Final Performance Report

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Project Title: Glycophorin A (*GPA*) Biodosimetry in I-131 Treated Patients

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List of Abbreviations

cGy	centiGray
cSv	centiSievert
DDREF	dose, dose-rate effectiveness factor
FISH	fluorescence *in situ* hybridization
GPA	glycophorin A
Gy	Gray
HLA-A	human leukocyte antigen A
HPRT	hypoxanthine phosphoribosyltransferase
mCi	milliCurie
MIRD	Medical International Radiation Dose
SD	standard deviation
Sv	Sievert
UPMC	University of Pittsburgh Medical Center
V_f	variant cell frequency ($x10^{-6}$)

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Significant Findings

This study was undertaken to further explore the short-term and persistent response of the glycophorin A (*GPA*) somatic cell mutation assay, which quantitates human *in vivo* somatic mutation in bone marrow erythroid progenitor cells, under conditions of low dose/low dose rate exposure to ionizing radiation. Thyroid cancer patients, treated with ablative ^{131}I therapy, were studied for radiation-induced somatic mutation by applying the assay to a time-series of blood samples collected prior to, and 14, 30, 50, 90, 120, 180, and 365 days after administration of 97.5 - 203 (mean 126) mCi of ^{131}I. A consistent short-term induction and partial long-term persistence of the frequency of *GPA* allele-loss variant cells (V_f) was observed suggestive of radiation-induced *GPA* locus somatic mutation in both committed erythroid lineage and bone marrow stem cell populations resulting from a calculated mean marrow dose of 34 cSv. The persistent mean increase of *GPA* V_f, measured one year after exposure, was 1.9 per million cells compared to 8.5 predicted from the dose-response relationship derived from the previous high dose/high dose rate studies, suggesting a DDREF of ~4.5 associated with this exposure. This result, consistent with assay data obtained previously in populations of Sellafield nuclear and Chernobyl cleanup workers with protracted radiation exposures, demonstrates that, like other experimental and biodosimetric systems, the *GPA* assay yields a substantially reduced biological response to low dose/low dose rate exposures.

Usefulness of Findings

The experimental design of this study, in which time-series pre- and post-therapy blood sampling is performed on thyroid disease patients treated with ^{131}I, was designed to focus on the time kinetics and dose response of the *GPA* assay to this internal exposure to ionizing radiation. This calibration, performed in subjects receiving clinically well-defined doses of whole-body ionizing radiation, is critical in defining and quantitating the dose response of the assay to low dose/low dose rate exposures relevant to the application of the assay to occupational/environmental biomonitoring of populations exposed to radiation. In addition, these data provide additional insights into the radiobiological effects of ionizing radiation on human bone marrow cells.

Abstract

The glycophorin A (*GPA*)-based human *in vivo* somatic cell mutation assay, because of it's demonstrated long-term biological memory of past human exposures to ionizing radiation, together with it's relatively low cost and high sample thruput, is unique among presently available human biomarker assays for practical use as a retrospective radiation biodosimeter in epidemiological investigations of large human populations in environmental or occupational exposure settings. This study was undertaken to further validate the response of the assay as a biodosimeter of low dose/low dose rate radiation exposure in a longitudinal study of patients receiving ^{131}I therapy for thyroid disease. These patients are excellent subjects for this investigation as they receive clinically well-characterized doses ranging typically from 10 to 100 cSv of whole body bone marrow exposure to ionizing radiation resulting from the radioactive decay of administered ^{131}I. The study design entailed the collection and analysis of multiple peripheral blood samples from patients drawn prior to, during, and following therapy to follow the induction, accumulation, and persistence of radiation-induced somatic mutation at the *GPA* locus in bone marrow stem cells. These mutations in nucleated bone marrow progenitor cells give rise to erythrocytes in the peripheral circulation expressing a *GPA* allele-loss variant phenotype. These variants are directly enumerated in the assay using immunolabeling with *GPA* allele-specific monoclonal antibodies and flow cytometry. Based on *GPA* assay results obtained in populations with high/high dose rate radiation exposures, this study was designed to primarily investigate the radiation-dose response of the assay over a range of doses that surround the extrapolated doubling dose over background response of the assay of approximately 30 cGy. The longitudinal design of the study, applied to patients receiving relatively low doses of ^{131}I, permitted a critical examination of the practical ultimate sensitivity of the assay by comparing *GPA* variant cell frequencies (V_f) in post-therapy samples to those observed in pre-therapy samples within individual patients. The results of the study provide critical information to assess the power of the assay to demonstrate, or to estimate the upper limits of, radiation exposures in population surveys of environmentally-exposed populations or occupationally-exposed workers. To date, 17 thyroid cancer patients, treated with ablative ^{131}I therapy, have been enrolled in the study and complete *GPA* assay data has been obtained and analyzed for eight patients who provided a time-series of blood samples collected prior to, and 14, 30, 50, 90, 120, 180, and 365 days after administration of 97.5 - 203 (mean 126) mCi of ^{131}I. A consistent short-term induction and partial long-term persistence of *GPA* allele-loss V_f was observed suggestive of radiation-induced *GPA* locus somatic mutation in both committed erythroid lineage and bone marrow stem cell populations resulting from a calculated mean marrow dose of 34 cSv. The persistent mean increase of GPA V_f, measured one year after exposure, was 1.9 per million cells compared to 8.5 predicted from the dose-response relationship derived from the previous high dose/high dose rate studies, suggesting a DDREF of ~4.5 associated with this exposure. This result, consistent with assay data obtained previously in populations of Chernobyl cleanup workers with protracted radiation exposures, demonstrates that, like other experimental and biodosimetric systems, the *GPA* assay yields a substantially reduced biological response to low dose/low dose rate exposures.

Background

Biological Dosimetry

Epidemiological studies of radiation-exposed populations, most notably those of Hiroshima and Nagasaki atomic bomb survivors, have provided compelling evidence for radiation-induced cancer in humans (BEIR V, 1990) and the quantitative relationship between radiation dose and induced cancer incidence derived from these studies has been used to define acceptable dose limits for occupational/environmental radiation exposures (BEIR V, 1990). Exquisitely sensitive physical methods of radiation monitoring are normally employed to assure compliance with such limits but in populations where such physical dosimetry is not available, e. g., historic or accidental exposures, or in cases of incomplete, missing, or questionable data, retrospective biologically-based dosimetric methods can be employed. These methods can provide estimates of received radiation doses to individuals by quantitating a biological endpoint, preferably mechanistically relevant to the etiology of carcinogenesis, that has long-term "biological memory" of past and cumulative radiation doses and responds in a predictable dose-dependent manner to such exposures.

There are presently two validated biodosimetry methods that fulfill these criteria: 1) quantitation of stable translocations in peripheral blood lymphocyte metaphase chromosomes, and 2) enumeration of glycophorin A (*GPA*) allele-loss variant erythrocytes in peripheral blood. The biological foundation of these assays results from the rapidly emerging understanding of the molecular genetics underlying human cancer where accumulated cytogenetic changes in normal somatic cells ultimately give rise to clonal proliferation of malignant cells. One major process of oncogenesis involves activation by point mutation, chromosomal translocation, or amplification of proto-oncogenes resident in normal cells (Bishop, 1987). A second process involves several mechanisms of somatic mutation including point mutation, gene/chromosome deletion, or somatic recombination leading to the loss or inactivation of so-called "tumor suppressor genes" coding for proteins involved in the regulation of cell growth and/or differentiation (Green, 1988). Most recently, a third process implicates the loss or inactivation of genes coding for DNA mis-match repair function leading to cells with a "somatic mutator" phenotype wherein the process of accumulation of further genetic changes is greatly accelerated (Leach *et al.*, 1993).

Cytogenetic Assays

The quantitation of stable chromosome translocations is the most widely applied and best understood bioassay and represents the historical "gold standard" biodosimeter of human radiation exposure. While other cytogenetic endpoints such as unstable chromosome aberrations, dicentric chromosomes, and micronucleus frequencies determined in peripheral blood lymphocytes all respond in a dose-dependent manner to ionizing radiation in the short term, these assays all lack long-term "biological memory" and cannot be used to quantitate protracted or historic exposures (Bender *et al.*, 1988; Littlefield *et al.*, 1990). In contrast, significant radiation dose-dependent increases in the

frequency of cells bearing stable translocations have been demonstrated in blood samples from medically-irradiated patients and atomic bomb survivors drawn years to decades after exposure (Buckton *et al.*, 1978; Awa, 1983). The significant drawbacks of this method are that a relatively large volume of peripheral blood is required (~10 ml), the lymphocytes must be isolated and placed in culture within 48 hours of sampling, and the laboratory procedures required for preparing, staining, and particularly scoring of metaphase chromosomes on microscope slides are labor intensive and costly. Also, in studies involving low radiation doses, where only a small increase in radiation-induced aberrations is expected over background levels, large numbers of metaphases need to be examined thus limiting sample thruput. These technical factors make the method impractical for application in studies of large populations with low radiation exposures. The recent development and application of the fluorescence *in situ* hybridization (FISH)-based "chromosome painting" technique (Pinkel *et al.*, 1986: Lucas *et al.*, 1992) has simplified and increased the speed of translocation scoring, albeit at increased cost, and coupled advances in automated metaphase-finding technology offer additional increases in sample thruput in the future (Piper *et al.*, 1994). However, until these recent technical advances are fully implemented, this method cannot serve as a primary biodosimetry screening assay in large population surveys. It can and should be used however as an important validation assay in selected subsets of individuals drawn from such populations.

