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Abstract: Background: While advances in the treatment of myeloid neoplasms have led to improved patient survival, this has been accompanied by an increased risk of second primary cancer. To gain insight into risk of second primary cancer, we conducted a bi-directional analysis of risk of second primary cancer, i.e., risk of second primary cancer after myeloid neoplasia and risk of myeloid neoplasia as second primary cancer.
Patients & Methods: Using the Swedish Family-Cancer Database, we identified 35,928 individuals with primary myeloid malignancy, including myeloproliferative neoplasms, acute myeloid leukemia, chronic myeloid leukemia chronic myeloid leukemia and myelodysplastic syndrome diagnosed between 1958 and 2015. The primary endpoint was the assessment of relative risks (RRs) for second primary cancer, which were calculated using the generalized Poisson regression model.
Findings: Overall relative risk of second primary cancers was significantly increased after acute myeloid leukemia (RR = 1.29), chronic myeloid leukemia (1.52), myelodysplastic syndrome (1.42) and all myeloproliferative neoplasm (1.37) relative to the incidence of these cancers as first primary cancer. For myeloid neoplasia as second primary cancer, risks were significantly increased for acute myeloid leukemia (1.56), chronic myeloid leukemia (1.26) and myelodysplastic syndrome (1.54) relative to the incidence of these myeloid neoplasia as first primary cancers. Risk of upper aerodigestive tract and squamous cell skin cancers and non-Hodgkin lymphoma were increased as SPCs following all four types of myeloid neoplasia relative to their incidence as first primary cancers. High risks of myelodysplastic syndrome and acute myeloid leukemia as second primary cancers were found after hematological malignancies (RRs between 5.08 and 10.04).

Interpretation: The risks of second primary cancer are clinically important for the long-term management of patients with myeloid malignancies. The bi-directional associations of myeloid malignancies with many cancers suggest a number of candidate mechanisms that might contribute to the etiology of second primary cancer, many of which remain elusive; these might include immune dysfunction as well as the effects of therapy, thus laying out the groundwork for future investigations.
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RISK OF SECOND PRIMARY CANCER FOLLOWING MYELOID NEOPLASIA AND RISK OF MYELOID NEOPLASIA AS SECOND PRIMARY CANCER A NATION-WIDE FOLLOW-UP STUDY

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ABSTRACT

Background: While advances in the treatment of myeloid neoplasms have led to improved patient survival, this has been accompanied by an increased risk of second primary cancer. To gain insight into risk of second primary cancer, we conducted a bi-directional analysis of risk of second primary cancer, i.e., risk of second primary cancer after myeloid neoplasia and risk of myeloid neoplasia as second primary cancer.

Patients & Methods: Using the Swedish Family-Cancer Database, we identified 35,928 individuals with primary myeloid malignancy, including myeloproliferative neoplasms, acute myeloid leukemia, chronic myeloid leukemia chronic myeloid leukemia and myelodysplastic syndrome diagnosed between 1958 and 2015. The primary endpoint was the assessment of relative risks (RRs) for second primary cancer, which were calculated using the generalized Poisson regression model.

Findings: Overall relative risk of second primary cancers was significantly increased after acute myeloid leukemia (RR = 1.29), chronic myeloid leukemia (1.52), myelodysplastic syndrome (1.42) and all myeloproliferative neoplasm (1.37) relative to the incidence of these cancers as first primary cancer. For myeloid neoplasia as second primary cancer, risks were significantly increased for acute myeloid leukemia (1.56), chronic myeloid leukemia (1.26) and myelodysplastic syndrome (1.54) relative to the incidence of these myeloid neoplasia as first primary cancers. Risk of upper aerodigestive tract and squamous cell skin cancers and non-Hodgkin lymphoma were increased as SPCs following all four types of myeloid neoplasia relative to their incidence as first primary cancers. High risks of myelodysplastic syndrome and acute myeloid leukemia as second primary cancers were found after hematological malignancies (RRs between 5.08 and 10.04).

Interpretation: The risks of second primary cancer are clinically important for the long-term management of patients with myeloid malignancies. The bi-directional associations of myeloid malignancies with many cancers suggest a number of candidate mechanisms that might contribute to the etiology of second primary cancer, many of which remain elusive; these might include immune dysfunction as well as the effects of therapy, thus laying out the groundwork for future investigations.

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INTRODUCTION

All myeloid malignancies originate from the same hematopoietic lineage and are characterized by excessive proliferation, abnormal self-renewal and differentiation defects[1]. The distinct clinical phenotypes comprise *BCR-ABL1*-negative myeloproliferative neoplasms, polycythemia vera, essential thrombocythemia, myelofibrosis, chronic myeloid leukemia, myelodysplastic syndrome and acute myeloid leukemia. Polycythemia vera is characterized by a high erythrocyte count, essential thrombocythemia by a high platelet count, and myelofibrosis by bone marrow failure. Myelodysplastic syndrome is characterized by ineffective hematopoiesis, morphologic dysplasia in hematopoietic cells and cytopenia [2]. While each myeloproliferative neoplasm subtypes are essentially independent diseases, transformation from myeloproliferative neoplasms and myelodysplastic syndrome to acute myeloid leukemia can occur [1,3].

The etiology of myeloid diseases is poorly understood with few identified risk factors, which for acute myeloid leukemia include exposure to ionizing radiation, chemicals such as benzene, and cytotoxic chemotherapy [1]. For myelodysplastic syndrome risk factors include autoimmunity and immunological aberrations [4-6]. A number of rare high penetrance mutations in cancer susceptibility genes, such as Janus kinase 2 (*JAK2*), calreticulin (*CALR*), and myeloproliferative leukemia virus oncogene (*MPL*), have been identified and have been increasingly incorporated in disease classification, for example in myeloproliferative neoplasms [2,7]. Therapy for myeloproliferative neoplasm aims at reducing the risk for progression and has relied on compounds such as hydroxyurea, inhibiting DNA synthesis, and cytokine interferon alpha modulating immune and other functions; more recently specific *JAK-2* inhibitors have been included [8]. For instance, acute myeloid leukemia patients may receive induction therapy with DNA synthesis inhibitors cytarabine and anthracycline, supplemented with hydroxyurea. Consolidation therapy may include cytarabine administration or bone marrow transplantation. Chronic myeloid leukemia used to be treated earlier with hydroxycarbamide, interferon alpha and allogeneic hematopoietic stem-cell transplantation but from around year 2000, tyrosine kinase inhibitors were introduced into standard of care for *BCR-ABL1*-positive patients [9]. The choice of therapy for myelodysplastic syndrome, however, depends on various diagnostic findings and symptoms, and may involve multiple modalities.

Recent advances in the management of all forms of myeloid malignancies, including personalised approaches, have led to a markedly improved survival in patients. This has come at the cost of an increasing number of second primary cancers and other treatment-related complications, such as cardiovascular and neurological symptoms. A number of studies have estimated the risks of second

primary cancers following myeloid malignancy but have confined their analyses mainly to acute myeloid leukemia and chronic myeloid leukemia, with limited data on myeloproliferative neoplasms [8,10-13]. This appears to be largely because other myeloid malignancies are not curated by most cancer registries, thus limiting population-based epidemiological studies. To address this deficiency, we have conducted an analysis of all myeloid malignancies, using the Swedish Cancer Registry and including 35,928 patients, where we also report second primary cancers following myeloid malignancies. Additionally, we have conducted a novel analysis to assess myeloid malignancies diagnosed as second primary cancers following the occurrence of any non-myeloid first cancer. The assessment of bi-directional associations between myeloid malignancies and other cancers, i.e., risk of cancer after myeloid neoplasia and risk of myeloid neoplasia after cancer, may provide insight into possible mechanistic bases of second primary cancer development beyond therapeutic side effects and shared risk factors

METHODS

Study design and participants

The Swedish Family-Cancer Database includes the Swedish population organized in families and linked to the national Cancer Registry with more than 2 million cancers registered since 1958.The registry relies on separate compulsory notifications from clinicians who diagnosed the neoplasms and from pathologists/cytologists. The coverage is estimated at more than 90% of all cancer diagnoses in Sweden [14]. The registry counts tumors not patients, except for skin and urinary tract tumors diagnosed at the same topological area (https://www.ancr.nu/dyn/resources/File/file/7/4247/1412940269/total_document_survey_optimeret.pdf). To classify cancer types, the Swedish Cancer Register has used International Classification of Disease (ICD)-7 since 1958, ICD-9 since 1987, SNOMED (ICD-O/2) since 1993, and ICD-O/3 since 2005. The degree of histological verification of myeloid malignancies vary from close to 100% for acute myeloid leukemia and chronic myeloid leukemia to about 95% for polycythemia vera and myelofibrosis [15]. An *ad hoc* study on the diagnostic accuracy of second neoplasms in the Swedish Cancer Registry found 98% of these to be correctly classified; no recorded second primary cancer was found to be a metastasis upon re-examination [16].

The Cancer Registry orders any reported cancers as the first, second, third etc. primary cancer and we followed exactly this ordering. We included all patients with first primary acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome, all myeloproliferative neoplasm,

polycythemia vera, essential thrombocythemia, myelofibrosis and myeloproliferative neoplasm (not otherwise specified) between 1958 and 2015 for the risk of second primary cancer. While assessing the risk of myeloid malignancies as second primary cancer, the patients with any first primary cancer except leukemia were included. We used a combination of ICD-7 and ICD-O/2 codes to distinguish between different hematological malignancies and its subtypes. Myeloproliferative neoplasms were classified according to the 2016 WHO classification (https://doi.org/10.1038/s41408-018-0054-y, last accessed in June 2018). Myeloproliferative neoplasm (not otherwise specified), myelofibrosis and myelodysplastic syndrome were distinguished in ICD-O/2 and available since 1993. We excluded rare myeloproliferative neoplasms such as chronic eosinophilic leukemia and chronic neutrophilic leukemia from our subtype analysis. We did not consider myeloid diseases following myeloid diseases (609 patients) because of transformation between tumor types. The ICD codes used by the Swedish Cancer Registry do not specify therapy related cancers, which according to literature now account for 7% of acute myeloid leukemia [17].

Outcomes and statistical analysis

Relative risks (RRs) were assessed by means of incidence rate ratios assuming cancer diagnoses to follow waiting time distribution, regressed over a fixed effects generalized Poisson model (https://doi.org/10.1002/sim.4780122406, last accessed in June 2018). Regression was assessed on case numbers scaled on person-years. This reduced case numbers comparable to the reference fractions in each person-year category. Person-year calculation for the background population assured large case numbers in each covariate bands and met the large number assumption for the Poisson distribution. RRs for second primary cancer (cancer X) were obtained by comparing incidence rates for each second primary cancer in myeloid malignancy patients with respective population background rates for the first primary cancer X. Conversely, for the other-way round analysis considering myeloid malignancies as second primary cancers, the incidence rates were compared against background population incidence of the first primary myeloid neoplasia. Sex, age group, calendar-period, socio-economic status and residential areas were treated as potential confounders and were adjusted for in the regression model. Confidence intervals (CIs) were calculated for 5%, 1% and 0.1% level of significance. All analyses were performed in SAS (v9.4) or R (v3.3.4).

Subhayan Chattopadhyay and Guoqiao Zheng carried out primary statistical analysis.

The study was approved by the Ethical Committee of Lund University, Sweden and conducted in accordance with the tenets of the Declaration of Helsinki.

Role of the funding sources

The funding sources had no role in the study. K.H. had full access to all data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Of the 35,928 patients diagnosed with primary myeloid malignancy from 1958 till 2015, 2,631 (7.3%) developed a second primary cancer with a median follow-up time of 4 years (Table 1). Acute myeloid leukemia was the most common diagnosis but only 3.3% of 12,832 acute myeloid leukemia patients were diagnosed with second primary cancer. Among 6,636 polycythemia vera patients, 13.8% were diagnosed with second primary cancer. Out of the 940,811 other cancer patients, 3,873 developed a myeloid malignancy as second primary cancer with a median follow-up of 6 years.

