

Relationship between Radiosensitivity and Normal Tissue Complications in Saudi Cancer Patients Treated with Radiotherapy

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ABSTRACT

Purpose: To assess the variations in radiosensitivity, its relationship with clinical complications and the potential application of predictive testing in Saudi radiotherapy patients.

Materials and Methods: Forty-one patients included in this study, during (17) or after (24) their radiation treatment for head and neck (26), breast (9), gynecological (3) or other (3) cancer. Skin fibroblasts were established and radiosensitivity was measured. The surviving fraction at 2 Gy (SF2) was calculated and compared to the maximum grade of acute (erythema, desquamation, mucositis, ulceration) and late reactions (atrophy, fibrosis, xerostomia, telangiectasia). Follow-up ranged between 12 and 178 months (median 30).

Results: SF2 ranged between 0.16 and 0.56 (mean 0.34). The inter-patients coefficient of variation (CV) was 26%. The intra-patient CV was 18%. There was a statistically significant correlation between fibroblasts SF2 and the maximum grade of late ($p = 0.012$; 40 patients), but not acute complications ($p = 0.70$; 36 patients). There was no correlation between acute and late reactions ($p > 0.05$; 34 patients).

Conclusions: These data revealed wide variations in cellular radiosensitivity that correlated with late reactions to radiation treatment. Radiotherapy patients, particularly those at risk to sustain severe complications may well benefit from individualizing the doses prescription. However, a predictive test alternative to SF2 is required.

Key Words: Fibroblasts - Radiosensitivity - SF2 - Normal tissue reactions - Predictive assays.

INTRODUCTION

Radiotherapy (RT) is one of the most important available treatment options of cancer therapy. Most cancer patients (50-70%) receive RT during the management of their disease.

Recent advances in imaging and optimization of tumor targeting and irradiation delivery are expected to improve patient outcome and allow for dose escalation. However, the tolerance of normal tissues constitutes the limiting factor for dose escalation in RT. Patients vary considerably in their normal tissue response to RT even after similar treatment [7,28]. Therefore, much interest in normal tissue radiosensitivity has emerged and raised the possibility of developing predictive assays for radiosensitivity. This trend has been endorsed by the demonstration of a possible positive therapeutic gain from applying knowledge of radiosensitivity to treatment planning [17,25,35]. This assumption is supported by two case-report studies where RT dose prescriptions were successfully reduced to account for the increased radiosensitivity of the patients [2,18]. Although many factors could influence the severity of reactions to RT [9,33,37, 38], large parts of inter-patient variability is attributed to individual differences in cellular intrinsic radiosensitivity, which is determined by genetic variations between patients [3,38].

A number of studies have been carried out to evaluate the predictive value of cellular radiosensitivity. Although results were varied, the overall picture lends support to the hypothesis and establishes a strong evidence for a correlation between late radiation-induced normal tissue complications and cellular radiosensitivity even though the in vitro test remains elusive [1,5,17,20,22,23,29]. Radiosensitivity studies in radiation oncology patients in the Middle East are still lacking. In this study we set out to test the hypothesis of the relationship between

RT-induced normal tissue complications and in vitro cellular radiosensitivity of skin fibroblasts, measured by the surviving fraction at 2 Gy (SF2).

MATERIALS AND METHODS

1- Patients' population and clinical data:

A total of 41 patients were recruited for this study. The age of patients ranged between 18 and 77 years with a median of 51. There were 24 males and 17 females. Patients were enrolled in this study during their course of RT treatment (17) or retrospectively selected (24) from those already treated in the Radiation Oncology Department at King Faisal Specialist Hospital and Research Centre. The patients were treated by definitive radiotherapy for Head & Neck (26), Breast (9), Gynecologic (3) or other (3) cancers. Although late radiation-induced side effects in skin, mucosa and deep tissues were directly scored at the time of follow-up, some acute reactions' data were retrospectively retrieved from the medical records of the patients. Scoring followed the RTOG/EORTC grading system. Records of acute reactions were available for 36 patients while late reactions were obtained for 40 patients (one patient died 5 months after the completion of RT). Although irradiation regimens varied according to the cancer site, the treatment was fairly standardized and conformed to the acceptable limit of tolerance of normal tissues. Furthermore, none of the late reactions could be attributed to a physical overdose. For groups comparison, the patients with no or minimal reactions (grade 0-1) were referred to as control and the patients with moderate to severe reactions (grade 2-4) were referred to as radiosensitive. For the retrospectively enrolled patients, an effort was made to balance selected patients between these two groups taking into account the tumor sites and the total RT dose received. The institutional basic and ethics research committees approved the study and all patients signed the informed consent.