Somatic Mutation Assays

The second method, the *GPA in vivo* somatic cell mutation assay, has both the biological properties and practical utility needed for retrospective biodosimetry studies of large populations. As detailed below, the assay also has long-term "biological memory" of past radiation exposures and displays radiation dose-dependent increases in the frequency of allele-loss variant erythrocytes detectable in peripheral blood samples from individuals exposed to whole-body ionizing radiation. In addition, the assay offers practical utility; only small volumes (~1 ml) of blood are required, freshly-drawn samples can be stored for one to two weeks prior to analysis, no cell culture is required, and the immunolabeling and flow cytometry-based system provides for rapid analysis and high sample thruput that is approximately one order of magnitude greater than that presently achievable with FISH-based translocation scoring. It's only limitations are that, due to the genetics of the system, only approximately one-half of a selected population can be assayed and, since peripheral blood erythrocytes are terminally differentiated and contain no genetic material, cells of variant phenotype cannot be clonally expanded or analyzed at the DNA level to reveal the nature of the underlying mutation in the *GPA* gene.

The other well developed and widely applied human *in vivo* mutation system, that based on the enumeration of peripheral blood T-lymphocytes expressing mutations in the X-linked HPRT gene (Albertini *et al.*, 1990) while demonstrably sensitive to mutation induction by radiation, appears to have limited "biological memory" of past exposures. HPRT T-lymphocyte mutant frequencies show only a shallow residual dose response in Hiroshima atomic bomb survivors (Hakoda *et al.*, 1988) and longitudinal sampling of individuals with 100 to 700 cGy exposures from the ^{137}Cs source accident in Goiânia, Brazil reveals an abrupt decline in initially high HPRT mutant frequencies over three

years (da Cruz and Glickman, 1994). Since this assay is also a cell culture-based system, it is not particularly well suited for large population surveys.

The Glycophorin A (GPA) In Vivo Somatic Cell Mutation Assay

In regard, to mechanistic relevance of the *GPA* system, as outlined above, the genetic events occurring *in vivo* in somatic cells that underlie the process of human tumorigenesis have been demonstrated to occur by many more molecular mechanisms than simple classical mutation, i.e. defined as gene-specific point mutations, insertions, deletions and translocations. We have designed the *GPA* assay, to detect, in peripheral blood erythrocytes, a wide range of potentially inactivating mutations at the *GPA* locus. Over the last decade, our laboratory has pioneered the development, validation, and clinical and epidemiological application of the *GPA*-based assay now one of the most widely applied assays for measuring human *in vivo* somatic cell mutation (Grant and Bigbee, 1993). The assay uses immunolabeling and flow cytometry to enumerate, in peripheral blood samples, erythrocytes of allele loss variant phenotypes resulting from locus-specific and chromosomal genetic alterations in erythroid progenitor cells in the bone marrow. As such, the frequency of these variant erythrocytes reflects the level of genetic damage at this reporter locus in an accessible tissue that represents an estimate of both the accumulated and constitutive endogenous level of genomic alterations in all tissues of the body. The GPA assay is based on allele-specific loss of expression at the single-copy autosomal GPA gene coding for the major erythroid cell-specific sialoglycoprotein (Loken, 1987). This locus is part of a family of related genes, including *GPB* and *GPE* (Cartron *et al.*, 1990; Kudo and Fukuda, 1990) that have been mapped to chromosome 4q28-31 (Rahuel *et al.*, 1988) The human *GPA* locus has been cloned and extensively characterized to reveal a mammalian gene of typical size and complexity consisting of seven coding exons and six introns extending over 40 kb (Tate and Tanner, 1988; Cartron *et al.*, 1990) The gene is transcribed and processed to yield a 1.7 kb major mRNA (Cartron *et al.*, 1990). *GPA* expression is detectable at the early erythroblast stage of erythropoiesis and is complete at the normoblast stage of differentiation (Loken, 1987). Bone marrow and peripheral blood reticulocytes as well as mature erythrocytes maintain a stable level of 0.5-1.0 x 10^6 *GPA* molecules on the cell surface (Merry *et al.*, 1986; Langlois *et al.*, 1985). Two common *GPA* alleles are present in the human population, coding for proteins that differ at the first and fifth positions of a sequence of 131 amino acids. These two allelic forms of *GPA* determine the M,N blood group and, in heterozygous $GPA^{M/N}$ individuals (~50% of the population), the alleles are co-dominantly expressed resulting in equal synthesis in all normal erythroid cells (Furthmayr, 1978; Langlois *et al.*, 1986).

Operationally, the assay uses murine monoclonal antibodies specific for the M and N forms of the *GPA* protein and tagged with distinguishable fluorophores, to label erythrocytes in blood from $GPA^{M/N}$ heterozygous individuals. Flow cytometry is then used to rapidly screen the large population of two-color, doubly-labeled cells to enumerate single-color variants with allele-loss phenotypes. Results are reported as the frequency of variant cells detected per million total erythrocytes analyzed (V_f). Two distinguishable populations of variant cells are detected; cells of hemizygous phenotype that lack

expression of one GPA allele and express the remaining allele at a single-copy level, and variants of homozygous phenotype that also lack expression of one allele but express the remaining allele at a two-copy level (Langlois *et al.*, 1986, 1990). The assay design requires that the homologous allelic protein be expressed thus precluding the involvement of genetic or epigenetic events that would affect both alleles (Jensen *et al.*, 1991).

Thus, as illustrated in Figure 1, the assay quantitates classical allele-expression loss ("null") mutations arising by mutational mechanisms that include base-pair substitutions, gene-specific deletions and insertions, cytogenetically-detectable chromosome deletions and chromosome loss to produce variant cells of hemizygous phenotype. In addition, other chromosomal events including chromosome loss and duplication and somatic recombination may lead to homozygosity at the GPA locus, resulting in variants of homozygous phenotype. Hence the assay is potentially sensitive to a wide spectrum of mutational and segregational mechanisms, all of which have been implicated in the process of human oncogenesis.

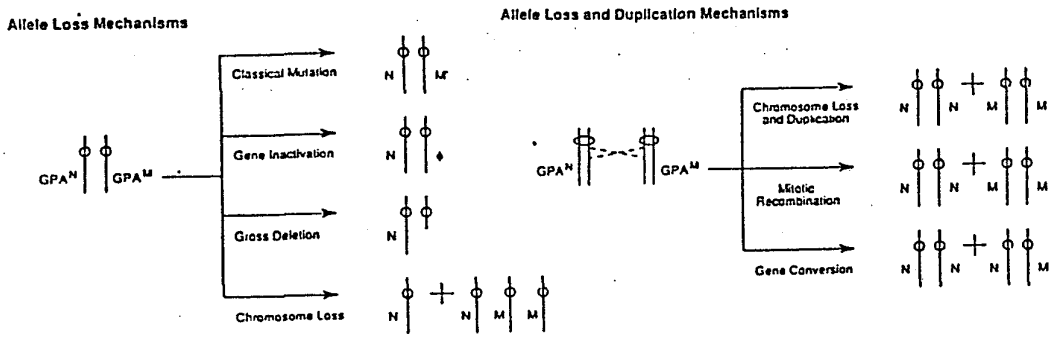

Figure 1. Allele loss and loss and duplication at the *GPA* locus on the long arm of chromosome 4. In the figure on the left, four possible mechanisms of loss of the GPA^M allele are shown, including: 1) mutation at the GPA^M allele resulting in either loss of expression or loss of monoclonal antibody binding; 2) inactivation of the GPA^M allele by an epigenetic mechanism such as DNA methylation; 3) deletion of a region of chromosome 4 including the GPA^M allele; or 4) loss of the entire copy of chromosome 4 carrying the GPA^M allele. The latter event is shown as occurring via non-disjunction during mitosis, with the simultaneous generation of a daughter cell trisomic for chromosome 4, although other mechanisms are possible. In the figure on the right, three potential mechanisms leading to homozygosity for the GPA^N allele are described, including: 1) chromosome loss and duplication, which may occur via two successive non-disjunctional events, or by a single aberrant disjunction in which paired chromatids segregate into daughter cells; 2) mitotic recombination occurring between the centromere and the GPA locus, leading to the indicated daughter cells 50% of the time, depending on the chromosomal segregation at mitosis; and 3) a putative gene conversion event, in which the DNA from one chromatid is actually replicated by base pairing with the homologous chromosome, with the result that one allele, in this case the GPA^M allele, is not replicated.

