiation and Health, 21–24 September 2014, Las Vegas, Nevada, USA

❖ Grant EJ, Sugiyama H, Brenner AV, Ozasa K, RERF-NCI Cancer Risk Analysis Group. Solid cancer incidence among atomic bomb survivors: Overview of dosimetry, follow-up, and risk updates. 21st International Meeting Conference on Radiation and Health, 21–24 September 2014, Las Vegas, Nevada, USA

❖ Sadakane A, Sakata R, Grant EJ, Utada M, Ozasa K. Evaluation of medical radiation exposures among atomic bomb survivors. 21st International Meeting Conference on Radiation and Health, 21–24 September 2014, Las Vegas, Nevada, USA

❖ Sakata R, Rajaraman P, RERF-NCI Cancer Risk Analysis Group. Stomach cancer radiation risks in the LSS. 21st International Meeting Conference on Radiation and Health, 21–24 September 2014, Las Vegas, Nevada, USA

❖ Kodama K. RERF's biosample resources—Issues need to be resolved for future use. 60th Annual Meeting of the Radiation Research Society, 21–24 September 2014, Las Vegas, Nevada, USA (related to *Adult Health Study* and *F_1 Studies*)

❖ Shore RE. How much risk from CT examinations of children? 60th Annual Meeting of the Radiation Research Society, 21–24 September 2014, Las Vegas, Nevada, USA

❖ Takahashi I, Shimizu Y, Grant EJ, Sakata R, Sadakane A, Ozasa K. Heart disease mortality among the atomic-bomb survivors—The Life Span Study, 1950–2008. 60th Annual Meeting of the Radiation Research Society, 21–24 September 2014, Las Vegas, Nevada, USA

❖ Ozasa K, Sakata R, Sugiyama H, Utada M, Takahashi I, Sadakane A, Grant EJ. What we can say from the epidemiological data of the atomic-bomb survivors. 57th Annual Meeting of the Japan Radiation Research Society, 1–3 October 2014, Kagoshima

❖ Ozasa K, Takahashi I, Shimizu Y, Sakata R, Sugiyama H, Sadakane A, Utada M, Grant EJ, Kodama K. Mortality risk of heart diseases in the atomic-bomb survivor's life span study. 6th International MELODI (Multidisciplinary European Low Dose Initiative) Workshop, 7–9 October 2014, Barcelona, Spain

❖ Ozasa K. Long-term health effects of exposure to radiation. 67th Meeting of the Hiroshima Medical Association, 9 November 2014, Hiroshima

❖ Kodama K. Radiation health effects; Lessons learned from the study of atomic-bomb survivors. Public Lecture, 57th Annual Meeting of the Japan Thyroid Association, 15

November 2014, Osaka (related to *Adult Health Study*)

❖ Shore RE. LSS updated studies of radiation and cancer incidence. NAS BEIR VIII Planning Meeting, 17 November 2014, Washington, DC, USA (related to *Special Cancer Studies*)

❖ Shore RE. Does age at exposure affect radiation disease risk among atomic bomb survivors? NIRS-WHO Joint Symposium, 7–9 December 2014, Tokyo (related to *Special Cancer Studies*)

❖ Sadakane A, Sakata R, Grant EJ, Sugiyama H, Utada M, Ozasa K. Evaluation of medical radiation exposures among atomic bomb survivors. 25th Annual Scientific Meeting of the Japan Epidemiological Association, 21–23 January 2015, Nagoya

❖ Ozasa K. Late health effects of radiation exposure and gastric cancer. 87th Annual Meeting of Japanese Gastric Cancer Association, 6 March 2015, Hiroshima

❖ Ozasa K. Long-term health effects of the atomic-bomb radiation. Epidemiology Conference for Emergency Workers of Tokyo Electric Power's Fukushima I Nuclear Power Plant, 11 March 2015, Kitakyushu (related to *F1 Studies*)

❖ Cologne JB, Izumi S, Sakata R, Yamada M. Interaction between exposure and age in cohort-based risk models: effect modification or age dependence of the excess rate? 2015 Annual Meeting of Applied Statistics Society, 14 March 2015, Kyoto (related to *Special Clinical Studies*)

Research Protocols 1-15, 2-14, 2-11, 7-10, 7-09, 2-75 (Platform Protocol), A6-12, A3-09

Adult Health Study (AHS)

RP 1-15 Effects of Ionizing Radiation on Impairments of Glucose and Lipid Metabolism and Impact on Risks of Arteriosclerotic Diseases and Cancers

Tatsukawa (C), Hida A (CN), Yamada M (C), Kim Y-M (S), Cullings HM (S), Sera N, Sakata R (E), Ohishi W (C), Nakanishi S, Yoneda M

Impairment of glucose metabolism and lipid metabolism are major risk factors for arteriosclerotic diseases, but the effects of atomic bomb radiation on those conditions are unclear.

The Adult Health Study (AHS) cohort of atomic bomb survivors, which has been followed by biennial health examinations since 1958 and has obtained medical information at each examination cycle, is comprised of suitable subjects for assessing the effects of radiation on conditions such as diabetes and dyslipidemia. Furthermore, the AHS cohort was expanded in 2008 to include about 1,900 younger survivors (<10 years old at the time of bombing [ATB]), and various biomarkers for glucose and lipid metabolism have been measured in them as well as in the original cohort. Since the expansion group includes a large number of lightly exposed individuals relative to the original cohort, it will provide more precise information on dose response. In this proposed research protocol (RP), we will use a longitudinal study format to examine whether radiation exposure is associated with the development of diabetes and whether any such association is influenced by the city of exposure and age ATB.

It is well known that radiation exposure is associated with increased risk for many cancers. Recent studies have also suggested an association between radiation exposure and the risk of arteriosclerotic diseases; however, the underlying mechanism remains elusive. Diabetes and dyslipidemia are major risk factors for arteriosclerotic diseases. Recent epidemiological studies have also shown that diabetes is associated with risk for not only arteriosclerotic diseases but some cancers as well. This RP will also investigate whether radiation exposure is associated with increased risks of arteriosclerotic disease and cancer through impaired glucose and/or lipid metabolism, using 'causal modeling' to evaluate intermediate variables. We will use a cross-sectional study design, to investigate an association between radiation exposure and HbA1c, insulin resistance, lipid levels (such as HDL cholesterol), and biomarkers involving glucose and lipid metabolism (such as adiponectin).

RP 2-14 Study of heart disease in the Adult Health Study population using echocardiography

Takahashi I (CH), Haruta D (CN), Hidaka T, Tsuneto A, Kajimura J (R), Hayashi T(R), Furukawa K (S), Imaizumi M (CN), Hida A (CN), Ohishi W (CH), Kihara Y

Objectives: Our main purpose is evaluating radiation effects on diastolic heart failure, one subtype of heart failure, among Adult Health Study (AHS) participants aged 15 or less at the time of bombing. The study will also provide

information relevant to valvular and ischemic heart disease.
Background and Significance: Recent Life Span Study (LSS) reports demonstrated an excess risk of heart disease mortality. Reports from radiotherapy patients and experimental data have indicated that high-dose radiation may cause heart damage and development of cardiac dysfunction, especially diastolic dysfunction. But the epidemiological data about radiation-related heart dysfunction at low-to-moderate doses are limited.
Study Methods: Based on echocardiograph assessment and blood markers in the Adult Health Study (AHS), we will determine heart failure in subjects who were 15 years of age or younger at the time of bombings and categorize it into its subtypes. The expected number of subjects for the study is about 2,700 AHS members (including *in utero* survivors) in Hiroshima and Nagasaki.
Study Progress: Echocardiographic assessment and blood sampling was started in June 2014 and is now underway. An addendum RP planned to conduct a more sophisticated speckle-tracking analyses is now in the internal review process.
Results and Conclusions: None yet.

RP 2-11 Study of arteriosclerosis in the Adult Health Study population (Part 2. Analysis of the cytokine network regulating differentiation of mesenchymal stem cells in artery)

Takahashi I (CH), Ohishi W (CH), Hayashi T (R), Cologne JB (S), Takahashi T, Kusunoki Y (R), Ozasa K (EH), Kihara Y, Matsumoto M, Fujiwara S
Objectives: To test the hypothesis that arteriosclerosis develops due to abnormalities in mesenchymal tissues because cell damage from radiation exposure triggers the cytokine network involved in repair of such tissue damage in arteries.
Background and Significance: AHS data have suggested a relationship between radiation exposure and the prevalence or incidence of stroke, ischemic heart disease, and aortic calcification. However, the pathogenetic mechanism(s) underlying arteriosclerosis due to radiation exposure is unclear. Some have hypothesized that arteriosclerosis is primarily an inflammatory disease, but it appears to have a complex pathology, of which inflammation is only one component. We hypothesize that atherosclerosis develops due to abnormalities in mesenchymal tissues because cell damage from radiation exposure triggers the cytokine network involved in repair of such tissue damage in arteries.
Study Methods: This is a cross-sectional study among 2,000 AHS subjects (including those exposed at young ages) in Hiroshima. We are measuring several multi-functional cytokines involved in the "cytokine network" that are possibly related to radiation-induced atherogenesis and will relate them to physiological measurements of atherosclerotic cardiovascular outcomes (based on RP 7-09). We will then examine if the "cytokine network" functions to either moderate or mediate the radiation effect upon atherosclerotic cardiovascular outcomes.
Study Progress: Data collection began in April 2011. We finished collecting blood samples in March 2014, and almost completed measuring blood cytokine levels related to CVD such as pentraxin-3, osteopontin, osteoprotegerin, high mobility group box (HMGB)-1, VEGF, and apolipoprotein (Apo)-J, in December 2014 for about 2,100 AHS subjects.
Results and Conclusions: None yet. Results expected in 2016.

RP 7-10 Study of body composition of the Hiroshima Adult Health Study population

Tatsukawa Y (CH), Fujiwara S, Harris TB, Misumi M (S), Ohishi W (CH), Yamada M (CH), Kasagi F
Objectives: The major objectives of this research protocol are to test whether radiation exposure is related to an increased incidence of arteriosclerotic diseases and their risk factors through modifications in body composition.
Background and Significance: Recent studies of childhood cancer survivors have suggested that radiation exposure causes modifications in body composition (such as higher body fat mass and lower lean mass). These findings reflect high exposures from radiotherapy (e.g., Hodgkin disease treatment), but extrapolation to lower doses is unknown. It is important to examine the association between radiation dose and body composition, and to determine whether radiation exposure is related to an increased incidence of arteriosclerotic diseases and their risk factors partly through modifications of body composition.
Study Methods: Study subjects total approximately 2,200 Hiroshima AHS participants who underwent whole-body composition examination by dual energy x-ray absorptiometry (DEXA) during the period 1994–1996. With those data it is possible to evaluate the whole-body/regional (trunk, limb, etc.) fat mass and lean mass and to relate those to other AHS data on cardiovascular diseases.
Study Progress: We have started further analysis to examine the associations of body composition, such as appendicular lean mass and trunk-to-limb fat ratio, with development of diabetes.
Results and Conclusions: A-bomb radiation dose was negatively associated with BMI and appendicular lean mass (a surrogate marker for muscle mass) in both sexes. It was positively associated with trunk-to-limb fat ratio in women who were less than 15 years old at the time of exposure. In conclusion, significant associations of radiation dose with BMI and body composition were observed 50 years after A-bomb exposure. (*Int J Obesity* 2013; 37:1123–8)

RP 7-09 Study of arteriosclerosis in the Adult Health Study population (Part 1. Physiological Indices of arteriosclerosis)

Takahashi I (CH), Hida A (CN), Kohata M, Yamada M (CH), Ohishi W (CH), Cologne JB (S), Misumi M (S), Takahashi T, Kihara Y, Matsumoto M, Fujiwara S
Objectives: The goal is to study one of the potential mechanisms by which radiation may promote cardiovascular disease. We will evaluate acceleration of arterial stiffness by radiation among AHS subjects (including the expanded group of younger survivors).
Background and Significance: Past studies have reported a significant association between radiation exposure and atherosclerotic disease mortality/morbidity among A-bomb survivors.

Atherosclerosis conceptually has two aspects: atherosis

(fatty degeneration) and sclerosis (arterial stiffness). While acceleration of arterial stiffness might be caused by radiation-induced structural changes in arterial walls, it has not been fully investigated. In this study, we will evaluate the associations of radiation and arterial stiffness taking into account correlations among stiffness indices and atheromatous disease indices/risk factors.

Study Methods: This is a cross-sectional study among all AHS subjects in Hiroshima and Nagasaki. The associations of radiation and the arterial stiffness indices (brachial-ankle pulse wave velocity [baPWV], augmentation index [AI]) will be analyzed with consideration of atheromatous disease indices (ankle-brachial blood pressure index [ABI], carotid intima-media wall thickness [CIMT], aortic calcification, and left ventricular hypertrophy) and atherosclerosis risk factors (Framingham risk scores).

Study Progress: Data collection began in April 2010 and was completed in March 2014. We are now in the process of cleaning data, and analyses will be started in 2015 on 3,787 AHS subjects.

Results and Conclusions: None yet. Results expected in 2016.

RP 2-75 Research plan for RERF Adult Health Study (AHS), Hiroshima and Nagasaki

Ohishi W (CH), Yamada M (CH), Tatsukawa Y (CH), Takahashi I (CH), Ueda K (CH), Mitsui F (CH), Hida A (CN), Sera N, Imaizumi M (CN), Haruta D (CN), Soda M (EN), Fujiwara S

Objectives: To evaluate in a systematic fashion the age and radiation exposure-dependent changes in the clinical status of long-term survivors (Adult Health Study [AHS] cohort) of the atomic bombings, and to provide extensive biological specimens and information concerning lifestyle or other potential risk factors for many fields of study, which include cytology, genetics, immunology, radiobiology, and medical dosimetry.

Background and Significance: The AHS program of biennial comprehensive medical examinations is the only program in the world to provide long-term health screening of a representative irradiated population of all exposure ages and a wide range of doses, and therefore can provide unique longitudinal information on subclinical and clinical disease risk, plus biosamples for numerous clinical and radiobiology studies. It began in 1958 with a targeted population of about 20,000 survivors and controls in the contact areas of Hiroshima and Nagasaki, was enriched in 1978 with about 2,400 additional higher-dose subjects and all available (~1,000) persons who were exposed *in utero,* and expanded again in 2008 with over 1,900 young exposed subjects (<10 years old at the bombings) to increase our ability to document radiation effects among those exposed when young.

Study Methods: The study examines differences in rates of a wide variety of diseases or pre-clinical disorders by level of radiation exposure. During the 28th cycle (August 2012–June 2015 in Hiroshima, February 2013–December 2015 in Nagasaki), 2,247 individuals among the original 1958–1978 AHS cohort were examined, representing approximately 65% of that cohort who were still living in the catchment area, and 1,556 individuals in the 2008 expanded AHS group.

Study Progress: Health examinations are continuing. The biological samples collected are used for clinical determinations and stored for ongoing and future studies. Evaluation of possible interactions has been assessed between radiation and various biological factors based on stored samples with respect to health outcomes; the factors include infectious agents, hormones, inflammatory markers, and a variety of other phenotypic and genetic factors. Ongoing collaborative studies include ones on: the interactions between radiation and infectious agents or hormones in the development of hepatocellular carcinoma (HCC), gastric cancer, and breast cancer, the association between thyroid function and various endpoints (the association between thyroid function and kidney disease or anemia were initiated recently), and the association of progressive cardiac conduction defect and weight fluctuation with cancer and cardiovascular disease (CVD). Because significant associations between radiation exposure and atherosclerotic disease mortality/morbidity have been reported in past studies, we are evaluating arterial stiffness, atherosis, and several multi-functional cytokines to study potential mechanisms by which radiation may promote cardiovascular disease. We recently initiated an echocardiographic study to detect heart failure and several other kinds of heart disease, plus measuring markers for incipient heart failure (NT-proBNP) and several other biomarkers of cardiac risk factors. Informed consent has been obtained for more specific permission for preservation and later use of clinical information and blood and urine samples collected at current and previous examinations.

Results: New findings were reported on the associations of radiation exposure with thyroid nodules among younger A-bomb survivors, and on the interaction of radiation with IL-6 on HCC risk.

RP-A6-12 Choice reaction time of middle-aged and elderly Japanese as a predictor of cardiovascular mortality: Radiation Effects Research Foundation Adult Health Study

Shimizu M, Misumi M (S), Yamada M (CH), Ohishi W (CH), Yamamoto H, Kihara Y

Objectives: The association between choice reaction time and subsequent coronary heart disease (CHD) and stroke mortality in middle-aged and elderly individuals based on a roughly 35-year follow-up of participants of the Adult Health Study (AHS) will be studied.

Background and Significance: Reaction time, certain biological indices that exhibit aging-related changes, and an index of cognitive function were reported to predict life prognosis and vascular disease mortality. However, large-scale studies of general populations and cohort studies with a broad age range were limited in number, and there are few reports from studies on Asian cohorts.

Study Methods: In the AHS, reaction time was measured by Bogitch's flash reaction test for about 5,000 Hiroshima participants aged 35–74 years old, between 1970 and 1972. Vital status of the subjects was followed up from the time of baseline reaction time measurement until the end of 2007, with causes of death recorded in death certificates coded using the International Classification of Diseases (ICD). The association between reaction time and circulatory disease

mortality will be investigated.
Study Progress: A manuscript was submitted for journal publication.
Results and Conclusions: Reaction time and grip strength, alone and in combination, predicted CVD mortalities, which suggests that both cognitive and physical functions may be useful for CVD prognosis.

RP-A3-09 The association between chronic kidney disease and cardiovascular disease among atomic bomb survivors

Haruta D (CN), Tsuneto A, Takahashi I (CH), Hida A (CN), Sera N, Imaizumi M (CN), Yamada M (CH), Ohishi W (CH), Tatsukawa Y (CH), Nakashima E (S), Misumi M (S), Fujiwara S, Akahoshi M

Objectives: To clarify whether radiation is associated with chronic kidney disease (CKD) and whether CKD is involved in the mechanism(s) linking radiation exposure and cardiovascular disease (CVD).
Background and Significance: The association between atomic-bomb radiation exposure and CVD has recently been drawing attention. CKD has recently been recognized as a risk factor for CVD. CKD and CVD share many common risk factors such as obesity, insulin resistance, impaired glucose tolerance, hypertension, dyslipidemia, and nephritis. This is the first RERF study to examine whether CKD serves as a CVD risk factor and the possible involvement of A-bomb radiation exposure in the disease process.
Study Methods: In this analysis, we will identify prevalent cases of CKD diagnosed during the four-year period between January 1988 and December 1991 (baseline period) and incident cases of CKD diagnosed between January 1992 and December 2006 (follow-up period) in the AHS cohort. We will also identify both prevalent and incident cases of CVD during the above-mentioned periods, respectively. CVD includes coronary heart disease (CHD) and stroke in this RP. Based on these data, we will determine whether the effects of A-bomb radiation exposure can be observed for several endpoints, with adjustment for other risk factors: (1) Associations of CKD prevalence or incidence with radiation dose and CKD risk factors. (2) Associations of CHD and stroke prevalence with radiation dose, CKD risk factors, and previous prevalent or incident CKD.
Study Progress: The data to analyze the association of prevalent cases of CKD with radiation dose and CKD risk factors and the incidence data on CKD have been collected but the incidence data on CVD need to be compiled and cleaned.
Results and Conclusions: None yet. Results expected in 2015.

Adult Health Study Publications

RERF Reports (RR)

♦ Imaizumi M, Ohishi W, Nakashima E, Sera N, Neriishi K, Yamada M, Tatsukawa Y, Takahashi I, Fujiwara S, Sugino K, Ando T, Usa T, Kawakami A, Akahoshi M, Hida A: Association of radiation dose with prevalence of thyroid nodules among atomic bomb survivors exposed in childhood (2007–2011). JAMA Intern Med 2015 (February); 175(2):228–36. **(RR 9-14)** (related to *Special Clinical Studies*)

[Study Findings] A thyroid study conducted among A-bomb survivors 62–66 years after exposure to radiation in childhood (at less than 10 years of age) revealed a significant association between the prevalence of larger thyroid nodules (those with a diameter of 10 mm or more, or those with past surgery for a nodule) and A-bomb radiation dose to the thyroid; however, no association was observed for small nodules of less than 10 mm in diameter.
[Explanation] Adult Health Study (AHS) participants were examined to investigate the association between thyroid nodules and A-bomb radiation in survivors exposed in childhood (at less than 10 years of age). The AHS has examined the health status of A-bomb survivors in Hiroshima and Nagasaki since 1958 through biennial medical assessments. ***Objectives*** It is known that the risk of thyroid cancer is higher after radiation exposure in childhood compared with exposure in adulthood. Determining whether or not the effects of radiation exposure on the thyroid appear among adults many years after exposure in childhood is an important public health issue. On the other hand, thyroid ultrasonography can detect thyroid nodules in 17–67% of the general population who have had little exposure to radiation. This study aims to elucidate the association between thyroid nodules and A-bomb radiation among A-bomb survivors more than 60 years after exposure in childhood. ***Methods*** Thyroid examinations, including thyroid ultrasonography, were conducted on 3,087 A-bomb survivors participating in the AHS between 2007 and 2011 who were less than 10 years old at the time of exposure. The study investigated the association of thyroid dose with the prevalence of larger thyroid nodules (diameter of 10 mm or more, or post-surgical cases), as well as small thyroid nodules (diameter of less than 10 mm) among 2,688 survivors for whom A-bomb radiation dose to the thyroid had been estimated. The mean age of participants was 68.2 years; 1,213 were males and 1,455 females; the median dose was 0.182 Gy (182 mGy); and the dose range was 0–4.040 Gy. ***Results*** (1) The prevalence (17.6% of those examined) of larger thyroid nodules (diameter of 10 mm or more, or post-surgical cases) was significantly associated with thyroid radiation dose; the estimated excess odds ratio per gray (Gy) of thyroid dose was 1.65 (95% Confidence Interval (CI): 0.89, 2.64). We conducted the analyses by classifying the thyroid nodules into solid nodules and cysts, and then further classifying the solid nodules as malignant tumors or benign nodules. Respective analyses detected a significant association between nodule prevalence and thyroid dose. (2) The study revealed that age at exposure significantly affected the association between the prevalence of larger thyroid nodules (diameter of 10 mm or more, or post-surgical cases) and radiation, and that the effects of radiation were greater with earlier childhood exposure. On the other hand, the radiation risk did not vary substantially by sex, family history of thyroid disease, presence of antithyroid antibodies, or intake of seaweed. (3) No significant association was observed between small thyroid nodules (diameter of less than 10 mm) and radiation dose to the thyroid.

More than 60 years after exposure to radiation in childhood, instances of larger thyroid nodules (diameter

of 10 mm or more, or post-surgical cases) were associated with radiation dose; however, such an association was not observed for small thyroid nodules (diameter of less than 10 mm). The term thyroid nodule refers to a variety of clinical states, including cancers, benign tumors, hyperplasia, and thyroiditis-related changes. The difference in effects of radiation in relation to the size of nodules may provide clues to the mechanisms through which radiation exposure affects the clinical state of thyroid nodules.

♦ Nakashima E, Neriishi K, Hsu WL: Radiation may indirectly impair growth resulting in reduced standing height via subclinical inflammation in atomic-bomb survivors exposed at young ages. Epidemiol Res Int 2015 (January); Volume 2015 (Article ID 295958):10 pages. doi: 10.1155/2015/295958. **(RR 17-11)** (*A summary explanation was not created for this RR.*)

Adult Health Study Oral Presentations

❖ Shore RE. Human radiation biology studies: issues and RERF biosamples. 5th EpiRadBio Contractors Meeting, 28–30 April 2014, Lisbon, Portugal (related to *Life Span Study*)

❖ Itakura K, Takahashi I, Nakashima E, Yanagi M, Kawasaki R, Neriishi K, Wang JJ, Wong TY, Hida A, Ohishi W, Kiuchi Y. Atomic bomb radiation exposure and the prevalence of age-related macular degeneration: The Hiroshima-Nagasaki atomic bomb survivor study. Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO), 4–8 May 2014, Orlando, Florida, USA

❖ Tatsukawa Y, Yamada M, Ohishi W, Nakanishi S. Types of obesity and development of diabetes. 57th Annual Scientific Meeting of the Japan Diabetes Society, 22–24 May 2014, Osaka

❖ Ohishi W, Ueda K, Tatsukawa Y, Nakashima E, Yamada M, Kohata M, Takahashi I, Tsuge M, Chayama K. Study of factors associated with prevalence and disease progressions in nonalcoholic fatty liver disease. 50th Annual Meeting of the Japan Society of Hepatology, 29–30 May 2014, Tokyo (related to *Special Clinical Studies*)

❖ Hayashi T, Hu Y, Furukawa K, Ohishi W, Hayashi I, Yoshida K, Kajimura J, Kyoizumi S, Kusunoki Y, Nakachi K. Age and radiation effects on intracellular reactive oxygen species in the blood cells of atomic-bomb survivors and their association with immune-related biomarkers. 12th Japan-Korea Cancer and Aging Symposium, 19–21 June 2014, Inacho, Saitama (related to *Immunology Studies*)

❖ Imaizumi M, Ohishi W, Nakashima E, Sera N, Neriishi K, Yamada M, Tatsukawa Y, Takahashi I, Fujiwara S, Ando T, Usa T, Kawakami A, Akahoshi M, Hida A. Thyroid study in Hiroshima and Nagasaki atomic-bomb survivors exposed in childhood: Radiation dose-response relationships for thyroid nodules (2007–2011). 16th International Congress of Endocrinology & The Endocrine Society's 96th Annual Meeting & Expo, 21–24 June 2014, Chicago, Illinois, USA (related to *Special Clinical Studies*)

❖ Tatsukawa Y, Yamada M, Ueda K, Takahashi I, Ohishi W, Nakanishi S. Association between the distribution of body fat and the incidence of diabetes mellitus among elderly Japanese. 16th International Congress of Endocrinology & The Endocrine Society's 96th Annual Meeting & Expo, 21–24 June 2014, Chicago, Illinois, USA

❖ Nakashima E. Radiation dose-response estimation with emphasis on low dose range: Application of restricted cubic spline models to all solid cancer mortality data, 1950–2003, in atomic-bomb survivors. 27th International Biometric Conference, 6–11 July 2014, Florence, Italy

❖ Nakashima E, Neriishi K. Assessing acceleration of failure time with grouped poison regression model. 27th International Biometric Conference, 6–11 July 2014, Florence, Italy

❖ Takahashi I. Stroke study among the Japanese atomic-bomb survivors—Association between radiation and stroke incidence. 15th Hiroshima Clinical Conference of Cerebral Circulation and Metabolism, 17 July 2014, Hiroshima

❖ Furukawa K, Takahashi I. A multi-stage multiple imputation in a large-scale cohort study. 35th Annual Conference of the International Society for Clinical Biostatistics, 24–28 August 2014, Vienna, Austria

❖ Hayashi T, Hu Y, Morishita Y, Sasaki K, Maki M, Furudoi K, Nagamura H, Yoshida K, Kajimura J, Ohishi W, Hida A, Hayashi I, Kyoizumi S, Kusunoki Y, Nakachi K. Functional significance of *IL6R* genotype and colorectal cancer risk by genotype among atomic-bomb survivors. 21st Annual Meeting of the Japanese Society of Immunotoxicology, 11–12 September 2014, Tokushima (related to *Immunology Studies*)

❖ Tatsukawa Y, Yamada M, Ohishi W, Yoneda M. Relationship between body fat distribution and cardiometabolic risk factors in nonobese Japanese subjects. 9th Metabolic Syndrome, Type 2 Diabetes and Atherosclerosis Congress, 12–14 September 2014, Kyoto

❖ Hayashi T, Hu Y, Morishita Y, Sasaki K, Maki M, Furudoi K, Nagamura H, Ohishi W, Hida A, Hayashi I, Yoshida K, Kajimura J, Kyoizumi S, Kusunoki Y, Nakachi K. Relationship of intracellular reactive oxygen species in blood cells with age, radiation and immune- and inflammation-related gene polymorphisms among atomic-bomb survivors. 23rd Annual Meeting of the Japanese Society for Histocompatibility and Immunogenetics, 13–15 September 2014, Nagasaki (related to *Immunology Studies*)

❖ Kodama K. RERF's biosample resources—Issues need to be resolved for future use. 60th Annual Meeting of the Radiation Research Society, 21–24 September 2014, Las Vegas, Nevada, USA (related to *Life Span Study* and *F1 Studies*)

❖ Ueda K, Ohishi W, Nakashima E, Tatsukawa Y, Yamada M, Kohata M, Takahashi I, Tsuge M, Chayama K. Study of factors associated with prevalence of nonalcoholic fatty liver disease in non-obese subjects. 22nd Japan Digestive Disease Week, 23–26 October 2014, Kobe (related to *Special Clinical Studies*)

❖ Tatsukawa Y, Yamada M, Ohishi W, Yoneda M. Prevalence of metabolic abnormality and development of diabetes among elderly population. 52nd Chugoku and Shikoku District Meeting of the Japan Diabetes Society, 24–25 October 2014, Hiroshima

❖ Imaizumi M, Ohishi W, Nakashima E, Sera N, Neriishi K, Yamada M, Tatsukawa Y, Takahashi I, Fujiwara S, Sugino K, Ando T, Usa T, Kawakami A, Akahoshi M, Hida A. Thyroid Study in Hiroshima and Nagasaki atomic-bomb survivors exposed in childhood: Thyroid function and autoimmune thyroid disease. 57th Annual Meeting of the Japan Thyroid

Association, 13–15 November 2014, Osaka (related to *Special Clinical Studies*)

❖ Kodama K. Radiation health effects; Lessons learned from the study of atomic-bomb survivors. Public Lecture, 57th Annual Meeting of the Japan Thyroid Association, 15 November 2014, Osaka (related to *Life Span Study*)

❖ Hayashi T, Hu Y, Yoshida K, Ohishi W, Hida A, Kyoizumi S, Kusunoki Y. Functional significance of *IL6R* genotype and effects thereof on colorectal cancer risk among atomic bomb survivors. 43rd Annual Meeting of the Japanese Society for Immunology, 10–12 December 2014, Kyoto (related to *Immunology Studies*)

❖ Tatsukawa Y, Misumi M, Kim YM, Yamada M, Ueda K, Takahashi I, Ohishi W, Yoneda M. Body composition and development of diabetes in a Japanese population. Endocrine Society's 97th Annual Meeting and Expo, 5–8 March 2015, San Diego, California, USA

Research Protocols 4-10 (Platform Protocol), A3-12
F_1 Clinical Study

RP 4-10 Longitudinal clinical study of the F_1 offspring of A-bomb survivors

Ohishi W (CH), Tatsukawa Y (CH), Furukawa K (S), Cologne JB (S), Yamada M (CH), Takahashi I (CH), Ueda K (CH), Mitsui F (CH), Hida A (CN), Imaizumi M (CN), Haruta D (CN), Sera N, Takahashi N (RC), Satoh Y (G), Kusunoki Y (R), Grant EJ (EH), Ozasa K (EH), Cullings HM (S), Kodama Y (BC/G), Katayama H (IT), Watanabe T (EH), Nakamura N, Fujiwara S

Objectives: The objective of this study is to elucidate the effects of parental exposure to A-bomb radiation on the development of polygenic, multifactorial diseases and subclinical conditions among the F_1 offspring.