We carried out stratified risk analyses on second primary cancer incidence after myeloid neoplasia by stratifying over period, sex, age at first cancer diagnosis and follow-up time (Appendix pages 2 to 5). Appendix page 2 shows that for myelodysplastic syndrome and myeloproliferative neoplasm (not otherwise specified) no cases were available in the early periods and the coding system for case identification started in 1993. RRs for second primary cancer did not differ between the periods (i.e., 95%CIs were overlapping) with the exception of a high RR of 1.81 for myeloproliferative neoplasm in 1971-1985, contributed by the high RR for essential thrombocythemia. Sex did not influence risk for second primary cancer (Appendix page 3) but early diagnosis (<65 y) of myeloid neoplasia was a strong risk modifier, except for myelodysplastic syndrome. Follow-up time did not systematically influence risks of second primary cancer, and significantly increased risks were noted after each myeloid neoplasia over most follow-up periods. For myelodysplastic syndrome follow-up was relatively short, 23 years. We analyzed in more detail risks of second primary cancer after chronic myeloid leukemia because the standard treatment changed with the introduction of tyrosine kinase inhibitors around year 2000 (Appendix page 6). Although case numbers for individual second primary cancers were small (mostly <10) , significant increases in second primary cancer risk for upper aerodigestive tract, thyroid and connective tissue cancers were

documented in the period between 2001-2015 relative to the background risk of these cancers as first primaries in 2001-2005 .

Table 2 details the RRs of second primary cancer following diagnosis of a myeloid malignancy and for all myeloid malignancies as second primary cancer. Overall, the risk of second primary cancer after all myeloid malignancies was increased 1.36 times relative to the background risk of any first primary cancer diagnosis. An increased risk was shown for 15 cancer sites, notably for nasal cancer (RR=3.10), squamous cell skin cancer (skin SCC, RR=2.80) and Hodgkin lymphoma (RR=2.63). Note that these RRs were significant at the 0.1% level. Myeloid malignancy as a second primary cancer showed an RR of 1.32. Individually, 15 cancers were associated with an increased risk of myeloid malignancy primarily, most increased after cancers of the hematopoietic system, non-Hodgkin lymphoma (RR=3.31), Hodgkin lymphoma (RR=4.47), and myeloma (RR= 4.78). Bi-directional increases were observed for 9 myeloid neoplasia-cancer pairs, including cancers of the small intestine, kidney, bladder, skin (both melanoma and SCC), connective tissue and hematopoietic tissue (Table 2).

Table 3 shows the RRs of all non-myeloid cancers following diagnosis of acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome or myeloproliferative neoplasm. The overall RRs for second primary cancers after each of these 4 diseases were 1.29, 1.52, 1.42 and 1.37, respectively. The respective numbers of second primary cancers with increased RRs were 8, 7, 8 and 12 compared to the background risk of first primary cancer. Risk of upper aerodigestive tract cancer, skin SCC and non-Hodgkin lymphoma was increased irrespective of myeloid primary type. Risk of kidney cancer was increased in chronic myeloid leukemia, myelodysplastic syndrome and myeloproliferative neoplasm patients relative to the background risk. For acute myeloid leukemia, high RRs were noted for anal cancer (RR=3.92) and Hodgkin lymphoma (RR=3.60). The RR for skin SCC was 5.54 after chronic myeloid leukemia. Myelodysplastic syndrome associated with a high risk of non-Hodgkin lymphoma (RR=3.17). After myeloproliferative neoplasm, the highest RRs were noted for Hodgkin lymphoma (RR=2.77), followed by endocrine (RR=2.55), nasal (RR=2.51), kidney (RR=2.22) and nervous system (RR=2.11) cancers relative to the background first primary cancer risk.

Reciprocal analysis, examining risk of each myeloid malignancy following a diagnosis of a non-myeloid cancer, revealed that overall RRs were increased for acute myeloid leukemia (RR=1.57), chronic myeloid leukemia (RR=1.26) and myelodysplastic syndrome (RR=1.54) (Table 4). An increased risk of acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome

and myeloproliferative neoplasm was seen following the diagnosis of 17, 5, 8 and 5 of the 31 primary cancers relative to background risk of myeloid neoplasms as first primary cancer. The largest RRs were seen for acute myeloid leukemia and myelodysplastic syndrome following diagnosis of hematological malignancy, non-Hodgkin lymphoma (RRs 5.16 and 6.03), Hodgkin lymphoma (RRs 7.69 and 10.04) and myeloma (RRs 8.71 and 9.45) relative to the reference rate of first cancers. Risks of acute myeloid leukemia, chronic myeloid leukemia and myelodysplastic syndrome were increased after prostate and testicular cancers relative to the population background rate.

In a vectored underpinning, we compared RRs between a cancer-pair (bi-directional RR) interrogating Table 3 and 4. Increased bi-directional RRs were found among acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome and myeloproliferative neoplasms for 6, 2, 4 and 4 cancers, respectively. The RRs for both of the reciprocal associations of acute myeloid leukemia and hematological malignancies and anal cancer were high relative to respective background rates.

We assessed RRs for second primary cancers following subtypes of myeloproliferative neoplasms in Table 5. The overall RRs for diagnosis of any second primary cancer after polycythemia vera, essential thrombocythemia, myelofibrosis and myeloproliferative neoplasm (not otherwise specified) were 1.34, 1.39, 1.88 and 1.50, respectively, compared to risk of any first primary cancer diagnosis rate. After polycythemia vera, risk of 11 second primary cancers were increased, after each of essential throbocythemia and myelofibrosis, risk of 7 SPC were increased, and after myeloproliferative neoplasm (not otherwise specified) risk of 9 cancer sites were increased relative to respective population background rates. Skin SCC was the only site which showed an increased risk after all myeloproliferative neoplasms, most after myelofibrosis (RR=3.71). Risks of nervous system cancer and non-Hodgkin lymphoma were increased after three myeloproliferative neoplasm subtypes. Risk of other female genital cancers (RR=3.31) was increased only in essential thrombocythemia patients. Hodgkin lymphoma reached an RR of 12.26 in myelofibrosis patients.

We assessed the risk of myelioproliferative neoplasms as second primaries after other cancers in Appendix page 7. With the exception of kidney and thyroid cancers, all other increased RRs were found for single myeloproliferative neoplasm subtypes against background rates. Similar to the vectored process discussed above reciprocal associations were observed between Tables 5 and Appendix page 7 and increased bi-directional risk were found for 6 unique cancer-myeloproliferative neoplasm pairs.

DISCUSSION

Our analysis provides further evidence that survivorship from a myeloid malignancy is associated with a significant risk of a second primary cancer. A major strength of our study as compared to other analyses is that we have avoided ascertainment bias in patient selection because our cohort analysis was based on the entire Swedish population, for which there is high case registration with long-term follow-up. We applied for the first time systematic bi-directional analysis for second primary cancer after myeloid malignancy and myeloid malignancy as second primary cancer. The analysis benefited from the largest population of myeloid neoplasms yet published [8]. Novel findings of the present study are that we were able to demonstrate that the increased risk is not confined to a specific myeloid malignancy, and includes myelodysplastic syndrome which has not been the subject of a previous cohort analyses. The risk of upper aerodigestive tract cancer, skin SCC and non-Hodgkin lymphoma constituted the major second primary cancers following diagnosis of acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome and myeloproliferative neoplasm. Our results thus provide new systematically analysed information on the risk of diverse types of second primary cancers as well as validate and expand previous observations [8-13]. The limitations include unavailability of some myeloid neoplasms before year 1993 and no information on treatment, in addition to the inherent weaknesses of observational studies in deducing disease causation.

Therapy-related side effects are generally considered to be the cause of many second primary cancers following treatment for a myeloid malignancy. Such a mechanism is not, however, likely to be solely responsible for all second primary cancer risk and there may be several possible mechanisms for the increased second primary cancer risk in patients. Although treatment for the myeloid malignancies is heterogeneous and has changed over time, many patients, certainly those within the earlier part of the patient cohort diagnosed before year 2000, are likely to have been in receipt of one or more types of cytoreductive treatment during their disease course [18]. While not universal, hydroxyurea has been employed as first line of cytoreductive treatment in myeloproliferative neoplasm patients. An increased risk of non-melanoma skin cancer during hydroxyurea treatment has been previously reported and it has been proposed that hydroxyurea may act as a photosensitizer and thus in combination with ultraviolet irradiation exposure increasing the risk of skin SCC [19]. However, this mechanism is not likely for myeloproliferative neoplasm following skin SCC because skin cancer is treated by surgery. In our study, side effects of other chemotherapeutic agents, such as anthracyclines, may have contributed to the risk of second

primary cancers which were increased after myeloid neoplasia, such as those of the lung and upper aerodigestive tract. We showed a possible increase in upper aerodigestive tract cancers after chronic myeloid leukemia in period 2001 to 2015 relative to the earlier follow-up periods, which in a previous small Swedish study was described as an increase in 'nose and throat cancer', possibly associated with use of tyrosine kinase inhibitors [9]. Testicular cancer was followed by an increased risk of acute myeloid leukemia, chronic myeloid leukemia and myelodysplastic syndrome. Testicular cancer is treated by radiotherapy and a combination of DNA damaging drugs bleomycin, etoposide and cisplatin whereby iatrogenic causes may be a likely explanation for the increased risk of second myeloid neoplasia [20].

Myeloid neoplasms were associated with all hematological malignancies for which acquired immune dysfunction due to cytotoxic therapy and bone marrow transplantations may be an underlying mechanism[21]. Inherited mechanisms may also contribute through master regulators of hematopoiesis, including transcriptional regulators of critical steps in cell development, such as STAT5, CEBPα, PU.1, CITED2 , among others[22]. Their expression is controlled at the genetic or epigenetic level and aberrations may have pleiotropic effects on several cell lineages. *GATA-2* is an example of a genetic regulator, loss of which has been shown to suppress hematopoiesis and contribute to immunodeficiency [23]. Other epigenetic regulators include *IDH*, *TET2* and *BCAT1* genes with possibly similar effects [24]. Given that many cancer susceptibility genes have pleiotropic effects, it is plausible that part of the risk of second primary cancer is influenced by inherited genetic factors, either through high penetrance alleles or co-inheritance of multiple common risk variants. Myeloid neoplasia patients have an increased risk of non-hematologic malignancies prior to their myeloproliferative neoplasm diagnosis and first-degree relatives of myeloproliferative neoplasm patients have a significantly increased risk of myeloproliferative neoplasms and chronic lymphocytic leukemia as well as melanoma and brain cancer, consistent with germline susceptibility to myeloproliferative neoplasm and other malignancies [25,26]. While risks of other cancers are yet to be established, common genetic variants of *TERT* associated with myeloproliferative neoplasm risk have also been shown to influence glioma and other cancers [27]. The Swedish Family-Cancer Database could be used to assess the possible contribution of familial risks to the present associations. However, as we here do not consider concordant associations between myeloid neoplasia but discordant associations between 7 myeloid neoplasia and 32 cancers we would need to consider 224 discordant familial pairs of cancers. We have recently published such results between all common cancers and shown that discordant familial risks do exist but RRs are small, of the order of 1.1-1.2 for most cancer pairs [28]. The present results may stimulate a range of other studies assessing risks of second primary cancer between selected sets of cancers.

From another angle, since myeloid diseases and their treatment can be associated with a certain degree of immunosuppression, this raises the possibility that impaired tumor immunosurveillance could play a role in the observed associations but this warrants further investigation [21]. Immune dysfunction in these cancers might also be compounded by germline susceptibility, as in the case of *GATA2*, where germline mutations have been reported to contribute to immune deficiency in hematological malignancies, including familial myelodysplastic syndrome/acute myeloid leukemia[29]. We have provided evidence of bi-directional associations between myeloid malignancies and lymphomas, and between lymphomas and skin SCC, kidney and bladder cancers. It is likely that the explanation of the increased risk of second primary cancer in patients with myeloid malignancies is multifactorial where a combination of cytoreductive treatment, genetic predisposition, as well as immune-related effects all may contribute to an increased cancer risk.

In conclusion, we have provided a comprehensive analysis of cancer risks associated with myeloid malignancies. Although a limitation of our study in terms of understanding the mechanistic basis of myeloproliferative neoplasm and second primary cancers is reliance on observational data on cancer registry information, we propose putative mechanisms involved in these processes. Such contributing effects could include immune disturbances acting together with cytotoxic and genetic effects; however, future studies are clearly warranted to investigate such potential mechanisms in detail, for example by assessing immune competence as a predictor of second primary cancers and consequences of immune therapy on the risk of second primary cancers Our findings further substantiate the significant cancer risks associated with survivorship from each of the myeloid malignancies which may be informative in defining the long-term management of patients successfully treated for these tumors when monitoring the occurrence of second primary cancers.

