Gastrointestinal malignancies in high-risk populations = Gastro-intestinale maligniteiten in hoog-risico populaties

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3 DNA COPY NUMBER ABERRATIONS IN SECOND PRIMARY SMALL AND LARGE BOWEL MALIGNANCIES AFTER TREATMENT FOR HODGKIN LYMPHOMA OR TESTICULAR CANCER

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ABSTRACT

Background

Hodgkin lymphoma (HL) and testicular cancer (TC) survivors have an increased risk of second primary bowel malignancies (both colorectal cancer (CRC) and small bowel adenocarcinoma (SBA)). We aimed to determine differences in genetic characteristics and mismatch repair (MMR) status of primary and second primary bowel malignancies.

Methods

Copy number aberrations (CNAs) generated by shallow whole-genome sequencing (WGS) were collected from previous studies of second primary (sp) CRC (n=39), primary CRC (pCRC, n=90) and primary SBA (pSBA, n=14). In addition, seven new samples from second primary SBA (spSBA) in HL/TC survivors, identified through the Dutch national pathology registry, were available. MMR status was evaluated by immunohistochemistry.

Results

Overall, CNA patterns of spCRC and spSBA were similar to those in pCRC and spSBA. Losses of 21q22.2 were observed more frequently (p=0.057) in spCRC compared with pCRC, while in spSBA gains of 10p15.3-15.1 and losses of 18q12.1-23 were significantly more frequently detected compared with pSBA. One spSBA was MMR deficient, by unexplained mechanism.

Conclusions

spCRC and spSBA show comparable CNAs as pCRC and pSBA, respectively. This suggests that the pathogenesis of spCRC/SBA tumours in these cancer survivors is largly similar to that of pCRC and pSBA, despite exposure to previously applied DNA damaging cancer treatments.

INTRODUCTION

Cancer survivors are at increased risk of developing different types of second primary malignancies. Chemotherapy and/or infradiaphragmatic radiotherapy are associated with an increased risk of small and large bowel malignancies in Hodgkin lymphoma (HL) and testicular cancer (TC) survivors.[1-5] Especially, chemotherapy containing procarbazine for HL survivors and platinum-based chemotherapy for TC survivors seems to increase this risk.[5,6] The combination of chemotherapy and radiotherapy shows the highest association with an increased risk of colorectal cancer (CRC) and small bowel adenocarcinoma (SBA).[4-12] HL survivors have a two to seven times higher risk of developing CRC compared with the general population.[4,6,10,11] In the general population, SBA is rare but an increased risk has been reported in HL survivors (relative risk of 11 to 16) and in TC survivors (standardized incidence ratio (SIR) of 4.3).[13-16] These increased risks for developing second primary bowel malignancies persist up to 40 years after treatment for HL or TC.[4,7]

It has been suggested that the pathogenesis and molecular profile of second primary malignancies may differ from primary malignancies. Supporting this, specific mutational signatures have been shown to be associated with certain cancer treatments.[17] Moreover, HL treatment can induce single nucleotide variance leading to cancer susceptibility, as an association between certain polymorphisms and the risk of subsequent malignancy has been described.[17,18] Moreover, our group has previously detected a higher frequency of mismatch repair (MMR) deficiency in CRC in HL survivors, compared with CRC in the general population (24% vs. 11%). This MMR deficiency was due to somatic biallelic inactivation (mutations/loss of heterozygosity) in MMR genes.[19] So far, no information is available about the MMR status of second primary SBA. Furthermore, for both primary CRC and SBA, copy number aberrations (CNAs) have been described. However, whether the CNAs in second primary bowel malignancies (CRC or SBA) in cancer survivors differ from primary bowel malignancies is unknown.

Our aim was to determine whether differences in CNAs and MMR status occurred between second primary and primary bowel malignancies. We evaluated the frequency of CNA and MMR status in second primary bowel malignancies (both CRC and SBA) in HL and TC survivors in comparison with primary CRC and SBA.

MATERIALS AND METHODS

Patients and tissue samples

Four groups of tumours were evaluated; i) second primary CRC (n = 39), ii)

primary CRC in general population with average risk of developing CRC (n = 90), iii) second primary SBA (n = 8) and iv) primary SBA developed in the general population(n = 33).