Background and Significance: The initial examination of the longitudinal F_1 clinical cohort from 2002 to 2006 provided no evidence for an increased prevalence of adult-onset multifactorial diseases due to parental radiation exposure, but the study subjects were still quite young. The rationale for this study is that definitive human data can be obtained only if a high-quality clinical study is continued until the subjects become elderly, when many multifactorial diseases develop. Self-selection bias also tends to be minimized when prospective longitudinal data are obtained, because such data allow estimates of disease incidence.

Study Methods: This prospective study will conduct quadrennial health examinations for up to 12,500 subjects who responded favorably by mail or telephone to participate in health examinations between May 2000 and November 2008. Multifactorial diseases detected via these examinations will be analyzed in relation to the radiation doses of their parents, taking into consideration confounding factors.

Study Progress: The second examination cycle of the longitudinal F_1 clinical study cohort began in November 2010. We sent brochures that provide an overview of the health examinations to 13,210 potential study subjects and have contacted them by telephone to request their participation in the health examination. Of those, 10,019 subjects have undergone health examinations, and 64 subjects are due to participate in health examinations (participation rate of 76.3%). Among those who were examined in 2002–2006, 82.9% are participating again.

Results and Conclusions: The initial examination of the F_1 clinical cohort provided no evidence of a risk from parental radiation exposure for hypercholesterolemia, hypertension, diabetes, angina, myocardial infarction, or stroke when the various endpoints were examined individually (*J Radiol Prot* 2013; 33:281–93).

RP-A3-12 Continued preservation of frozen fresh thyroid samples obtained from children of A-bomb survivors

Imaizumi M (CN), Ohishi W (CH), Sera N, Hida A (CN), Yamada M (CH), Hamatani K (R), Akahoshi M

Objectives: The purpose of the present study is continued preservation of the existing frozen fresh thyroid tissues from thyroid cancer cases detected among the subjects of the Health Effects Study of the Children of A-bomb Survivors

in preparation for future molecular studies.

Background and Significance: Thyroid cancer is one of the cancers affected by radiation among A-bomb survivors. Even though evidence linking parental radiation exposure with solid cancer incidence among the offspring of A-bomb survivors has proved elusive, these offspring are just now entering their cancer-prone years. It is necessary to consider the possibility that future epidemiological research may suggest such effects. Furthermore, mechanisms behind thyroid cancer development are not yet fully understood. Under the present circumstances, preservation of as many frozen thyroid cancer samples as possible is indispensable for future molecular research of thyroid carcinogenesis. At RERF, thyroid ultrasound screening performed in the Health Effects Study of the Children of A-bomb Survivors during the period 2002–2006 detected dozens of cases of thyroid cancer, which were then referred for surgical removal. From among those cases, frozen fresh thyroid samples from 36 cases are currently stored at RERF. Continued preservation of the relevant frozen fresh thyroid samples will be valuable for future molecular research.

Study Methods: We continue to store the frozen fresh thyroid samples using liquid nitrogen. Sample data (research identification number, date of surgery, site [tumor or tumor-free], quantity of sample) have been stored in a database.

Study Progress: We are continuing to store the frozen fresh thyroid samples.

Results and Conclusions: None. This RP is only for continued preservation of frozen fresh samples from thyroid cancer cases detected among the F_1 population in preparation for future molecular research. A research protocol using the samples will be separately prepared in the future.

F_1 Clinical Study (FOCS) Publications

Journal Publication

♦ Tatsukawa Y, Cologne JB, Yamada M, Ohishi W, Hida A, Furukawa K, Takahashi N, Nakamura N, Ozasa K, Akahoshi M, Fujiwara S, Shore RE: Association between parental radiation exposure and prevalence of multifactorial diseases: F_1 Clinical Health Study (2nd Report). Hiroshima Igaku [J Hiroshima Med Assoc] 2014 (April); 67(4):375–8. (Proceedings of the 54th Late A-bomb Effects Research Meeting, 2013) (Japanese) (related to *F_1 Studies*)

Research Protocols 5-09, 4-09, 3-09, 4-04 and 5-04, 1-03, 4-02, 2-97, 2-90, 7-87, B48-09 and P2-11, B44-06, P1-14, P1-13

Immunology Studies

RP 5-09 Effects of radiation exposure and aging on hematopoietic stem cells (HSCs) and dendritic cells (DCs)—Analyses of numerical and functional changes

Kusunoki Y (R), Kyoizumi S (R), Kajimura J (R), Yoshida K (R), Hayashi T (R), Geyer SM, Misumi M (S), Ohishi W (CH), Ozasa K (EH), Hirabayashi Y, Iwama A, Yasutomo K, Inoue T, Inaba K, Manley NR, van den Brink MRM, Sempowski GD, Nikolich-Zugich J, Weng N-P, Murasko D, Seed TM, Nakachi K (RC)

Objectives: To delineate the long-term consequences of prior A-bomb irradiation and advancing age on homeostatic control of HSCs and DCs, we will analyze numerical and functional changes within the circulating HSC and DC pools among A-bomb survivors.

Background and Significance: There is accumulating evidence that suggests accelerated immunosenescence in A-bomb survivors. However, the mechanisms of radiation-associated immunosenescence are poorly understood. We hypothesize that radiation exposure induces premature aging of HSCs, resulting in reduced numbers and impaired self-renewal abilities that in turn accelerate loss of lymphoid potential. We also hypothesize that A-bomb irradiation affects innate and adaptive immunity, possibly by altering DC populations toward a T-cell suppressor phenotype. Animal models will also be explored for a better understanding of the reconstitution of hematopoietic and immune systems following radiation-induced damage.

Study Methods: Numerical and functional changes are analyzed in relation to radiation dose within the circulating HSC and DC pools among about 260 AHS subjects in Hiroshima. In order to confirm the results obtained in the A-bomb survivor studies, we have developed a series of *in vitro* assay systems and mouse experimental models to determine the functional and differentiation status of HSC and DC populations following exposure to ionizing radiation.

Study Progress: Functional analyses of circulating HSCs and DCs among 246 and 243 AHS subjects, respectively, have been completed. Statistical evaluation of the data obtained from the analyses is underway.

Results and Conclusions: Results expected in 2015.

RP 4-09 Effects of ionizing radiation exposure and aging on vaccination responses

Hayashi T (R), Kusunoki Y (R), Yoshida K (R), Ohishi W (CH), Ozasa K (EH), Geyer SM, Hirabayashi Y, Iwama A, Yasutomo K, Inoue T, Inaba K, Manley NR, van den Brink MRM, Sempowski GD, Nikolich-Zugich J, Weng N-P, Murasko D, Seed TM, Nakachi K (RC)

Objectives: It is important to examine whether the radiation-impaired immune system modifies health-related status, in this case the vaccine response, in aging A-bomb survivors. The purpose of this study is to evaluate the effects of prior A-bomb radiation exposure on the immunological capacity of aging individuals to respond to influenza vaccination.

Background and Significance: RERF's epidemiology

and clinical studies have long indicated increased risks of age-related and immune/inflammation-related diseases among A-bomb survivors. Further, the noted radiation effects on the immune system are similar to those associated with aging.

Study Methods: Following a pilot study in FY2010 with 50 Adult Health Study (AHS) subjects, full-scale studies were conducted in FY2011 and FY2012 with a total of 300 AHS subjects, who were selected by stratified random sampling of dose and age groups and gender. The primary endpoint is change in anti-influenza virus antibody titer levels before and three weeks after influenza vaccination. Secondary endpoints include plasma cytokines and inflammation-related proteins, lymphocyte subsets, and intracellular activation markers. The responses of peripheral blood mononuclear cells to stimulation were also determined by comparing cytokine concentrations in culture supernatants between stimulated and unstimulated cells.

Study Progress: Updated analyses of the vaccine antibody titer level responses after vaccination are in progress with the revised bone marrow dose estimates. We are now conducting analyses to assess differential vaccine response between groups of interest, based on gender, age at vaccination, age at exposure, as well as radiation exposure.

Results and Conclusions: For selected antigens, men had higher chances of seroprotection response than women, especially for H3N2 in the first vaccination year (2011) and for both years for the B antigen. For second year subjects' response to strains that were different from previous year (H3N2 and B), we saw a tendency for the high exposure subjects to have higher odds of achieving seroprotection response.

RP 3-09 Development of an integrated scoring system for human immune competence as it relates to age and ionizing radiation

Hayashi T (R), Hu Y (R), Kusunoki Y (R), Yoshida K (R), Ohishi W (CH), Ozasa K (EH), Furukawa K (S), Geyer SM, Hirabayashi, Iwama A, Yasutomo K, Inoue T, Inaba K, Manley NR, van den Brink MRM, Sempowski GD, Nikolich-Zugich J, Weng N-P, Murasko D, Seed TM, Nakachi K (RC)

Objectives: The objective of this study is to develop an integrated scoring system for evaluating the immunological and inflammatory status of individuals as a function of age and radiation dose, and predicting the effects of radiation on the immune system and somatic mutation in exposed subjects.

Background and Significance: This immunology study, unique to RERF, consists of repeated observations of various immunological parameters in A-bomb survivors with long-term follow-up, which may demonstrate significant radiation-related alterations in the immune system among the survivors, even 65 years after the atomic bombings.

Study Methods: A cross-sectional analysis includes about 1,800 Hiroshima AHS subjects with measurements of numerous immunological and inflammation-related markers such as blood inflammatory cytokines and lymphocyte subsets. A longitudinal analysis also is being conducted on a subset of 300 AHS subjects, selected so as to balance the subset on age and radiation dose. Plasma biomarkers are measured using cytokine multiplex assays on two sets of samples collected from the 300 AHS subjects 10 years apart, along with measurement of leukocyte telomere length. The results will be utilized to construct an integrated scoring system.

Study Progress: The biomarker measurements for the cross-sectional study were completed and the data set was finalized in 2014. We have also completed measurements of plasma biomarkers for the longitudinal study as well as telomere length measurement among 415 subjects (329 healthy and 86 illness) with two samples. The image data of DNA electrophoresis patterns were created at RERF and sent to Dr. Nan-ping Weng's laboratory (U.S. National Institute on Aging) for the evaluation of telomere length.

Results and Conclusions: An analytical result from the telomere length study showed that telomere lengths in A-bomb survivors exposed to >700 mGy were significantly shortened compared with those in survivors with <5 mGy when their ages ATB were <12 years.

RP 4-04 Relationship between cancer development and genetic polymorphisms among A-bomb survivors, focusing on immune-related genes
RP 5-04 Identification of cancer-related gene polymorphisms and immunological markers (Addendum to RP 4-04)

Hayashi T (R), Hu Y (R), Yoshida K (R), Cologne JB (S), Misumi M (S), Ohishi W (CH), Hida A (CN), Kusunoki Y (R), Nakachi K (RC)

Objectives: This study aims to assess whether immunogenetic backgrounds of A-bomb survivors affect susceptibility to cancer.

Background and Significance: Epidemiological studies have demonstrated long-lasting impacts of A-bomb radiation on the incidence/mortality of inflammation-related cancers such as gastrointestinal cancer. Although enhanced inflammation has been consistently observed among A-bomb survivors, roles of inflammatory responses in radiation carcinogenesis are not understood. Therefore, we are investigating relationships among risks of radiation-associated cancers, individuals' genetic backgrounds, and radiation exposure. Since our follow-up study observed that the slope of the radiation dose response curve of *glycophorin A* (*GPA*) mutation frequency was significantly higher in the cancer group than in the cancer-free group, relationships between somatic mutability and individuals' genetic backgrounds are also being examined.

Study Methods: Using DNA from 4,690 A-bomb survivors including 1,359 cancer cases, we have been conducting a series of case-cohort and case-control studies of various radiation-associated cancers in terms of polymorphisms of immune/inflammation-related genes and other cancer-associated genes such as DNA repair genes and drug-metabolizing enzyme genes, which are also known to be involved in inflammatory responses. In this study, risks of cancers are evaluated as functions of both radiation dose and genotypes.

Study Progress: We examined the association between colorectal cancer (CRC) risk and radiation exposure based on *IL6R* genotypes in the immunogenome (IMG) study cohort (a total of 4,673 individuals at baseline generating 222 CRC cases; part of the Adult Health Study) for which RERF

has followed-up cancer incidence in 1981–2005.
Results and Conclusions: The relative risk of CRC was the largest for the *IL6R-A/G* or *IL6R-G/G* group exposed to the highest dose category, as compared to the reference group (non-exposed *IL6R-A/A*). These results suggest the possibility that the *IL6R* genotypes are associated with the inter-individual variance of incidence risk of radiation-related CRC among A-bomb survivors.

RP 1-03 A study of gene polymorphisms and their possible role in the development of diabetes mellitus in the Adult Health Study population

Hayashi T (R), Hu Y (R), Yoshida K (R), Nakashima E (S), Tatsukawa Y (CH), Ohishi W (CH), Hida A (CN), Kusunoki Y (R), Nakachi K (RC)
Objectives: This study aims to assess the effect of radiation and various genetic factors on the development of diabetes mellitus (DM) in the AHS cohort, and determine whether differences in frequencies of any particular genotypes between Hiroshima and Nagasaki survivors may account for why a significant association between risk of DM and radiation dose is observed in A-bomb survivors in Hiroshima but not Nagasaki.
Background and Significance: Although early studies did not show associations between radiation exposure and DM, data on AHS subjects in 1992–1994 indicated a significant positive radiation-DM association in Hiroshima but not in Nagasaki, after adjusting for gender, age, and body mass index. This somewhat puzzling finding may reflect genetic differences between the Hiroshima and Nagasaki populations. Our preliminary results suggest that radiation may persistently impair the immune system and that the radiation-DM association may be especially relevant to a sub-group of A-bomb survivors who have a specific *HLA* class II haplotype.
Study Methods: We investigated the relationships between *HLA*-related genetic factors, risk of DM, and radiation dose in the AHS subjects in a case-control study. A total of 3,242 AHS subjects, comprising DM cases (569 in Hiroshima and 307 in Nagasaki) and controls (1,138 in Hiroshima and 1,228 in Nagasaki, matched on city, sex, two age-ATB groups, and three radiation-dose groups), were selected by the Department of Statistics and genotyped for *HLA* and *HLA*-related gene polymorphisms by RME.
Study Progress: We have determined *DRB1/DQB1/DQA1*, *A/B/C*, and *MICA/MICB* genotypes for 864/860/864, 823/782/790, and 842/800 DM cases, and 2,331/2,317/2,331, 2,195/2,096/2,109, and 2,205/2,180 controls, respectively. We have also determined 162 SNPs of 38 immune-/inflammation-related genes for DM cases and control subjects.
Results and Conclusions: None yet. Results expected in 2015.

RP 4-02 Perturbation of T-cell homeostasis in atomic-bomb survivors

Yoshida K (R), Kajimura J (R), Hayashi T (R), Ohishi W (CH), Misumi M (S), Cologne JB (S), Nakashima E (S), Kodama Y (BC/G), Nakachi K (RC), Kusunoki Y (R)
Objectives: In this study we are testing the hypothesis that radiation exposure may perturb T-cell homeostasis, consequently elevating risks of various diseases.
Background and Significance: Perturbed homeostasis of the T-cell immune system is believed to be a primary cause of increased morbidity from selected infectious and inflammation-related diseases, which include both acute and chronic diseases. So far, we have found four dose-dependent changes in T-cell profiling of A-bomb survivors: 1) reduced sizes of both naïve CD4 and naïve CD8 T-cell populations; 2) decreased repertoire of T-cell receptors in memory CD4 T cells; 3) increased percentages of functionally weak memory CD4 T cells; and 4) inverse associations between the relative frequency of naïve CD4 T cells and plasma levels of inflammatory cytokines. These results are consistent with the above hypothesis. We will further examine additional indicators of T-cell homeostasis, and relationships between changes in T-cell profiles and subsequent disease development. We anticipate that this study will contribute to determining how, and to what degree, such T-cell immunity perturbations are involved in disease development in A-bomb survivors.
Study Methods: Among the AHS subjects, we analyze 1) the percentages of peripheral blood lymphocyte subsets and T-cell receptor repertoire by flow cytometry, 2) the numbers of T-cell receptor excision circles (TRECs) in CD4 and CD8 T-cell fractions by real-time PCR, 3) the telomere lengths of T-cell populations by flow fluorescent *in situ* hybridization, and 4) associations between the T-cell parameters and clinical phenotypes including obesity and disease development.
Study Progress: Statistical data analyses are being conducted for direct effects of radiation on T-cell subsets and repertoire, TRECs, and T-cell telomere lengths, and for interactions between radiation exposure and energy metabolism, which can modify radiation responses as well as T-cell homeostasis.
Results and Conclusions: Results expected in 2015.

RP 2-97 Lyophilization of blood samples to be obtained from Adult Health Study subjects in Hiroshima and Nagasaki for later DNA extraction (Addendum to RP 2-90)

Hayashi T (R), Hu Y (R), Yoshida K (R), Kusunoki Y (R), Ohishi W (CH), Hida A (CN), Nakachi K
Objectives: The purpose of this study is to supplement RP 2-90 by providing a means of storing DNA from stock sources that otherwise would be wasted so that DNA would be available for multiple small-scale molecular analyses (i.e., biospecimens from RP 2-90).
Background and Significance: In studying the late effects of radiation exposure in A-bomb survivors, analyses of gene alterations due to radiation are essential in investigations of genomic instability, genetic susceptibility, and molecular oncology. With new technologies, investigations can now be conducted with extremely small amounts of DNA.
Study Methods: Blood samples, destined for DNA extraction and subsequent molecular analyses, are lyophilized and stored for use in current and future studies.
Study Progress: We have thus far cryopreserved 29,001 and 15,173 blood sample vials from AHS participants in Hiroshima and Nagasaki, respectively, including the

expanded AHS participants who were exposed to radiation at the age of nine years or younger. The PCR amplification of DNA extracted from laboratory control blood stored at –80°C for 17 years on paper showed no recognizable degradation of DNA after the long-term storage.
Results and Conclusions: This project provides a valuable resource for future molecular and genetic studies of radiation effects.

RP 2-90 Cryopreservation of blood cells from Hiroshima and Nagasaki Adult Health Study participants

Hayashi T (R), Hu Y (R), Kusunoki Y (R), Yoshida K (R), Hida A (CN), Ohishi W (CH), Nakachi K
Objectives: The purpose of this study is to cryopreserve viable blood mononuclear cells from all AHS participants to ensure that appropriate materials will be available for ongoing studies of the late effects of A-bomb radiation exposure as well as future analyses using advanced techniques.
Background and Significance: Various aspects of the health effects of A-bomb radiation exposure have been studied at ABCC-RERF. It is reasonable to expect that technological improvements in methodologies will continue and that studies of effects that were previously not practical will become feasible in the future.
Study Methods: Peripheral blood mononuclear cells are separated from 4 ml of heparinized blood using the Ficol/Hypaque density gradient centrifugation technique. Peripheral blood mononuclear cells are preserved in a tank of liquid nitrogen.
Study Progress: We have cryopreserved blood cells from Hiroshima and Nagasaki AHS participants. We have also collected lymphocyte samples from the expanded group of A-bomb survivors since October 2008 (those who were exposed to radiation at the age of nine years or younger).
Results and Conclusions: In FY2014, we have cryopreserved blood cells from 1,244 AHS participants from Hiroshima and 762 from Nagasaki, including the expanded group members who were exposed to radiation at the age of nine years or younger. We confirmed that the viability of cryopreserved cells for 24 years was more than 80% and that thawed lymphocytes expressed normal surface antigens and immunological functions.

RP 7-87 X-ray radiosensitivity of lymphocytes *in vitro* from A-bomb survivors. Part 3: Transformation of B cells by Epstein-Barr virus and their cryopreservation (Addendum to RP 3-86)

Hayashi T (R), Hu Y (R), Kusunoki Y (R), Yoshida K (R), Hida A (CN), Ohishi W (CH), Nakachi K
Objectives: The initial purpose of this project was to cryopreserve Epstein-Barr virus (EBV)-transformed B-cell lines from high-dose and control survivors for new cell biology studies, e.g., those dealing with radiosensitivity. The resulting B-cell lines are also of considerable use in studying radiation effects related to immune functions and the role of genetic backgrounds underlying disease development.
Background and Significance: Based on a recommendation by the Multinational Peer Review Panel for the radiobiology program (1998), we have been cryopreserving EBV-transformed B-cell lines from high-dose (≥1 Gy) and control A-bomb survivors for molecular epidemiological, immunological, and other genomic and proteomic studies. About 500 samples that overlapped with the F_1 study have been immortalized and are being stored in the Department of Genetics.
Study Methods: The AHS subjects for this study are the high dose (≥1 Gy) and control (<0.005 Gy) groups. The total number of participants in those groups is about 3,500 in Hiroshima and Nagasaki. Peripheral blood lymphocytes from A-bomb survivors are being transformed by EBV and cryopreserved in liquid nitrogen.
Study Progress: We have completed EBV transformation of mononuclear cells from 2,743 AHS subjects, including nearly all who are currently participating in the AHS.
Results and Conclusions: The immortalization of lymphocytes from Hiroshima AHS subjects is nearing completion (1,895 subjects), and lymphocytes from 856 Nagasaki AHS participants have also been successfully transformed. We have thus far cryopreserved 7,904 and 3,563 blood sample vials from Hiroshima and Nagasaki AHS participants, respectively. Samples numbering 2,735 have been distributed to both Hiroshima and Nagasaki storage facilities.

RP-B48-09 Evaluation of the existence and utility of the thymus autopsy specimens available in the RERF tissue archives for future analyses of thymus architecture and function in response to aging and exposure to ionizing radiation
RP-P2-11 Evaluation of the existence and utility of the thymus autopsy specimens that are available from LSS subjects who died at 70 or more years old in the RERF tissue archives and that are available from LSS subjects in Hiroshima University Hospital (Addendum to RP-B48-09)

Yoshida K (R), Kusunoki Y (R), Ozasa K (EH), Ito R (R), Kajimura J (R), Kyoizumi S (R), Hayashi T (R), Misumi M (S), Arihiro K, Geyer SM, Sempowski GD, Manley NR, van den Brink MRM, Douple EB, Nakachi K

RP-B44-06 Establishment of new assay systems to facilitate evaluation of genetic instability in human hematolymphoid cells by flow cytometry

Kusunoki Y (R), Kajimura J (R), Hamasaki K (G), Yoshida K (R), Hayashi T (R), Imai K, Furukawa K (S), Cologne JB (S), Nakachi K

RP-P1-14 Longitudinal analysis of peripheral blood T-cell receptor repertoire among in-house volunteers using next-generation sequencing technology

Yoshida K (R), Cologne JB (S), Misumi M (S), Matsui H, Kanai A, Hayashi T (R), Robins H, Kusunoki Y (R)

RP-P1-13 Establishment of methods for analyzing transcriptome in human blood cells using high-throughput sequencing

Yoshida K (R), Kyoizumi S (R), Hayashi T (R), Kajimura J (R), Misumi M (S), Matsui H, Kanai A, Inaba T, Ikeo K, Gojobori T, Nakachi K, Kusunoki Y (R)

Immunology Studies Publications

RERF Report (RR)

♦ Kyoizumi S, Kubo Y, Kajimura J, Yoshida K, Hayashi T, Nakachi K, Young LF, Moore MA, van den Brink MRM, Kusunoki Y: Linkage between dendritic and T cell commitments in human circulating hematopoietic progenitors. J Immunol 2014 (June); 192(12):5749–60. **(RR 7-13)**

[Study Findings] Dendritic cells (DCs) play an indispensable role in the differentiation and functional expression of T cells. These two types of cells differentiate from hematopoietic stem cells (HSCs), and the current study finds that the frequencies of T-cell and DC precursors are significantly linked with each other in stem cells existing in blood, whereas they are not linked to the precursor frequency (PF) of natural killer (NK) cells.

