

Single Nucleotide Polymorphisms, Apoptosis, and the Development of Severe Late Adverse Effects After Radiotherapy

David Azria,^{1,2} Mahmut Ozsahin,⁴ Andrew Kramer,³ Sheila Peters,⁵ David P. Atencio,⁵ Nigel E.A. Crompton,⁹ Françoise Mornex,¹⁰ André Pèlerin,¹ Jean-Bernard Dubois,^{1,2} René-Olivier Mirimanoff,⁴ and Barry S. Rosenstein^{5,6,7,8}

Abstract Purpose: Evidence has accumulated in recent years suggestive of a genetic basis for a susceptibility to the development of radiation injury after cancer radiotherapy. The purpose of this study was to assess whether patients with severe radiation-induced sequelae (RIS; i.e., National Cancer Institute/CTCv3.0 grade, ≥ 3) display both a low capacity of radiation-induced CD8 lymphocyte apoptosis (RILA) *in vitro* and possess certain single nucleotide polymorphisms (SNP) located in candidate genes associated with the response of cells to radiation.

Experimental Design: DNA was isolated from blood samples obtained from patients ($n = 399$) included in the Swiss prospective study evaluating the predictive effect of *in vitro* RILA and RIS. SNPs in the *ATM*, *SOD2*, *XRCC1*, *XRCC3*, *TGFB1*, and *RAD21* genes were screened in patients who experienced severe RIS (group A, $n = 16$) and control subjects who did not manifest any evidence of RIS (group B, $n = 18$).

Results: Overall, 13 and 21 patients were found to possess a total of < 4 and ≥ 4 SNPs in the candidate genes. The median (range) RILA in group A was 9.4% (5.3-16.5) and 94% (95% confidence interval, 70-100) of the patients (15 of 16) had ≥ 4 SNPs. In group B, median (range) RILA was 25.7% (20.2-43.2) and 33% (95% confidence interval, 13-59) of patients (6 of 18) had ≥ 4 SNPs ($P < 0.001$).

Conclusions: The results of this study suggest that patients with severe RIS possess 4 or more SNPs in candidate genes and low radiation-induced CD8 lymphocyte apoptosis *in vitro*.

Radiation injury may develop months to years after radiotherapy (RT) with the manifestation of diverse pathologic lesions such as fibrosis, necrosis, atrophy, and vascular damages (1). In recognition of the large number of people who survive for many years after a cancer diagnosis, the National Cancer

Institute has identified cancer survivorship and the development of adverse late sequelae resulting from the use of therapeutic modalities, including RT, as an important area that has been under researched (2).

A variety of patient, tumor, treatment, cellular, and molecular factors contribute to the variability in severity of normal tissue reactions exhibited after RT. In addition, it has been hypothesized that individual intrinsic radiosensitivity may influence the development of adverse radiation responses (3). Therefore, efforts have been made to develop assays capable of predicting susceptibility for the development of radiation injury that would allow customization of RT protocols on an individual basis (3, 4). By doing so, it has been estimated that a significant improvement in the therapeutic index could be achieved. In the clinic, two complementary approaches have been explored with encouraging results: the lymphocyte assay (4) and single nucleotide polymorphism (SNP) screening (3).

Lymphocyte apoptosis was developed as a rapid tool for characterization of normal tissue radiosensitivity (5-8), particularly due to the ease of blood collection in a standardized, patient-convenient manner. In a prospective study, blood samples were obtained from 399 patients treated with curative intent and tested using CD8 lymphocyte apoptosis after an X-ray dose of 8 Gy. Apoptosis was assessed by associated condensation of DNA in lymphocytes. The 2-year cumulative incidence for grade 2 or 3 late toxicity was 70%, 32%, and 12% for patients with absolute changes in CD8 T-lymphocyte apoptosis of $\leq 16\%$, 16% to 24%, and $> 24\%$, respectively (4).