AUTHOR CONTRIBUTIONS

Design: KH, AS, SC. KH was in charge of the overall project supervision and management.
Acquisition of data: JS, KS
Statistical analysis and interpretation: SC, GZ, AS, HY, KH.
Manuscript writing: KH, RSH, AS, AH, AF.
Approval of the final text: All authors

COMPETING INTERESTS STATEMENT

A.H. is shareholder in Targovax ASA. A.H. is employee and shareholder in TILT Biotherapeutics Ltd. Other authors declared no conflict of interest.

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Research in context

Evidence before this study

Notifications about second primary cancers have increased in many cancer registries and they exceed 20% of all notifications in some cancer registries. Improved survival is an important contributor to an increasing number of second primary cancers. Distinct clinical phenotypes of myeloid neoplasia comprising myeloproliferative neoplasms, polycythemia vera, essential thrombocythemia, myelofibrosis, chronic myeloid leukemia, myelodysplastic syndrome and acute myeloid leukemia. Treatment for these malignancies is heterogeneous and has changed over time, but many patients have received cytoreductive treatment during their disease course. Therapy-related side effects are generally considered to be the cause of many second primary cancers following treatment for a myeloid malignancy. Such a mechanism is not, however, likely to be solely responsible for all second primary cancer risk and there may be several possible mechanisms for the increased second primary cancers risk.

Added value of this study

The present study applied two novel approaches. Firstly, using the resources of the Swedish Cancer Registry and 35,928 individuals with myeloid neoplasia we were able to include all main types of primary myeloid malignancy, including myelodysplastic syndrome for which no earlier data were available. Secondly, we carried systematic analysis of risk of second primary cancers after myeloid neoplasms and analysis of risk of myeloid neoplasms after any of 32 cancers; we refer to this two-way analysis as bi-directional. As a novel finding, we were able to demonstrate that the increased risk of second primary cancers is not confined to a specific myeloid malignancy, and that it also includes myelodysplastic syndrome. Bi-directional increases of risk were observed for 9 myeloid neoplasia-cancer pairs, including cancers of the small intestine, kidney, bladder, skin (both melanoma and squamous cell), connective tissue and hematopoietic tissue. The risk of upper aerodigestive tract cancer, squamous cell skin cancer and non-Hodgkin lymphoma constituted the major second primary cancers following diagnosis of acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome and myeloproliferative neoplasm.

Implications of all available evidence

Second primary cancers are increasingly being recognized as a major impediment in efforts to boost survival in patients with cancer. The current result with the available data points to heterogeneous mechanisms underlying development of second primary cancers. Side effects of chemotherapeutic agents may have contributed to the risk of second primary cancers when these were increased after

myeloid neoplasia, such as lung cancer. Risks of myeloid neoplasms were associated with all hematological malignancies, in which immune dysfunction due to cytotoxic therapy and bone marrow transplantations might be an underlying mechanism, but this remains to be investigated. . Increased risks of myeloid malignancies after melanoma, squamous cell skin and kidney cancers, all treated primarily by surgery, raise the possibility that immune dysfunction may be a contributing factor but the demonstration of which requires experimental studies. Therapy for myeloid neoplasms has undergone many recent changes and therapeutic successes need to be weighed against side effects, such as risk of second primary cancer.

RISK OF SECOND PRIMARY CANCER FOLLOWING MYELOID NEOPLASIA AND RISK OF MYELOID NEOPLASIA AS SECOND PRIMARY CANCER A NATION-WIDE FOLLOW-UP STUDY

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Key words: second primary cancer, myeloproliferative neoplasms , acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome.
Running title: Myeloid neoplasia

Words: 3433 (text), 350 (abstract).

ABSTRACT

Background: While advances in the treatment of myeloid neoplasms have led to improved patient survival, this has been accompanied by an increased risk of second primary cancer ~~(SPC)~~. To gain insight into risk of second primary cancer,~~SPC~~ we conducted a bi-directional analysis of risk of second primary cancer~~SPC~~, i.e., risk of second primary cancer ~~-~~after myeloid neoplasia and risk of myeloid neoplasia as second primary cancer~~SPC~~.

Patients & Methods: Using the Swedish Family-Cancer Database, we identified 35,928 individuals with primary myeloid malignancy, including myeloproliferative neoplasms ~~(MPN)~~, acute myeloid leukemia ~~(AML)~~, chronic myeloid leukemia ~~(cml~~chronic myeloid leukemia~~)~~ and myelodysplastic syndrome ~~(MDS)~~diagnosed between 1958 and 2015. The primary endpoint was the assessment of r~~R~~elative risks (RRs) for second primary cancer~~SPC~~, which were calculated using the generalized Poisson regression model.

Findings: Overall relative risk of second primary cancers~~SPCs~~ was significantly increased after ~~aml~~acute myeloid leukemia (RR = 1.29), ~~cml~~chronic myeloid leukemia (1.52), ~~mds~~myelodysplastic syndrome (1.42) and all ~~MPN~~myeloproliferative neoplasm (1.37) relative to the incidence of these cancers as first primary cancer.~~….~~ For myeloid neoplasia as second primary cancer~~SPC~~, risks were significantly increased for ~~AML~~acute myeloid leukemia (1.56), ~~CML~~chronic myeloid leukemia (1.26) and ~~MDS~~myelodysplastic syndrome (1.54) relative to the incidence of these myeloid neoplasia as first primary cancers.~~….~~ Risk of upper aerodigestive tract and squamous cell skin cancers and non-Hodgkin lymphoma were increased as SPCs following all four types of myeloid neoplasia relative to their incidence as first primary cancers.~~….~~ High risks of ~~MDS~~myelodysplastic syndrome and ~~AML~~acute myeloid leukemia as second primary cancers ~~SPCs~~were found after hematological malignancies (RRs between 5.08 and 10.04).

Comment [CS1]: Numbers?

Interpretation: The risks of second primary cancer~~SPC~~ are clinically important for the long-term management of patients with myeloid malignancies. The bi-directional associations of myeloid malignancies with many cancers suggest a number of candidate mechanisms that might contribute to ~~responsible for~~ the etiology of second primary cancer~~SPCs~~, many of which remain elusive; ~~-~~these might~~aside from~~ include immune dysfunction as well as the effects of therapy~~including immune dysfunction~~, thus laying out the groundwork for future investigations.

Funding: Supported by Deutsche Krebshilfe, Jane and Aatos Erkko Foundation, Sigrid Juselius Foundation, Finnish Cancer Organizations, Swedish Research Council, ALF from Region Skåne and Bloodwise.

INTRODUCTION

All myeloid malignancies originate from the same hematopoietic lineage and are characterized by excessive proliferation, abnormal self-renewal and differentiation defects[1]. The distinct clinical phenotypes comprise ~~of~~ *BCR-ABL1*-negative myeloproliferative neoplasms ~~(MPN)~~, polycythemia vera ~~(PV)~~, essential thrombocythemia ~~(ET)~~, myelofibrosis ~~(MF)~~, chronic myeloid leukemia ~~(CML)~~, myelodysplastic syndrome ~~(MDS)~~ and acute myeloid leukemia ~~(AML)~~. ~~pv~~Polycythemia vera is characterized by a high erythrocyte count, ~~ET~~ essential thrombocythemia by a high platelet count, and ~~MF~~myelofibrosis by bone marrow failure. ~~MDS~~ Myelodysplastic syndrome is characterized by ineffective hematopoiesis, morphologic dysplasia in hematopoietic cells and cytopenia [2]. While each ~~MPN~~myeloproliferative neoplasm subtypes are essentially independent diseases, transformation from m~~MPN~~yeloproliferative neoplasms and ~~MDS~~myelodysplastic syndrome to ~~AML~~acute myeloid leukemia can occur [1,3].

The etiology of myeloid diseases is poorly understood with few identified risk factors, which for ~~aml~~acute myeloid leukemia include exposure to ionizing radiation, chemicals such as benzene, and cytotoxic chemotherapy [1]. For ~~MDS~~myelodysplastic syndrome ~~autoimmunity and immunological aberrations are~~ risk factors includ~~ing~~e autoimmunity and immunological aberrations [4-6]. A number of rare high penetrance mutations in cancer susceptibility genes, such as Janus kinase 2 (*JAK2*), calreticulin (*CALR*), and myeloproliferative leukemia virus oncogene (*MPL*), have been identified ~~which~~ and have been increasingly ~~been~~ incorporated in disease classification, for example in myeloproliferative neoplasms [2,7]. Therapy for ~~MPN~~myeloproliferative neoplasm aims at reducing the risk for progression and ~~takes use of~~has relied on compounds such as hydroxyurea, inhibiting DNA synthesis, and cytokine interferon alpha modulating immune and other functions; ~~and~~ more recently specific *JAK-2* inhibitors have been included [8]. For instance, ~~AML~~acute myeloid leukemia patients may receive ~~an~~ induction therapy with DNA synthesis inhibitors cytarabine and anthracycline, supplemented with hydroxyurea. Consolidation therapy may include cytarabine administration or bone marrow transplantation. C~~CML~~hronic myeloid leukemia ~~was~~ used to be treated earlier with hydroxycarbamide, interferon alpha and allogeneic hematopoietic stem-cell transplantation but from around year 2000, tyrosine kinase inhibitors were introduced into standard of care for *BCR-ABL1*-positive patients [9]. The choice of t~~T~~herapy for ~~MDS~~myelodysplastic syndrome, however, ~~-~~depends on various diagnostic findings and symptoms, and may involve multiple modalities.

Comment [CS2]: Please see editorial decision letter; please spell out.

Comment [CS3]: Such as?

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Comment [CS5]: Administered how? And what do these compounds target?

Comment [CS6]: Which target what?

Recent advances in the management of all forms of myeloid malignancies, including ~~precision agents~~personalised approaches, have led to a markedly improved survival in patients. This has come at the cost of an increasing number of second primary cancers ~~(SPCs)~~ and other treatment-related complications, such as cardiovascular and neurological symptoms. A number of studies have estimated the risks of second primary cancers~~SPC~~ ~~after~~ following myeloid malignancy but have confined their analyses mainly to ~~he AML~~acute myeloid leukemia and ~~CML~~chronic myeloid leukemia, with limited data on ~~MPN~~myeloproliferative neoplasms [8,10-13]. This ~~is~~ appears to be largely because other myeloid malignancies are not curated by most cancer registries, thus limiting population-based epidemiological studies. To address this deficiency, we have conducted an analysis of all myeloid malignancies, using the Swedish Cancer Registry and including 35,928 patients ~~and~~, where we also report second primary cancers ~~SPCs~~following myeloid malignancies. Additionally, we ~~report~~ have conducted a novel analysis ~~considering~~ to assess myeloid malignancies diagnosed as second primary cancers~~SPCs~~ ~~after~~ following the occurrence of any non-myeloid first cancer. The assessment of bi-directional associations between myeloid malignancies and other cancers, i.e., risk of cancer after myeloid neoplasia and risk of myeloid neoplasia after cancer, ~~-~~may provide ~~s~~ insight into ~~the~~ possible mechanistic ~~basis~~ bases of second primary cancer ~~SPC~~ development ~~,~~ beyond therapeutic side effects and shared risk factors

Comment [CS7]: Based on what supportive evidence?

Comment [CS8]: Please define here exactly this term.

Comment [CS9]: Please indicate what the objective and hypothesis of this study was.

