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ARTICLE

Genetics and Genomics

Somatic hits in mismatch repair genes in colorectal cancer among non-seminoma testicular cancer survivors

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BACKGROUND: Non-seminoma testicular cancer survivors (TCS) have an increased risk of developing colorectal cancer (CRC) when they have been treated with platinum-based chemotherapy. Previously we demonstrated that among Hodgkin lymphoma survivors (HLS) there is enrichment of rare mismatch repair (MMR) deficient (MMRd) CRCs with somatic hits in MMR genes. We speculate that this phenomenon could also occur among other cancer survivors. We therefore aim to determine the MMR status and its underlying mechanism in CRC among TCS (TCS-CRC).

METHODS: Thirty TCS-CRC, identified through the Dutch pathology registry, were analysed for MMR proteins by immunohistochemistry. Next-generation sequencing was performed in MMRd CRCs without *MLH1* promoter hypermethylation ($n = 4$). Data were compared with a male cohort with primary CRC (P-CRC, $n = 629$).

RESULTS: MMRd was found in 17% of TCS-CRCs vs. 9% in P-CRC ($p = 0.13$). MMRd was more often caused by somatic double or single hit in MMR genes by mutation or loss of heterozygosity in TCS-CRCs (3/30 (10%) vs. 11/629 (2%) in P-CRCs ($p < 0.01$)).

CONCLUSIONS: MMRd CRCs with somatic double or single hit are more frequent in this small cohort of TCS compared with P-CRC. Exposure to anticancer treatments appears to be associated with the development of these rare MMRd CRC among cancer survivors.

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BACKGROUND

Testicular cancer (TC) survivors have an increased risk of developing colorectal cancer (CRC) [1–7]. This increased risk appears to be associated with platinum-based chemotherapy, which was associated with a hazard ratio (HR) for CRC of 3.9 (95% confidence interval (CI) 1.7–8.9) [8, 9]. Such an association between platinum-based treatment and risk of second primary gastrointestinal (GI) malignancies has also been described in childhood cancer survivors [10].

The increased risk of second primary CRC in TC survivors (TCS-CRC) may be due to mutagenic and genome destabilising effects of cancer treatment on normal colonic mucosa [11]. These changes can result in premature ageing of the colonic mucosa and/or cancer development at an earlier age among cancer survivors [12, 13]. These treatment-induced changes may also activate pathogenetic processes that result in molecular profiles that are different from those of primary CRC. Previously, we have shown that Hodgkin lymphoma (HL) survivors treated with abdominal radiotherapy and/or procarbazine-containing chemotherapy have a higher frequency of mismatch repair (MMR) deficient (MMRd) CRC compared with CRC patients in the general population [14]. This higher frequency was due to the enrichment of somatic double hit in MMR genes by either mutations or loss of heterozygosity (LOH). Also, MMRd cases with somatic single hit occurred in this group. These findings suggested a novel association of prior anticancer therapy with somatic MMR gene mutations or LOH. We hypothesise that this association may not be specific to the context of HL. Instead, we contemplate that this phenomenon could also occur in other cancer survivors that received other types of anticancer treatments. To examine this hypothesis, we evaluated whether MMR status and the underlying mechanism of MMRd in TCS-CRC differs from CRC occurring in the general population (primary CRC, P-CRC).

METHODS

Patients and tissue samples

The population-based Netherlands Cancer Registry (NCR) was used to identify CRC after non-seminoma TC, diagnosed before the age of 50 years, irrespective of non-seminoma treatment. Patients were diagnosed with non-seminoma TC between 1989 and 2011. This range is caused by the fact that CRC develops predominately 10 years after treatment for TC, and therefore CRC was still diagnosed in 2019. A total of 36 CRC were identified

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at least one year after the diagnosis of non-seminoma TC. These cases were subsequently linked to the PALGA (the nationwide network and registry of histopathology and cytopathology) registry to obtain pathology reports and formalin-fixed paraffin-embedded (FFPE) material [15]. Tissue from 30 TCS-CRCs was available for analyses. Non-seminoma TC treatment data were retrieved through the NCR. All data collection and analyses were pseudonymised.

Histopathology

Histopathology of 30 of 36 (83%) retrieved samples was reassessed on haematoxylin & eosin (H&E)-stained slides according to standard protocol by an experienced gastrointestinal pathologist (PS). One patient had a metachronous CRC, of which both CRCs were completely evaluated, leading to 30 CRCs in 29 TC patients.