Authors' Affiliations: ¹Inserm, U896, Institut de Recherche en Cancérologie de Montpellier, ²Department of Radiation Oncology, and ³Biostatistics Unit, CRLC Val d'Aurelle-Paul Lamarque, Montpellier, France; ⁴Department of Radiation Oncology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; Departments of ⁵Radiation Oncology, ⁶Dermatology, and ⁷Community and Preventive Medicine, Mount Sinai School of Medicine and, ⁸Department of Radiation Oncology, NYU School of Medicine, New York, New York; ⁹Laboratory of Noninvasive Imaging and Radiation Biology, Van Andel Research Institute, Grand Rapids, Michigan; and ¹⁰Department of Radiation Oncology, Centre Hospitalier Universitaire Lyon Sud, Pierre Benite, France

Received 3/16/08; revised 6/2/08; accepted 6/18/08.

Grant support: US Department of the Army (grant DAMD 17-02-1-0503), the Swiss Cancer League (KFS 00539-9-1997 and SKL 00778-2-1999), the Programme Hospitalier de Recherche Clinique 2005 from the French "Institut National du Cancer", and was included in the Gene-PARE project (*Genetic Predictors of Adverse Radiotherapy Effects*).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: D. Azria and M. Ozsahin contributed equally to this work.

Requests for reprints: Barry S. Rosenstein, Box 1236, Department of Radiation Oncology, Mount Sinai School of Medicine, One Gustave Levy Place, New York, NY 10029. Phone: 212-241-9408; Fax: 212-996-8927; E-mail: barry.rosenstein@mssm.edu.

©2008 American Association for Cancer Research.
doi:10.1158/1078-0432.CCR-08-0700

Translational Relevance

The radiation-induced CD8 lymphocyte apoptosis assay combined with detection of single nucleotide polymorphisms associated with clinical radiosensitivity may provide a basis for an assay to predict which patients diagnosed with cancer are at greatest risk for developing adverse effects resulting from radiotherapy. For these patients, use of a standard treatment protocol may be contraindicated and use of a radiotherapy technique that limits the volume and radiation dose to normal tissues may be critical to the success of their cancer therapy. Alternatively, a strictly surgical treatment, if possible, may be most appropriate for such patients. Such a predictive assay may also permit identification of patients at reduced risk for developing radiation injury after radiotherapy and allow dose escalation for such individuals in an effort to enhance the probability for tumor cure.

Consistent with these results are those reported by Svensson et al. (9) who examined the expression of a series of genes associated with radiation response in RT patients with or without late radiation toxicity. It was reported that the majority of the discriminative genes and gene sets belonged to the ubiquitin, apoptosis, and stress signaling networks. In addition, it has been reported that possession of variants in genes, the products of which play a role in radiation response, may be associated with the development of adverse effects after RT (3). Candidate genes that possess SNPs associated with radiation-induced sequelae include *ATM*, *TGFB1*, *XRCC1*, *XRCC3*, *SOD2*, and *RAD21*. The selection of these specific six genes for SNP screening was based on previous results indicating that these genes possess SNPs associated with the development of adverse effects from RT (3, 10–14).

Here, we present evidence that patients with severe radiation-induced sequelae display both a low radiation-induced CD8 lymphocyte apoptosis (RILA) *in vitro* and possess SNPs located in candidate genes associated with the response of cells to radiation.

Materials and Methods

Blood samples. Between April 1998 and October 2001, a total of 399 patients with miscellaneous cancers were included in the KFS 00539-9-1997/SKL 00778-2-1999 prospective study evaluating the predictive value of CD4 and CD8 T-lymphocyte apoptosis on the development of radiation-induced late side effects (4). Among them, 28 patients (7%) developed grade 3 sequelae. These latter patients experienced 32 grade 3 late side effects: 14 subcutaneous, 5 skin, 9 salivary gland, 1 brain, 1 pharynx, 1 ear, and 1 mucous membrane. At the 2-y visit, 24 patients maintained the same grade 3 late toxicity. Grade 3 late toxicities were mainly observed for patients with breast (9%), and head and neck (16%) cancers. These patients were invited to participate in the Gene-Pare project (3) for genetic profile evaluations (see details below). Sixteen patients (group A) accepted this new study approved by the Lausanne University Ethical Committee and came for a medical visit where informed consent were signed before sending blood samples to the Mount Sinai hospital in New York (Pr Rosenstein) for genetic screening. Details of all patients were described (4) but briefly, 9 of 16 (56%) and 6 of 16 (38%) breast and head and neck

