Second Malignancies in Patients with Multiple Myeloma

Risk Factors and Impact on Survival

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Thesis for the degree of Philosophiae Doctor

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Lýðgrunduð rannsókn á þróun annarra krabbameina hjá sjúklingum með mergæxli

Áhættuþættir og áhrif á lifun

Guðbjörg Jónsdóttir

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Ágrip

Inngangur: Með tilkomu áhrifaríkari lyfja í meðhöndlun sjúklinga með mergæxli síðastliðna áratugi hefur lifun þeirra stóraukist. Þessi aukna lifun hefur leitt til aukinnar áherslu rannsakennda á langtíma afleiðingum í formi þróun annarra krabbameina. Sjúklingar með mergæxli eru í aukinni áhættu á að þróa með sér ákveðnar tegundir krabbameina svo sem bráða hvítblæði og mergmisþoska. Undirliggjandi ástæður þessa eru óljósar og áhættuþættir lítt þekktir. Vísindamenn telja að ástæður seinni krabbameina hjá sjúklingum með mergæxli séu líklega fjölþættar og samanstandi af þáttum sem tengjast meðferð, sjúkdómnum sjálfum, erfðum, umhverfinu og lífsstíl. Meginmarkmið þessarar ritgerðar var að kanna áhrif annarra krabbameina á lifun sjúklinga með mergæxli og leitast er við að kanna áhættuþætti fyrir þróun þeirra með sérstaka áherslu á bráðahvítblæði og mergmisþroska.

Efniviður og aðferðir: Við framkvæmdum lýðgundaða rannsókn þar sem notast var við gögn frá sænsku krabbameinsskránni sem samanstóð af öllum sjúklingum sem greinst höfðu með mergæxli í Svíþjóð á tveimur mismunandi tímabilum, annars vegar frá 1. janúar 1958 til 31. december 2011 (n=26,627) og hins vegar frá 1. janúar 1973 til 31. desember 2010 (n=19,791). Úr fyrri hópinn fundum við alla sjúklinga sem höfðu greinst með annað krabbamein eftir mergæxlisgreiningu (n=1,547). Þeir voru paraðir eftir aldri, kyni og dagsetningu mergæxlisgreiningar við einn til þrjá mergæxlissjúklinga, sem ekki höfðu þróað með sér annað krabbamein, og þeir valdir með slembiúrtaki (n=4,019). Við úrvinnslu gagnanna var meðal lifun sjúklinga milli þessarra tveggja hópa borin saman með Cox líkani. Til þess að kanna áhættuþætti fyrir bráðahvítblæði/mergmisþoska sértaklega fundum við þá einstaklinga sem höfðu geinst með bráðahvítblæði/mergmisþoska úr fyrri hópnum (n=124) og pöruðum við einn sjúkling sem ekki hafði þróað með sér annað krabbamein. Nákvæmar upplýsingar um meðferð og rannsóknarniðurstöður við greiningu, meðal annars blóðrannsóknir og niðurstöður beinmergsskoðunar voru skráðar úr sjúkraskrám. Útsetning fyrir samanlögðum skammti lyfjameðferðar fyrir hvert lyf var borin var saman milli hópa ásamt rannsóknarniðurstöðum við greiningu með einhliða fervikagreiningu, Kí-kvaðratsprófi og Kruskal-Wallis prófi. Við könnuðum samanlagða skammta af melphalani milli hópa með lógistískri aðhvarfsgreiningu. Þýði úr seinni hópnum var notað til að kanna áhrif fyrra krabbameins á þróun annarra krabbameina eftir mergæxlisgreiningu. Áhætta á að þróa með sér annað krabbamein eftir mergæxlisgreiningu var borin

saman milli sjúklinga sem höfðu greinst með krabbamein fyrir mergæxlisgreiningu og þeirra sem höfðu ekki sögu um fyrra krabbamein með Cox líkani.

Niðurstöður: Þróun annars krabbameins í sjúklingum með mergæxli var tengt verri horfum (HR 2.3; 95% CI 2.1-2.5). Meðaltals lifun var 1.1 ár (95% CI 1.0-1.2) eftir annað krabbamein og 3 ár (95% CI 2.8-3.1) fyrir mergæxlissjúklinga sem ekki höfðu þróað með sér annað krabbamein ($p<0.001$). Við sýndum fram á, að þrátt fyrir aukna lifun á árunum 2001-2011 þá eru áhrif annarrra krabbameina á lifun þessara sjúklinga mikil því þeir sjúklingar sem greindust eftir aldarmótin með annað krabbamein höfðu skertar lífslíkur samanborið við sjúklinga sem greindir voru fyrir aldarmótin og höfðu ekki greinst með annað krabbamein (1958-2000) (HR 1.1; 95% CI 1.1-1.5). Við sýndum fram á að lifun sjúklinga með mergæxli sem þróa með sér bráðahvítblæði/mergmisþoska er sérstaklega slæm og þeir eru í 70% aukinni áhættu á að deyja borið saman við sjúklinga með bráðahvítblæði/ mergmisþoska sem ekki höfðu sögu um fyrra krabbamein. Við fundum jákvætt samband milli sögu um fyrra krabbamein og þróun annars krabbameins eftir mergæxlisgreiningu (HR 1.4; 95% CI 1.2-1.7) sem gæti bent til erfðafræðilegs þáttar í þeirri þróun. Við fundum að fyrra krabbamein hafði neikvæð áhrif á lifun (HR 1.2; 95% CI 1.1-1.3) og að þeir sjuklingar sem greindust með fleiri en eitt fyrra krabbamein höfðu enn verri horfur (HR 1.3; 95% CI 1.2-1.5). Hærri samanlagður skammtur af melphalan lyfjameðferð tengdist aukinni áhættu á þróun bráðahvítblæðis/mergmisþoska (OR 2.8; 95% CI 1.7-5.2). Miðgildistími frá mergæxlisgreiningu fram að greiningu á bráðahvítblæði/mergmisþoska var 3.8 ár (IQR 2.8-5.8). Við fundum ekki samband milli þróunnar bráðahvítblæði/mergmisþoska og eftirfarandi sjúkdómsþátta: M-prótein ísótýpu, blóðleysi, nýrnabilun, kalkofhleðslu og beinúrátu.

Ályktun: Niðurstöður þessara lýðgrunduðu rannsókna gefa til kynna að meðferðartengdir og erfðarfræðilegir þættir eiga mögulega þátt í þróun annarra krabbameina hjá sjúklingum með mergæxli. Skert lifun þessara sjúklinga undirstrikar mikilvægi þess að greina áhættuþætti og líffræðilega ferla sem valda þessari þróun. Aukin þekking á áhættuþáttum og orsökum annarra krabbameina hjá sjúklingum með mergæxli gæti haft áhrif á leiðbeiningar varðandi eftirfylgd og ákvarðanartöku um mismunandi meðferðir í framtíðinni.

Lykilorð: Mergæxli, annað krabbamein, bráðahvítblæði/mergmisþoski, melphalan, lifun

Abstract

Introduction: Over the past two decades, increased access to modern effective therapies has improved survival and clinical outcomes for patients with multiple myeloma (MM). However, improved survival has led to concerns regarding long-term complications such as development of second malignancies. Patients with MM have been found to have increased risk of developing certain types of second malignancies such as acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) although exact biological mechanisms and risk factors are not well-established. A multifactorial model has been proposed to explain the etiology of second malignancies in MM patients, possibly including treatment-, host-, disease-, environmental-, and lifestyle-related factors. The overall aim of this thesis was to determine the effects of second malignancies on survival in patients with MM and identify risk factors for second malignancy development with focus on patients who develop AML/MDS.

Methods: Data was obtained on all patients diagnosed with MM in Sweden reported to the Swedish Cancer Registry, for two different time periods. The first cohort included patients diagnosed from January 1,1958 to December 31, 2011 (n=26,627) and the second included patients diagnosed from January 1, 1973 to December 31, 2010 (n=19,791). In the first cohort, we subsequently identified all patients with MM who developed a second malignancy (n=1,547) and randomly matched them by age, sex and date of MM diagnosis with one to three MM patients who had not developed a second malignancy (n=4,019). Median overall survival (OS) was compared between the groups using the Kaplan-Meier method. Cox proportional hazard model for matched data was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs). We looked further into the clinical implication of AML/MDS by comparing survival in patients with MM and subsequent AML/MDS to patients with *de novo* AML/MDS. To identify risk factors for AML/MDS specifically, patients who developed AML/MDS from the first MM cohort were identified (n=124) and matched to one patient without a subsequent second malignancy diagnosis. Detailed clinical and treatment data was obtained from medical records located at >50 hospitals in Sweden. Exposure to treatment, including cumulative doses of alkylating chemotherapy was compared between the groups as well as baseline disease-related factors by using one-way ANOVA, Chi-Square, and Kruskal-Wallis tests. We further investigated cumulative melphalan exposure between

cases and controls, reported as odds ratios (OR). The second cohort was used to assess impact of prior malignancies on second malignancies and survival. All cancer diagnoses prior to and after the MM diagnosis were identified. Prior malignancy was hypothesized to be a proxy for inherent gentetic susceptibility (host-related factor). Risk of developing a second malignancy and survival was compared between patients with MM who had a history of a prior malignancy diagnosis compared to MM patients who did not, using a Cox proportional hazard model.

Results: Second malignancy development was associated with inferior surivival in patients with MM (HR 2.3; 95% CI 2.1-2.5). Median OS was 1.1 years (95% CI 1.0-1.2) after a second malignancy diagnosis and 3.0 years (95% CI 2.8-3.1) for MM patients without a second malignancy ($p<0.001$). We found that despite the survival improvement seen in 2001-2011, the impact of second malignancies is such that patients diagnosed after the millennia had a worse outcome than patients without second malignancies before the introduction of modern MM therapy (1958-2000; HR 1.3; 95% CI 1.1-1.5). We observed that patients with the combination of MM and subsequent AML/MDS have a dismal outcome, and a 70% higher risk of dying when compared to patients with *de novo* AML/MDS. History of prior malignancy was associated with increased risk of developing subsequent malignancy (HR 1.4; 95% CI 1.2-1.7), suggesting a possible role of inherent genetic susceptibility. Additionally, we found that prior malignancy negatively impacts survival (HR 1.2; 95% CI 1.1-1.3) and that more than one prior malignancy reduces survival even further (HR 1.3; 95% CI 1.2-1.5). Higher cumulative melphalan exposure was associated with increased risk of developing AML/MSD (OR 2.8; 95% CI 1.7-5.2). The median time to AML/MDS development was 3.8 years (IQR 2.8-5.8). We showed that the risk of AML/MDS was not statistically altered by M protein isotype, anemia, renal failure, hypercalcemia, or lytic bone lesion.

Conclusion: The results of our studies suggest that treatment- and host-related factors might play a role in second malignancy development in MM patients. Our findings of inferior survival in patients with second malignancies highlight the importance of identification of risk factors as well as biological mechanisms that result in second malignancies. Increased knowledge of second malignancy risk in MM patients could affect surveillance protocols and treatment decisions in the future.