METHODS

Study design and participants

Comment [CS10]: Please see decision letter regarding structure

Comment [S.c11]: All the elements of observational study are included

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The Swedish Family-Cancer Database includes the Swedish population organized in families and linked to the national Cancer Registry with more than 2 million cancers registered since 1958.The registry relies on separate compulsory notifications from clinicians who diagnosed the neoplasms and from pathologists/cytologists. The coverage is estimated at more than 90% of all cancer diagnoses in Sweden [14]. The registry counts tumors not patients, except for skin and urinary tract tumors diagnosed at the same topological area (https://www.ancr.nu/dyn/resources/File/file/7/4247/1412940269/total_document_survey_optimeret.pdf). To classify cancer types, the Swedish Cancer Register has used International Classification of Disease (ICD)-7 since 1958, ICD-9 since 1987, SNOMED (ICD-O/2) since 1993, and ICD-O/3 since 2005. The degree of histological verification of myeloid malignancies vary from close to 100% for ~~AML~~acute myeloid leukemia and ~~CML~~chronic myeloid leukemia to about 95% for ~~PV~~polycythemia vera and ~~MF~~myelofibrosis [15]. An *ad hoc* study on the diagnostic accuracy of

second neoplasms in the Swedish Cancer Registry found 98% of these to be correctly classified; no recorded second primary cancer was found to be a metastasis upon re-examination [16].

The Cancer Registry orders any reported cancers as the first, second, third etc. primary cancer and we followed exactly this ordering. We included all patients with first primary ~~AML~~acute myeloid leukemia, ~~CML~~chronic myeloid leukemia, ~~mds~~myelodysplastic syndrome, all ~~mpn~~myeloproliferative neoplasm, ~~pv~~polycythemia vera, essential thrombocythemia~~ET~~, ~~MF~~myelofibrosis and myeloproliferative neoplasm (not otherwise specified) ~~(MPN-NOS)~~ between 1958 and 2015 for the risk of second primary cancer~~SPC~~. While assessing the risk of myeloid malignancies as second primary cancer~~SPC~~, the patients with any first primary cancer except leukemia were included. We used a combination of ICD-7 and ICD-O/2 codes to distinguish between different hematological malignancies and its subtypes. ~~mpn~~Myeloproliferative neoplasms were classified according to the 2016 WHO classification (https://doi.org/10.1038/s41408-018-0054-y, last accessed in June 2018). ~~mpn~~Myeloproliferative neoplasm ~~NOS~~ (not otherwise specified), ~~MF~~myelofibrosis and ~~mds~~myelodysplastic syndrome were distinguished in ICD-O/2 and available since 1993. We excluded rare ~~mpn~~myeloproliferative neoplasms such as chronic eosinophilic leukemia and chronic neutrophilic leukemia from our ~~mpn~~ subtype analysis. We did not consider myeloid diseases following myeloid diseases (609 patients) because of transformation between tumor types. The ICD codes used by the Swedish Cancer Registry do not specify therapy related cancers, which according to literature now account for 7% of acute myeloid leukemia [17].

Comment [h12]:

Outcomes and statistical analysis

Relative risks (RRs) were assessed by means of incidence rate ratios assuming cancer diagnoses to follow waiting time distribution, regressed over a fixed effects generalized Poisson model (https://doi.org/10.1002/sim.4780122406, last accessed in June 2018). Regression was assessed on case numbers scaled on person-years. This reduced case numbers comparable to the reference fractions in each person-year category.~~,~~ Person-year calculation for the background population assured large case numbers in each covariate bands and met the large number assumption for the Poisson distribution. RRs for second primary cancer~~SPC~~ (cancer X) were obtained by comparing incidence rates for each second primary cancer~~SPC~~ in myeloid malignancy patients with respective population background rates for the first primary cancer X. Conversely, for the other-way round analysis considering myeloid malignancies as second primary cancers ~~SPCs~~, the incidence rates were compared against background population incidence of the first primary myeloid neoplasia. Sex, age group, calendar-period, socio-economic status and residential areas were treated as

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potential confounders and were adjusted for in the regression model. Confidence intervals (CIs) were calculated for 5%, 1% and 0.1% level of significance. All analyses were performed in SAS (v9.4) or R (v3.3.4).

Subhayan Chattopadhyay and Guoqiao Zheng carried out primary statistical analysis.

The study was approved by the Ethical Committee of Lund University, Sweden and conducted in accordance with the tenets of the Declaration of Helsinki.

Role of the funding sources

The funding sources had no role in the study. K.H. had full access to all data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Of the 35,928 patients diagnosed with primary myeloid malignancy from 1958 till 2015, 2,631 (7.3%) developed a ~~SPC~~ second primary cancer with a median follow-up time of 4 years (Table 1). ~~aml~~Acute myeloid leukemia was the most common diagnosis but only 3.3% of 12,832 ~~and 16.2% of aml~~acute myeloid leukemia patients were diagnosed with second primary cancer~~SPC~~. Among 6,636 ~~pv~~polycythemia vera patients, 13.8% ~~34.8%~~ were diagnosed with second primary cancer~~SPC~~. Out of the 940,811 other cancer patients, 3,873 developed a myeloid malignancy as second primary cancer~~SPC~~ with a median follow-up of 6 years.

We carried out stratified risk ~~some basic~~ analyses on second primary cancer~~SPC~~ ~~risks~~ incidence after myeloid neoplasia by stratifying ~~according to some relevant variables, including~~over period, sex, age at first cancer diagnosis and follow-up time (Appendix- pages~~…~~ 2 to 5~~Supplementary Table 1 to 4~~). ~~Supplementary Table 1~~Appendix -page~~….~~ 2 shows that for ~~mds~~myelodysplastic syndrome and ~~MNP~~myeloproliferative neoplasm ~~NOS~~ (not otherwise specified) no cases were available in the early periods and the coding system for case identification ~~was~~ started in 1993. RRs for second primary cancer~~SPC~~ did not differ between the periods (i.e., 95%CIs were overlapping) with the exception of a high RR of 1.81 for ~~mpn~~myeloproliferative neoplasm in 1971-1985, contributed by the high RR for ~~ET~~essential thrombocythemia. Sex did not influence risk for second primary cancer~~SPC~~ (~~Supplementary Table 2~~Appendix page ~~…~~3) but early diagnosis (<65 y) of myeloid neoplasia was a strong risk modifier, except for ~~mds~~myelodysplastic syndrome. Follow-up

Comment [CS14]: Please see editorial decision letter for structure.

Comment [CS15]: The results section reads a little bit like a descriptive list. Wherever possible, please include rationale for each analysis.

Comment [CS16]: From when to when?

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Comment [CS18]: Include the rationale

time did not systematically influence risks of second primary cancer~~SPC~~, and significantly increased risks were noted after each myeloid neoplasia over most follow-up periods. For ~~mds~~myelodysplastic syndrome follow-up was relatively short, 23 years. We analyzed in more detail risks of second primary cancer~~SPC~~ after ~~cml~~chronic myeloid leukemia because the standard treatment changed with the introduction of tyrosine kinase inhibitors ~~changed the treatment at~~ around year 2000 (Appendix page ~~…~~6~~Supplementary Table 5~~). ~~Case~~ Although case numbers for individual second primary cancers~~SPCs~~ were small (mostly <10) ~~but~~ , ~~the only~~ significant increases in second primary cancer risk for upper aerodigestive tract, thyroid and connective tissue cancers ~~relative to….~~ were documented in the period between 2001-2015 relative to the background risk of these cancers as first primaries in 2001-2005.

Table 2 details the RRs of second primary cancer~~SPC~~ following diagnosis of a myeloid malignancy and for all myeloid malignancies as ~~an~~ second primary cancer~~SPC~~. Overall, the risk of second primary cancer~~SPC~~ after all myeloid malignancies was increased 1.36 times ~~.~~relative to~~….~~ the background risk of any first primary cancer diagnosis. An increased risk was shown for 15 cancer sites, notably for nasal cancer (RR=3.10), squamous cell skin cancer (skin SCC, RR=2.80) and Hodgkin lymphoma (RR=2.63). Note that these RRs were significant at the 0.1% level. Myeloid malignancy as a second primary cancer~~SPC~~ showed an RR of 1.32. Individually, 15 cancers were associated with an increased risk of myeloid malignancy primarily, most increased after cancers of the hematopoietic system, non-Hodgkin lymphoma (RR=3.31), Hodgkin lymphoma (RR=4.47), and myeloma (RR= 4.78). Bi-directional increases were observed for 9 myeloid neoplasia-cancer pairs, including cancers of the small intestine, kidney, bladder, skin (both melanoma and SCC), connective tissue and hematopoietic tissue (Table 2).

Table 3 shows the RRs of all non-myeloid cancers following diagnosis of ~~aml~~acute myeloid leukemia, ~~cml~~chronic myeloid leukemia, ~~mds~~myelodysplastic syndrome or ~~mpn~~myeloproliferative neoplasm. The overall RRs for second primary cancers~~SPCs~~ after each of these 4 diseases were 1.29, 1.52, 1.42 and 1.37, respectively. The respective numbers of second primary cancers~~SPCs~~ with increased RRs were 8, 7, 8 and 12 ~~relative to….~~compared to the background risk of first primary cancer. Risk of upper aerodigestive tract cancer, skin SCC and non-Hodgkin lymphoma was increased irrespective of myeloid primary type. Risk of kidney cancer was increased in ~~cml~~chronic myeloid leukemia, ~~mds~~myelodysplastic syndrome and ~~mpn~~myeloproliferative neoplasm patients relative to~~…~~ the background risk. For ~~aml~~acute myeloid leukemia, high RRs were noted for anal cancer (RR=3.92) and Hodgkin lymphoma (RR=3.60). The RR for skin SCC was 5.54 after ~~cml~~chronic myeloid leukemia. ~~mds~~Myelodysplastic syndrome associated with a high risk of non-

Comment [CS19]: Please be specific

Comment [CS20]: Changed the treatment how? This is different from what you stated before.

Comment [CS21]: Please be specific.

Comment [CS22]: Increases in what? Please be specific.

Comment [CS23]: In what MDS?

Comment [CS24]: In what MDS?

Comment [S.c25]: Case number and case distribution of MDS did not warrant for period specific analysis

Comment [CS26]: Unclear what this means. Please be more specific.

Comment [CS27]: What and where is the data for this?

Comment [CS28]: Please include implications for these findings in the discussion section.

Hodgkin lymphoma (RR=3.17). After ~~mpn~~myeloproliferative neoplasm, the highest RRs were noted for Hodgkin lymphoma (RR=2.77), followed by endocrine (RR=2.55), nasal (RR=2.51), kidney (RR=2.22) and nervous system (RR=2.11) cancers relative to~~…~~ the background first primary cancer risk.

Reciprocal analysis, examining risk of each myeloid malignancy following a diagnosis of a non-myeloid cancer, revealed that overall RRs were increased for ~~aml~~acute myeloid leukemia (RR=1.57), ~~cml~~chronic myeloid leukemia (RR=1.26) and ~~mds~~myelodysplastic syndrome (RR=1.54) (Table 4). An increased risk of ~~aml~~acute myeloid leukemia, ~~cml~~chronic myeloid leukemia, ~~mds~~myelodysplastic syndrome and ~~mpn~~myeloproliferative neoplasm was seen following the diagnosis of 17, 5, 8 and 5 of the 31 primary cancers relative to~~…~~ background risk of myeloid neoplasms as first primary cancer. The largest RRs were seen for ~~aml~~acute myeloid leukemia and ~~mds~~myelodysplastic syndrome following diagnosis of hematological malignancy, non-Hodgkin lymphoma (RRs 5.16 and 6.03), Hodgkin lymphoma (RRs 7.69 and 10.04) and myeloma (RRs 8.71 and 9.45) relative to~~…~~ the reference rate of first cancers. Risks of ~~aml~~acute myeloid leukemia, ~~cml~~chronic myeloid leukemia and ~~mds~~myelodysplastic syndrome were increased after prostate and testicular cancers relative to~~…~~ the population background rate.

In a vectored underpinning, we compared RRs between a cancer-pair (bi-directional RR) interrogating Table 3 and 4. ~~Bi~~Increased bi-directional ~~risks~~ RRs ~~Table 3 and 4~~ were found among ~~aml~~acute myeloid leukemia, ~~cml~~chronic myeloid leukemia, ~~mds~~myelodysplastic syndrome and ~~MNP~~ myeloproliferative neoplasms for 6, 2, 4 and 4 cancers, respectively. The RRs for both of the reciprocal associations of ~~aml~~acute myeloid leukemia and hematological malignancies and anal cancer were high relative to respective background rates.