[Explanation] ***Objectives*** DCs are a type of leukocyte with dendrites. They initiate an adaptive immune response by conveying antigen information about viruses and bacteria that have invaded the body to T cells, which serve as a 'control tower' for the adaptive immune response. DCs also prevent the generation of self-antigen-responsive T cells in the thymus as a negative selection. Because self-antigen-responsive T cells recognize substances that make up their host body as antigens, these cells are detrimental. DCs are thus essential for the differentiation and functional expression of T cells. Based on this understanding, we hypothesized that there may be a correlation between the potency of HSCs to differentiate into DCs and their potency to differentiate into T cells, suggesting a linkage between DC and T-cell commitments. (HSCs are the least differentiated cells that can give rise to all blood cell types, are long lived, and have the ability to self-replicate.) Verifying this hypothesis was considered to provide important findings for ongoing studies of A-bomb survivors involving T-cell progenitors and DCs. DCs can be classified as conventional DCs (cDCs, which inform T cells of antigens to initiate direct attack) or plasmacytoid DCs (pDCs, which produce type-I interferon and induce anti-viral infection immunity). In the present study, we analyzed DC progenitors of both types. ***Methods*** It is possible to separate hematopoietic progenitor cells (HPCs, cells with the potency to differentiate into T cells and DCs) in human peripheral blood using a cell sorter, and then to differentiate HPCs into T cells and NK cells using the culture method reported in Kyoizumi *et al.*: *J Immunol* 2013; 190:6164–72. NK cells are a type of cytotoxic lymphocyte that is important for the innate immune system. Since the present study confirmed that this culture method could induce differentiation of HPCs into DCs, we used it as an experimental system to simultaneously assay T cells, NK cells, and DC progenitors. Blood samples were collected, based on informed consent, from 20 RERF in-house volunteer donors aged 26–65 years, and we measured the PFs of T cells and NK cells, as well as cDCs and pDCs, based on the culture method, to examine correlations among the PFs of these cells. Furthermore, we examined the potencies of single precursor cells to differentiate into T cells and NK cells, as well as cDCs and pDCs, based on precursor cell clonal culture. ***Results*** (1) The PFs of cDCs and pDCs in HPCs were found to correlate significantly with T-cell PFs, but not with NK-cell PFs. (2) The precursors producing T cells and NK cells were classified into clones producing T/NK dual-, T single-, and NK single-lineage precursors. The clones of T/NK dual- and T single-lineage precursors produced cDCs or pDCs at high frequencies. The clones of NK single-lineage precursors, however, produced very few cDCs or pDCs. ***Consideration and Conclusion*** These findings show that T-cell and DC commitments are linked with each other, as indicated with the green lines below. On the other hand, this research suggests that NK-cell commitment is induced independently from DC commitment. In addition, the linkage between T-cell and DC precursors might be intrinsically imprinted in long-lived HSCs existing in bone marrow that are self-replicating, since the T-cell and DC progenitors in the human body have short life spans.

Other Journal Publications

♦ Hirabayashi Y, Tsuboi I, Nakachi K, Kusunoki Y, Inoue T: Experimentally induced, synergistic late effects of a single dose of radiation and aging: significance in LKS fraction as compared with mature blood cells. J Appl Toxicol 2015 (March); 35(3):230–40.

♦ Hu Y, Yoshida K, Kajimura J, Kyoizumi S, Kusunoki Y, Cologne JB, Ohishi W, Hayashi I, Nakachi K, Hayashi T: The relationship between *CD14* gene polymorphism and risks of colorectal cancer subsites among atomic bomb survivors. Nagasaki Igakkai Zasshi [Nagasaki Med J] 2014 (September); 89(Special issue):257–61. (Proceedings of the 55th Late A-bomb Effects Research Meeting, 2014) (Japanese)

♦ Kusunoki Y, Yoshida K, Kubo Y, Yamaoka M, Kajimura J, Hayashi T, Kyoizumi S, Nakashima E, Ohishi W: Effects of atomic-bomb radiation on human immune responses. Report 27: Inverse associations between obesity indicators and thymic T-cell production levels in A-bomb survivors. Nagasaki Igakkai Zasshi [Nagasaki Med J] 2014 (September); 89(Special issue):288–91. (Proceedings of the 55th Late A-bomb Effects Research Meeting, 2014) (Japanese)

Immunology Studies Oral Presentations

❖ Hayashi T, Cologne JB, Hu Y, Yoshida K, Ohishi W, Hayashi I, Kajimura J, Kyoizumi S, Kusunoki Y, Nakachi K. Effects of *IL10* haplotypes and atomic-bomb radiation exposure on risks of gastric cancer subtypes. 105th Annual Meeting of the American Association for Cancer Research (AACR), 5–9 April 2014, San Diego, California, USA

❖ Hu Y, Yoshida K, Kajimura J, Kyoizumi S, Kusunoki Y, Cologne JB, Ohishi W, Hayashi I, Nakachi K, Hayashi T. *CD14* gene polymorphisms associated with development of colorectal cancer subtypes among atomic bomb survivors in Japan. 105th Annual Meeting of the American Association for Cancer Research (AACR), 5–9 April 2014, San Diego, California, USA

❖ Hayashi T, Hu Y, Furukawa K, Ohishi W, Geyer SM, Weng NP, Hayashi I, Yoshida K, Kajimura J, Kyoizumi S, Kusunoki Y, Nakachi K. The association of reactive oxygen species in blood cells with age, past radiation exposure, and *IL-6R* gene polymorphisms. Immunology 2014 American

Association Immunologists Annual Meeting, 2–6 May 2014, Pittsburgh, Pennsylvania, USA

❖ Hu Y, Yoshida K, Kajimura J, Kyoizumi S, Kusunoki Y, Cologne JB, Ohishi W, Hayashi I, Nakachi K, Hayashi T. The relationship between *CD14* gene polymorphism and risks of colorectal cancer subtypes among atomic bomb survivors. 55th Late A-bomb Effects Research Meeting, 1 June 2014, Nagasaki

❖ Kusunoki Y, Yoshida K, Kubo Y, Yamaoka M, Kajimura J, Hayashi T, Kyoizumi S, Nakashima E, Ohishi W. Effects of atomic-bomb radiation on human immune responses. Report 27: Inverse associations between obesity indicators and thymic T-cell production levels in A-bomb survivors. 55th Late A-bomb Effects Research Meeting, 1 June 2014, Nagasaki

❖ Cologne JB, Yoshida K, Misumi M, Kyoizumi S, Hayashi T, Kusunoki Y. DNA repair gene SNPs and radiation exposure: assessing gene-environment interaction in somatic mutation and cancer using kernel regression. Scientific Meeting for Cancer Prevention 2014 Tokyo, 13–14 June 2014, Tokyo

❖ Hu Y, Hayashi T, Yoshida K, Kajimura J, Kyoizumi S, Kusunoki Y, Nakachi K, Cologne JB, Ohishi W, Hayashi I. *CD14* and *IL18* gene polymorphisms associated with development of colorectal cancer subtypes among atomic bomb survivors. Scientific Meeting for Cancer Prevention 2014 Tokyo, 13–14 June 2014, Tokyo

❖ Yoshida K, Kusunoki Y, Cologne JB, Kyoizumi S, Nakachi K, Maki M, Hu Y, Hayashi T. A genetic polymorphism-based approach to explicating individual differences in radiation-dose response of *glycophorin A* mutant fractions in erythrocytes. Scientific Meeting for Cancer Prevention 2014 Tokyo, 13–14 June 2014, Tokyo

❖ Hayashi T, Hu Y, Furukawa K, Ohishi W, Hayashi I, Yoshida K, Kajimura J, Kyoizumi S, Kusunoki Y, Nakachi K. Age and radiation effects on intracellular reactive oxygen species in the blood cells of atomic-bomb survivors and their association with immune-related biomarkers. 12th Japan-Korea Cancer and Aging Symposium, 19–21 June 2014, Inacho, Saitama (related to *Adult Health Study*)

❖ Hu Y, Yoshida K, Kajimura J, Kyoizumi S, Kusunoki Y, Cologne JB, Ohishi W, Hayashi I, Nakachi K, Hayashi T. Possible involvement of *CD14* polymorphisms in radiation-associated colorectal cancer in Japan. 40th Annual Meeting of Korean Cancer Association, 19–20 June 2014, Seoul, South Korea

❖ Hayashi T. Molecular epidemiology study of cigarette smoking effects on immunity- and inflammation-related biomarkers and lifestyle-related disease development. FY2013 Research Meeting of Smoking Research Foundation, 15 July 2014, Tokyo

❖ Shore RE. Epidemiologic studies of radiation cataract risk: Dose and dose rate. 2014 Expert Meeting on Radiation Effects, 5 September 2014, Tokyo (related to *Special Clinical Studies*)

❖ Hayashi T, Hu Y, Morishita Y, Sasaki K, Maki M, Furudoi K, Nagamura H, Yoshida K, Kajimura J, Ohishi W, Hida A, Hayashi I, Kyoizumi S, Kusunoki Y, Nakachi K. Functional significance of *IL6R* genotype and colorectal cancer risk by genotype among atomic-bomb survivors. 21st Annual Meeting of the Japanese Society of Immunotoxicology, 11–12 September 2014, Tokushima (related to *Adult Health Study*)

❖ Hayashi T, Hu Y, Morishita Y, Sasaki K, Maki M, Furudoi K, Nagamura H, Ohishi W, Hida A, Hayashi I, Yoshida K, Kajimura J, Kyoizumi S, Kusunoki Y, Nakachi K. Relationship of intracellular reactive oxygen species in blood cells with age, radiation and immune- and inflammation-related gene polymorphisms among atomic-bomb survivors. 23rd Annual Meeting of the Japanese Society for Histocompatibility and Immunogenetics, 13–15 September 2014, Nagasaki (related to *Adult Health Study*)

❖ Hayashi T, Kyoizumi S, Kusunoki Y, Ohishi W, Nakachi K. Colorectal cancer risk in atomic-bomb survivors based on *IL6R* gene polymorphism and radiation exposure dose. 73rd Annual Meeting of the Japanese Cancer Association, 25–27 September 2014, Yokohama

❖ Hayashi T, Hu Y, Yoshida K, Ohishi W, Hida A, Hayashi I, Kajimura J, Kyoizumi S, Kusunoki Y, Nakachi K. Impact of *ATM* genetic polymorphisms on radiation-associated breast cancer risks among atomic bomb survivors. 57th Annual Meeting of the Japan Radiation Research Society, 1–3 October 2014, Kagoshima

❖ Yoshida K, Yamaoka M, Kubo Y, Kyoizumi S, Kajimura J, Hu Y, Hayashi T, Ohishi W, Kusunoki Y. Metabolic modulation of radiation effects on T-cell receptor Vβ repertoire in peripheral blood CD4T cells. 57th Annual Meeting of the Japan Radiation Research Society, 1–3 October 2014, Kagoshima

❖ Wang C, Kusunoki Y, Kyoizumi S, Nakachi K, Iwama A. Effects of non-lethal ionizing radiation on repopulation and differentiation potentials of human HSCs in humanized mice. 76th Annual Meeting of the Japanese Society of Hematology, 31 October–2 November 2014, Osaka

❖ Hayashi T, Hu Y, Yoshida K, Ohishi W, Hida A, Kyoizumi S, Kusunoki Y. Functional significance of *IL6R* genotype and effects thereof on colorectal cancer risk among atomic bomb survivors. 43rd Annual Meeting of the Japanese Society for Immunology, 10–12 December 2014, Kyoto (related to *Adult Health Study*)

❖ Hu Y, Yoshida K, Kyoizumi S, Kusunoki Y, Ohishi W, Hayashi T. Immune/inflammation-related *CD14* and *IL18* gene polymorphisms and risks of colorectal cancer subtypes among atomic bomb survivors. 43rd Annual Meeting of the Japanese Society for Immunology, 10–12 December 2014, Kyoto

❖ Kusunoki Y. Radiobiology studies using biosamples from A-bomb survivors—Immunological analyses and molecular oncology studies. Epidemiology Conference for Emergency Workers of Tokyo Electric Power's Fukushima I Nuclear Power Plant, 11 March 2015, Kitakyushu (related to *Cell Biology Studies*)

Research Protocols 3-11, 2-10, 6-08, 3-05, 2-99, 9-92, 5-92, 3-89, A1-15, A3-14, A1-14, A5-13, A4-13, A3-13, A2-12, A1-12, A4-11, A14-08, A13-08, A10-08

Special Clinical Studies

RP 3-11 Radiation exposure in children and *in utero* survivors of the atomic bombings of Hiroshima and Nagasaki and late-life neurocognitive function

Yamada M (CH), Hida A (CN), Kasagi F, Cologne JB (S), Landes R (S), Nagano Y, Matsumoto M, Tsujino A, Mimori Y, Sasaki H, Nakamura S, Krull KR

Objectives: Objectives, are (1) to examine associations between radiation exposure at ages 0–12 or *in utero* and neurocognitive function in late life as assessed by neuropsychological examinations, (2) to investigate the effects of sex, age, attained education, lifestyle, and comorbidity on neurocognitive function as risk factors or modifiers of radiation effects, and (3) to collect baseline data on cognitive function for the investigation of longitudinal progression in cognitive decline and the occurrence of dementia with advancing age.

Background and Significance: The atomic bomb study and various studies of childhood radiotherapy have shown that the brain is susceptible to radiation damage *in utero* and in early childhood. The proposed study is a unique opportunity to examine the prenatal and early childhood effects of radiation exposure on cognitive function more than 65 years later. To our knowledge, no other data exist regarding late-life cognitive function after radiation exposure in childhood or *in utero*.

Study Methods: Study subjects are survivors exposed at ages 0–12 or *in utero*. We will evaluate neurocognitive function for about 1,050 subjects in Hiroshima and Nagasaki using the Cognitive Abilities Screening Instrument (CASI) and the Childhood Cancer Survivors Study (CCSS) Neurocognitive Questionnaire (NCQ) during the period 2011–2015. For the CASI, assessment is based on face-to-face interviews by trained nurse technicians during routine health examinations in the AHS and includes performance items related to neurocognitive function (e.g, short-term memory). The self-administered NCQ, which elicits neurocognitive symptoms, is conducted by mail survey.

Study Progress: Evaluation of neurocognitive function using CASI and NCQ was started in 2011. About 1,250 subjects have been investigated using CASI. About 1,300 subjects have answered the NCQ.

Results and Conclusions: None yet.

RP 2-10 Evaluation of retinal arteriolosclerosis and age-related macular degeneration using stored retina images with standardized measurements in relation to glaucoma development in atomic bomb survivors and to association with aortic arteriosclerosis (Addendum to RP 1-05)

Takahashi I (CH), Yanagi M (Hiroshima Univ.), Misumi M (S), Itakura K, Kawasaki R, Nakashima E (S), Yokoyama T, Takamatsu M, Kinoshita H, Tsuiki E, Uematsu M, Kumagami T, Kiuchi Y, Kitaoka T, Ohishi W (CH), Hida A (CN), Neriishi K

Objectives: To investigate if retinal arteriolosclerosis is associated with radiation dose and is involved as an intermediate risk factor in radiation-associated glaucoma.

Background: Retinal vessel caliber is a well-established, non-invasive marker of microvascular disorders, which may contribute to the initiation and/or progression of radiation-induced cardiovascular diseases. Furthermore, alterations of ocular perfusion could cause ischemia and poor perfusion of the optic disc with subsequent glaucomatous damage. Preliminary analyses of the glaucoma study during 2006–2008 suggested a relationship between radiation and an increased prevalence of normal-tension glaucoma among A-bomb survivors. To investigate the pathological background of glaucoma, we planned to calculate retinal vessel diameters, which are possibly involved in glaucoma genesis through eye-circulation impediment. In addition, we will be able to investigate the relationship between radiation and age-related macular degeneration (AMD), which will be assessed using the same retinal images.

Study Methods: This is a cross-sectional study of AHS subjects for whom retinal photography was performed during 2006–2008. Vessel calibers and AMD were assessed from the digitized retinal images at the University of Melbourne using a computer-assisted program.

Study Progress: As for retinal vessel caliber, we analyzed smoking effects on retinal vessel calibers and published the findings. Retinal vessel calibers are also being analyzed in relation to radiation dose. The analyses about the association between radiation exposure and AMD prevalence were completed and the manuscript was submitted for journal publication.

Results: In women, central retinal vein equivalent (CRVE) caliber showed a consistent increase with number of cigarettes smoked/day, consistent with previous findings by others. However, females who quit smoking ≥10 years ago, had similar CRVE calibers to never smokers, a new finding in the literature. There was no association of smoking and arterial caliber (*Invest Ophthalmol Vis Sci* 2014; 55:405–11). No significant association of radiation dose was found for either early or late AMD, while it was indicated that the number of large drusen (retinal precursor to AMD development) increased with dose.

RP 6-08 Liver stiffness study using elastometer in Hiroshima atomic-bomb survivors

Ohishi W (CH), Tatsukawa Y (CH), Ueda K (CH), Mitsui F (CH), Fujiwara S, Nakashima E (S), Kohata M, Yamada M (CH), Ozasa K (EH), Tsuge M, Chayama K

Objectives: The hypothesis behind this study is that radiation exposure may accelerate the severity of liver fibrosis irrespective of hepatitis virus infection. The objective is to determine whether A-bomb radiation exposure has increased liver stiffness, which serves as a marker of liver fibrosis severity, and to investigate the possibility that liver fibrosis is involved in the development of atherosclerotic diseases by inducing insulin resistance.

Background and Significance: Liver fibrosis sometimes progresses into liver cirrhosis and hepatocellular carcinoma (HCC), among chronic type B or C liver disease and nonalcoholic steatohepatitis cases. The RERF data have shown that both chronic liver disease and liver cirrhosis are related to radiation dose. In the LSS and AHS populations,

radiation effects on the incidence of atherosclerotic diseases, such as cardiovascular disease (CVD) and CVD risk factors, have also been observed.

Study Methods: We will examine the relationship between liver stiffness and radiation dose, in order to determine whether this is a plausible pathway by which radiation exposure is involved in increased liver disease including fatty liver disease, chronic hepatitis, and liver cirrhosis. We will also examine whether an increase in liver fibrosis is involved, through insulin resistance, in the development of atherosclerotic diseases, in order to elucidate mechanisms of radiation effects underlying these diseases.

Study Progress: Cleaning of the data set, which includes measurement of liver stiffness by elastometer and blood cytokine levels such as TNF-α, IL-6, MCP-1, adiponectin, leptin, and IGF-1 for 2,911 Hiroshima AHS participants, was performed. Preliminary analyses for younger A-bomb survivors exposed before age 10 years were conducted.

Results and Conclusions: Increased prevalence of nonalcoholic fatty liver disease (NAFLD) was associated independently with radiation dose, after adjustment for BMI, diabetes, liver function, and total adiponectin levels. Severity of liver fibrosis in NAFLD was associated independently with, age, obesity, smoking, diabetes, and elevated levels of total adiponectin, but not associated with radiation dose.

RP 3-05 Inflammation and cancer incidence in atomic bomb survivors

Tatsukawa Y (CH), Cologne JB (S), Nakashima E (S), Little MP, Ozasa K (EH), Soda M (EN), Yamada M (CH)

Objectives: To explore effects of inflammation levels on radiation risk for cancer development.

Background and Significance: Experimental and epidemiological studies report a relationship between inflammation and cancer. Because A-bomb survivors have radiation dose-dependent increases in inflammatory biomarkers, we are investigating the relationship between selected biomarkers and cancer incidence among 12,870 Adult Health Study (AHS) participants followed from 1965 to 1999 and their possible role in radiation risk for cancer.

Study Methods: A number of inflammation biomarkers have been measured in the AHS cohort. Since associations between radiation, biomarkers, and cancer outcomes are complex, we are using several statistical analysis approaches to characterize them.

Study Progress: Analyses of the joint effects of white blood cell (WBC) and radiation or smoking on all solid cancer incidence using a causal model were completed and an internal research document was approved after internal review.

Results and Conclusions: Longitudinal trends in WBC counts over time are elevated among subjects exposed to ≥2 Gy radiation (result published in the *Journal of Radiation Research* in 2010). There is a significant mediation effect by the longitudinal WBC count upon the radiation risk for all solid cancer, with the proportion of total radiation effect attributed to mediation by WBC being about 7%. Whether there is mediation by WBC of the radiation risk for specific cancer sites could be determined statistically for only certain common cancers; the mediation proportion for lung cancer was much higher (27%) than for all solid cancers.

RP 2-99 Thyroid diseases in Hiroshima and Nagasaki atomic-bomb survivors

Imaizumi M (CN), Ohishi W (CH), Usa T, Akahoshi M, Neriishi K, Sera N, Yamada M (CH), Nakashima E (S), Sugino K, Hida A (CN)

Objectives: To investigate radiation effects on thyroid diseases in the AHS cohort, and to examine how frequently thyroid cancer has developed among subjects with thyroid nodules detected in an earlier study (1984–1987).

Background and Significance: An earlier study of thyroid disease in the Nagasaki AHS cohort in 1984–1987 showed an approximately linear association between thyroid radiation dose and the prevalence of thyroid nodules and suggested an association between autoimmune hypothyroidism and radiation dose (*JAMA* 1994; 272:364). The current thyroid study is conducted in both Hiroshima and Nagasaki with AHS cohort members to clarify the radiation dose response for thyroid diseases. A second objective is to examine how frequently thyroid cancer develops among irradiated subjects with thyroid nodules detected in the previous study (1984–1987).

Study Methods: Thyroid examinations are performed (blinded as to radiation dose) in AHS cohort members and dose responses for thyroid disorders are analyzed. For the second objective, incident thyroid cancer is ascertained among those with thyroid nodules in 1984–1987.

Study Progress: We performed thyroid examinations in AHS cohort members between 2000 and 2003. We are continuing the follow-up of AHS subjects for detecting the development of thyroid cancers. We conducted thyroid examinations in AHS subjects exposed at younger ages (<10 years old at exposure) between 2008 and 2011 (RP 3-07), and a manuscript regarding dose-responses for thyroid nodules has been published.

Results and Conclusions: We found that both malignant thyroid tumors and benign nodules were increased with radiation dose. On the other hand, autoimmune hypothyroidism and Graves' disease were not associated with radiation dose (*JAMA* 2006; 295(9): 1011–22). No significant dose responses for thyroid diseases were observed among those exposed *in utero* (*J Clin Endocrinol Metab* 2008; 93:1641–8). We more frequently detected cancer in subjects with solid thyroid nodules than in nodule-free controls (*J Clin Endocrinol Metab* 2005; 90:5009–14). Among AHS subjects exposed at younger ages (<10 years old at exposure), significant linear dose-response relationships were observed for the prevalence of thyroid nodules, while radiation exposure is not associated with small thyroid nodules (*JAMA Internal Medicine* 2015; 175:228–36).

RP 9-92 Study of liver diseases in the Adult Health Study sample: Relationship between radiation dose and infection by hepatitis B and C viruses

Ohishi W (CH), Cologne JB (S), Ueda K (CH), Mitsui F (CH), Cullings HM (S), Nakashima E (S), Yoshida K (R), Kusunoki Y (R), Hayashi T (R), Hida A (CN), Fujiwara S, Chayama K

Objectives: The hypothesis behind this study is that radiation may increase the incidence of hepatocellular carcinoma (HCC) either by increasing the rates of chronic

hepatitis B (HBV) and C (HCV) virus infection, or by accelerating the disease progression after hepatitis virus infection. The objective of the study is to investigate the relationship between radiation dose and the natural history of HBV or HCV infection among the Adult Health Study (AHS) cohort.

Background and Significance: Our previous studies demonstrated that the prevalence of hepatitis HBsAg increased with radiation dose among the AHS. The percent among subjects who were unable to clear the virus increased significantly with radiation dose among those receiving blood transfusions. No relationship was found between radiation dose and the prevalence of anti-HCV, but the radiation-dose response for chronic liver disease among anti-HCV-positive subjects was suggestively greater than that among anti-HCV-negative subjects.

Study Methods: We will examine (1) the relation between radiation dose and HBV activity ascertained by measuring hepatitis B e-antigen (HBeAg) and HBV DNA, and the HBeAg or HBsAg seroconversion rates, (2) the relation between radiation dose and the natural history of chronic type B or C liver diseases, and (3) the effects of clinicopathological features, immunogenetic background, and radiation dose on the course following hepatitis virus infection among the AHS cohort.

Study Progress: We have updated the analysis data set using the latest HCC follow-up data, HBV/HCV infection status data, and other risk factor information. Using the updated data set, an association between radiation dose and HBV/HCV infection status was investigated.

Results and Conclusions: Subjects with persistent HCV infection showed Th1-dominant immunological features. Increased Th1 cell percentages were significantly associated with accelerated progression of liver fibrosis, while Tc1 and NK cell percentages were inversely associated with fibrosis progression (*Hum Immunol* 2011; 72:821–6).

RP 5-92 Study of senile dementia among the Adult Health Study subjects

Yamada M (CH), Mimori Y, Cologne JB (S), Landes R (S), Sasaki H, Matsumoto M, White LR

Objectives: In this study, we are examining the effects of radiation exposure on cognitive function, the prevalence and incidence of dementia, and other age-related physiologic variables such as reaction time in older age among adult survivor members of the Adult Health Study (AHS).

Background and Significance: This study investigates the hypothesis that the effects of ionizing radiation on the mature central nervous system could possibly be manifested as accelerated neurological aging. In the late 1980s, a collaborative study of dementia using standardized procedures to compare Japanese-Americans living in Seattle and Honolulu with the Adult Health Study (AHS) cohort was initiated to identify whether the prevalence, incidence, and causes of dementia were the same across cultures (the NI-HON-SEA study).

Study Methods: Study subjects were survivors exposed at ≥13 years of age. We evaluated cognitive performance for about 3,113 subjects in Hiroshima and Nagasaki with the Cognitive Abilities Screening Instrument (CASI) during the period 1992–1998. The prevalence of dementia and its subtypes was assessed among 2,648 Hiroshima AHS subjects aged 60 years or older at baseline examination (1992–1996). A total of 2,286 dementia-free subjects at baseline examination have been followed up to assess dementia incidence and results were published.

Study Progress: The database of the CASI longitudinal examinations had been restructured. Longitudinal analyses regarding trajectories of cognitive function and effects of demographic factors or radiation exposure have been analyzed in cooperation with the Department of Statistics.

Results and Conclusions: No association was found between previous radiation exposure and cognitive impairment and/or development of dementia among subjects exposed at ≥13 years of age. Additional longitudinal analyses regarding radiation effects on cognitive decline did not show significant radiation effects. Manuscripts are prepared for journal publication.

RP 3-89 Osteoporosis in Hiroshima atomic-bomb survivors

Fujiwara S, Takahashi I (CH), Ohishi W (CH), Masunari N, Furukawa K (S), Nakamura T, Yoshimura N, Fukunaga M, Orimo H

Objectives: To determine the relationship between ionizing radiation and the prevalence and severity of osteoporosis as a potential, long-term health consequence of prior radiation exposure.

Background and Significance: Our working hypothesis is that acute ionizing radiation might accelerate the aging process as manifested by increased osteoporosis. To date, preliminary analyses of bone mineral density (BMD) in long-term atomic-bomb survivors do not suggest radiation exposure-related changes in BMD after adjusting for age, weight, and age at menopause. We are utilizing accumulated data on BMD and fracture for national and international collaborative studies that provide new insights regarding BMD and guidelines for health maintenance of atomic bomb survivors and others.

Study Methods: Longitudinal follow-up study of BMD as a part of routine health examinations.

Study Progress: We are conducting international and national collaboration studies using accumulated data and information related to this RP and have published several papers.

Results and Conclusions: As a collaborative study with the WHO working group, we have published a Japanese version of the WHO fracture risk assessment tool including age, sex, BMD, prior fracture, smoking, alcohol drinking, and so on as risk factors (*Osteoporosis Int* 2008; 19:429–35). Our papers from the AHS have contributed to development of a WHO fracture risk assessment tool, Japanese guidelines for prevention and treatment, guidelines for treatment of steroid-induced osteoporosis, and so on.

RP A1-15 The association between radiation and atrial fibrillation among atomic bomb survivors

Haruta D (CN), Reid D. Landes (S), Hida A (CN), Sera N, Imaizumi M (CN), Ichimaru S (CN), Takahashi I (CN), Ohishi W (CH), Akahoshi M, Maemura K

Objectives: Our main objective is to evaluate the impact of ionizing radiation on incidence of atrial fibrillation (AF)

while controlling for established risk factors of AF among atomic bomb survivors.