As mentioned above, the *GPA* assay can be usefully applied to large population surveys given it's relative speed and economy. Since the assay directly enumerates erythrocyte variants in peripheral blood samples, only a small volume of fresh blood (~1 ml) is needed for each assay. Finally, our recent development of a new and more precise version of the *GPA* assay does not require cell sorting and is designed to run on cheaper and simpler commercially-available flow cytometry instrumentation such as the Becton Dickinson FACScan™ cytometer (Langlois *et al.*, 1990; Jensen and Bigbee, 1996). The only practical limitation of the assay is the fact that it requires sampled individuals to be of heterozygous M/N blood type. Since the GPA^M and GPA^N alleles occur at approximately equal frequencies in human populations, about one-half of selected individuals can be studied with the *GPA* assay.

GPA Studies of Populations Exposed to Ionizing Radiation

The *GPA* assay has been demonstrated to be a quantitative biodosimeter in studies of populations acutely exposed to high levels of ionizing radiation. These cohorts have included A-bomb survivors (Langlois *et al.*, 1987, 1993; Kyozumi *et al.*, 1989), ^{137}Cs source (Straume *et al.*, 1991; Bigbee *et al.*, 1996) and Chernobyl accident victims (Jensen *et al.*, 1995). Consistent, and highly significant, dose-associated levels of *GPA* allele-loss variant erythrocytes have been observed of ~25 induced variants per Sv per million cells.

Following these initial investigations of the response of the *GPA* assay to acute and sub-acute high dose human exposures to ionizing radiation, we and others have undertaken several additional studies focused on the response of the assay to both chronic and low dose/low dose rate exposures. In collaboration with Dr. J.D. Boice, Jr., we have conducted a study of an occupationally-exposed population in which the *GPA* assay was employed as the primary biodosimetric endpoint supplemented with selected analyses using the FISH-based chromosome translocation assay for additional validation. This study was an extensive biodosimetric and leukemia incidence survey of individuals from Estonia, Latvia, and Lithuania that were assigned to Chernobyl as cleanup workers shortly after the accident in 1986 (Bigbee *et al.*, 1997). Most of these workers received a summary report of their putative cumulative exposures that range from 0 to 30 cSv (mean dose approximately 15 cSv) although these physical dose estimates are quite uncertain. We compiled extensive questionnaire information on these workers together with *GPA* and selected FISH-based chromosome translocation biodosimetry data in order to provide more reliable estimates of received doses. No significant difference in *GPA* allele-loss V_f between the exposed and control subjects, nor any significant correlation with individual physical dose estimates, was observed in our study (Bigbee *et al.*, 1997). Recently, a second study of Chernobyl cleanup workers from Russia employing the *GPA* assay as a biodosimeter was published (Moore *et al.*, 1997). Similar to our results in the cleanup workers from the Baltic countries, no significant biological responses in the *GPA* assay was observed in this cohort with a mean physical dose estimate of ~13 cSv. Lastly, a biodosimetric study employing the *GPA* assay applied to a group of nuclear fuel reprocessing workers and matched unexposed controls at Sellafield, U.K. who have

received well-documented cumulative radiation doses ranging from 15 to 110 cSv over their working lifetimes has been published (Tucker *et al.*, 1997). A shallow, and non-significant, regression of *GPA* allele-loss V_f was reported in this study. Together these studies strongly suggest a substantially reduced biological effectiveness of the induction of *GPA* locus mutations from low-dose-rate versus acute γ radiation exposure.

Proposed Study of GPA Mutation in Thyroid Disease Patients Treated with ^{131}I

This study was designed to test the utility of the *GPA*-based assay as a biodosimeter of bone marrow radiation exposure using blood samples from patients with thyroid disease who receive whole body *in vivo* exposure to ionizing radiation when treated with the radioisotope ^{131}I. The radioactive half-life of ^{131}I is 8.04 days decaying by emission of low energy β radiation and high energy γ radiation. The biologically effective half-life in the body is about six days so that 95% of the dose is delivered in a protracted sub-acute exposure of about four weeks (Holm *et al.*, 1991). Patients treated for hyperthyroidism and thyroid cancer receive total systemic ^{131}I doses, given either in single or multiple administrations, usually ranging from 5 to 500 mCi . A number of clinical methods have been used to determine the radiation doses to blood and/or red bone marrow resulting from this exposure. "Classical" methods have used sequential sampling of blood and/or urine as well as external whole-body counting (Benua *et al.*, 1962; Leeper and Shimoka, 1980; Akabani and Poston, 1991); most recently the use of whole-body counting following administration of a 2 to 5 mCi pre-therapy diagnostic dose of ^{131}I has been proposed and validated (Thomas *et al.*, 1993). For these calculations, patients are classified into three groups: 1) those with metastatic thyroid cancer detectable with the diagnostic dose, 2) those with residual functional thyroid tissue, and 3) those with no indication of radioiodine concentrating tissue. Bone marrow dose estimates for these three groups using both methods are in close agreement with an overall range of 0.24 to 0.65 cSv/mCi (Thomas *et al.*, 1993). The mean dose to red bone marrow from the ^{131}I treatment for hyperthyroidism is estimated to be 0.59 cSv/mCi (McEwan, 1977).

Thyroid Cancer Patients Treated with ^{131}I

In collaboration with Drs. J.C. Reynolds and J. Robbins at the NIH, we have recently completed a pilot study of five thyroid cancer patients treated with relatively high doses of ^{131}I (Jensen *et al.*, 1997). One newly-diagnosed patient was selected for the study and blood samples from this individual were obtained prior to therapy and at approximately 5, 10, and 15 months following therapy. Four previously-treated patients, scheduled for additional therapy, were enrolled whose previous therapies were completed from approximately 4 to 108 months prior to commencing the study. Initial blood samples were obtained from these patients prior to the additional therapy and at approximately 5 months following therapy. Clinical estimates of cumulative doses to bone marrow ranged from 61 to 394 cSv. A regression of *GPA* hemizygous Ø/N V_f measured in these blood samples against these cumulative doses yielded a *GPA* dose response of 10.9×10^{-6} per Sv for this exposure, which is approximately one-half of that observed in the acutely-exposed Hiroshima, Chernobyl, and Goiânia populations. As inferred from the published studies of the Chernobyl cleanup and Sellafield nuclear workers, this difference may well reflect

the reduced "dose-rate effectiveness factor" of low-dose-rate versus acute γ radiation exposure estimated to be as large as 2 to 5 (BEIR V, 1990).

Specific Aims

This study was designed to critically evaluate the potential utility of the glycophorin A- (*GPA*) based *in vivo* somatic cell mutation assay as a cumulative biodosimeter of low dose/low dose rate radiation exposure by applying the assay to a population of patients with thyroid disease who received protracted uniform whole-body exposure to ionizing radiation attendant to their therapy with ^{131}I. To this end the specific aims of this study included:

- Identify and enroll a population of newly-diagnosed patients with thyroid disease who are to receive ablative ^{131}I therapy.

- Obtain initial blood samples from these patients and perform M,N blood group serotyping to identify those patients of heterozygous MN blood type required for application of the glycophorin A- (*GPA*) based *in vivo* somatic cell mutation assay. Approximately 100 patients of MN blood type will be selected for the study with clinical doses of ^{131}I expected to range from 50 to 500 mCi corresponding to approximately 10 to 100 cSv of total body exposure.

- Perform the *GPA* assay on blood samples from these patients obtained prior to, during, and up to approximately one year after ^{131}I therapy.

- Cryopreserve sphered and fixed erythrocyte preparations from these samples as a resource for replicate *GPA* assays and for future erythrocyte-based assays.

- Isolate and cryopreserve viable lymphocytes from these samples for potential follow-up studies using lymphocyte-based cytogenetic and mutation assays.

- Correlate *GPA* somatic mutation variant cell frequencies with both total administered ^{131}I and clinical determinations of blood and bone marrow doses to construct the radiation dose response of the assay over this dose range that spans the estimated doubling dose over background of approximately 30 cSv. This analysis will yield the shape of the assay dose response and repeated measures analyses of the longitudinal responses observed in individual donors will provide an estimate of the useful limit of sensitivity of the assay for prospective monitoring of radiation-exposed populations.

- Compare the *GPA* assay responses among patients receiving similar doses to assess the inter-individual variability in biological response to radiation exposure.

Procedures

Selection of Newly-Diagnosed Patients with Thyroid Disease Scheduled for ^{131}I Therapy

Newly-diagnosed thyroid cancer patients were identified and enrolled by clinical collaborators at the University of Pittsburgh Medical Center (UPMC). These collaborators included Manuel L. Brown, M.D., Professor, Department of Radiology, Director, Nuclear Medicine, Lynn A. Burmeister, M.D., Assistant Professor, Department of Endocrinology, Sally E. Carty, M.D., Assistant Professor, Department of Surgery, Charles G. Watson, M.D., Professor, Department of Surgery, School of Medicine, University of Pittsburgh, and Dennis Swanson, M.S., School of Pharmacy, University of Pittsburgh. Ms. Lisa Streb, R.N., Department of Surgery, School of Medicine, University of Pittsburgh served as the clinical coordinator for the study. All procedures and protocols used in the study were approved by the University of Pittsburgh Institutional Review Board (IRB) prior to the initiation of the study (IRB approval letters are included in the Appendix of this report). Patient recruitment and enrollment procedures followed a written IRB-approved clinical protocol and informed consent document (included in the Appendix to this report).