Firstly, DNA of CRC in HL survivors was obtained from a previous study in which HL survivors developed CRC at least five years after the diagnosis HL (referred to as second primary CRC) as described previously.[20] HL patients were treated with chemotherapy (including procarbazine) and/or infradiaphragmatic radiotherapy. In that study, MMR status of 54 CRC in HL survivors was already evaluated, and DNA of 39 second primary CRCs was available for shallow whole-genome sequencing (WGS). We did not include CRC of TC survivors in this analysis.

Secondly, data of shallow WGS and MMR status of 90 primary CRC cases was obtained as previously described.[21] Patients had no history of previous malignancy.

Thirdly, HL and TC survivors who were diagnosed with SBA at least five years after the diagnosis of HL or TC (referred to as second primary SBA) were included. Both HL and TC patients were treated with chemotherapy and/or radiotherapy. Cases were selected using two methods. I) Patients were collected from two Dutch multicenter cohorts of five-year cancer survivors. One cohort included HL survivors treated in the period between 1965-2000 (N=3905).[4] The other cohort included TC survivors with a treatment period between 1976-2007 (N=5848).[9] Data was obtained through revision of medical records, questionnaires sent to general practitioners and record linkage with the Netherlands Cancer Registry since 1989, when nationwide coverage was reached.[4,9] II) Additional patients with a second primary SBA after either HL or TC (seminoma or non-seminoma) were identified through the PALGA registry (a Dutch nationwide network and registry of histology and cytopathology).[22] To receive information about treatment for HL or TC, hospitals were contacted through PALGA to provide this data. Pathology reports and formalin-fixed paraffin-embedded (FFPE) were requested through PALGA for all patients.[22] Clinical information was obtained from the Netherlands Comprehensive Cancer Organisation (IKNL) after linkage with the PALGA database. Second primary SBA occurred in 13 cancer survivors, of which five were excluded (cancer of the ampulla of Vater (n=4) and insufficient tissue available for analysis (n=1)).

Finally, we included primary SBA cases from a previous study of our group comparing the molecular pathogenesis of sporadic/non-celiac and celiac SBA (referred to as primary SBA).[23] Patients with celiac disease, M.Crohn and hereditary tumor syndromes including Lynch syndrome and Familial adenomatous polyposis were excluded in the present study since these patients have an increased risk for developing SBA.[23,24] DNA was available for 14/33

primary SBAs for shallow WGS sequencing analysis.
This study was approved by the Institutional Review Board of the Netherlands Cancer Institute (study number CFMPB307). Collection, storage and use of patient-derived tissue and data were performed in compliance with 'Code of conduct for responsible use', Dutch Federation of Dutch Scientific Societies, the Netherlands. All analyses were collected and analysed on anonymous basis.

Histopathology
The histopathology of second primary CRC, primary CRC and primary SBA was reassessed previously.[19,21,23] Of second primary SBA, the histopathology was reassessed according to standard protocol on hematoxylin & eosin (H&E) stained slides to confirm the diagnosis of SBA.

Immunohistochemistry
The MMR status of second primary CRC and primary SBA was previously determined by immunohistochemistry (IHC) of MLH1 and MSH2 and for primary SBA also MSI multiplex PCR was performed.[19,23] For second primary CRC the MMR status was evaluated as shown previously by IHC of all four MMR genes and MSI testing.[19] For second primary SBA, tissue microarrays (TMAs) were made when resection specimens were available and used for IHC. Whole slides were used when only biopsy material was available. IHC was performed for the four MMR proteins according to standard protocols for Ventana immunostainer (Roche, United States) using MLH1 (Agilent / DAKO, clone ES05), MSH2 (Roche / Ventana, clone G219-1129), MSH6 (Epitomics, clone EP49) and PMS2 (Roche / Ventana, clone A16-4) antibodies in second primary SBA. Samples with positive staining for all MMR proteins were considered MMR proficient.

