Secondar cancer after prostate
Early biomarkers related to secondary primary cancer risk in radiotherapy treated prostate cancer patients: IMRT versus IMAT

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\textbf{A B S T R A C T}
Purpose: To investigate whether rotational techniques (Volumetric Modulated Arc Therapy – VMAT) are associated with a higher risk for secondary primary malignancies compared to step-and-shoot Intensity Modulated Radiation Therapy (ss-IMRT). To this end, radiation therapy (RT) induced DNA double-strand-breaks and the resulting chromosomal damage were assessed in peripheral blood T-lymphocytes of prostate cancer (PCa) patients applying γH2AX foci and G\textsubscript{0} micronucleus (MN) assays.

Methods and materials: The study comprised 33 PCa patients. A blood sample was taken before start of therapy and after the 1st and 3rd RT fraction to determine respectively the RT-induced γH2AX foci and MN. The equivalent total body dose ($D_{T\text{ETB}}$) was calculated based on treatment planning data.

Results: A linear dose response was observed for γH2AX foci yields versus $D_{T\text{ETB}}$ while MN showed a linear-quadratic dose response. Patients treated with large volume (LV) VMAT show a significantly higher potential risk for RISM because it requires more radiation fields involving a larger volume of normal tissue exposed to low radiation doses and because it requires more monitor units (MU) to deliver a specified dose causing a larger total body dose due to head leakage and collimator scatter [2,3,5]. Using Volumetric Modulated Arc Therapy (VMAT), dose rate, shape of the beam and speed of rotation can be changed during gantry rotation enabling to give the RT fraction in one single rotation. Variations on the VMAT principle are RapidArc (Varian Medical Systems, Inc., Palo Alto, CA, USA) and IMAT (Intensity Modulated Arc Therapy) [6]. No information is available regarding RISM following arc therapy.

In this paper, a biomarker approach is applied to compare the potential risk for radiation induced RISM in prostate cancer (PCa) patients treated with ss-IMRT and VMAT (small volume and high volume). To this end RT induced DNA double-strand-breaks (γH2AX foci) and the resulting chromosomal damage (MN) were assessed in PBLs.

\textbf{Materials and methods}
\textbf{Patient population and blood sampling}

The study population consisted of 33 PCa patients treated at the Department of Radiation Oncology (Ghent University Hospital, Belgium) between October 2010 and August 2011. Heparinised blood samples were obtained from patients treated with 3 different RT modalities: (1) ss-IMRT (Elekta Synergy linear accelerator) ($n=12$), (2) small volume (SV) VMAT (RapidArc; Varian Clinac linear accelerator) ($n=9$) and (3) large volume (LV) VMAT (IMAT;...
Elekta SL18 linear accelerator). The latter population was further subdivided in a group receiving normofractionation \((n=8)\) and a group receiving hypofractionation \((n=4)\). In these groups the dose per fraction was respectively 2.09, 2.02, 1.83 and 2.85 Gy. Details regarding patient population and treatment modalities are summarised in Table 1. Before each fraction, imaging was applied to verify position of patient and fields: Electronic Portal Imaging Device (EPID) for LV-VMAT and Cone Beam Computed Tomography (CBCT) for ss-IMRT and SV-VMAT. After obtaining signed informed consent, blood samples were taken at different time points, as shown in Fig. 1. Blood samples taken a few minutes after exposure reflect the average dose given to the lymphocyte pool in the peripheral blood [7]. Blood sampling for the \(\gamma\)H2AX foci was performed before and after the first fraction, blood sampling for the MN assay was performed before and after the third fraction. In previous work it was shown that the number of radiation induced MN is proportional to the equivalent total body dose \(D_{ETB}\) up to three fractions of RT treatment [8].

### Table 1 Overview of patient population and treatment modalities considered in this study.

<table>
<thead>
<tr>
<th>RT technique</th>
<th>Age (y)</th>
<th>MU/fraction</th>
<th>Photon energy (MV)</th>
<th>PTV (dm(^3))</th>
<th>Dose/fraction (Gy)</th>
<th>Total tumour dose (Gy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMRT</td>
<td>Mean</td>
<td>61.6</td>
<td>328.9</td>
<td>6</td>
<td>0.136</td>
<td>2.09</td>
</tr>
<tr>
<td>Range</td>
<td>47.5–72.2</td>
<td>305–379</td>
<td></td>
<td></td>
<td>0.086–0.160</td>
<td>1.92–2.25</td>
</tr>
<tr>
<td>SV-VMAT</td>
<td>Mean</td>
<td>64.9</td>
<td>378.8</td>
<td>6</td>
<td>0.137</td>
<td>2.02</td>
</tr>
<tr>
<td>Range</td>
<td>50.8–77.3</td>
<td>322–468</td>
<td></td>
<td></td>
<td>0.062–0.211</td>
<td>2.00–2.10</td>
</tr>
<tr>
<td>LV-VMAT</td>
<td>Mean</td>
<td>64.8</td>
<td>428.1</td>
<td>6</td>
<td>0.231</td>
<td>1.83</td>
</tr>
<tr>
<td>Range</td>
<td>57.6–73.5</td>
<td>275–734</td>
<td></td>
<td></td>
<td>0.083–1.048</td>
<td>1.27–2.09</td>
</tr>
<tr>
<td>Hypofractionation</td>
<td>Mean</td>
<td>67.7</td>
<td>733.8</td>
<td>18</td>
<td>0.17</td>
<td>2.85</td>
</tr>
<tr>
<td>Range</td>
<td>60.5–77.3</td>
<td>532–1064</td>
<td></td>
<td></td>
<td>0.138–0.203</td>
<td>2.83–2.87</td>
</tr>
</tbody>
</table>

