9. Summary and general discussion
SUMMARY
This thesis evaluates the pathogenesis of gastrointestinal neoplasia in Hodgkin lymphoma survivors and the indication for prevention program for colorectal cancer in Hodgkin lymphoma survivors, by evaluating the yield of advanced colorectal neoplasia of a first surveillance colonoscopy.

Patients who survived Hodgkin lymphoma (HL) have an increased risk of developing second primary malignancies. These malignancies are an important cause of excess morbidity and mortality in HL survivors. 1,2

Gastrointestinal cancer contributes to 20% of the absolute excess risk of cancer in HL survivors (24 excess cases per 10,000 person-years). 3 Reported increased risks (relative risks or standardized incidence ratios) for site-specific gastrointestinal cancers range from 3-31 for esophageal cancer, 3-11 for gastric cancer, 12-16 for small bowel cancer and 2-7 for colorectal cancer (CRC). 3-10 These increased risks are strongly associated with cancer treatment, including abdominal radiotherapy and alkylating agents, especially procarbazine.

Previous reports on a large Dutch multicenter cohort of HL survivors provide important insights into gastrointestinal cancers diagnosed in HL survivors. 3,11 In this cohort, the risk of gastrointestinal cancer was 4.6-fold increased compared with the general population. Gastrointestinal cancer risk was increased from 10 years up to 40 years after treatment for Hodgkin lymphoma compared with the general population. In addition, the risk of gastrointestinal malignancies did not appear to have decreased over HL treatment periods between 1965-2000, a period in which HL treatments changed substantially. This persistently increased risk cannot be explained.

Because of the strong association of this increased risk with prior cancer treatment, the tumors that developed in cancer survivors are here referred to as “therapy-related” tumors. However, it should be noted that a substantial proportion of these tumors are not related to prior cancer treatment, but are sporadic tumors (approximately 20% of gastrointestinal cancers, estimated by subtracting the attributable risk from 1 (attributable risk=((relative risk-1)/relative risk).
This epidemiologic evidence suggests that clinical interventions, such as prevention programs, may be warranted for HL survivors at increased risk of gastrointestinal cancer. Population-based screening programs for CRC are considered insufficient for high-risk population programs, as the detection rates of precursor lesions at risk for malignant development is low. When considering the implementation of surveillance, knowledge of the presence and types of precursor lesions, carcinogenesis and types of carcinomas is essential. Prior to this thesis, the evidence on the pattern of development of gastrointestinal cancer after abdominal radiotherapy or alkylating agents was limited to cellular experiments, mouse models and case series. 12-18 This thesis provides comprehensive insights into the pathogenesis of and preventive options for gastrointestinal neoplasia in HL survivors.

The clinical aspects of gastrointestinal neoplasia in HL survivors are investigated in Part I.

Chapter 2 describes three cancer survivors – of HL, testicular cancer and nephroblastoma – who were treated with abdominal radiotherapy. All three patients were diagnosed with intestinal adenomatous polyposis. One of these patients also developed gastric cancer and one developed rectal cancer. No predisposing germline mutations were identified in these patients, which led us to conclude that abdominal radiotherapy may be associated with the development of intestinal neoplasia.

Consequently, indications for a gastrointestinal cancer surveillance program should be evaluated in these patients. The efficacy of gastrointestinal cancer surveillance is associated with multiple factors that are evaluated in this thesis, including the pathogenesis, tumor characteristics and survival of gastrointestinal cancer in HL survivors.

In chapter 3, we evaluate whether survival of HL survivors who subsequently developed gastrointestinal cancer differs from survival of first primary gastrointestinal cancer patients. For this purpose, a total of 104 gastrointestinal cancer patients in a HL survivor cohort (including esophageal, gastric, small
intestinal and CRC) was compared with a cohort of 1,025 first primary gastrointestinal cancer patients. This comparison cohort was generated by case matching based on tumor site, gender, age at and year of diagnosis. Compared with first primary gastrointestinal cancer patients, overall and disease-specific survival of HL survivors who developed gastrointestinal cancer was worse (hazard ratio (HR) 1.30 (95% confidence interval (CI) 1.03-1.65) P=0.03 and HR 1.29 (95% CI 1.00-1.67) P=0.049, respectively). After adjustment for gastrointestinal cancer stage, grade of differentiation, surgery, radiotherapy, and chemotherapy for gastrointestinal cancer, this survival difference remained present (HR 1.33 (95% CI 1.05-1.68) P=0.02 and HR 1.33 (95% CI 1.03-1.72) P=0.03, respectively). Therefore, the survival difference could not be explained by these factors. Mortality from other causes was non-significantly increased in HL survivors who developed gastrointestinal cancer compared with first primary gastrointestinal cancer patients (HR 1.44 (95% CI 0.81-2.56) P=0.22).