DNA isolation
The AllPrep DNA/RNA FFPE extraction kit (QIAGEN, Germany) was used to isolate DNA of FFPE material from second primary SBA, following the manufacturer instructions. The Qubit 2.0 Fluorometer with the Qubit dsDNA Assay (Provenience) measured the DNA concentrations.

Microsatellite instability status analysis
Microsatellite instability (MSI) was examined in second primary SBA by a pentaplex PCR-based assay using fluorescent labelled primes of five mononucleotide repeat targets (BAT25, BAT26, NR24, NR21, NR27). Subsequent fragment analysis was performed. Tumours were considered MSI when instability of two or more markers occurred. Promoter methylation of the *MLH1* gene

was evaluated by a multiplex ligation-dependent probe amplification (MLPA) kit (ME011-B2 kit; MRC Holland, Amsterdam, the Netherlands). Positivity was defined when at least 3 of the 5 probes had a value superior to the cut-off of 0.2, at probe level.
In case of MMR deficiency without *MLH1* promoter methylation, further analysis was performed to screen the MMR genes for mutations, (loss of heterozygosity) LOH and CNV via Next Generation Sequencing (NGS) as described[25] using the msCRCv2 panel with supplier's materials and protocols (Life Technologies, Carlsbad, CA, USA). Details of the panel can be found at https://www.palga.nl/datasheet/LUMC/MMR_Panel_MSCRCv2_LUMC.pdf.

Shallow whole genome sequencing (WGS)
Shallow WGS data was already available for primary CRC.[21] DNA of second primary CRC, second primary SBA and primary SBA was used to evaluate CNAs of samples from all three remaining cohorts by performing shallow WGS (0.2x). Single-read sequences with a sequencing length of 65 basepairs were obtained using HiSeq 2500 High Output (Illumina, Cambridge, United Kingdom) system. Of the samples with low quality additionality HiSeq 4000 with 50 basepairs was performed.

Statistical analyses
Data was analysed using IBM SPSS V.22.0 database software. The χ^2 tests or Fisher's exact tests were used to analyse binary or categorical data and Kruskal Wallis tests for continuous data. Two-sided significance level was defined at $p<0.05$.
The algorithms and settings to call chromosomal copy number gains and losses and to determine chromosomal regions were performed as described previously.[26] Calls obtained from the shallow WGS data were processed by CGHregions (v1.34) with default settings. This resulted differences in 270 genomic regions. A permutation-based chi-squared test was performed to test whether frequencies in DNA CNAs between two groups (second primary malignancy versus primary malignancy) had significant differences. To account for multiple testing, False Discovery Rates (FDRs) were calculated using the Benjamini-Hochberg procedure. CGHtest (v 1.1) was used with the number of permutations set to 10.000 and the parameter 'af' set to 0.1.[27]

RESULTS

Characteristics of cancer survivors with second primary small and large bowel malignancies and of patients with primary small and large bowel malignancies

Characteristics of HL treatment in HL survivors who developed CRC have been described previously.[19] Median age at diagnosis of second primary CRC was 57 years,[19] compared with 72 years in the primary CRC cohort (p<0.01, Table 1).[21]

Table 1 | Baseline characteristics of patients with second primary colorectal cancer (CRC) in Hodgkin lymphoma (HL) survivors, primary CRC, second primary small bowel adenocarcinoma (SBA) in HL survivors and testicular cancer (TC) survivors and primary SBA.

CRC	**Second primary CRC (n = 54)**	**Primary CRC (n =122)**	**p-value**
Male	33 (61%)	70 (57%)	0.64
Age at diagnosis (median, standard deviation (SD), y)	57 (SD 16.2)	71.8 (SD 9.1)	<0.01
Location			<0.01
Proximal	24 (45%)	75 (61%)	
Distal colon	8 (15%)	26 (22%)	
Rectum	21 (39%)	20 (17%)	
Unknown	1	1	
SBA	**Second primary SBA (n = 8)**	**Primary SBA (n = 33)**	**p-value**
Male	7 (88%)	14 (42.4%)	0.045
Age of diagnosis SBA (median, minimum-maximum, y)	48 (26-68)	61 (29-87)	0.025
Interval between primary cancer and SBA (median, min-max)	20 (5-27)	N/A	N/A
Year of diagnosis SBA (range, y)	1997-2014	Unknown	N/A
Material (N, %)			N/A
Biopsy	5 (63%)	Unknown	
Resection	3 (37%)	Unknown	
Location			0.10
Duodenum	6 (75%)	11 (33%)	
Jejunum	1 (12%)	12 (37%)	
Ileum	1 (13%)	10 (30%)	