Keywords: multiple myeloma, second malignancies, melphalan, survival, AML/MDS

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Contents

List of abbreviations

MM	Multiple Myeloma
M-protein	Monoclonal protein
IMWG	International Myeloma Working Group
MP	Melphalan and Prednisone
OS	Overall Survival
US	United States
HDM	High Dose Melphalan
ASCT	Autologous Stem Cell Transplantation
NCI	National Cancer Institiute
SEER	Surveillance, Epidemioloigy, and End Results program
AML	Acute Myeloid Leukemia
MDS	Myelodysplastic Syndromes
SIR	Standardized Incidence Ratio
CIBMTR	Center for International Blood and Marrow Transplant research
PFS	Progression Free Survival
VRd	Velcade, Revlimide and Dexamethasone
HR	Hazard Ratio
CI	Confidence Interval
SNP	Single Nucleotide Polymorphisms
ICD 7	International Classification of Diseases, revision 7
CR	Complete Response
sCR	Stringent Complete Response
VGPR	Very Good Partial Response
PR	Partial Response
SD	Stable Disease

PD	Progressive Disease
UV	Ultraviolet
IQR	Interquartile Range
NA	Not Applicable
MVP	Melphalan, Bortezomib and Prednisone

List of figures

List of tables

List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I-III):

I. **Survival in multiple myeloma patients who develop second malignancies: a population-based cohort study.** Jonsdottir G, Lund SH, Bjorkholm M, Turesson I, Blimark C, Hultcrantz M, Porwit A, Landgren O, and Kristinsson SY. *Haematologica*. 2016; 101(4): e145-e148.

II. **The impact of prior malignancies on second malignancies and survival in MM patients: a population-based study.** Jonsdottir G, Lund SH, Bjorkholm M, Turesson I, Hultcrantz M, Porwit A, Jethava YS, Landgren O, and Kirstinsson SY. *Blood advances*. 2017; 1(25): 2392-2398.

III. **Cumulative exposure to melphalan chemotherapy and subsequent risk of developing acute myeloid leukemia and myelodysplastic syndromes in patients with multiple myeloma**. Jonsdottir G, Bjorkholm M, Turesson I, Hultcrantz M, Diamond B, Porwit A, Landgren O, and Kirstinsson SY. *Accepeted for publication in the European Journal of Haematology.*

In addition, some unpublished data may be presented. All papers are reprinted by kind permission of the publishers.

Declaration of contribution

I, Guðbjörg Jónsdóttir, planned the research work for the papers that form the foundation for this thesis in close collaboration with my supervisor. The research work included spending six weeks in Sweden collecting data from medical records at multiple different Swedish hospitals. A statistician conducted the analyses for the first study. I conducted statistical analyses for the second study under the guidance of a statistician. I conducted all statistical analyses in the third study. I drafted all manuscripts and revised in close collaboration with my co-authors. I wrote this thesis under the guidance of my supervisor.

1 Introduction

Multiple myeloma (MM) is a malignant plasma cell disorder that accounts for approximately 10% of hematological malignancies. It is characterized by clonal proliferation of malignant plasma cells in the bone marrow (Figure 1),[1] monoclonal protein (M-protein) in the blood or urine, and associated organ dysfunction.[2] It has been shown that MM evolves from an asymptomatic premalignant state called monoclonal gammopathy of undetermined clinical signficiance (MGUS). Complex genetic and environmental changes then lead to the transformation of these cells into a malignant neoplasm.[3] MGUS is associated with low risk of malignant conversion or ~1% per year.[4] Smoldering multiple myeloma (SMM) is an intermediate clinical stage between MGUS and MM with much higher risk of progression to malignant conversion or ~10% per year.[5] Therefore, intitially MM is thought to arise from MGUS that progresses to SMM and finally to symptomatic MM. This is a disease of the elderly with median age at diagnosis around 70 years and in Western countries the annual age adjusted incidence is approximately 6.7 cases per 100,000.[6]

The diagnostic criteria for MM have evolved throughout the years and were most recently updated in 2014 by the International Myeloma Working Group (IMWG). Traditionally, the diagnosis required the presence of at least 10% of clonal plasma cells in the bone marrow and M-protein in serum or urine in addition to evidence of end-organ damage that was attributable to the malignant plasma cell clone: hypercalcemia, renal failure, anemia, or osteolytic bone lesions, commonly referred to as the CRAB criteria.[7] The diagnosis of MM according to the revised IMWG criteria in 2014 includes the above features but additionally incorporated three specific biomarkers: clonal bone marrow plasma cells ≥60%, serum free light chain (FLC) ratio ≥100, and more than one focal lesion on magnetic resonance imaging as independent diagnostic criteria. These additional criteria were implemented to identify patients within the SMM group with high risk of progression to symptomatic end-organ damage who could benefit from earlier treatment.[8]

After the diagnosis of MM is made, two main staging systems have been used to stratify patients according to disease burden and prognosis. In 1975, Durie and Salmon introduced a staging system using the level and type of M-protein, serum hemoglobin, serum calcium, serum creatinine, and number of bone lesions.[9] This system remained the most widely used staging system

for the next 25 years. In 2005, based on a study including 10,750 patients with MM, the IMWG reported that the combination of serum β2-microglobulin and serum albumin were powerful predictors of survival and introduced a new three-stage classification called The International Staging System (ISS).[10] The median survival off stage I, II and III patients was 62 months, 44 months and 29 months, respectively. The ISS was later revised in 2015 to incorporate two further prognostic factors, genetic risk assessed by fluorescence in-situ hybridization (FISH) and level of serum lactate dehydrogenase.[11]

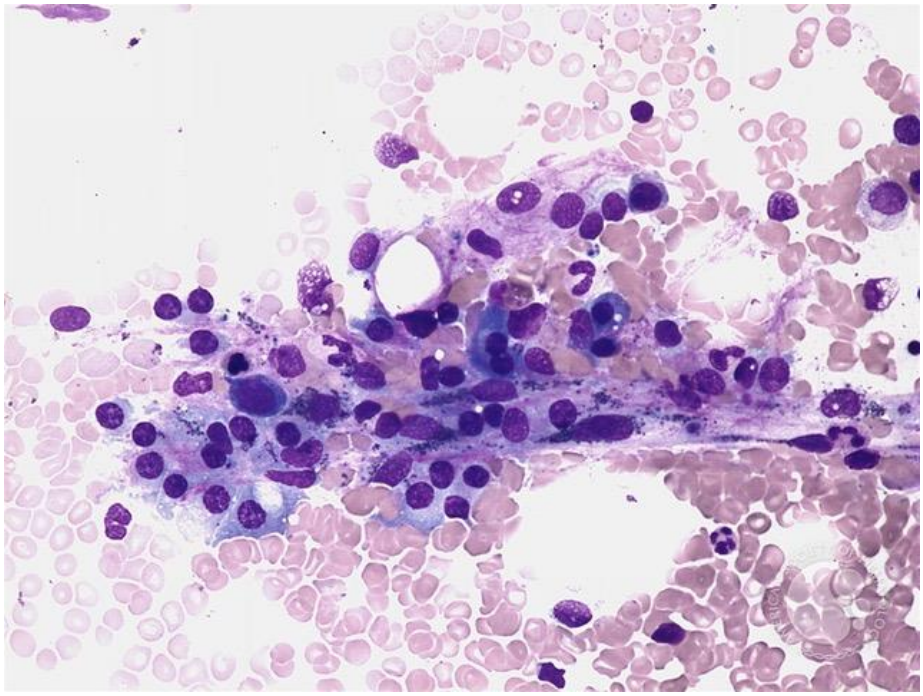

Figure 1. Abnormal plasma cells in a bone marrow aspirate from a patient with MM

MM is a heterogenous disease and the clinical course can be markedly different in patients with similar staging and host factors. Cytogenetic abnormalities have been found to be a major prognostic factor in patients with MM and by analysing bone marrow plasma cells using FISH tecnique tumors can be stratified according to outcome. Cytogenetic abdnomalities such as t(4;14), del(17/17p), t(14;16), t(14;20), nonhyperdiploidy, and gain(1q) have been found to predict for a poor prognosis.[12]

1.1 Treatment and survival

In the early 1960s, alkylating treatment with melphalan was found to have an anti-MM effect and was until early 2000s the mainstay of treatment for MM in combination with prednisone (MP).[13] Before the introduction of alkylating agents the median overall survival (OS) for patients with MM was <1 year.[14] In an attempt to improve outcomes from 1960s and onwards, varous combination chemotherapy regimens were used for treatment of MM. These included combinations of vincristine, melphalan, cyclophosphamide and prednisone (VMCP), and vincristine, carmustine, doxorubicine and prednisone (VBAP). However these combination chemotherapy regimens were not found to improve median OS compared to treatment with MP.[15] In 1979 a case series was published showing promising benefits of interferon monotherapy in previously untreated MM patients.[16] Over the next two decades multiple randomized trials were performed on interferon-chemotherapy induction treatment as well as on interferon therapy for maintenance with conflicting results.[17-19] In 2000, the results from a meta-analysis of 30 randomized trials among 3948 MM patients evaluating interferon treatment was published and was only found to be associated with a small OS benefit.[20] High dose melphalan with autologous stem cell transplantation (HDM-ASCT) was introduced in the treatment of MM in the late 1980s,[21] and in 1996 Attal *et al*. published a landmark study demonstrating that HDM-ASCT was assicated with improved response rate, event-free surivial and OS in MM patients.[22] The five-year estimated survival rate was 52% in the HDM-ASCT group and 12% in the conventional chemotherapy group. To date, HDM-ASCT remains the treatment of choice for patients younger than 70 who are otherwise in good health.[23] Over the past decades progress in understanding the pathobiology of MM has transformed the treatment paradigm and patient outcomes.[24-26] In the early 2000s, immunomodulatory imide drugs (IMiDs), lenalidomide, and thalidomide,[27,28] as well as the proteasome inhibitor bortezomib[29] were introduced in the treatment of MM and were associated with significant survival benefit. Other new agents in recent years include the proteasome inhibitors carfilzomib and ixazomib,[30,31] pomalidomide (IMiD),[32] the deacetylase inhibitor panabinostat,[33] and the nuclear export inhibitor selinexor.[34] Another new targeted drug class that has emerged is monoclonal antibodies, including daratumumab, elotuzumab and isatuximab.[35,36] Daratumumab targets CD38 on plasma cells and was first approved in 2015 as a monotherapy for patients with relapsed/refractory disease.[37] In addition, multiple new drugs are currently in development.[38] This remarkable progress has led to significantly improved outcomes and currently in the United States (US) the median OS for otherwise healthy younger patients with MM is approximately 10 years.[39]

1.2 Second malignancies

With improved survival in patients with MM, the development of second malignancies started to gain clinical and scientific attention.[40,41] Interestingly, in the US, second or higher order malignancies are the third most common cancer diagnosed and an overall 14% higher risk of developing a new malignancy was observed in cancer survivors compared to the general population.[42] The prevalence of second malignancies in MM patients is expected to increase due to improved survival and possibly from MM treatment itself. This has been observed in other cancers with favorable outcomes such as Hodgkin's lymphoma, where second malignancies have a significant impact on survival, and after 15 years of follow-up the mortality due to second malignancies exceeds that of Hodgkin's lymphoma.[43-45] Importantly, a study conducted on 33,229 MM patients using data from the NCI SEER database in 2011 showed that the cumulative risk of death from MM significantly outweighed the risk of developing second malignancies.[40]

1.2.1 Incidence of second malignancies in MM

The incidence of second malignancies, both solid and hematological, has been investigated in population-based registry studies, retrospective studies and prospective clinical trials (Table 1). Significant heterogeneity has been observed in risk by second malignancy type, age and sex, with the most significant risks noted for acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS).[46-50] Razavi *et al.* reported an approximately seven-fold increased risk of AML in a population-based study from the National Cancer Institute (NCI) Surveillance, Epidemiology, and End Results (SEER) program that included 36,491 MM patients.[51] They did not find an increased overall risk of second malignancies (standardized incidence ratio [SIR] 1.0). The risk of breast (SIR 0.8), prostate (SIR 0.7), esophageal (SIR 0.5), and lung/bronchus (SIR 0.9) cancer was significantly decreased while the risk of melanoma (SIR 1.4), bladder (SIR 1.2), kidney (SIR 1.3), thyroid (SIR 1.6) and colorectal cancers (SIR 1.5) was significantly increased. In a population-based study from Sweden including 8,740 patients with MM, a 26% overall increased risk of developing any second malignancy was observed compared to the general population, as was an 11-fold increased risk of developing AML/MDS.[52] Additionally, in this study the authors reported an increased risk of gastrointestinal malignancies (SIR 1.3) and non-melanoma skin cancer (SIR 2.2). Increased risk of developing hematological malignancies other than AML/MDS has also been published, including non-Hodgkin's lymphoma (SIR 1.7),[53] and CML (SIR 2.3).[54]

The cumulative incidence of second malignancies has been reported to be as high as 11.6-15.9% at 10 years, [50,55-57] however this needs to be considered in relation to the cumulative incidence of cancer per life-year in the general population studied. Another challenge is that clinical trials are often not designed to capture second malignancies as patients with prior/secondary malignancies are often excluded; therefore, there can be substantial underreporting in these studies. Due to differences in study designs and populations, the true incidence of second malignancies is difficult to estimate, and while the risk of solid tumor second malignancies has been reported as both decreased and increased, the increased risk of AML/MDS has been consistently observed.

