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BIOLOGICAL EFFECTS INDUCED BY SWIFT HEAVY IONS OF LITHIUM ON AQUEOUS SOLUTION OF PLASMID pMTa4

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Biological samples exposed to swift heavy ions sustain damage on different components. Damage to DNA, a critical component of a living system, has considerable biological implications. In this study aqueous solution of plasmid pMTa4 was exposed to varying fluence of swift ^{7}Li ions and its different topological forms were analysed by agarose gel electrophoresis to study the induced damage. To monitor radiation labile nucleotide sequence the ^{7}Li ions exposed plasmid was degraded by three different restriction endonucleases and also analysed by agarose gel electrophoresis. The results show that ^{7}Li ions predominantly induced double strand breaks in the plasmid DNA in a dose-dependent manner and affected preferentially the GC-rich motifs of the DNA. The results suggest that ^{7}Li ions induce premutagenic lesions at an enhanced frequency in segments of the DNA which are rich in CG content as compared to GC-poor segments.

Keywords: ^{7}Li ion; pMTa4 plasmid DNA; *In vitro*; Restriction endonuclease; Nucleotide sequence

1 INTRODUCTION

Radiation produces damage to various components of cells, tissues and organisms. The damage includes a variety of changes which, among others, may result in the observed biological endpoints such as mutation, chromosomal aberration, oncogenic transformation and cell death [1–3]. These events and their molecular mechanisms are to a great extent understood in the case of low-linear energy transfer (LET) radiation. On the other hand, the situation remains far from clear for high-LET radiation, notwithstanding their reasonably well-characterized biophysical features [4]. Understandably, the elucidation of complex radiobiological behavior at molecular level following swift ion bombardment continues to be of global interest [5].

The damage to DNA by radiation is generally considered to be the prime lesion in a living system. Owing to its implication in major biological events, extensive studies have

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subsequently been carried out with different types of radiation. Indeed, considerable insights into DNA damage induced by radiation have been gained in the process. However, the intricacy of damage mechanisms has prevented the understanding of the quality and nature of DNA damage and its correlation with the eventual biological response. In order to gain further insight a simple plasmid DNA model was designed to study damage of DNA. Using a defined plasmid DNA, pMTa4, and restriction endonucleases it has been shown that γ-radiation induced damage in pMTa4 DNA was influenced by a specific nucleotide sequence [6]. Since high-LET radiations are known to cause more severe impacts than low-LET radiations, swift heavy ions of ^{7}Li were used in this preliminary investigation to examine its effects on pMTa4 DNA *in vitro*.

2 EXPERIMENTAL DETAILS

The work was carried out at the Nuclear Science Centre, New Delhi, India using the bio-beam line of the Pelletron accelerator facility. ^{7}Li ions (LET $\sim$101 keV/μm) at approximately 0.9×10^{6} ions cm^{-2} were used for exposing the aqueous solution of plasmid DNA.

Ten μl of aqueous solution of pMTa4 (10 μg DNA) was prepared as previously described [6]. The solution was exposed in polypropylene membrane pouches (1.5×1.5 cm^2 with a thickness of 40×10^{-3} cm) to accumulate varying numbers of ^{7}Li ions (1×10^{6}, 5×10^{6}, 1×10^{7} and 1×10^{8} cm^{-2}). The pouches were kept on ice, except during the exposure. A non-exposed pouch was maintained in similar conditions as the control. After the exposure, 5 μl plasmid solution (5 μg DNA) from each of the pouches was recovered and processed for analysis of damage.

A part of the plasmid DNA samples was analysed by agarose gel electrophoresis for monitoring the extent of strand breaks induced by exposure to ^{7}Li ions. Simultaneously, another part of the plasmid DNA samples was fragmented with restriction endonucleases *Bgl I*, *Hinf I* and *Hae II* in conditions as per the manufacturer's recommendation (Genei, Bangalore, India) and was also analysed by agarose gel electrophoresis. The gel electrophoresis used 1% agarose gel in TAE buffer (40 mM tris-acetate, 1 mM EDTA) and ran for 60 min at a constant voltage of 90 V in a horizontal electrophoresis apparatus (Stratagene). Gels were stained in 0.3 μg ml^{-1} ethidium bromide for 10 min. After 30 min of destaining in double distilled water, the fluorescence of DNA-intercalated ethidium bromide was visualized on a UV trans-illuminator and was captured in a gel documentation system.