Background and Significance: Atrial fibrillation (AF) is a common arrhythmia seen in clinical practice. AF prevalence and incidence increases with age. AF is a main cause of cardiovascular disease (CVD), especially congestive heart failure and stroke. It has been established that various factors such as aging, male gender, hypertension, obesity, diabetes mellitus, hyperthyroidism, alcohol consumption, smoking, chronic kidney disease, and organic heart diseases are associated with the development of AF. AF shares many common risk factors with CVD. The Life Span Study (LSS) showed that increases in atomic-bomb radiation doses were associated with increases in CVD mortality. To date, there has been no systematic report about the effects of radiation on AF. Whether a positive relationship between radiation dose and AF is involved in the radiation-related CVD increase is not known. Understanding of the radiation-AF relationship will increase understanding of the radiation-CVD relationship.

Study Methods: The two-year period from July 1, 1967 to June 30, 1969 was used as the baseline. A total of 11,252 subjects (4,022 men and 7,230 women) were examined at baseline. Baseline AF cases, subjects without estimated dose and without additional follow-up examinations, and NIC subjects were excluded; the remaining about 7,400 subjects were followed up for the development of AF until December 31, 2009. We identified AF cases using ECG and clinical diagnosis databases. We use Cox regression analysis to estimate the radiation effects on AF with adjustment made for AF risk factors.

Study Progress: The dataset has been completed. We have started the statistical analyses.

Results and Conclusions: None yet.

RP-A3-14 The relation between low thyroid function and anemia, and the impact of both conditions on adverse outcomes (Addendum to RP-A10-08)

den Elzen W, Imaizumi M (CN)

Objectives: To study 1) the co-occurrence of anemia and low thyroid function, 2) the association between low thyroid function and changes in hemoglobin level or hematocrit during follow-up, and 3) the impact of a combination of both conditions on adverse health outcomes.

Background and Significance: An underactive thyroid gland (hypothyroidism) and anemia are common disorders in the elderly. Hypothyroidism and anemia have similar clinical symptoms and both lead to decreased quality of life. Anemia of various types can be associated with hypothyroidism, especially long-standing and severe disease. However, the effect of a hypothyroid state on the progression of anemia over time has not been studied in large community based populations. Furthermore, studies on the association between subclinical hypothyroidism and anemia are scarce. By using combined data from the Thyroid Studies Collaboration, we will study the association of low thyroid function with anemia and the impact of a combination of both conditions on adverse health outcomes.

Study Methods: We will perform an individual participant pooled data analysis from 15 prospective cohorts of adults with measurement of baseline thyroid function and hematological data.

Study Progress: We are collecting individual participant data from cohorts.

Results and Conclusions: Not yet. Results expected in 2015.

RP-A1-14 Association of chronic kidney disease and albuminuria with cardiovascular diseases among A-bomb survivors

Sera N, Hida A (CN), Haruta D (CN), Imaizumi M (CN), Takahashi I (CH), Yamada M (CH), Tatsukawa Y (CH), Nakashima E (S), Ohishi W (CH), Akahoshi M, Kawakami A

Objectives: Using both urinary albumin and estimated glomerular filtration rate (eGFR), we will make diagnoses and detailed categorizations of chronic kidney disease (CKD) for AHS participants in Hiroshima and Nagasaki to review their association with cardiovascular diseases (CVD) prevalence and radiation dose for each of the CKD categories.

Background and Significance: The association between atomic-bomb radiation exposure and CVD has recently been drawing attention. CKD has recently been recognized as a risk factor for CVD. CKD and CVD share many common risk factors such as obesity, impaired glucose tolerance, hypertension, dyslipidemia, and nephritis. A study within the Nagasaki AHS has also indicated the association of severe renal dysfunction (eGFR < 30ml/min/1.73m^2) and CKD (eGFR < 60ml/min/1.73m$^{2)}$) with radiation dose. That study used only eGFR for diagnosis of CKD, but we have used urinary albumin since 2008. Albuminuria is less likely to be affected by age and is considered as a marker of glomerular capillary endothelial damage.

Study Methods: Between October 2008 and December 2014, 5,077 AHS subjects were measured for both serum creatinine and urinary albumin in Hiroshima and Nagasaki. We diagnosed CKD with eGFR calculated with serum creatinine and urine albumin-to-creatinine ratio (UACR), and categorized six stages by eGFR and three stages by UACR. We will analyze the association between radiation and CKD first, and analyze the association between radiation and CVD considering CKD and other CVD risk factors.

Study Progress: The data for analysis were compiled and cleaned.

Results and Conclusions: None yet. Results expected in 2015.

RP-A5-13 The association between thyroid status and the progression of renal function over time

den Elzen W, Meuwese CL, Gussekloo J, Rodondi N, Imaizumi M (CN), Ohishi W (CH)

Objectives: The study hypothesis is that thyroid function is associated with the progression of renal dysfunction over time. The objectives of the present analysis are: 1) to study the association between thyroid function and renal function cross-sectionally. 2) to study the association between thyroid function and changes in renal function and the development of chronic kidney disease (CKD) over time, and 3) to study age as an effect-modifier in the previous associations.

Background and Significance: The prevalence of CKD increases with age. Throughout all age categories, CKD is

associated with an increased risk of adverse cardiovascular outcomes. Like CKD, the prevalence of overt and subclinical hypothyroidism increases with age. In the general population, overt hypothyroidism and subclinical hypothyroidism are both associated with an increased cardiovascular risk, which could be attributed to various cardiovascular effects of thyroid hormones. End-stage renal disease is frequently accompanied by thyroid hormone alterations in the absence of primary hypothalamic-pituitaric-thyroidal dysfunction. Conversely, small observational studies have indicated deterioration in renal function due to the effects of overt and subclinical hypothyroidism. By using combined data from the Thyroid Studies Collaboration, it will be possible to study the effects of a low thyroid function on renal function.

Study Methods: At this point, 15 large international cohorts including the Hiroshima and Nagasaki AHS have renal function data. We will perform a pooled analysis of individual participants in the studies to determine whether there is an association between low thyroid function and renal function.

Study Progress: Analyses are underway.

Results and Conclusions: None yet. Results expected in 2015.

RP-A4-13 Subclinical thyroid dysfunction and adverse bone outcomes (Addendum to RP-A10-08)

Blum MR, da Costa B, Imaizumi M (CN), Rodondi N

Objectives: To study: (1) the association between subclinical thyroid dysfunction and subsequent fracture risk (hip, non-spine), (2) the association between subclinical thyroid dysfunction and a greater bone loss as assessed by serial measurements of hip and spine bone mineral density (BMD), (3) the association between thyroid stimulating hormone (TSH) levels within the normal range and/or thyroxine therapy and increased fracture risk (hip, non-spine) and/or lower BMD, and (4) if fracture risk in subclinical thyroid dysfunction is mediated by its effects on BMD.

Background and Significance: Overt hyperthyroidism is known to be associated with osteoporosis and fractures, and overt hypothyroidism has been shown to decrease bone turnover. Excess thyroid hormone (hyperthyroidism) affects osteoclasts and osteoblasts and leads to bone loss, clinically resulting in osteoporosis and increased fracture risk. Excess TSH (hypothyroidism) also has direct effects on bone remodeling by directly or indirectly acting on osteoclast and osteoblast activity in experimental studies. Subclinical hyperthyroidism (SHyper) is characterized by low TSH and normal to high-normal free T4 levels, which could induce loss of BMD and increased fracture risk. Conversely, subclinical hypothyroidism (SHypo) could cause adverse bone outcomes by direct effects of TSH on bone turnover with subsequent change of bone quality. However, studies on the association of SHyper or SHypo with BMD and fractures are limited and controversial. By using combined data from the Thyroid Studies Collaboration, we want to study the associations of serum TSH levels and the risk of bone fracture or BMD.

Study Methods: We will perform an individual participant pooled data analysis from all available prospective cohorts of adults with measurement of baseline thyroid function and fracture outcomes and BMD.

Study Progress: Analyses were finished and the manuscript was submitted for publication.

Results and Conclusions: Subclinical hyperthyroidism was associated with increased risk of hip and other fractures, particularly for TSH < 0.10 mIU/L. Risks were similar after adjustment for osteoporotic fracture risk factors. Endogenous subclinical hyperthyroidism (excluding users of thyroid-altering medications) was associated with increased risk for all fractures. No association was found between subclinical hypothyroidism and fracture risk.

RP-A3-13 Serum TSH levels and the risk of stroke (Addendum to RP-A10-08)

van Dijk B, Chaker L, Peeters RP, Franco O, Imaizumi M (CN)

Objectives: (1) To study the association between suppressed or elevated thyroid stimulating hormone (TSH) levels and stroke, (2) to study the associations between low-normal or high-normal TSH levels and stroke, and (3) to study if the relationship between suppressed TSH and stroke is mediated via atrial fibrillation, and if the relationship between elevated TSH and stroke is driven via atherosclerosis (using carotid intima-media thickness as an index).

Background and Significance: Subclinical thyroid disease has been associated with a higher risk of cardiovascular disease in various studies. The underlying hypothesis is that subclinical hypothyroidism may increase the risk of atherosclerosis, whereas subclinical hyperthyroidism is related to an increased risk of atrial fibrillation. Both atherosclerosis and atrial fibrillation are known risk factors for the development of stroke, suggesting that subclinical thyroid disease may also be associated with an increased risk of stroke. However, only very few epidemiological studies evaluated subclinical thyroid disease as a risk factor for stroke. At this point, 14 cohorts with stroke data have joined the Thyroid Studies Collaboration, providing considerable statistical power to assess the association between both high and low TSH levels and the risk of stroke.

Study Methods: We will perform a pooled analysis of individual participants in large international cohort studies to determine whether there are associations of serum TSH levels and the risk of stroke.

Study Progress: Analyses were finished and the manuscript was submitted for publication.

Results and Conclusions: Although there was no overall effect of subclinical hypothyroidism on the risk of stroke, our data suggest that subclinical hypothyroidism is associated with an increased risk of stroke events or fatal stroke in subjects younger than 65 years of age and those with a TSH concentration of 7.0 to 9.9 mIU/L.

RP-A2-12 Weight fluctuation and cancer and cardiovascular disease incidence and mortality in Japanese.

Nanri A, Mizoue T, Sera N, Haruta D (CN), Takahashi I (CH), Soda M (EN), Ozasa K (EH), Cologne JB (S), Araki Y, Cullings HM (S), Ohishi W (CH), Hida A (CN), Akahoshi M

Objectives: We identify patterns of weight fluctuation and

examine their association with subsequent morbidity and mortality of cancer and cardiovascular disease in the AHS cohort.

Background and Significance: Being obese or underweight at a single point in time, as well as experiencing weight gain and loss, have been reported to be associated with increased mortality. Some studies have also found an association between weight fluctuation and mortality. However, measures of weight fluctuation in previous studies have had limited ability to capture weight fluctuation patterns in terms of the frequency and magnitude of weight change.

Study Methods: In this study, we first calculate a classical measure of weight fluctuation and, to address weight fluctuation patterns, we will further develop innovative measures that facilitate examining the relationship of longitudinal patterns in weight change to morbidity and mortality. We will prospectively examine the association of the new indicators of weight fluctuation with morbidity and mortality from all causes, cancer, and cardiovascular disease using Cox regression. Analyses will be based on 5,790 subjects in the Adult Health Study (AHS) cohort who were aged 20 to 49 years old at baseline (1958) and had health examination seven times or more between baseline and start of follow-up 20 years later (1978). The analysis should provide improved estimates of the risk of weight fluctuation based on enhanced conceptual and statistical approaches compared with those used in previous studies.

Study Progress: We started analyses in 2013.

Results and Conclusions: None yet. Results expected in 2016.

RP-A1-12 Study for the epidemiological and genetic basis of progressive cardiac conduction defect

Makita N, Akahoshi M, Haruta D (CN), Maemura K, Ohishi W (CH), Hida A (CN), Nakashima E (S)

Objectives: We will focus on progressive cardiac conduction defect (PCCD), which is a hereditary lethal arrhythmia.

Background and Significance: PCCD is a hereditary lethal arrhythmia characterized by electrocardiographic findings of atrioventricular block and bundle branch block caused by progressive degenerative fibrosis of cardiac conduction system. Sudden death and pacemaker implantation are outcomes of PCCD and three genes have been reported as responsible for PCCD. We will determine the possible PCCD cases that progressed to sudden death or pacemaker implantation from bundle branch block in the Adult Health Study (AHS) and conduct gene analysis in these possible PCCD cases.

Study Methods: Among 16,170 individuals who underwent health examinations in the AHS in Nagasaki and Hiroshima between 1967 and 2010, we will extract those who were diagnosed with right bundle branch block (RBBB; 828 cases). They will be categorized into two groups: a group (non-PCCD) who did not show progression of the bundle branch block, and a case group (PCCD) that progressed into sick sinus syndrome (SSS) or complete atrioventricular block (AVB). The latter group includes individuals who have undergone pacemaker implantation. Endpoints of the study are pacemaker implantation (32 cases).

At Nagasaki University, genomic DNA will be extracted from peripheral lymphocytes of subjects in the case group, and genetic screening will be conducted on the following genes: connexin genes, which modulate the electrical connection between cardiac cells (connexin 40, 43, 45), Na channels (*SCN10A, SCN4B*), which have been posited as candidate genes affecting cardiac conduction in genome-wide association analysis, and the cytoskeletal protein lamin, which plays a role in maintenance of structure of cellular nucleus and modulation of transcription. Exons of the respective genes will be amplified by the PCR method, and the base sequences will be analyzed with an ABI 3130 sequencer.

Study Progress: Part of this epidemiological study has been published.

Results and Conclusions: The risk of pacemaker implantation for RBBB was 4.79 (95% confidence interval [CI] 1.89–12.58; P = 0.001), 3.77 (95% CI, 1.09–13.07; P = 0.036), and 6.28 (95% CI, 1.24–31.73, P = 0.026), when implantation was for all causes, AV block, and SSS, respectively. RBBB, especially with axis deviation (AD), progresses to AV block and SSS that requires pacemaker implantation; the mechanisms by which the conduction defect progresses differ among patients with and without AD.

RP-A4-11 Role of visceral fat and its effects in the association between radiation dose and cardiovascular disease

Cullings HM (S), Nakashima E (S), Carter RL, Hida A (CN), Imaizumi M (CN), Akahoshi M

Objectives: We plan to investigate the causal pathways and associations among atomic bomb (A-bomb) radiation, visceral fat accumulation and its sequelae (fatty liver, hypertension, hyperlipidemia, type 2 diabetes mellitus), and cardiovascular disease (CVD).

Background and Significance: It has been reported that A-bomb radiation exposure increases the risk of CVD. In addition, A-bomb radiation is related to fatty liver, hypertension, abnormal lipid profiles, impaired glucose tolerance, and inflammation, which are all related to visceral fat accumulation. Free fatty acids (FFAs) and several adipokines (inflammatory cytokines), adiponectin, angiotensinogen, and plasminogen activator inhibitor-1 [PAI-1]) are secreted from visceral fat (adipocytes and macrophages in fat tissue). FFAs and adipokines, along with visceral fat accumulation, may contribute to the mechanism(s) of radiation-induced CVD. Thus, visceral fat accumulation and metabolic and/or inflammatory parameters relating to FFAs, as well as adipokines may be part of the causal pathway that explains the association between A-bomb radiation and CVD.

Study Methods: We examined 1,366 Nagasaki Adult Health Study (AHS) subjects (521 males, 845 females) from 2004 through 2007 and collected (1) the data of surrogate markers of visceral fat accumulation and atherosclerosis, and (2) metabolic or inflammatory data relating to FFAs and adipokines. We also identified cases of hypertension, type 2 diabetes, hyperlipidemia, metabolic syndrome, angina pectoris, myocardial infarction, and stroke, based on standard diagnostic criteria, after taking consideration of medication history. Using this dataset, we plan to investigate the potential causal pathways and associations among A-bomb radiation, visceral fat accumulation and its sequelae

(fatty liver, hypertension, hyperlipidemia, type 2 diabetes), as well as CVD. The mediating effects of visceral fat accumulation on the radiation-CVD association will be investigated using the conceptual model: radiation + visceral fat accumulation → inflammation → metabolic function → atherosclerosis → CVD.

Study Progress: We have conducted preliminary demographic analyses and found positive associations of ischemic heart disease with hypertension (p = 0.0019) and type 2 diabetes mellitus (p = 0.0065). Dr. Cullings agreed to take over the RERF portion of this RP due to Dr. Sera's departure from RERF.

Results and Conclusions: None yet. Results expected in 2015.

RP-A14-08 The incidence and prognostic value of the early repolarization electrocardiogram pattern

Haruta D (CN), Tsuneto A, Nakashima E (S), Akahoshi M

Objectives: To examine the incidence of the early repolarization pattern (ERP) and its prognostic value in terms of mortality from unexpected death, cardiac disease mortality, and total mortality among Nagasaki AHS subjects.

Background and Significance: The majority of sudden cardiac deaths are caused by ventricular tachyarrhythmias that occur in persons with a structurally normal heart. ERP has been considered benign, but one experimental study reported its potential arrhythmogenicity (Gussak I et al., *J Electrocardiol* 2000; 33:299–309), suggesting the possibility that ERP is a cause of idiopathic ventricular fibrillation leading to sudden death.

Study Methods: We reviewed all the ECG records of 5,976 AHS subjects who were examined at least once between 1958 and 2004. Brugada-type ECG cases were excluded. We identified those who showed ERP and calculated the incidence during the follow-up period after excluding prevalent ERP cases. We assessed the cause of death using death certificates and calculated the risk of: 1) unexpected deaths including sudden death or unexplained accidental death; 2) cardiac disease mortality; and 3) total mortality in ERP cases by means of Cox proportional hazards analysis with adjustment for age, sex, and underlying cardiovascular diseases.

Study Progress: We have finished analyses of the associations of ERP with unexpected death, cardiac death, and total mortality.

Results and Conclusions: ERP incidence was 715 per 100,000 person-years, with ERP observed in 35.5% of unexpected death cases. ERP had an elevated risk of unexpected death (HR 1.83, 95% CI 1.12–2.97), and a decreased risk of cardiac death (HR 0.75, 95% CI 0.60–0.93), and all-cause death (HR 0.83, 95% CI 0.78–0.93). ERP has significant public health implications to prevent unexpected death, but given the negative association for all cardiac death, confirmatory data are needed.

RP-A13-08 Prognostic significance of VPCs in taking consideration of their origins

Haruta D (CN), Nakashima E (S), Ohishi W (CH), Hida A (CN), Akahoshi M

Objectives: Ventricular premature contractions (VPC) in regular 12-lead electrocardiogram (ECG) recordings have again gained attention as a predictive variable for cardiovascular mortality in clinical populations. Thus, we would like to evaluate the significance of VPC in regular 12-lead ECG recordings as a risk factor for cardiovascular mortality in our representative, general population.

Background and Significance: Since the CAST trials found no evidence for the theory that VPC suppression reduces risk, VPC has generally been ignored on routine ECG examinations. However, some recent reports have indicated that the presence of VPC in regular 12-lead ECG recordings is a significant and independent predictor of cardiovascular mortality.

Study Methods: We will identify VPC cases from AHS subjects (4,092 in Hiroshima and 2,642 in Nagasaki) who underwent a regular 12-lead ECG recording during the period from 1990 to December 1993. We will identify subjects with VPC, and classify it into three groups according to VPC morphology: (1) left bundle branch block (LBBB) type that originates from the right ventricle; (2) right bundle branch block (RBBB) type that originates from the left ventricle, and; (3) unidentified type. We will compare the basic characteristics at VPC diagnosis, and the underlying diseases between those with and without VPC and also among VPC cases with LBBB type, RBBB type, and unidentified type. Information on mortality and cause of death until December 2005 will be used to assess the prognostic significance for cardiovascular mortality between those with and without VPC. We will conduct a Cox proportional hazards analysis to assess the prognostic significance of VPC, frequency of cardiovascular mortality according to VPC diagnosis, and morphology of VPC, with adjustment for age, sex, and underlying diseases.

Study Progress: We prepared a manuscript and it is under review by the International Heart Journal.

Results and Conclusions: No VPCs were associated with all-cause and cardiac mortality, but LBBB type was significantly associated with coronary heart disease (CHD) mortality (Hazard ratio, 2.73; 95% CI, 1.11–6.73), after adjustments were made for other risk factors. LBBB type VPC was associated with increased CHD mortality. Larger studies are needed to confirm the effect of morphology, as it might help to predict risk.

RP-A10-08 The association between subclinical thyroid dysfunction and cardiovascular disease and mortality: An individual participant pooled analysis of large international cohort studies

Rodondi N, Gussekloo J, Imaizumi M (CN)

Objectives: 1) To assess the relationship between subclinical thyroid dysfunction and coronary heart disease (CHD) and mortality, 2) to assess whether these relationships persist after adjusting for important potential confounders, and 3) to determine whether these relationships differ according to age and gender, race, TSH levels, or presence or absence of prevalent cardiovascular disease.

Background and Significance: Subclinical hypothyroidism has been reported to be associated with elevated cholesterol and increased risk for atherosclerosis. We previously reported on risk for ischemic heart disease and all-cause mortality in subclinical hypothyroidism (*J Clin*

Endocrinol Metab 2004; 89:3365). In this study, we found a significant association between the prevalence of ischemic heart disease and subclinical hypothyroidism and a possible increased mortality in men with subclinical hypothyroidism by using the data of 2,856 Nagasaki AHS subjects examined between 1984 and 1987. However, several reports from other cohorts on the relationship between subclinical hypothyroidism and CHD events and mortality are conflicting. Only a pooled analysis of the large cohort studies with individual participant data might confirm these associations, explore the potential differences, and clarify the conflicting data.

Study Methods: We are performing a pooled analysis of individual participants in large international cohort studies to determine whether there is an association between subclinical thyroid dysfunction and cardiovascular disease and mortality.

Study Progress: We are evaluating the association between subclinical hypothyroidism and cardiovascular risk factors according to age based on the small-scale RP related to this RP. Three manuscripts of related studies (RP-A7-12, A3-13, and A4-13; addendums to this RP) have been submitted for publications and a related study (A3-14) has started as an addendum to this RP.

Results and Conclusions: Subclinical hypothyroidism is associated with an increased risk of CHD events and CHD mortality in those with higher TSH levels, particularly in those with a TSH concentration of 10 mIU/L or greater (Rodondi et al. [including Imaizumi M], *JAMA*, 2010; 304(12): 1365–74).

Special Clinical Studies Publications

RERF Reports (RR)

♦ Imaizumi M, Ohishi W, Nakashima E, Sera N, Neriishi K, Yamada M, Tatsukawa Y, Takahashi I, Fujiwara S, Sugino K, Ando T, Usa T, Kawakami A, Akahoshi M, Hida A: Association of radiation dose with prevalence of thyroid nodules among atomic bomb survivors exposed in childhood (2007–2011). JAMA Intern Med 2015 (February); 175(2):228–36. **(RR 9-14)** (refer to summary explanation in *Adult Health Study* Publications)

♦ Kusumoto S, Kawano H, Makita N, Ichimaru S, Kaku T, Haruta D, Hida A, Sera N, Imaizumi M, Nakashima E, Maemura K, Akahoshi M: Right bundle branch block without overt heart disease predicts higher risk of pacemaker implantation: The study of atomic-bomb survivors. Int J Cardiol 2014 (June); 174(1):77–82. **(RR 6-13)**

[Study Findings] Right bundle branch block (RBBB) is a common disorder observed in electrocardiograms. However, the prognosis of RBBB patients without overt heart disease has not been studied. We demonstrated that atrioventricular (AV) block and sick sinus syndrome (SSS) develop among RBBB patients even with no overt heart disease, and that the risk of pacemaker implantation was higher for these subjects than for those without RBBB. The risk was especially pronounced in subjects with axis deviation (AD). There was no significant association between A-bomb radiation exposure and either RBBB or pacemaker implantation.

[Explanation] In the Adult Health Study, which has been conducted for many years to follow the health of A-bomb survivors in Hiroshima and Nagasaki, electrocardiograms are obtained during biennial examinations. Electrocardiograms were used for diagnosis of RBBB, and subsequent clinical developments were examined. ***Objective*** The long-term prognosis of RBBB is generally benign, and it has been reported that the incidence of progression to AV block is 0–0.3%. However, in previous studies, the number of cases was limited, and no consideration was given to the presence or absence of AD. In this study, we clarified the long-term course of RBBB, using a population that has been followed for many years. ***Methods*** Among the 16,170 A-bomb survivors in Hiroshima and Nagasaki who participated in the Adult Health Study from 1967 to 2010, 520 participants developed RBBB during the study without overt heart disease. To estimate the comparative risk (hazard ratios) of pacemaker implantation, we selected 1,038 subjects without RBBB, matched for age at RBBB diagnosis and sex, to act as controls. ***Results*** (1) Risk of progression to pacemaker implantation: Hazard ratios (95% confidence interval) of pacemaker implantation in the RBBB group were significantly higher than that in the control group: when implantation was for all causes (AV block, SSS, and unclear cause), 4.79 (1.89–12.58); for AV block, 3.77 (1.09–13.07); and for SSS, 6.28 (1.24–31.73). (2) Association between implantation risk and the presence or absence of AD: RBBB subjects with AD had a higher risk of pacemaker implantation (for all causes) than subjects without AD (hazard ratio, 3.03; 95% confidence interval, 1.00–9.13). RBBB subjects with AD were younger than subjects without AD at the time of RBBB diagnosis (59.4 ± 7.6 and 74.4 ± 3.1 years old, respectively), and their progression to pacemaker implantation took significantly longer. (3) Association with radiation: We conducted an analysis of the association between A-bomb radiation and RBBB or pacemaker implantation, but did not observe any significant correlation. It has been reported that high-dose radiotherapy can cause cardiac conduction disturbance. In this study, however, estimated A-bomb radiation doses were substantially lower than those experienced during high-dose radiotherapy. This suggests that lower-dose radiation exposures have no effect on the cardiac conduction system.

We demonstrated a significantly increased risk of pacemaker implantation resulting from AV block and SSS in RBBB subjects, especially when AD was present. The study also suggested that the mechanism behind the progression to conduction defect differ between patients with AD and those without AD.

Other Journal Publication

♦ Imaizumi M, Furukawa K: Thyroid disorders in Hiroshima and Nagasaki atomic-bomb survivors. Hiroshima Igaku [J Hiroshima Med Assoc] 2014 (April); 67(4):284–6. (Proceedings of the 54th Late A-bomb Effects Research Meeting, 2013) (Japanese) (related to *Special Cancer Studies*)

Manuscripts in Press

⌘ Haruta D, Nakashima E, Ohishi W, Hida A, Akahoshi M: Prognostic significance of VPCs in taking consideration of their origins. Ann Noninvasive Electrocardiol.

⌘ Yamada M, Landes RD, Mimori Y, Nagano Y, Sasaki H:

Trajectories of cognitive function in dementia-free subjects: Radiation Effects Research Foundation Adult Health Study. J Neurol Sci.