Table 1. Selected studies focusing on second malignancies in patients with MM

Author	Design/Study period	No. of patients	% of any second malignancy	% of hematological second malignancies	% of solid second malignancies
Randomized phase III trials					
Attal M *et al.*[58]	Len v placebo after HDM-ASCT	608	8.5 v 3.6	4.2 v 1.7	5.0 v 2.0
McCarthy *et al.*[46]	Len v placebo after HDM-ASCT	460	7.8 v 2.6	3.5 v 0.4	4.3 v 2.2
Palumbo *et al*.[59]	Len v placebo after MP -/+ Len	455	8 v 6 v 3	5.0 v 3.3 v 0.7	3.3 v 2.6 v 2.0
Population-based registry studies					
Dong *et al*.[53]	1958-1996	8,656	5.5	1.0	4.5
Mailankody *et al.*[52]	1986-2005	8,740	6.6	0.8	5.8
Razavi *et al*.[51]	1973-2008	36,491	5.5	0.7	4.7
Engelhardt *et al.*[47]	1997-2011	744	6.6	2.3	4.3
Tzeng *et al.*[60]	1997-2009	3,970	1.8	0.9	0.9
Rafkin *et al.*[61]	2009-2012	1,443	5.1	1.0	2.6
Retrospective studies					
Barlogie *et al.*[62]	1989-2007	2,418	1.1	1.1	NR
Usmani *et al.*[49]	1998-2009	1,148	6.4	3.1	3.2
Cuzick *et al.*[63]	1964-1975	648	1.9	1.9	NR
Hasskarl *et al.*[50]	1997-2008	589	3.1	1.0	2.0

Munker *et al.*[56]	1995-2010	197	2.5	0.5	2.0
Finnish Leukemia Group[64]	1979-1985	432	9.3	3.9	5.3
Fenk *et al.*[57]	1994-2009	313	5.8	2.8	2.8

Len: Lenalidomide, v: Versus, MP: Melphalan and prednisone, HDM-ASCT: High dose melphalan with autologous stem cell transplantation, NR: Not reported

1.2.2 Alkylating therapy

Myeloid malignancies following chemo- or radiation therapy are defined as therapy-related according to the World Health Organization classification.[65] The exact biological mechanisms for the increased risk of AML/MDS in MM patients are not well established. Traditionally, the excess risk has been mainly attributed to treatment-related factors, including the use of melphalan and other alkylating agents.[66,67] The mechanism for development of AML/MDS after alkylating treatment have been suggested to be related to DNA damage that induces mutations in hematopoietic progenitor cells.[68] In 1970, Kyle and colleagues reported on the development of acute AML in four patients who had been treated with prolonged melphalan for MM or primary amyloidosis.[69] The time from MM diagnosis to AML development ranged from 2.5 to 4.8 years. In 1979, Bergasagel *et al.* found a greater than expected incidence of AML among 364 patients treated for MM with low-dose MP in various combinations with other alkylating agents, where 3.8% of patients developed AML.[48] The authors concluded that it was not possible to determine whether treatment with leukemogenic agents increased the risk of AML in patients with MM as the incidence in untreated patients was not known. In a study conducted by the Medical Research Council, the 5- and 8-year prevalence of MDS or AML in MM patients treated with melphalan or cyclophosphamide was 3% and 10%, respectively. The majority of patients were diagnosed with AML/MDS after 5 years from the MM diagnosis.[63] In this study, the cumulative melphalan dose recieved 3 years before the AML/MDS diagnosis was considered to be the most important determinant of risk. However, this association has not held true in all studies. For example, in a retropsective cohort study from the Finnish Leukemia Group published in 2000 the authors found no assciation between cumulative doses of melphalan or duration of melphalan treatment.[64] The reported cumulative doses of melphalan were 1440mg and 1400mg, respectively, for patients who developed AML/MDS and those who did not.

1.2.3 HDM-ASCT

In 1986, Barlogie *et al.* published a study on the use of HDM-ASCT in patients with MM that showed limited benefit.[21] A decade later, Attal *et al.* published a randomized prospective study reporting the benefit of HDM-ASCT compared to conventional chemotherapy, and showed significantly improved event-free and OS.[22] There have been conflicting reports in the literature regarding HDM-ASCT and the potential increase in risk of second malignancies. Gorvindarajan *et al.* tried to answer this question in a study assessing the risk of AML/MDS in 188 MM patients who underwent tandem HDM-ASCT.[70] In Group 1, median duration of pre-transplant therapy was 7.6 months compared to 24 months in Group 2. All seven patients developing MDS belonged to Group 2. The authors concuded that preceeding treatments rather than the myeloablative treatment was assocated with development of MDS. In a retrospective analysis conducted on data from the Center for International Blood and Marrow Transplant Research (CIBMTR) from 1990 to 2010 on patients receiving a first auto-transplant, the incidence of second malignancies was similar to age- race- and gender-adjusted comparison subjects; however, AML and melanoma were observed at higher than expected rates.[71] In another study by Radivoyevitch *et al.*, using data from CIBMTR the authors found significantly increased risk of AML/MDS following autotransplant in patients with MM, non-Hodgkin‘s lymphoma and Hodgkin lymphoma compared to patients with similar diagnoses in the NCI SEER registry.[72] The risk factors reported for subsequent AML/MDS development in the MM patients undergoing HDM-ASCT were age, sex and number of prior lines of chemotherapy. In 2017, a large randomized clinical trial for newly diagnosed MM patients compared the median progression-free survival (PFS) in patients treated with five cycles of bortezomib,lenalidomide and dexamethasone (VRd) including HDM-ASCT and followed by one year of lenalidomide maintenance therapy versus eight cycles of VRd without upfront HDM-ASCT and followed by one year of lenalidomide maintenance.[73] The investigators found a 14-month longer median PFS in the treatment arm including HDM-ASCT and no significant differences between groups were observed in the rates of second malignancies.

1.2.4 Immunomodulatory drugs (IMiDs)

In 2010, three randomized, phase III trials reported an excess of hematological malignancies among MM patients who received lenalidomide maintenance therapy after either ASCT or induction treatment compared to patients who did not receive lenalidomide maintenance.[46,58,59] In 2014, a

meta-analysis was published by Palumbo *et al.* using data from seven clinical trials and a total of 3,254 MM patients of whom 2,620 had received lenalidomide and 589 had not.[74] The cumulative five-year incidences of hematological second malignancies were 3.1% in the lenalidomide group and 1.4% in the group who had not received lenalidomide, respectively (hazard ratio [HR] 3.8). Additionally, lenalidomide plus oral melphalan significantly increased hematological second malignancy risk compared to melphalan alone (HR 4.9). There was no increased risk of hematological second malignancies with lenalidomide plus cyclophosphamide (HR 1.3) or lenalidomide plus dexamethasone (HR 0.9) compared to melphalan alone. It has been shown that the survival benefit of lenalidomide maintenance outweighs the risk of second malignancy development.[75] To date, no increased risk for second malignancies has been observed in association with other newer drug treatments in MM such as proteasome inhibitors or monoclonal antibodies, although long-term follow-up remains limited.[76-78]

1.2.5 Radiotherapy

Ionizing radiation is a well-known carcinogen[79] although data regarding the role of radiation treatment and risk of second malignancies in patients with MM is limited. Studies focusing on this aspect in other malignancies such as breast cancer and Hodgkin‘s lymphoma have reported a dose-response relationship between risk of second malignancies and radiation dose to the surrounding tissue.[80,81] Depending on study population, the proportion of patients with MM who receive radiation treatment ranges from 25 to 55%.[82] A recent study conducted on data from the US Connect MM registry found no association between radiotherapy and second malignancy incidence.[61]

1.2.6 Disease-related factors

It has been postulated that the development of second malignancies in MM patients is likely a multifactorial process including a complex interaction between treatment-, disease-, host-, environmental- and lifestyle-related factors (Figure 2).[67,83,84] Suggested disease-related factors include heterogeneity in MM cytogenetics and MM subtypes. In a population-based study conducted on 5,652 patients with MGUS, an 8-fold increased risk of developing AML/MDS was observed.[52] Interestingly this study showed that the risk varied between M-protein isotypes and size of the M protein. Patients with M-protein concentration >1.5g/dl had a higher risk compared to patients with <1.5 g/dl and the risk was confined to IgG and IgA isotypes. In another large population-based study conducted by the Mayo Clinic which included 605 MGUS patients, MGUS was associated with increased risk of developing

MDS (HR 2.4) but not AML.[85] In this study, MDS occurred in patients with all M-protein isotypes. The authors suggested that these findings supported a mechanism for inherent increased risk of AML/MDS in plasma cell dyscrasias. Results from studies investigating heterogeneity in plasma cell cytogenetics and risk of second malignancy have been conflicting. Usmani *et al.*, using data from the TT2, TT3A and TT3GB trials, reported that cytogenetic abnormalities were associated with increased incidence of second malignancies.[49] In contrast, in a large registry study including 744 patients with MM followed for 25 years, the authors found that MM cytogenetics in patients with subsequent second malignancy development were predominantly favorable, suggesting that long disease latency due to less aggressive MM may have allowed for manifestation of additional malignancies.[47]

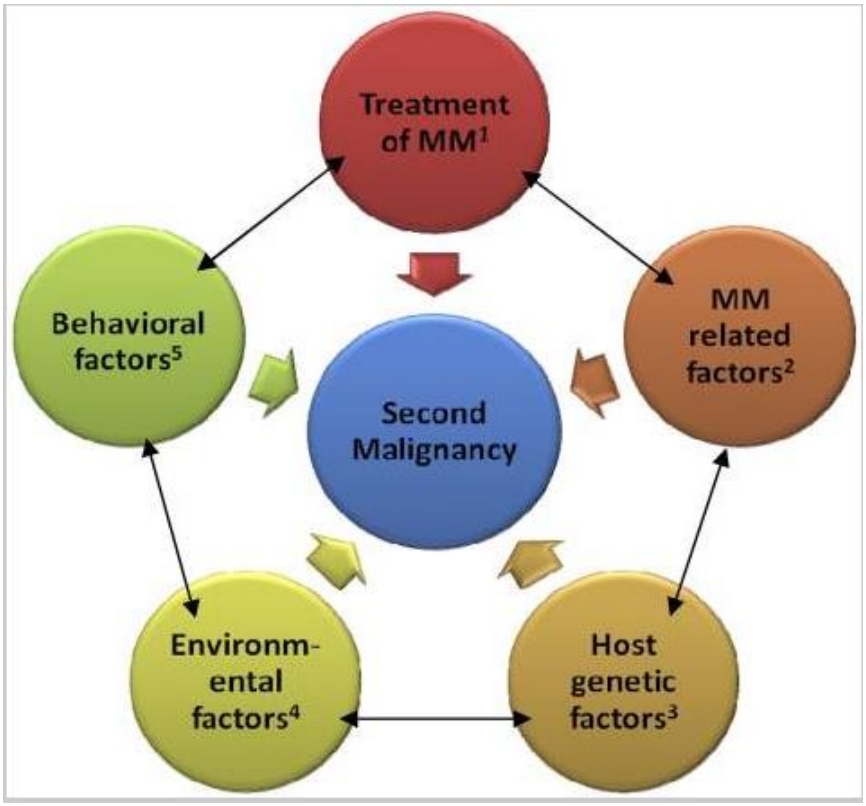

Figure 2. Proposed model of second malignancies after MM

Multiple different immune system defects have been described in MM patients, including T- and B-cell abnormalities, abnormal cytokine production, as well as NK-cell and dendritic cell defects. Furthermore, multiple lines of treatment that are given to patients with relapsed/refractory disease may result in cumulative immunosuppression.[86] Researchers have postulated that tumor-induced immunodeficiency and chronic inflammation may play a role in second malignancy development in MM patients. In support of this hypothesis it has been reported that chronic antigen stimulation and immune dysfunction are associated with development of both MM and AML.[87-89]