Densitometric images of different topological forms of pMTa4 on the gels were quantified for the relative amounts of DNA using Molecular Analyst^{TM}/PC Windows software (BioRad), which calculated the sum of the pixel intensities of the DNA bands.

3 RESULTS AND DISCUSSION

Figure 1 shows the effect of increasing fluence of accelerated ^{7}Li ions on pMTa4. The electropherogram of the plasmid DNA after exposure to ^{7}Li ions shows different quantities of three topological forms of the plasmid (Fig. 1A). Figure 1B depicts the plots of the topological forms of plasmid as quantified by densitometry. While the native, covalently closed (CC) form of plasmid decreased, its linear (L) form increased in a dose-dependent manner indicating that ^{7}Li ions primarily induced double strand breaks (DSB). There was no apparent increase in the open circle (OC) form (Fig. 1B). The linear form of plasmid DNA is produced largely due to a single DSB or occasionally due to 2 single strand breaks (SSB) in close

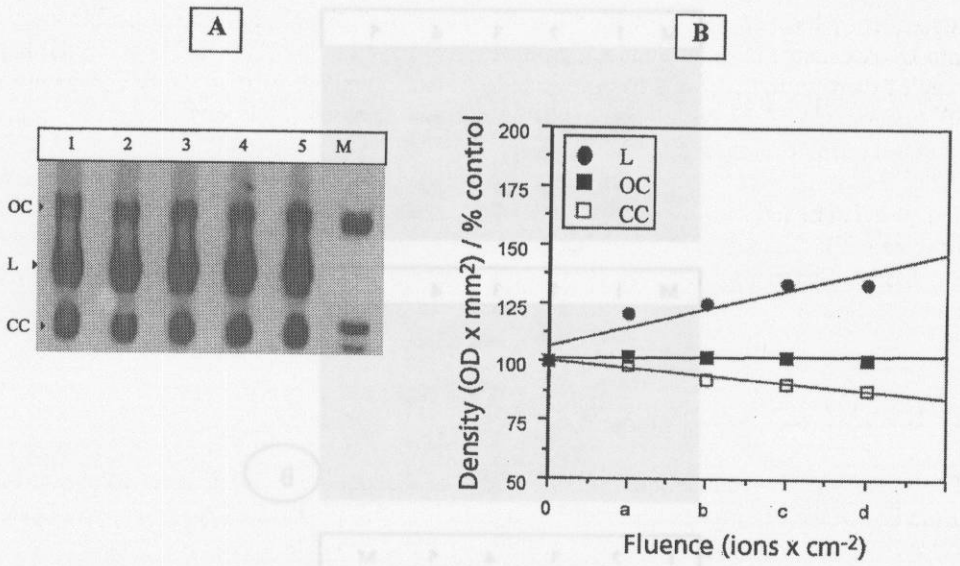

FIGURE 1 Effect of ^{7}Li ion bombardment on plasmid pMTa4. Panel A shows different topological forms of the plasmid without (lane 1) and following (lanes 2–5) ^{7}Li ion exposure. L, OC and CC represent linearised, open circular and covalently closed forms of the plasmid, respectively. Plasmids exposed to fluences of 1×10^{6}, 5×10^{6}, 1×10^{7} and 1×10^{8} ions cm^{-2} delivered at LET of ~101 keV/μm were loaded on lanes 2, 3, 4 & 5, respectively. Lane M represents lambda DNA double digest marker. The densitometric analysis of topological forms of the plasmid has been plotted in panel B. Fluence of 1×10^{6}, 5×10^{6}, 1×10^{7} and 1×10^{8} ions cm^{-2} delivered at LET of ~101 keV/μm are represented by a, b, c and d, respectively, on the x-axis (see text for details).

vicinity. High-LET radiation like ^{7}Li ion causes a dense ionization track, which is why it induces more DSB than SSB [4]. Therefore, the percentage of the linear (L) topological form of pMTa4 in the sample can be taken as a measure of the frequency of DSB induction.