WHO classification (N,%)			N/A
Adenocarcinoma	7 (88%)	Unknown	
Signet-ring cell carcinoma	1 (12%)	Unknown	
Differentiation grade (N,%)			N/A
Well/moderate	6 (75%)	Unknown	
Poor	2 (25%)	Unknown	

Second primary SBA was diagnosed in five (63%) in TC survivors and three (37%) in HL survivors (Figure 1). The received treatment for the primary malignancy consisted of abdominal radiotherapy and/or chemotherapy in six (75%) patients; for two (25%) patients the treatment was unknown (Supplementary Table 1).

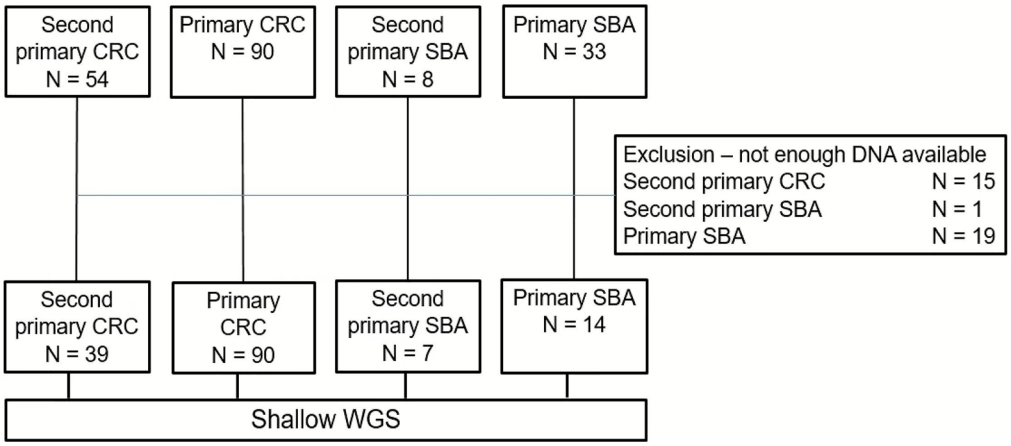


Figure 1 | Cohorts of colorectal cancer (CRC) and small bowel adenocarcinoma (SBA) where shallow coverage sequencing (WGS) was performed.

The median age of SBA diagnosis was 48 years for cancer survivors and 61 years for primary SBA (p=0.03). The locations of second primary SBA were duodenum in 6 (75%), jejunum in 1 (12.5%) and ileum in 1 (12.5%), while primary SBA was located in the duodenum in 11 (33%), jejunum 12 (37%) and ileum in 10 (30%) (p=0.10). All baseline characteristics of second primary SBA and primary SBA are described in Table 1.

Mismatch repair (MMR) status in small bowel malignancies in cancer survivors

MMR deficiency analysed by IHC in all seven second primary SBAs of which tissue was available revealed one case (13%) with MLH1 and PMS2 deficiency without *MLH1* promoter hypermethylation. MSI PCR was evaluated in six

out of seven (86%) cases. MSI was detected in the same case that lacked MLH1/PMS2 expression. This MMR deficiency was detected in a TC survivor, of whom details on the treatment of TC was unknown. Additional somatic mutation analysis of the MMR genes by NGS revealed no somatic pathogenetic mutation in the MMR genes. There was also no MMR mutation found in normal tissue, which excludes Lynch syndrome. As such the MMR deficiency could neither be explained by biallelic somatic inactivation nor Lynch syndrome and was regarded as unexplained. In primary SBA, three out of 33 cases (9%) were MMR deficient .[23] All these three MMR deficient tumors showed *MLH1* promoter hypermethylation. In the seven second primary SBA there was one MMRd case, which was not explained by NGS analyses.