1.2.7 Host-related factors

Suggested host-related factors include both genetic and non-genetic factors. Among reported predisposing non-genetic factors are advanced age, male sex and African American ethnic group.[54,58,74,90,91] Genetic alterations have been suggested to contribute to familial and individual predisposition to MM and possibly second malignancies. Inherited genetic susceptibility for developing MM has been supported by genome-wide association studies that have identified single-nucleotide polymorphisms (SNP) localized to several genomic regions that are associated with increased risk.[92] Furthermore, studies on germline mutations have reported that mutations in the CDKN2A gene predisposes to MM and other cancers,[93] and the G/G phenotype of the SNP rs1617640 in the erythropoietin promoter gene has been associated with MDS development in MM patients.[94] Familial studies on patients with MM and their first-, second-, and third-degree relatives have shown a significant excess of cases of prostate cancer and melanoma.[95-97]

Information regarding prior malignancies and their impact on MM patients is limited. For instance, patients with prior malignancies are often excluded from clinical trials,[22,98] thus making it difficult to generalize current literature findings to this group and results of previously published studies on the effect of prior malignancies have been conflicting.[47,61]

1.2.8 Environmental- and lifestyle-related factors

Proposed environmental- and lifestyle-related risk factors for second malignancy development include smoking, ultraviolet (UV) light exposure, pesticide exposure, obesity and alcohol use.[67,99,100] Tobacco use, for example, has a strong association with lung, head and neck as well as upper gastrointestinal malignancies, although it has not been associated with development of MM.[101] Obesity has been associated with developing MM and its precursor state MGUS and has been associated with the development

of other malignancies.[102,103] In a retrospective registry analysis, alcohol was not associated with increased risk of second malignancy development in MM patients.[47] There is no comprehensive assessment available in the literature regarding these proposed factors in MM patients specifically and their potential influence might be difficult to quantify in the context of competing risks.

2 Aims

2.1 Survival

The aims of study I in this thesis were to:

Determine the effects of second malignancies on survival of patients with MM.

Establish the clinical impact of subseqent AML/MDS in MM.

2.2 Prior malignancies

In study II we aimed to determine the impact of prior malignancies on second malignancies and survival.

2.3 Risk factors for AML/MDS

The aim of study III in this thesis was to increase our understanding of risk factors for AML/MDS development in patients with MM by systematically comparing disease-related factors, cumulative drug doses and other exposure risk factors in patients with and without subseqent AML/MDS.

3 Materials and Methods

3.1 Central registers

All residents in Sweden have equal access to health care under a largely decentralized, taxpayer-funded system. All cancer diagnoses are reported to the centralized nationwide Swedish Cancer Register, which was established in 1958.[104] The diagnostic accuracy and overall completeness of the Swedish Cancer Register is high.[105,106] Pathologists and physicians in Sweden are obliged by law to report each cancer diagnosis to this register. Within the register, information on cancer type, sex, date of birth, and date of diagnosis are stored. All deaths and dates of death are centrally registered in the Swedish Cause of Death Register.[107]

3.2 Patient cohort study I

We identified all patients diagnosed with MM in Sweden from January 1, 1958 to December 31, 2011 reported to the Swedish Cancer registry. Information was gathered on sex, date of birth and date of MM diagnosis. All subsequent second malignancy diagnoses were identified through record-linkage within the registry, and the type and date documented. For each MM patient with a second malignancy diagnosis, one to three patients without a second malignancy diagnosis from the MM cohort were randomly selected and matched by age (+/- 3 years), sex, and date of MM diagnosis (+/- 1 year) (Figure 3). The matching criteria also requried that all patients without a second maligancy were alive when the corresponding matched MM patient developed a second malignancy. Patients diagnosed with MM or second malignancy at autopsy, patients with prior malignancy and patients with non-identifiable match (5%) were excluded. Information on date of death was gathered from the Swedish Cause of Death register.

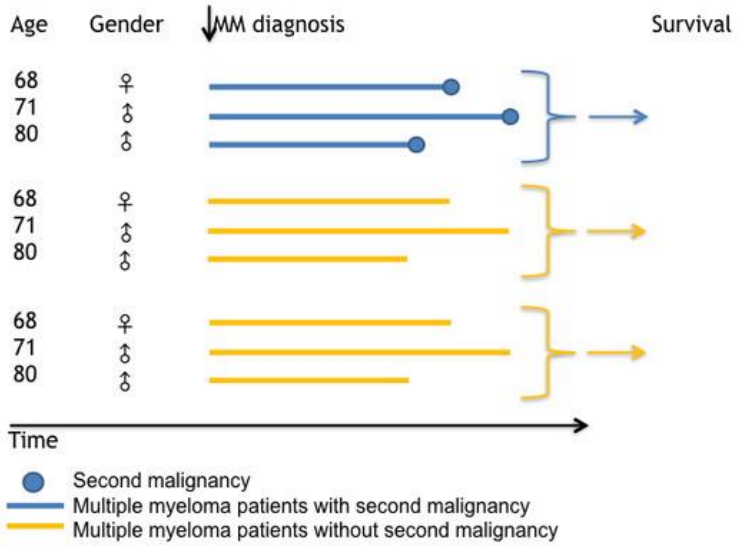

Figure 3. Schematic overview of the design for study I

Second malignancy type was classified according to the International Classification of Disease version 7 (ICD 7; Appendix A) to the following cancer subtypes: 1. Hematological, 2. Gastrointestinal, 3. Male reproductive, 4. Female reproductive, 5. Breast, 6. Kidney and urinary tract, 7. Non-melanoma skin, 8. Melanoma, 9. Respiratory, 10. Oral, nasal and pharyngeal, 11. Endocrine, 12. Nervous system, 13. Bone and cartilage, 14. Soft tissue and mediastinal, and 15. Unspecified.

From the group of MM patients with a hematological second malignancy, we identified all patients with AML/MDS. Each patient with MM and AML/MDS was matched by age (+/-3 years), sex and year of AML/MDS diagnosis with four patients having *de novo* AML/MDS, and four patients diagnosed with AML/MDS as a second malignancy, excluding patients with non-melanoma skin cancer and MM as the primary cancer diagnosis. They are hereafter referred to as having "secondary AML/MDS".

3.3 Patient cohort study II

All patients diagnosed with MM between January 1, 1973 and December 31, 2010 were identified from the Swedish Cancer Registry. Information was gathered on sex, date of birth, date of MM diagnosis. All cancer diagnoses prior to and after the MM diagnosis were identified through cross-linkage within the Swedish Cancer Registry, and the type and date of the cancer documented (Figure 4). Date of death was obtained from the Cause of Death Regiser. Prior and subsequent malignancies were classified according to ICD-7 to the same cancer subtypes as described earlier for the patient cohort in study I. End of study was December 31, 2013.

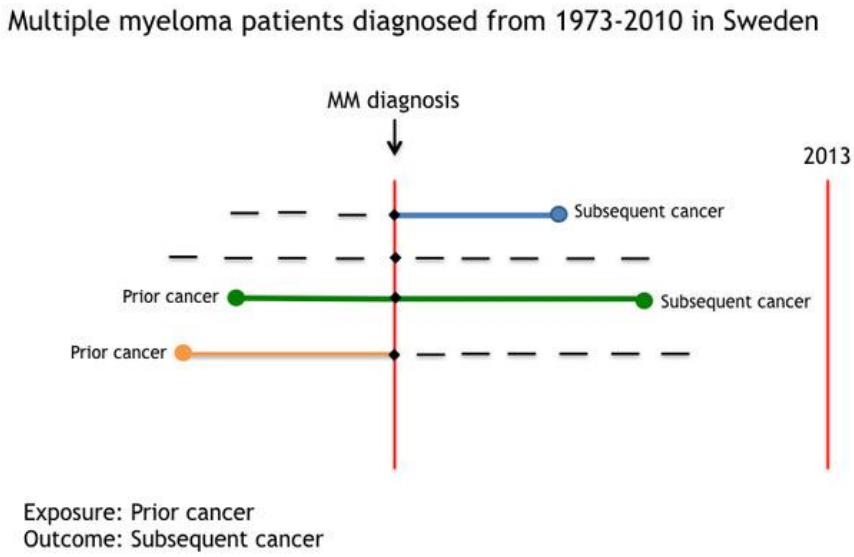

Figure 4. Schematic overview of the design for study II

3.4 Patient cohort study III

The same cohort of patients as described for study I was used—all patients diagnosed with MM in Sweden from January 1, 1958 to December 31, 2011. A record linkage was performed within the Swedish Cancer Registry to obtain information on all subsequent AML/MDS diagnoses within the MM cohort. For each patient with MM and a subsequent AML/MDS diagnosis (cases), one patient without a subsequent second malignancy diagnosis was randomly selected from the MM cohort and matched by age (+/- 3 years), sex, and date

of MM diagnosis (+/- 1 year; controls). Additionally, it was required that all controls were alive when the corresponding matched MM patient developed AML/MDS.

3.5 Data collection for study III

For both cases and controls, we obtained detailed clinical and treatment information from medical records. These included history of smoking as well as the following laboratory variables at diagnosis: complete blood count, chemistry panel, serum albumin and β2-microglobulin values. Serum protein electrophoresis, serum immunofixation, urine electrophoresis, and urine immunofixation findings were also recorded. Skeletal X-ray results were reviewed, and bone involvement was considered if a patient had lytic lesions, pathological compression fractures or severe osteopenia. Furthermore, bone marrow examination (at MM diagnosis and at AML/MDS development for cases), type of therapy received, and cumulative doses of each chemotherapy agent were documented. Cumulative doses of radiation were documented for each patient when data was available. Cumulative doses of chemotherapy and radiation received were calculated from date of MM diagnosis until AML/MDS development for cases and corrisponding date for controls. Response to treatment was categorized as follows: complete response (CR), stringent complete response (sCR), very good partial response (VGPR), partial response (PR), stable disease (SD), and progressive disease (PD) according to the international uniform response criteria for MM.[108] The study period was divided into the following five calendar periods: 1958-1970, 1971-1980, 1981-1990, 1991-2000, and 2001-2011. End of follow-up was December 31, 2012.

3.6 Inclusion and exclusion criteria study III

The patients in this study had received treatment at more than 50 hospitals in Sweden and medical records were found for 97 of the 124 (78%) AML/MDS patients. After review of pathology results, a total of 10 patients were excluded—6 had a primary diagnosis of solitary plasmacytoma and four were excluded as their secondary diagnosis was not consistent with AML/MDS—leaving 87 cases included in the study analyses. Of the 87 matched controls, medical records were found for a total of 69 patients (79%; Figure 5). The total number of patients with a diagnosis of MM during each calendar period who later developed AML/MDS was: 1958-1970, 3 (3%); 1971-1980, 15 (17%); 1981-1990, 31 (36%); 1991-2000, 24 (28%), and 2001-2011, 14 (16%).