2- Fibroblast primary culture:

Methodology followed previously described procedures [1] with little modifications. Briefly, a punch skin biopsy (3 mm in diameter) was obtained under local anesthesia from the arm of each patient. The biopsy was collected in minimal essential medium (MEM), supplement-

ed with high concentration of antibiotic (5%: penicillin, streptomycin, and amphotricin B). The biopsy was minced into very fine fragments and inoculated into two 25 cm² tissue culture flasks. One milliliter of fresh media supplemented with 20% fetal bovine serum (FBS) and 1% antibiotic was gently added and the fragments were incubated at 37°C in humidified atmosphere with 95% air and 5% CO₂. The media volume was progressively increased to 5 ml over the following 2-3 weeks, at which point fibroblast outgrowth was abundant to allow for trypsinization and subculturing.

3- Radiosensitivity measurements:

Experiments were carried out using cells between passage 2 and 7. Clonogenic survival was assessed using fixed number of seeded cells (tested + feeder) of about 1000 cells/cm². Feeder cells from the same cell line were irradiated by a lethal dose of 30 Gy. They were trypsinized and seeded in appropriate numbers in previously labeled culture flasks, 24-48 hours before receiving the tested cells. The tested cells were prepared from fresh contact-inhibited culture that were trypsinized, counted, diluted and seeded in appropriate number to yield 50 colonies in each of 3-4 flasks. For the first experiment, called pilot experiment, the number of cells seeded was calculated assuming a theoretical survival curve having a surviving fraction at 2 Gy (SF2) of 0.50, and a plating efficiency (PE) of 25%. Different number of cells was seeded in 3-4 flasks to take into account the unknown radiosensitivity and PE assuming an overall change in survival between 0.5 and 3 fold. For the remaining experiments, called confirming experiments, the results of the pilot experiment were used for the calculation of the number of cells seeded. After seeding the test cells, the flasks were returned to the incubator for 3-4 hours before being irradiated by single radiation doses that ranged between 1 and 4 Gy. The cells were then incubated at 37°C in a humidified atmosphere with 5% CO₂. Two to 3 weeks later, the cells were fixed and stained using crystal violet. Colonies of at least 50 cells were scored as survivors. Ideally, three flasks containing up to 75 colonies were obtained per dose and per experiment. Three to five independent experiments were carried out for each cell line. The same investigator carried out all survival experiments.

4- Irradiation of cultured cells:

All irradiations were performed at room temperature using Co-60 gamma-rays at a dose rate that ranged between 97 and 112 cGy/min.

5- Data analysis:

The average survival per dose and per experiment was calculated. The means of replicate experiments were pooled and fitted to the linear quadratic model. The SF2 was calculated from the whole survival curve and used to characterize the cellular radiosensitivity, compare between the different cell strains and correlate with clinical endpoints of acute and late radiation reactions. Statistical analysis used the Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks to check for significant differences in SF2 between patients having different grades of radiation reactions. For groups' comparison, the unpaired t-test was used to check for differences between control (grade 0-1) and hypersensitive (grade 2-4) after passing the tests of normality of data distribution and the equality of the variance; otherwise, the Mann-Whitney rank sum test was used. The Spearman's rank correlation test was used to check for correlation between the maximum grade of acute and late reactions. All statistical analysis was carried out using the SigmaStat platform (Version 3.0, SPSS Science, IL, USA).