We assessed RRs ~~Data~~ for second primary cancers~~SPCs~~ following subtypes of ~~mpn~~myeloproliferative neoplasms ~~are shown~~ in Table 5. The overall RRs for diagnosis of any second primary cancer after ~~pv~~polycythemia vera, ~~et~~essential thrombocythemia, ~~mf~~myelofibrosis and ~~mpn~~myeloproliferative neoplasm ~~nos~~ (not otherwise specified) were 1.34, 1.39, 1.88 and 1.50, respectively, compared to risk of any first primary cancer diagnosis rate. After ~~pv~~polycythemia vera, risk of 11 second primary cancers~~SPCs~~ were increased, after each of essential thrombocythemia~~ET~~ and ~~MF~~myelofibrosis, risk of 7 SPC were increased, and after ~~mpn~~myeloproliferative neoplasm ~~nos~~ (not otherwise specified) risk of 9 ~~SPCs~~ cancer sites were increased relative to respective population background rates. Skin SCC was the only ~~SPC~~ site which showed an increased risk after all ~~MPN~~myeloproliferative neoplasms, most after ~~MF~~myelofibrosis

Comment [CS29]: Please include implications for these findings in the discussion section.

Comment [CS30]: Please include implications for these findings in the discussion section.

Comment [CS31]: RR?

Comment [CS32]: This analysis is completely obscure. Please clarify the exac approach. What was the question being asked? Please revise thisi sentence.

Comment [CS33]: Please be specific. And high relative to…?

Comment [CS34]: Please include implications for these findings in the discussion section.

Comment [CS35]: What data? What was the question being asked?

(RR=3.71). Risks of nervous system cancer and non-Hodgkin lymphoma were increased after three ~~mpn~~myeloproliferative neoplasm subtypes. Risk of other female genital cancers (RR=3.31) was increased only in essential thrombocythemia~~ET~~ patients. Hodgkin lymphoma reached an RR of 12.26 in ~~MF~~myelofibrosis patients.

We assessed the risk of myelioproliferative neoplasms as second primaries after other cancers in Appendix page 7.~~Reverse analysis for the subtypes of mpns is shown in Supplementary Table 1Appendix page…~~. With the exception of kidney and thyroid cancers, all other increased RRs were found for single ~~mpn~~myeloproliferative neoplasm subtypes ~~relative to…~~against background rates. Similar to the vectored process discussed above r~~R~~eciprocal associations were observed between Tables 5 and Appendix page 7 and increased bi-directional risk were found for 6 unique cancer-~~mpn~~myeloproliferative neoplasm pairs.~~ between Tables 5 and Supplementary Table 6Appendix page…~~

DISCUSSION

Our analysis provides further evidence that survivorship from a myeloid malignancy is associated with a significant risk of a second primary cancer~~SPC~~. A major strength of our study as compared to other analyses is that we have avoided ascertainment bias in patient selection because our cohort analysis was based on the entire Swedish population, for which there is high case registration with long-term follow-up. We applied for the first time systematic bi-directional analysis for second primary cancer~~SPC~~ after myeloid malignancy and myeloid malignancy as second primary cancer~~SPC~~. The analysis benefited from the largest~~and most complete~~ population of myeloid neoplasms yet published [8]. Novel findings of the present study are that we were able to demonstrate that the increased risk is not confined to a specific myeloid malignancy, and includes ~~mds~~myelodysplastic syndrome which has not been the subject of a previous cohort analyses. The risk of upper aerodigestive tract cancer, skin SCC and non-Hodgkin lymphoma constituted the major second primary cancers~~SPCs~~ following diagnosis of ~~aml~~acute myeloid leukemia, ~~cml~~chronic myeloid leukemia, ~~mds~~myelodysplastic syndrome and ~~mpn~~myeloproliferative neoplasm. Our results thus provide new systematically analysed information on the risk of diverse types of second primary cancers~~SPC~~ as well as validate and expand previous observations [8-13]. The limitations include unavailability ~~lacking of data on~~of some myeloid neoplasms before year 1993 and no information on treatment, in addition to the inherent weaknesses of observational studies in deducing disease causation.

Comment [CS37]: Please ensure that you add comparators when reporting increased risks!

Comment [CS38]: Please include implications for these findings in the discussion section.

Comment [CS39]: What exactly does this mean?

Comment [CS40]: In what?

Comment [CS41]: Which?

Comment [CS42]: Please include implications for these findings in the discussion section.

Comment [CS43]: Unclear phrasing, please revise. What do you mean between tables

Comment [CS44]: It's unclear why this type of analysis is important. Please provid a rationale.

Comment [CS45]: Please tone down

Comment [CS46]: Please describe and include references

Comment [CS47]: Namely what? Please restate for the benefit of the reader

Comment [CS48]: Incorrect grammar, please revise

Comment [CS49]: There are more limitations than thi

Therapy-related side effects are generally considered to be the cause of many second primary cancerssSPCs following treatment for a myeloid malignancy. Such a mechanism is not, however, likely to be solely responsible for all second primary cancerSPC risk and there may be several possible mechanisms for the increased second primary cancerSPC risk in patients. Although treatment for the myeloid malignancies is heterogeneous and has changed over time, many patients, certainly those within the earlier part of the patient cohort diagnosed before year 2000, are likely to have been in receipt of one or more types of cytoreductive treatment during their disease course [18]. While not universal, hydroxyurea has been employed as first line of cytoreductive treatment in mpnmyeloproliferative neoplasm patients. An increased risk of non-melanoma skin cancer during hydroxyurea treatment has been previously reported and it has been proposed that hydroxyurea may act as a photosensitizer and thus in combination with ultraviolet irradiation exposure increasing the risk of skin SCC [19]. However, this mechanism is not likely for myeloproliferative neoplasm following skin SCC because skin cancer is treated by surgery. In our study, sSide effects of other chemotherapeutic agents, such as anthracyclines, may have contributed to the risk of second primary cancersSPCs which were increased after myeloid neoplasia, such as those of the lung and upper aerodigestive tract. We showed a possible increase in upper aerodigestive tract cancers after cmlchronic myeloid leukemia in period 2001 to 2015 relative to the earlier follow-up periods, …, which in a previous small Swedish study was described as an increase in 'nose and throat cancer', possibly associated with use of tyrosine kinase inhibitors [9]. Testicular cancer was followed by an increased risk of acute myeloid leukemia, chronic myeloid leukemia and myelodysplastic syndrome. Testicular cancer is treated by radiotherapy and a combination of DNA damaging drugs bleomycin, etoposide and cisplatin whereby iatrogenic causes may be a likely explanation for the increased risk of second myeloid neoplasia [20].

Bi-directional increases require other mechanistic explanations, particularly for cancer primarily treated with surgery, such as melanoma, skin SCC and kidney cancer. Further investigations are thus warranted.

Myeloid neoplasms were associated with all hematological malignancies for which, amlacute myeloid leukemia bi-directionally, mpnmyeloproliferative neoplasm as first neoplasm only and mdsmyelodysplastic syndrome as SPC only. acquired immune dysfunction due to cytotoxic therapy and bone marrow transplantations may be an underlying mechanism for what…? [21]. One might also consider the influence of Inherited mechanisms may also contribute that may also operate through master regulators of hematopoiesis, which areincluding transcriptional regulators of critical steps in cell development, such as and include STAT5, CEBPα, PU.1, CITED2 , among others[22]. Their expression is controlled at the genetic or epigenetic level and aberrations may have pleiotropic

Comment [CS50]: Because?

Comment [CS51]: Such as?

Comment [CS52]: And? Implications?

Comment [CS53]: And? This sentence is a non sequitur. Please revise.

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Comment [CS54]: Why?

Comment [CS55]: This needs to be better understood. Please explain further and rephrase for clarity.

Comment [CS56]: Again, this term is completely obscure

Comment [CS57]: Please rephrase for clarity.

Comment [CS58]: And? What does this mean?

Comment [CS59]: Based on what supportive evidence?

Comment [CS60]: Because? Based on what supportive evidence?

Comment [CS61]: To affect what?

effects on several cell lineages. *GATA-2* is an example of a genetic regulator, loss of which has been shown to suppresses hematopoiesis and contributes to immunodeficiency as pointed out above [23]. Other eEpigenetic master regulators can also include *IDH*, *TET2* and *BCAT1* genes with possibly similar effects but these are often somatically mutated[24].. Given that many cancer susceptibility genes have pleiotropic effects, it is plausible that part of the risk of second primary cancer is influenced by inherited genetic factors, either through high penetrance alleles or co-inheritance of multiple common risk variants. Myeloid neoplasia patients have an increased risk of non-hematologic malignancies prior to their myeloproliferative neoplasm diagnosis and first-degree relatives of myeloproliferative neoplasm patients have a significantly increased risk of myeloproliferative neoplasms and chronic lymphocytic leukemia as well as melanoma and brain cancer, consistent with germline susceptibility to myeloproliferative neoplasm and other malignancies [25,26]. While risks of other cancers are yet to be established, common genetic variants of *TERT* associated with myeloproliferative neoplasm risk have also been shown to influence glioma and other cancers [27]. The Swedish Family-Cancer Database could be used to assess the possible contribution of familial risks to the present associations. However, as we here do not consider concordant associations between myeloid neoplasia but discordant associations between 7 myeloid neoplasia and 32 cancers we would need to consider 224 discordant familial pairs of cancers. We have recently published such results between all common cancers and shown that discordant familial risks do exist but RRs are small, of the order of 1.1-1.2 for most cancer pairs [28]. The present results may stimulate a range of other studies assessing risks of second primary cancer between selected sets of cancers.

Testicular cancer was followed by increased risks of amlacute myeloid leukemia, cmlchronic myeloid leukemia and mdsmyelodysplastic syndrome. Testicular cancer is treated by radiotherapy and a combination of DNA damaging drugs bleomycin, etoposide and cisplatin whereby iatrogenic causes are likely [24].

From another angle, sSince all of the myeloid diseases and their treatment can be associated with a certain degree of immunosuppression, this raises the possibility of immune dysfunction andthat impaired tumor immunosurveillance playing could play a role in the observed associations but this warrants further investigation ……?[21].Any such immune suppression may be but this warrants further investigation Immune dysfunction in these cancers might also be compounded by germline susceptibility, for exampleas in the case of *GATA2*, where germline gene mutations are known to leadhave been reported to contribute to immune deficiency in hematological malignancies, including familial mdsmyelodysplastic syndrome/amlacute myeloid leukemia[29]. We have provided evidence of Thebi-directional associations in…we have demonstrated,between myeloid malignancies and lymphomas, and between lymphomas and skin SCC, kidney and bladder cancers

Comment [CS62]: And? What is the point you are trying to make?

Comment [CS63]: And? This sentence is a non sequitur. Please revise.

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~~are all cancers typified by having a significant immune response~~ [26,27]. ~~Thus the results are compatible with immune dysfunction, either acquired or inherited, playing a role~~ It is ~~however most~~ likely that the explanation of the increased risk of second primary cancer~~SPC~~ in patients with myeloid malignancies is multifactorial where a combination of cytoreductive treatment, ~~and~~ genetic predisposition, as well as immune-related effects all may contribute to an increased cancer risk.

~~Given that many cancer susceptibility genes have pleiotropic effects, it is plausible that part of the risk of SPC is influenced by inherited genetic factors, either through high penetrance alleles or co-inheritance of multiple common risk variants. A number of rare high penetrance mutations in cancer susceptibility genes have been identified which for mpnmyeloproliferative neoplasm include mutually exclusive Janus kinase 2 (*JAK2*), calreticulin (*CALR*), and myeloproliferative leukemia virus oncogene (*MPL*) mutations [7]. Myeloid neoplasia patients have an increased risk of non-hematologic malignancies prior to their mpnmyeloproliferative neoplasm diagnosis and first-degree relatives of mpnmyeloproliferative neoplasm patients have a significantly increased risk of mpnmyeloproliferative neoplasm and CLL as well as melanoma and brain cancer, consistent with germline susceptibility to mpnmyeloproliferative neoplasm and other malignancies [28,29]. Germline mutations predisposing to mdsmyelodysplastic syndrome/amlacute myeloid leukemia include the *CEBPA, DDX41, RUNX1, ANKRD26, ETV6, SRP72* and *GATA2* genes [2]. While risks of other cancers are yet to be established, common genetic variants of TERT associated with mpnmyeloproliferative neoplasm risk have also been shown to influence glioma and other cancers [30]. The Swedish Family-Cancer Database could be used to assess the possible contribution of familial risks to the present associations. However, as we here do not consider concordant associations between myeloid neoplasia but discordant associations between 7 myeloid neoplasia and 32 cancers we would need to consider 224 discordant familial pairs of cancers. We have recently published such results between all common cancers and shown that discordant familial risks do exist but RRs are small, of the order of 1.1-1.2 for most cancer pairs [31]~~

In conclusion, we have provided a comprehensive analysis of cancer risks associated with ~~the~~ myeloid malignancies. Although a limitation of our study in terms of understanding the mechanistic basis of myeloproliferative neoplasm and second primary cancers is reliance on observational data on cancer registry information,~~by implementing a novel bs~~. ~~We~~ we have been able to propose ~~a number of possible~~ we propose putative mechanisms ~~responsible~~ involved in~~for~~ these processes. Such contributing effects could include immune disturbances acting together with cytotoxic and genetic effects; however, future studies are clearly warranted to investigate such potential mechanisms in detail, for example by assessing immune competence as a predictor of second primary cancers and consequences of immune therapy on the risk of second primary cancers.~~.~~ Our findings further substantiate the significant cancer risks associated with survivorship from each of

Comment [CS64]: This is completely speculative and not a strong argument. Please delete.