Approval was obtained from the Regional Ethical Review Board in Stockholm for the studies in this thesis. Informed consent was waived because we had no contact with study patients (Appendix B).

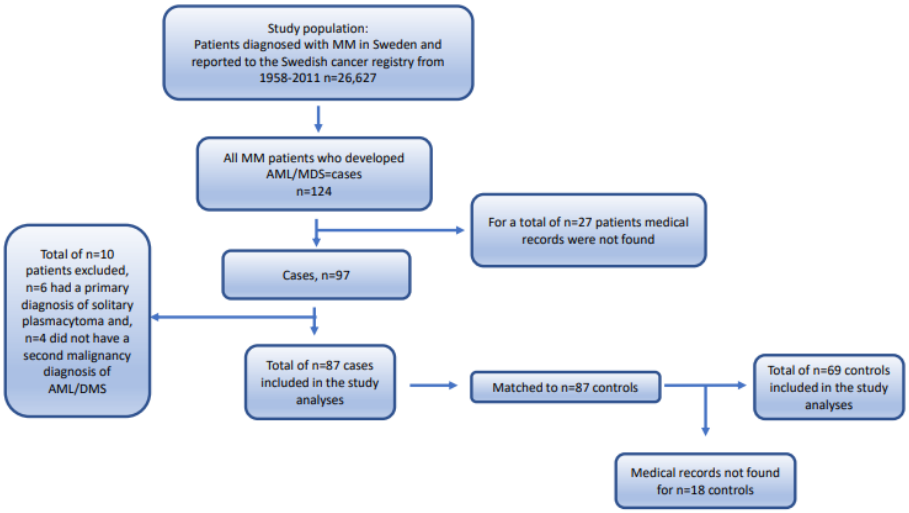

Figure 5. Flow chat of the population for study III with inclusion and exclusion criteria

3.7 Statistical analyses study I

Survival was estimated from the date of the second malignancy diagnosis and corresponding date for matched MM patients without second malignancy until death, emigration, or end of study (December 31, 2012), whichever occurred first. The Kaplan-Meier method was used to compare the median OS between groups as well as to estimate one- and five-year survival. Cox proportional hazard model for matched data was used to calculate HRs and 95% CIs. Two-sided p-values of <0.05 were considered statistically significant. Analyses were performed for the cancer types described above. Additionally, survival in MM patients with AML/MDS was compared to matched MM patients without a second malignancy.

A separate analysis was performed for patients with MM and AML/MDS (n=95) compared to matched patients with *de novo* AML/MDS (n=380) and to matched patients with secondary AML/MDS (n=380).

To assess survival patterns before and after the introduction of modern MM therapy in Sweden,[13] survival analyses were conducted for two different

time periods, 1958-2000 and 2001-2011. MM patients without a second malignancy diagnosed 1958-2000 (n=2,968) were compared to MM patients without a second malignancy 2001-2011 (n=1,051). Patients with MM and a second malignancy diagnosed 1958-2000 (n=1,164) were compared to patients with MM and a second malignancy diagnosed 2001-2011 (n=383). MM patient without a second malignancy diagnosed 1958-2000 were compared to MM patients with a second malignancy diagnosed 2001-2011. Cox proportional hazard model was used to calculate HRs and 95% CIs, adjusting for age, sex and year of MM diagnosis. Inference on these four comparisons was done simultaneously with a general linear hypothesis test.[109]

Several sensitivity analyses were performed where overall HRs were calculated as well as HRs for cancer subtypes. During the long study period, changes in diagnostic techniques and criteria might have influenced our results; therefore, an analysis was conducted where all patients diagnosed with MM before the year 1980 were excluded. Plasmacytoma was first registered in the Swedish Cancer Registry in 1987 as a separate diagnosis to MM; to take this into account, an analysis was performed where all MM patients diagnosed before the year 1987 were excluded as well as all patients with a plasmacytoma. A separate analysis was performed where patients diagnosed at autopsy with second malignancy were included.

3.8 Statistical analyses study II

The exposure was the binary categorical variable of a first malignancy diagnosis before MM diagnosis and the outcome was the binary categorical variable of a primary malignancy diagnosis after MM diagnosis. Demographic characteristics were compared between groups by using frequency measures and percentages as well as median values. A cox regression model was used to calculate HRs and 95% CIs where prior malignancy was compared between MM patients who developed a subsequent malignancy and those who did not, adjusting for age at MM diagnosis, date of MM diagnosis, and sex. Two-sided p-values of <0.05 were considered statistically significant. All MM patients in the study were censored either at date of death, at the time of first subsequent malignancy diagnosis, or at the end of study period (December 31, 2013), whichever occured first. All malignancy diagnoses from autopsies were excluded. Malignancies in the same patient with excactly the same ICD diagnosis code were both included if they were registered with more than a 5 year interval (new disease). Time from first prior malignancy diagnosis to MM diagnosis (T1) was compared between MM

patients who developed a subsequent primary malignancy and those who did not with the Mann-Whitney U test. Time from MM diagnosis to subsequent malignancy diagnosis (T2) was compared between those who had a prior malignancy diagnosis and those who did not with the Mood's median test.

A subgroup analysis was conducted with the same Cox regression model as described above to assess the risk of developing a specific subsequent malignancy subtype in MM patients with a prior malignancy diagnosis compared to those without.

Sensitivity analyses were performed where carcinogen induced malignancies were excluded, both for UV) light induced malignancies (non melanoma-skin cancer, melanoma skin cancer) and smoking related (respiratory malignancies). Additionally, a sensitivity analysis where patients diagnosed with prior or second malignancy within 30 days from MM diagnosis were excluded was performed. In a separate sensitivity analysis including the whole cohort we used a Fine & Gray model for competing risk, where death was modeled as a competing risk factor for developing a subseqent malignancy.

Survival was estimated from the date of the MM diagnosis until death, emigration, or end of study (December 31, 2013), whichever occurred first. The Kaplan-Meier method was used to estimate survival in MM patients with and without a prior malignancy diagnosis. Cox proportional hazard model was used to calculate HRs and 95% CIs. Two-sided p-values of <0.05 were considered statistically significant. A dose-response relationship, analyzing the effect of increasing number of malignancies on survival was estimated using the same method, adjusting for age at MM diagnosis, date of MM diagnosis, and sex.

3.9 Statistical analyses study III

Demographic characteristics at MM diagnosis, disease-related factors, frequency of radiation therapy received, cumulative radiation doses, HDM-ASCT, cumulative chemotherapy doses and response to treatment were compared between groups using frequency measures and percentages as well as median and mean values. Mean results were accompanied with standard deviation (SD) and median values with interquartile range (IQR). One-way ANOVA, Chi-square and Kruskal-Wallis tests were used to perform statistical analyses. Statistical analyses for treatment comparisons were performed when 10 or more patients in each group received the respective treatment. Additionally, we further investigated cumulative melphalan

exposure between cases and controls, reported as odds ratios (OR) and 95% CIs derived from logistic regression. Two-sided p-values <0.05 were considered statistically significant. Follow-up was estimated from date of MM diagnosis until date of death, emigration or end of study period, whichever occurred first.

All statistical analyses were done with R versions 3.1.1-3.6.1. for the studies in this thesis (R foundation for Statistical Computing, Vienna, Austria).

4 Results

4.1 Study I

4.1.1 Survival in MM patients with second malignancies

A total of 26,627 patients were diagnosed with MM in Sweden from January 1, 1958 to December 31, 2011. Of these, 1,547 (5.8%) patients developed a second malignancy and were matched to 4,019 MM patients without a second malignancy (Table 2). Median age at MM diagnosis was 70 years and 74 years at second malignancy diagnosis. Fifty-nine percent of patients who developed a second malignancy were male. The median time to second malignancy diagnosis was 2.7 years. The number of patients with each type of second malignancy diagnosis is shown in Table 2.

Overall, MM patients with a second malignancy had a statistically significant 2.3-fold (95% CI 2.1-2.5, p <0.001) increased risk of death in comparison to MM patients without a second malignancy (Table 3 and Figure 6). Median survival was 1.1 years (95% CI 1.0-1.2) after second malignancy diagnosis and 3.0 years (2.8-3.1) after corresponding date for MM patients without a second malignancy (p <0.001).

4.1.2 Surival in MM patients according to type of second malignancy

A statistically significant increased risk of death was observed in MM patients with (compared to without) the following cancer types: hematologic, gastrointestinal, male reproductive, female reproductive, kidney and urinary tract, non-melanoma skin cancer, respiratory, oral, nasal and pharyngeal, nervous system, soft tissue and mediastinal, and unspecified tumors shown in Table 3. There was no significant effect on survival in MM patients with subsequent breast cancer, melanoma, endocrine, or bone and cartilage cancer. One- and five-year estimated survival for each second malignancy type are reported in Table 3.

Table 2. Patient characteristics study I

	MM patients with second malignancies	MM patients without second malignancies
Patients, n (%)	1,547 (5.8)	4,019
Sex, male, n (%)	916 (59)	2, 382 (59)
Age, median (years)	70	71
Calendar year of MM diagnosis, n (%)		
1958-2000	1,164 (75.2)	2,968 (73.8)
2000-2011	383 (24.8)	1,051 (26.2)
Second malignancy type, n (%)		
Hematologic	200 (13.0)	NA
Gastrointestinal	364 (23.5)	NA
Male reproductive	220 (14.2)	NA
Female reproductive	60 (3.9)	NA
Breast	95 (6.1)	NA
Kidney and urinary tract	112 (7.2)	NA
Non-melanoma skin cancer	229 (14.8)	NA
Melanoma	62 (4.0)	NA
Respiratory	68 (4.4)	NA
Oral, nasal, and pharyngeal	20 (1.3)	NA
Endocrine	25 (1.6)	NA
Nervous system	35 (2.3)	NA
Bone and cartilage	5 (0.3)	NA
Soft tissue and mediastinal	15 (1.0)	NA
Unspecified tumors	37 (2.4)	NA
Age at second malignancy diagnosis, median, years	74	NA
Calendar year of second malignancy diagnosis, n (%)		
1958-2000	983 (63.5)	NA
2001-2011	564 (36.5)	NA
Time to second malignancy diagnosis, median, years	2.7	NA

Abbreviations: MM: multiple myeloma, n: number of patients, NA: not applicable

Table 3. Risk of death in MM patients with second malignancy compared to patients without second malignancy

	HR	95% CI	p-value	1 year[a] (%)	5 year[b] (%)
Overall MM with a second malignancy	2.3	2.1-2.5	<0.001	52 vs. 81	18 vs. 30
Hematologic	4.9	3.8-6.4	<0.001	27 vs. 82	9 vs. 33
Gastrointestinal	3.4	2.8-4.1	<0.001	39 vs. 82	13 vs. 30
Male reproductive	1.3	1.1-1.6	0.011	70 vs. 79	24 vs. 26
Female reproductive	2.2	1.4-3.4	<0.001	57 vs. 87	29 vs. 39
Breast	1.3	0.9-1.8	0.176	78 vs. 84	31 vs. 37
Kidney and urinary tract	1.9	1.4-2.6	<0.001	55 vs. 77	12 vs. 30
Non-melanoma skin cancer	1.4	1.2-1.8	<0.001	70 vs. 79	20 vs. 28
Melanoma	1.3	0.9-1.9	0.236	81 vs. 83	23 vs. 36
Respiratory	5.2	3.2-8.2	<0.001	25 vs. 81	8 vs. 32
Oral, nasal, and pharyngeal	2.9	1.4-6.3	0.006	55 vs. 85	15 vs. 34
Endocrine	1.1	0.6-2.0	0.792	68 vs. 82	47 vs. 28
Nervous system	5.1	2.7-9.8	<0.001	37 vs. 85	19 vs. 33
Bone and cartilage	0.7	0.2-2.6	0.558	80 vs. 69	40 vs. 8
Soft tissue and mediastinal	5.8	2.1-16.4	<0.001	53 vs. 89	8 vs. 34
Unspecified tumors	14.2	6.0-33.9	<0.001	14 vs. 79	0 vs. 23

Abbreviations: MM: multiple myeloma, HR; hazard ratio, CI: confidence interval.
Risk of death in MM patients with a second malignancy (n=1 547) compared to matched MM patients without a second malignancy (n=4 019). Survival was estimated from second malignancy diagnosis and corresponding date for matched MM patients without a second malignancy diagnosis. Patients with each second malignancy type are compared to matched MM patients without a second malignancy.
[a]One-year survival is reported for second malignancy type vs. matched MM patients without a second malignancy.
[b]Five-year survival is reported for second malignancy type vs. matched MM patients without a second malignancy.