cancer patients, respectively, experienced grade 3 subcutaneous fibrosis. One patient (6%) treated for a localized meningioma presented a severe intracerebral edema with long term treatment of corticoids.

The second step was to include “control” patients (group B) from this trial treated for breast and head and neck cancers and meningioma without any long term clinical signs of radiation-induced toxicities. We stopped accrual when a “matched” sample size was obtained in comparison with group A, i.e., 10 breast, 7 head and neck, and 1 meningioma. The selection of the “matched” patients was made from the entire population included initially in the prospective trial. Just before the genetic screening, but after identifying patients who suffered from severe side effects, we selected patients who did not present any radiation-induced sequelae. The selection was made according to the type and location of the tumor, initial tumor size, total radiation dose, irradiated volume, concurrent tamoxifen (breast), or cisplatin (head and neck) administration. No statistical difference was found between the two groups. Blood samples were sent to the Mount Sinai hospital in New York for genetic screening. The human investigations were done after approval by the local institutional review board.

Toxicity assessments. Toxicities were graded according to the RTOG/European Organization for Research and Treatment of Cancer system in all patients by the same physician without knowledge of any translational studies so as not to bias the evaluation (15). The timing of late side effects from 6 wk post-RT up to 2 y corresponds to the time of observation of the worst late toxicity grade. The exposure to concomitant RT chemotherapy (head and neck cancer patients), concomitant hormonotherapy, and post-RT chemotherapy (mainly breast cancer patients) as well as all technical RT modalities (irradiated volume, total dose, dose at the surface of the skin, and dose fractionation) were also taken into account to evaluate late side effects (4, 16).

Genetic screening. The lymphocyte isolation, DNA extraction, and denaturing high-performance liquid chromatography procedures were done as previously described (12). All PCR products were subjected to either denaturing high-performance liquid chromatography or the Surveyor nuclease assay using a Transgenomic WAVE High Sensitivity Nucleic Acid Fragment Analysis System. PCR primers for the DNA amplicons encompassing the SNPs of interest were designed using the genomic sequence obtained from National Center for Biotechnology Information¹¹ and the online primer design program Primer3.¹² The SNPs in *ATM* were identified using denaturing high-performance liquid chromatography, whereas the SNPs in all other candidate genes were detected using the Surveyor nuclease assay, which was done as described (17–20).

Briefly, a mixture was added to each 25 μ L PCR reaction product consisting of 0.5 μ L 10 \times buffer, 1 μ L Surveyor nuclease W, 1 μ L Enhancer W, and 2.5 μ L double-distilled water (Transgenomic Surveyor kit). The reaction was incubated at 42 $^{\circ}$ C for min. At this time, the reaction was terminated by adding 5 μ L of a mixture of 3 μ L Stop Buffer and 2 μ L double-distilled water. For each primer set, a gradient was chosen using Navigator software (Transgenomic) based on the size of the PCR fragment. The “DS multiple fragments” setting was used at 45 $^{\circ}$ C with the addition of the dye WAVE-HS1 (Transgenomic). The fragment length minimum was set to 25 bp, and the fragment length maximum was set to roughly 50 to 75 bp larger than the size of the fragment. The resulting traces were examined for the presence of multiple peaks suggestive of heterozygosity for the SNP, comparing the sizes against a low molecular weight DNA ladder (New England Biolabs). To identify patients who may be homozygous for the minor allele for each SNP, an equal amount of DNA derived from a subject known to be homozygous for the major allele was mixed with each DNA sample before the PCR to create a potentially heterozygous SNP if that patient had been homozygous for the minor

