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

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12 GENERAL DISCUSSION AND FUTURE PERSPECTIVES

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Early detection of gastrointestinal (GI) malignancies and its precursor lesions in high-risk populations is of great importance as this will lead to reduced incidence and/or mortality. Currently, we offer surveillance to high-risk populations after identification of these high-risk individuals. The awareness about the late adverse events of cancer treatment, including the development of second primary malignancies, is increasing.[1-10] Focus should be on earlier diagnosis and preventive measures for those high-risk populations in order to reduce the incidence, morbidity and mortality of second primary malignancies in cancer survivors.

The discussion of this thesis focusses firstly on the molecular profiles and risk factors for GI malignancies in cancer survivors. Explanations for the differences and similarities between the pathogenesis of primary and second primary GI cancer will be discussed. Secondly, we consider methods to identify high-risk cancer survivors for second primary GI malignancies and which colorectal cancer (CRC) surveillance recommendations should be offered for cancer survivors. Furthermore, we discuss mismatch repair (MMR) deficiency testing and Lynch syndrome from a few angles. Finally, we provide recommendations for future research and clinical implications.

Molecular profiles and risk factors of gastrointestinal malignancies in cancer survivors

In this thesis, we investigated the molecular profiles of GI malignancies among Hodgkin lymphoma (HL) and testicular cancer (TC) survivors.[11-14] It has been previously shown that differences in molecular profiles exist between when second primary cancers are compared to primary cancers.[15-21] Our group has confirmed the hypothesis that the molecular profile of second primary cancers differ from the profile in primary cancers.[22] Contradictorily though, studies have also been emerging where limited or no differences have been found.[23,24] This includes our own findings in **chapters 2 and 3**. Here below these seemingly conflicting results will be discussed and a joint hypothesis formulated.

Limited molecular differences between primary and second primary GI cancer; role of premature ageing

We aimed to detect differences in the molecular profile between second primary GI carcinomas in HL and TC survivors and sporadic carcinomas. However, in **chapters 2 and 3** we did not detect many differences, neither with regard to RNA expression profiles in esophageal squamous cell cancer (ESCC) nor with regard to the copy number aberrations (CNA) profiles in CRC and

small bowel adenocarcinoma (SBA). Previously, for other types of second primary malignancies, also no distinct pattern from those of the primary cancers have been described.[23,24] These findings suggest that the pathogenesis of second primary GI malignancies in cancer survivors may actually largely overlap with that of primary GI malignancies.

There are at least two main ways of explaining the limited differences between primary and second primary GI malignancies. Firstly, cancer is the result of a long and complex pathogenetic process. Differences present in the initiation and early steps of carcinogenesis may disappear or be overshadowed by the complex molecular changes that accumulate (often stochastically) on top of the early changes. In other words, molecular differences between primary and second primary GI cancers may in general be subtle and hard to detect.

A second possible explanation could be the phenomenon of premature aging occurring in cancer survivors.[25,26] According to current paradigms there is a direct link between age-related biological changes and cancer risk.[27] It could be hypothesized that anti-cancer therapy simply shifts the risk of developing cancer to an earlier age through induction of premature ageing. There is evidence in the literature to support this. Cancer survivors appear to develop age-related diseases and frailty sooner than the general population.[28-30] Besides DNA damage, it has been shown that cancer treatment induces damage to non-neoplastic normal tissues, which accelerates processes associated with aging.[29] Additionally, *in vivo* it has been shown that the normal colorectal epithelium in a lymphoma survivor treated with chemotherapy had a three to fivefold higher mutational burden than expected for his age. The authors concluded that the normal colorectal epithelium of the lymphoma survivor was 200 to 300-year-old, while the patient was only 66-years old when the specimen was obtained.[15]

Similarly, in **chapter 2** we showed that non-neoplastic squamous tissue in HL survivors had more resemblance to tumor tissue of the ESCC in HL survivors than to non-neoplastic squamous tissue of sporadic ESCC at the RNA level. This corroborates accumulating evidence that treatment-induced changes already occur in normal tissue exposed to radiotherapy and/or chemotherapy.