Variation in the fragmentation capabilities of three restriction endonucleases (RE) towards pMTa4 was observed following ^{7}Li ion exposure (Fig. 2). In *Bgl I* and *Hinf I* restricted plasmid there were no observable changes in its restriction patterns between unexposed and ^{7}Li ion-exposed plasmid samples (Fig. 2A and 2B, respectively). In contrast, the exposed plasmid restricted with *Hae II* showed additional slow migrating bands representing large DNA pieces on the gel, which were entirely absent in the unexposed control (Fig. 2C). This shows that the efficiency of the plasmid restriction by *Hae II* was reduced following ^{7}Li exposure but remained unchanged for *Hinf I* and *Bgl I*. This could result in partial restriction of pMTa4 by *Hae II*. It is likely that plasmid DNA sustained certain alteration or modification in nucleotides of the *Hae II* restriction site such that the site became less efficient for its complete restriction. Incomplete cutting, therefore, produced larger DNA fragments, which are seen on the gel as slow migrating bands (Fig. 2C). By examining the specific nucleotide sequences of the restriction sites for *Hae II* in pMTa4, it was possible to find a clue for such partial fragmentation (Tab. I). The *Hae II* restriction site, unlike those for *Bgl I* or *Hinf I*, is GC-rich and only *Hae II* produced 100% GC staggered-ended DNA pieces after restriction (Tab. I). *Bgl I* and *Hinf I*, which also generated staggered-ended pieces, did not show the GC-motif feature (Tab. I). The nucleotide compositions in the flanking region around the restriction sites for *Hae II* also showed a higher GC content [6, 7]. The results, therefore, suggest that GC-rich regions of pMTa4 were more affected or modified upon exposure to ^{7}Li ions than GC-poor regions. The alteration in the nucleotide sequence may cause reduced efficiency of *Hae II* restriction of pMTa4. This is also supported by restriction analysis of pMTa4 with a range of other RE with qualitatively different restriction sites [7].

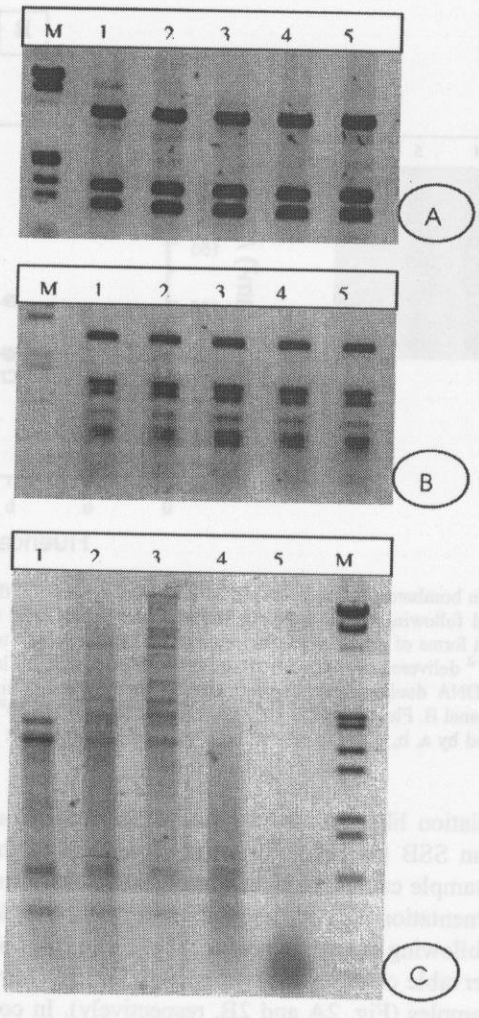

FIGURE 2 Agarose gel showing plasmid pMTa4 restricted by *Bgl I* (A), *Hinf I* (B) and *Hae II* (C) under standard condition (see text for details). Unexposed control (lane 1) and ^{7}Li ion exposed plasmids (lanes 2, 3, 4 & 5 at fluences of 1×10^{6}, 5×10^{6}, 1×10^{7} and 1×10^{8} ions cm^{-2} delivered at LET of ~101 keV/μm, respectively) and lambda DNA double digest marker (lane M) were run as described in the text.