Patient Eligibility Criteria:

- Diagnosis of thyroid cancer to be treated with administration of ^{131}I
- Age of 18 years or older
- Willingness to provide informed consent to participate in the study and to provide up to eight (8) blood samples of approximately 50 ml each over a period of one (1) year
- M/N blood type to be determined from serotyping of the pre-therapy sample
- Normal complete blood count (CBC)

Patient Exclusion Criteria:

- Under 18 years of age
- Previous chemotherapy or radiotherapy
- Blood transfusion within the last year
- Pregnancy
- M/M or N/N blood type to be determined from serotyping of the pre-therapy sample. Approximately one-half of potential research subjects will be excluded by this criteria

Patient Enrollment Protocol

Participating UPMC clinical collaborators and/or the clinical coordinator initially discussed the opportunity to participate in the study with each newly-diagnosed thyroid cancer patients scheduled for therapy with ^{131}I. The patient's physician and/or the clinical coordinator reviewed with the patients the inclusion and exclusion criteria to determine if the patient could be enrolled in the study. Those patients expressing an interest in participating and who satisfied the study inclusion and exclusion criteria were then furnished with a copy of the study consent form (included in the Appendix to this report).

After reading the form, the patients was then afforded an opportunity to ask questions regarding the study of the collaborating physician, clinical coordinator, and/or the principal investigator. Those patients wishing to be enrolled in the study and who were willing to provide informed consent did so by initialing each page of the consent form and signing the final page of the form in the presence of their physician, the clinical coordinator, and/or principal investigator of the study. The consent form was also signed and dated by the clinical collaborator, clinical coordinator, or the principal investigator and a witness as indicated on the final page of the consent form. The original copy of the completed consent form is kept by the principal investigator with copies retained by the patient's physician and by the clinical coordinator. The patient was also supplied with a copy of the completed consent form. No collection of blood samples or abstraction of clinical data from a patient was obtained prior to the patient's witnessed signature of the consent form as required by the University of Pittsburgh IRB. Every effort was made, within the constraints imposed by the populations served by the UPMC, to include appropriate numbers of minorities and women in the study population.

Personal Information and Clinical Data

With informed consent at the time of enrollment, personal information regarding potentially confounding factors including age, sex, ethnicity, and smoking history was obtained by from each patient. Appropriate clinical data regarding medical history, ^{131}I dosimetry, and disease status were abstracted from clinical records by the clinical coordinator. These specific data include:

- Name
- Address
- Home and work telephone numbers
- Sex
- Age
- Height
- Weight
- Ethnicity
- Attending physician
- Diagnosis
- Clinical treatment regimen, including dose and schedule of ^{131}I administration
- ^{131}I retention from whole body counting measurements
- CBC results
- All medications
- All other significant medical conditions and history

Blood Sampling Procedures

Blood Sampling Schedule

To carefully follow individual patient responses to therapy, blood samples were obtained prior to therapy and at approximately 14, 31, 50, 90, 120, 180, and 365 days

following therapy. The pre-therapy sample and samples collected at 14, 31 and 50 days following therapy were obtained as part of the standard clinical care of the patients. Samples at 90, 120, 180 and 365 days following therapy were drawn expressly for the purpose of the research study.

Blood Sample Collection and Storage

Fresh blood samples from these patients and samples from local healthy controls were obtained with informed consent and processed for the *GPA* assay and for lymphocyte isolation as detailed below. Typical blood sample volumes were approximately 50 ml. Samples were collected by standard arm veinipuncture by a licensed M.D. or trained phlebotomist with a licensed M.D. in attendance at the UPMC or other clinical center of convenience for the subject into five (5) 10 ml Vacutainer® tubes containing sodium heparin anticoagulant. Each tube was labeled only with the patient's ID number that was assigned by the clinical coordinator as detailed below and with the draw date. The samples were stored a room temperature (~22°C) and then delivered to the laboratory in insulated and sealed shipping containers for processing within 48 hours of collection.

Blood Sample Encoding

To insure subject anonymity and to blind laboratory personnel, the blood samples were labeled with a study ID number assigned by the clinical coordinator. The ID number was composed of the prefix PTS (for Pittsburgh Thyroid Study) and the last four (4) digits of the subject's social security/hospital number followed by a "dash" and two (2) or three (3) digits corresponding to the scheduled draw day of the sample, e.g., PTS2875-00 (for pre-therapy sample), PTS2875-31, PTS2875-180 (for days 31 and 180, respectively), etc. An encoded and password-protected computer file containing the patient's name and ID numbers and dates of all blood samples collected from each patient was maintained by the clinical coordinator who, on a monthly basis, supplied a copy of the file to the principal investigator.

Methodology

GPA Assay

Cell fixation, immunolabeling, and flow cytometric analysis procedures for the *GPA* assay were performed using previously published methods (Langlois *et al.*, 1990, Jensen and Bigbee, 1996). Briefly, blood samples were first serotyped for the M,N blood group using commercial anti-M and anti-N sera (Ortho Diagnostic, Raritan NJ). Samples from heterozygous $GPA^{M/N}$ donors were fixed with formalin, then immunolabeled with two monoclonal antibodies specific for the GPA^M (6A7) and GPA^N (BRIC157) allelic forms of the *GPA* protein. Distinguishable green (BRIC157-fluorescein)(BRIC157-F) and orange (6A7-phycoerythrin) (6A7-PE) fluorescent labels are conjugated to the antibodies so that normal erythrocytes, which express both the GPA^M and GPA^N alleles, are doubly labeled. The two fluorescence intensities of each of 5×10^6 cells per sample were quantified using a Becton Dickinson FACScan™ flow cytometer. Erythrocytes with normal expression of both GPA alleles bind both antibodies and are doubly labeled. A small number of variant cells are detected which bind the normal level of GPA^N-specific antibody, but exhibit no binding of the GPA^M-specific antibody, indicating that these cells have lost expression of the GPA^M allele. These variants are designated allele-loss "hemizygous Ø/N" cells. A second discrete population of rare variant cells appear to have lost expression of the GPA^M allele, but express the GPA^N allele at twice the normal level. These variants are designated as allele-loss and duplication "homozygous N/N" cells. Variant frequencies (V_f) for both classes of variants were directly measured by counting the number of both variant and normal cells and are expressed as variants per 10^6 cells. A typical analysis that simultaneously determines the V_f of both cell classes requires ~30 minutes to examine the 5×10^6 total cells at a rate of ~3000 cells/sec.

Blood samples from healthy controls were prepared and analyzed with each batch of patient samples as quality controls and to monitor the long-term baseline stability of the assay. These control samples were drawn from a local population of normal donors whom we have sampled repeatedly and for whom *GPA* V_f are precisely known.

Erythrocyte Cryopreservation

Formalin-fixed SDS sphered erythrocytes prepared as outlined above were pelleted by centrifugation then resuspended in erythrocyte freezing media consisting of RPMI 1640 and 5% dimethylsulfoxide. The suspension was then divided into 200 μL aliquots in Corning 1 ml cryovials. The vials are placed on dry ice for one hour, then transferred to a −150°C freezer for long-term storage. These cryopreserved specimens were maintained for replicate GPA assay measurements, if needed, and will be retained for future studies employing additional erythrocyte-based assays when they may become available.

Lymphocyte Isolation and Cryopreservation

Lymphocytes were prepared from ~50 ml of heparinized blood using the established method of centrifugation through a continuous density gradient on Ficoll-Paque™

(Pharmacia, Inc., Piscataway, NJ). For freezing, the cells were resuspended in 90% fetal calf serum, 10% dimethylsulfoxide, distributed into five aliquots, frozen using a controlled 1°C/minute automated freezer, and stored at -150°C. To insure adequate growth of the cells, these procedures were performed within 48 hours of delivery.

These separated and cryopreserved viable lymphocytes will serve as a valuable resource for future use in lymphocyte-based cytogenetic and/or somatic mutation assays and as a source of DNA for molecular studies. These assays could include micronucleus, dicentric and/or stable chromosome translocation analyses and/or *HPRT* and *HLA-A* somatic mutation biomarker assays as well as new lymphocyte-based assays developed in the future.

Calculation of Bone Marrow Doses

Thyroid cancer patients received ^{131}I as orally administered sodium iodide. The iodide is converted to iodine (I_2) in the gut and absorbed from the gastrointestinal tract directly into the blood stream or into the lymphatic circulation (Alpen 1990; Calvalieri 1997). Although most ^{131}I concentrates in the thyroid gland, there is deposition in other tissues, including the salivary glands, digestive tract, kidneys and bladder the amount depending on their metabolic activity, biological accessibility, and the competing excretion of the isotope in urine and feces (Edwards et al., 1986; Alpen 1990). Once incorporated in the thyroid gland, iodine is excreted slowly or removed by radioactive decay (Alpen 1990). The two types of radiation emitted from decay of ^{131}I are β particles that are locally absorbed and penetrating γ radiation. Since iodine is loosely bound to blood plasma albumin, radioiodine rapidly comes to a specific activity equilibrium with the iodine of the blood plasma. ^{131}I decays to stable ^{131}Xe so there are no radioactive daughter products of the physical decay that contribute to radiation dose (Radiobiological Health Handbook, 1970; Alpen 1990).