The processes of pathobiology behind accelerated aging include the role of telomeres, senescent cells, epigenetic modifications and micro RNA.[26] In an exploratory study in TC survivors treated with at least three cycles of BEP (bleomycine, etoposide and platinum) chemotherapy, it has been shown that already at an median age of 27 years an immunological phenotype associated with *immunosenescence* exists and the expression of an aging biomarker (*p16^{INK4a}*) in CD3+ lymphocytes was increased compared with healthy controls.[31] This reinforces the hypothesis that certain anti-cancer treatments in-

duce premature aging, which could explain the development of a malignancy at a relatively younger age in cancer survivors.
Better understanding potential premature aging is of importance to improve therapeutic options and mitigate the late effects in cancer survivors.[32] Additionally, cancer treatment has improved over the past decades, resulting in new therapies and new treatment regimes, of which the long-term effects are yet unknown. It would be interesting to determine whether the premature aging is detectable for the procarbazine-containing chemotherapy, platinum-based chemotherapy and infradiaphragmatic radiotherapy, for instance in mouse models. For example, the mutational burden may be determined in CRC mouse models, also determining the effects on non-neoplastic tissue. Furthermore, as we did not detect differences on the RNA profiles expression and CNA level, it would be interesting to perform whole-genome sequencing which might help in identifying differences in molecular profiles in these second primary GI cancers. Moreover, other molecular analyses like epigenetic alterations and non-coding RNA regulation could be evaluated in order to identify why these cancer survivors are more prone to developing second primary GI cancers.

Evidence for differences between primary and second primary GI cancers

Contrasting aforementioned results showing only limited differences between primary and second primary GI cancers, our group has previously demonstrated that a rare subgroup of MMR deficient CRC, i.e. cases based on biallelic and monoallelic somatic MMR gene mutations, occurs more frequently in CRC in HL survivors.[22] In other words, molecular differences do occur, at least in a subset of cases. In **chapter 5** we demonstrated a similar phenomenon among TC survivors as MMR deficiency was more frequently explained by somatic biallelic and monoallelic inactivation of the MMR genes. This finding implies that different types of anti-cancer treatment may cause the development of this rare MMR deficient CRC subtype among cancer survivors. The explanation for this phenomenon is unknown but may involve pre-existing MMR deficient cells or crypts that are vulnerable to the mutagenic effect of anti-cancer treatment, perhaps irrespective of the type of treatment. The phenomenon of therapy-induced MMR deficient carcinomas may also be tissue specific as our group was neither able to detect this in small bowel (**chapter 3**) nor gastro-esophageal adenocarcinomas.[37]
Finally, it should also be mentioned that differences in molecular profiles of primary versus second primary cancers have been described at an epigenetic level where alterations in DNA methylation and histone modification have been detected.[38-40]

Joint hypothesis for contradictory results; primary and secondary tumors are largely similar, partly different. Large differences are detectable in non-neoplastic tissue

We suggest that differences in the molecular profiles between second primary GI cancers and primary GI cancers do occur but predominately in a subset of cases and perhaps tissue specific. For the remaining cases the molecular profiles on DNA and RNA level appear so far to be largely similar – due to reasons as explained above.

Emerging data seem to indicate that the biggest and perhaps most important differences – when comparing cancer and carcinogenesis among cancer survivors versus the general population – are found in morphologically normal tissue. These differences in normal tissue can be explained since chemotherapy and radiotherapy will not only affect neoplastic cells, but also surrounding cells/tissue and therefore can induce alterations in non-neoplastic/normal tissue of cancer survivors.[17] Understanding the earliest phases of carcinogenesis in second primary malignancies could be of guidance for risk assessment, prevention and early detection of cancer among cancer survivors.

Risk factors for developing second primary gastrointestinal malignancies and mortality risk in cancer survivors

The development of second primary GI carcinomas may not only be explained by the effect of cancer treatment on the molecular profile of the (precursor of) malignancies, but also by the presence of certain risk factors for developing a malignancy. These risk factors could include age, smoking, alcohol use, obesity and single nucleotide polymorphism, among others. It is suggested that in cancer survivors, the development of second primary (precursor lesions of) malignancies is predominately related to the previous cancer treatment since a dose-dependent relationship has been described.[11-13] On the other hand, research based on the Surveillance, Epidemiology, and End Results (SEER) registries in the United States showed that smoking- and obesity were important risk factors for the development of second primary neoplasms among survivors of adult-onset cancers.[8] However, this study involved many types of primary malignancies. In contrast, in **chapter 4** we found no association between the known risk factors for CRC with the development of (advanced) neoplasia among HL survivors. Only a longer follow-up period between HL diagnosis and colonoscopy was associated with a higher prevalence of (advanced) neoplasia whereas smoking and obesity were not. A possible explanation for the different results between these two studies is that we investigated the association of risk factors with precursor lesions, while the SEER database was used to investigate the association of risk factors with cancer. Since only a subset of precursor lesions will progress to cancer it is possible that obesity