**Abbreviations:** MU, number of monitor units; MV, mega voltage; PTV, planned target volume; Gy, gray.

\(\gamma\)H2AX foci and \(G_0\) MN assays

The procedure for the \(\gamma\)H2AX foci assay on T-lymphocytes is described in detail in a previous paper [9]. The Metacyte software module of the Metafer 4 scanning system (MetaSystems, Altussheim, Germany) was applied for automated scanning of the slides, for spot detection and foci scoring [10].

The procedure for the \(G_0\) MN assay can be found elsewhere [11]. The MSearch software module of the Metafer 4 scanning system (MetaSystems) was applied for automated scanning of binucleated (BN) cells and scoring of MN. Gallery images of the positive as well as the negative class of BN cells were checked and scores were adjusted when necessary.

**In vitro irradiations**

For the determination of the \(\gamma\)H2AX foci and MN in vitro dose response curves, whole blood samples of 3 healthy donors were exposed to different doses up to 500 mGy of Co-60 \(\gamma\)-rays. The same procedures as used for the patient samples were applied.

### Calculation of the equivalent total body dose

The equivalent total body dose \(D_{ETB}\) was calculated for each patient based on the treatment planning data. To this end the mean dose within the skin contour of the scanned volume was normalised to the patient mass. For the \(\gamma\)H2AX foci assay \(D_{ETB}\) has to be interpreted as the equivalent total body blood dose. As liver, heart/large blood vessels and lungs contain together 38.5% of the total blood volume it was assumed that 61.5% of the blood pool is distributed uniformly over the rest of the body. In the \(D_{ETB}\) calculation the contribution of the medical imaging, CBCT and EPID was also taken into account.

### Statistical analysis

Statistical analysis of the data was performed using the Statistical Package for Social Sciences (SPSS) version 20.0. The 2-tailed Mann–Whitney test was performed to investigate the significance of differences in biomarker scores. The statistical power of the obtained results was assessed using G*Power version 3.1.4 software [12].

### Results

**\(\gamma\)H2AX foci and MN induced in vivo by different RT modalities for prostate cancer**

Fig. 2 represents the number of radiation induced (background corrected) \(\gamma\)H2AX foci/T-lymphocyte plotted against \(D_{ETB}\) after one RT fraction for each patient. Taking into account all foci data, the number of \(\gamma\)H2AX foci increases linearly with \(D_{ETB}\) but with a relatively large scatter of the data \((R^2 0.67)\). For the IMRT patient group two separated clouds of data points can be noticed in the dose ranges 0–20 mGy and 40–60 mGy. Compared to the in vitro dose response patient foci-\(D_{ETB}\) data are generally higher, especially for the hypofractionated LV-VMAT. However, differences are not statistically significant \((p 0.12)\).

The mean values of \(D_{ETB}/\)fraction and number of radiation induced \(\gamma\)H2AX foci averaged over the patients for each RT technique applied are summarised in Table 2 and represented graphically in Fig. 2 by the \(\times\) symbols with standard deviations as error bars. The

### Blood sampling

![Blood sampling](image-url)

**Fig. 1.** Time schedule of the blood samplings of the study.
The number of γH2AX foci was borderline significantly higher in IMRT compared to SV VMAT which is in line with the DETB values (p = 0.039). On the other hand, the mean number of foci induced by LV-VMAT was significantly higher compared to both IMRT and SV-VMAT (p < 0.001). Significance remains even when the LV-VMAT patients are subdivided according to the normo- and hypofractionation regime (0.001 < p < 0.005). The same trend is seen for the DETB values. The statistical significance of differences in foci numbers obtained among these rather small patient populations was supported by a post hoc power analysis which indicated a very good power (range 0.916–0.999) except for the comparison between IMRT and SV-VMAT (0.512).