Immunohistochemistry

Immunohistochemistry (IHC) was performed for MMR proteins according to standard protocols for Ventana immunostainer (MLH1 (Agilent/DAKO, Cat. # M3640), MSH2 (Roche/Ventana, Cat. # 8033684001), MSH6 (Epitomics, cat. # AC-0047EU), PMS2 (Roche/Ventana, Cat. # 8033692001). IHC was performed on tissue microassay when available. In case of biopsy material, whole sections were cut for IHC.

Molecular analyses

The AllPrep DNA/RNA FFPE extraction kit (QIAGEN, Germany) was used to isolate DNA of FFPE material of CRC in TC survivors following the manufacturer's instructions. The concentrations were measured using the Qubit 2.0 Fluorometer with the Qubit dsDNA Assay Kit (Provenience).

Additionally, we evaluated the mutational status in common CRC-related genes, i.e. *KRAS*, *NRAS*, *BRAF* and *PIK3CA*, using a gene panel (Sequenom Massarray, Agena Bioscience, San Diego, California, USA) that also included *AKT1*, *DDR2*, *EGFR* and *MEK1*.

Due to very high concordance of MMR IHC and MSI PCR between between MMR status and microsatellite status in colorectal cancer [16–19], we did not perform MSI PCR.

Assessment of mechanism behind MMR deficiency

Promoter methylation of MMR genes was evaluated in MMRd tumours by a multiplex ligation-dependent probe amplification (MLPA) kit (ME011-B2 kit; MRC Holland, Amsterdam, the Netherlands). This probemix included a total of 25 probes for the promoter region of six different MMR genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *MSH3*, *MLH3*). Gene positivity was defined as 33% of probes per gene with a cut-off for positivity of 0.2 at probe level.

In case of MMRd without *MLH1* promoter methylation, further analysis was performed on both tumour tissue and normal tissue to screen MMR genes for mutations and LOH via Next Generation Sequencing (NGS) using the msCRCv2 panel with supplier's materials and protocols (Life Technologies, Carlsbad, CA, USA) as described previously [20]. Details of the panel can be found at https://www.palga.nl/datasheet/LUMC/MMR_Panel_MSCRCv2_LUMC.pdf.

The mechanism underlying MMRd was classified as follows: (1) *MLH1* promoter methylation, (2) Lynch syndrome, (3) somatic double hit by mutations or LOH and (4) somatic single hit by mutation or LOH. For statistical analysis, cases with somatic double or single hit were grouped together. We included all cases of MMRd in our analysis, including MMRd explained by Lynch syndrome to provide an overview on all MMRd subgroups.

Control group of CRC <70 years in the general population

The frequencies of MMRd and its mechanism of inactivation were compared to data of sporadic CRC in a general population cohort, referred to as primary CRC (P-CRC) [21, 22]. This included 1117 patients prospectively collected between 2007 and 2009 at ages ≤70. For this study, we selected male patients ($n = 629$) only to ensure comparability with our cohort. This control group was selected because it was a relatively young cohort within the general population and because of the availability of the required data (MSI status, MMR status, *MLH1* promoter methylation, etc).

Statistical analyses

Data was analysed using IBM SPSS V.22.0 database software. Data were compared between groups using χ^2 tests or Fisher's exact tests for categorical data and Mann–Whitney *U*-test for continuous data that were not normally distributed. The significance level was defined as two-sided $p \leq 0{\cdot}05$.

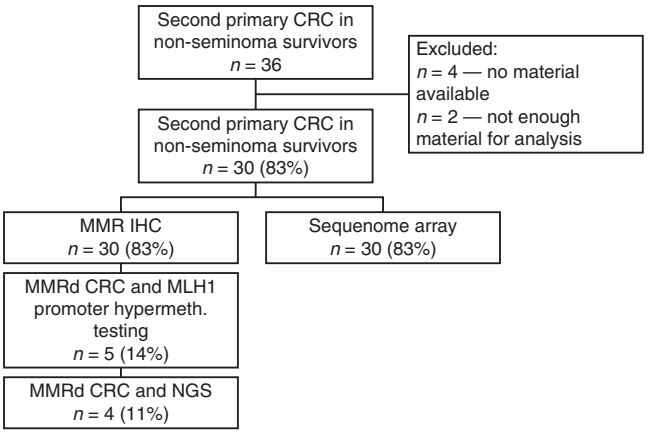

Fig. 1 Study flowchart. The flowchart of colorectal cancer (CRC) diagnosed in non-seminoma testicular cancer survivors treated with platinum-based chemotherapy.