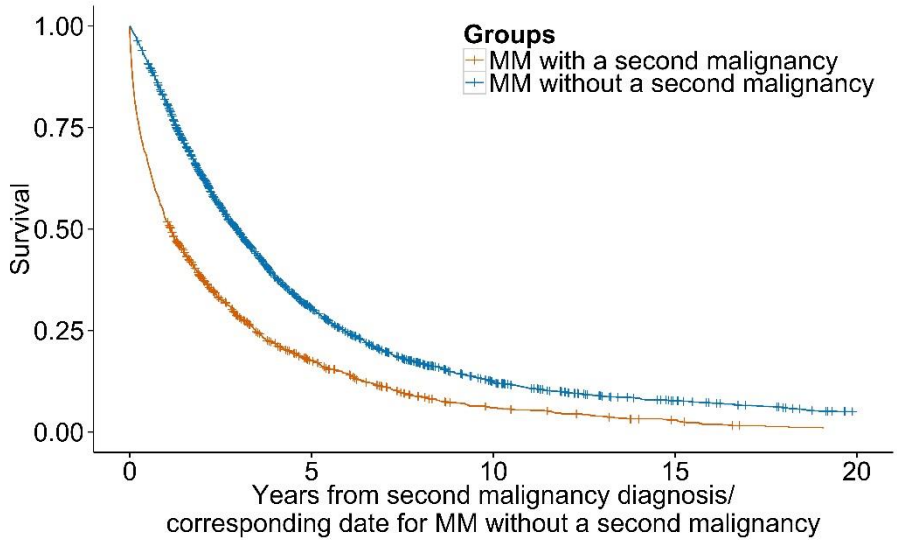

Figure 6. Kaplan-Meier curve of survival in MM patients with and without a second malignancy

4.1.3 Sensitivity analyses

Results from sensitivity analyses performed for different time periods of MM diagnosis and where patients diagnosed at autopsy were included are reported in Table 4.

Table 4. Risk of death in MM patients with a second malignancy compared to patients without a second malignancy over different time periods, and including patients diagnosed at autopsy

	1980-2011[a] HR (95% CI)	1987-2011[b] HR (95% CI)	Including autopsy[c] HR (95% CI)
Overall	2.1 (1.9-2.3)	1.9 (1.7-2.1)	2.6 (2.4-2.8)
Hematological	5.4 (4.0-7.3)	4.8 (3.4-7.0)	5.1(4.0-6.7)
Gastrointestinal	2.8 (2.3-3.4)	2.5 (2.0-3.2)	4.0 (3.3-4.7)
Male reproductive	1.1 (0.9-1.4)	1.1 (0.8-1.5)	1.7 (1.4-2.0)
Female reproductive	2.1 (1.3-3.5)	1.9 (1.0-3.4)	2.2 (1.4-3.4)
Breast	1.3 (0.9-1.9)	1.1 (07-1.2)	1.3 (0.9-1.8)
Kidney and urinary tract	1.5 (1.1-2.1)	1.4 (1.0-2.0)	2.3 (1.8-3.0)
Non-melanoma skin cancer	1.4 (1.2-1.8)	1.4 (1.1-1.7)	1.4 (1.2-1.8)
Melanoma	1.3 (0.8-2.1)	1.2 (0.7-2.0)	1.3 (0.9-1.9)
Respiratory	5.5 (3.2-9.3)	5.9 (3.0-11.9)	7.3 (4.7-11.4)
Oral, nasal, and pharyngeal	2.7 (1.1-6.8)	3.5 (0.8-15.2)	2.9 (1.4-6.3)
Endocrine	0.7 (0.3-1.4)	0.6 (0.2-1.3)	1.6 (0.9-2.7)
Nervous system	4.4 (2.2-8.7)	4.4 (2.0-9.6)	8.6 (4.7-15.7)
Bone and cartilage	NA	NA	0.7 (0.2-2.6)
Soft tissue and mediastinal	4.9 (1.7-14.1)	3.2 (1.0- 10.1)	7.3 (2.9-18.3)
Unspecified tumors	13.4 (5.1-34.9)	11.3 (3.8-33.6)	15.7(6.6-37.2)

Abbreviations: MM: multiple myeloma, HR: hazard ratio, CI: confidence interval, NA: not applicable
[a]1980-2011: Patients diagnosed before 1980 were excluded from the main analysis
[b]1987-2011: Patients diagnosed before 1987 and all patients with plasmacytomas were excluded from the main analysis
[c]Autopsy: The main analysis including all patients diagnosed with second malignancy at autopsy

4.1.4 Risk of death in MM and AML/MDS, *de novo* AML/MDS and secondary AML/MDS

MM patients with AML/MDS had an 8.5-fold (95% CI 5.5-13.2, p <0.001) increased risk of death compared to matched MM patients without a second malignancy (Table 5). The median overall survival was 2.4 months (range 1.7-3.6) in MM patients with AML/MDS and one-year survival was 16%.

Patients with MM and AML/MDS had a statistically significant 1.7-fold (95% CI 1.2-2.1, p <0.001) increased risk of death compared to matched patients with *de novo* AML/MDS. Patients with MM and AML/MDS did not have a statistically significant increased risk of death (HR 1.2; 95% CI 0.9-1.5, p=0.180) compared to matched patients with secondary AML/MDS (Table 5 and Figure 7).

Table 5. Comparison of risk of death in MM patients with AML/MDS to MM patients without AML/MDS, patients with *de novo* AML/MDS and patients with secondary AML/MDS

	HR	95% CI	p-value	1 year[a] (%)
MM with AML/MDS vs. MM without AML/MDS	8.5	5.5-13.2	<0.001	16 vs. 84
MM with AML/MDS vs. *de novo* AML/MDS	1.7	1.3-2.1	<0.001	16 vs. 34
MM with AML/MDS vs. secondary AML/MDS	1.2	0.9-1.5	0.180	16 vs. 28

Abbreviations: MM: Multiple myeloma, AML: Acute myeloid leukemia, MDS: Myelodysplastic syndromes, HR: hazard ratio, CI: confidence interval.
[a]One-year survival is reported for MM patients with AML/MDS vs. matched MM patients without a second malignancy, patients with *de novo* AML/MDS and secondary AML/MDS.

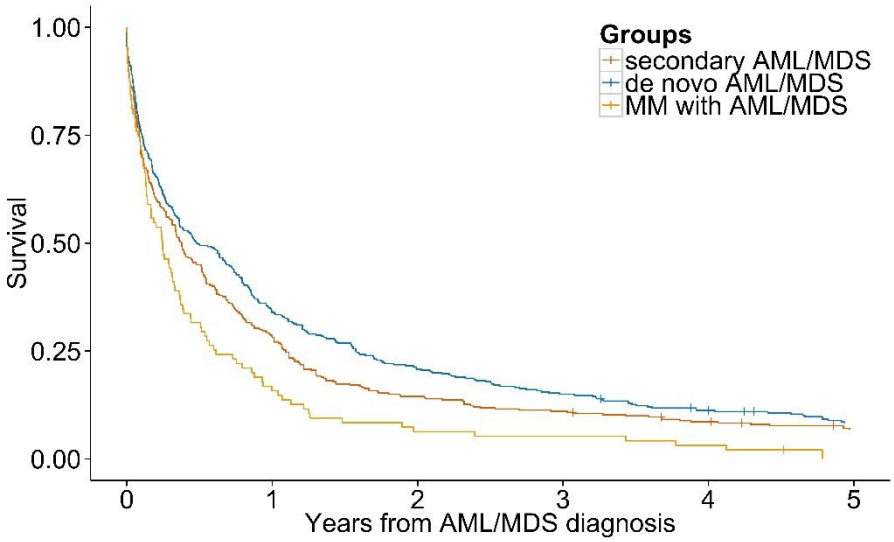

Figure 7. Kaplan-Meier curve of survival in patients with MM and AML/MDS, patients with secondary AML/MDs and patients with *de novo* AML/MDS

4.1.5 Patterns of survival between different time periods

MM patients without a second malignancy diagnosed 1958-2000 (median age, 70 years) had a statistically significant 1.3-fold (95% CI 1.1-1.5, p <0.001) increased risk of death compared to MM patients without a second malignancy diagnosed 2001-2011 (median age, 72 years). When comparing MM patients with a second malignancy, there was a statistically significant 1.5-fold (95% CI 1.3-1.8, p <0.001) increased risk of death in patients diagnosed 1958-2000 (median age, 70 years) compared to patients diagnosed 2001-2011 (median age, 73 years). MM patients with a second malignancy diagnosed 2001-2011 had a statistically significant 1.3-fold (95% CI 1.1-1.5, p=0.005) increased risk of death in comparison to MM patients without a second malignancy diagnosed 1958-2000 (Table 6 and Figure 8).

Table 6. Comparison of risk of death in MM patients with and without a second malignancy according to different time periods.

	HR	95% CI	p-value
MM without second malignancy 1958-2000 vs. MM without second malignancy 2001-2011	1.3	1.1-1.5	<0.001
MM with second malignancy 1958-2000 vs. MM with second malignancy 2001-2011	1.5	1.3-1.8	<0.001
MM with second malignancy 2001-2011 vs. MM without second malignancy 1958-2000	1.3	1.1-1.5	0.005

Abbreviations: MM: Multiple myeloma, HR: hazard ratio, CI: confidence interval.

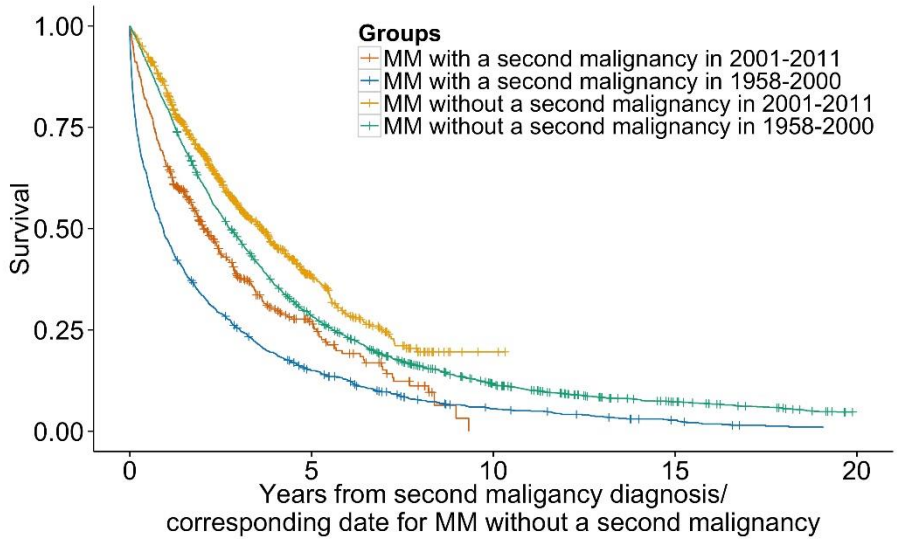

Figure 8. Kaplan-Meier curve of survival in MM patients with and without a second malignancy, before and after the introduction of modern MM therapy.