Two methods were used to determine the radiation dose to the bone marrow from the administered ^{131}I. The first used the methodology developed by Edmonds and Smith (1986) based on ^{131}I dose per unit administered activity (rad/mCi) and percent thyroid uptake. This model is based on the MIRD 1975 principles (Synder et al., 1975; Edmonds and Smith, 1986). The second dosimetry model utilized a derived regression equation for bone marrow dose (cSv) = 1.54 + 0.27 x administered ^{131}I activity (mCi) derived from our previous study of ^{131}I-treated thyroid cancer patients (Jensen *et al.*, 1997). In this model, body and blood retained concentrations of ^{131}I were measured experimentally at 0, 2, 4, 6, 10, 24, 72, and 96 hours following ^{131}I administration. MIRD schema were then used to calculate the radiation dose to the bone marrow. The derived bone marrow dose using both of these models for each patient in the study is presented in Table 2.

Data Analysis of GPA V_f and Correlation with Clinical Dose Parameters

Comparison of *GPA* hemizygous Ø/N and homozygous N/N V_f measured in groups of patients treated with similar ^{131}I doses and between *GPA* V_f data obtained at each blood sampling point were be compared and statistical inferences drawn using both parametric (*t*

test) and non-parametric (χ^2 test and Mann-Whitney U test) procedures on transformed and nontransformed V_f data, respectively. Appropriate transformations, e.g. logarithmic, were selected by examining normal probability plots of ranked residuals after fitting the V_f by linear regression.

GPA hemizygous Ø/N and homozygous N/N V_f determined in patients at each sampling time were compared by multiple logistical regression analysis of variance techniques and repeated measures analysis using untransformed and/or appropriately transformed *GPA* V_f data. Multiple regression and ANOVA were used to identify and assess the significance of a particular factor, here the calculated bone marrow dose from administered ^{131}I, on *GPA* V_f after taking into account the contributions of all other potentially confounding variables both nominal and continuous. For this study, potentially confounding factors include age, sex, ethnicity, smoking history, and disease status.

In addition, at each sampling time the measured *GPA* V_f for hemizygous Ø/N and separately for homozygous N/N V_f were averaged for all patients to yield a sampling interval V_f. These sampling interval V_f were then plotted against the time in days relative to ^{131}I treatment. This averaging procedure across all of the patients in the study at a given sampling interval was performed to minimize inter-individual variation and to identify any changes in group V_f related to treatment.

To examine and determine the dose response of the *GPA* assay, linear and nonlinear curve fitting of patient-averaged *GPA* V_f versus clinically-determined bone marrow doses were performed using the least squared error curve fitting method.

Results and Discussion

Study Population

Seventeen newly-diagnosed thyroid cancer patients provided informed consent and participated in the study. Nine of these patients were heterozygous (M/N) at the *GPA* locus. One of these patients died between the fourth and fifth sampling interval and hence did not complete the study. The remainder of these patients completed the one year course of the study and provided all eight blood samples for *GPA* analysis. The demographic summary of the study population is presented in Table 1. The average of the patients was 43.2 years (SD 13.4 years) ranging from 26 to 66 years. Seven of the eight were female. This age and gender distribution was not unexpected as thyroid cancer patients are most likely to be middle-aged females (Mazzaferri, 1997). Six of the eight were nonsmokers, with one ex-smoker and one current smoker.

Table 1. Demographic Data on *GPA* M/N Heterozygous Thyroid Cancer Patients

Patient #	Age	Sex	Smoking Status[1]	Height (In)	Weight (Lbs)
3	35	F	NS	63	126
4	38	F	NS	61	156
5	58	M	NS	70	160
8	55	F	NS	61	135
10	38	F	NS	65	120
12	27	F	NS	66	117
14	31	F	ES (7 Yr)	69	130
15	66	F	S (40 Yr)	65	152

[1]NS, nonsmoker, ES, ex-smoker, S, active smoker

Clinical Data

As detailed in Table 2, the mean administered ^{131}I dose to the seven patients was 125 ± 39 mCi (mean ± SD) resulting in a mean red marrow dose of 31 ± 8 cSv (Model 1) or 36 ± 11 (Model 2).

GPA Assay Data

GPA Ø/N and N/N V_f measured in each blood sample obtained from each of the patients are presented in Tables 3 and 4, respectively.

Table 2. Clinical, Therapeutic and Marrow Dose Summary on *GPA* M/N Heterozygous Thyroid Cancer Patients

Patient #	Diagnosis	^{131}I Dose (mCi)	Thyroid Uptake (%)	Marrow Dose (cSv)[1] Model 1	Model 2
3	Papillary	100	7.9	30	29
4	Papillary	203	0	45	57
5	Papillary	131.4	0.67	30	37
8	Multicentric papillary	102	7.1	31	29
10	Follicular	149.7	0.99	34	42
12	Papillary	102.5	0.85	24	29
14	Papillary	122.7	0.8	28	35
15	Well differentiated papillary	97.49	0.58	22	28

[1]Model 1, Edmonds and Smith, 1986; Model 2, Jensen et al., 1997

Table 3. *GPA* Ø/N V_f for Thyroid Cancer Patients

Sampling Time (Days)	Patient # 3	4	5	8	10	12	14	15
0	5.2	7.8	13.4	12.8	2.0	11.2	3.2	3.8
14	8.6	12.6	14.2	20.2	5.2	13.2	5.2	6.0
31	8.8	7.6	25.0	21.4	6.4	13.4	6.0	7.6
50	6.6	8.8	19.4	19.6	7.8	16.2	4.8	9.4
90	6.6	7.2	12.6	15.6	10.2	12.0	7.8	9.6
120	6.4	7.0	22.6	16.0	6.6	10.2	5.4	6.2
80	7.8	5.8	18.7	13.0	4.2	13.2	3.4	7.6
365	7.2	8.3	17.4	15.8	5.2	11.6	4.0	7.2

Table 4. *GPA* N/N V_f for Thyroid Cancer Patients

Sampling Time (Days)	Patient # 3	4	5	8	10	12	14	15
0	3.4	4.6	153.6	5.8	3.2	4.4	3.2	6.4
14	4.4	4.4	146.0	5.4	5.2	5.6	4.4	7.8
31	3.2	3.2	148.2	5.0	5.8	6.2	1.8	4.8
50	7.4	5.0	157.8	6.0	4.4	5.8	4.0	5.0
90	4.2	5.6	155.4	7.0	5.2	5.8	3.6	5.6
120	4.4	3.8	159.0	5.2	5.4	5.2	5.2	5.4
80	5.8	6.8	156.8	6.8	5.6	5.8	3.8	5.0
365	5.0	7.4	159.9	6.0	7.6	6.4	7.2	6.2

These results, for *GPA* Ø/N and N/N V_f are presented graphically below in Figures 2 and 3, respectively.

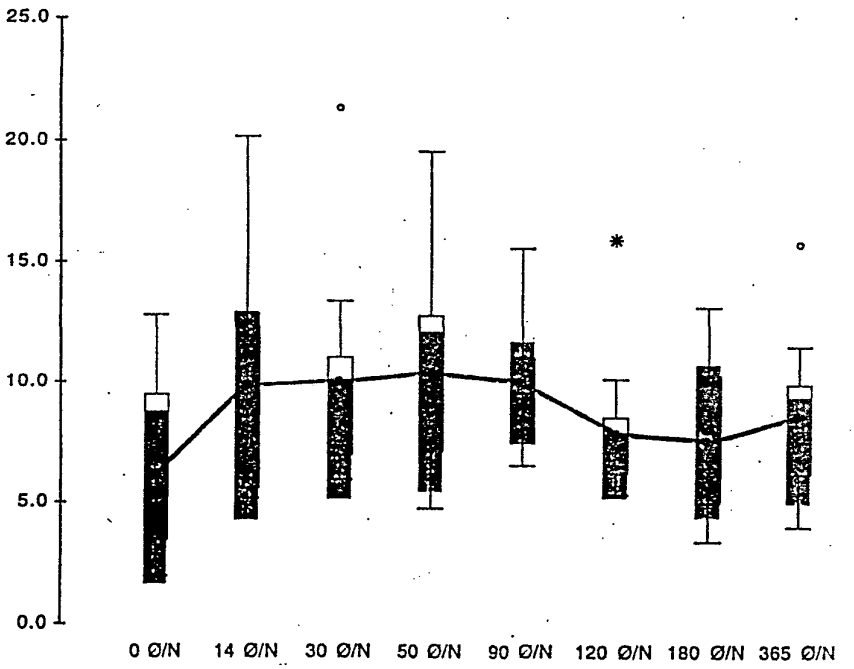

Figure 2. Box plots of *GPA* Ø/N V_f in combined analyses of thyroid cancer patients at each sampling point. The solid line connects the mean V_f observed in the seven patients at each sampling time.