Table 1. Baseline characteristics of non-seminoma testicular cancer (TC) survivors with second primary colorectal cancer (CRC).

	***N* (%) (*N* = 29)[a]**
Age of non-seminoma TC diagnoses	
Median (range)	39 (22–45)
Treatment period	
1989–1999	22 (76%)
2000–2011	7 (24%)
Stage non-seminoma	
I	9 (40%)
II	3 (15%)
III	4 (20%)
IV	4 (20%)
Unknown	9
Treatment non-seminoma	
Chemotherapy only	8 (89%)
Radiotherapy + chemotherapy	1 (11%)
Unknown	20

[a]Only characteristics of those patients from whom samples were retrieved are presented in the table of which one patient developed two CRCs.

RESULTS

Patient characteristics

FFPE material of 30 out of 36 TCS-CRCs (83%) was available for analyses (Fig. 1). One TC survivor had developed a second CRC after 1 year. The non-seminoma TC were diagnosed at a median age of 39 years (IQR 22–45 years) in the 29 patients (Table 1). In most cases, data on TC therapy could not be retrieved. Of patients for whom data could be retrieved ($n = 9$), all had received platinum-based chemotherapy (8/9 cisplatin and 1/9 carboplatin). Patient characteristics of the non-seminoma TC are described in Table 1.

The median interval between non-seminoma TC diagnosis and CRC was 19 years (IQR 2–29 years). Median age at diagnosis of TCS-CRC was 55 years (range 35–68), which was significantly younger than the median age at diagnosis of the P-CRC

Table 2. Characteristics of second primary colorectal cancer (CRC) in non-seminoma survivors and primary CRC.

	Second primary CRC in non-seminoma survivors ($n = 29$)	**Primary CRC <70 years ($n = 629$)**	***p* value**
Interval between TC diagnosis and CRC (median, range, years)	19 years (2–29 years)	N/A	–
Age at diagnosis of CRC (median, range, years)	55 years (35–68 years)	61 years (27–71 years)	<0.01
Year of CRC diagnosis (range)	1994–2019	2007–2009	N/A
	Total CRC $n = 30$ (n, (%))	**Total CRC $n = 629$ (n, (%))**	
Location			0.59
Proximal[a]	8 (29%)	153 (25%)	
Distal	12 (43%)	218 (36%)	
Rectum	8 (28%)	228 (38%)	
Unknown	1	30	
Stage			0.18
I	10 (50%)	123 (28%)	
II	3 (15%)	123 (28%)	
III	6 (30%)	173 (39%)	
IV	1 (5%)	25 (6%)	
Unknown	9	184	
MMR status			0.13
Proficient	25 (83%)	575 (91%)	
Deficient	5 (17%)	54 (9%)	
MMR staining			0.20
Staining present	25 (83%)	576 (92%)[b]	
MLH1 and PMS2 deficiency	3 (10%)[a]	38 (6%)	0.38
MSH2 and/or MSH6 deficiency	2 (7%)	14 (2%)	0.12
Mechanism of MMR deficiency			0.02
Somatic *MLH1* hypermethylation	1 (3%)	30 (5%)	0.18
Lynch syndrome	1 (3%)	13 (2%)	0.64
Somatic double or single hit in MMR genes	3 (10%)	11 (2%)	<0.01

MMR mismatch repair.
[a]In one there was loss of MLH1 and PSM2 staining, which also included secondary loss of MSH6 staining.
[b]One case with MMR proficient IHC result while MSI PCR showed MSI.

(diagnosed ≤70 years) (61 years, IQR 27–71 years, $p < 0.01$). The tumour location did not significantly differ between TCS-CRC and P-CRC. All TCS-CRC ($n = 30$) were conventional adenocarcinomas. *KRAS*, *NRAS* and *BRAF* mutation occurred in 35, 7 and 3% of TCS-CRCs, respectively. Patient and CRC characteristics are described in Table 2.

MMR status of second primary colorectal cancer in non-seminoma survivors

MMRd occurred in 17% (5/30) of TCS-CRC compared with 9% (54/629) in P-CRC ($p = 0.13$). Three of five MMRd cases (60%) demonstrated combined absence of MLH1 and PMS2 staining. One of these cases also showed absence of MSH6 staining, which is recognised as secondary inactivation resulting in loss of MSH6 on IHC [23]. The remaining two cases demonstrated either isolated absence of MSH6 staining or combined absence of MSH2 and MSH6 staining. Of all five MMRd cases, treatment given for non-seminoma TC was unknown.

