Project 2.7: Interim Final Technical Progress Report, November 2006

Summary

Our goal (see Project Objectives) is to deliver a dosimetry system which will enable both a Pu body burden of 0.3 kBq, and a 30 cGy γ ray dose, to be separately estimated with a confidence limit of ±30%. In terms of the numbers analyzed and the data we have accrued, we have direct quantitative evidence that we are on track to providing such a comprehensive independent dosimetry system for Mayak workers.

Project Objectives

We had previously demonstrated that intra-chromosomal aberrations measured in peripheral blood lymphocytes can be used as a sensitive long-lived low-background quantitative biomarker of densely-ionizing radiation dose in individuals exposed many years ago. We propose to calibrate the system such that it can be used to estimate both the densely-ionizing internal plutonium exposure and the sparsely-ionizing external exposure in Mayak workers exposed to different combinations of these over a prolonged period, mostly many years ago.

Our objective is to deliver a dosimetry system (set of calibration parameters) which will enable both a comparatively low Pu body burden of 0.3 kBq, and a comparatively low 30 cGy γ ray dose (one of these or both of these) to be estimated with a confidence limit of ±30% (higher doses will of course be estimated with smaller confidence intervals and still lower doses with larger confidence intervals).

1) Draw blood, make metaphase slides and measure numbers of intra- and inter-chromosome aberrations (using the mBAND and mFISH techniques) in…

A) 255 healthy former Mayak workers who were exposed various combinations of internal and external exposures. The individuals were chosen from the data base of healthy former workers through computer simulation to optimize estimates of the dosimetry parameters. The individuals have both internal and external dose estimates from the SUBI Doses-99 system.

B) 85 healthy non-exposed controls with ages, gender, and smoking status, chosen based on computer simulation to optimize estimates of the dosimetry calibration parameters.

2) To assess the time course of loss of aberrations with age, measure intra-and inter-chromosomal aberrations in archival slides from 5 live healthy individuals from whom blood has been repeatedly drawn over periods of more than 20 years.

3) Analyze the above results using appropriate regression techniques to provide a deliverable dosimetry system in which a measurement of intra- and inter chromosomal aberrations in a blood sample taken from a previously exposed individual will yield separate estimates of both the internal and the external dose.

4) Training: Significant effort will be dedicated to training our SUBI colleagues in the use of the systems we have developed.

5) Quality Assurance: QA is central to this project.

6) Investigate variants of the current methodology to further enhance the practicality and ease of use of the dosimetry system.

Summary of progress regarding our six goals:

Goal 1A. "Draw blood, make metaphase slides and measure numbers of intra- and inter-chromosome aberrations (using the mBAND and mFISH techniques) in 255 healthy former Mayak workers who were exposed various combinations of internal and external exposures. The individuals are chosen from the data base of healthy former workers through computer simulation to optimize estimates of the dosimetry parameters. The individuals have both internal and external dose estimates from the SUBI Doses-99 system."

- As of October 2006, we have made measurements on 115 workers.

Goal 1B: " 85 healthy non-exposed controls with ages, gender, and smoking status, chosen based on computer simulation to optimize estimates of the dosimetry calibration parameters."

- As of October 2006, we have made measurements on 65 control individuals.

- In summary, we have made measurements on slides from 25 workers since the last semi-annual progress report. In total, we have now made measurements on 180 individuals out of our target of 340 individuals, in the first 40 months of this project.

Goal 2. "To assess the time course of loss of aberrations with age, measure intra-and inter-chromosomal aberrations in archival slides from 5 live healthy individuals from whom blood has been repeatedly drawn over periods of more than 20 years."

This goal is independent of our goal to create an mFISH/mBAND based dosimetry system for SUBI, but was an opportunistic goal based on our "discovery" in SUBI of slides of chromosome spreads for the same individuals, taken over the course of many years. We are pleased to report continual progress in this Goal. We earlier reported that we had been able to hybridize only 2 out the 12 slides that we have analyzed from individuals 2 and 3. In the last reporting period, we have been able to hybridize 50% of the slides from these two individuals, all of the slides being more than 25 years old, and we believe that we now have an optimal "recipe" for the hybridization.

Goal 3 "Analyze the above results using appropriate regression techniques to provide a deliverable dosimetry system in which a measurement of intra- and inter chromosomal aberrations in a blood sample taken from a previously exposed individual will yield separate estimates of both the internal and the external dose."

According to the protocol, Doses-99 dose estimates assigned to individuals are not available to us until repeat measurements are made in 60% of each batch of samples. We have not yet reached this value for our most recent batches of samples (we are now at 53%), thus we cannot as yet undertake dose-effect analyses of our most recent data.