Comment [CS65]: You were just alluding to this a couple of paragraphs before. Please remove repetitions and also consolidate with the section that discusses this. Re-writing the discussion in this regar is required.

Comment [CS66]: Take home message? Implications?

Comment [CS67]: There are many more limitations; please thoroughly discuss

Comment [CS68]: Consisting of? Which has allowed us to determine that……

the myeloid malignancies ~~and are~~which may be informative in defining the long-term management of patients successfully treated for these tumors ~~in respect of surveillance for~~when monitoring the occurrence of second primary cancers.

Comment [CS69]: It is really not clear at all how your findings will advance the field and how they may inform such strategies. What exactly do you envision happening from a practical perspective?

AUTHOR CONTRIBUTIONS

Design: KH, AS, SC. KH was in charge of the overall project supervision and management.

Acquisition of data: JS, KS

Statistical analysis and interpretation: SC, GZ, AS, HY, KH.

Manuscript writing: KH, RSH, AS, AH, AF.

Approval of the final text: All authors

COMPETING INTERESTS STATEMENT

A.H. is shareholder in Targovax ASA. A.H. is employee and shareholder in TILT Biotherapeutics Ltd. Other authors declared no conflict of interest.

ACKNOWLEDGEMENT

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Comment [CS70]: 25 AND 26 ARE REVIES JOURNALS.

Research in context

Evidence before this study

Notifications about second primary cancers have increased in many cancer registries and they exceed 20% of all notifications in some cancer registries. Improved survival is an important contributor to an increasing number of second primary cancers. Distinct clinical phenotypes of myeloid neoplasia comprising myeloproliferative neoplasms ~~(MPN)~~, polycythemia vera ~~(PV)~~, essential thrombocythemia ~~(ET)~~, myelofibrosis ~~(MF)~~, chronic myeloid leukemia ~~(CML)~~, myelodysplastic syndrome ~~(MDS)~~ and acute myeloid leukemia ~~(AML)~~. Treatment for these malignancies is heterogeneous and has changed over time, but many patients ~~within the earlier part of the cohort~~ have received cytoreductive treatment during their disease course. Therapy-related side effects are generally considered to be the cause of many second primary cancers~~SPCs~~ following treatment for a myeloid malignancy. Such a mechanism is not, however, likely to be solely responsible for all second primary cancer~~SPC~~ risk and there may be several possible mechanisms for the increased second primary cancers ~~SPC~~ risk.

Added value of this study

The present study applied two novel approaches. Firstly, using the resources of the Swedish C~~c~~ancer R~~r~~egistry and 35,928 individuals with myeloid neoplasia we were able to include all main types of primary myeloid malignancy, including ~~mds~~myelodysplastic syndrome for which no earlier data were available. Secondly, we carried systematic ~~out a bi-directional~~ analysis of risk of second primary cancers after myeloid neoplasms and analysis of risk of myeloid neoplasms after any of 32 cancers; we refer to this two-way analysis as bi-directional. ~~, assessing risks for any SPC following diagnosis of myeloid subtypes, and, in reverse order, assessing risk of…for myeloid subtypes following diagnosis of any of 32 different cancers.~~ As a novel finding~~s~~, we were able to demonstrate that the increased risk of second primary cancers~~SPC~~ is not confined to a specific myeloid malignancy, and that it also includes ~~mds~~myelodysplastic syndrome. Bi-directional increases of risk were observed for 9 myeloid neoplasia-cancer pairs, including cancers of the small intestine, kidney, bladder, skin (both melanoma and squamous cell), connective tissue and hematopoietic tissue. The risk of upper aerodigestive tract cancer, squamous cell skin cancer and non-Hodgkin lymphoma constituted the major second primary cancers~~SPCs~~ following diagnosis of ~~aml~~acute myeloid leukemia, ~~cml~~chronic myeloid leukemia, ~~mds~~myelodysplastic syndrome and ~~mpn~~myeloproliferative neoplasm.

Implications of all available evidence

Comment [CS71]: Please be aware that information listed in the RIC panel needs to be discussed in the main text with citations added there (main text).

Comment [CS72]: Please spell out this term throughout entire manuscript

Comment [CS73]: Such as?

Comment [CS74]: Please elaborate on this in the main text.

Comment [CS75]: What exactly do you mean?

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Comment [CS77]: This approach needs to be listed clearly in the main text

Comment [CS78]: Please double-check that this is still the case

Comment [CS79]: And what is the inmplication of this?

Second primary cancers~~PCs~~ are increasingly being recognized as a major impediment in efforts to boost survival in patients with cancer. The current result with the available data points to heterogeneous mechanisms underlying development of second primary cancers~~SPCs~~. Side effects of chemotherapeutic agents may have contributed to the risk of second primary cancers~~SPCs~~ when these were ~~uni-directionally~~ increased after myeloid neoplasia, such as lung cancer. Risks of m~~M~~yeloid neoplasms were associated with all hematological malignancies, in which ~~acquired~~ immune dysfunction due to cytotoxic therapy and bone marrow transplantations ~~may~~ might be an underlying mechanism, but this remains to be investigated. ~~The bi-directional associations between myeloid malignancies and squamous cell skin and kidney cancers and lymphomas are all typified by having a significant immune response suggesting that immune dysfunction, either acquired or inherited, plays a role. It is however most likely that the explanation of the~~an increased risk of ~~SPC in patients with myeloid malignancies is multifactorial where a combination of cytoreductive treatment, and genetic predisposition, as well as immune-related effects all may contribute to an increased risk of SPC. Our findings further substantiate the significant cancer risks associated with survivorship from each of the myeloid malignancies and are informative in defining the long-term management of patients successfully treated for these tumors in respect of surveillance for SPC~~. Increased risks of myeloid malignancies after melanoma, squamous cell skin and kidney cancers, all treated primarily by surgery, raise the possibility that immune dysfunction may be a contributing factor but the demonstration of which requires experimental studies. Therapy for myeloid neoplasms has undergone many recent changes and therapeutic successes need to be weighed against side effects, such as risk of second primary cancer.

Comment [CS80]: This is very obscure and needs to be revised

Comment [CS81]: Occurrence? Risk? Please be very specific

Comment [CS82]: Associaitons in what?

Comment [CS83]: This paraphrases verbatim the main text. Please revise.

Table

Table 1 Distribution of patients with myeloid malignancies

Total number of individuals	15,329,616		
Men	7,762,777 (50.6)[a]		
Women	7,566,839 (49.4)		
Myeloid malignancies as first primary cancer			
	First primary cancer	**[†]Second primary cancer**	**Median follow-up time in years**
Sex			
Men	18,816 (52.4)	1,405 (53.4)	-
Women	17,112 (47.6)	1,226 (46.6)	-
Calendar year			
1958 – 1970	8,334	132 (5.0)	-
1971 – 1985	7,775	542 (20.6)	-
1986 – 2000	8,551	923 (35.1)	-
2001 – 2015	11,268	1,034 (39.3)	-
Patients			
Acute myeloid leukemia	12,832	427 (3.3)	3 [2-5] [b]
Chronic myeloid leukemia	5,567	306 (5.5)	3 [2-6]
Myelodysplastic syndrome	3,520	259 (7.4)	1 [0-5]
Polycythemia vera	6,636	916 (13.8)	6 [3-9]
Essential thrombocythemia	4,081	422 (10.3)	4 [3-10]
Myelofibrosis	1,454	118 (8.1)	2 [0-5]
Chronic neutrophilic leukemia/ Chronic eosinophilic leukemia	204	10 (4.9)	2 [0-3]
Myeloproliferative neoplasm (not otherwise specified)	1,634	173 (10.6)	3 [1-6]
Myeloid malignancies as second primary cancer			
	Second primary cancer		**Median follow-up time in years**
Acute myeloid leukemia	1,463 (37.7)		5 [2-11]
Chronic myeloid leukemia	380 (9.8)		6 [3-13]
Myelodysplastic syndrome	750 (19.4)		6 [3-11]
Polycythemia vera	474 (12.2)		8 [3-15]
Essential thrombocythemia	392 (10.1)		10 [3-18]
Myelofibrosis	171 (4.4)		6 [2-14]
Chronic neutrophilic leukemia/ Chronic eosinophilic leukemia	22 (0.6)		10 [5-14]
Myeloproliferative neoplasm (not otherwise specified)	221 (5.7)		5 [2-13]

[a] Percentages in parentheses
[b] Inter-quartile ranges in square brackets
[†]Second primary cancers after primary myeloid malignancies.

Table 2

Table 2. Risk of second primary cancers in patients with myeloid malignancies (A) and risk of second primary myeloid neoplasia in patients with other cancers (B)

Cancer site	A. Risk of SPC after myeloid neoplasia				B. Risk of myeloid neoplasia as SPC			
	N	RR	CI $_{Lower}$	CI $_{Upper}$	N	RR	CI $_{Lower}$	CI $_{Upper}$
Upper aerodigestive tract	77	***1.95***	1.56	2.43	94	1.15	0.94	1.40
Esophagus	26	1.38	0.95	2.00	6	0.75	0.34	1.66
Stomach	75	0.93	0.74	1.17	66	1.00	0.79	1.27
Small intestine	15	**1.71**	1.03	2.84	17	**1.66**	1.03	2.67
Colorectum	248	0.97	0.86	1.10	362	1.06	0.95	1.17
Anus	9	***2.31***	1.24	4.30	8	1.37	0.69	2.75
Liver	76	1.21	0.96	1.52	8	**0.47**	0.24	0.94
Pancreas	64	1.04	0.82	1.33	7	**0.45**	0.21	0.94
Nose	8	***3.10***	1.61	5.97	5	0.94	0.39	2.26
Lung	223	***1.40***	1.23	1.60	73	1.11	0.88	1.39
Breast	184	0.98	0.84	1.13	624	***1.13***	1.05	1.23
Cervix	12	0.81	0.45	1.47	71	0.91	0.72	1.15
Endometrium	45	1.05	0.78	1.40	179	1.11	0.96	1.28
Ovary	36	1.12	0.80	1.55	110	***1.53***	1.27	1.85
Other female genitals	6	0.81	0.39	1.70	17	1.22	0.76	1.96
Prostate	419	1.05	0.96	1.16	695	***1.30***	1.20	1.40
Testis	3	1.23	0.40	3.80	32	***1.91***	1.35	2.70
Other male genitals	2	0.58	0.14	2.31	6	0.78	0.35	1.73
Kidney	107	***2.07***	1.71	2.51	112	***1.54***	1.28	1.85
Bladder	126	***1.31***	1.10	1.56	237	***1.40***	1.23	1.59
Melanoma	104	***1.84***	1.52	2.23	169	***1.27***	1.09	1.47
Skin (SCC)	301	***2.80***	2.50	3.14	195	***1.47***	1.28	1.70
Eye	4	0.72	0.23	2.25	15	1.33	0.80	2.21
Nervous system	90	***2.23***	1.82	2.73	88	1.12	0.91	1.38
Thyroid gland	14	1.42	0.86	2.36	73	***2.06***	1.64	2.59
Endocrine glands	51	***2.24***	1.71	2.94	100	**1.25**	1.03	1.52
Bone	4	2.14	0.80	5.70	9	1.50	0.78	2.88
Connective tissue	21	***1.99***	1.30	3.05	29	**1.47**	1.02	2.11
non-Hodgkin lymphoma	119	***1.95***	1.62	2.34	239	***3.31***	2.91	3.76
Hodgkin lymphoma	15	***2.63***	1.59	4.37	59	***4.47***	3.46	5.77
Multiple myeloma	49	***1.60***	1.21	2.12	122	***4.78***	3.78	6.04
Cancer of unknown primary	89	1.23	1.00	1.51	26	0.98	0.67	1.45
All	2631	***1.36***	1.30	1.42	3873	***1.32***	1.28	1.37

Abbreviations:
SPC, second primary cancer; N, frequency; RR, relative risk; CI, 95% confidence interval; SCC, squamous cell carcinoma;
Bold, italicized and underlined indicate statistical significance at 5%, 1% and 0.1% level respectively

Table 3

Table 3. Risk of second primary cancer among survivors of myeloid malignancy.