RESULTS

1- Clinical data:

All patients received standard conventional treatment as practiced in our hospital. The total dose and overall treatment time did vary according to the tumor histological type and primary site. An effort was made, however, to balance patients for these variables in each radiosensitivity group. The dose distribution was verified from the treatment planning isodose curves to rule out treatment calculation error as a possible source of complications. Acute reactions included erythema, moist desquamation, confluent mucositis, xerostomia and ulceration. Late reactions were atrophy, hair loss, hyperpigmentation, telangiectasia, xerostomia and fibrosis. Although more than one type of reactions were scored per patient, only the maximum grade of any of these complications was used to correlate with cellular radiosensitivity. This approach was deemed most appropriate because of the differences in tumor sites and radiation fields

used for each patient [16]. Furthermore, the RTOG/EORTC is a semi-quantitative grading system and to some extent subjective. Nevertheless, this may only have influenced the scoring of complications in the intermediate range, particularly grade 2 as there are no clear-cut boundaries in the biological endpoints under observation. We accepted a minimal follow-up of 12 months because we noticed that most severe late reactions appeared within this period of time after the treatment. Five patients were treated with Co-60 gamma-rays. Those patients showed a trend toward higher grade of complications as compared to patients treated with higher energy irradiation. No attempt was made to stratify patients according to age, gender, cancer primary site, field size, total dose received or other patients or treatment characteristics because of the overall small number of patients enrolled in this cohort.

2- Fibroblasts radiosensitivity:

All experiments were carried out using optimized methodology. The PE ranged between 0.05 and 0.86 with a mean of 0.32 ± 0.17 . In general, PE decreased with increasing passage number but there were no measurable consequences on radiosensitivity (data not shown). A few experiments yielded very low PE (0.02 or less) particularly with slowly growing cells; these were discarded and the experiments were repeated until a satisfactory PE was obtained. The survival curves showed a wide range of radiosensitivity (Fig. 1). The SF2 ranged between 0.16 and 0.56 with a mean of 0.34. The inter-patients coefficient of variation (CV) was 26%. The intra-patient CV was 18%. Three ataxia telangiectasia mutated (ATM) cell strains were concurrently studied and used as control for extreme radiosensitivity (Fig. 1). The ATM cells showed a typical SF2 of about 0.02.

3- The correlation between radiation reaction and in vitro radiosensitivity:

The relationship between the maximum grade of acute reactions and SF2 is shown in the left panels of Fig. (2). There was no tendency toward a correlation even after excluding the 5 patients treated by Co-60 gamma-ray. The Kruskal-Wallis test showed no significant difference in the median values of SF2 among the different grades of acute radiation reactions ($p=0.70$). To check further we separated the patients into two groups: control (grade 0-1)

and radiosensitive (grade 2-4). The unpaired t-test confirmed the lack of a statistical difference between the two groups ($p=0.73$). The relationship between the maximum grade of late reactions and SF2 is shown in the right panels of Fig. (2). Although there was a wide scatter in the data points, a trend was apparent where the lower the SF2 the higher the grade of late reactions. The Kruskal-Wallis test showed significant difference between the median values of SF2 of the different grades of late radiation reactions ($p=0.012$). The difference was enhanced when the 5 patients treated by Co-60 gamma-ray were excluded ($p=0.002$; Fig. 2, B, right panel). This observation may argue for the consideration of the parameter of RT dose as a confounding factor in this study that included different cancer sites. However, a brief analysis of the total doses received by the Head and Neck cancer patients, who constitute the majority of the patients reported here, showed no difference between the control (65 ± 4.5 Gy) and the radiosensitive (66 ± 5.7 Gy) subgroups. Meanwhile, the difference in SF2 was comparable to the following whole groups' comparison (Fig. 2, C, right panel). The mean SF2 of the control group was 0.40 ± 0.08 . The mean SF2 of the radiosensitive group was 0.31 ± 0.08 . The unpaired t-test showed that the difference between the means of the two groups is statistically significant ($p=0.001$). Similar results were obtained when the same clinical endpoint (grade of fibrosis, 31 patients) was tested ($p=0.02$).

4- The correlation between acute and late reactions:

The relationship between the maximum grade of acute and late reactions is shown in Fig. (3). There was no correlation of statistical significance between these two clinical endpoints (Spearman's rank correlation test: $p > 0.05$; $R2 = 0.04$).