As discussed earlier, however, our earlier data, which have had the doses "unblinded" to us, are very encouraging. As reported earlier, we compared our aberration-based Pu dose estimates with their Pu doses which were estimated using Mayak-based urinalyses (Doses-99) system. The Pu doses as estimated in these two independent ways were highly correlated (R^2 correlation coefficient = 81%). The average percent difference between the urine-based Pu dose estimates

and our aberration-based Pu dose estimates was 37%, which was very encouraging. An encouraging aspect was the agreement between the urine-based and the aberration-based plutonium dose estimates for the lower-dose plutonium workers (<40 cGy). A key point here is the aberration-based plutonium dose estimates were made despite large variations in the external gamma-ray doses. Overall, these results clearly demonstrate that we will be able to deliver a plutonium dosimetry system with overall uncertainties less than 30%, as proposed.

Goals 4/5. Training / Quality Assurance: Significant effort will be dedicated to training our SUBI colleagues in the use of the systems we have developed. Quality assurance in this regard will be central to the success of the Project.

At SUBI, having previously optimized the mFISH system, in the last 6 months, the mBAND system has been tested and optimized. The mFISH and now mBAND spreads made at SUBI are of indistinguishable quality to those made in New York. To date, hybridization of chromosomal slides using probes from the Vysis and MetaSystems companies was carried out at SUBI by mFISH for 17 individuals and by mBAND for 8 individuals. Images of metaphase spreads were obtained and analysis using Metasystems software was carried out for all the slides.

Goal 6: Investigate variants of the current methodology to further enhance the practicality and ease of use of the dosimetry system.

In collaboration with MetaSystems, Germany, we have been optimizing a new single-arm mFISH system, which will enable us to measure intra-chromosomal pericentric inversions in all chromosomes simultaneously. The use of single-arm mFISH is expected to considerably speed up our analysis, and in the last 6 months, we have quantified this, taking into account the probe cost, and the scoring of one chromosome for pericentric and paracentric intra-chromosomal aberrations (mBAND), vs. scoring all chromosomes for pericentric inversions. Our conclusion is that, per unit dollar, one can gain an approximately 30% decrease in the standard deviation of the measured frequency of intra-chromosomal aberrations, by using single-arm mFISH compared with mBAND.

Acquisition of Samples

For the reported period we identified 48 Mayak PA workers from the "Clinics" DB according to the required characteristics (age, gender, doses of external and internal exposure). Of these identified workers, 25 were invited to take part in the study. With each participant the questionnaire and the signing of the Informed Consent about voluntary participation in the study were undertaken. The blood sample was taken from each volunteer, cultivation of peripheral blood lymphocytes was carried out, and the chromosomal slides were prepared.

Biophysical analysis to identify Pu-239 body burden was carried for each participant. The medical information for each participant which was obtained from the medical documents and interviewing study participants was collected and entered into the database "Clinics". Doses of external and internal exposure were provided for SUBI from the system Doses-2005 Mayak PA (Mayak "Dose-2005").

Data Sharing

We are sharing our data with Elaine Ron's group at the NCI Radiation Epidemiology Branch, who are doing some molecular analyses of the blood from the same individuals; Dr Ron has referred to the combination of our cytogenetic analyses and her molecular analyses as "FISH and Chips". We also have an agreement to share results and samples with Dr Janet Tawn at Sellafield, who is doing dosimetric analyses of British plutonium workers (Tawn EJ, Whitehouse CA, Riddell AE. *FISH chromosome analysis of plutonium workers from the Sellafield nuclear facility.* Radiat Res. May 2006;165:592-7).

Publications

1. D. J. Brenner, N. Okladnikova, P. Hande, L. Burak, C. R. Geard and T. Azizova, Biomarkers specific to densely-ionising (high LET) radiations. *Radiat. Prot. Dosim.* **97**, 69-73 (2001).
2. M. P. Hande, T. V. Azizova, C. R. Geard, L. E. Burak, C. R. Mitchell, V. F. Khokhryakov, E. K. Vasilenko and D. J. Brenner, Past exposure to densely ionizing radiation leaves a unique permanent signature in the genome. *Am. J. Hum. Genet.* **72**, 1162-1170 (2003).
3. C. R. Mitchell, T. V. Azizova, M. P. Hande, L. E. Burak, J. M. Tsakok, V. F. Khokhryakov, C. R. Geard and D. J. Brenner, Stable intrachromosomal biomarkers of past exposure to densely ionizing radiation in several chromosomes of exposed individuals. *Radiat. Res.* **162**, 257-263 (2004).
4. D. J. Brenner, Comments on "Chromosome Intrachanges and Interchanges Detected by Multicolor Banding in Lymphocytes: Searching for Clastogen Signatures in the Human Genome". *Radiat. Res.* 162:600 (2004)
5. M. Prakash Hande, Tamara V. Azizova, Ludmilla E. Burak, Valentin F. Khokhryakov, Charles R. Geard, and David J. Brenner. Complex Chromosome Aberrations Persist in Individuals Many Years after Occupational Exposure to Densely-Ionizing Radiation: An mFISH Study. *Genes, Chromosomes, Cancer* 44:1-9 (2005)