SPC site	Acute myeloid leukemia		Chronic myeloid leukemia		Myelodysplastic syndromes		Myeloproliferative neoplasms	
	N	RR (95% CI)	N	RR (95% CI)	N	RR (95% CI)	N	RR (95% CI)
Upper aerodigestive tract	16	***2.19*** (1.34 - 3.58)	10	**2.10** (1.13 - 3.91)	9	**2.29 (1.19 - 4.41)**	42	**1.43** (1.06 - 1.94)
Esophagus	2	0.55 (0.14 - 2.22)	4	1.83 (0.69 - 4.87)	5	**2.64 (1.10 - 6.35)**	15	1.16 (1.06 - 1.93)
Stomach	10	0.73 (0.39 - 1.36)	14	1.35 (0.80 - 2.27)	5	1.04 (0.43 - 2.49)	46	0.92 (0.69 - 1.23)
Small intestine	2	1.25 (0.31 - 4.98)	2	2.07 (0.52 - 8.26)	2	2.12 (0.53 - 8.48)	9	1.50 (0.78 - 2.88)
Colorectum	40	0.91 (0.67 - 1.24)	32	1.24 (0.88 - 1.75)	23	0.83 (0.55 - 1.24)	153	0.88 (0.75 - 1.03)
Anus	3	**3.92** (1.26 - 12.16)	0	-	1	2.02 (0.28 - 14.35)	5	1.71 (0.71 - 4.11)
Liver	20	***1.82*** (1.18 - 2.83)	6	0.87 (0.39 - 1.93)	5	1.09 (0.46 - 2.63)	45	1.14 (0.85 - 1.53)
Pancreas	12	1.09 (0.62 - 1.92)	4	0.56 (0.21 - 1.50)	2	0.43 (0.11 - 1.72)	46	1.21 (0.90 - 1.61)
Nose	0	-	1	2.75 (0.39 - 19.56)	2	***8.14*** (2.03 - 32.62)	5	**2.51** (1.05 - 6.05)
Lung	36	1.26 (0.91 - 1.75)	18	1.06 (0.67 - 1.68)	30	***1.99*** (1.39 - 2.85)	139	***1.38*** (1.17 - 1.63)
Breast	31	0.88 (0.62 - 1.26)	13	0.60 (0.35 - 1.04)	9	0.61 (0.32 - 1.17)	131	0.98 (0.83 - 1.17)
Cervix	2	0.72 (0.18 - 2.89)	2	0.90 (0.23 - 3.61)	0	-	8	0.92 (0.46 - 1.85)
Endometrium	6	0.73 (0.33 - 1.64)	13	***2.55*** (1.48 - 4.40)	0	-	26	0.85 (0.58 - 1.25)
Ovary	10	1.60 (0.86 - 2.98)	2	0.45 (0.11 - 1.82)	2	1.11 (0.28 - 4.44)	22	1.04 (0.68 - 1.57)
Other female genitals	0	-	0	-	1	1.30 (0.18 - 9.25)	5	0.81 (0.34 - 1.94)
Prostate	72	0.92 (0.73 - 1.15)	50	1.20 (0.91 - 1.58)	35	**0.60** (0.43 - 0.84)	262	1.07 (0.95 - 1.21)
Testis	0	-	1	1.73 (0.24 - 12.25)	1	**8.94** (1.26 - 63.57)	1	0.83 (0.12 - 5.89)
Kidney	9	0.98 (0.51 - 1.88)	16	**2.61** (1.60 - 4.25)	9	**2.14** (1.11 - 4.11)	73	***2.12*** (1.69 - 2.67)
Bladder	21	1.27 (0.83 - 1.95)	12	1.28 (0.73 - 2.25)	17	1.42 (0.88 - 2.29)	76	1.08 (0.86 - 1.35)
Melanoma	17	1.55 (0.96 - 2.49)	6	0.92 (0.42 - 2.06)	11	1.43 (0.79 - 2.59)	70	***1.75*** (1.38 - 2.21)
Skin (SCC)	37	***2.18*** (1.58 - 3.01)	47	**5.54** (4.16 - 7.37)	47	***2.34*** (1.76 - 3.12)	170	***1.94*** (1.67 - 2.26)
Nervous system	22	***2.69*** (1.77 - 4.09)	9	1.63 (0.85 - 3.13)	4	1.29 (0.48 - 3.43)	55	***2.11*** (1.62 - 2.76)
Thyroid gland	3	1.56 (0.50 - 4.83)	3	2.23 (0.72 - 6.91)	0	-	8	1.13 (0.57 - 2.26)
Endocrine glands	7	1.57 (0.75 - 3.30)	2	0.71 (0.18 - 2.85)	1	0.46 (0.07 - 3.29)	41	***2.55*** (1.88 - 3.46)
Bone	1	2.68 (0.38 - 19.06)	0	-	0	-	3	2.59 (0.83 - 8.03)
Connective tissue	4	2.06 (0.77 - 5.48)	4	**3.23** (1.21 - 8.60)	2	1.93 (0.48 - 7.70)	11	1.55 (0.86 - 2.80)
non-Hodgkin lymphoma	18	**1.67** (1.05 - 2.64)	14	***2.27*** (1.35 - 3.84)	22	***3.17*** (2.09 - 4.82)	65	***1.60*** (1.25 - 2.04)
Hodgkin lymphoma	4	**3.60** (1.35 - 9.60)	1	1.20 (0.17 - 8.53)	0	-	10	***2.77*** (1.49 - 5.15)
Multiple myeloma	11	**2.03** (1.13 - 3.67)	1	0.31 (0.04 - 2.17)	6	2.03 (0.91 - 4.51)	31	**1.58** (1.11 - 2.25)
Cancer of unknown primary	7	0.54 (0.26 - 1.14)	15	**2.00** (1.20 - 3.31)	7	0.97 (0.46 - 2.04)	60	**1.31** (1.02 - 1.69)
All	427	***1.29*** (1.17 - 1.41)	306	***1.52*** (1.35 - 1.69)	259	***1.42*** (1.26 - 1.59)	1639	***1.37*** (1.30 - 1.43)

Abbreviations:
N, frequency; RR, relative risk; CI, confidence interval; SCC, squamous cell carcinoma;
Bold, italicized and underlined indicate statistical significance at 5%, 1% and 0.1% level respectively

Table 4. Risk of myeloid malignancies after first primary cancer

Cancer site	Acute myeloid leukemia		Chronic myeloid leukemia		Myelodysplastic syndrome		Myeloproliferative neoplasm	
	N	RR (95%CI)	N	RR (95%CI)	N	RR (95%CI)	N	RR (95%CI)
Upper aerodigestive tract	39	**1.42 (**1.03-1.94**)**	11	1.14 (0.63-2.07)	16	1.48 (0.91-2.42)	28	0.81 (0.56-1.17)
Esophagus	2	0.71 (0.18-2.85)	2	2.26 (0.57-9.05)	2	1.69 (0.42-6.75)	0	
Stomach	21	0.85 (0.56-1.31)	10	1.18 (0.64-2.20)	11	1.45(0.8-2.62)	24	0.97 (0.65-1.45)
Small intestine	5	1.51 (0.63-3.63)	2	1.90 (0.47-7.59)	4	2.37 (0.89-6.32)	6	1.38 (0.62-3.07)
Colorectum	135	**1.21 (**1.02-1.44**)**	40	1.15 (0.84-1.58)	73	1.25 (0.99-1.58)	115	0.80 (0.67-0.96)
Anus	7	***3.88*** (1.85-8.13)	0	-	0		1	0.39 0.05-2.77)
Liver	4	0.65 (0.24-1.73)	3	1.61 (0.52-4.99)	1	0.41 (0.06-2.93)	0	
Pancreas	1	0.17 (0.02-1.18)	1	0.53 (0.07-3.74)	2	1.04 (0.26-4.17)	3	0.53 (0.17-1.64)
Nose	3	1.66 (0.53-5.14)	0	-	0		2	0.92 (0.23-3.66)
Lung	26	1.16 (0.79-1.70)	9	1.28 (0.66-2.46)	18	**1.79** (1.13-2.85)	20	0.74 (0.48-1.15)
Breast	249	***1.43*** (1.26-1.62)	74	**1.29** (1.03-1.63)	83	0.94 (0.76-1.17)	219	0.92 (0.80-1.05)
Cervix	42	***1.61*** (1.19-2.18)	5	0.49 (0.20-1.18)	9	0.96 (0.50-1.85)	15	**0.46** (0.28-0.76)
Endometrium	72	***1.41*** (1.11-1.77)	25	**1.53** (1.03-2.27)	32	1.16 (0.82-1.64)	50	**0.72** (0.54-0.95)
Ovary	61	***2.55*** (1.98-3.28)	8	0.97 (0.49-1.95)	16	1.58 (0.97-2.59)	25	0.83 (0.56-1.23)
Other female genitals	9	**1.92** (1.00-3.69)	1	0.64 (0.09-4.52)	3	1.49 (0.48-4.61)	4	0.69 (0.26-1.85)
Prostate	219	***1.31***(1.15-1.50)	73	***1.57*** (1.24-1.98)	159	***1.45*** (1.23-1.70)	244	1.06 (0.93-1.20)
Testis	14	***2.58*** (1.53-4.37)	6	**2.60** (1.17-5.79)	5	**2.50** (1.04-6.02)	7	0.98 (0.47-2.06)
Kidney	33	1.35 (0.96-1.90)	14	**1.76** (1.04-2.97)	14	1.28 (0.76-2.17)	51	***1.68*** (1.28-2.21)
Bladder	85	***1.57*** (1.27-1.95)	23	1.38 (0.91-2.08)	44	***1.49*** (1.11-2.01)	85	1.16 (0.94-1.44)
Melanoma	38	0.95 (0.69-1.30)	16	1.24 (0.76-2.02)	29	1.20 (0.83-1.73)	86	***1.45*** (1.17-1.79)
Skin (SCC)	45	1.09 (0.81-1.46)	19	1.51 (0.96-2.38)	49	***1.92*** (1.45-2.55)	82	***1.45*** (1.17-1.81)
Nervous system	36	**1.39** (1.00-1.92)	4	0.45 (0.17-1.20)	12	1.03 (0.58-1.81)	36	1.09 (0.79-1.52)
Thyroid gland	20	**1.72** (1.11-2.67)	5	1.23 (0.51-2.97)	9	1.80 (0.94-3.47)	39	***2.61*** (1.91-3.58)
Endocrine gland	25	0.99 (0.67-1.47)	10	1.26 (0.68-2.35)	13	0.94 (0.54-1.62)	52	***1.48*** (1.13-1.95)
Bone	5	**2.44** (1.02-5.87)	1	1.32 (0.19-9.34)	1	1.26 (0.18-8.98)	2	0.82 (0.20-3.26)
Connective tissue	10	1.55 (0.83-2.88)	1	0.45 (0.06-3.21)	5	1.68 (0.70-4.03)	13	1.55 (0.90-2.66)
non-Hodgkin lymphoma	119	***5.16*** (4.31-6.19)	10	1.42 (0.76-2.46)	76	***6.03*** (4.80-7.57)	34	1.10 (0.79-1.55)
Hodgkin lymphoma	35	***7.69*** (5.52-10.72)	1	0.55 (0.08-3.89)	15	***10.04*** (6.05-16.68)	8	1.50 (0.75-2.99)
Myeloma	76	***8.71*** (6.95-10.91)	1	0.36 (0.05-2.58)	35	***9.45*** (6.77-13.19)	10	0.96 (0.51-1.78)
Cancer of unknown primary	11	1.16 (0.64-2.10)	3	1.00 (0.32-3.09)	5	1.37 (0.57-3.28)	7	0.68 (0.32-1.43)
All	1463	***1.57*** (1.48-1.65)	380	***1.26*** (1.13-1.40)	750	***1.54*** (1.42-1.67)	1280	1.02 (0.96-1.08)