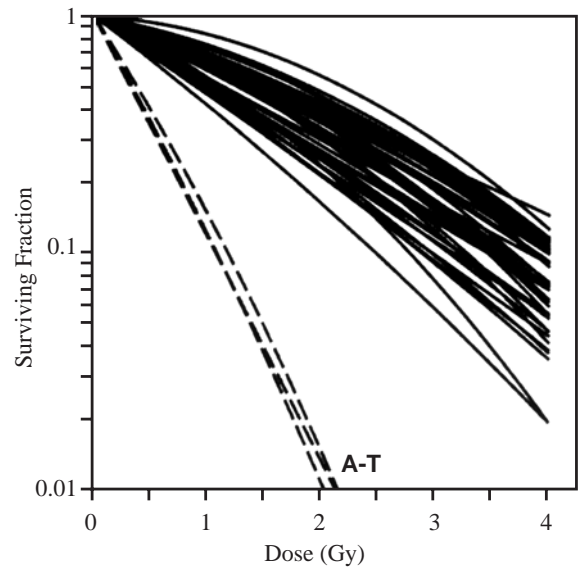


Fig. (1): Survival curves of 41-fibroblast cell strains derived from cancer patients treated by radiotherapy (Solid lines). Dashed lines: survival curves of 3 ataxia telangiectasia cell strains used as control for extreme radiosensitivity. Data points and error bars were removed for clarity.

Fig. (2): The relationship between cellular radiosensitivity (SF2) and reactions to radiotherapy: A. All available data, B. Excluding 5 patients treated by Co60 gamma-ray, C. By grouping to control (Grade 0-1) and radiosensitive (Grade 2-4). Left panels: SF2 versus maximum grade of acute reactions. Right panels: SF2 versus maximum grade of late reactions. The error bars represent the 95% confidence interval.

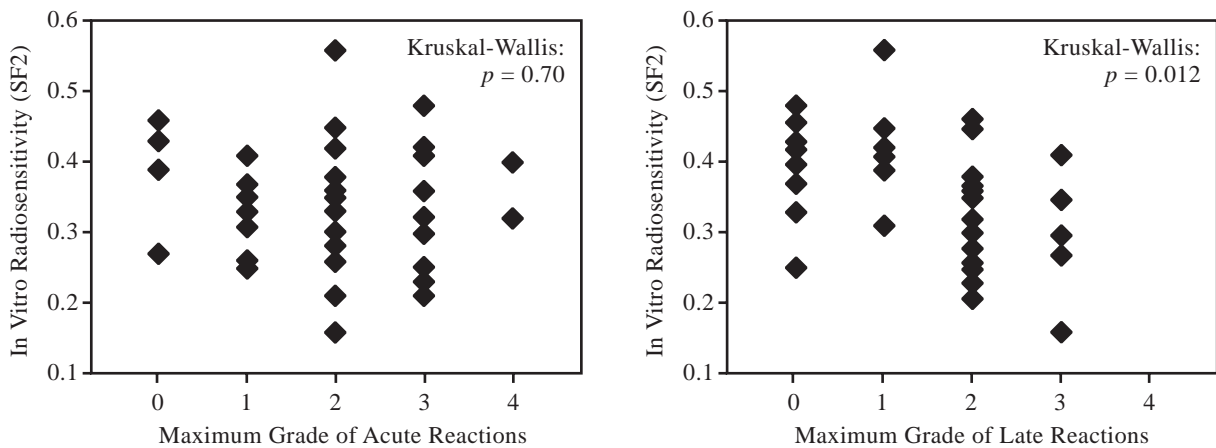


Fig. (2-A): All available data.