Special Clinical Studies Oral Presentations

❖ Blum MR et al. (RERF: Imaizumi M). Subclinical thyroid dysfunction and adverse bone outcomes: An individual participant data analysis of prospective cohorts. 37th Annual Meeting of the Society of General Internal Medicine (SGIM), 23–26 April 2014, San Diego, California, USA

❖ Fujiwara S, Masunari N, Takahashi I, Ohishi W. Sarcopenia and increased mortality among elderly men. European Calcified Tissue Society 2014, 17–20 May 2014, Prague, Czech

❖ Ohishi W, Ueda K, Tatsukawa Y, Nakashima E, Yamada M, Kohata M, Takahashi I, Tsuge M, Chayama K. Study of factors associated with prevalence and disease progressions in nonalcoholic fatty liver disease. 50th Annual Meeting of the Japan Society of Hepatology, 29–30 May 2014, Tokyo (related to *Adult Health Study*)

❖ Yamada M, Landes RD, Mimori Y, Nagano Y, Sasaki H. Cognitive decline among a dementia-free Japanese elderly population: Radiation Effects Research Foundation Adult Health Study. Asia Pacific Geriatric Conference, 6–8 June 2014, Taipei, Taiwan

❖ Yanagi M, Takahashi I, Misumi M, Neriishi K, Kiuchi Y. Association between retinal vessel diameter and glaucoma in atomic bomb survivor in Hiroshima and Nagasaki, Japan. 11th European Glaucoma Society Congress, 7–10 June 2014, Nice, France

❖ Kusumoto S, Kawano H, Koide Y, Ikeda S, Takeno M, Eguchi M, Yonekura T, Haruta D, Akahoshi M, Maemura K. Right bundle branch without overt heart disease has higher risk of pacemaker implantation: the study of atomic-bomb survivors. 56th Annual Meeting of Japan Geriatrics Society, 12–14 June 2014, Fukuoka

❖ Imaizumi M, Ohishi W, Nakashima E, Sera N, Neriishi K, Yamada M, Tatsukawa Y, Takahashi I, Fujiwara S, Ando T, Usa T, Kawakami A, Akahoshi M, Hida A. Thyroid study in Hiroshima and Nagasaki atomic-bomb survivors exposed in childhood: Radiation dose-response relationships for thyroid nodules (2007–2011). 16th International Congress of Endocrinology & The Endocrine Society's 96th Annual Meeting & Expo, 21–24 June 2014, Chicago, Illinois, USA (related to *Adult Health Study*)

❖ Shore RE. Epidemiologic studies of radiation cataract risk: Dose and dose rate. 2014 Expert Meeting on Radiation Effects, 5 September 2014, Tokyo (related to *Immunology Studies*)

❖ Fujiwara S, Masunari N, Takahashi I, Ohishi W. Prevalence and mortality of Sarcopenia among elderly. 16th Annual Meeting of the Japan Osteoporosis Society, 23–25 October 2014, Tokyo

❖ Ueda K, Ohishi W, Nakashima E, Tatsukawa Y, Yamada M, Kohata M, Takahashi I, Tsuge M, Chayama K. Study of factors associated with prevalence of nonalcoholic fatty liver disease in non-obese subjects. 22nd Japan Digestive Disease Week, 23–26 October 2014, Kobe (related to *Adult Health Study*)

❖ Imaizumi M, Ohishi W, Nakashima E, Sera N, Neriishi K, Yamada M, Tatsukawa Y, Takahashi I, Fujiwara S, Sugino K, Ando T, Usa T, Kawakami A, Akahoshi M, Hida A. Thyroid Study in Hiroshima and Nagasaki atomic-bomb survivors exposed in childhood: Thyroid function and autoimmune thyroid disease. 57th Annual Meeting of the Japan Thyroid Association, 13–15 November 2014, Osaka (related to *Adult Health Study*)

❖ Yanagi M, Takahashi I, Neriishi K, Ohishi W, Kiuchi Y. Association between retinal vessel diameter and glaucoma in atomic bomb survivor in Hiroshima and Nagasaki, Japan. 68th Annual Congress of Japan Clinical Ophthalmology, 13–16 November 2014, Kobe

❖ Shore RE. Epidemiologic studies of radiation cataract risk: Dose and dose rate. NAS BEIR VIII Planning Meeting, 17 November 2014, Washington, DC, USA

❖ Cologne JB, Izumi S, Sakata R, Yamada M. Interaction between exposure and age in cohort-based risk models: effect modification or age dependence of the excess rate? 2015 Annual Meeting of Applied Statistics Society, 14 March 2015, Kyoto (related to *Life Span Study*)

Research Protocols 1-12, 5-89 (Platform Protocol), A2-08
Histopathology Studies

RP 1-12 Development of an archival system for surgical cancer samples from atomic-bomb survivors

Ozasa K (EH), Sugiyama H (EH), Soda M (EN), Yasui W, Arihiro K, Fujihara M, Arita K, Nishisaka T, Matsuura H, Nakashima M, Shigematsu K, Takahara O, Kusunoki Y (R), Katayama H (IT)

Objectives: To develop an archival system for surgical cancer samples from the atomic-bomb survivors in collaboration with pathologists of major hospitals in Hiroshima and Nagasaki.

Background and Significance: To clarify the mechanisms of site-specific differences in cancer risks, the shapes of dose-response curves, and the effects of age at the time of bombing and attained age or time since exposure, pathological studies and potentially molecular biological studies of carcinogenesis mechanisms are conducted. These studies should contribute to improved health care for the atomic-bomb survivors and all those suffering from radiation damage in the future.

Study Methods: The major hospitals in Hiroshima and Nagasaki will be collaborators and they will keep the surgical samples derived from LSS cohort members in accordance with common procedures that are defined in the RP. The guidelines to use the stored samples for research are also defined in the RP.

Study Progress: Preparation of an operations protocol for the procedures between individual hospitals and RERF is being planned.

Results and Conclusions: None yet.

RP 5-89 Pathology studies in Hiroshima and Nagasaki, revised research plan (Formerly RP 3-75)

Ozasa K (EH), Sadakane (EH), Yonehara S, Fujihara M, Soda M (EN), Kodama K (CS)

Objectives: This RP is a revised platform protocol for the conduct of pathology studies at RERF. Pathologists in community and university hospitals are involved in the site-specific cancer incidence studies.

Background and Significance: The RERF autopsy and surgical programs (ABCC TR 4-61 and RERF RP 3-75) were terminated and this RP is their replacement.

Study Methods: Intensive efforts are made to seek the cooperation of pathologists at other medical institution in providing pathological materials. We are collecting microscopic slides for those whose autopsies were performed at local hospitals in Hiroshima and Nagasaki since 1989 after terminating the autopsy program at RERF.

Study Progress: A series of site-specific studies has been conducted. We are taking an inventory of formalin-fixed paraffin-embedded tissues that are stored at RERF. Those specimens are from around 6,700 autopsies and 6,500 surgical operations. We collected seven new autopsy cases in Nagasaki this year.

Results and Conclusions: Essential methods of collecting and storing pathological materials were established.

RP-A2-08 Histopathological identification of multiple primary cancers occurring in Nagasaki atomic-bomb survivors

Nakashima M, Soda M (EN), Utada (EH), Sadakane (EH), Furukawa K (S), Sekine I, Yamashita S, Shibata Y, Ozasa K (EH)

Objectives: The purpose of this protocol is to identify true multiple primary cancers (MPC) diagnoses by modern histopathological methods as an initial step in evaluating the relationship between MPC and radiation exposure in A-bomb survivors.

Background and Significance: A recent analysis at the Atomic Bomb Disease Institute of the Nagasaki University Graduate School of Biomedical Sciences, using similar histopathological methods, found a strong association between MPC rates and distance from the hypocenter, especially for exposure at young ages. The present study is being conducted within the LSS so that actual dose estimates can be applied to the risk estimation.

Study Methods: Based on immunohistochemistry for cytokeratin-7 (CK7) and CK20 (cytoskeleton proteins), thyroid transcription factor-1 and prostatic specific antigen (tissue-specific markers), lung surfactant PE-10, and ovarian cancer marker CA125, MPC of various sites can be distinguished from metastatic tumors. Pathological review by the principal investigator intends to discriminate metastases from second primary cancers by determining the cell type or tissue of origin.

Study Progress: Among the Nagasaki Life Span Study (LSS) cohort (n = 38,107), there were a total of 6,305 primary-cancer patients between 1958 and 2003. After reviewing the HE-stained tissue specimens and immunohistochemistry findings among 648 patients who had two or more cancers, 595 cases were identified as MPC. Results of the review have been interpreted by the pathologist.

Results and Conclusions: Not yet.

Research Protocols 1-14, 1-13, 1-11 and 2-12, 5-10, 5-02, B49-10, P1-11

Cell Biology Studies

RP 1-14 A study of the biological significance of the *EML4-ALK* fusion gene in radiation-associated thyroid carcinogenesis using conditional transgenic mice

Hamatani K (R), Ito R (R), Taga M (R), Kim YM (S), Hayashi Y, Eguchi H, Kusunoki Y (R)

Objective: This study aims to clarify the biological significance of the *EML4-ALK* fusion gene in radiation-related papillary thyroid carcinogenesis using conditional transgenic mice, based on the hypothesis that the *EML4-ALK* fusion gene plays an important role in causing papillary thyroid cancer (PTC) and is a result of the radiation exposure.

Background and Significance: We found the *EML4-ALK* fusion gene in PTC cases exposed to radiation doses of 5 mGy and over, but not in those with radiation doses less than 5 mGy. This fusion gene occurred in a manner fundamentally exclusive of *RET, NTRK1, BRAF*, and *RAS* gene alterations. PTC bearing this fusion gene-positive had characteristic solid/trabecular architectures at a high frequency in cancerous regions. Elucidation of the biological significance of the *EML4-ALK* fusion gene in development of PTC will shed light on the molecular mechanisms of radiation-related papillary thyroid carcinogenesis.

Study Methods: We will examine morphological changes of thyroid tissue prior to tumor development and histological features of PTC developed in transgenic mice following doxycycline-induced *EML4-ALK* expression. We will compare irradiated and non-irradiated conditional transgenic mice with regard to the time required for PTC generation and histological features including the cancer's aggressiveness.

Study Progress: To make thyroid tissue-specific and time-specific conditional transgenic mice, we constructed a single doxycycline-inducible gene expression vector having cDNA of the *EML4-ALK* fusion gene. Since transgenic mice cannot be made at RERF, we are contracting out production of the conditional transgenic mice (F0 and F1 mice) to a commercial laboratory (UNITECH Co., Chiba, Japan). Six lines of founder mice were produced by microinjection of target DNA into C57BL/6 fertilized eggs. F1 transgenic mice were produced from four lines of F0 mice. These F1 mice are in doxycycline treatment to select one F1 line showing high thyroid-specific and high-level expression of *EML4-ALK*.

Results and Conclusions: None yet. Results for histological examination of PTC developed in transgenic mice will be expected in 2015.

RP 1-13 Analyses of molecular characteristics of lung cancer among atomic-bomb survivors

Taga M (R), Hamatani K (R), Ito R (R), Niwa Y (R), Grant EJ (E), Ozasa K (E), Katayama H (IT), Misumi M (S), Sui H, Harris CC, Yasui W, Kusunoki Y (R)

Objectives: The aim of this study is to clarify molecular oncological characteristics of lung cancer among A-bomb survivors.

Background and Significance: Molecular mechanisms of how radiation exposure is associated with the development of lung cancer are unclear. We hypothesize that radiation exposure may affect the profile of genetic and/or epigenetic alterations associated with lung carcinogenesis, working together with cigarette smoking.

Study Methods: Using surgical and autopsy lung cancer tissue specimens from the Life Span Study (LSS) cohort, we analyze genetic and epigenetic alterations associated with lung carcinogenesis in relation to patho-epidemiological factors including radiation dose and cigarette smoking history. For instance, we examine LOH of *p53*, *p16* and *RASSF1A*, point mutations of *p53*, *EGFR*, and *K-ras*, and methylation of *p16*, *RASSF1A*, and LINE1, as well as Alu retrotransposones (a marker of global methylation in genomic DNA). In addition, we examine selected gene rearrangements (e.g., rearranged *ALK*) in LSS lung adenocarnoma specimens that have been reported in sporadic lung adenocarcinoma.

Study Progress: 1) Thus far we have collected 20 radiation-exposed (dose > 0) and 18 non-exposed (dose = 0) LSS surgical non-small cell lung cancer (NSCLC) tissue specimens. We found that *p53* mutation frequency and *RASSF1A* methylation levels in exposed NSCLC cases were suggestively higher and lower than that in non-exposed NSCLC, respectively, although not statistically significant. Gene rearrangements were also examined in lung adenocarcinoma cases. No *RET* rearrangements were found, and *ALK* rearrangements were detected in two adenocarcinoma cases among those thus far examined. Analysis of the autopsy lung cancer specimens stored at RERF has started.

Results and Conclusions: Analyses of genetic and epigenetic alterations in the LSS NSCLC cases will be completed in 2018, and those of *ALK* and *RET* rearrangements in the LSS lung adenocarcinoma cases will be completed in 2015.

RP 1-11 Study of Radiation-induced Circulatory Diseases Using Animal Models
RP 2-12 A Study of Circulatory Diseases Using Animal Models Irradiated with Lower Doses (Addendum to RP 1-11)

Takahashi N (RC), Niwa Y (R), Murakami H. (R), Ohishi W (CH), Misumi M (S), Kusunoki Y (R), Inaba T, Nagamachi A, Tanaka I, Tanaka S

Objectives: The purpose of this study is to assess the relationship between radiation and circulatory diseases (CD). Data from pathological analysis and measurement of blood biomarkers may provide mechanistic information on the relationship between radiation exposure and development of CD.

Background and Significance: Based on the data from the LSS and AHS, we hypothesize that radiation exposure may cause an elevated risk of CD. For assessing this hypothesis, we are conducting animal studies where spontaneous hypertensive rat (SHR) rats and stroke prone-SHR (SHRSP) rats irradiated with moderate doses are used. Through this study, we expect to obtain information on the mechanisms of radiation-related CD.

Study Methods: In this SHRSP study, male rats are irradiated by gamma ray with 0.25, 0.5, 0.75 and 1 Gy, as well as a 0 Gy control. This study is conducted in two ways: 1) measurement of the time to show symptoms related to stroke, and 2) pathological analyses and blood biomarker

measurements in fresh samples before symptoms appear. In the study of SHR rats (who become hypertensive but are resistant to stroke) irradiated with 0, 1, 2 and 4 Gy, blood pressure and their body weights were measured weekly until 30 weeks after irradiation.

Study Progress: In this SHRSP study, stroke onset and lifespan experiments with a statistical analysis have been completed, and biomarker measurements and pathological analyses are underway. In the SHR study of rats irradiated with 0, 1, 2 and 4 Gy, we examined four endpoints; 1) blood pressure, 2) pathological phenotypes, 3) body weight, and 4) blood biomarkers, and completed them except for the measurement of blood biomarkers including cytokines.

Results and Conclusions: The symptoms related to stroke in irradiated SHRSP, even at 0.25 Gy, were significantly earlier than that observed in the controls, and lifespan was shortened with increasing dose. Systolic blood pressure level of irradiated SHR was higher than that of unirradiated SHR. The body weight of irradiated SHR was significantly lower than that of unirradiated SHR. Pathological analyses demonstrated that fatty changes of the liver were observed in only irradiated rats. Conclusions for the entire study will be obtained in 2015.

RP 5-10 Analyses of molecular characteristics of colorectal cancer among atomic-bomb survivors

Ito R (R), Hamatani K (R), Taga M (R), Imai K (R), Ozasa K (EH), Katayama H (IT), Cologne JB (S), Misumi M (S), Izumi S, Oue N, Yasui W, Nakachi K (RC), Kusunoki Y (R)

Objectives: The aim of this study is to clarify molecular oncological characteristics of colon and rectal cancers among A-bomb survivors.

Background and Significance: Radiation exposure is associated with an increased risk of colon cancer, but not rectal cancer. In colorectal cancer, two major phenotypes, chromosomal instability (CIN) and microsatellite instability (MSI), characterize different carcinogenic pathways. Our hypothesis is that the MSI pathway may preferentially occur in colorectal cancer among A-bomb survivors. MSI and CIN are not necessarily exclusive, and whether or not radiation exposure is associated with CIN-positive colon and rectal cancers is yet to be addressed. This study will help elucidate mechanisms to explain the observed increased risk of colon cancer, but not rectal cancer, among A-bomb survivors.

Study Methods: We use LSS archival surgical and autopsied colorectal cancer tissue specimens that are preserved at RERF as well as those from the Department of Molecular Pathology, Hiroshima University Graduate School of Biomedical Sciences. Using DNA extracted from the micro-dissected cells, we examine MSI and CIN status and their related gene alterations.

Study Progress: We have thus far analyzed 77 LSS colorectal cancer cases of which 14 MSI-high cases were found to have no significant association with radiation exposure status. In addition, no association between *MLH1* alterations and radiation exposure status was found. However, *MLH1* mutation, methylation, and LOH statuses in the MSI-high cases differed by latency period and gender. We also examined CpG island methylator phenotype (CIMP) status in 33 cases, using a bisulfite restriction analysis method combined with new CIMP markers.

Results and Conclusions: Results on the characteristics of exposed cases with MSI-high status expected in FY2015.

RP 5-02 Papillary thyroid carcinomas in residents of Hiroshima and Nagasaki who were exposed to A-bomb radiation as children: A study of *RET* gene rearrangements and other DNA changes potentially responsible for the origins and/or development of these tumors

Hamatani K (R), Taga M (R), Ito R (R), Cologne JB (S), Soda M (EN), Imai K, Nakachi K, Kusunoki Y (R)

Objective: To elucidate the mechanisms of adult-onset papillary thyroid carcinogenesis among A-bomb survivors, we will clarify characteristics of gene alterations that occur at an early stage of thyroid carcinogenesis. We will use archival cancer tissue specimens from Life Span Study (LSS) subjects.

Background and Significance: Rearrangements of the *RET* gene are induced in human thyroid cells by *in vitro* and *in vivo* x-ray irradiation. *RET* rearrangements occur at a low frequency of about 5–10% of sporadic adult-onset papillary thyroid cancer (PTC) cases. On the other hand, we observed that the relative frequency of PTC cases with *RET* rearrangements significantly increased with radiation dose, while the frequency of PTC with point mutations, typically $BRAF^{V600E}$, significantly decreased with dose.

Study Methods: Using DNA and RNA from archival tissue specimens of PTC from the LSS cohort, we examine various gene alterations including *RET/PTC* and *ALK* rearrangements and the $BRAF^{V600E}$ point mutation.

Study Progress: In addition to the PTC cases carrying *RET/PTC* and *NTRK1* rearrangements, we have thus far found *EML4-ALK* fusion genes in 10 exposed PTC cases. This fusion was fundamentally exclusive of any *RET*, *NTRK1*, *BRAF*, or *RAS* gene alterations. To further assess gene alterations in exposed PTC cases, fifteen PTC cases with no known gene alterations were examined for acylglycerol kinase (*AGK*)/*BRAF* and the transcription factor ETS variant 6 (*ETV6*)/*NTRK3* rearrangements, which were recently reported in post-Chernobyl childhood thyroid tumors; however, no such rearrangements were found in these 15 cases. Since an *STRN-ALK* fusion gene formed by a deletion was reported in an aggressive form of thyroid cancer, we also started working to detect this fusion gene. To detect unknown partner genes of fusion genes, we established an inverse PCR method in which specificity and sensitivity were enhanced.

Results and Conclusions: Chromosomal rearrangements, such as *RET*, *NTRK1*, and *ALK* rearrangements, likely play an important role in radiation-associated adult-onset thyroid carcinogenesis.

RP-B49-10 Establishment of methods for transfection of expression vectors into primary cultured epithelial cells of mouse thyroid tissues

Hamatani K (R), Niwa Y (R), Kusunoki Y (R)

RP-P1-11 Aging effects on epigenetic status in blood cell subsets

Niwa Y (R), Hamatani K (R), Taga M (R), Ito R (R), Yoshida K (R), Kusunoki Y (R)

Cell Biology Study Publications

RERF Report (RR)

♦ Hamatani K, Eguchi H, Koyama K, Mukai M, Nakachi K, Kusunoki Y: A novel *RET* rearrangement (*ACBD5/RET*) by pericentric inversion, inv(10)(p12.1;q11.2), in papillary thyroid cancer from an atomic bomb survivor exposed to high-dose radiation. Oncol Rep 2014 (November); 32(5):1809–14. **(RR 4-14)**

[Study Findings] Our analysis has shown that a novel type of *RET* rearrangement (*ACBD5-RET* fusion gene) that was identified in papillary thyroid cancer (PTC) cells of an atomic bomb survivor constitutively activates the MAPK-signaling pathway and is tumorigenic, as is the case with other types of *RET* fusion genes.

[Explanation] Through chromosomal inversion or translocation, radiation-related PTC cases such as adult PTC among A-bomb survivors and childhood PTC that developed after the Chernobyl nuclear accident often exhibit rearranged *RET* genes, that is fusion genes such as *CCDC6-RET* and *NCOA4-RET*, between the *tyrosine kinase domain* of the *RET* gene and other gene sites. These fusion genes are believed to be significantly involved in papillary thyroid carcinogenesis. We analyzed the structure and function of a novel RET rearrangement (*ACBD5-RET* fusion gene) found in the PTC of an A-bomb survivor exposed to high-dose radiation. ***Objectives*** To elucidate, through *in vitro* and *in vivo* experiments, whether the *ACBD5-RET* fusion gene found in the PTC of the A-bomb survivor activates the MAPK-signaling pathway and is tumorigenic. ***Methods*** (1) Expression vectors, i.e., plasmid DNA with a promoter sequence needed for gene expression, containing cDNA (DNA synthesized from an RNA template by reverse transcriptase and complementary to mRNA) of the *ACBD5-RET* fusion gene were introduced into mouse *NIH3T* cells (immortalized fibroblasts established from mouse skin cells). This process was undertaken to determine, using Western-blotting, whether phosphorylation (or, activation) of protein kinase in the MAPK-signaling pathway is enhanced. (2) We introduced the cDNA of the *ACBD5-RET* fusion gene into *NIH3T3* cells, injected the resultant *NIH3T3* cells subcutaneously into nude mice, and examined tumor formation to evaluate the tumorigenicity of the *ACBD5-RET* fusion gene. ***Results*** (1) We determined that the *ACBD5-RET* fusion gene is formed via inverse binding between a break point in the *tyrosine kinase* domain of the *RET* gene located in the long arm of chromosome 10 and the 5' end of the *ACBD5* gene located in the short arm of the same chromosome (pericentric inversion). (2) Accelerated phosphorylation of protein kinase was observed in the *NIH3T3* cells with an *ACBD5-RET* fusion gene. (3) After injection into the nude mice, the *NIH3T3* cells with an *ACBD5-RET* fusion gene continued proliferating and formed tumors. This study showed that the *ACBD5-RET* fusion gene found in the PTC of an A-bomb survivor is significantly involved in papillary thyroid carcinogenesis, as is the case with other *RET* fusion genes such as *CCDC6-RET* and *NCOA4-RET*. *RET* gene rearrangement induced either directly or indirectly by radiation is therefore suggested to be closely involved in papillary thyroid carcinogenesis among PTC patients exposed to relatively high doses of radiation.

Other Journal Publications

♦ Hamatani K: Molecular oncology study using preserved solid cancer tissue specimens developing in A-bomb survivors—With a focus on papillary thyroid cancer. Nagasaki Igakkai Zasshi [Nagasaki Med J] 2014 (September); 89(Special issue):195–201. (Proceedings of the 55th Late A-bomb Effects Research Meeting, 2014) (Japanese)

♦ Ito R, Hamatani K, Yano S, Shinohara T, Takahashi K, Yasui W, Nakachi K, Kusunoki Y: Microsatellite instability (MSI) and MLH1 alteration in colorectal cancer among A-bomb survivors. Hiroshima Igaku [J Hiroshima Med Assoc] 2014 (April); 67(4):356–60. (Proceedings of the 54th Late A-bomb Effects Research Meeting, 2013) (Japanese)

♦ Taga M, Mukai M, Koyama K, Ito R, Misumi M, Nakachi K, Kusunoki Y, Yasui W, Hamatani K: Analysis of *ALK* gene rearrangements in lung adenocarcinomas of atomic-bomb survivors—A pilot study. Hiroshima Igaku [J Hiroshima Med Assoc] 2014 (April); 67(4):361–4. (Proceedings of the 54th Late A-bomb Effects Research Meeting, 2013) (Japanese)

Cell Biology Study Oral Presentations

❖ Ito R, Hamatani K, Yano S, Shinohara T, Takahashi K, Oue N, Yasui W, Nakachi K, Kusunoki Y. Characteristics of high microsatellite instability (MSI-H) cases in colorectal cancers among A-bomb survivors. 103rd Annual Meeting of the Japanese Society of Pathology, 24–26 April 2014, Hiroshima

❖ Hamatani K. Molecular oncology study using preserved solid cancer tissue specimens developing in A-bomb survivors: With a focus on papillary thyroid cancer. 55th Late A-bomb Effects Research Meeting, 1 June 2014, Nagasaki

❖ Takahashi N, Murakami H, Ohishi W, Misumi M, Kusunoki Y, Nagamachi A, Inaba T, Oghiso Y, Tanaka S, Tanaka I, Niwa Y. Study of radiation related circulatory diseases using an animal model: Introducing spontaneous hypertensive rat (SHR) and stroke prone SHR (SHRSP) as animal models. 60th Annual Meeting of the Radiation Research Society, 21–24 September 2014, Las Vegas, Nevada, USA

❖ Hamatani K, Taga M, Kusunoki Y. *EML4-ALK* fusion gene induction in immortalized human thyroid epithelial cells following *in vitro* X-ray irradiation. 73rd Annual Meeting of the Japanese Cancer Association, 25–27 September 2014, Yokohama

❖ Taga M, Hamatani K, Ito R, Misumi M, Kusunoki Y. Analysis of *ALK* fusion genes in lung adenocarcinoma among atomic-bomb survivors. 57th Annual Meeting of the Japan Radiation Research Society, 1–3 October 2014, Kagoshima

❖ Takahashi N, Murakami H, Ohishi W, Misumi M, Kusunoki Y, Nagamachi A, Inaba T, Oghiso Y, Tanaka S, Tanaka I, Niwa Y. Study of radiation related circulatory diseases using animal models: Appropriateness of spontaneous hypertensive rat (SHR) strains as animal models. 57th Annual Meeting of the Japan Radiation Research Society, 1–3 October 2014, Kagoshima

❖ Niwa Y, Murakami H, Ohishi W, Misumi M, Kusunoki Y, Inaba T, Nagamachi A, Tanaka S, Tanaka I, Takahashi N. Study of radiation related circulatory diseases using animal

models, Part II: Evaluating feasibility of spontaneous hypertensive rat (SHR) as an animal model. 6th International MELODI (Multidisciplinary European Low Dose Initiative) Workshop, 7–9 October 2014, Barcelona, Spain

❖ Hamatani K, Koyama K, Yano S, Taga M, Kusunoki Y. *In vitro* X-ray irradiation induces *ALK* gene rearrangement in human thyroid epithelial cells. 84th Annual Meeting of the American Thyroid Association, 29 October–2 November 2014, Coronado, California, USA

❖ Ito R, Hamatani K, Yano S, Shinohara T, Takahashi K, Oue N, Yasui W, Nakachi K, Kusunoki Y. Characteristics of high microsatellite instability (MSI-H) cases in colorectal cancers among A-bomb survivors. 60th Autumn Annual Meeting of the Japanese Society for Pathology, 20–21 November 2014, Urazoe

❖ Kusunoki Y. Radiobiology studies using biosamples from A-bomb survivors—Immunological analyses and molecular oncology studies. Epidemiology Conference for Emergency Workers of Tokyo Electric Power's Fukushima I Nuclear Power Plant, 11 March 2015, Kitakyushu (related to *Immunology Studies*)

Research Protocols 2-13, 4-11, 1-10, 5-85 and 1-01, P2-14

Biochemical Genetics Studies

RP 2-13 Estimation of genetic risk of radiation on mature oocytes of mice by using next generation sequencer

Satoh Y (G), Furukawa K (S), Cullings HM (S), Nakamura N, Nishimura M, Shimada Y, Asakawa J (G)

Objectives: The purpose of this study is to examine the mutation induction rate following 4-Gy gamma irradiation in mature mouse oocytes by whole genome sequencing.

Background and Significance: Whole genome sequencing provides more detailed information on the genome compared to previous techniques used in the Department of Genetics. In this study, using a next generation sequencer we are conducting whole-genome sequencing of mouse parents and their offspring born following 4-Gy irradiation of mature oocytes. We are determining the mutation frequency and spectrum of small deletions/insertions (indels) that are not detectable by previous techniques. We also evaluate the feasibility of human studies using this method.

Study Methods: A total of eight genomic DNA (the parents and three F_1 mice born before and three F_1 mice born after irradiation of the mother) were sequenced with a next generation sequencer. The data were analyzed using a super computer at the National Institute of Genetics, Japan in collaboration with Dr. Sese, National institute of Advanced Industrial Science and Technology, Japan.