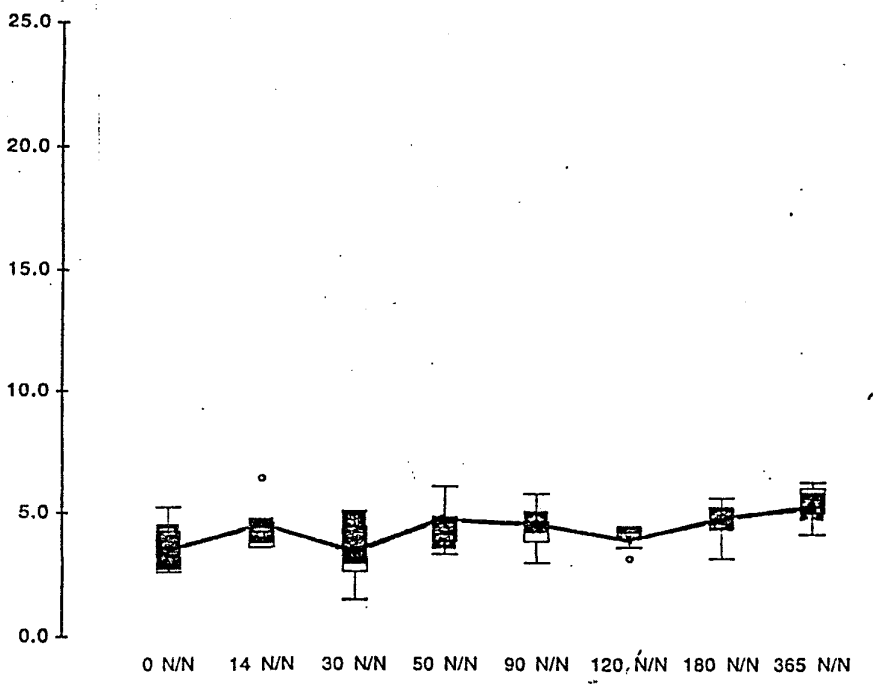

Figure 3. Box plots of *GPA* N/N V_f in combined analyses of thyroid cancer patients at each sampling point. The solid line connects the mean V_f observed in the seven patients at each sampling time.

From the previously obtained acute high dose response function of the *GPA* assay of ~25 induced Ø/N variants per Sv per million cells, this dose would be predicted to induce a persistent mean increase of ~8 Ø/N variants per million cells over the mean pre-therapy level. As illustrated in Figure 2, a much smaller, and non-significant, effect was obtained in these patients with an observed increase of only 1.9 Ø/N variants per million cells (mean V_f of 6.6 at baseline versus 8.5 at 365 days ($P = 0.31$). No significant induction of *GPA* N/N variant cells was apparent in these data. These results are consistent with recent radiation epidemiology studies which suggest a reduced biological effectiveness in the assay for chronic occupational exposures to ionizing radiation (Bigbee *et al.*, 1997; Moore *et al.*, 1997; Tucker *et al.*, 1997).

The time series design of this study with multiple blood sampling shortly after ^{131}I administration permitted, for the first time, the observation of the temporal response of the *GPA* assay immediately following exposure to ionizing radiation. Interestingly, the level of Ø/N variants appears to increase in blood samples collected at times around one red cell lifetime (~120 days). We interpret this result to suggest that the exposure induces *GPA* locus mutations in the relatively large committed erythroid progenitor cell population as well as the smaller long-lived stem cell population in the bone marrow.

This induction would thus produce a wave of erythrocyte variants shortly after exposure followed by a small, but persistent, increase due to the expression of *GPA* locus mutations in the pluripotent bone marrow progenitor cells. No such trend was apparent in the levels of N/N variants consistent with previous observations that ionizing radiation predominantly induces the allele-loss (Ø/N) class of *GPA* variants. These data suggest that the *GPA* assay may be sensitive to the mutagenic effects of ionizing radiation in the short term and thus may have utility as a sensitive biomonitor of very recent accidental radiation exposures.

Inter-individual Variability in GPA Assay Response among Patients

Inter-patient variability in *GPA* assay response as a measure of inter-individual variability to similar radiation exposures could not be usefully addressed in this study. For this group of patients who received relatively low administered doses of ^{131}I, the minimal responses observed in the GPA assay did not allow a rigorous test of individual differences in response. This question of important biological and epidemiologic significance can only be addressed in a group of patients all receiving substantially higher administered ^{131}I doses. We are currently pursuing this follow-up study and are exploring this study design with several nuclear medicine clinical sites, most prominently Mt. Sinai Medical Center and Memorial Sloan Kettering Cancer Center, to identify sufficient numbers of newly-diagnosed and/or relapsed patients to be treated with ^{131}I doses two-three times higher than in the present study.

Conclusions

The results of this biodosimetric study of thyroid cancer patients assayed repetitively before and after ablative ^{131}I therapy demonstrate that the response of the *GPA* assay to low dose/low dose rate ionizing radiation exposure is shallower than that predicted from acute high dose exposure consistent with a DDREF of 4-5. This observation in this study, with well characterized doses to the target cells in the bone marrow, confirms this same observation in earlier population-based studies of occupational ionizing radiation exposures with less reliable dosimetry. Together these results suggest that the *GPA* assay is, like other experimental and biodosimetric systems, substantially less sensitive to chronic ionizing radiation exposures than suggested from earlier acute dose studies, effectively limiting the sensitivity of the assay to exposures exceeding 50 cSv.

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Present and Planned Publications

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Jensen, R.H., Reynolds, J.C., Robbins, J., Bigbee, W.L., Grant, S.G., Langlois, R.G., Pineda, J.D., Lee, T., and Barker, W.C. Glycophorin A as a biological dosimeter for radiation dose to the bone marrow from iodine-131. *Radiation Research* 147: 747-752 (1997).

Livingston, G.K. and Bigbee, W.L. Persistent cytogenetic and mutagenic effects in peripheral blood cells following I-131 radiotherapy. *International Journal of Radiation Biology* (submitted).

Henry, B.J., Burmeister, L.A., Carty S.E., Brown, M., Streb, L.A., Sussman, N.B., Claycamp, H.G., Bigbee, W.L. Glycophorin A (*GPA*) *in vivo* somatic mutation frequencies in radioiodine-treated thyroid cancer patients. *Radiation Research* (manuscript in preparation).

Appendix

University of Pittsburgh Institutional Review Board (IRB) Approval Letters

Patient Informed Consent Form

Clinical Protocol

University of Pittsburgh

Health Sciences
Institutional Review Board for Biomedical Research

219 Nese Barkan Building Annex
c/o WPIC, 3811 O'Hara Street
Pittsburgh, Pennsylvania 15213
412-647-7644

MEMORANDUM

TO: William Bigbee, Ph.D.

FROM: Richard L. Cohen, M.D., Chairman

DATE: December 21, 1994

RE: IRB #9407121: Glycophorin A Biodosimetry in I-131 Treated Patients

The Biomedical Institutional Review Board has reviewed the recent modifications to your protocol and consent form(s) and find them acceptable for expedited review. These changes are noted in your memo of 12/16/94.

The approval date on your consent form(s) should remain the same 7/27/94. Therefore, the protocol and consent form(s) together with a brief progress report must be resubmitted within a year from that time for annual review as required by the General Assurance No. M1259 given to DHHS by the University of Pittsburgh.

If any untoward events occur, please report them at once to the Board. Please refer to the above IRB number on all correspondence or telephone inquiries.

Two copies of your protocol and consent form(s) will be filed in our office. If your protocol involves the CRC, it is your responsibility to immediately forward to them a copy of your IRB approval and a complete protocol/consent form(s) packet.

If your research proposal involves an investigational drug, it is necessary for you to forward a copy of this approval letter along with a copy of the Cover Sheet, protocol, consent form(s) and drug brochure to Anna Giordana, R.Ph., Coordinator, Investigational Drug Service, A131 Scaife Hall.

RLC:jmb

University of Pittsburgh

Health Sciences
Institutional Review Board for Biomedical Research

219 Nese Barkan Building Annex
c/o WPIC, 3811 O'Hara Street
Pittsburgh, Pennsylvania 15213
412-647-7644

MEMORANDUM

TO: William L. Bigbee, Ph.D.

FROM: Samuel Gershon, M.D., Chairman

DATE: August 23, 1995

RE: IRB #9508119: Glycophorin A Biodosimetry in I-131 Treated Patients

Your renewal with modifications of the above protocol has been approved by expedited review by the Chairman of the Institutional Review Board for Biomedical Research.

Please type the approval date (approved: 8/23/95, Biomedical IRB, University of Pittsburgh) on the upper right corner of the consent form before copies are made for patient/normal subject's signature. Please be sure that each patient/subject signs three copies: one for your file, one for the patient's chart, and one for the patient/subject to keep. If the consent form is more than one page, please be sure that each page is signed or initialed, and that each page is numbered.

If any untoward reactions are encountered, please report them at once to the Board.

The protocol and consent form together with a brief progress report must be resubmitted within a year for annual review as required by the Assurance, No. M1259, given to DHHS by the University of Pittsburgh.

Two copies of your protocol and consent form will be filed in our office.

As a reminder, please use the new reference number in all your correspondence and telephone inquiries.