Underlying mechanism of MMR deficiency in colorectal cancer in non-seminoma survivors

Of the three cases with MLH1/PMS2 deficiency, the first one had somatic hypermethylation of the *MLH1* promoter. The second was explained by Lynch syndrome (germline *MLH1* mutation accompanied by second somatic hit) and the third case by somatic double hit in the *MLH1* gene by mutation and LOH (Table 3). In the fourth case, which demonstrated MSH2/MSH6 deficiency on IHC, there was somatic single hit in the *MSH2* gene by LOH. In this case, we also detected LOH of *MSH6*, but these genes are in close proximity of each other on chromosome 2. It was therefore classified as a somatic single hit. Finally, for the case with isolated MSH6 deficiency, we found three mutations in the *MSH6* gene (Table 3). These three mutations included one frameshift mutation with known pathogenicity and two missense mutations of unknown pathogenicity. Therefore, we classified this case as having somatic single hit.

The distribution of molecular mechanisms underlying the MMRd was different between TCS-CRC and P-CRC ($p = 0.02$; Table 2). This difference was primarily due to enrichment of MMRd cases showing somatic double or single hit in MMR genes by mutation/LOH (10 vs. 2%, $p < 0.01$). The frequency of *MLH1* promoter hypermethylation was similar to the P-CRC cohort (resp. 3 vs. 5%, $p = 0.18$). Also, the frequency of Lynch syndrome was similar in TCS-CRC compared with P-CRC (resp. 3 vs. 2%, $p = 0.48$).

DISCUSSION

In this study, we aimed to determine whether TCS-CRC have different pathogenesis compared to P-CRC for which we evaluated the MMR status and its underlying mechanism. We have found

Table 3. Outcome of next-generation sequencing (NGS) of the four mismatch repair deficient (MMRd) colorectal cancer (CRC) in non-seminoma testicular cancer (TC) survivors (exclusion of the MMRd CRC explained by *MLH1* hypermethylation).

No.	Age at TC	Age at CRC	IHC loss	Material	Chr:ChrPos	Gene	HGVS Coding	Class[b]	Type	LOH	Conclusion of MMR	Mechanism of MMR deficiency
1[a]	37	40	MLH1 PMS2	Tumour	3:37053310 3:37038205	*MLH1* *MLH1*	NM_000249.3:c.546-1G>A NM_000249.3:c.207+5G>A	4 4	Splice-site Splice-effect	No LOH	2 mutations	Lynch syndrome
				Normal	3:37053310	*MLH1*	NM_000249.3:c.546-1G>A	4	Splice-site	No LOH	1 mutation	
2	31	57	MLH1 PMS2	Tumour	3:37053595	*MLH1*	NM_000249.3:c.677+5G>T	4	Splice-site	LOH of *MLH1*	1 mutation + LOH	Somatic double hit
				Normal		No pathogenic mutations						
3	22	50	MSH2 MSH6	Tumour		No pathogenic mutation				LOH of *MSH2* and *MSH6*	LOH	Somatic single hit
				Normal		No pathogenic mutation						
4	26	35	MSH6	Tumour	2:48026606	*MSH6*	NM_000179.2:c.1484delG NM_000179.2:c.890C>A NM_000179.2:c.728G>A	4 3 3	Frameshift Missense Missense	No LOH	1 mutation	Somatic single hit
				Normal		No pathogenic mutations						

TC testicular cancer, *CRC* colorectal cancer, *IHC* immunohistochemistry of mismatch repair (MMR) genes, *Chr:ChrPos* chromosome and chromosome position, *HGVS* a series of variance on one chromosome, *LOH* loss of heterozygosity.

[a]Of one CRC, two samples of FFPE material were available.

[b]Class: prediction of pathogenicity of gene variant (benign (1), likely benign (2), uncertain (3), likely pathogenic (4) or definitely pathogenic (5)).

that 17% of TCS-CRC are MMRd. MMRd status is significantly more often caused by double or single somatic hit compared to P-CRC (10 vs 2%, $p < 0.01$). In other words, we have shown that a rare subgroup of CRC with MMR deficiency, i.e. CRC with somatic double or single hit in MMR genes by mutation or LOH, is more common in TCS-CRC. Cases explained by *MLH1* promoter hypermethylation or Lynch syndrome are equally frequent in both cohorts.