Study Progress: We identified about 6 million SNPs/indels per mouse. We detected a total of 72 *de novo* base substitution mutation candidates using stringent filtering conditions for detection of mutations. Thirty-one mutations in the three F1 mice born after irradiation and 16 in the three F1 mice born before irradiation were found to be true mutations by Sanger-sequencing validation.

Results and Conclusions: Not yet obtained.

RP 4-11 Genetic study of atomic bomb radiation by using HD-microarray CGH analysis

Asakawa J (G), Kodaira M (G), Satoh Y (G), Furukawa K (S), Nakamura N

Objectives: The purpose of this proposed study is to examine whether parental exposure to A-bomb radiation has induced deletion/amplification mutations in the genomes of the children.

Background and Significance: The heritable genetic effects of A-bomb radiation (trans-generational effect) have not been fully elucidated due to the low rates of induction of both spontaneous mutations and radiation-induced mutations. We have established a comparative genome hybridization (CGH) approach as a reliable mutation screening method by improving repeatability, resolution, accuracy, and efficiency. Our improved CGH approach can now detect from small (3–5 kb) to large (~10 Mb) deletions with high accuracy. Since radiation-induced mutations are primarily deletions initiated with DNA double-strand breaks, we are conducting a CGH study using high-density microarrays.

Study Methods: We estimated the mutation induction rate

by examining 688 DNA samples from both parents of 184 families (exposure is restricted to either parent) and their 320 offspring (160 from paternally and 160 from maternally exposed families) using high-density microarrays with 1.4 million probes. We conducted the CGH experiments two times (the same set of DNA samples were labeled with Cy3 or Cy5 fluorescent dye in a reversed combination).

Study Progress: We have finished the CGH experiments on the 688 DNA samples. We have finished most of the CGH data and established a database of polymorphic copy number variants among 300 Japanese (parents only). We have identified about 40 mutation candidates and have been conducting PCR-based molecular validation on these candidates. Currently, we identified 10 deletion mutations and 4 amplification mutations among 220 offspring.

Results and Conclusions: Not yet obtained.

RP 1-10 Estimation of genetic effects of radiation in male germ cells of mice: Study for assessment of a high-density microarray CGH platform

Asakawa J (G), Kodaira M (G), Shimada Y, Cullings HM (S), Nakamura N

Objectives: The purpose of the study is, as an animal model of human male exposure, to estimate the mutation induction rate following 4 Gy of gamma irradiation of mouse spermatogonia, and to molecularly characterize the mutations.

Background and Significance: This study will provide crucial information necessary for planning future genetic studies. Interpretation of the results will be important in order to determine the feasibility of using this approach to examine the DNA obtained (e.g., whether or not duplications occur as frequently as deletions and whether both are of equal importance) from the offspring of Hiroshima and Nagasaki survivors whose radiation doses are much smaller than those used in animal studies.

Study Methods: We estimated the mutation induction rate by examining DNA samples of 100 F_1 mice derived from the spermatogonia of male mice irradiated with 4 Gy of γ rays and 100 F_1 mice in the control group by an HD-array CGH technique.

Study Progress: A total of 20 mutations, 10 in the exposed group (8 deletions in 7 mice, 2 amplifications in 2 mice) and 10 mutations in the controls (6 deletions in 6 mice, 4 amplifications in 3 mice, i.e., 1 mouse had 2 amplifications) were detected. We molecularly characterized all the mutations, including junction sequencing and identification of parental origin by SNP analyses. Among the 8 deletion mutations in the exposed group, 6 were paternally derived mutations occurring on the exposed paternal alleles. In contrast, among the 6 deletion mutations in the controls only one was paternally derived, with the remaining 5 being maternally derived mutations.

Results and Conclusions: The results imply that the mean response of a genome to transgenerational effect of radiation is far lower than expected when compared with the mean response at specific loci in mice (Russell 7-locus data).

RP 5-85 Culture of lymphoblastoid cell lines as sources of biological samples for investigation of genetic effects of radiation on children of atomic bomb survivors

RP 1-01 The acquisition of signed informed consent forms from the donors (or their proxies) for whom permanent cell lines have been established (Addendum to RP 5-85)

Satoh Y (G), Takahashi N (RC), Ohishi W (CH), Katayama H (IT), Hida A (CN)

Objectives: To archive untreated cells and to establish lymphoblastoid cell lines by transformation of B-cells from members (parents and children) of families as biological resources for current and future genetic studies, and to obtain written informed consent from them.

Background and Significance: Blood cells have been obtained from 908 parent-child "trios" (including 1,500 children) and lyphoblastoid cell lines have been established. Moreover, as the new F_1 clinical follow-up study started in November 2010, we have started to re-collect blood samples from participants in order to increase the numbers of untreated cells, which will be useful in the future for analyses with newly developed technologies.

Study Methods: An aliquot of lymphocytes separated from the donated blood was transformed by Epstein-Barr virus to establish cell lines while the remaining lymphocytes and poly-nuclear cells were cryo-preserved without culturing. In

Table. Total number of children with informed consent and cell lines

Father's dose (Gy)	Mother's dose (Gy)							
	>2.00[a]	1.50–1.99	1.00–1.49	0.50–0.99	0.01–0.49	0&Null[c]	Unknown[d]	Total
>2.00	2(2[b])	3		1	4(3)	67(22)	1	78(27)
1.50–1.99					3	44(15)	2	49(15)
1.00–1.49		1	7	5(5)	17(1)	96(34)	3	129(40)
0.50–0.99		2(1)	1(1)	13(4)	8(1)	132(50)	4	160(57)
0.01–0.49	4(1)	2	2	11	40(10)	115(44)	5(5)	179(60)
0&Null[c]	27(5)	44(10)	117(36)	294(85)	137(57)	654(134)	51	1,324(327)
Unknown[d]	2			7(3)	4(2)	39(2)		52(7)
Total	35(8)	52(11)	127(37)	331(97)	213(74)	1,147(301)	66(5)	1,971(533)

[a] The doses shown in the table are rounded to the second decimal place.

[b] The numbers in parentheses are the number of children whose blood samples have been re-collected.

[c] The "null" individuals were spouses of the atomic-bomb survivors who were not in city or more than 10,000 m from the bomb and were not included in the LSS cohort.

[d] Distances from the hypocenter were known, but shielding conditions were unknown for individuals in this column.

the new F_1 clinical study, additional uncultured lymphocytes and poly-nuclear cells will be cryo-preserved.

Study Progress: We collected new blood samples from 102 individuals this year.

Results and Conclusions: In the course of this project, we have established cell lines from 4,374 individuals based on their informed consent. The total number of children who gave us informed consent and whose cell lines were established is 1,971, as summarized in the Table in relation to parental dose. We have re-collected 533 specimens in the new F_1 clinical study. The numbers of re-collected blood samples are summarized inside the parentheses of the Table.

RP-P2-14 Whole exome sequencing based genetic study of atomic-bomb radiation by using next generation sequencer: A small-scale mouse study to establish experimental methods and analysis pipeline of exome sequencing

Asakawa J, Cullings HM (S), Fujimoto A, Misumi M (S)

Biochemical Genetics Studies Publications

RERF Report (RR)

♦ Asakawa J, Kamiguchi Y, Kamiya K, Nakamura N: Mutagenic effects of ionizing radiation on immature rat oocytes. Radiat Res 2014 (October); 182(4):430–4. **(RR 6-14)**

[Study Findings] This study, using rats, examined deletion mutations, which serve as a marker for determining genetic effects of radiation. We screened more than one million DNA fragments from the offspring (F_1) of female rats whose immature oocytes had received 2.5 Gy of radiation and a similar number from the offspring of non-irradiated controls. We did not find any deletion mutations that were derived from the irradiated female rats. These results suggest that immature oocytes are less sensitive than mature oocytes to mutation induction.

[Explanation] Risk assessment of genetic effects of radiation in humans is mainly based on the results of mouse experiments. In male mice, much data are available after radiation exposure of their spermatogonial cells. In female mice, the immature oocytes, which are the analogous female target of study, are highly sensitive to radiation and die in a process called apoptosis, leaving the F_1 offspring derived from the surviving immature oocytes essentially mutation-free. In the case of human females, however, such high sensitivity to radiation-induced apoptosis is not observed in immature oocytes. Given that apoptotic death eliminates damaged oocytes in mice and prevents viable offspring with mutations, the possibility exists that mutations may occur at a high frequency in situations where such a clearance mechanism does not function (e.g., in humans). To explain the results of RERF's genetic studies of A-bomb survivors and their offspring (F_1), experimental data from female animals are indispensable, and hence we have searched over many years for appropriate experimental animals that can be used as a model for radiation exposure in human females. Since our investigation into this issue revealed that rat immature oocytes are not as sensitive to apoptosis as mouse immature oocytes and can therefore serve as an animal model, we conducted a genetic effects study on F_1 populations derived from irradiated female rats and nonirradiated female rats utilizing two-dimensional electrophoresis (2DE method). ***Objectives*** To determine the mutation rates induced by radiation in immature oocytes in rats irradiated with 2.5 Gy and to characterize the mutations observed. ***Methods*** (1) Materials: Female Sprague-Dawley (SD) rats were irradiated with 2.5 Gy of gamma radiation. To exclude offspring derived from mature or maturing oocytes at the time of irradiation, irradiated females were mated with nonirradiated Brown Norway (BN) males starting at 80 days following irradiation. These F_1 rats comprised the irradiated group, while the F_1 rats born to non-irradiated pairs were considered controls. At three weeks of age, the spleen, kidney, and liver were collected from the F_1 rats, quickly frozen with liquid nitrogen, and stored at –80°C. (2) Two-dimensional electrophoresis: DNA isolated from the spleen of F_1 rats was digested with *Not*I and *Eco*RV restriction enzymes, and the breakpoints were isotope labeled. Two types of gels containing *Not*I-*Eco*RV fragments of 1–4 kb and 4–10 kb were prepared. We conducted two-dimensional electrophoresis of these gels, dried them, and visualized the DNA fragments on X-ray film by autoradiography. Computer-based analysis of the obtained electrophoresis images was conducted to detect aberrant fragments that would indicate a deletion mutation. ***Results*** (1) Selection of DNA fragments to be examined: Detection of a total of about 3,000 DNA fragments (spots) is possible with use of the aforementioned two types of autoradiograms. From among the spots, 162 from female SD rats, 179 from male BN rats, and 1,387 shared by both SD and BN strains were found to be suitable for mutation screening (equivalent to testing of 1,549 and 1,566 gene loci, respectively, for SD and BN rats). With regard to a total of about 3,000 images obtained from 750 rats each in the F_1 group (which was derived from 2.5 Gy-irradiated female rats) and the control group, we examined DNA fragments by means of computer image analysis. Radiation emitted from the isotope contained in DNA fragments hitting silver particles turned them black on the film, and hence we determined as possible mutation cases those that had lost spot intensity by one-half as well as cases that had lost DNA fragments unique to SD or BN. (2) Detected mutations: About 1.13 million spots were screened in each of the irradiated group and the control group. As a result, a total of 50 germ cell mutations, 18 and 32 mutations in the irradiated group and control group, respectively, were detected. A majority of the 50 mutations were caused by changes in number of repeats of 2–8 core sequences that constitute genetically unstable loci called microsatellites, which our previous research suggest were not caused by radiation. A total of three mutations, two in the control group and one in the irradiated group, were base-change mutations at restriction enzyme recognition sites. This type of mutation is also less sensitive to radiation, and hence radiation exposure is unlikely to have been involved.

With regard to deletion mutations, which serve as a marker for determining genetic effects of radiation, we detected one and three such mutations, respectively, in the control group and the 2.5 Gy-irradiated group, none

of which could be established as being derived from the irradiated females (two of the three mutations in the irradiated group were derived from males, and the parental origin of the remaining mutation could not be determined). The aforementioned results suggest that the paucity of mutations in "mouse" immature oocytes is not attributable to apoptotic elimination of radiation-damaged cells, because rat immature oocytes that are more resistant did not indicate a higher radiation sensitivity for mutation induction. Therefore, immature oocytes may be refractory (in other words, resistant) by nature to mutation induction. Due to the limited number of mutations observed, however, we plan to conduct research in the future using a different method that would enable screening of a larger number of genomic sites.

Biochemical Genetics Studies Oral Presentations

❖ Asakawa J, Kamiguchi Y, Kamiya K, Nakamura N. Mutagenic effects of ionizing radiation on immature rat oocytes. 39th Annual Meeting of the Chugoku Area Radiation Research Society, 18 July 2014, Hiroshima

❖ Satoh Y. Whole genome sequencing of F1 mice born after irradiation to female mouse. Expanded Group Meeting, 2014, Scientific Support Programs for Genome Science, 20–21 August 2014, Kobe

❖ Miura A, Tsuji T, Imanaka M, Kitamura J, Kaneko J, Nakamoto Y, Nishikori E, Satoh Y, Furukawa K, Kodaira M, Nakamura N, Asakawa J. Genetic study of atomic bomb radiation by using HD-microarray CGH analysis. 57th Annual Meeting of the Japan Radiation Research Society, 1–3 October 2014, Kagoshima

❖ Satoh Y, Sese J, Nishimura M, Shimada Y, Nakamura N, Asakawa J. Estimation of genetic effects of radiation on mature oocytes of mice by using whole genome sequencing. 57th Annual Meeting of the Japan Radiation Research Society, 1–3 October 2014, Kagoshima

Research Protocols 6-11, 6-09, 1-08, 6-00, 8-93, A4-09, A2-09

Cytogenetics Studies

RP 6-11 A study of chromosome aberration frequency in thyroid cells following fetal exposure to ionizing radiation in mice

Hamasaki K (G), Noda A (G), Nakamura N, Landes RD (S), Kodama Y (BC/G)

Objectives: Aims of this study are to determine whether chromosome aberrations induced following *in utero* exposure to radiation persist in mouse thyroid cells and to better understand the tissue-specific characteristics of radiation-induced chromosome aberrations following fetal irradiation.

Background and Significance: Studying the biological effects of radiation in the fetuses is essential for understanding the cancer risk to the fetus. We have previously reported that translocation frequencies following fetal exposure to radiation may differ among different types of tissues; namely, such frequencies are low in lymphoid cells while remaining high in mammary epithelial cells in rats. To understand the mechanisms of the tissue dependency, we are examining thyroid cells of mice following fetal irradiation. The results obtained in this study may help to clarify potential cancer risks in A-bomb survivors who were exposed *in utero*.

Study Methods: To test the hypothesis that chromosome aberration frequency following fetal irradiation may differ by tissue, we are examining translocation frequencies in thyroid cells from three groups of mice: 1) mice irradiated as fetuses; 2) mothers irradiated while pregnant; and 3) non irradiated mice. Also, to evaluate alterations of translocation frequencies during fetal development, pregnant mice are being irradiated at 6.5 days post coitus (dpc) (before organogenesis) or at 15.5 dpc (after organogenesis). Using chromosome slides prepared following primary culture of thyroid epithelial cells, translocation frequencies are being determined and evaluated using 2 color FISH (fluorescence *in situ* hybridization) labels chromosome 1 (green) and 3 (red). More than 500 metaphase cells will be analyzed in each group.

Study Progress: We have obtained the results for radiation-induced translocation frequencies in thyroid cells as a function of time of fetal development. Frequencies following irradiation at 6.5 dpc were very low (3/502, 0.6%) while a significant increase in translocations were observed in mice irradiated at 15.5 dpc (30/1155, 2.6%). However, in spleen lymphocytes, only a few translocations were observed in both 6.5 and 15.5 dpc irradiated mice, as were seen in previous studies.

Results and Conclusions: Observed results suggest that radiation-sensitivity of the stem cells may vary with fetal stage, i.e. before or after organogenesis. In other words, present observations suggest that apparent tissue differences in frequency of translocations in adult mice following fetal irradiation may depend on whether tissue stem cell have entered into their niches at the time of irradiation.

RP 6-09 Evaluation of the nonmelanoma skin cancer risk among heterozygotes bearing a founder mutation allele unique to a Japanese population at *xeroderma pigmentosum group A (XPA)* gene

Hirai Y (G), Nakamura N, Noda A (G), Cullings HM (S), Ozasa K (EH), Yonehara S, Fujihara M, Moriwaki S, Nishigori C, Mabuchi K, Kraemer KH, Land CE, Kodama Y (BC/G)

Objectives: To evaluate the relative risk of developing skin cancers among carriers of an inactive allele of the *XPA* gene.

Background and Significance: The frequency of patients with cancer-prone recessive hereditary disorders, such as xeroderma pigmentosum (XP), is very rare, but carriers (heterozygotes) are not rare. However, there are few data regarding cancer risk in heterozygote carriers, as they are generally difficult to identify. This study will focus on a founder mutation allele of the *XPA* gene, which is an inactive allele known to cause severe disease phenotypes under homozygous conditions, and the mutation heterozygotes in Japanese are fairly common (in about 1% of the population). This high frequency provides a unique advantage in effective screening of such carriers.

Study Methods: We are screening about 1,000 nonmelanoma skin cancer specimens and an additional 500 chromosome slides as controls to estimate the frequency of XPA heterozygotes using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Study Progress: We have screened DNA from additional 150 non-melanoma skin cancers, and found four additional XPA heterozygotes among them.

Results and Conclusions: Thus far, we found five XPA heterozygotes among 682 chromosome slides, and 16 XPA heterozygotes among 1,007 non-melanoma skin cancers.

RP 1-08 Establishment of a recombinant mouse model for assessment of genetic effects of radiation at low doses

Noda A (G), Hidaka M (G), Sekihara K (G), Hirai Y (G), Hamasaki K (G), Kodama Y (BC/G), Cullings HM (S), Landes RD (S), Nakamura N

Objectives: To make novel animal model systems which will allow us to estimate the genetic risk (mutagenic risk) of radiation at low doses. Risks of somatic mutations and genomic instability in various tissues will also be evaluated in irradiated individuals as well as F_1 populations born to irradiated parents.

Background and Significance: Since the genetic risk of radiation is difficult to assess at low doses, we plan to generate new mouse *in vivo* systems to detect and measure radiation-induced mutant frequencies occurring in germ cells, as well as in various somatic cells, at low doses. In these genetically engineered mice, mutant cells that are naturally occurring, or radiation-induced, become fluorescent in living tissues. That breakthrough in classical genetics required analysis of millions of F_1 mice. With regards to somatic tissue, mutagenic risk assessment for target organs of radiation carcinogenesis will be validated *in situ*, without destroying tissue architecture.

Study Methods: By applying transgenic/knock-in techniques to mouse embryonic stem (ES) cells, recombinant mice will be generated. We plan to create two kinds of mouse strains. One is a gene reversion system (first-generation knock-in): mice carrying a partial duplication of the *HPRT* gene in conjunction with the *GFP* gene at the 3′ end of the duplicate ($HPRT^{dup}GFP$ mice). In this case, HR-mediated reversion from the duplicate produces HPRT-GFP fusion proteins, thereby making the mutant cells fluorescent. The other is a gene forward mutation system (second-generation knock-in): activating mutations of oncogenes, or inactivating mutations of tumor suppressor genes, making the cells GFP positive. Based on previous studies by multiple investigators, we hypothesize that this will lead to the development of tumors. We plan to make such systems using the *ras* or *p53* gene.

Study Progress: Our first generation knock-ins, $HPRT^{dup}GFP$ mice, are now at the 14th generation. In these mice, spontaneously arising and radiation-induced mutant cells (revertants) in the body could be detected clearly by observation of dissected tissues with a fluorescent microscope. We submitted the first paper. However, highly variable inter-individual and tissue specific difference in mutant frequencies in both control and irradiated mice became apparent. Reduction of background mutant frequencies is the next task. As to second-generation knock-in mice, p53-GFP knock-in or transgenic mice were planned to be developed. Both the chimera and transgenic mice have been born, yet the germ line transmission of the transgene has not been confirmed.

Results and Conclusions: We evaluated the HPRT-dup-GFP mice and found that a reduction of backgrounds is important for future large-scale or collaborative study. We may successfully generate p53-GFP knock-in mouse.

RP 6-00 Molecular alterations in early-onset breast and ovarian cancers among atomic bomb survivors

Hirai Y (G), Nakamura N, Cologne JB (S), Mabuchi K, Land CE

Objectives: To test the hypothesis that the high incidence of early-onset breast cancers among A-bomb survivors may be due to the damaging effect of A-bomb radiation on the normal allele of a breast cancer susceptibility gene in women who are heterozygous carriers of germline mutations of the gene.

Background and Significance: Breast cancer is one of the most radiogenic tumors among A-bomb survivors. Further, the risk is particularly high in those who were exposed at ages below 20 and developed breast cancer before age 35 (early onset). We hypothesized that the high risk is due to the heterozygous inheritance of mutated breast cancer susceptibility genes and that radiation exposure had caused loss of the function of the remaining wild-type allele.

Study Methods: We are examining the plausibility of specific single nucleotide polymorphisms (SNPs) that have been suggested as contributing to early-onset breast cancers.

Study Progress: We reexamined TP53 codon72 polymorphism among 25 cases, because we could not obtain clearly defined results from them at the first examination.

Results and Conclusions: None yet. Results will be summarized in 2015.

RP 8-93 Cytogenetic study in the Adult Health Study population by fluorescence *in situ* hybridization (FISH)

Kodama Y (BC/G), Hamasaki K (G), Noda A (G), Kodaira M (G), Kusunoki Y (R), Shimizu Y (EH), Cullings HM (S), Misumi M (S), Nakamura N

Objectives: The purpose of this study is to quantitatively examine radiation-induced chromosome aberrations (mainly translocations) in blood lymphocytes of atomic-bomb (A-bomb) survivors in the Adult Health Study (AHS) cohort using the fluorescence *in situ* hybridization (FISH) technique.

Background and Significance: A cytogenetic investigation following ionizing radiation exposure is regarded as the most reliable tool for biological dosimetry. Blood lymphocytes can be considered as a natural, *in situ* dosimeter for the exposed individual. A further advantage is that the chromosome test is free from any recall bias, whereas physical dose reconstruction requires precise information on location and shielding conditions for individual dose estimation that are critical but are often unavailable or subject to recall biases.

Study Methods: We have employed 2-color-FISH for detection of translocations involving chromosomes 1, 2, and 4. As a routine procedure, we score 500 FISH-stained metaphases per sample. All blood samples are coded so that the chromosome analyses are being performed without any knowledge of individual physical doses.

Study Progress: In FY2014, blood samples were examined from 18 survivors (7 from Hiroshima and 11 from Nagasaki). To date, 1,197 survivors in Hiroshima and 728 survivors in Nagasaki have been examined using FISH. Blood sample collections were almost completed.

Results and Conclusions: The dose response from current FISH data showed a wide scattering of individual translocation frequencies, as we observed in the previous solid Giemsa staining study. However, the two sets of data (solid Giemsa and FISH) for the same individuals agreed well with each other.

RP-A4-09 Detection of unrepairable DNA damage (DNA double strand breaks) in cells and tissues post-irradiation

Noda A (G), Hirai Y (G), Hidaka M (G), Sekihara K (G), Landes RD (S), Nakamura N, Kodama Y (BC/G)

Objectives: Since we have seen persistent γH2AX/53BP1 foci in human cells and mouse tissues long after irradiation, we have hypothesized that radiation-induced unrepairable DNA double strand breaks (DSB) are permanently retained in non-apoptotic, non-dividing quiescent cells. We are attempting to find sensitive methods to detect such unrepairable DSB *in vitro* and *in vivo*, and to apply these methods to archival tissues of A-bomb survivors so that retrospective dose estimation can be made.

Background and Significance: Following irradiation of cells *in vitro*, unrepairable DSBs form large foci consisting of repair and stress-signal proteins in the nuclei. We hypothesize that such unrepairable DSBs may remain for years post-irradiation. If we are able to distinctly detect such foci in the tissues long after irradiation (we believe that they remain permanently if the cells are non-dividing and have a very long life span, e.g., neuronal or pancreatic cells), it will help us to estimate radiation doses, as well as to elucidate causative mechanisms of the late effects of radiation in irradiated tissues.

Study Methods: (1) Biochemical identification of the specific components of the repair foci, (2) expression array screenings of the genes specifically expressed in cells bearing unrepairable DSBs, and (3) isolation of monoclonal antibodies that distinctly react with unrepairable DSBs, have been carried out.

Study Progress: Even long after exposure to ionizing radiation (up to one year) we have been able to successfully detect unrepairable DSB-foci in a dose-dependent manner in normal human fibroblasts that were kept under quiescent conditions. We found that the formation of such foci was decreased by treatment with farnesyl-inhibitors, and increased by hypertonic treatment, or SIRT1 activation, suggesting that nuclear architecture and chromatin compaction are involved. Transcriptome and proteome analyses revealed several candidate genes (proteins) that might be specific to the cells. Trials to generate mouse monoclonal antibodies against such proteins have been performed.

Results and Conclusions: Our results indicate that the changes in nuclear architecture may be involved in DSB repair, especially for damage that is difficult to be repaired. Comprehensive screenings to identify the genes (proteins) distinctly localized in unrepairable DSBs are still needed.

RP-A2-09 Comprehensive analysis of radiation-induced genetic damage in human peripheral blood T cells using comparative genomic hybridization (CGH) and cytogenetic techniques

Honma M, Ukai A, Hamasaki K (G), Kodama Y (BC/G), Kusunoki Y (R)

Objectives: Comprehensive analysis of damaged genome regions following radiation exposure in human peripheral blood T-cell populations.

Background and Significance: Ionizing radiation induces various types of large structural alterations in the genome, and some genetic alterations may lead to cell transformation. However, little is known about how large chromosomal regions are altered and what types of genetic alterations preferentially remain in a normal cell following ionizing irradiation with a given dose. This approach may provide a novel way to seek possible mechanisms of somatic gene mutations leading to radiation-related cancers.

Study Methods: To determine structural alterations in the genome following radiation exposure, we have evaluated damaged genome regions in human peripheral blood T-cell populations that were clonally propagated after *in vitro* x-irradiation. By comparing DNA extracted from each irradiated T-cell clone with reference DNA also extracted from unirradiated peripheral blood mononuclear cells (PBMCs), damaged genome regions were identified by a comparative genomic hybridization (CGH) assay using a 244K human genome array that allows comprehensive analysis over the entire genome. G-banding and multi-color chromosome analyses have also been conducted for T-cell clones identified with gene deletions to confirm damaged genome regions at the chromosomal level.

Study Progress: All assays have been completed. One manuscript is in preparation.

Results and Conclusions: Among 33 T-cell clones from peripheral blood mononuclear cells irradiated with 1 Gy of x-rays *in vitro*, 11 (33%) showed structural changes including deletions (14 kb–130 Mb) or amplification (28 kb) in at least one chromosome, with one clone showing trisomy. Molecular and cytogenetic analyses demonstrated that the majority of deletions were simple interstitial ones, and that one clone had a large terminal deletion (130 Mb) accompanying an unbalanced translocation. Fourteen non-irradiated T-cell clones were similarly analyzed: One showed trisomy and the other 13 showed no structural changes. These results indicate that genome damage induced by x-rays in normal human blood cells is usually repaired by non-homologous end-joining, resulting in simple interstitial deletions. Effects of radiation dose and dose rate as well as other DNA-damaging agents can be evaluated using a similar assay system.