¹¹ <http://www.ncbi.nlm.nih.gov>

¹² <http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.www.cgi>

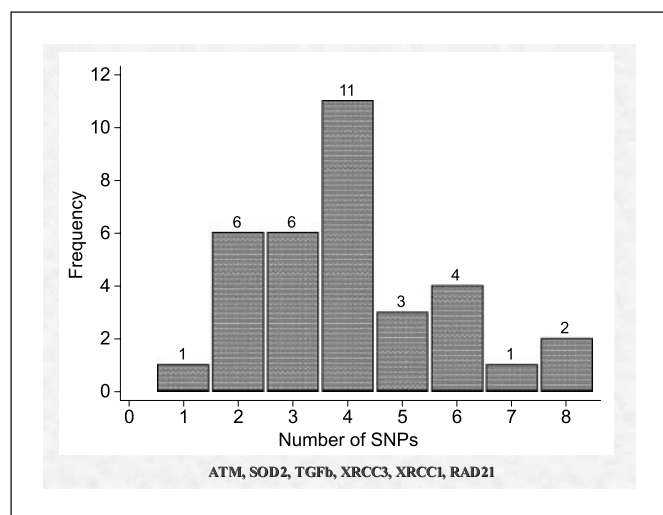


Fig. 1. Distribution of the number of SNPs in the study population. The candidate genes were selected from previous studies as *ATM*, *SOD2*, *TGFβ*, *XRCC3*, *XRCC1*, and *RAD21*. Thirteen and 21 patients presented less than three and four or more SNPs, respectively.

allele. Any samples for which the chromatogram differed from that obtained for DNA homozygous for the major allele were subjected to DNA sequencing using an ABI BigDye Terminator Version 3.1 Ready Reaction Cycle Sequencing kits and an ABI 3730xl DNA Analyzer. All analyses were blinded to treatment, side effects, and RILA yield.

RILA. As described (4, 5, 8), heparinized whole blood collected before starting RT was diluted 1:10 in RPMI 1640 containing 20% fetal bovine serum, irradiated with 0 and 8 Gy, and incubated for 48 h. The cells were then labeled with FITC-conjugated anti-CD8 monoclonal antibodies, RBC were lysed, and the DNA of the remaining cells stained with propidium iodide. Samples were measured using a FACScan flow cytometer, and data analysis was done using CellQuest software (Becton Dickinson). Apoptotic CD8 T-lymphocytes were defined as those cells staining positively for their cell type-specific antibodies and displaying reduced propidium iodide fluorescence and cell size. These cells were previously shown to be apoptotic using the terminal deoxynucleotidyl-transferase-mediated dUTP nick-end labeling assay (8). Data from at least 10,000 cells per sample were acquired.

In our earlier study (4), a decreased percentage of grade 2 or more late toxicity was observed for increasing values of CD4 and CD8. No grade 3 side effects were observed for patients with CD4 apoptosis of >15% and CD8 apoptosis of >24%. A multivariate receiver-operating characteristics analysis for CD4 and CD8 was done among the patients who were evaluated at 2 y, or who experienced a grade ≥ 2 before 2 y. The area under the receiver-operating characteristics curve was greater for CD8 [0.827; 95% confidence interval (95% CI), 0.78-0.87] than for CD4 (0.714; 95% CI, 0.66-0.77). The addition of CD4 into the model did not contribute significantly in separating the two groups (area under the curve, 0.84). Therefore, we decided to take into account only CD8 results for the present study. In the prospective trial (4), all apoptosis yields were blindly collected by a technician before final analyses.

Statistical methods. Fisher's exact test was used for the comparison of 2×2 contingency tables. An odds ratio was estimated from the probabilities of developing grade ≥ 3 toxicity between groups A and B. 95% CIs for probabilities were obtained from the binomial distribution. Youden's index associated with receiver-operating characteristics curves was obtained from sensitivity (Se) and specificity (Sp) as $Se+Sp-1$. This index was used to determine the best cutoff point for the number of SNPs. Median values for continuous variables were compared between groups with the Wilcoxon nonparametric test.