Copy number variation in second primary and primary colorectal cancer

Shallow WGS was performed in 39 second primary CRC and 90 primary CRC (Figure 1). Frequency plots of CNAs in both groups are shown in Figure 2A and 2B, respectively. Overall, second primary CRC and primary CRC revealed a similar pattern of CNAs. No significant differences were observed for gains between the two groups. Losses within chromosomal region 21q22.2, were observed more frequently in second primary CRC (FDR = 0.06) (Table 2).

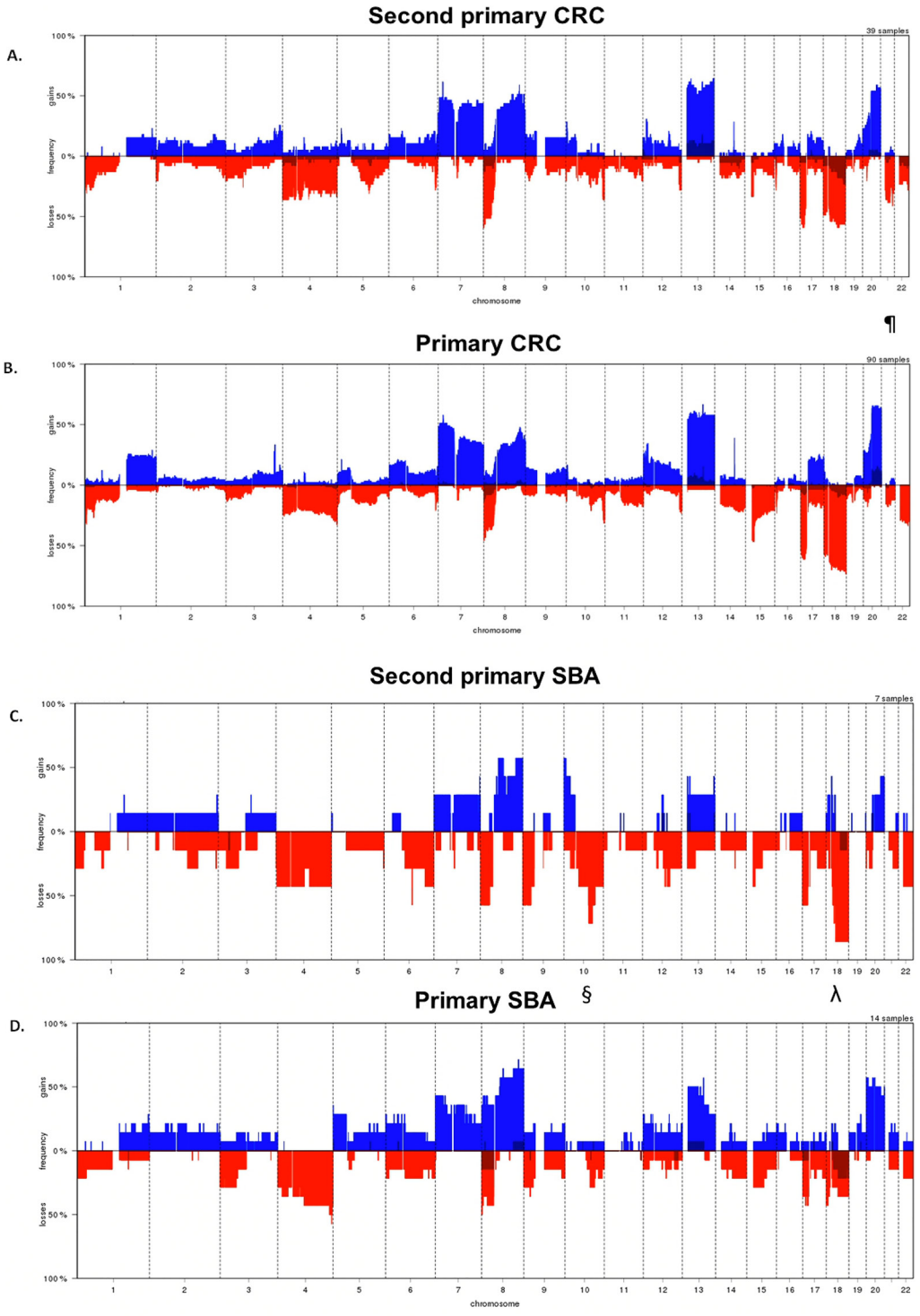
Copy number variation in second primary and primary small bowel carcinoma