In a previous study on HL survivors, we demonstrated a significant enrichment of somatic double hit as cause of MMRd (7/54, 13%) compared to the general population (8/1111, 0.7%) [14]. In that study, we primarily focussed on cases demonstrating somatic double hit, but we also found significantly more cases with somatic single hit (3/54, 6%) compared to CRC in the general population (3/1111, 0.3%, $p < 0.01$). The combined frequency of these two rare MMRd subgroups was 19% (10/54), which is much higher than in the general population reference cohort for that study (11/1111, 1%, $p < 0.01$).

The present data show an enrichment of a rare subgroup of MMRd cases, i.e. with somatic double or single hit in MMR genes, as previously observed in the study on HL survivors [14]. This enrichment becomes more apparent when comparing these frequencies to data from a recent meta-analysis taking all age-groups into account which showed that somatic double and single hit in MMR genes only occurs in 1.8% and 0.7% of all CRCs, respectively [24]. This underscores the rarity of this MMR subgroup in CRC in the general population and contrasts the frequency among second primary CRC. These data are of great importance, because the repeated link between anticancer treatment and the occurrence of these rare MMRd CRC among cancer survivors raises the question whether various anticancer treatments may cause the development of this MMRd subgroup among cancer survivors. The patient cohort with HL survivors was predominately treated with alkylating agents such as procarbazine and/or radiotherapy, while the large majority of patients with non-seminoma TC are treated with platinum-based chemotherapy [25]. In the current study, we unfortunately did not have information on treatment of patients with MMRd CRC. Also, experimental data explaining the mechanisms underlying these associations is lacking. Still, there is a link between the MMR system and cisplatin exposure, as it was shown that the MMR mechanism is important in repairing DNA damage caused by cisplatin [26–30]. Furthermore, a link between the MMR system, radiotherapy and alkylating agents has been described [14]. We previously hypothesised that pre-existing epithelial intestinal cells with some level of MMR dysfunction are targeted by anticancer treatments, which could then lead to the development of MMRd CRC.

Previously, patients with MMRd CRC have been referred to as having Lynch-like syndrome (LLS) when neither *MLH1* promoter hypermethylation nor germline mutations in MMR genes were detected. Since then, it has become clear that in a significant part of these cases, acquired somatic double or single hit in MMR genes can be found [31]. Cases with double hit in MMR genes can be regarded as fully clarified. However, MMR deficient cases with only a single detectable hit in an MMR gene are not fully clarified. Since inactivation of both alleles is necessary to result in complete loss of expression of MMR genes it can be deduced that a second hit is present although it was not identified. The lack of second hit is most likely explained by genetic alterations that are not detected by the methods used, such as certain types of LOH, epigenetic alterations or complex genomic alterations resulting in silencing of the other MMR gene. In studies examining patients with LLS, there also remains a subgroup where no somatic changes can be detected [31].

In our analysis, we found one TC survivor with corresponding MMR gene mutation both in CRC tumour tissue as well as in normal colonic tissue. Therefore, this single patient was regarded to have Lynch syndrome. The remaining patients did not carry

MMR mutations in normal colonic tissue. For these patients it could therefore be concluded that the MMR gene hits were unique to the CRC and not involved in the carcinogenesis of the prior testicular cancer. An increased risk of testicular cancer among Lynch syndrome patients has never been reported [32] and 97% of germ cell tumours from various locations among Lynch syndrome patients are microsatellite stable [33]. Also, the rate of MMRd in testicular cancers has been reported to be very low, i.e. much less than 1% [34, 35]. These observations contrast the relatively high percentage of MMRd in second primary CRC among TC survivors and agree with our finding that second primary MMRd CRC of TC survivors are largely unrelated to Lynch syndrome. This is also analogous to our previous findings on second primary MMRd CRC among Hodgkin lymphoma survivors [14].

Limitations of this study are the small sample size and the incomplete information on prior treatment for non-seminoma TC. Studies on MMRd CRC with somatic double or single hit usually lack information on whether these patients received previous anticancer therapy [31, 36–38]. However, when combining results from three recent studies with a total of 30 patients with MMRd due to somatic double hit, one of these patients had a previous history of HL and another of leukaemia [39–41]. None of these studies reported other prior cancer types or anticancer therapies. Even though treatment for TC was unknown in most cases in the present study, a large majority of non-seminoma TC patients do receive treatment with platinum-based chemotherapy, as the relapse risk varies between 15 and 50% depending on the presence of lymph-vascular invasion [25]. Clinical experience shows that a majority of the patients treated for TC will have received chemotherapy and, to a lesser extent, radiotherapy. The increased risk for developing CRC appears to be associated with the dosage of platinum-based chemotherapy in TC survivors [1–10, 42]. An elevated risk of developing CRC was even present 35 years after treatment [4, 5, 42]. We suggest that platinum-containing chemotherapy is associated with this increased risk, especially since platinum levels in serum remain elevated for a long period after treatment and is still detectable in tissues of various organs [43–47]. However, whether long-term retention in colorectal tissue, a fast-turnover tissue, is possible, remains unknown.