Results

Genotype distributions and severe radiation-induced sequelae. The genotype distributions are depicted in Figs. 1 and 2. A putative model for estimation of fibrosis risk based on multiple SNPs was used as has been described (13). Overall, 13 and 21 patients were found to possess a total of <4 and ≥ 4 SNPs in the candidate genes screened (*ATM*, *TGFβ*, *SOD2*, *XRCC1*, *XRCC3*, and *RAD21*). Among the 16 patients who experienced grade ≥ 3 sequelae (group A), 15 (94%; 95% CI, 70-100%) carried ≥ 4 SNPs compared with 6 of 18 (33%; 95% CI: 13-59%) patients without any clinical signs of RT-induced late toxicity ($P < 0.001$).

The probability for the development of grade ≥ 3 toxicity (Fig. 3) was significantly higher (odds ratio 9.3; 95% CI, 1.4-62; $P = 0.003$) for patients with ≥ 4 SNPs (0.71; 95% CI, 0.48-0.89) compared with those with <4 SNPs (0.08; 95% CI, 0.01-0.36). In terms of sensitivity and specificity, as can be seen from Fig. 2, the cutoff at 4 or more SNPs gave the largest values for Youden's

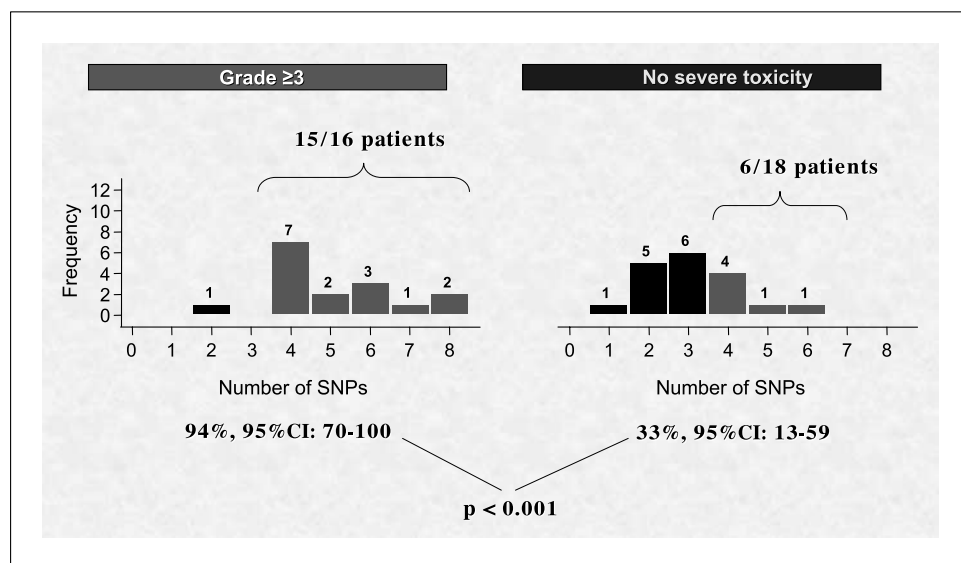


Fig. 2. Distribution of the number of SNPs according to radiation-induced late effects (grade ≥ 3 versus grade <3). Ninety-four percent of (15 of 16) patients presented ≥ 4 SNPs in the grade ≥ 3 toxicity group compared with 33% (6 of 18) in the control group without any severe clinical radiation-induced toxicity ($P < 0.001$).