Shallow WGS was also performed in seven second primary SBA and 14 primary SBA (Figure 1). Frequency plots of CNAs in both groups are shown in Figure 2C and 2D, respectively. Overall, the pattern of gains and losses between the two groups was comparable. In second primary SBA, gains were more frequent within chromosomal region 10p15.3-15.1 as well as losses in chromosomal region 18q12.1-q23, compared with the primary SBA (FDRs ranging from 0.02 to 0.06) (Table 2).

Table 2 | List of chromosomal regions which were found to have significant differences in copy number aberattions (CNAs) as determine by CGH test between second primary colorectal cancer (CRC) in HL survivors (n = 39) versus primary CRC (n = 90) and second primary small bowel adenocarcinoma (SBA) in cancer survivors (HL and testicular cancer (TC) survivors, n = 7) versus primary SBA (n = 14).

Second primary CRC vs. primary CRC					
Gains vs. no gains					
Chromosome	Location	Start (bp)	End (bp)	p-value	FDR
-	-	-	-	-	-
Losses vs. no losses					
Chromosome	Location	Start (bp)	End (bp)	p-value	FDR
21	q22.2	41100001	42400001	4,00E-04	0.0572

Second primary SBA vs. primary SBA					
Gains vs. no gains					
Chromosome	Location	Start (bp)	End (bp)	p-value	FDR
10	p15.3	100001	1100001	0.005	0.0544
10	p15.2	3100001	3300001	0.005	0.0544
10	p15.2-15.1	3400001	5700001	0.005	0.0544
Losses vs. no losses					
Chromosome	Location	Start (bp)	End (bp)	p-value	FDR
18	q12.1-q12.2	32200001	37000001	0.0027	0.0151
18	q12.2-q12.3	37100001	37200001	0.0027	0.0151
18	q12.3	37300001	39700001	0.0027	0.0151
18	q12.3-q21.1	39800001	43800001	0.0034	0.0151
18	q21.1	43900001	44600001	0.0034	0.0151
18	q21.1	44700001	46100001	0.0034	0.0151
18	q21.1	46200001	47400001	0.0034	0.0151
18	q21.1	47500001	48000001	0.0034	0.0151
18	q21.2	48300001	48400001	0.0034	0.0151
18	q21.2	48500001	48900001	0.0094	0.0447
18	q21.2-q21.32	49000001	56600001	0.0094	0.0447
18	q21.32-q22.3	56700001	71700001	0.0094	0.0447
18	q22.3-q23	71800001	75600001	0.016	0.0645
18	q23	75700001	78000001	0.0034	0.0151



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Losses occurred more frequently on chromosomal region 21q22.2 in second primary CRC vs. primary CRC; §: in second primary SBA vs. primary SBA gains occurred more frequently on chromosomal region 10p15.1-15.3; λ: on chromosomal region 18q12.1-23 losses were more frequently detected for second primary SBA vs. primary SBA.

Figure 2 | Frequency plot of DNA copy number variations in (A) second primary colorectal cancer (CRC) in cancer survivors (n = 39), (B) primary CRC (n = 90), (C) second primary small bowel adenocarcinoma (SBA) (n = 7, cancer survivors) and (D) primary SBA (n = 14). The frequency of gains (blue) and losses (red) are shown on the y-axis, sorted in chromosomal order and by chromosomal position on the x-axis.