The overall and disease-specific survival differences between HL survivors who developed gastrointestinal cancer and first primary gastrointestinal cancer patients, albeit not large, remained unexplained. These differences might result from a worse treatment response because of a difference in either pathogenesis or comorbidity between patients with therapy-related gastrointestinal cancer and sporadic gastrointestinal cancer patients.

**Chapter 4** describes the protocol of a prospective cohort study with the primary aim to evaluate the diagnostic yield of the detection of advanced colorectal neoplasia by a first surveillance colonoscopy in HL survivors who are at increased risk of developing CRC. This study invited HL survivors for colonoscopy who were treated with abdominal radiotherapy and/or procarbazine at least eight years after HL treatment. The results were compared with a Dutch general population cohort (n=1276 asymptomatic individuals between 50-75 years of age) that underwent a primary screening colonoscopy. The results of the interim analyses, after inclusion of 101 HL survivors, are presented in **Chapter 5**. Despite the fact that the general population cohort was nearly a decade older than the HL survivors in this study (median 51 years vs. 60
years, $P<0.001$), the prevalence of advanced colorectal neoplasia was higher in HL survivors than in the comparison cohort (25% vs. 12%, $P<0.001$). Advanced colorectal neoplasia consisted of advanced adenomas (prevalence of 14% in HL survivors and 9% in the comparison cohort, $P=0.08$), advanced serrated lesions (12% in HL survivors vs. 4% in the comparison cohort, $P<0.001$) and CRC (0% in HL survivors vs. 0.6% in the comparison cohort, $P=0.42$).

When evaluating patients between 50-70 years of age, the prevalence of advanced colorectal neoplasia was 33% in HL survivors and 11% in the comparison cohort ($P<0.001$).

In male HL survivors, the prevalence of advanced adenomas was higher than in male controls (23% vs. 10%, $P=0.002$), whereas the prevalence of advanced serrated lesions was similar in both groups (5% in HL survivors vs. 4% in controls, $P=0.64$). Opposite results were found in females, as the prevalence of advanced adenomas was not significantly different in HL survivors and controls (2% vs. 8%, $P=0.24$), and the prevalence of advanced serrated lesions was evidently higher (21% in HL survivors vs. 4% in controls, $P<0.001$).

An unexpected finding was that a total of 6% of HL survivors were diagnosed with serrated polyposis syndrome. This rare syndrome, with the highest reported prevalence in the general population of 0.09%, has an unknown etiology and gives rise to a very high risk of developing CRC. Thus, prior anticancer treatment may be a predisposing factor for serrated polyposis syndrome.

In combination with the increased risk of CRC, the high prevalence of advanced colorectal neoplasia in HL survivors suggests that colonoscopy surveillance should be recommended. Our current recommendation is that colonoscopy surveillance should start after at least eight to ten years of follow up since HL treatment, as the increased CRC risk starts after approximately 10 years. Surveillance should not start before the age of 35 years, as HL survivors have an increased risk of CRC that becomes manifest at 40 years of age, and we did not have sufficient patient numbers to evaluate the prevalence of advanced colorectal neoplasia before the age of 40 years. Similar to the recommendations for other high-risk groups, such as familial CRC, a five-yearly colonoscopy is advised after a negative
colonoscopy. In the presence of neoplasia, standard post-polypectomy follow-up guidelines should be followed. Further research is necessary to establish the optimal surveillance interval in this high-risk group.

As the efficacy of a CRC surveillance program is also influenced by patients’ perception and attendance rate, we evaluate the burden and acceptance of the first surveillance colonoscopy in participating HL survivors in chapter 6. Results were compared with the previously mentioned Dutch general population cohort in which a primary screening colonoscopy was performed.\(^{26}\)

In HL survivors and in the comparison cohort, approximately 80% of participants returned both the expected and perceived burden questionnaires. A total of 38% of HL survivors were rather or extremely reluctant to undergo a colonoscopy. In HL survivors, the colonoscopy was perceived less embarrassing, painful and burdensome than expected (P<0.001, P=0.03, P=0.009, respectively). Compared with controls, expected burden (38% vs 24% P=0.004) and perceived burden (17% rather/extreme burdensome vs. 7%, P<0.001) of the total procedure were higher in HL survivors. However, HL survivors were more frequently inclined to definitely undergo another colonoscopy (77% vs. 66% in controls, P=0.04).