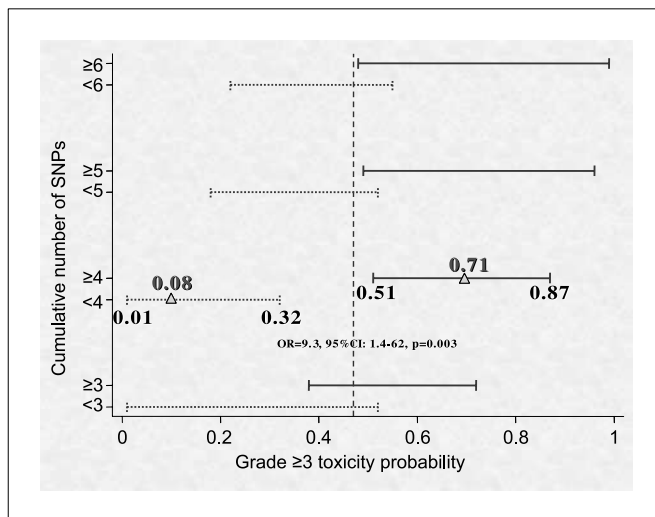


Fig. 3. The probability of grade 3 or more toxicity as a function of the cumulative number of SNPs. Four and more SNPs are strongly correlated with the risk of grade ≥ 3 toxicity; odds ratio, 9.3, 95% CI, 1.4-62; $P = 0.003$.

index with sensitivity (true positives) of 94% (15 of 16) and specificity (true negatives) of 67% (12 of 18) corresponding to an index of 0.61. At the 4 or more cutoff value, the positive predictive value was 71% and the negative predictive value was 92%. Few patients (10 of 34) had 5 or more SNPs in our study. Youden's index was lower and equal to 0.39 (sensitivity, 50%; specificity, 89%) and a positive predictive value of 80% and negative predictive value of 67%.

Taken individually, the total numbers of SNPs detected in *ATM* 5557 G>A (number of patients, $n = 15$), *SOD2* 47 T>C ($n = 22$), *TGFB1* -509 C>T ($n = 18$) and 870 T>C ($n = 24$), *XRCC1* 1321 G>A ($n = 15$), *XRCC3* 1075 C>T ($n = 18$), and *RAD21* 1625 T>C ($n = 10$) were greater in group A compared with group B (Table 1).

Low RILA, number of SNPs, and radiation-induced sequelae. Grade 3 late toxicities were mainly observed for patients with breast (9%), and head and neck (16%) cancers with an inverse relationship between the incidence of late side effects and percent CD8 apoptosis. The median (range) RILA in group A was 9.4% (5.3-16.5), and 94% (95% CI, 70-100) of patients (15 of 16) had ≥ 4 SNPs. In our control population (group B), median (range) RILA was 25.7% (20.2-43.2), and 33% (95% CI, 13-59) of patients (6 of 18) had ≥ 4 SNPs ($P < 0.001$; Fig. 2).

Discussion

Normal-tissue radiobiology has achieved notable advances mainly through an improved mechanistic understanding of molecular pathogenesis (21). Radiation-induced tissue remodeling is a complex process driven by intercellular communication via cytokines and growth factors, which are induced during the radiation response of participating cells (22).

Mechanistically, the early steps of radiation-induced fibrosis are similar to those of a wound-healing response characterized by an up-regulation of cytokines with proinflammatory recruitment of cells within the surrounding irradiated tissue (21). Transforming growth factor- β is secreted in a latent form and may be activated directly in the extracellular space by a triggering event such as ionizing radiation (23). The second key process characteristic of radiation fibrogenesis is damage of endothelial cells (24), which activates the apoptosis death program and also leads to release of pro-fibrotic cytokines, mainly transforming growth factor- β (21).

We reported that radiation-induced T-lymphocyte apoptosis can be predictive for grade 2 and 3 late effects ($P < 0.0001$). Patients with grade 3 late effects showed CD8 radiation-induced apoptosis significantly ($P < 0.0001$) below the median (4).

Regarding the relationship between genetic variation and clinical radiosensitivity (3, 13), we screened for SNPs in candidate genes that are associated with the response of cells to radiation: *ATM*, *TGFB1*, *SOD2*, *XRCC3*, *XRCC1*, and *RAD21*. It has been reported (8) that ataxia telangiectasia patients, who are homozygous for mutations in *ATM*, display a low apoptotic response of CD4 and CD8 lymphocytes. Therefore, it is plausible that SNPs in genes that encode proteins involved with apoptosis may affect the apoptotic response in those cells.