DISCUSSION

Cancer survivors have an increased risk of developing CRC and SBA.[1-5] A likely hypothesis is that these second primary malignancies result from long-term DNA damage caused by prior chemo- and/ or radiotherapy. These therapies (among which procarbazine, cisplatin and/or radiation) cause double-stranded DNA breaks and may hence lead to chromosomal aberrations, including gains and losses, which could result in different molecular profiles when compared with molecular profiles of primary bowel malignancies. Our aim was to compare the MMR status and CNAs between second primary bowel malignancies of HL/TC survivors and primary bowel malignancies in order to provide insight into the pathogenesis of small and large bowel malignancies in cancer survivors. Different from our hypotheses, we detected a comparable pattern of CNAs in both CRC and SBA when comparing second primary bowel malignancies with primary bowel malignancies, suggesting that in general the carcinogenesis is similar at the CNA level. Some difference in the CNAs were detected. Furthermore, we observed that biallelic somatic mutation leading to MMR deficiency did not occur more frequently in our small cohort of second primary SBA compared with primary SBA, in contrast to what has been observed in the second primary CRCs.[19]

CNAs frequently described for primary CRC are gains located in chromosome 1q, 7, 8q, 13q and 20q and losses located in chromosome 1p, 4, 8p, 14q, 15q, 17p and 18q.[28] We detected this same pattern for both primary and second primary CRC. However, although not significant, losses occurred more frequently in chromosomal region 21q22.2 in second primary CRC in HL survivors versus primary CRC. A potential interesting locus at this genomic region is the long noncoding RNA DSCAM-AS1 (Down Syndrome Cell Adhesion Molecule antisense 1) as it has previously been associated with CRC. DSCAM-AS1 downregulation is correlated with (lymph node) metastasis, advanced stage

of CRC and poor prognosis, as this locus negatively regulates proliferation, migration and invasion.[29-31] One hypothesis could be that DSCAM-AS1 more often plays a role in secondary primary CRC. However, the prognosis between second primary and primary CRC does not differ.[32]

The pathogenesis of primary SBA is still poorly understood, but gains on chromosome 5p, 7, 8, 9q, 12, 13q, 16p, 19q and 20q and losses on chromosome 2, 4, 5q, 6q, 8, 9, 15q, 17p, 18 and 21 have been described.[15,23,33,34] Although, with some differences, SBA shares many CNAs with CRC, as described previously.[15]

We observed overall a similar pattern in chromosomal aberrations in second primary SBA compared to primary SBA except for a higher frequency of gains detected in chromosomal regions 10p15.3-15.1 and losses in 18q12.1-23 in second primary SBA, respectively. For a gene on chromosome 10p15.3-15.1 an association with CRC has been described such as *KLF6* and *AKR1C4*,[35,36] but, to our best knowledge, this has not been described previously for SBA. A previous study showed that deletions of 18q occurred frequently in primary SBA affecting the gene SMAD4, located at 18q21-q22.[33] SMAD4 is associated with the progression from adenoma to carcinoma in SBA and plays a central role in the TGF-β signalling pathway.[37] We hypothesize that TGF-β signalling may be more frequently involved in pathogenesis of SBA in cancer survivors. The gene DCC (deleted in colorectal carcinoma) is also present on chromosome 18q and encodes for a transmembrane protein (netrin-1 receptor) which plays a tumour suppressive role in CRC and esophageal cancer.[38] Although DCC gene mutations in SBA are rarely described, it could still be related to the development of second primary SBA.[37,39]

Previous studies have shown that CNAs of SBA are more comparable to CRC than to gastric cancer.[15,37] Prior to this study, we hypothesized that a specific different pattern in CNAs in second primary bowel malignancies could be detected in comparison with primary bowel malignancies, since the treatment for HL and TC may induce specific alterations. Surprisingly, no specific different CNAs were detected between second primary and primary bowel malignancies, nor were specific CNAs common in the second primary cancers (both CRC and SBA). Although the numbers are limited, these findings suggest that whatever specific alterations induced by the previous treatments, these are not reflected at the DNA copy number level. This suggests that the pathogenesis of first primary malignancies and second primary bowel malignancies in cancer survivors are similar. One possible explanation for this could be that, through therapy-related premature ageing, the process of carcinogen-

esis leading to second primary cancer is similar, but might start at an earlier age. It is known that prior cancer treatments lead to premature aging of tissues.[40,41] And in fact, both CRC and SBA were diagnosed in cancer survivors at a significantly younger age compared with primary CRC and SBA.[19] A previous study showed that TC survivors at a median age of 27 years, treated with at least three cycles of BEP chemotherapy, express an immunological phenotype associated with immunosenescence and increased expression of an aging biomarker (p16INK4a in CD3+ lymphocytes), which may indicate premature aging of the immune cells.[42] An alternative explanation is that prior treatments would lead to attenutation of the immune system (or immunosenescence)[43] resulting also in the earlier development of bowel malignancies.