To conclude, somatic double or single hit in MMR genes is significantly more frequent in secondary CRCs that develop in non-seminoma TC survivors compared to primary CRC in the general population. Since similar results were shown in HL survivors, this may suggest an association between prior anticancer treatment and MMRd with double or single hit in MMR genes. Furthermore, our results could imply that this phenomenon is neither specific to a certain primary cancer nor a single type of prior anticancer treatment. These findings need confirmation in larger cancer survivor cohorts.

DATA AVAILABILITY

Data are available upon request from the corresponding author.

REFERENCES

1. Fossa SD, Langmark F, Aass N, Andersen A, Lothe R, Borresen AL. Second non-germ cell malignancies after radiotherapy of testicular cancer with or without chemotherapy. Br J Cancer. 1990;61:639–43.
2. Horwich A, Fossa SD, Huddart R, Dearnaley DP, Stenning S, Aresu M, et al. Second cancer risk and mortality in men treated with radiotherapy for stage I seminoma. Br J Cancer. 2014;110:256–63.
3. Ondrus D, Ondrusova M, Friedova L. Second malignancies in long-term testicular cancer survivors. Int Urol Nephrol. 2014;46:749–56.
4. Travis LB, Curtis RE, Storm H, Hall P, Holowaty E, Van Leeuwen FE, et al. Risk of second malignant neoplasms among long-term survivors of testicular cancer. J Natl Cancer Inst. 1997;89:1429–39.
5. Travis LB, Fossa SD, Schonfeld SJ, McMaster ML, Lynch CF, Storm H, et al. Second cancers among 40,576 testicular cancer patients: focus on long-term survivors. J Natl Cancer Inst. 2005;97:1354–65.
6. van Leeuwen FE, Stiggelbout AM, van den Belt-Dusebout AW, Noyon R, Eliel MR, van Kerkhoff EH, et al. Second cancer risk following testicular cancer: a follow-up study of 1,909 patients. J Clin Oncol. 1993;11:415–24.
7. Richiardi L, Scelo G, Boffetta P, Hemminki K, Pukkala E, Olsen JH, et al. Second malignancies among survivors of germ-cell testicular cancer: a pooled analysis between 13 cancer registries. Int J Cancer. 2007;120:623–31.
8. van den Belt-Dusebout AW, de Wit R, Gietema JA, Horenblas S, Louwman MW, Ribot JG, et al. Treatment-specific risks of second malignancies and cardiovascular disease in 5-year survivors of testicular cancer. J Clin Oncol. 2007;25:4370–8.
9. Groot HJ, Lubberts S, de Wit R, Witjes JA, Kerst JM, de Jong IJ, et al. Risk of solid cancer after treatment of testicular germ cell cancer in the platinum era. J Clin Oncol. 2018;36:2504–13.
10. Henderson TO, Oeffinger KC, Whitton J, Leisenring W, Neglia J, Meadows A, et al. Secondary gastrointestinal cancer in childhood cancer survivors: a cohort study. Ann Intern Med. 2012;156:757–66. W-260
11. Pich O, Muinos F, Lolkema MP, Steeghs N, Gonzalez-Perez A, Lopez-Bigas N. The mutational footprints of cancer therapies. Nat Genet. 2019;51:1732–40.
12. Cupit-Link MC, Kirkland JL, Ness KK, Armstrong GT, Tchkonia T, LeBrasseur NK, et al. Biology of premature ageing in survivors of cancer. ESMO Open. 2017;2:e000250.
13. Armenian SH, Gibson CJ, Rockne RC, Ness KK. Premature aging in young cancer survivors. J Natl Cancer Inst. 2019;111:226–32.
14. Rigter LS, Snaebjornsson P, Rosenberg EH, Atmodimedjo PN, Aleman BM, Ten Hoeve J, et al. Double somatic mutations in mismatch repair genes are frequent in colorectal cancer after Hodgkin's lymphoma treatment. Gut. 2018;67:447–55.