Thus, despite the fact that colonoscopy surveillance is burdensome in HL survivors, the perceived burden did not reduce their intention to definitely undergo a second surveillance colonoscopy. These findings indicate that colonoscopy surveillance may be feasible in HL survivors.

**Part II** involves research on histopathological and molecular characteristics of therapy-related gastrointestinal cancer, focusing on the question whether the pathogenesis of these tumors differs from sporadic gastrointestinal cancer. The histopathologic and molecular characteristics of 54 therapy-related CRCs diagnosed in a HL survivor cohort were evaluated in chapter 7. The analyses involved immunohistochemistry for mismatch repair proteins and molecular analyses for oncogene mutation status and microsatellite instability (MSI). Frequencies of MSI were compared with a general population cohort of CRC patients diagnosed between 2007-2009 at the age of 70 or younger (n=1111).\(^{27}\)
Heterogeneous molecular characteristics were present in therapy-related CRCs, including *KRAS* and *BRAF* mutations in 41% and 15%, respectively. Compared with CRCs in the general population, therapy-related CRCs had a higher MSI frequency (24% versus 11%, *p*=0.003) and more frequent loss of MSH2/MSH6 staining (13% versus 1%, *p*<0.001). Loss of MLH1/PMS2 staining and *MLH1* promoter methylation were equally common in therapy-related CRCs and CRC in the general population. In MSI CRCs without *MLH1* promoter methylation, double somatic mismatch repair gene mutations (or loss of heterozygosity as second hit) were detected in 7/10 (70%) therapy-related CRCs and 8/36 (22%) CRCs in the general population (*p*=0.008). MSI therapy-related CRCs could not be ascribed to Lynch syndrome. Thus, in contrast with MSI CRC in the general population, MSI in therapy-related CRC was frequently caused by double somatic mutations in mismatch repair genes. This is the first association of prior anticancer treatment with somatic mismatch repair gene mutations in CRC.

Finally, in *chapter 8*, we evaluated if a similar pathogenesis was present in therapy-related gastric cancer diagnosed in HL survivors or testicular cancer survivors. In addition, we classified these gastric cancers into (surrogate) molecular subtypes based on immunohistochemical and molecular analyses: Epstein-Barr virus (EBV), mismatch repair deficiency or MSI, strong nuclear staining (≥70%) of p53 as a surrogate for chromosomal instability (sCIN), and a surrogate for genomic stability (sGS) for cancers without these aberrations. Results were compared with gastric cancer in the general population described in literature.

Molecular subtyping of 90 therapy-related gastric cancers resulted in 3% EBV, 8% MSI, 36% sCIN and 53% sGS. Three MSI therapy-related gastric cancers were not explained by *MLH1* promoter methylation, including two with double somatic mutations in mismatch repair genes. Therapy-related gastric cancers were more frequently sGS than gastric cancer in the general population (53% vs. 38%, *P*=0.04). Furthermore, therapy-related gastric cancer was more frequently sGS in HL and testicular cancer patients treated before 1990 - when this treatment was more intensive - than after 1990 (63% vs. 38%, *P*=0.03). Therapy-related gastric cancers located in the antrum, an area that receives high irradiation doses, were also more
frequently sGS (61% vs. 28% for antral gastric cancer in the general population, P=0.02).

Thus, therapy-related gastric cancers were more frequently classified as the sGS subtype than gastric cancer in the comparison cohort. The pathogenesis of these therapy-related gastric cancers of the sGS subtype remains unknown. In contrast with therapy-related CRC, the frequency of MSI was not increased in therapy-related gastric cancers. However, double somatic mismatch repair gene mutations were detected in two MSI therapy-related gastric cancers. These results suggest that at least a subset of therapy-related gastric cancers may have a different pathogenesis than gastric cancer in the general population.

In conclusion, this thesis demonstrates that in at least a subset of HL survivors, the pathogenesis of gastrointestinal neoplasia is different from the pathogenesis in the general population. Prior evidence on the increased risk of CRC in HL survivors, in combination with the evidence provided by this thesis, suggest that colonoscopy surveillance should be recommended for HL survivors who were treated with abdominal radiotherapy and/or procarbazine. For gastric cancer, further research is necessary.