Abbreviations:
N, frequency; RR, relative risk; CI, confidence interval; SCC, squamous cell carcinoma;
Bold, italicized and underlined indicate statistical significance at 5%, 1% and 0.1% level respectively

Table 5. Risk of second primary cancers among patients diagnosed with subtypes of myeloproliferative neoplasms

SPC site	Polycythemia vera		Essential thrombocythemia		Primary myelofibrosis		MPN - not otherwise specified	
	N	RR (95% CI)	N	RR (95% CI)	N	RR (95% CI)	N	RR (95% CI)
Upper aerodigestive tract	20	1.44 (0.93 - 2.23)	10	1.66 (0.89 - 3.09)	5	**2.82** (1.17 - 6.77)	7	***3.27*** (1.56 - 6.85)
Esophagus	10	1.41 (0.76 - 2.62)	5	1.61 (0.67 - 3.88)	0	-	0	-
Stomach	32	1.01 (0.71 - 1.43)	12	1.26 (0.71 - 2.21)	1	0.30 (0.04 - 2.11)	1	0.32 (0.04 - 2.26)
Small intestine	3	1.02 (0.33 - 3.15)	4	**2.93** (1.10 - 7.81)	0	-	2	**4.09** (1.02 - 16.38)
Colorectum	77	0.89 (0.71 - 1.12)	38	0.94 (0.68 - 1.29)	13	1.19 (0.69 - 2.05)	24	**1.66** (1.11 - 2.48)
Anus	4	**2.93** (1.10 - 7.81)	1	1.34 (0.19 - 9.53)	0	-	0	-
Liver	30	1.29 (0.90 - 1.84)	6	0.70 (0.31 - 1.56)	5	1.78 (0.74 - 4.27)	4	1.39 (0.52 - 3.70)
Pancreas	23	1.02 (0.68 - 1.54)	13	1.40 (0.79 - 2.46)	4	1.45 (0.54 - 3.86)	6	2.04 (0.91 - 4.53)
Nose	3	2.94 (0.95 - 9.12)	1	2.42 (0.34 - 17.23)	0	-	1	6.99 (0.98 - 49.70)
Lung	86	***1.57*** (1.27 - 1.94)	32	1.31 (0.93 - 1.86)	6	0.85 (0.38 - 1.89)	15	**1.73** (1.04 - 2.87)
Breast	76	1.23 (0.98 - 1.54)	39	1.18 (0.86 - 1.61)	6	0.85 (0.38 - 1.90)	9	0.83 (0.43 - 1.60)
Cervix	3	0.66 (0.21 - 2.03)	3	1.59 (0.51 - 4.94)	0	-	2	3.54 (0.88 - 14.14)
Endometrium	15	1.04 (0.63 - 1.73)	9	1.18 (0.62 - 2.27)	1	0.59 (0.08 - 4.19)	1	0.40 (0.06 - 2.81)
Ovary	12	1.03 (0.58 - 1.81)	5	1.05 (0.44 - 2.51)	1	0.76 (0.11 - 5.43)	3	2.03 (0.65 - 6.29)
Other female genitals	0	-	5	***3.31*** (1.38 - 7.96)	0	-	0	-
Prostate	156	***1.25*** (1.07 - 1.47)	63	1.15 (0.90 - 1.48)	21	1.11 (0.72 - 1.70)	20	0.87 (0.65 - 1.35)
Testis	1	1.97 (0.28 - 13.99)	0	-	0	-	0	-
Kidney	46	***2.51*** (1.87 - 3.36)	18	***2.44*** (1.52 - 3.93)	5	2.22 (0.92 - 5.33)	4	1.65 (0.62 - 4.38)
Bladder	49	***1.53*** (1.15 - 2.02)	19	1.24 (0.79 - 1.94)	2	0.49 (0.12 - 1.94)	6	1.07 (0.48 - 2.38)
Melanoma	37	***2.27*** (1.65 - 3.14)	23	***2.26*** (1.50 - 3.40)	4	1.66 (0.62 - 4.42)	6	1.60 (0.72 - 3.56)
Skin (SCC)	73	***2.19*** (1.74 - 2.76)	52	***2.57*** (1.95 - 3.38)	16	***3.71*** (2.27 - 6.05)	26	***3.45*** (2.35 - 5.06)
Nervous system	29	***2.17*** (1.51 - 3.13)	16	***2.63*** (1.61 - 4.29)	6	***3.29*** (1.48 - 7.32)	4	1.88 (0.71 - 5.01)
Thyroid gland	2	0.58 (0.15 - 2.34)	3	1.94 (0.63 - 6.02)	0	-	3	***5.59*** (1.80 - 17.33)
Endocrine glands	26	***3.49*** (2.38 - 5.13)	11	***3.10*** (1.72 - 5.61)	1	0.94 (0.13 - 6.70)	3	2.41 (0.78 - 7.49)
Bone	2	3.27 (0.82 - 13.08)	0	-	1	**12.31** (1.73 - 87.47)	0	-
Connective tissue	6	1.70 (0.76 - 3.79)	2	1.25 (0.31 - 5.01)	2	**4.35** (1.09 - 17.38)	1	1.75 (0.25 - 12.40)
non-Hodgkin lymphoma	31	**1.59** (1.12 - 2.26)	14	1.42 (0.84 - 2.40)	7	**2.64** (1.26 - 5.53)	12	***3.29*** (1.87 - 5.80)
Hodgkin lymphoma	3	1.55 (0.50 - 4.80)	2	2.74 (0.69 - 10.98)	3	***12.26*** (3.95 - 38.03)	2	**8.19** (2.05 - 32.76)
Multiple myeloma	17	**1.64** (1.02 - 2.64)	0	-	0	-	2	1.21 (0.30 - 4.84)
Cancer of unknown primary	33	1.30 (0.93 - 1.83)	13	1.16 (0.67 - 2.00)	6	1.86 (0.84 - 4.14)	8	**1.98** (1.09 - 3.95)
All	916	***1.34*** (1.25 – 1.43)	422	***1.39*** (1.27 – 1.54)	118	***1.88*** (1.64 – 2.17)	173	***1.50*** (1.28 – 1.76)

Abbreviations:
N, frequency; RR, relative risk; CI, confidence interval; SCC, squamous cell carcinoma;
Bold, italicized and underlined indicate statistical significance at 5%, 1% and 0.1% level respectively

Necessary Additional Data

Click here to download Necessary Additional Data: Supplementary appendix.docx

***Reply to Reviewers Comments**

EDITORIAL COMMENTS

1. I have made some specific remarks and performed some recommended developmental and line-editing changes on a great portion of the document (see attached Word file in a subsequent email). Please carefully go over these changes, paying special attention to scientific accuracy.

>>> Please refer to the draft

2. Rewording of the manuscript will be required (please see attached word file). In addition, in some instances, implications and take-home messages are needed. A more in-depth interpretation of the findings and clinical relevance by disease subtype in this study needs to be included. What is the usefulness of the findings? A major limitation is that molecular and clinical characteristics were not addressed, and this needs to be pointed in the discussion. Indeed, the limitations of this study need to be thoroughly discussed.

>>> Please refer to the Discussion

3. The manuscript reads with difficulty. Thus, I would ask that a native English speaker proofread this article in depth before resubmitting your revision.

>>> We have tried to simplify the text

4. From reviewer 2: “The incidence of SPCs may be substantially affected by the type of therapy for myeloid malignancies or other primary cancers, which might have substantially differed according to the era. Unfortunately, the type and doses of therapy were not described in the registry, which is a potential limitation of the study. Meanwhile, it would be interesting to look at the effect of the year of diagnosis of initial cancer on the risk of PSC and myeloid malignancy.” This limitation needs to be addressed in the discussion.

>>> Included

5. From reviewer 2: “If these positive RRs are real, authors may expect high RRs between two non-hem cancers; for example, if positive RRs are noted between AML and PSC A and between AML and PSC B, it might be that RRs between PSC A and PSC B might be expected and if so, the results of the current study would be substantially intensified. >>>We are of course interested about such hypotheses and deal with them on separate studies.” Please add this information in the discussion.

>>> Included

6. From Reviewer 2: “Among many positive association (or significant RRs), it should be noted which has been previously reported and which is new, with providing appropriate literatures. >>>We added some words of explanation to the first paragraph of Discussion. This is the first study on MDS which was already stated there.”

>>> Already included

7. From Reviewer 3: “Please add more information on which associations have already been demonstrated and for what specifically, with appropriate references .Please clarify how the authors can distinguish second primary cancer from therapy-related cancer. Second primary may imply that there is no effect of previous therapy. How is this handled in the study? >>>The ICD codes used by the Swedish Cancer Registry do not specify therapy related cancers, which according to literature now account for 7% of AML (Heuser M, Hematology 2016).” Please further discuss this and include this information.

>>> Included

8. From Reviewer 3: “"Bi-directional increases" is used across the manuscript. What does it actually mean and where is it defined? This is a new term that oncologists may need to be familiarized with, and I still did not understand it after reading the manuscript. Please define very clearly so that we know what this means. This should be probably defined in the Introduction and clarified early on. >>> Last paragraph of Introduction explains the bi-directional approach.” I still agree that this term remains very obscure throughout the manuscript and needs to be clearly defined and elaborated upon further, and in several places.

>>> We have included explanation in several instances now

9. From Reviewer 3: “In the Introduction, paragraph 3, last 3 lines. Novel analysis. Does this refer to the bi-directional analysis? Why is it new if it considers myeloid malignancies after any first cancer? >>> The novelty of the analyses lies in interpretation of excess risk observed in both direction for a given cancer pair which indicates biological and mechanistic underpinnings rather than the popularly accepted notion of ‘effect of therapy’ between first and second cancers.” This needs to be included in the manuscript.

>>> Now included

10. You talk about TKI inhibitors in the intro, etc. is there anything else that can be included in your results and interpretations based on supplemental table 5?

>>> We lack data to further implicate analyses

11. From Reviewer 3: “Table 5. What is the difference between Table 5 and Table 3? Both discuss second primary cancers among patients with myeloid malignancy. This appears to selectively discuss MPN. Any unique findings that require this additional Table? >>> As incidence rates and in some cases definition of disease changes over the long follow-up time in our study, it is desirable to look at each of the subtypes of MPN separately as diagnoses of these neoplasia are not identical.” Your explanation needs to be included in the main text.

>>> Included now

12. “>>> We have acknowledged the limitation due to unavailability of data on treatment or tumor-molecular and genetic characteristics.” This needs to be further elaborated upon in the discussion section.

>>> We elaborated this point in discussion

13. From Reviewer 6: “It would be useful for the authors to explain why they did not (or where unable) to link the treatments data (since this seems to be routine for many studies across many disease areas using the Scandinavian routine datasets)? >>> The reason was lack of treatment data in the Cancer Registry.” This needs to be included in the main text.

>>> Included in limitation statement

14. From Reviewer 6: “it would be useful if the authors gave more insight into why the generalised version was needed and indeed what assumptions they were then making? >>> To avoid inflation in variance, we adapted a generalized model where the assumption lies on expectation of estimated variance.” … and “>>> The regression is assessed on case numbers scaled on person-years. This reduces case numbers to sizable fractions comparable to the reference fractions in each person-year category.” Please include this information in the methods section.

>>> Included in methods

15. “The ICD codes used by the Swedish Cancer Registry do not specify therapy related cancers, which according to literature now account for 7% of AML (Heuser M, Hematology 2016).” Please include this information in the main text.

>>> Included as discussed above

We have also carefully rectified formatting related issues of the text, tables and appendix.