15. Casparie M, Tiebosch AT, Burger G, Blauwgeers H, van de Pol A, van Krieken JH, et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. Cell Oncol. 2007;29:19–24.
16. Loughrey MB, McGrath J, Coleman HG, Bankhead P, Maxwell P, McGready C, et al. Identifying mismatch repair-deficient colon cancer: near-perfect concordance between immunohistochemistry and microsatellite instability testing in a large, population-based series. Histopathology. 2021;78:401–13.
17. Hissong E, Crowe EP, Yantiss RK, Chen YT. Assessing colorectal cancer mismatch repair status in the modern era: a survey of current practices and re-evaluation of the role of microsatellite instability testing. Mod Pathol. 2018;31:1756–66.
18. Bartley AN, Luthra R, Saraiya DS, Urbauer DL, Broaddus RR. Identification of cancer patients with Lynch syndrome: clinically significant discordances and problems in tissue-based mismatch repair testing. Cancer Prev Res (Philos). 2012;5:320–7.
19. Moreira L, Balaguer F, Lindor N, de la Chapelle A, Hampel H, Aaltonen LA, et al. Identification of Lynch syndrome among patients with colorectal cancer. JAMA. 2012;308:1555–65.
20. Suerink M, Kilinc G, Terlouw D, Hristova H, Sensuk L, van Egmond D, et al. Prevalence of mismatch repair deficiency and Lynch syndrome in a cohort of unselected small bowel adenocarcinomas. J Clin Pathol. 2021;74:724–9.
21. Geurts-Giele WR, Leenen CH, Dubbink HJ, Meijssen IC, Post E, Sleddens HF, et al. Somatic aberrations of mismatch repair genes as a cause of microsatellite-unstable cancers. J Pathol. 2014;234:548–59.
22. van Lier MG, Leenen CH, Wagner A, Ramsoekh D, Dubbink HJ, van den Ouweland AM, et al. Yield of routine molecular analyses in colorectal cancer patients </=70 years to detect underlying Lynch syndrome. J Pathol. 2012;226:764–74.
23. Shia J, Zhang L, Shike M, Guo M, Stadler Z, Xiong X, et al. Secondary mutation in a coding mononucleotide tract in MSH6 causes loss of immunoexpression of MSH6 in colorectal carcinomas with MLH1/PMS2 deficiency. Mod Pathol. 2013;26:131–8.
24. Eikenboom EL, van der Werf-'t Lam AS, Rodriguez-Girondo M, Van Asperen CJ, Dinjens WNM, Hofstra RMW, et al. Universal immunohistochemistry for Lynch syndrome: a systematic review and meta-analysis of 58,580 colorectal carcinomas. Clin Gastroenterol Hepatol. 2022;20:e496–507.
25. Honecker F, Aparicio J, Berney D, Beyer J, Bokemeyer C, Cathomas R, et al. ESMO Consensus Conference on testicular germ cell cancer: diagnosis, treatment and follow-up. Ann Oncol. 2018;29:1658–86.
26. Fink D, Nebel S, Aebi S, Zheng H, Cenni B, Nehme A, et al. The role of DNA mismatch repair in platinum drug resistance. Cancer Res. 1996;56:4881–6.
27. Sawant A, Kothandapani A, Zhitkovich A, Sobol RW, Patrick SM. Role of mismatch repair proteins in the processing of cisplatin interstrand cross-links. DNA Repair (Amst). 2015;35:126–36.
28. Honecker F, Wermann H, Mayer F, Gillis AJ, Stoop H, van Gurp RJ, et al. Microsatellite instability, mismatch repair deficiency, and BRAF mutation in treatment-resistant germ cell tumors. J Clin Oncol. 2009;27:2129–36.
29. Lin X, Ramamurthi K, Mishima M, Kondo A, Christen RD, Howell SB. P53 modulates the effect of loss of DNA mismatch repair on the sensitivity of human