These results are similar to the earlier findings after γ-irradiation [6]. The observations show that even with ^{7}Li ions, there was no apparent alteration in GCCGTTT/GGGC and G/ANTC sequences that would change the restriction efficiency of *Bgl I* and *Hinf I*, respectively (Tab. I). On the other hand, ^{7}Li ions influenced GGCGC/(TC), the restriction site for *Hae II* (Tab. I), in an ion fluence-dependent manner. While the lowest fluence of 1×10^{6} ions cm^{-2} used in this investigation showed little effect on pMTa4 (Fig. 2C, lane 2), exposure to 5×10^{6} ions cm^{-2} or more (Fig. 2C, lanes 3–5) prominently affected the extent of restriction by *Hae II*. At the fluence of 5×10^{8} ions cm^{-2}, the whole plasmid was fragmented into very small pieces of DNA (Fig. 2C, lane 5). The sizes of the slow migrating fragments were calculated and were found to be similar to those resulting from γ-radiation [6, 7]. These observations may mean that though the extent of damage induced by high- and low-LET radiations may differ, the molecular basis of the damage may follow a similar mechanism for both types of radiation.

TABLE I Characteristics of the Different Restriction Endonucleases (RE) Used in this Investigation.

Restriction endonuclease (RE)	*Restriction site*	*Fragment-end generated by RE cleavage*	*Effect on the restriction following radiation*
Bgl I	−GCCGTTT|GGGC− −CGGC|AAACCCG−	−GCCGTTT −CGGC	−
Hae II	−GGCGC|(T,C)− −C|CGCG(A,G)−	−GGCGC −C	+
Hinf I	−G|ANTC− −CTNA|G−	−G −CTNA	−

Even though the observations made above are in the realm of RE action, they indicate the likelihood of a specific nucleotide sequence being more susceptible to radiation-induced damage. Regardless of the type and nature of the modification, the results indicate that GC-rich motifs of DNA should be more frequently modified or damaged upon exposure to ^{7}Li ion. Such indications may have important bearings on the stability and integrity of the genomic DNA. Structural alterations in the DNA due to base modification may lead to conformational changes in the DNA which may in turn retard the efficiency of DNA polymerase and other DNA repair enzymes. The modified forms of C and G have been implicated in mutagenesis, carcinogenesis and ageing [8, 9]. Several studies have reported higher mutagenicity at GC than AT [10–14]. With such evidence of GC-vulnerability to mutation, it is likely that radiomodified GC nucleotides would form important premutagenic lesions. While further investigations would be required, this report suggests that inherent radiosensitivity may be at least partly determined by the GC-richness of the nucleotide sequence of the DNA.

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INT. J. RADIAT. BIOL 2003, VOL. 79, NO. 3, 217–219

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Meeting Report

Preparedness to respond to possible acts of nuclear terrorism: Some strategies and recommendations

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1. Background

The unprecedented terrorist attack on the World Trade Centre, New York, on 11 September 2001 and the turn of events across the globe since then have heightened the perception of the threat to the civilized world. The fear of a repeat of such events with increased intensity and the possible use of weapons of mass destruction in such acts is not an impossible reality. Prompted by such possibilities, the scientific communities of the world have started to address the issue of planning appropriate strategies to minimize loss of life and property. One important workshop, 'Interagency Workshop on the Radiobiology of Nuclear Terrorism', was held at Bethesda, MD, USA in December 2001 (Moulder 2002a) to address the nuclear-related issues in the post-11 September era. Incidentally, about a year earlier, another workshop, 'Modifying Normal Tissue Damage Postirradiation', also addressed issues relevant to radiation damage (Stone *et al.* 2002). Both workshop deliberations have many overlaps, understandably due to common concerns, in the domain of the existing knowledge base of the consequences of human exposure to moderate doses (1–10 Sv) of radiation. The Bethesda workshop, in addition, outlined possible strategies to be adopted over the next 5 years to improve our ability to diagnose, triage, prevent and treat radiation injuries (Moulder 2002b). While the academic contents and recommendations of these meetings are relevant to the nuclear disaster scenario, one has to keep in mind that in a nuclear tragedy situation large segments of population will likely be simultaneously exposed to a range of doses. Therefore, there is a need to look into the biological response to different qualities as well as to variable quantities of radiation. Further, the context of discussion in the two meetings mentioned above related to situations in developed countries where medical, paramedical and first-response teams are relatively better trained and the awareness level of the population is relatively high. The circumstances in a large number of underdeveloped, Third World counties, including India, are quite different. The response management and medical infrastructures are relatively poor and the awareness level of the population is low. Thus, it is important that other aspects, including the psychological state of the population in distress, are also discussed and evaluated so that the immediate impact of such a disaster could be kept to a minimum. With this in view, the Indian Society for Radiation Biology (ISRB) took the lead in organizing a multinational, multi-agency workshop, 'Radiation Risk in the Age of Nuclear Terrorism', in collaboration with the Research Center, Juelich (Germany), Health Canada, Ottawa (Canada) and the School of Life Sciences, Jawaharlal Nehru University (JNU), New Delhi (India).