Analogous to the current findings, we previously did not detect differences when comparing second primary to primary esophageal cancer.[44] We did previously, however, detect a higher prevalence of MMR deficiency in second primary CRC due to biallelic somatic inactivation (mutations/loss of heterozygosity) in the MMR genes (24%) compared with CRC diagnosed at before the age of 70 years in the general population (11%, $p<0.01$).[19,45] This might be related to treatment with methylating chemotherapeutic drugs, which can select for MMR deficiency. In second primary SBA we detected only one case (13%; 1 out 8) with MMR deficiency, while in primary SBA MMR deficiency has been reported to range from 8-35% (reported in studies both including and excluding MMR deficiency caused by Lynch syndrome).[46-53] In a recent large Dutch study, MMR deficiency was detected in 22% of the resected SBA (n = 332). In this study no data about presence of Lynch syndrome, *MLH1* hypermethylation or biallelic somatic mutations as explanation for the MMR deficiency was presented.[52] Although the numbers are very small, our data suggest that MMR deficiency has less influence on the development of second primary SBA compared with second primary CRC. However, it should be noted that procarbazine-containing chemotherapy, which may select for MMR deficiency, has only been received by two of our patients with second primary SBA.

The present study has several limitations. First of all, the sample size of SBA is small. This is due to the low incidence of primary SBA and specifically even lower incidence of second primary SBA. Secondly, other risk factors for developing bowel malignancies were not considered including diet, smoking and alcohol use. Thirdly, we only evaluated MMR status and CNAs, but differences in molecular profiles could also relate to other mutational signatures or altered gene expression levels.

CONCLUSIONS

In this study, we evaluated differences in CNAs in second primary bowel malignancies compared with primary bowel malignancies. In general, there were no major differences in the CNA patterns between second primary and primary cancers, nor a common pattern between second primary CRC and SBA vs. primary CRC and SBA was found. This suggests that whatever specific alterations may be induced by the previous anti-cancer treatments, these are not reflected at the DNA copy number level. Further research, for example looking at mutations, epigenetic changes and non-coding RNA, both in the tumours as well as in normal mucosa, is necessary to better understand the carcinogenesis of bowel malignancies in cancer survivors.

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SUPPLEMENTARY DOCUMENT

Supplementary Table 1 | Baseline characteristics of primary malignancy in patients who developed small bowel adenocarcinoma (SBA, n = 8).

Primary malignancy	**Hodgkin lymphoma survivors**	**Testicular cancer survivors**
Primary malignancy (N, %)	3 (37%)	5 (63%)
Age of diagnosis primary malignancy (median, min-max, y)	31 (13-36)	34 (21-44)
Year of diagnosis primary malignancy (min-max, y)	1980 – 1984	1979 – 2009
Histology TC (N, %) Non-seminoma Seminoma	 N.A. N.A.	 1 (20%) 4 (80%)
Stage primary malignancy (N,%) I II III IV Unknown	 1 (34%) 1 (33%) 1 (33%) - -	 - - 1 (100%) - 4
Treatment, radiotherapy (N,%) Radiotherapy Yes Mantle field Abdominal Para-aortal, iliac Retroperitoneal Unknown No Missing	 3 (100%) 1 (33%) 2 (67%) -	 2 (67%) - - 1 (100%) 1 1 (33%) 2
Treatment, chemotherapy (N, %) Yes MOPP Consisting of procarbazine BEP Unknown/missing No Missing	 3 (100%) 2 (100 %) 2 (100%) - 1 - -	 2 (67%) - - 1 (100%) 1 1 (33%) 2

Legend: MOPP: Mustargen + oncovin + procarbazine + prednisone; BEP: bleomycin + etoposide + platinum (cisplatin).