The correlation between the number of radiation induced (background corrected) MN/1000BN and γH2AX foci/1000 T-lymphocytes for the patient population is represented in Fig. 4. A linear-quadratic relationship between both biomarkers of radiation damage results in an excellent fit (R^2 = 0.86). The mean baseline level of the γH2AX foci was 0.15 ± 0.14 and for the MN was 17.45 ± 5.82. No correlation between the baseline levels of both biomarkers could be demonstrated (R^2 = 0.07).

### Table 2

Mean values of DETB after 1 and 3 fractions, mean number of radiation induced γH2AX foci and mean number of radiation-induced MN/1000BN averaged over the patients for each RT technique applied.

<table>
<thead>
<tr>
<th></th>
<th>DETB/fraction (mGy)</th>
<th>γH2AX foci/T-lymphocyte</th>
<th>DETB/3 fractions (mGy)</th>
<th>MN/1000BN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Range (mGy)</td>
<td>Mean (SD)</td>
<td>Range</td>
</tr>
<tr>
<td>IMRT a</td>
<td>36.06 (20.11)</td>
<td>9.14–63.13</td>
<td>0.36</td>
<td>0.18–0.59</td>
</tr>
<tr>
<td>CBCT</td>
<td>3.93</td>
<td></td>
<td>19.17</td>
<td></td>
</tr>
<tr>
<td>Low volume arc therapy a</td>
<td>31.18 (7.03)</td>
<td>18.83–37.77</td>
<td>0.22</td>
<td>0.09–0.47</td>
</tr>
<tr>
<td>CBCT</td>
<td>2.77</td>
<td></td>
<td>13.51</td>
<td></td>
</tr>
<tr>
<td>High volume arc therapy a (Normofractionation)</td>
<td>72.92 (19.61)</td>
<td>55.19–104.48</td>
<td>0.83</td>
<td>0.39–1.19</td>
</tr>
<tr>
<td>EPID</td>
<td>106.59 (11.09)</td>
<td>92.74–116.09</td>
<td>1.39</td>
<td>1.27–1.59</td>
</tr>
<tr>
<td>High volume arc therapy a (Hypofractionation)</td>
<td>5.2</td>
<td></td>
<td>21.9</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Abbreviations: DETB, equivalent total body dose; CBCT, Cone Beam Computed Tomography; EPID, Electronic Portal Imaging Device.

- a Including the CBCT/EPID dose contribution.
Discussion

In present study, a biomarker approach was used to compare the risk for development of RISM in PCa patients receiving ss-IMRT, SV-VMAT and LV-VMAT in a normo- and hypofractionation regime. The patients' γH2AX foci and MN data show in general a systematic increase with $D_{ETB}$. Foci yields were borderline significantly higher in IMRT compared to SV-VMAT treated patients but in line with the $D_{ETB}$ values. It is often stated that SV-VMAT reduces the daily treatment time compared to IMRT resulting in a smaller...
amount of scatter radiation from the treatment head, which should lower the risk of RISM [13]. On the other hand Aznar et al. indicated that the mean dose to healthy tissues was slightly increased for SV-VMAT treatment compared to 5-field IMRT [13,14]. Our data do not indicate a significant difference in risk of RISM inherent to the technique itself.

For IMRT treated patients, receiving a \( D_{\text{ETR}}/\text{fraction} \) less than 20 mGy, the number of observed \( \gamma \)-H2AX foci was 3–5× higher than expected from the in vitro dose–response. A comparison of the low dose IMRT data with the in vivo dose response determined over the total dose range resulted in a borderline significant difference (\( p = 0.046 \)). Possible explanations are underestimation of \( D_{\text{ETR}} \) by low dose radiation scatter and/or head leakage not included in the calculation of \( D_{\text{ETR}} \) or the bystander effect [15,16]. Such an effect was not observed for the micronuclei and also not for the in vitro irradiated samples.

A systematic comparison of in vivo and in vitro data shows higher scores for both biomarkers after in vivo exposure especially for LV-VMAT produced by 18 MV X-rays. This difference may be explained by the factors just mentioned but for the LV-VMAT data also photo-neutrons, which have a high RBE, may play a role [5].

The used biomarkers can also be considered as early biomarkers of secondary cancer risk after RT. In a radiosensitive Patched-1 mouse model \( \gamma \)-H2AX foci induced in shielded cerebellum of mice irradiated in vivo were linked to medulloblastoma formation [17]. MN as marker for chromosomal damage are linked to carcinogenesis due to the causal role of balanced chromosome rearrangements in early stages of carcinogenesis and the increased MN frequency seen in cancer patients [18].

Conclusion

A comparative study of \( \gamma \)-H2AX foci and MN as radiation effect biomarkers in PCA patient groups treated with IMRT and VMAT (small and large volume) reveal that the biomarker response was governed by dose and irradiated volume of normal tissue. However, no significant differences between IMRT and rotational therapy inherent to the technique itself were observed.

Conflict of interest

None of the authors have conflicts of interests to report concerning the manuscript.

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References