2. Issues of discussion at the workshop

The workshop was held on 16 November 2002 at the School of Life Sciences, JNU. The 40 participants formed a multinational team of radiation biologists and allied scientists, including scientists from Japan—the only country with first-hand experience of the management of the aftermath of nuclear bombings (for details, see ISRB Participants 2002). In his opening remarks, Professor R. N. Sharan highlighted the need and urgency of the workshop and discussed its scope in light of the increasing threat perception of nuclear or 'dirty' devices falling into the hands of terrorists. Professor Emeritus P. N. Srivastava (JNU), in his inaugural address, recalled the chronological events of the past where large segments of populations across the globe were exposed to doses of radiation. He impressed upon the participants the need to come up with suitable recommendations that may

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form part of the strategies of various governments to tackle radiation emergencies with dexterity. Professor K. A. Dinshaw (Tata Memorial Center, Mumbai) delivered a lucid thematic talk, '"Managing Radiation Emergencies"—a physician's view', covering all aspects of medical and health management during and after a nuclear disaster. The next four technical sessions covered in depth various aspects of the consequences of radiation exposure, new approaches to biological dosimetry, biological responses and radioprotection strategies. Dr A. Trivedi (Health Canada) presented new strategies for radiation risk assessment in the dose range 1–10 Sv—doses that may not be lethal but can potentially cause acute effects. Dr K. P. Mishra (Bhabha Atomic Research Center, Mumbai) detailed the need for more extensive research to elucidate the role of free radicals in the induction of membrane and DNA damage that may provide a better understanding of apoptotic death and bystander effects. Professor R. N. Sharan (NEHU, Shillong) presented evidence of the nucleotide sequence determined vulnerability of segments of genomic DNA suggesting that genome instability or inherent radiosensitivity may be, at least in part, determined by the primary sequence of nucleotides. Professor Emeritus M. S. Sasaki (Kyoto University) presented a new model of biological dosimetry. The strategy takes into account the fact that dose distribution is unlikely to be homogeneous in those exposed to radiation in a nuclear disaster. Thus, the score of chromosome aberration recorded in lymphocytes is spread over a mixed Poisson distribution into dose components to get the most probable dose distribution profile and a realistic biological dose assessment. Dr F. H. A. Schneeweiss (Institute of Medicine, Research Center, Juelich) offered an alternative to this approach in which early cellular response could be assessed by analyses of lymphocyte proteins by two-dimensional differential gel electrophoresis and mass-spectrophotometry coupled with COMET-FISH analysis of DNA damage. Professor P. Uma Devi (J.N. Cancer Hospital, Bhopal) presented results of extensive research involving prenatal exposures of mice and cancer incidence. Prenatal exposure was shown to increase genome instability significantly. Professor R. K. Kale (JNU) presented evidence of the xanthine oxidoreductase system producing free radicals in the post-irradiation period suggesting, thereby, that inhibition of the system may reduce radiation damage. Dr B. S. Dwarkanath (Institute of Nuclear Medicine and Allied Sciences, Delhi) discussed the possible use of minor groove binding DNA ligands, such as Hoechst 33258 and 33342, for protection of radiation-induced DNA damage. The Hoechst ligands were shown to scavenge free radicals as well as afford stabilization to the DNA superstructure. Dr A. Chatterjee (NEHU, Shillong) elaborated on the use of the endogenous radioprotector, GSH, in reducing post-irradiation damage to proliferating cells.