Recent clinical investigations have suggested that fibrosis risk is not only dependent on the type of the SNPs but also on the number of "risk alleles" (14). The results reported in the present article reinforce this hypothesis and indicate that possession of multiple SNPs associated with radiosensitivity correlates with an increased probability for developing severe radiation-induced sequelae. Of particular note may be the inverse correlation between RILA and the number of SNPs in the candidate genes screened, whose products are involved in both fibrosis and apoptosis after irradiation (3, 9, 11, 25). It is our hypothesis that radiation-induced late effects are exacerbated in tissues with reduced apoptosis because a diminished apoptotic cell death response may

Table 1. Number of patients presenting specific single nucleotide polymorphisms (SNPs) according to both groups (A, grade ≥ 3 late effects and B, no severe late effects)

Type of SNPs	Number of patients (%)	
	Grade ≥ 3 late effects group, $n = 16$	No severe late effects group, $n = 18$
<i>ATM</i> 5557 G>A	9 (56%)	6 (33%)
<i>SOD2</i> 47 T>C	13 (81%)	9 (50%)
<i>TGFB1</i> -509 C>T	11 (69%)	7 (39%)
<i>TGFB1</i> 870 T>C	15 (94%)	9 (50%)
<i>XRCC1</i> 1321 G>A	8 (50%)	7 (39%)
<i>XRCC3</i> 1075 C>T	9 (56%)	9 (50%)
<i>RAD21</i> 1625 T>C	7 (44%)	3 (17%)

enhance the probability for induction the cytokine cascade induced by radiation that result in a proinflammatory response resulting in long-term adverse responses. We should be able to verify this hypothesis in our on-going COHORT trial (NCI PDQ: NCT00208273) with 150 patients specifically designed to evaluate this association.

Further investigations are strongly warranted to identify more comprehensively the SNPs that are associated with radiation-induced sequelae and may provide the basis for an assay to predict which patients are at greatest risk for the development

of radiation injury, particularly in the current era of combined modality treatments (16, 26, 27).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Michèle Ben Sta and Eliane Cottin for their technical assistance.