colon cancer cells to the cytotoxic and mutagenic effects of cisplatin. Cancer Res. 2001;61:1508–16.
30. Lin X, Trang J, Okuda T, Howell SB. DNA polymerase zeta accounts for the reduced cytotoxicity and enhanced mutagenicity of cisplatin in human colon carcinoma cells that have lost DNA mismatch repair. Clin Cancer Res. 2006;12:563–8.
31. Lefol C, Sohier E, Baudet C, Naibo P, Ruano E, Grand-Masson C, et al. Acquired somatic MMR deficiency is a major cause of MSI tumor in patients suspected for "Lynch-like syndrome" including young patients. Eur J Hum Genet. 2021;29:482–8.
32. Huang D, Matin SF, Lawrentschuk N, Roupret M. Systematic review: an update on the spectrum of urological malignancies in Lynch syndrome. Bladder Cancer 2018;4:261–8.
33. Latham A, Srinivasan P, Kemel Y, Shia J, Bandlamudi C, Mandelker D, et al. Microsatellite instability is associated with the presence of Lynch syndrome pan-cancer. J Clin Oncol. 2019;37:286–95.
34. Dum D, Steurer S, Simon R, Zimmermann PV, Burandt E, Clauditz TS, et al. Mismatch repair deficiency occurs very rarely in seminomas. Transl Androl Urol. 2021;10:1048–55.
35. Bonneville R, Krook MA, Kautto EA, Miya J, Wing MR, Chen HZ, et al. Landscape of microsatellite instability across 39 cancer types. JCO Precis Oncol. 2017;2017;PO.17.00073.
36. Pico MD, Castillejo A, Murcia O, Giner-Calabuig M, Alustiza M, Sanchez A, et al. Clinical and pathological characterization of Lynch-like syndrome. Clin Gastroenterol Hepatol. 2020;18:368.e1–74.e1.
37. Guillerm E, Svrcek M, Bardier-Dupas A, Basset N, Coulet F, Colas C. Molecular tumor testing in patients with Lynch-like syndrome reveals a de novo mosaic variant of a mismatch repair gene transmitted to offspring. Eur J Hum Genet. 2020;28:1624–8.
38. Adan-Merino L, Aldeguer-Martinez M, Alonso-Gamarra E, Valentin-Gomez F, Zaera-De la Fuente C, Martin-Chavarri S. Diagnosis and clinical behavior in patients with Lynch-like syndrome. Rev Gastroenterol Mex (Engl Ed). 2018;83:470–4.
39. Wang T, Lee LH, Vyas M, Zhang L, Ganesh K, Firat C, et al. Colorectal carcinoma with double somatic mismatch repair gene inactivation: clinical and pathological characteristics and response to immune checkpoint blockade. Mod Pathol. 2019;32:1551–62.
40. Xicola RM, Clark JR, Carroll T, Alvikas J, Marwaha P, Regan MR, et al. Implication of DNA repair genes in Lynch-like syndrome. Fam Cancer. 2019;18:331–42.
41. Golubicki M, Diaz-Gay M, Bonjoch L, Franch-Exposito S, Munoz J, Cuatrecasas M, et al. Comprehensive genomic characterization of fifteen early-onset Lynch-like syndrome colorectal cancers. Cancers. 2021;13:1259.
42. Hemminki K, Liu H, Sundquist J. Second cancers after testicular cancer diagnosed after 1980 in Sweden. Ann Oncol. 2010;21:1546–51.
43. Brouwers EE, Huitema AD, Beijnen JH, Schellens JH. Long-term platinum retention after treatment with cisplatin and oxaliplatin. BMC Clin Pharmacol. 2008;8:7.
44. Gietema JA, Meinardi MT, Messerschmidt J, Gelevert T, Alt F, Uges DR, et al. Circulating plasma platinum more than 10 years after cisplatin treatment for testicular cancer. Lancet. 2000;355:1075–6.
45. Poirier MC, Reed E, Litterst CL, Katz D, Gupta-Burt S. Persistence of platinum-ammine-DNA adducts in gonads and kidneys of rats and multiple tissues from cancer patients. Cancer Res. 1992;52:149–53.
46. Tothill P, Klys HS, Matheson LM, McKay K, Smyth JF. The long-term retention of platinum in human tissues following the administration of cisplatin or carboplatin for cancer chemotherapy. Eur J Cancer. 1992;28A:1358–61.
47. Travis LB, Beard C, Allan JM, Dahl AA, Feldman DR, Oldenburg J, et al. Testicular cancer survivorship: research strategies and recommendations. J Natl Cancer Inst. 2010;102:1114–30.

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AUTHOR CONTRIBUTIONS

All authors contributed to the study concept and design. BLMY performed acquisition of the data. PS contributed to histopathological evaluation of tissue samples and TvW performed NGS. BLMY, PS and MEvL wrote the manuscript and all authors reviewed and approved the manuscript. PS and MEvL supervised the study.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Institutional Review Board of the Netherlands Cancer Institute (CFMPB703). Collection, storage and use of patient-derived tissue and data were performed in compliance with the 'Code of conduct for responsible use', Dutch Federation of Dutch Scientific Societies, the Netherlands.

ADDITIONAL INFORMATION

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