and smoking are predominately important as risk factors in the progression of colorectal precursor lesions to carcinoma. This might also mean that anti-cancer treatment is mainly important in leading to an increased number of precursor lesions (with corresponding increase in cancer risk), after which other risk factors come into the picture and add to the risk of further progression to cancer. Previous studies have shown that even radiotherapy with dosage below 30 Gray resulted in a higher prevalence of adenomatous polyps.[41,42] The higher prevalence of colorectal neoplasia has also been described in other cancer survivors than HL.[41,43,44]
These epidemiological data inform us on the importance of informing cancer survivors about these risk factors and discussing preventive measurements such as smoking cessation, aiming for a normal BMI and the option of surveillance procedures.

Methods of identification of high-risk cancer survivors for second primary gastrointestinal malignancies

Due to the increased risk of developing GI malignancies in HL and TC survivors, it is of importance to offer surveillance endoscopy in concordance with surveillance in other high-risk groups. As mentioned earlier, it might be possible to use molecular changes in morphologically normal tissue to identify high-risk individuals prone to develop a therapy-related/induced second primary GI malignancy. It can be hypothesized that certain molecular profiles may be found in non-neoplastic tissue, and our data show that the non-neoplastic tissue of HL survivors with squamous cell carcinoma of the esophagus already shows resemblance with neoplastic tissue. This could indicate (or even select for) an increased risk of developing a second primary malignancy. These individuals could benefit from more intense follow-up compared with individuals in whom these alterations in non-neoplastic tissue are not detectable or at a lower level.[45] Another possibility may be to measure for biomarkers for cancer in blood (for example by circulating tumor DNA – which is now predominately used in the follow-up of cancer treatment),[46-49] determine an aging biomarker[31] or evaluate the microbiome in order to identify individuals at risk for a malignancy.[50] However, currently, this is not being investigated in this high-risk population.

Specifically for TC survivors, we hypothesize that the level of platinum in the plasma may select high-risk TC survivors for developing CRC, but also for other second primary malignancies. A higher level of platinum has been described in the plasma until 20 years after treatment.[51] Currently, no correlation between platinum levels and the risk of developing a second primary malignancy has been described. We have initiated a study on the diagnostic

yield of colonoscopy surveillance in TC survivors treated with platinum-based chemotherapy (**chapter 11**). Additionally, the level of platinum in plasma will be correlated with the colonoscopy result in order to determine an association between platinum levels and the prevalence of advanced neoplasia. A high level of platinum may result in more DNA damage and/or premature aging, resulting in a higher prevalence of colorectal neoplasia. These results may also have implications for other cancer survivors who received platinum-based chemotherapy, and potentially also for other types of second primary malignancies or their precursor lesions.

Surveillance for colorectal cancer in high-risk populations

The aim of CRC surveillance in high-risk populations is to prevent the development of CRC. This CRC surveillance strategy should differ between the different high-risk groups, since the risk for CRC and all-cause mortality varies between the different groups.[52,53] The Dutch guideline for individuals with familial CRC risk advises a colonoscopy every five years from age 45 years.[54,55] This strategy has been shown to reduce the CRC incidence and mortality.[56] The relative risk of developing CRC in these individuals with a familial CRC risk is higher than 2.5 compared with the general population, resulting in a lifetime risk of >10% for patients that fulfil the criteria of familial CRC.[57] HL survivors treated with procarbazine-based chemotherapy and/or infradiaphragmatic radiotherapy have 2- to 7-fold higher risk for developing CRC in comparison with the general population. For TC survivors treated with platinum-based chemotherapy, a hazard ratio for developing CRC of 3.9 has been described. FIT surveillance may have a role for CRC surveillance in this risk group. In a systematic review and meta-analysis,[58] a pooled sensitivity of 93% of FIT for CRC was shown in individuals with either a personal or familial history of CRC. However, the sensitivity for detecting advanced neoplasia was lower (48%).[58] Currently, the diagnostic accuracy of FIT and mt-sDNA test is being evaluated in individuals with a colonoscopy surveillance indication – familial risk of CRC or history of polypectomy or CRC.[59] However, it might be suggested that stool test surveillance will not be offered to individuals with an increased risk of CRC, due to the low sensitivity of detecting advanced neoplasia and a false negative FIT result in patients with CRC or around 10%. Especially since polypectomy has been shown to prevent CRC deaths.[60] Furthermore, it has been described that a false-negative FIT result occurred more frequently in individuals with a family history of CRC than in those without.[61] However, further research is necessary, as it has been shown that repeating the stool test would result in a higher sensitivity.[62]