4.2 Study II

4.2.1 Prior and subsequent malignancies in MM patients

A total of 19,791 patients were diagnosed with MM in Sweden from January 1, 1973 to December 31, 2010. Of these, 2,469 (12.5%) patients had one or more prior malignancy diagnoses at the time of MM diagnosis and 17,322 (87.5%) patients had no prior history of malignancy. A total of 216 (8.8%) MM patients with a prior malignancy developed subsequent malignancies. The

number of MM patients without a prior malignancy who developed a subsequent malignancy was 1,257 (7.3%). Baseline patient characteristics of these groups are compared in Table 7. Types of both prior and subsequent malignancies in MM patients are presented in Figure 9.

4.2.2 Risk of second malignancy in MM patients with a prior malignancy

MM patients with a prior malignancy diagnosis had an increased risk of developing a subsequent malignancy after MM diagnosis compared to MM patients without a prior malignancy (HR 1.42; 95% CI 1.23-1.65, p <0.001). In a subgroup analysis, any prior malignancy in MM patients was associated with an increased risk of developing hematological (HR 1.59; 95% CI 1.04-2.42, p=0.032), malignant melanoma (HR 2.67; 95% CI 1.43-5.00, p=0.002), non-melanoma skin cancer (HR 1.99; 95% CI 1.47-2.71, p <0.001) and malignancies of the respiratory tract (HR 3.24; 95% CI 1.79-5.88, p <0.001) (Table 8). In sensitivity analyses where carcinogen induced malignancies were excluded the HR was 1.25 (95% CI 1.05-1.47 p=0.01) when melanoma and non-melanoma skin cancers were excluded and 1.41 (95% CI 1.22-1.64, p <0.01) when respiratory malignancies were excluded. In a sensitivity analysis where patients with a prior and second malignancy diagnosed within a month of their MM diagnosis were excluded the HR was 1.31 (95% CI 1.06-1.64, p=0.015). In an additional sensitivity analysis, using the Fine & Gray model for competing risks, where death was modeled as a competing risk factor for developing a subseqent malignancy the overall HR was 1.19 (95% CI 1.07-1.39, p=0.01) for developing a subsequent malignancy in patients with prior malignancy compared to patients who did not have a prior malignancy.

4.2.3 Time to malignancy development

The median time from the first prior malignancy diagnosis to MM diagnosis was 7.1 years both in the group that did not develop a subsequent malignancy and in the group that did develop a subsequent malignancy (p=0.732). The median time to first subsequent malignancy diagnosis was 2.3 years (range 0.02-21.5) among patients who had a prior malignancy diagnosis compared to 3.2 years (range 0.003-37.5) in patients who did not have a prior malignancy diagnosis (p <0.003).

Table 7. Patient characteristics study II

	MM with prior malignancies	MM without prior malignancies
Patients, n (%)	2,469 (12.5)	17,322 (87.5)
Age at MM diagnosis in years, median (range)	75 (34-98)	71 (19-99)
Sex, n (%) (Male)	1,220 (49.4)	9,483 (54.5)
Year of MM diagnosis by category, n		
1973 - 1980	197	3,188
1981 - 1990	463	4,657
1991 - 2000	720	4,644
2001 - 2010	1,089	4,833
Year of MM diagnosis, median (range)	1999 (1973-2010)	1992 (1973-2010)
Follow-up in years, median (range)	2.0 (0.003-29.4)	2.6 (0.003-40.9)
Time to subsequent malignancy (range)	2.3 (0.02-21.5)	3.2 (0.003-37.5)
Prior malignancy type, n of patients (%)		
Hematological	163 (6.6)	-
Gastrointestinal	323 (13.1)	-
Male reproductive	427 (17.3)	-
Female reproductive	401 (16.2)	-
Breast	329 (13.3)	-
Kidney and urinary tract	186 (7.5)	-
Melanoma	125 (5.1)	-
Non-melanoma skin cancer	235 (9.5)	-
Respiratory	43 (1.7)	-
Oral, nasal, and pharyngeal	46 (1.8)	-
Endocrine	93 (3.8)	-
Nervous system	61 (2.4)	-
Bone and cartilage	6 (0.2)	-
Soft tissue and mediastinal	17 (0.7)	-
Unspecified tumors	14 (0.6)	-
Number of prior malignancy diagnoses		
1	2,158	-
≥2	311	-
Patients with subsequent malignancies, n (%)	216 (8.8)	1,257 (7.3)

Abbreviations: n: Number of patients, MM: Multiple myeloma.

Table 8. The risk of developing a certain subsequent malignancy subtype in MM patients with a prior malignancy compared to those without

	HR	95% CI	p-value
Overall	1.42	1.23-1.65	<0.001
Hematological (n=193)	1.59	1.04-2.42	0.032
Gastrointestinal (n=318)	1.13	0.81-1.58	0.475
Male reproductive (n= 204)	0.74	0.44-1.26	0.276
Female reproductive (n=58)	0.71	0.28-1.79	0.468
Breast (n=83)	1.16	0.61-2.22	0.643
Kidney and urinary tract (n=105)	1.40	0.80-2.41	0.234
Melanoma (n=58)	2.67	1.43-5.00	0.002
Non-melanoma skin cancer (n=256)	1.99	1.47-2.71	<0.001
Respiratory (n=64)	3.24	1.79-5.88	<0.001
Oral, nasal, and pharyngeal (n=18)	1.30	0.29-5.86	0.731
Endocrine (n=24)	0.78	0.18-3.37	0.736
Nervous system (n=35)	0.86	0.26-2.87	0.808
Bone and cartilage (n=5)	NA	NA	NA
Soft tissue and mediastinal (n=13)	1.91	0.40-9.03	0.415
Unspecified tumors (n=39)	1.28	0.49-3.33	0.618

Abbreviations: HR: Hazard ratio, CI: Confidence interval, n: Number of patients, NA: Not applicable

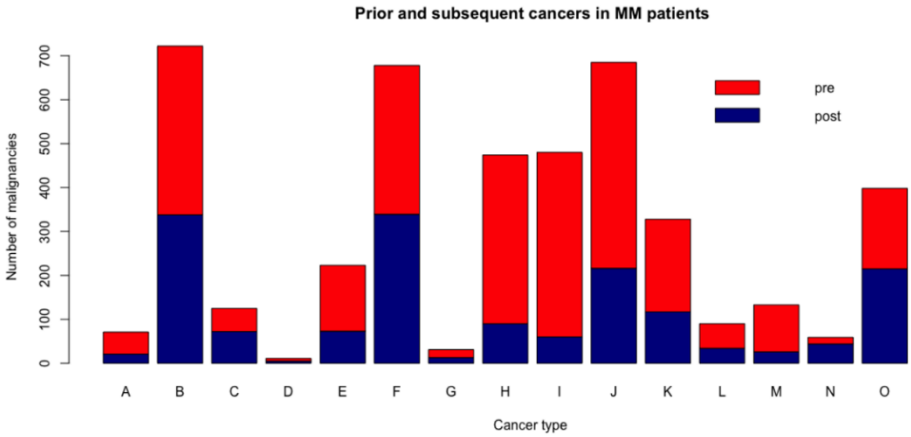

Figure 9. Number of prior and subsequent malignancies in MM patients according to malignancy types

Group letters: A, Oral, nasal, and pharyngeal; B, Gastrointestinal; C, Respiratory; D, Bone and cartilage; E, Melanoma; F, Non-melanoma skin cancer; G, Soft tissue and mediastinal; H, Breast; I, Female reproductive; J, Male reproductive; K, Kidney and urinary tract, L, Nervous system, M, Endocrine, N, Unspecified tumors; and O, Hematological.

4.2.4 Survival in MM patients with and without a prior malignancy

MM patients with a prior malignancy diagnosis had a statistically significant 21% increased risk of death (HR 1.21; 95% CI 1.15-1.26, p <0.001) compared to MM patients without a prior malignancy diagnosis. In a dose-response analysis, MM patients with ≥2 malignancy diagnoses had a 34% increased risk of death (HR 1.34; 95% CI 1.19-1.52, p <0.001) compared to MM patients without a prior malignancy diagnosis (Figure 10).

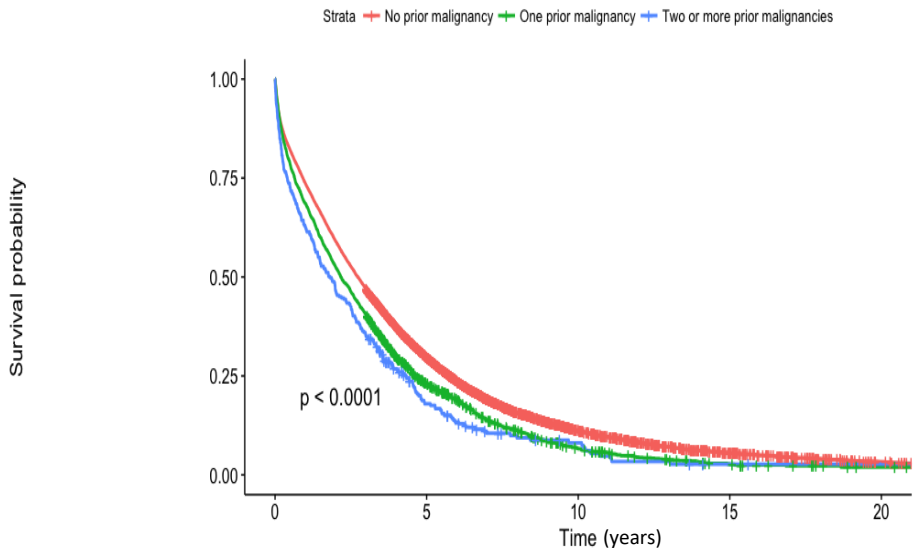

Figure 10. Survival in MM patients with and without prior malignancies. Survival was compared between patients with no prior malignancy, one prior malignancy, and two or more prior malignancies.

4.3 Study III

4.3.1 AML/MDS in patients with MM

A total of 26,627 patients with newly diagnosed MM were reported to the Swedish Cancer Registry during the study period. Of these, 124 (0.5%) developed subsequent AML/MDS (cases). The median age at MM diagnosis was 73 years (IQR 63-78) for cases and 70 years (IQR 60-77) for controls

(Table 9). The median follow-up time for cases was 4.1 years (IQR 3.1-6.3) and 5.8 years (IQR 4.1-8.5) for controls. The median time from MM diagnosis to AML/MDS diagnosis was 3.8 years (IQR 2.8-5.8).

4.3.2 Treatment exposure and risk of AML/MDS

The cumulative melphalan exposure was 3-fold higher (OR 2.8, 95% CI 1.7-5.2; p<0.001) among MM patients who subsequently developed AML/MDS (median 988 mg; IQR 644-1,640) compared to MM patients who did not develop AML/MDS (median 578 mg; IQR 360-967). The median cumulative cyclophosphamide dose was 6,900 mg (IQR 3,650-9,900) for cases and 4,000 mg (IQR 2,250-9,375) for controls (p=0.26). The median cumulative doxorubicin dose was 140 mg (IQR 80-240) for cases and 243 mg (IQR 194-345) for controls (p=0.17). The median cumulative vincristine dose was 18 mg (IQR 8-48) for cases and 6 mg (IQR 5-21) for controls (p=0.10). A total of five patients (6%) in the case group underwent HDM-ASCT and six in the control group (9%). Radiation treatment was administered to 24% of patients in the case group and 23% of patients in the control group, respectively. The median cumulative radiation dose administered in the case group was 33 Gy (IQR 30-51) and 38 Gy (IQR 30-50) in the control group (p=0.28). A PR response or better was observed in 62% of patients in the case group and 53% of patients in the control group (p=0.36; Table 10).