Cytogenetics Studies Oral Presentations

❖ Noda A, Hirai Y, Hamasaki K, Kodama Y, Landes RD, Nakamura N. Examination of somatic mutations in CAG-HPRTdupGFP mice. 39th Annual Meeting of the Chugoku Area Radiation Research Society, 18 July 2014, Hiroshima

❖ Noda A. Development of model systems for *in vivo* and *in situ* detecting somatic mutant cells at whole body level. 16th Annual Meeting of Society of Evolutionary Studies, 21–24 August 2014, Osaka

❖ Noda A, Suemori H, Hirai Y, Hamasaki K, Kodama Y, Mitani H, Landes RD, Nakamura N. Evaluation of HPRT-dup-GFP mice for *in vivo* and *in situ* somatic mutation analysis. 45th Annual Meeting of Environmental Mutagenesis and Genomics Society, 13–17 September 2014, Orlando, Florida, USA

❖ Hamasaki K, Noda A, Nakamura N, Kodama Y. Relationship between irradiation time and chromosome aberration frequency in adult spleen and thyroid cells following fetal exposure to radiation. 57th Annual Meeting of the Japan Radiation Research Society, 1–3 October 2014, Kagoshima

❖ Hidaka M, Noda A, Kodama Y. Activation of SIRT1 increases the number of unrepairable DSBs induced by radiation exposure. 57th Annual Meeting of the Japan Radiation Research Society, 1–3 October 2014, Kagoshima

❖ Noda A, Hirai Y, Hamasaki K, Nakamura N, Kodama Y. Biology of radiation-induced unrepairable DSBs. 57th Annual Meeting of the Japan Radiation Research Society, 1–3 October 2014, Kagoshima

❖ Noda A, Suemori H, Hirai Y, Hamasaki K, Kodama Y, Mitani H, Landes RD, Nakamura N. New approaches to develop *in vivo* model systems that detect somatic and germ line mutations (report IV). 57th Annual Meeting of the Japan Radiation Research Society, 1–3 October 2014, Kagoshima

❖ Sekihara K, Sakuma T, Yamamoto T, Noda A. New approaches to generate p53-GFP knock-in mice. 57th Annual Meeting of the Japan Radiation Research Society, 1–3 October 2014, Kagoshima

❖ Sekihara K, Sakuma T, Yamamoto T, Noda A. New approaches to generate p53-GFP knock-in mice. Radiation Biology Center, Kyoto University "Exchange Meeting with Students and Overseas PI," 18–19 February 2015, Kyoto

Research Protocol 4-75 (Platform Protocol)
F_1 Studies—Genetic Effects of Atomic Radiation on Children Born to Bombing Survivors

RP 4-75 Research plan for RERF studies of the potential genetic effects of atomic radiation; Hiroshima and Nagasaki. Part 1. Mortality and cancer incidence study of children born to atomic-bomb survivors

Grant EJ (EH), Furukawa K (S), Sakata R (EH), Cullings H (S), Shimizu Y, Ozasa K (EH), Kodama K (CS), Cologne JB (S)

Objectives: To determine whether parental radiation exposure affects mortality and/or cancer incidence rates in their offspring.

Background and Significance: Animal studies have reported effects of radiation on mutation rates in the F_1 generation in accordance with the genetic hypothesis. This population provides one of the very few opportunities in the world to study the heritable genetic risks in humans into adulthood from exposure to ionizing radiation. As population exposures to medical radiation procedures increase before and during childbearing years, the public health implications of the results from this study continue to be timely and important.

Study Methods: The F_1 mortality cohort target sample consisted of 76,814 subjects. They were selected from the children born from May 1946 through December 1984 to parents with a variety of A-bomb radiation exposures, ranging from those known not to be in the city at the time of the bombing to those who were heavily exposed to acute radiation. The average age of the cohort as of 2008 was 53 years; 91% of the cohort was still alive as of 2009.

Study Progress: A previous manuscript was re-designed to include additional analyses and has been resubmitted for internal RERF review.

Results and Conclusions: To date, we have found no significantly increased rates of noncancer or cancer mortality, or cancer incidence associated with paternal or maternal dose.

F_1 Studies Publications

Journal Publication

♦ Tatsukawa Y, Cologne JB, Yamada M, Ohishi W, Hida A, Furukawa K, Takahashi N, Nakamura N, Ozasa K, Akahoshi M, Fujiwara S, Shore RE: Association between parental radiation exposure and prevalence of multifactorial diseases: F_1 Clinical Health Study (2nd Report). Hiroshima Igaku [J Hiroshima Med Assoc] 2014 (April); 67(4):375–8. (Proceedings of the 54th Late A-bomb Effects Research Meeting, 2013) (Japanese) (related to *F_1 Clinical Study [FOCS]*)

F_1 Studies Oral Presentations

❖ Kodama K. RERF's biosample resources—Issues need to be resolved for future use. 60th Annual Meeting of the Radiation Research Society, 21–24 September 2014, Las Vegas, Nevada, USA (related to *Adult Health Study* and *Life Span Study*)

❖ Ozasa K. Long-term health effects of the atomic-bomb radiation. Epidemiology Conference for Emergency Workers of Tokyo Electric Power's Fukushima I Nuclear Power Plant, 11 March 2015, Kitakyushu (related to *Life Span Study*)

Research Protocols 7-11, 2-09, 1-09, 5-08 and 6-10, 4-07, 1-06, 2-04, 1-04, 6-02, 3-94, 9-88, 29-60, A2-14, A5-12, A5-10, A3-10, A12-08,

Special Cancer Studies

RP 7-11 Preservation of fresh thyroid samples obtained from Adult Health Study participants (Addendum to RP 2-86)

Imaizumi M (CN), Ohishi W (CH), Sera N, Hida A (CN), Yamada M (CH), Hamatani K (R), Ozasa K (EH), Akahoshi M

Objectives: To preserve fresh thyroid samples removed from thyroid tumor cases detected among AHS subjects for future genetic and mechanistic studies on thyroid radiation carcinogenesis.

Background and Significance: Thyroid cancer shows an increased incidence with radiation dose among A-bomb survivors. However, the mechanisms of radiation induction of thyroid cancer are not fully understood. Collection of as many thyroid cancer samples as possible could contribute to the mechanistic elucidation of thyroid cancer development and radiation carcinogenesis. Furthermore, since significant dose response is observed even for benign thyroid tumors, it is also important to compare cancer and benign tumors at the molecular level for elucidation of carcinogenic mechanisms. In accordance with an earlier research protocol, RP 2-86, RERF has worked on the collection and cryopreservation of fresh thyroid cancer tissues from A-bomb survivors and non-exposed controls. Thyroid ultrasound examination has been performed in AHS health examinations since 2000, thereby leading to the detection of a large number of thyroid nodules. Due to the rapid decrease in number of A-bomb survivors, it is important to systematically collect as many thyroid samples as possible, both benign and malignant thyroid tumors, from AHS subjects. The present RP, an addendum to the aforementioned RP 2-86, aims at the systematic collection of fresh tissue from both benign and malignant thyroid tumors removed from AHS subjects.

Study Methods: When we are notified that thyroid surgery is to be performed on an AHS subject, we obtain advance consent from the donor subject and visit the hospital to obtain the fresh sample. One to two pieces of the sample are rapidly frozen in liquid nitrogen.

Study Progress: We are continuing sample collection. We have obtained fresh samples from a total of 32 thyroid tumor cases (including samples collected based on RP 2-86).

Results and Conclusions: None. This RP is only for collection of frozen fresh samples from thyroid tumor cases detected among AHS subjects in preparation for future molecular research. Research protocols using the samples will be prepared separately in the future.

RP 2-09 Study on secondary cancer risks after radiotherapy among A-bomb survivors

Yoshinaga S, Soda M (EN), Akahane K, Doi K, Moriwaki H (EH), Hida A (CN), Yamada M (CH), Katayama H (IT), Shimada Y, Fujiwara S, Kasagi F, Sugiyama (EH), Sadakane A (EH), Ozasa K (EH)

Objectives: To evaluate the combined effects of A-bomb radiation and subsequent therapeutic radiation.

Background and Significance: Very limited information is available from epidemiological studies on the effects of exposure to multiple radiation sources. The RP focuses on secondary cancer risk of a subset from the LSS who had undergone radiotherapy subsequent to A-bomb radiation.

Study Methods: The subjects include 1,501 A-bomb survivors who were confirmed to have undergone radiotherapy and whose organ or tissue doses from that radiotherapy will be approximated. Secondary cancer risks from radiotherapy doses will be calculated adjusting for A-bomb radiation dose and also modifying A-bomb radiation effects.

Study Progress: Analysis is ongoing at the National Institute of Radiological Sciences (NIRS). NIRS scientists periodically visit RERF to discuss analysis and interim results.

Results and Conclusions: None yet.

RP 1-09 A nested case-control study of factors contributing to acceleration of the development of hepatocellular carcinoma using stored sera (Addendum to RP 1-04)

Ohishi W (CH), Cologne JB (S), Fujiwara S, Ueda K (CH), Mitsui F (CH), Hida A (CN), Niwa Y (R), Sakata R (EH), Ozasa K (EH), Tsuge M, Chayama K

Objectives: The hypothesis behind this study is that chronic inflammation and/or insulin resistance may be involved in increased risk of radiation-related hepatocellular carcinoma (HCC). The objective of this study is to examine the contribution of inflammation and/or insulin resistance to HCC risk, taking into account radiation exposure, hepatitis virus infection, lifestyle-related factors, and severity of liver fibrosis.

Background and Significance: This research protocol is an addendum to RP 1-04. In the previous study, we demonstrated that hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, obesity, and alcohol consumption are independent risk factors for HCC. The combination of HCV infection and increased body mass index (BMI) exerted a synergistic effect on risk of HCC.

Study Methods: We have measured blood cytokine levels related to chronic inflammation and/or insulin resistance that are considered important factors contributing to acceleration of the development of HCC using stored sera before HCC diagnosis among subjects of this nested case-control study (RP 1-04), and examined their contribution to HCC risk.

Study Progress: We have worked on a statistical model for the biomarkers of chronic inflammation, insulin resistance, and liver fibrosis such as CRP, TNF-α, IL-6, adiponectin, leptin, resistin, type 4 collagen, and platelet count.

Results and Conclusions: Higher serum levels of IL-6 were associated with increased HCC risk, independently of hepatitis virus infection, lifestyle-related factors, and radiation exposure. Interaction of obesity with IL-6 levels in relation to HCC risk was significant, but that of radiation with IL-6 levels was not observed (*Int J Cancer* 2014; 134:154–63).

RP 5-08 Breast cancer incidence among atomic-bomb survivors, 1950–2005
RP 6-10 Intrinsic subtypes of breast cancer among atomic-bomb survivors (Addendum to RP 5-08)

Yonehara S, Nishisaka T, Nakashima M, Furukawa K (S), Soda M (EN), Sekine I, Mabuchi K, Preston DL, Sadakane A (EH), Ozasa K (EH), Kodama K (CS)

Objectives: To clarify the relationship between radiation and breast cancer incidence based on histologically reviewed diagnoses according to current WHO classification criteria, and to clarify the immunohistochemical (IHC) characteristics of radiation-induced breast cancer. This study updates the past study by adding cases diagnosed during the period 1991–2005 and will provide more precise estimates of radiation effects.

Background and Significance: Breast cancer shows one of the highest associations with radiation, but no differences in the histological distributions were observed in the past between exposed and control cases. In recent years, research has identified important differences in the risk factors, pathogenesis and prognosis for subtypes of breast adenocarcinomas defined by their estrogen and progesterone receptor and Her2 status. It therefore is important to determine whether radiation exposure bears different associations with the various breast cancer subtypes. Histological reviews according to a new histological classification system along with IHC subtyping will enable us to determine whether radiation risk differs according to histological or IHC subtypes of breast cancer.

Study Methods: Potential breast cancer cases diagnosed during 1950–2005 collected from cancer registries and other available sources will be histologically reviewed by pathologists (RP 5-08). Among histologically confirmed breast cancer cases, suitable samples will undergo IHC staining to determine intrinsic subtypes of breast cancer according to presence or absence of estrogen and progesterone receptors (ER/PR) and Her2 (RP 6-10).

Study Progress: The pathology panel has completed the review and confirmed histological diagnosis of all cases based on the WHO Classification of Tumors of the Breast, Fourth Edition (2013). Histological grade, which has been reported to be partly related to gene expression patterns, is being evaluated. The IHC staining has been completed for all 1,362 suitable cases among the histologically confirmed breast cancer cases. Evaluation of intrinsic subtypes will be completed by early 2015.

Results and Conclusions: We identified 1,615 histologically confirmed female breast cancer cases. Among these cases, 81% were invasive ductal carcinoma, 2% invasive lobular carcinoma, 16% other epithelial tumors and 1% non-epithelial tumors. Preliminary analysis indicated that there was no difference in distribution of histological types between the levels of radiation exposure.

RP 4-07 Pathology study of malignant tumors of soft tissue and bone among A-bomb survivors, 1957–2003

Brenner A, Berrington de Gonzalez A, Mabuchi K, Preston DL, Sadakane A (EH), Ozasa K (EH), Kodama K (CS)

Objectives: To clarify the relationship between radiation and the incidence of soft tissue and bone tumors based on histologically reviewed diagnoses, and to clarify the histological characteristics of radiation-induced soft tissue and bone tumors.

Background and Significance: Sarcomas of the soft tissues and bones are known to be associated with high therapeutic doses of radiation. The broad classification of "sarcoma" showed a suggestive association with radiation in the recent LSS tumor incidence report. This study will provide more definitive evidence regarding the effects of radiation on histologically reviewed soft tissues and bone tumors.

Study Methods: Potential cases of soft tissue and bone tumors collected from cancer registries and other available sources will be histologically reviewed by pathologists.

Study Progress: A pathologist panel histologically confirmed a total of 91 cases with malignant tumor of soft tissue and bone, and another 35 probable cases were included based on their medical records with histological examinations. Data analysis is being conducted in collaboration with the U.S. NCI.

Results and Conclusions: Among the total of 126 eligible cases, 89 were soft tissue and 37 were bone tumors. Soft tissue tumors consisted of 20 fibroblastic/myofibroblastic tumors, 18 so-called fibrohistiocytic tumors, 10 adipocytic tumors, and 41 other miscellaneous tumors. Bone tumors consisted of 17 osteosarcomas, 4 chondrosarcomas and 16 other miscellaneous tumors. In a case-only analysis, there was no apparent association of tumor type with radiation dose.

RP 1-06 Study on cancer of the uterus among A-bomb survivors, 1950–2003 (Addendum to RP 8-85)

Fujihara M, Matsuo T, Nishisaka T, Nakajima H, Hirai Y (G), Soda M (EN), Sekine I, Preston DL, Mabuchi K, Sadakane A (EH), Ozasa K (EH), Kodama K (CS)

Objectives: To clarify the relationship between radiation and uterine cancer incidence based on histologically reviewed diagnoses, and to clarify the histological characteristics of uterine cervix and corpus cancer associated with radiation.

Background and Significance: In the recent cancer incidence analyses, there was some suggestion that radiation may be associated with uterine corpus cancer, especially in subjects exposed at young ages, but no association was seen for cervical cancer. Other factors in the pathogenesis of cervical and corpus cancers include HPV infection and estrogens, respectively.

Study Methods: Uterine corpus cancer is given priority in this study. Potential corpus cancer cases collected from cancer registries and other available sources will be histologically reviewed by pathologists.

Study Progress: The first screening of case information sheets for a total of 1,592 possible cases of uterine corpus cancer has been completed and 381 cases were accepted for histological review. We are reorganizing the methods of review and the study period.

Results and Conclusions: None yet.

RP 2-04 A case-control study of atrophic gastritis and gastric cancer using frozen sera and genomic DNA: Identification of new biomarkers for chronic gastritis associated with gastric cancer

Ohishi W (CH), Ueda K (CH), Cullings HM (S), Fujiwara S, Hayashi T (R), Mitsui F (CH), Hida A (CN), Ozasa K (EH), Tahara E

Objectives: To determine whether the radiation exposure-dependent gastric cancer risk seen in the atomic bomb survivors is related to chronic tissue inflammation associated with H. pylori infection.

Background and Significance: Three major classes of factors—environmental (diet, smoking), host (age, H. pylori infection), and genetic—jointly affect the genesis of gastric cancer. We are investigating interactions between radiation exposure and these risk factors in developing gastric cancer to gain new insights into radiation risk susceptibility.

Study Methods: A nested case-control study was performed in the longitudinal cohort of atomic bomb survivors using stored sera obtained before diagnosis. Enrollees included about 300 gastric cancer cases and three controls per case selected from cohort members matched on age, gender, city, time of serum storage, and type of serum storage, and counter-matched on radiation dose to increase statistical power.

Study Progress: A draft manuscript on the joint effects of radiation and chronic atrophic gastritis upon gastric cancer risk is currently in the final stages of revision.

Results and Conclusions: H. pylori infection, chronic atrophic gastritis (CAG), and smoking are all independent predictors of gastric cancer. Higher radiation relative risks were noted with the diffuse type of gastric cancers, after adjusting for these risk factors (*Cancer Epidemiol Biomarkers Prev* 2007; 16:1224–8). The LTA 252 genotype is associated with diffuse-type noncardia gastric cancer, and this genotype was an effect modifier for radiation dose (*Helicobacter* 2009; 14:571–9). In preliminary analysis, radiation exposure was associated with diffuse-type noncardia gastric cancer without CAG, independently of H. pylori infection and smoking.

RP 1-04 A nested case-control study of hepatocellular carcinoma among atomic-bomb survivors using stored sera

Ohishi W (CH), Fujiwara S, Cologne JB (S), Ueda K (CH), Mitsui F (CH), Hida A (CN), Ozasa K (EH), Chayama K

Objectives: The primary objective of this study is to investigate the relationship between radiation exposure and the risk of hepatocellular carcinoma (HCC) among A-bomb survivors after taking into account hepatitis virus infection.

Background and Significance: An increased risk of liver cancer with radiation dose has been reported based on mortality studies and tumor registries among A-bomb survivors, but hepatitis virus infection status was not taken into account. Other previous studies at RERF provided suggestive evidence of a possible interaction, i.e., they reported supermultiplicative effects between radiation exposure and chronic HCV infection in the etiology of HCC without cirrhosis. These questions are especially important because they may help explain differences in the magnitude of radiation-HCC risk seen in this population compared with western populations where HCV infection is uncommon.

Study Methods: The study included 224 HCC cases and 644 controls that were matched to the cases on gender, age, city, time and method of serum storage, and counter-matched on radiation dose.

Study Progress: Analyses of the joint effects of radiation and intermediate risk factors such as hepatitis virus infection is underway, along with the development of statistical methods.

Results and Conclusions: HBV and HCV infections, obesity, and alcohol consumption of ≥40g of ethanol per day are independent risk factors for HCC (*Cancer Epidemiol Biomar Prev* 2008; 17:846–54). After adjusting for alcohol consumption, smoking habit, and body mass index (BMI), radiation exposure is associated independently with increased HCC risk. In particular, radiation exposure was a significant risk factor for non-B, non-C HCC with no apparent confounding by alcohol consumption, smoking habit, or BMI (*Hepatology* 2011; 53:1237–45).

RP 6-02 A nested case-control study of breast and endometrial cancer in the cohort of Japanese atomic bomb survivors

Ohishi W (CH), Grant EJ (EH), Cologne JB (S), Ozasa K (EH), Sharp GB, Eguchi H, Nakachi K (RC), Nakashima E (S), Izumi S, Key TJ, Stevens RG, Berrington de Gonzalez A

Objectives: To characterize the joint effects of radiation and serum-based indicators of hormonal status, oxidative stress, and phytoestrogen consumption in breast and endometrial cancer development.

Background and Significance: It is not known to what extent established breast-cancer risk factors may be directly or indirectly involved in radiation-induced cancer. Assessing the joint effects of radiation and other factors using causal models may shed light on the mechanisms of radiogenic breast and endometrial cancer etiology.

Study Methods: Serum measurement from 243 breast cancer cases whose blood samples were collected up to 30 years before cancer diagnosis and 486 age-matched controls, selected with counter-matching on radiation dose, were conducted in 2007–2008. Statistical analyses and manuscript preparations are ongoing, addressing a number of specific mechanistic hypotheses.

Study Progress: Manuscripts were published on breast cancer risk related to radiation exposure and ferritin levels and on hormone levels in relation to radiation dose. A manuscript on radiation risk for breast cancer with hormone levels as an effect-modifier or mediating variable was submitted for internal review. Additional analyses and publications are being considered.

Results and Conclusions: Serum levels of estradiol (E2) and testosterone increased with radiation dose in cancer-free postmenopausal women (*Radiat Res* 2011; 176:678–87). The mechanism of the joint effects of ferritin and radiation on postmenopausal breast cancer risk could not be assessed (*Cancer Sci* 2011; 102:2236–40). Radiation exposure, higher levels of post-menopausal E_2, testosterone and progesterone, and classically known reproductive risk factors were all positively associated with postmenopausal breast cancer risk. An estimate of the interaction between radiation and E_2

was large and positive but not statistically significant. Some portion of the risk of radiation exposure appears to be mediated through raised levels of post-menopausal bioavailable estrogen.

RP 3-94 Incidence of lymphoid malignancies among the atomic-bomb survivors, 1950–90

Fujihara M, Namba K, Tokunaga M, Takahara O, Soda M (EN), Dohy H, Kamada N, Tomonaga M, Preston DL, Mabuchi K, Sakata R (EH), Ozasa K (EH), Kodama K (CS)

Objectives: To clarify the relationship between radiation and lymphoid malignancy incidence based on histologically reviewed diagnoses, and to clarify the histological characteristics of radiation-induced lymphoid malignancies.

Background and Significance: There have been controversial findings on the effects of radiation on lymphoid malignancies. It is necessary to thoroughly analyze the data on lymphoid malignancies in A-bomb survivors through histological reviews based on current standardized diagnostic criteria.

Study Methods: Potential lymphoid malignancies collected from various available sources including cancer registries and others are histologically reviewed by pathologists.

Study Progress: A total of 476 definite and probable cases of malignant lymphomas (ML) from the period 1950–1995 were identified for analyses. A paper on analysis of diagnosed cases is being drafted. An analysis of radiation risks by histological type is being conducted.

Results and Conclusions: Among T-cell lymphomas, mean age at exposure, age at disease onset, and latent period (defined as years from exposure to onset) appeared to be younger or shorter in the cases exposed to high doses of radiation although there were no such tendencies in B-cell lymphoma.

RP 9-88 Guidelines for the conduct of site-specific cancer incidence studies among A-bomb survivors, Hiroshima and Nagasaki

Ozasa K (EH), Sekine I, Soda M (EN), Tokunaga M, Mabuchi K, Cullings HM (S), Kodama K (CS)

Objectives: To establish guidelines for pathological studies on the incidence of specific cancers in the LSS population. Guidelines include specifying basic study designs, methodology, and procedures to maintain uniformity. These guidelines are intended to simplify the preparation of subsequent research plans for site-specific cancer incidence studies and simultaneously provide for uniformity in basic design and operation of the studies.

Background and Significance: Some uncertainties have been detected in the accuracy of diagnoses based on death certificates or tumor and tissue registries in the LSS cohort studies. Standardized pathological reviews are therefore valuable. These guidelines are intended to simplify the preparation of subsequent research plans for site-specific cancer incidence studies and to provide uniformity in the basic design and conduct of studies.

Study Methods: The framework for the site-specific cancer studies includes three major areas: case ascertainment, pathological review, and data analysis.

Study Progress: Research protocols have been written for cancers and/or tumors of the liver (RP 5-90), salivary glands (RP 1-91), skin (RP 2-91, updated to RP 2-02), ovary (RP 2-92), thyroid (RP 6-91), central nervous system (RP 4-92), breast (RP 6-93, updated to RP 5-08, and RP 6-10 for intrinsic subtypes), lung (RP 1-94), lymphoid system (RP 3-94), uterus (RP 1-06), and soft tissue and bone (RP 4-07). Among them, studies of the liver, salivary gland, central nervous system, ovary, skin, thyroid gland, and lung have been completed and others are underway.

Results and Conclusions: Essential methods for site-specific cancer incidence studies with histology reviews were established.

RP 29-60 Detection of leukemia and related disorders

Ozasa K (EH), Soda M (EN), Sugiyama H (EH), Kodama K (CS), Tomonaga M, Kimura A, Kamada N, Dohy H, Miyazaki Y, Cologne JB (S)

Objectives: The purpose of the study is to determine the incidence and risks of leukemia and related hematological disorders in the radiation-exposed persons of Hiroshima and Nagasaki. Primary questions are those concerning the leukemogenic effects of ionizing radiation.

Background and Significance: A significant excess risk of radiation-induced leukemia was observed within five years after the bombings, and the latest data suggest the possibility of a small, continued excess of leukemia and an increased risk of myelodysplastic syndrome (MDS). Leukemia was specifically surveyed in the Leukemia Registry (TR 5-65) by ABCC from 1946 to the early 1990s, and cases have been reclassified using a modern leukemia classification system. Therefore, leukemia has been more completely surveyed than other malignancies. Currently leukemia cases are collected through usual population-based cancer registries.

Study Methods: Incident cases of leukemia and related hematological disorders have been collected through the Leukemia Registry and the population-based cancer registries in both cities.

Study Progress: After a major paper of risk assessment was published in 2013, routine collection of case information has continued.

Results and Conclusion: No results from the updated data.

RP-A2-14 A proposal to join the pooled analysis of premenopausal breast cancer

Sadakane A (EH), Grant EJ (EH), Sugiyama H (EH), Soda M (EH), Sakata R (EH), Ohishi W (CH), Hida A (CN), Ozasa K (EH)

Objectives: To investigate risk factors for premenopausal breast cancers with a focus on time since last birth, body mass index (BMI), and exercise by assembling a large cohort dataset.

Background and Significance: Etiology and risk factors may differ between pre- and post-menopausal breast cancers. However, cohort studies for premenopausal breast cancer are more limited than that for postmenopausal cancers. Pooled analysis of risk factors for premenopausal breast cancer focusing on three specific risk factors—time since last birth, BMI, and exercise, has been proposed by Dr. Swerdlow of the Institute of Cancer Research (ICR), UK

and Dr. Nichols of National Institute of Environmental Health Sciences (NIEHS), USA. RERF will provide data for all analyses. Pooled analysis, in which individual-level data from cohorts are pooled and assembled, enables us to identify stronger and more significant associations, to conduct detailed analyses by subgroups, and to perform full investigations of confounding and effect modification with high statistical power.

Study Methods: Data offered by participating cohorts will be sent to the principal investigators of ICR and NIEHS and harmonized for pooled analyses. Initial analyses will explore risks of premenopausal breast cancer in relation to the three primary risk factors of interest: time since last birth, BMI, and exercise. To avoid any concern of bias due to radiation exposure, only data from subjects exposed to less than 100 mGy were provided to NCI.

Study Progress: Cohorts from 18 populations have been recruited, resulting in over 15,000 premenopausal breast cancer cases among more than one million women aged less than 55 years. Data on possible risk factors and incident cases of premenopausal breast cancer among female LSS subjects with <100 mGy of radiation exposure are being prepared at RERF.

RP-A5-12 A proposal to join the diet and bladder cancer pooling project

Grant EJ (EH), Ozasa K (EH), Ohishi W (CH), Hida A (CN)

Objectives: The Diet and Bladder Cancer Pooling Project (DBCPP) is a collaborative pooling project designed to investigate the association of diet and bladder cancer using data from bladder cancer studies that have been conducted around the world. The Principal Investigator of the project is Dr. Maurice Zeegers of Maastricht University, the Netherlands (formerly of Cambridge University).

Background and Significance: Bladder cancer is the most expensive malignancy to treat from diagnosis until death, ranging 96,000–187,000 U.S. dollars per patient. With 400,000 new patients discovered annually worldwide, it is the 7th most common cancer. As is the case for many tumors, the development of bladder cancer is likely to be influenced by diet. However, the role of diet in bladder cancer could be even more pronounced, given that the bladder is an excretory organ. Previous research suggested that 30% of all bladder cancers might have been prevented by dietary modification. However, it is not yet clear which specific foods or nutrients are involved and therefore which dietary modifications should be recommended to prevent this disease. Efforts towards the prevention of this disease by dietary recommendations could lead directly to a substantial reduction in morbidity, mortality, and healthcare costs. A 30% reduction in bladder cancer in the USA would be equivalent to a decrease of 1.2 billion U.S. dollars in total medical care expenditures per year.

Study Methods: Pooled data from many different studies. Lifestyle data include smoking, alcohol consumption, education level, and food ingestion frequencies. Data will be harmonized as a first step. Later, pooled analyses will be performed.