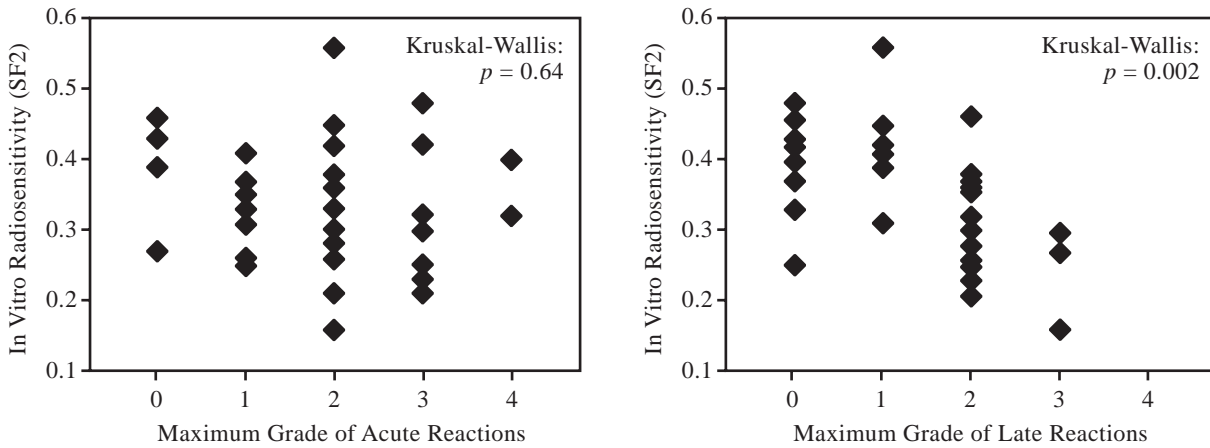


Fig. (2-B): Excluding Co-60 treated patients.

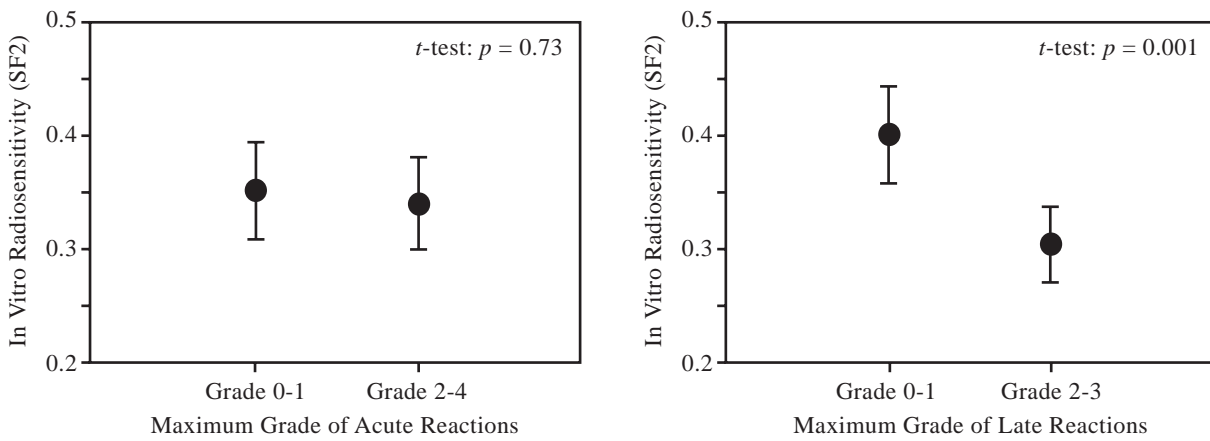


Fig. (2-C): Comparison between groups.

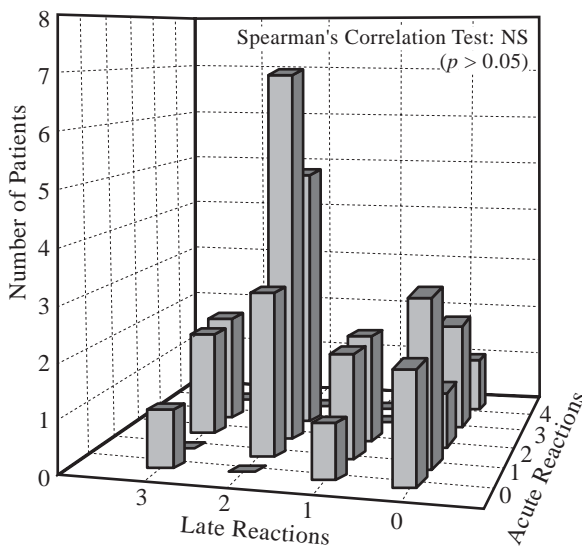


Fig. (3): The relationship between the maximum grade of acute and late reactions to radiotherapy. The length of the bars represents the integer number of patients.