SG/daw

University of Pittsburgh

Health Sciences
Institutional Review Board

219 Nese Barkan Building Annex
c/o WPIC, 3811 O'Hara Street
Pittsburgh, Pennsylvania 15213
412-647-7644

MEMORANDUM

TO: William L. Bigbee, Ph.D.

FROM: Samuel Gershon, M.D., Chairman

DATE: August 1, 1996

SUBJECT: IRB #9508119-9608: Glycophorin A Biodosimetry in I-131 Treated Patients

Your renewal with modifications of the above protocol has been approved by expedited review by the Administrative Vice Chair of the Institutional Review Board under 45 CFR 46.110 (#4).

Approval Date: 8/1/96

Expiration Date: 8/1/97

Please type the approval date on the upper right hand corner of the consent form before copies are made for patient/normal subject's signature. Please be sure that each patient/subject signs three copies: one for your file, one for the patient's chart, and one for the patient/subject to keep.

Any **serious or unexpected** adverse event involving drugs or devices must be reported to the IRB within 10 days of their observation at a University-affiliated site or within 30 days of receipt of notification of such event from the study sponsor. Please see the guidelines for further information on how to report such events.

The protocol and consent forms, along with a brief progress report must be resubmitted at least **one month prior** to the expiration date noted above for annual renewal as required by Assurance No. M1259, given to DHHS by the University of Pittsburgh. In the event the project is not renewed by that date, all research must be suspended until approval is secured. Please notify the IRB in writing when the project is complete so the file can be terminated.

Please use the IRB number noted above in all correspondence and telephone inquiries.

SG:th

University of Pittsburgh

Health Sciences
Institutional Review Board

219 Nese Barkan Building Annex
c/o WPIC, 3811 O'Hara Street
Pittsburgh, Pennsylvania 15213
412-647-7644

MEMORANDUM

TO: William L. Bigbee, Ph.D.

FROM: Samuel Gershon, M.D., Chairman S. Gershon /cm

DATE: September 15, 1997

SUBJECT: IRB #9508119: Glycophorin A Biodosimetry in I-131 Treated Patients

Your renewal with modifications has been approved by the Institutional Review Board.

Approval Date: September 10, 1997

Expiration Date: September 10, 1998

Please type the approval date on the upper right hand corner of the consent form before copies are made for patient/normal subject's signature. Please be sure that each patient/subject signs three copies: one for your file, one for the patient's chart, and one for the patient/subject to keep.

Any **serious or unexpected** adverse event involving drugs or devices must be reported to the IRB within 10 days of their observation at a University-affiliated site or within 30 days of receipt of notification of such event from the study sponsor. Please see the guidelines for further information on how to report such events.

The protocol and consent forms, along with a brief progress report must be resubmitted at least **one month prior** to the expiration date noted above for annual review as required by Assurance No. M1259, given to DHHS by the University of Pittsburgh. In the event the project is not renewed by that date, all research must be suspended until approval is secured. Please notify the IRB in writing when the project is complete so the file can be terminated.

Please be advised that your research study may be audited periodically by the Office of Research, Health Sciences.

As a reminder, please use the new reference number in all your correspondence and telephone inquiries.

SG/cm

University of Pittsburgh

Institutional Review Board

Liliane S. Kaufmann Building
Suite 1212
3471 Fifth Avenue
Pittsburgh, PA 15213
412-692-4370
Fax: 412-692-4332

MEMORANDUM:

TO: William L. Bigbee, Ph.D.

FROM: Dennis P. Swanson M.S., Vice Chairman

DATE: September 10, 1998

SUBJECT: IRB #9508119: Glycophorin A Biodosimetry in I-131 Treated Patients

Your renewal of the above-referenced proposal has received expedited review and approval by the Institutional Review Board. **This approval is for analysis of data only. In the event that recruitment is reinstituted, please submit a modification request.**

Approval Date: **September 10, 1998**
Expiration Date: **September 10, 1999**

The protocol and consent forms, along with a brief progress report must be resubmitted at least **one month prior** to the expiration date noted above for annual renewal as required by Assurance No. M1259, given to DHHS by the University of Pittsburgh.

Please be advised that your research study may be audited periodically by the Office of Research, Health Sciences.

DPS/sas

University of Pittsburgh

Graduate School of Public Health
Department of Environmental and Occupational Health

260 Kappa Drive
Pittsburgh, Pennsylvania 15238
412-967-6500
Fax: 412-624-1020

Approved: 08/01/96
Biomedical IRB #9508119-9808
University of Pittsburgh

CONSENT TO ACT AS A SUBJECT IN AN EXPERIMENTAL STUDY

TITLE: Glycophorin A Biodosimetry in I-131 Treated Patients

INVESTIGATOR: William L. Bigbee, Ph.D., Principal Investigator
Associate Professor
Department of Environmental and Occupational Health
Graduate School of Public Health
University of Pittsburgh
260 Kappa Drive RIDC Park
Pittsburgh, PA 15238
Telephone: (412) 967-6534

CLINICAL COLLABORATORS: Manuel L. Brown, M.D.
Professor of Radiology
Director of Nuclear Medicine
University of Pittsburgh Medical Center

Lynn A. Burmeister, M.D.
Assistant Professor of Endocrinology
University of Pittsburgh Medical Center

Donor's initials _____

Sally E. Carty, M.D.
Assistant Professor of Surgery
University of Pittsburgh Medical Center

Dennis Swanson, M.S.
School of Pharmacy
University of Pittsburgh Medical Center

Charles G. Watson, M.D.
Professor of Surgery
University of Pittsburgh Medical Center

SOURCE OF SUPPORT: Centers for Disease Control and Prevention (CDC)
National Institute for Occupational Safety and Health (NIOSH)

DESCRIPTION: I understand that I have been asked to participate in a research study investigating genetic changes in human blood cells due to exposure to radiation attendant to my clinical treatment with I-131. Both female and male subjects of all ages over 18 will be invited to participate. Volunteers are asked to participate during these studies over the next three years. Blood samples will be obtained by standard arm venipuncture by a licensed M.D or trained phlebotomist with a licensed M.D. in attendance. The maximum total amount of blood drawn for each sample will be 50 mL (approximately 1.7 fluid ounces).

RISK AND BENEFITS: Risks include the possibility of fainting, a bruise or soreness at the site of venipuncture, or a spasm with temporary loss of blood flow at the site. No direct personal or medical benefits are expected for any individual volunteer.

ALTERNATIVE TREATMENTS: None

Donor's initials _____

NEW INFORMATION: If new information, either good or bad, about this treatment comes to the attention of the investigator during the course of this study which may relate to my willingness to participate, it will be provided to me or my representative.

COSTS AND PAYMENTS: I will not be charged for my participation in this study. I understand that I can elect to receive $10 per blood sample that I donate to the study.

COMPENSATION FOR ILLNESS OR INJURY: I understand that I will not be compensated for any injury or illness resulting from this study, but any emergency medical treatment which may be necessary will be provided.

CONFIDENTIALITY: All personal information about me, such as gender, age, smoking, and medical history, will be maintained in paper form identified only by a code number. The blood samples I provide will also be identified only by this code number. The record linking my name and this code number, as well as the electronic database containing my personal information, will be maintained by the investigators as an encrypted computer file with access limited only to the investigators. This process will insure that any information about me will be treated in the same confidential manner as other hospital medical records. At no time will my name be linked to any of the personal data I provide or any assay results obtained using my blood sample. I consent to publication of any information for scientific purposes so long as my identity will not be revealed.

RIGHT TO WITHDRAW: I understand that I may refuse to participate in this study or withdraw at any time and that my decision will not adversely affect me in any way. I also understand that I may be withdrawn as a subject in the study by the investigators.

VOLUNTARY CONSENT: Dr. Bigbee, Dr. Brown, Dr. Burmeister, Dr. Carty, Mr. Swanson, Dr. Watson, or their representative has explained all of this to me and has answered all questions I have. I also understand that any future questions I have about this research will be answered by Dr. Bigbee who I may call at (412) 967-6534. Any questions I have about my rights as a research subject will be answered by the Office of the Senior Vice President for Health Sciences at the University of Pittsburgh. By signing this form, I agree to participate in this study.

Donor's initials _____

Signature

Witness

Date

INVESTIGATOR'S CERTIFICATION:

I declare that I have personally explained the above information to the volunteer subject.

Investigator's/Clinical Collaborator's signature

Witness

Date

CLINICAL PROTOCOL

UNIVERSITY OF PITTSBURGH MEDICAL CENTER (UPMC)

PROJECT TITLE: Glycophorin A Biodosimetry in ^{131}I Treated Patients

IRB APPROVAL #: #9508119
APPROVAL DATE: 9/10/98
EXPIRATION DATE: 9/10/99

PRINCIPAL INVESTIGATOR:

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Graduate School of Public Health
University of Pittsburgh
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Pittsburgh, PA 15238
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FAX: (412) 648-9551
E-mail: watson@pitt.med.edu

PROTOCOL COORDINATOR:

Lisa Streb, R.N.
497 Scaife Hall
University of Pittsburgh Medical Center
Telephone: (412) 648-1921, Beeper 6258
FAX: (412) 648-9551
E-mail: streb@pitt.med.edu

STUDY OVERVIEW

Time series peripheral blood samples from patients with thyroid disease treated with ^{131}I at UPMC will be analyzed using the glycophorin A (*GPA*) *in vivo* somatic cell mutation assay to determine the frequency of erythrocytes with *GPA* allele-loss variant phenotypes induced by this whole body radiation exposure. The frequency of these *GPA* erythrocytes in peripheral blood is a measure of the level of cumulative somatic mutation that has occurred at this locus in bone marrow erythroid progenitor cells. Previous studies using the assay have demonstrated a persistent and dose dependent increase in the frequency of *GPA* allele-loss variant cells in individuals exposed to ionizing radiation. The assay therefore has validated utility as a retrospective biodosimeter of human exposure to radiation.