3. Recommendations

The final session of the workshop was a round-table plenary discussion with panellists Professor P. N. Srivastava (India), Professor M. S. Sasaki (Japan), Dr F. H. A. Schneeweiss (Germany), Dr A. Trivedi (Canada) and Dr Vijaylaxami (USA). The session was initiated by a short presentation on 'Chemical, Biological, Radiological and Nuclear Research & Technology Initiative' by Dr Trivedi. All panellists and the participants interacted extensively and freely on various aspects of the proceedings of the day and made recommendations to define a strategy to handle a nuclear disaster scenario. Briefly, the main points of the recommendations were as follows (for details see, ISRB Recommendations 2002):

- International collaborations/partnerships: Closely interactive joint efforts be initiated and strengthened for free exchange of information and collaborative research among scientists and institutions engaged in radiobiological teachings and research across the globe.
- Preparedness: The participants felt strongly that 'preparedness' was an essential component of containing the damage of a nuclear disaster. It was felt that special initiatives were needed for school children and public awareness. The ISRB should play a proactive role in the design of such programmes to prevent misinformation and unfounded alarm to children and the public.
- First-response team: First-response teams should be created at various locations. The Fire Brigade, Police and paramilitary personnel should be given training and orientation on the effects of radiations and the handling of radiation accident/disaster situations including decontamination procedures. Preparation of emergency situation 'manuals' for different teams was recommended.
- Categorization of hospitals: Hospitals should be categorized and equipped for handling various categories of patients and different degrees of emergencies. Triage centres and teams, emergency centres, definitive care centres and specialized centres could be some of these categories of hospitals.
- Trauma control hospitals: Specialized hospitals with psychotherapists and psychoanalysts should be developed for trauma control.

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Diary of events

The *Journal* welcomes announcements of events from conference organizers for inclusion in this diary. Relevant information should be sent to the Editor.

2003

June 1–3 **2nd ESTRO Workshop on Biology in Radiation Oncology, Berg en Dal/Nijmegan, Netherlands.** *Contact: ESTRO, Avenue E. Mounier 83, Brussels, Belgium 1200; Tel: +32 2 775 93 40; E-mail: info@estro.be*

August 13–15 **5th Auger Symposium, An International Meeting on Physical, Molecular and Cellular Aspects of Auger Processes, Peter MacCallum Cancer Institute, St Andrews Place, East Melbourne, Victoria 3002, Australia.** *Contact: Dr Hooshang Nikjoo, MRC Radiation & Genome Stability Unit, Harwell, UK. Tel: +44-(0)1235-834776; Fax: +44-(0)1235-834776. URL: www.ragsu.har.mrc.ac.uk; E-mail: auger5har.mrc.ac.uk*

August 17–22 **12th International Congress of Radiation Research, Brisbane Australia.** *Contact: ICMS, PO Box 3496, South Brisbane, Queensland, Australia 4101. Tel: +61 7 3844 1138; E-mail: icrr2003@icms.com.au*

September 10–13 **The Sixth International Symposium on Chromosomal Aberrations, University of Essen, Germany.** The following topics will be discussed: 1) DNA Repair Related to Chromosomal Aberrations, 2) Molecular Cytogenetics, 3) Chromosomal Aberrations: Basic Aspects, 4) Chromosomal Aberrations: Applied Aspects, 5) Chromosomal Alterations and Human Diseases. *Contact: Prof. G. Obe, Dept. of Genetics, University of Essen, Universitaetsstrasse 5, 45117 Essen, Germany. Tel: +49 201 183 2688; Fax: +49 201 183 4397; URL: www.uni-essen.de/genetik; E-mail: guenter.obe@uni-essen.de*

September 21–25 **ECCO 12 – The European Cancer Conference, Copenhagen, Denmark.** *Contact: ECCO 12 Secretariat, Federation of European Cancer Societies, Avenue E. Mounier 83, Brussels, Belgium B-1200. Tel: +32 2 775 02 01; E-mail: ecco12@fecs.be*

October 19–23 **ASTRO: 45th Annual Meeting, Salt Lake City, UT, USA.** *Contact: American Society for Therapeutic Radiology and Oncology, 12500 Fair Lakes Circle, Suite 375, Fairfax, Virginia 22033-3 USA. Tel: +1 (703) 502 1550; E-mail: meetings@astro.org*