Study Progress: As of 2011, the DBCPP had recruited 18 studies from the United States of America, Belgium, the Netherlands, Sweden, Italy, Germany, France, the United Kingdom, Hungary, Romania, Slovakia, Spain, Singapore, and China representing more than 30,000 controls and 10,000 bladder cancer cases. RERF data were sent to Dr. Zeegers in October 2012.

Results and Conclusions: Awaiting final results from PI.

RP-A5-10 Methods for assessing joint effects of radiation and intermediate risk factors in nested case-control studies

Cologne J (S), Furukawa K (S), Kim YM (S), Grant E (EH), Ohishi W (CH), Cullings H (S)

Objectives: Extend regression methods for assessing mediation to nested case-control data that allow valid estimation of radiation risk and estimation of the amount of risk attributable to individual causal paths (especially mediation of radiation risk).

Background and Significance: Mediation of radiation risk by disease risk factors causally affected by radiation (e.g., radiation → HBV infection susceptibility → hepatocellular carcinoma; radiation → inflammation → heart disease) is now being explored in the atomic-bomb survivor studies. Regression models for assessing complex causal mechanisms are not immediately applicable to various study designs we use, e.g., the nested case-control study design, used in the RERF studies of liver cancer (RP 1-04), gastric cancer (RP 2-04), and breast cancer (RP 6-02), or the case-cohort study design used in the RERF cancer and immunogenome study (RP 4-04).

Study Methods: We are applying a combination of statistical theory, computer simulation, and comparative analyses using actual data to assess various approaches to fitting causal models to nested case-control data. Methods are being evaluated using cohort data from the AHS follow-up study of hepatitis virus and liver disease (RP 9-92) and by computer simulation.

Study Progress: A manuscript describing joint effects of radiation and serum sex hormones using path analysis in the nested case-control study of breast cancer has been revised following input from collaborators. In addition to a novel permutation test applied to path-analysis results to allow inference about mediation, we also implemented a new bias-corrected bootstrap confidence interval approach developed by Dr. Kim to obtain confidence intervals for mediation proportions. Analysis of AHS liver cancer follow-up data began using the new tumor registry data through the end of 2009 and plans have been formulated to conduct a simulation study of stratified nested case-control sampling in terms of extending cohort methods of mediation analysis to counter-matched, nested case-control data.

Results and Conclusions: We now have evidence of mediation of radiation risk by endogenous sex hormones in the case of breast cancer, based on the permutation test and bootstrap confidence intervals. Whether there is mediation of radiation risk by viral hepatitis in the case of liver cancer remains an open question; we are currently examining potential mediation in the AHS liver cancer follow-up data, and a review of chronic HCV infection status by Dr. Ohishi has provided more precise data on that outcome for assessing whether there is a radiation effect on chronic HCV infection (as there is for chronic HBV).

RP-A3-10 A proposal to join the Asia Cohort Consortium. *Project 1: Tobacco smoking, alcohol drinking, body mass index, and risk of rare cancers*

Grant EJ (EH), Sadakane A (EH), Ozasa K (EH), Ohishi W (CH), Hida A (CN), Shore RE (CR)

Objectives: This study created a framework by which RERF became a member of the Asia Cohort Consortium (ACC), a multi-centered project with a combined cohort of over one million Asians with lifestyle, cancer incidence, and mortality data. In addition, this RP initiated RERF's first collaborative project with the ACC entitled: *Tobacco smoking, alcohol drinking, body mass index and risk of rare cancers.*

Background and Significance: Current knowledge on the causes of rare neoplasms (i.e., neoplasms with age-adjusted incidence rate below 1.0/100,000 in most human populations) is hampered by the difficulty to assemble a large enough set of cases to study even potent carcinogens with a high prevalence of exposure, such as tobacco smoking. Investigators from China, India, Japan, Korea, Malaysia, Singapore, Taiwan, and other countries have combined their cohorts to create a pooled analysis cohort of over one million persons to address these and other questions relating to lifestyle and the etiology of disease among Asians.

Study Methods: The project will require the integration of data on exposure of interest, covariates, and outcome among the participating cohorts. The coordinating center moved in 2014 from the Fred Hutchinson Cancer Research Center in Seattle, Washington, USA, to the University of Tokyo, Japan. RERF data have been restricted to persons with less than 100 mGy shielded kerma exposure and to persons who participated in at least one lifestyle survey. Approximately 53,000 persons are included in the data contributed by RERF. Pooled methods for cohort data will be employed in the analyses.

Study Progress: This project has been successfully completed but this RP will remain open to provide a foundation for new projects.

RP-A12-08 Second collaborative analysis of radiation-associated thyroid cancers

Sakata R (EH), Veiga L, Lubin J, Sugiyama H (EH), Shore RE (D)

Objectives: To improve our understanding of radiogenic thyroid cancer and probe additional questions that remain regarding risk of radiation-related thyroid cancer associated with juvenile or adult exposure.

Background and Significance: A causal relationship between ionizing radiation and thyroid neoplasia has been well established. The association for thyroid cancer was first identified among A-bomb survivors in 1963 (Socolow et al., *N Engl J Med* 1963). However, data have been limited with regard to several important radiation risk issues: the slope of the dose-response curve, dose fractionation, age at exposure, length of time since exposure, host susceptibility and histologic cell type. The original pooled analysis (Ron E et al., *Radiat Res* 1995; 141:259–77) of the risk of developing thyroid cancer after external radiation exposure was based on seven major epidemiologic studies. A new, pooled analysis based on considerably more data will significantly add to what is known about radiation-related thyroid cancer and its modifying factors.

Study Methods: An updated and expanded pooled analysis of 16 studies of radiation exposure and thyroid cancer risk will be carried out. To carry out the Poisson regression analyses, data were cross-classified by attained age, age at exposure, calendar period, study population, dose, and other variables of interest.

Study Progress: Pooled analyses on thyroid cancer incidence following childhood exposure to external radiation were carried out at the NCI using data from 12 studies.

Results and Conclusions: A manuscript of the pooled analysis for thyroid cancer incidence risk after childhood exposure to external radiation is in preparation.

Special Cancer Studies Publications

RERF Reports (RR)

♦ Furukawa K, Preston DL, Misumi M, Cullings HM: Handling incomplete smoking history data in survival analysis. Stat Methods Med Res 2014 (October) doi:10.1177/0962280214556794 [Epub ahead of print]. **(RR 4-13)** (refer to summary explanation in *Life Span Study* Publications)

♦ Sugiyama H, Misumi M, Kishikawa M, Iseki M, Yonehara S, Hayashi T, Soda M, Tokuoka S, Shimizu Y, Sakata R, Grant EJ, Kasagi F, Mabuchi K, Suyama A, Ozasa K: Skin cancer incidence among atomic bomb survivors from 1958 to 1996. Radiat Res 2014 (May); 181(5):531–9. **(RR 16-12)**

[Study Findings] Researchers observed that exposure to radiation is associated with a significant risk of developing basal cell skin cancer, with a dose-response threshold of 0.63 Gy and risk increasing as the age at exposure decreases.

[Commentary] ***Objective*** Researchers have reported a clearly increased risk of skin cancer, especially basal cell cancer, in association with exposure to ionizing radiation, based on studies of A-bomb survivors, workers in the field of radiation medicine, and individuals with a history of radiation treatment. The Radiation Effects Research Foundation (RERF) previously reported radiation risks for skin cancer based on cases diagnosed during the period from 1958 to 1987 in the Life Span Study (LSS) population. In the present study, the observation period was extended for 9 years, and radiation risks for skin cancer according to histological type as well as the effects of ultraviolet radiation on radiation risks were investigated. ***Methods*** The subjects of this study were 80,158 of the 120,321 individuals in the LSS population who were alive in 1958, when both local cancer registries in Hiroshima and Nagasaki began operations, and whose radiation dose was estimated by the latest dosimetry system (DS02). Pathological review was conducted on potential skin tumors diagnosed from 1958 to 1996 to estimate radiation risks for first primary cancers by histological type. A Poisson regression model was used for the analysis. ***Results*** (1) Radiation risks for basal cell cancer: With regard to basal cell cancer (123 cases), the best fit was a linear-threshold model with a threshold dose of 0.63 Gy (95% confidence interval [CI]: 0.32, 0.89); the excess relative risk at 1 Gy of exposure (ERR_{1Gy}) was estimated to be 0.74 (95% CI: 0.26, 1.6) for those age 30 at exposure.

The risk was estimated to increase by 11% with each one-year decrease in age at exposure; thus the ERR_{1Gy} was estimated to be about 7 after exposure at age 10. On the other hand, attained age did not significantly affect radiation risks. (2) Relationship between ultraviolet radiation and radiation risks for basal cell cancer: The ERR_{1Gy} of basal cell cancer on skin areas likely to be exposed to ultraviolet radiation, such as the face and hands, was estimated at 0.6 (95% CI: <0, 2.1), and the ERR_{1Gy} of areas unlikely to be exposed to ultraviolet radiation, such as the trunk and extremities except for hands, was estimated as 2.3 (95% CI: 0.61, 6.7). Although there was no statistically significant difference between the two ($P = 0.15$), the results suggest that radiation risks were higher for the areas unlikely to be exposed to ultraviolet radiation. (3) Radiation risks for squamous cell cancer *in situ* (Bowen's disease): When a linear model was used, the ERR_{1Gy} of squamous cell cancer *in situ* (64 cases) was 0.71 (95% CI: 0.063, 1.9). However, by city, the ERR_{1Gy} of squamous cell cancer *in situ* (Bowen's disease) in Hiroshima (56 cases) was not significant, at 0.28 (95% CI: <0, 1.9), whereas that in Nagasaki (8 cases) was extremely high, at 17.6 (95% CI: 3.0, 149); and a statistically significant difference existed between the two ($P < 0.001$). However, the number of cases in Nagasaki was small, and there may have been a difference between Hiroshima and Nagasaki in the diagnosis of squamous cell cancer *in situ* and in the rate of reporting to local cancer registries. Therefore, it was difficult to draw a conclusion about whether any significant dose-response relationship existed. (4) Radiation risks for other histological types: There was no significant dose response relationship for malignant melanoma (10 cases), squamous cell cancer (114 cases), Paget disease (10 cases), or other skin cancers (15 cases). The null result for malignant melanoma with regard to radiation dose is especially notable since that is the most serious form of skin cancer.

The results of this study show that a linear dose-response relationship with a threshold dose of 0.63 Gy exists between basal cell cancer and A-bomb radiation, and that the lower the age is at exposure, the higher the radiation risk. There was no significant relationship between radiation exposure and malignant melanoma, squamous cell cancer, or Paget disease.

Other Journal Publications

♦ Imaizumi M, Furukawa K: Thyroid disorders in Hiroshima and Nagasaki atomic-bomb survivors. Hiroshima Igaku [J Hiroshima Med Assoc] 2014 (April); 67(4):284–6. (Proceedings of the 54th Late A-bomb Effects Research Meeting, 2013) (Japanese) (related to *Special Clinical Studies*)

♦ Zheng W et al. (RERF: Ozasa K, Ohishi W, Grant EJ): Burden of total and cause-specific mortality related to tobacco smoking among adults aged ≥45 years in Asia: A pooled analysis of 21 cohorts. PLoS Med 2014 (April); 11(4):e1001631.

Special Cancer Studies Oral Presentations

❖ Shore RE, Grant EJ, Milder C, Sadakane A. The role of other exposures in radiogenic breast cancer risk. 105th Annual Meeting of the American Association for Cancer Research (AACR), 5–9 April 2014, San Diego, California, USA (related to *Life Span Study*)

❖ Shore RE. LSS updated studies of radiation and cancer incidence. NAS BEIR VIII Planning Meeting, 17 November 2014, Washington, DC, USA (related to *Life Span Study*)

❖ Shore RE. Does age at exposure affect radiation disease risk among atomic bomb survivors? NIRS-WHO Joint Symposium, 7–9 December 2014, Tokyo (related to *Life Span Study*)

Research Protocol 18-61
Tumor and Tissue Registries, Hiroshima and Nagasaki

RP 18-61 Tumor and tissue registry study in Hiroshima and Nagasaki

Soda M (EN), Ozasa K (EH), Sugiyama H (EH), Grant EJ (EH), Shimizu Y (EH), Katayama H (IT), Kodama K (CS)

Objectives: The purpose of the study is to determine the incidence and risks of all types of neoplasms in the persons exposed to A-bomb radiation in Hiroshima and Nagasaki.

Background and Significance: Population-based tumor registries have been active in Hiroshima since 1957 and in Nagasaki since 1958. Tissue registry systems were established in Hiroshima in 1973 and in Nagasaki in 1974. ABCC-RERF has assisted in the collection of cases and the management and maintenance of those databases. The information and materials are available in the Epidemiology Department for incidence studies, special cancer studies, and case-control studies, and so on.

Study Methods: Information on tumor diagnosis is collected through: notifications from hospitals and other medical facilities, on-site abstraction of medical records by RERF personnel, and death certificates. Information on pathological diagnosis of tumor and materials is collected from hospitals and local pathology laboratories for the tissue registries. Every year the Epidemiology Department retrieves the information on RERF study subjects from the registries with permission of the registry authorities.

Study Progress: Case collection by notifications and death certificates has been nearly completed through 2012 in both Hiroshima and Nagasaki prefectures. On-site record abstraction is complete through 2011 in Hiroshima city and through 2013 in Nagasaki prefecture. Tissue diagnoses and samples are being collected through 2012 in Hiroshima prefecture and 2011 in Nagasaki prefecture. The Department of Epidemiology is linking registry information with RERF subjects. This is a laborious process and the datasets through 2009 have been completed.

Results and Conclusion: Annual reports on incidence data for 2011 in both Hiroshima and Nagasaki prefectures are scheduled for publication by March 2015. The cancer incidence information through 2009 in Hiroshima and through 2011 has been cross-checked with the database of the LSS, *in utero*, and F_1, and summarized. The new cancer incidence analysis of estimation of radiation-related ERR and EAR using the updated individual doses and lifestyle factors obtained by the mail surveys is being conducted in collaboration with the U.S. NCI.

Tumor and Tissue Registry Publications

Journal Publication

♦ Allemani C et al., the CONCORD Working Group (RERF: Sugiyama H, Utada M): Global surveillance of cancer survival 1995–2009: analysis of individual data for 25,676,887 patients from 279 population-based registries in 67 countries (CONCORD-2). Lancet 2015 (March); 385(9972):62038–9.

Tumor and Tissue Registry Oral Presentation

❖ Kaneko M, Sugiyama H, Ozasa K, Utada M, Kamada N, Arita K, Kajihara H, Yonehara S, Takeshima Y, Yasui W. Analysis of reported esophagus tumor cases in the Hiroshima prefecture tumor registries. 23rd Annual Meeting on Japanese Association of Cancer Registries, 12–13 June 2014, Tsu

Research Protocols 3-04, 1-92, 10-86, 18-59, A4-10
Atomic-bomb Dosimetry Studies

RP 3-04 ESR measurements of tooth samples from Nagasaki survivors (Addendum to RP 1-92)

Hirai Y (G), Nakamura N, Kodama Y (BS/G), Cullings HM (S), Hida A (CN), Tomonaga M, Iijima Y, Mine M

Objectives: To investigate the possibility that doses to Nagasaki survivors who were exposed in factories have been overestimated or misestimated.

Background and Significance: It is difficult to estimate doses for Nagasaki survivors who were exposed in factories because of possible partial shielding by nearby machinery. Biodosimetric estimates potentially can be used to validate or adjust the physical dose estimates. Tooth enamel from Nagasaki survivors, especially factory workers when available, is being measured by electron spin resonance (ESR) to evaluate radiation dose. Chromosome aberration frequency is being measured for the same donors. Based on the results, we are examining the validity of the factory worker DS02 dose estimates.

Study Methods: We use extracted teeth to measure the absorbed gamma-ray dose by ESR (^{60}Co gamma-ray equivalent dose). Measurements of chromosome aberration frequencies in blood lymphocytes are also being made from the same donors to compare the results.

Study Progress: We measured two molars in this fiscal year. Thus far, a total of 22 molars donated by 20 Nagasaki AHS participants have been measured.

Results and Conclusions: Because the number of tooth samples examined was limited, it was not possible to derive a conclusion about a possible dose bias thus far. Results are expected in 2016.

RP 1-92 Radiation dose estimates using tooth samples. Part 2: Use of electron spin resonance on tooth enamel from Hiroshima atomic bomb survivors

Hirai Y (G), Nakamura N, Kodama Y (BC/G), Wada T, Cullings HM (S), Ohishi W (CH), Wieser A

Objectives: To estimate individual doses using teeth for measurement by electron spin resonance (ESR) technique and to compare the results with DS02 dose, and with chromosome aberration frequencies in lymphocytes, from the same donors.

Background and Significance: This study plan is to measure CO_2^- radicals induced by gamma-ray exposure in tooth enamel by means of ESR technique (Ikeya et al., *Jpn J Appl Phy* 1984; 23:L697). For this purpose, we collected teeth according to RP 10-86. Since the ESR signal intensity is linearly related to gamma-ray dose, this technique enables us to estimate the gamma-ray dose of the survivors. ESR has proved to be a good choice in that the ESR-estimated doses agreed well with cytogenetically estimated doses and jointly these provide a good basis to evaluate the physical dose estimates (DS02).

Study Methods: We use tooth enamel to measure the absorbed gamma-ray dose by ESR (^{60}Co gamma-ray equivalent dose). Measurements of chromosome aberration frequencies in blood lymphocytes are made from the same donors and the results are compared.

Study Progress: We measured eight molars in this fiscal year. Thus far, a total of 291 molars donated by 220 Hiroshima AHS participants have been measured.

Results and Conclusions: None yet. Results expected in 2016.

RP 10-86 Radiation dose estimates using tooth samples. Part 1: Collection of tooth samples from A-bomb exposed people in Hiroshima and Nagasaki

Hirai Y (G), Nakamura N, Ohishi W (CH), Hida A (CN)

Objectives: To collect teeth from Adult Health Study (AHS) subjects who were exposed to the bomb within 2 km from the hypocenter and their controls (estimated dose <5 mGy).

Background and Significance: Electron spin resonance (ESR; also called electron paramagnetic resonance, EPR) was first used to measure the cumulative radiation dose to the enamel of A-bomb survivors' teeth by Okajima's group at Nagasaki University. As the ESR signal intensity is linearly related to gamma-ray dose, this technique enables us to estimate individual doses. The collection of teeth from A-bomb survivors is the first part of the project to estimate the radiation dose of A-bomb survivors.

Study Methods: The Department of Clinical Studies sends letters to AHS participants twice a year for health monitoring. A sentence in the letters expresses our continued interest in the collection of teeth.

Study Progress: A total of 18 teeth were received from Hiroshima AHS participants and 13 from Nagasaki AHS participants during FY2014.

Results and Conclusions: Thus far, we have collected 1,650 tooth samples from Hiroshima AHS participants during the past 27 years and 102 from Nagasaki AHS participants during the past 10 years. On average, nearly 20% of the collected samples are suitable for ESR measurement (i.e., molars that are not seriously decayed).

RP 18-59 Shielding survey and dosimetry study

Cullings HM (S), Grant EJ (EH), Watanabe T (EH), Funamoto S (S), Matsumoto N (S), Cologne JB (S)

Objectives: The purpose of this protocol is to refine dose estimates for Hiroshima and Nagasaki atomic-bomb survivors and to characterize the uncertainties in these estimates.

Background and Significance: Accurate and detailed dose estimates are essential to characterizing the radiation dose response of any health effect under study. Three successive systems for calculating survivor doses based on location and shielding have been developed and implemented: T65D, DS86, and DS02.

Study Methods: DS02 is based on calculations of the nuclear explosions and the generation, transport, and alteration by shielding of the neutrons and gamma rays that directly irradiated survivors. Methods have been developed at RERF to improve input data on survivor locations and shielding, including terrain shielding, to extend the systems to longer distances and less detailed shielding data than those for which the core system performs calculations, and to characterize uncertainty in individual dose estimates and reduce the impact of uncertainties on radiation risk estimates.

Study Progress: Pre-2014—In 2007 a new committee was initiated by the RERF Chairman, Dr. Toshiteru Okubo, to examine a number of issues (residual radiation, improvement of input data, factory worker doses, etc.). Dr. Okubo and others in the Dosimetry Committee devised methods to make new estimates of map coordinates for individual survivors using archival RERF data with current technology. Moreover, beginning in 2007, four new statistical collaborations on dose error and biodosimetry were begun with groups of external investigators.

The several years' work of re-estimating survivors' map coordinates and related terrain shielding was completed in 2014. Improved input data for individual survivors' terrain shielding calculations were estimated at the new locations for all survivors from new digital terrain elevation data, which were available on a horizontal grid with ~10 m spacing. This resulted in considerable improvement over the previous input data, which were either taken from work done in the 1960s (Nagasaki "globe terrain" cases) or were estimated for survivors behind mountains denoted as "distal terrain" in the implementation of DS02 using older digital elevation data on a ~50 m grid. Revised doses were made available for the LSS in the RERF internal database in 2014.

2014—The entire process of the re-estimation of those data will be described in a journal paper and additional details will be documented in an RERF Report; a related manuscript is currently under RERF internal review.

We continued collaborative research with several external researchers to develop and compare statistical methods to estimate dose uncertainties and minimize their effects on risk estimation, including use of biodosimetry data. A new manuscript was submitted by a collaborator team.

We continued work on residual radiation, and Dr. Cullings and Dr. Grant collaborated with Dr. Sakata of the Epidemiology Department on a published manuscript related to the survivor data on cancer risk of exposure to "fallout rain." We are collaborating with the Epidemiology Department on a similar manuscript by Dr. Ozasa on acute effects.

Results and Conclusions: Work in progress confirms the suggestion in DS86 that the population dose increment in person-gray from known sources of residual radioactivity is a small fraction of direct doses calculated by DS02. Preliminary geospatial analyses of various biological endpoints have not shown any patterns consistent with undocumented doses from residual radiation or other deficiencies in the dosimetry. Work on more sophisticated geospatial analyses and other aspects mentioned above is ongoing.

RP-A4-10 Semiparametric methods using radiation exposure and chromosome aberration data in atomic-bomb survivors studies

Wang C-Y, Cullings HM (S), Song X, Ozasa K (EH), Soda M (EN), Suyama A, Kodama Y (BC/G), Davis S, Kopecky KJ

Objectives: Use semiparametric statistical methods to adjust for the effects of radiation dose measurement error on the estimation of radiation dose responses, incorporating information from biodosimetric data available for a part of the cohort.

Background and Significance: Although some measurement error methods have been applied to adjust for radiation measurement error in RERF data, further development of semiparametric or nonparametric methods is important to understand radiation effects on cancer or other outcome variables. Dosimetry data may be considered as a surrogate variable for the unobserved underlying radiation exposure. Biomarkers such as percentage of stable chromosome aberrations can be treated as a type of instrumental variable for the unobserved radiation dose.

Study Methods: The AHS subcohort of about 4,000 participants who have DS02 radiation dose estimates, plus stable chromosome aberration data, and outcome data for diseases such as cardiovascular heart disease, stomach cancer, lung cancer, or breast cancer, will comprise the calibration sample. By using data from the calibration sample, we can estimate radiation dose responses for the entire LSS cohort, with an appropriate adjustment for the uncertainty in DS02 dose estimates. An important result here is that the measurement error standard deviation will not have an assumed value, but rather will be estimated from the data using an innovative method, even though the data do not include replicate measurements/estimates of radiation doses. We will consider logistic, Cox, and additive hazard regression models for the radiation dose response, with adjustment for smoking information, age at exposure, gender, education, and city.

Study Progress: A U.S. NIH grant was obtained (C-Y Wang, PI) to support the work. Statistical analysis is in progress. One related paper has been published and two others have been submitted by the non-RERF collaborators; these develop the method but do not use RERF data.

Results and Conclusions: Several methods have been developed that utilize different methods and different assumptions, for different situations in terms of available surrogate and instrumental variables and types of regression analysis.

Atomic-bomb Dosimetry Studies Publications

RERF Report (RR)

♦ Cullings HM, Pierce DA, Kellerer AM: Accounting for neutron exposure in the Japanese atomic bomb survivors. Radiat Res 2014 (December); 182(6):587–98. **(RR 9-13)**

[Study Findings] The atomic bomb radiation exposures consisted of primarily gamma rays and a much smaller amount of neutrons. It is well known that neutrons have a larger biological effect per unit of dose than gamma rays or x-rays. The degree to which the effect is greater is known as the relative biological effectiveness (RBE). Some experimental studies have indicated that the RBE values may be greater at low doses of neutrons and gamma rays than at higher doses (that is, a "variable RBE" according to dose level). This study showed that total doses calculated for the atomic bomb survivors studied by RERF do not depend critically on the RBE that is assumed for the neutron portion of the dose, even when a hypothetical variable RBE is applied. This may reduce some concerns raised by investigators who have advocated the importance of variable RBEs in estimating risk at low doses. Furthermore, in some cases the variable RBEs have not been correctly estimated for the mixed neutron and gamma-ray exposure of the atomic bomb survivors.

[Explanation] The atomic bomb survivors followed by RERF were directly exposed to both gamma rays and neutrons, with the absorbed dose from neutrons being a small fraction of that from gamma rays in all cases. The main interest of RERF studies is typically to estimate risks from the much larger gamma-ray component of dose, but the risk estimation must be based on an adjusted gamma-ray dose that is equivalent to the combined dose that the survivor received from both gamma rays and neutrons. This equivalent dose is calculated as the sum of the gamma-ray dose plus the neutron dose times a weighting factor that accounts for the greater biological effect of the neutrons per unit dose. The weighting factor used is the RBE of the neutrons. Because the neutron doses and gamma-ray doses received by the survivors were highly correlated, the neutron RBE cannot be reliably estimated from the survivors' data, and information from radiobiology must be invoked. For many years, RERF has used a constant neutron RBE value of 10, even though radiobiological studies indicate that the RBE takes on considerably larger values at low doses. The use of a constant RBE = 10 as an approximation assumes that if the RBE is variable it takes roughly this value in the range of total dose most relevant for estimating risk as a linear function of dose, namely about 1 Gy. This paper considers some possible RBE functions to explain the correct use and the impact of a dose-dependent RBE. However, the authors do not advocate a particular choice or even that a variable RBE be employed. Rather they show that the assumed neutron RBE, within a wide range of choices, is far less important to the outcome of risk assessment of the RERF data than has generally been believed. Some of these misperceptions have been related to the consideration of variable RBE functions without due attention to the fact that in the case of the A-bomb survivors' data, the mixed field of neutrons and gamma rays must be considered. In such mixed fields, the RBE value of neutrons is much lower than the RBE in pure neutron fields that are used in radiobiological experiments. This paper shows that applying the pure-neutron-field RBE to the mixed-field A-bomb radiation can lead to an overestimation of the actual neutron RBE for moderate total dose levels of 1 Gy by a factor of more than four. While in a pure neutron exposure the RBE depends on the neutron dose, and in the mixed field it generally depends on both components of exposure, this paper shows that in the RERF setting the RBE depends mainly on the accompanying gamma-ray dose. This is primarily because the individual neutron doses are so much smaller than the corresponding gamma-ray doses.

Atomic-bomb Dosimetry Studies Oral Presentations

❖ Cullings HM. Spatial distribution and other characteristics of reported severe epilation in the A-bomb survivors. Workshop on Residual Radiation from the Hiroshima and Nagasaki Atomic Bombings, 15–16 July 2014, Baltimore, Maryland, USA

❖ Cullings HM. LSS risk regression for solid cancer with added dose terms for putative external exposure to fallout gamma-rays. Workshop on Residual Radiation from the Hiroshima and Nagasaki Atomic Bombing, 15–16 July 2014, Baltimore, Maryland, USA

❖ Misumi M. Application of simulation-extrapolation (SIMEX) to the Radiation Effects Research Foundation Life Span Study data. 21st International Meeting Conference on Radiation and Health, 21–24 September 2014, Las Vegas, Nevada, USA