This study of ^{131}I treated patients is being undertaken to better characterize and calibrate the radiation dose response of the *GPA* assay over a range of whole-body doses received by individuals with occupational/environmental radiation exposures who are the subject of ongoing epidemiologic studies employing the assay as a retrospective biodosimeter. These study populations include cleanup workers at Chernobyl, populations living in contaminated areas near Chernobyl, x-ray technicians, and employees of nuclear fuel reprocessing facilities.

This protocol describes the criteria for selection and enrollment of ^{131}I treated UPMC patients, scheduling and acquisition of peripheral blood samples, and identification and collection of relevant clinical data.

PATIENT REQUIREMENTS

- Diagnosis of thyroid disease to be treated with administration of ^{131}I
- Age of 18 years or older
- Willingness to provide informed consent to participate in the study and to provide up to eight (8) blood samples of approximately 50 mL each over a period of one (1) year
- M/N blood type (to be determined from serotyping of the pre-therapy sample)
- Normal complete blood count (CBC)

PATIENT EXCLUSION CRITERIA

- Under 18 years of age
- Previous chemotherapy or radiotherapy
- Blood transfusion within the last year
- Pregnancy
- M/M or N/N blood type (to be determined from serotyping of the pre-therapy sample) Approximately one-half of potential subjects will be excluded by this criteria.

PATIENT ENROLLMENT PROTOCOL

Participating UPMC clinical collaborators and/or the protocol coordinator will initially discuss the opportunity to participate in the study with each newly-diagnosed thyroid disease patient scheduled for therapy with ^{131}I. The patient's physician and/or the study protocol coordinator will review with the patient the inclusion and exclusion criteria to determine if the patient can be enrolled in the study.

Those patients expressing an interest in participating and who satisfy the study inclusion and exclusion criteria will then be furnished with a copy of the study consent form. After reading the form, the patient will be afforded an opportunity to ask questions regarding the study of the collaborating physician, protocol coordinator, and/or the principal investigator. Those patients wishing to be enrolled in the study and who are willing to provide informed consent shall initial each page and sign the final page of the consent form in the presence of their physician, the principal investigator, or the clinical coordinator of the study. <u>The consent form must also be signed and dated by the principal investigator, clinical collaborator, or protocol coordinator and a witness as indicated on the final page of the form</u>.

The original copy of the completed consent form will be kept by the principal investigator, with copies retained by the patient's physician and by the protocol coordinator. The patient will also be supplied with a copy of the completed consent form.

<u>No collection of blood samples or clinical data from a patient can occur prior to the patient' s witnessed signature of the consent form</u>.

BLOOD SAMPLING METHOD AND SCHEDULE

Peripheral blood samples from patients enrolled in the study will be obtained prior to the administration of ^{131}I (pre-therapy sample) and at approximately 14, 31, 50, 90, 120, 180, and 365 days following therapy. The pre-therapy sample and samples collected at 14, 31 and 50 days following therapy are to be obtained as part of the standard clinical care of the patient. Samples at 90, 120, 180 and 365 days following therapy are to be drawn expressly for the purpose of this research study.

The eight (8) blood samples will be collected by standard arm veinipuncture by a licensed M.D or trained phlebotomist with a licensed M.D. in attendance at the UPMC or other clinical center of convenience for the patient. Scheduling arrangements for obtaining the required blood samples from each patient in the study will be the responsibility of the protocol coordinator. The volume of each blood sample will be approximately 50 mL drawn into five (5) 10 mL Vacutainer® tubes contained sodium heparin anticoagulant. Each tube will be labeled only with the patient's study ID number (to be assigned by the protocol coordinator as specified below) and with the draw date. The patient's name is not to appear on the blood sample tubes.

As soon as possible after the blood samples are obtained, the protocol coordinator will contact the analytical laboratory to arrange for transportation of the samples. The contact persons in the laboratory are:

Ms. Barbara J. Henry
Telephone: (412) 967-6591
FAX: (412) 624-1020
E-mail: wlbigbee+@pitt.edu

William L. Bigbee, Ph.D.
Telephone: (412) 967-6534
FAX: (412) 967-6576, (412) 624-1020
E-mail: wlbigbee+@pitt.edu

BLOOD SAMPLE ENCODING

To insure patient anonymity and to blind laboratory personnel, the blood samples will be labeled with a study ID number assigned by the protocol coordinator. The ID number will be composed of the prefix PTS (for Pitt Thyroid Study) and the last four (4) digits of the patient's social security/hospital number followed by a "dash" and two (2) or three (3) digits corresponding to the scheduled draw day of the sample, e.g., PTS2875-00 (for pre-therapy sample), PTS2875-31, PTS2875-180 (for days 31 and 180, respectively), etc. An encoded and password-protected computer file containing the patient's name, and ID numbers and dates of all blood samples collected from the patient will be maintained by the protocol coordinator who will monthly update a second copy of the file maintained by the principal investigator.

BLOOD SAMPLE STORAGE AND TRANSPORTATION

The blood samples are to be stored at room temperature (~22°C). The tubes should be labeled with the statement "STORE AT ROOM TEMPERATURE, DO NOT FREEZE". The samples are to be transported to the analytical laboratory, maintained at this same temperature in insulated shipping containers (with refrigerated cool packs if necessary) as soon as possible and in no case later than 48 hours after collection.

INITIAL M,N SEROTYPING

Since only approximately one-half (1/2) of the patients initially enrolled will be of the required M/N serotype required for the *GPA* assay, initial M,N typing results obtained in the analytical laboratory for the pre-therapy sample will be communicated as soon as possible to the protocol coordinator. The protocol coordinator will communicate this result to the patient to insure that patients of the required M/N serotype will be retained in the study and the additional post-therapy blood samples obtained. Patients not of M/N blood type, i.e. those of M/M and N/N blood type, will be informed that they are ineligible for the study.

CLINICAL DATA

The following personal and clinical data will be obtained by the protocol coordinator for each M/N patient enrolled in the study:

Name
Address
Home and work telephone numbers
Sex
Age
Height
Weight
Ethnicity
Attending physician
Diagnosis
Clinical treatment regimen, including dose and schedule of ^{131}I administration
^{131}I retention from whole body counting measurements
CBC results
All medications
All other significant medical conditions and history

GPA ASSAY METHODS AND DATA STORAGE

The *GPA* somatic cell mutation assay will be performed on the patient's peripheral blood samples, together with laboratory standard blood samples for the purpose of quality control, using the previously published DB6 version of the assay run on a Becton Dickinson FACScan™ flow cytometer. The results of analysis of each sample yielding the number of Ø/N and N/N variant red cells detected in 5×10^6 total cells will be recorded in a database maintained by the staff of the analytical laboratory and identified with the study ID number. This database will be also be maintained by the principal investigator and updated monthly by the staff of the analytical laboratory. In addition, hardcopy computer printouts of the results of each *GPA* analysis will be produced, and reviewed and retained by the principal investigator.

ERYTHROCYTE AND LYMPHOCYTE CRYOPRESERVATION

Formalin fixed and sphered erythrocyte preparations for each sample will be frozen using the standard "frozen spheres" protocol and cryopreserved at -70°C by the staff of the analytical laboratory. Viable lymphocytes will be prepared from the remainder of the ~50 mL sample of heparinized blood using the established method of centrifugation through a continuous density gradient of Ficoll-Paque™, frozen using an automated controlled-rate freezer, and stored at -150°C.

RESPONSIBILITIES OF THE PROTOCOL COORDINATOR:

- Maintain copies of current clinical protocol and IRB approval
- Maintain stock of patient consent forms
- Maintain stock of Styrofoam shipping containers and cool packs
- Be available to assist clinical collaborators with enrollment of new patients
- Maintain file of copies of completed patient consent forms
- Setup and maintain encoded and password-protected computer file containing patient data as detailed in the protocol
- Regularly communicate with clinical collaborators regarding availability of newly-diagnosed patients and scheduling of post-therapy appointments and blood sample collection
- Monitor time schedule of all enrolled patients and communicate with patients to schedule blood drawing appointments at UPMC or other convenient clinical centers as prescribed in the study protocol
- Arrange for proper storage and transportation of blood samples
- Make payments to the patients for blood samples and maintain receipts and record of payments
- Forward required patient data to the principal investigator as prescribed in the study protocol
- Participate in scheduled meetings of the principal investigator and clinical collaborators