An alternative CRC surveillance strategy besides colonoscopy could be bene-

ficial in cancer survivors for several reasons. Firstly, colonoscopy surveillance is burdensome and can result in complications for the patient.[63] Secondly, selecting for colonoscopy based on the amount of hemoglobin in feces will select the patient at highest risk for colorectal neoplasia resulting in a more cost-effective approach and thereby resulting in a more optimal use of the limited colonoscopy capacity. Especially when no hemoglobin was measured in the FIT, the risk of an interval CRC was very low in the Dutch population-based screening program.[64] Furthermore, not all neoplasia will develop into CRC and in only 10% of the surveillance colonoscopies advanced neoplasia is detected, thus the positive predictive value of a primary colonoscopy surveillance is limited.[65] By offering primary stool test CRC surveillance in order to select which high-risk-persons for CRC should undergo a colonoscopy, may eventually result in a higher neoplasia detection rate for FIT, simply because of the higher participation rate and higher adherence to follow-up colonoscopy in case of a positive FIT.

Our group has previously detected a higher prevalence of neoplasia and advanced neoplasia in HL survivors treated with infradiaphragmatic radiotherapy and/or procarbazine-containing chemotherapy.[66,67] The prevalence of advanced neoplasia was 25% in HL survivors compared with 12% in the general population. Especially more (advanced) serrated lesions, including serrated polyposis syndrome, were detected during the colonoscopy. However, the participation rate of colonoscopy surveillance was low in this study population (41%) while the uptake of FIT screening in the Dutch population-based screening program is over 70%.[55,67] We suggested that stool test surveillance could result in a higher participation rate and thereby a higher detection rate per invitee.[68] In **chapter 6**, we showed that FIT at a cut-off of 10 µg Hb/g feces had the highest sensitivity of 37% for advanced neoplasia. However, previously it has been shown that the sensitivity of FIT is low for (advanced) serrated lesions and proximal lesions.[69] The sensitivity of mt-sDNA test was 68% for advanced neoplasia in HL survivors, and thus higher compared to FIT sensitivity, especially because this test is more sensitive for serrated lesions and proximal lesions.[69] Furthermore, the sensitivity was based on a single stool test, while sensitivity can increase when repeating the stool test with an annual or biennual interval.[62] However, it has been previously reported that mt-sDNA surveillance was not cost-effective, as the reimbursement rate is 20 times higher compared with other stool test strategies[70-72]

In **chapter 7,** we found that FIT surveillance was the most optimal surveillance strategy for all HL treatment categories using a 100% adherence rate for all estimated strategies, since this test had the lowest incremental cost-ef-

fectiveness ratio. Indeed, the mt-sDNA test was not cost-effective in the microsimulation in HL survivors due to the high costs of the test. Colonoscopy was also not considered the most optimal CRC surveillance strategy when FIT surveillance was included. However, it could be debated whether FIT surveillance should indeed be implemented in the follow-up guideline for HL survivors, since it is questionable whether FIT surveillance is sensitive enough to detect the higher prevalence of (advanced) serrated lesions and the higher prevalence of proximal lesions. The microsimulation is based on the assumption that adenomas develop into CRC, but the pathway of serrated lesions is not (yet) implemented in this simulation. Therefore, it could be hypothesized that FIT surveillance is not effective in this population due to the high prevalence of (advanced) serrated lesions. Separate analyses were performed for a colonoscopy as surveillance strategy. However, the costs of that a colonoscopy surveillance program is high and as shown the participation rate was low in colonoscopy surveillance.

As described previously, specific CRC surveillance strategies should be implemented for different high-risk populations for developing CRC. In the microsimulation in **chapter 7**, we interestingly also detected that for HL survivors the most optimal surveillance strategy should stop at the age of 70, which can be explained by the higher all-cause mortality in HL survivors.[73] TC survivors treated with platinum-based chemotherapy have a lower mortality risk than HL survivors, and furthermore the risk of CRC is specific for each group.[13,74] Therefore, a separate cost-effectiveness analysis should be performed based on the results of the ongoing study regarding TC survivors.