DISCUSSION

The main purpose of the present report was to check in our local cancer population for variation in radiosensitivity and a possible correlation with the severity of acute and/or late reactions following radiotherapy. We used the classical clonogenic survival assay because it remains the gold standard to assess radiosensitivity as it measures the lethal effects of radiation. The analysis of survival curves of fibroblasts derived from 41 patients confirmed the existence of a significant inter-patients variation (3.5 fold differences) in cellular radiosensitivity. The mean SF2 (0.34) is in agreement with published data which showed a spectrum ranging between 0.24 and 0.46 [1,10,11,16,21,26,27,29,31,32]. The total variation (CV) in SF2 was 26%, which is comparable to many results published in the literature. Therefore, it is apparent that this group of patients demonstrates a consider-

able range of fibroblasts' radiosensitivity, comparable to other patients' population. The most sensitive cell strain ($SF2 = 0.16$) was derived from a patient who was treated by a radical radiation treatment alone (70 Gy in 35 fractions) for head and neck tumor. The patient developed severe late reactions, particularly grade 3 nasopharyngeal stenosis (fibrosis), which appeared 7 months after the radiation treatment, then persisted and required multiple sessions of dilatation. The most resistant cell strain ($SF2 = 0.56$) was derived from a patient with a similar head and neck tumor who was treated by 66 Gy in 33 fractions, plus a boost to the neck lymph node (6 Gy in 3 fractions), in addition to neoadjuvant and concurrent chemotherapy. The patient developed only minimal late reactions, namely grade 1 fibrosis (follow-up 42 months).

Data presented here showed a statistically significant correlation between the maximum grade of late radiation complications and cellular radiosensitivity (Kruskal-Wallis test: $p = 0.012$; unpaired t -test $p = 0.001$). This is in agreement with the published data [1]. The wide range of cellular radiosensitivity and clinical complications suggest that the patients would benefit from tailoring the dose prescription to each patient's radiosensitivity. Nevertheless, the overlap between the data points of different grades of late reactions (Fig. 2) and the considerable variability of repeated experiments for the same cell strain (intra-patient variations $CV = 18\%$), in addition to the time lapse of clonogenic assay and inter-laboratory variations preclude the usefulness of $SF2$ as determinant of radiosensitivity in routine clinical setting. Genetic testing can overcome these weaknesses [14]. In addition, how much of these variations are shared with the tumor cells would need to be determined, as the radiosensitivity of the tumor must be taken into account if dose reduction is deemed necessary to avoid the increased sensitivity of hypersensitive patients. Published data on the relationship between the radiosensitivity of normal and tumor cells are limited but do not support the assumption of a parallel decrease in the probability of tumor control when doses are reduced for hypersensitive patients [2]. Furthermore, confounding factors may contribute to the variability in the relationship between cellular radiosensitivity and reactions to RT. In this study, 9 patients had an associated disease (diabetes, hypertension, sys-

temic lupus erythematosus) that has been suggested to influence reactions to RT. However, no systemic trend was observed in this small group of patients. The five patients treated by Co-60 gamma-ray clearly incurred higher levels of both acute and late reactions. This was not associated with increased fibroblasts' radiosensitivity. The most probable explanation for developing complications in those 5 patients is probably the weak sparing effect of Co-60 gamma-ray irradiation on superficial tissues as compared to higher energy irradiations.

The lack of a correlation between acute reactions and fibroblasts' $SF2$ has generally been described [6,10,11,16,21,30]. Other cell types and/or other cellular radiosensitivity end points have shown various results [12]. The study by Oppitz and colleagues [26] showed a significant correlation between $SF2$ and acute reactions in 88 patients. The reason of the discrepancy between these results and other studies is not clear. It should be noted, however, that the experiments in the latter study were carried out using exponentially growing cells. These cells were irradiated at 4°C in suspension then seeded for colonies formation. It is possible that in these conditions a considerable percentage of cells were in the radioresistant S phase of the cell cycle. This could explain the elevated survival obtained by Oppitz et al. (mean $SF2$ was 0.46), which is the highest reported in these series of studies.

The lack of correlation between acute and late reactions is also in agreement with published data [8,10,31,36]. This lack of correlation may emanate from the inherent differences between acute and late reactions. These two types of reactions have different tissular manifestations, may involve diverse humoral regulators [4,13,15,19,24], show different patterns of response to fractionation and vary in their evolution. Late effects will remain the dose limiting factor in RT until they are controlled by individualizing RT treatment or prevented by using radioprotectors that could decrease radiation damage to critical normal tissues [34].

In conclusion, our data confirm the existence of wide variations in cellular radiosensitivity that correlated with late complications to radiation treatment in our cancer patients' population. Those patients could benefit from tailoring the dose to individual radiosensitivity. However,

a different approach than SF2 is required to screen patients according to their susceptibility to incur radiation complications. Newly proposed genetic testing may have a potential.

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