References

- Stone HB, Coleman CN, Anscher MS, McBride WH. Effects of radiation on normal tissue: consequences and mechanisms. *Lancet Oncol* 2003;4:529–36.
- Aziz NM. Cancer survivorship research: state of knowledge, challenges and opportunities. *Acta Oncol* 2007;46:417–32.
- Ho AY, Atencio DP, Peters S, et al. Genetic predictors of adverse radiotherapy effects: the Gene-PARE project. *Int J Radiat Oncol Biol Phys* 2006;65:646–55.
- Ozsahin M, Crompton NE, Gourgou S, et al. CD4 and CD8 T-lymphocyte apoptosis can predict radiation-induced late toxicity: a prospective study in 399 patients. *Clin Cancer Res* 2005;11:7426–33.
- Crompton NE, Miralbell R, Rutz HP, et al. Altered apoptotic profiles in irradiated patients with increased toxicity. *Int J Radiat Oncol Biol Phys* 1999;45:707–14.
- Crompton NE, Ozsahin M. A versatile and rapid assay of radiosensitivity of peripheral blood leukocytes based on DNA and surface-marker assessment of cytotoxicity. *Radiat Res* 1997;147:55–60.
- Crompton NE, Shi YQ, Emery GC, et al. Sources of variation in patient response to radiation treatment. *Int J Radiat Oncol Biol Phys* 2001;49:547–54.
- Ozsahin M, Ozsahin H, Shi Y, Larsson B, Wurgler FE, Crompton NE. Rapid assay of intrinsic radiosensitivity based on apoptosis in human CD4 and CD8 T-lymphocytes. *Int J Radiat Oncol Biol Phys* 1997;38:429–40.
- Svensson JP, Stalpers LJ, Esveltd-van Lange RE, et al. Analysis of gene expression using gene sets discriminates cancer patients with and without late radiation toxicity. *PLoS Med* 2006;3:e422.
- Hall EJ, Schiff PB, Hanks GE, et al. A preliminary report: frequency of A-T heterozygotes among prostate cancer patients with severe late responses to radiation therapy. *Cancer J Sci Am* 1998;4:385–9.
- Severin DM, Leong T, Cassidy B, et al. Novel DNA sequence variants in the hHR23 DNA repair gene in radiosensitive cancer patients. *Int J Radiat Oncol Biol Phys* 2001;50:1323–31.
- Iannuzzi CM, Atencio DP, Green S, Stock RG, Rosenstein BS. ATM mutations in female breast cancer patients predict for an increase in radiation-induced late effects. *Int J Radiat Oncol Biol Phys* 2002;52:606–13.
- Andreassen CN, Alsner J, Overgaard M, Overgaard J. Prediction of normal tissue radiosensitivity from polymorphisms in candidate genes. *Radiother Oncol* 2003;69:127–35.
- Andreassen CN, Alsner J, Overgaard J, et al. TGFB1 polymorphisms are associated with risk of late normal tissue complications in the breast after radiotherapy for early breast cancer. *Radiother Oncol* 2005;75:18–21.
- Cox JD, Stetz J, Pajak TF. Toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC). *Int J Radiat Oncol Biol Phys* 1995;31:1341–6.
- Azria D, Gourgou S, Sozzi WJ, et al. Concomitant use of tamoxifen with radiotherapy enhances subcutaneous breast fibrosis in hypersensitive patients. *Br J Cancer* 2004;91:1251–60.
- Qiu P, Shandilya H, D'Alessio JM, O'Connor K, Durocher J, Gerard GF. Mutation detection using Surveyor nuclease. *Biotechniques* 2004;36:702–7.
- Bannwarth S, Procaccio V, Paquis-Flucklinger V. Surveyor Nuclease: a new strategy for a rapid identification of heteroplasmic mitochondrial DNA mutations in patients with respiratory chain defects. *Hum Mutat* 2005;25:575–82.
- Shi R, Otomo K, Yamada H, Tatsumi T, Sugawara I. Temperature-mediated heteroduplex analysis for the detection of drug-resistant gene mutations in clinical isolates of *Mycobacterium tuberculosis* by denaturing HPLC, SURVEYOR nuclease. *Microbes Infect* 2006;8:128–35.
- Janne PA, Borrás AM, Kuang Y, et al. A rapid and sensitive enzymatic method for epidermal growth factor receptor mutation screening. *Clin Cancer Res* 2006;12:751–8.
- Bentzen SM. Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology. *Nat Rev Cancer* 2006;6:702–13.
- Rodemann HP, Blaese MA. Responses of normal cells to ionizing radiation. *Semin Radiat Oncol* 2007;17:81–8.
- Lawrence DA. Latent-TGF- β : an overview. *Mol Cell Biochem* 2001;219:163–70.
- Milliat F, Francois A, Isoir M, et al. Influence of endothelial cells on vascular smooth muscle cells phenotype after irradiation: implication in radiation-induced vascular damages. *Am J Pathol* 2006;169:1484–95.
- Moullan N, Cox DG, Angele S, Romestaing P, Gerard JP, Hall J. Polymorphisms in the DNA repair gene XRCC1, breast cancer risk, and response to radiotherapy. *Cancer Epidemiol Biomarkers Prev* 2003;12:1168–74.
- Azria D, Pelegrin A, Dubois JB, Mirimanoff RO, Ozsahin M. Radiation therapy and tamoxifen: concurrent or sequential? It's no longer the question! *J Clin Oncol* 2005;23:4239–41.
- Azria D, Rosenstein BS, Ozsahin M. Radiation-induced side-effects with or without systemic therapies: Primetime for prediction of individual radiosensitivity! *Int J Radiat Oncol Biol Phys* 2008;71:1293–4.