Testing of mismatch repair deficiency

MSI PCR and MMR IHC are two different ways to test for the same phenomenon, of which MMR IHC is currently more commonly used in the Netherlands. A high concordance between these two tests in CRC has been reported, varying between 96.1% to 99.6%.[75-77] However, the accuracy of MSI PCR has been questioned in malignancies other than CRC and endometrial cancer.[78] Latham et al[79] found that 30% of the non-colorectal and non-endometrial LS-associated tumors did not have a MSI-high result by PCR, but did show a MSI-indeterminate pattern. Similarly, in **chapter 9**, we showed that 67% of the cutaneous SCC diagnosed in Lynch syndrome individuals did not have MSI pattern by PCR, while by MMR IHC we demonstrated MMR deficiency in all cutaneous SCC, corresponding with the known germline mutation of the patient. This discordance between MSI PCR and MMR IHC has been previously described in sebaceous neoplasms,[80] and also in glioma, sarcoma, mesothelioma and adrenocortical neoplasms.[78] Explanations for this discordance may

be that a high tumor turnover is necessary to induce enough short nucleotides tandem repeats of small satellite DNA for it to be detectable on MSI PCR[81] or that the selection of microsatellites within the standard Pentaplex panel may not be sensitive for MSI detected by PCR in the context of tumor types other than colorectal and endometrial cancer.[78,82] Also, the role of tissue of origin (including micro-environment), histological tumor type, and the exact combination of biallelic MMR inactivation is likely to play a role. Our data support the growing notion that standard MSI PCR is less suitable to evaluate the MMR/MSI status for malignancies other than mainly CRC and endometrial cancer. We suggest that MMR IHC should be the main screening method for these non-colonic/endometrial malignancies. Recently, immunotherapy was approved for metastatic cutaneous SCC.
Besides IHC MMR and MSI PCR, the – relatively new – next generation or whole genome sequencing (NGS or WGS) computational MSI detection is possible. Data is accumulating showing that MSI tumors are very diverse and that this diversity, including highly variable level of microsatellite instability, is probably best captured by broad gene panels.[78] Still, routine MMR IHC testing will remain invaluable in screening of Lynch syndrome and MMR deficiency, especially when only FFPE material is available.

Additionally, MMR IHC has been shown to detect MMR deficiency in non-neoplastic colonic crypts, prior to the development of neoplasia.[83-85] This observation has been reported to be specific in individuals with Lynch syndrome, as this MMR deficiency was not detected in non-neoplastic colonic crypts in individuals with sporadic MMR deficient CRC.[85] For this purpose, in order to identify Lynch syndrome in individuals without a malignancy, MMR IHC may be valuble.[85] However, we were not able to detect MMR deficiency in normal colonic tissue in HL and TC survivors. In HL survivors, an increased frequency of MMR deficiency caused by biallelic somatic mutations in CRC has been described compared with the frequency of MMR deficiency in CRC in the general population.[22] Thus suggest an acquired MMR deficiency, not caused by Lynch syndrome. However, we were unable to detect MMR deficiency in the advanced precursor lesions of CRC in HL survivors, as described in **chapter 4**. This could suggest that the second hit of MMR deficiency is a late step in the development of these CRCs.

Recommendations for future research and clinical implications
Improving the knowledge about second primary malignancies in cancer survivors is of great importance, as the population is aging and the incidence of second primary malignancies is expected to increase. More research should be performed to better understand the effects of treatment on the patho-

genesis and the aging of organs and tissues at risk after cancer treatment. Furthermore, it should be studied whether changes in non-neoplastic tissue due to previous treatment could identify cancer survivors with the highest risk of developing specific second primary cancers. More precise risk assessment could be used to escalate or de-escalate surveillance programs for cancer survivors. Additionally, the effectiveness of FIT surveillance in HL and TC survivors should be investigated. All this data could result in reduction of the incidence and/or mortality of second primary malignancies in cancer survivors, not only for HL and TC survivors, but also for other types of malignancies.

Conclusion of the thesis

The pathogenesis of GI carcinomas in cancer survivors is complex and we are still in the early phase of understanding these tumors. Better understanding will help us to provide more adequate surveillance recommendations and aim for early detection. Furthermore, this thesis focused on CRC surveillance in HL and TC survivors and provided data for the implementation of CRC surveillance guidelines. Moreover, we evaluated the identification of Lynch syndrome in certain tumors. The results of this thesis serve as a contribution to personalized medicine among cancer survivors and other high-risk populations.

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