4.3.3 Disease-related and other factors

A total of 52% of cases and 46% of controls reported previous or current smoking history (p=0.68). Serum calcium, serum creatinine, hemoglobin concentration, and bone involvement (CRAB criteria) at MM diagnosis were not statistically different between cases and controls (Table 9). The median percentage of bone marrow plasma cells at diagnosis was 23% in the case group and 22% in the control group (p=0.87). In the case group, 58% of patients were found to have IgG M-protein at diagnosis, 34% had IgA, 2% IgM and 7% had light chain disease. In the control group, 66% of patients had IgG M-protein at diagnosis, 32% had IgA and 2% had IgM. No patients had light chain disease in the control group. The median serum M-protein concentration in the case group was 31 g/L (IQR 18-45) and 32 g/L (IQR 18-45) in the control group. Patients in the case group had a median urinary M-protein concentration of 1,050 mg (IQR 193-3,000) and 587 mg (IQR 150-1,300) in the control group and this difference was not statistically significant (p=0.15). A total of three patients (3%) in the case group and two patients (3%) in the control group had evidence of amyloidosis at diagnosis.

Table 9. Demographic and clinical characteristics of patients with MM who developed AML/MDS compared to patients who did not

Variable	MM-AML/MDS (n=87)	Controls (n=69)	p-values
Age at MM diagnosis, median (IQR)	73 (63-78)	70 (60-77)	0.19
Gender (M), n (%)	49 (56)	38 (55)	1.00
History of smoking, n (%)	32 (52)	18 (46)	0.68
Year of MM diagnosis, n (%)			
1958-1970	3 (3)	1 (1)	
1971-1980	15 (17)	9 (13)	
1981-1990	31 (36)	22 (32)	
1991-2000	24 (28)	23 (33)	
2001-2011	14 (16)	14 (20)	
Time to secondary malignancy, median years (IQR)	3.8 (2.8-5.9)	NA	
Diagnostic factors & Laboratory tests			
Hemoglobin (g/L), mean (SD)	112 (+/-20)	109 (+/-16)	0.37
Calcium (mmol/L), mean (SD)	2.6 (+/-0.5)	2.4 (+/-0.4)	0.10
Creatinine (µmol/L), mean (SD)	140 (+/-131)	125 (+/-75)	0.51
Bone involvement, n (%)	57 (66)	50 (73)	0.45
Albumin (g/L), median (IQR)	34 (30-39)	34 (29-39)	0.47
β2M concentration (mg/L), median (IQR)	4.1 (2.8-5.3)	5.1 (2.3-7.6)	0.64
Plasma cells in BM (%), median (IQR)	23 (13-30)	22 (10-35)	0.87
Serum M protein (g/L), median (IQR)	31 (18-45)	32 (22-49)	0.54
Type of M spike, n (%)			
IgG (κ or λ)	39 (57)	42 (67)	0.16
IgA (κ or λ)	23 (34)	20 (32)	
IgM (κ or λ)	1 (2)	1 (1)	
Light chain disease	5 (7)	0 (0)	
Urine M protein (mg/L), median (IQR)	1050 (193-3000)	587 (150-1300)	0.151
Kappa, n (%)	26 (60)	19 (65)	
Lambda, n (%)	18 (40)	10 (35)	
Amyloidosis, n (%)	3 (4)	2 (3)	1.00

Abbreviations: MM: Multiple myeloma; IQR: Inter Quartile Range, SD: Standard deviation, β2M: β2-microglobulin. BM: Bone marrow, MM-AML/MDS: All patients with MM who developed AML/MDS. Controls: Patients with MM who did not develop AML/MDS and were matched by year of birth (± 3 years), sex, and date of MM diagnosis (± 1 year) to MM patients who developed AML/MDS.

Table 10. Treatment administered and response to therapy in patients with MM who developed AML/MDS compared to patients who did not

Treatment	MM-AML/MDS n=87		Controls n=69		p-value
Chemotherapy, cumulative dose (po and/or IV)	Median (IQR)	n (%)	Median (IQR)	n (%)	
Melphalan, mg	988 (644-1640)	86 (98)	578 (360-967)	66 (96)	<0.001
Cyclophosphamide, mg	6900 (3650-9900)	27 (31)	4000 (2250-9375)	23 (33)	0.26
Carmustine, mg	190 (125-345)	11 (12)	360 (NA)	1 (1)	-
Doxorubicin, mg	140 (80-240)	18 (20)	243 (194-345)	14 (20)	0.17
Vincristine, mg	18 (8-48)	19 (22)	6 (5-21)	20 (29)	0.10
Interferon, million units	691 (246-1005)	11 (13)	137 (97-291)	6 (9)	-
Etoposide, mg	1320 (872-2350)	4 (5)	4140 (NA)	1 (1)	-
Lomustine, mg	540 (410-670)	2 (2)	100 (NA)	1 (1)	-
Thalidomide, mg	59400 (19600-125750)	7 (8)	4500 (2475-8675)	4 (6)	-
Bortezomib, mg	-	-	21 (NA)	1 (1)	-
Lenalidomide, mg	-	-	945 (NA)	1 (1)	-
Other types of therapy					
Radiation therapy received, n (%)	21 (24)		16 (23)		0.70
Cumulative dose of radiation (Gy), median IQR	33 (30-51)		38 (30-50)		0.28
HDM-ASCT, n (%)	5 (6)		6 (9)		-
Response to treatment, PR or better, n (%)					
Yes	54 (62)		37 (54)		0.36
No	8 (9)		10 (14)		
Unknown	25 (29)		22 (32)		

Abbreviations: MM: Multiple myeloma, Po: per os administration; IV: intra venous administration, SD: Standard deviation, PR: Partial response, MM-AML/MDS: All patients with MM who developed AML/MDS. Controls: Patients with MM who did not develop AML/MDS and were matched by year of birth (± 3 years), sex, and date of MM diagnosis (± 1 year) to MM patients who developed AML/MDS.

5 Discussion

5.1 Study I - Survival in patients with MM and second malignancies

5.1.1 Impact of second malignancies on survival

We found that second malignancies have a negative impact on survival in MM patients, with a median survival of only 1.1 years, which corresponds to a 130% higher risk of dying compared to MM patients without second malignancies. Few previous studies, with limited sample sizes or no control group, have assessed survival in MM patients with second malignancies. In a study including 18 patients with MM and a second malignancy, Hasskarl *et al.* reported that patients had a 150% higher risk of dying.[50] In another study, Munker *et al.* reported on survival in four patients with a cancer diagnosis after MM diagnosis, ranging from two months to two years.[56] A study based on data from the NCI SEER database assessed survival in patients with MM and second malignancies and reported that the median OS was 12 and 15 months for males and females, respectively, from the time of second malignancy diagnosis.[54] Our findings therefore confirm and expand on previous reports regarding poor survival in patients with MM who develop second malignancies. The inferior survival in MM patients with second malignancies is most likely multifactorial. It is possible that previous treatment with chemo- and radiation therapy leaves patients in a frail condition,[110] or that inherent factors specific to MM or the second malignancy are responsible. Also, patients already treated for MM who develop a second malignancy might only be able to receive sub-optimal treatment due to toxicity problems. Taken together, the impact of second malignancies on survival is significant and clinically relevant for the individual patient and warrants attention and further research.

We showed that second malignancies in MM patients such as hematological, gastrointestinal, nervous system, soft tissue/mediastinal, and respiratory were particularly aggressive, with a 200-500% higher risk of dying. It has been reported in other cancers, such as Hodgkin's lymphoma and head and neck cancers, that survival varies between second malignancy type, with certain types being more aggressive than others.[111,112] Also, it has been published that MM patients with a gastrointestinal cancer have a significantly inferior survival compared to MM patients with kidney and urinary tract cancer.[54] Our results resonate with these findings and additionally provide more in-depth data to add to the literature. The ability to stratify

survival in MM patients between different second malignancy type is important information for the treating physician and the individual patient, when treatment planning and counceling takes place in the event of second malignancy diagnosis.

5.1.2 Impact of AML/MDS development on survival

MM patients who developed AML/MDS in our study had a dismal outcome, even worse than reported in a study by Pemmaraju *et al.,* where 47 MM patients were diagnosed with therapy-related myeloid neoplasms and had a median OS of 6 months.[113] We found that MM patients with AML/MDS had a 70% higher risk of dying than patients having *de novo* AML/MDS. We also found that MM patients with AML/MDS did not have a higher risk of dying compared to patients with secondary AML/MDS. To our knowledge this comparison has not been investigated before in MM patients. Previous studies have reported that patients with therapy-related/secondary AML/MDS have a worse prognosis than patients with *de novo* AML/MDS,[114,115] with the cytogenetic profile thought to be one of the most important prognostic factors for survival.[116,117] In a case series of 41 patients with AML/MDS after MM, the authors found complex cytogenetics in 59.5% of patients.[118] The patients with MM and AML/MDS in our study might have had a more unfavorable cytogenetic profile than the patients with *de novo* AML/MDS, although further research is needed to build upon these findings.

5.1.3 Patterns of survival following modern MM treatment

Our results showed that survival in both MM patients with and without a second malignancy has been improving during the calendar periods from 1958-2000 to 2001-2011. A possible explanation for this could be that survival in the second malignancies has improved.[119,120] Another possibility is that new treatment options in MM with IMiDs and proteasome inhibitors are not as toxic and thus leave patients in a better condition to receive later treatment. Even though survival in MM patients with a second malignancy has been improving during the calendar periods in our study, we found that patients diagnosed with a second malignancy in recent years (2001-2011) had a 30% higher risk of dying than patients without a second malignancy diagnosed before the introduction of modern MM therapy (1958-2000). This is an important observation, given the expected increase in the number of patients with a second malignancy in the future with improving survival rates in MM.[13,121,122]

5.2 Study II – Prior malignancies

5.2.1 Prior and second malignancies in MM patients

We found that prior malignancies were more common than subsequent malignancies in MM patients and that solid malignancies were more common than hematological, both before and after the MM diagnosis. Many previous studies investigating second malignancies in MM patients have not included evaluation of prior malignancies in their analyses.[54,113] Our results support the findings of a registry analysis on 744 MM patients by Engelhardt *et al*.[47] who reported 11% prior/synchronus and 7% second malignancies, and results from a retrospective analysis including 305 MM patients seen at the Mayo Clinic, where the authors found that 18% of patients had a prior malignancy and 7% developed a second malignancy,[123] although another study by Hasskarl *et al*. has reported lower rates, 7% and 3%, respectively.[50]

Interestingly, 6.6% of prior malignancies and 12.5% of subsequent malignancies were hematological. This rate of subsequent hematological malignancies has been observed in hematological malignancies other than MM, such as Hodgkin's lymphoma.[124] The most frequently diagnosed malignancies prior to MM were female and male reproductive, non-melanoma skin, gastrointestinal, and breast cancers. This is in line with the report by Engelhardt *et al.*[47], although we found breast cancer to be more common compared to their study.

5.2.2 Impact of prior malignancy on second malignancy development

In our study a prior malignancy diagnosis in MM patients was associated with a 40% increased risk of developing a subsequent malignancy and these patients developed their subsequent malignancy almost a year sooner than those without a history of prior malignancy. There is limited data regarding this association and two recent registry studies reported conflicting results.[47,61] The Connect MM registry study by Rifkin *et al.* included 1,430 MM patientsand reported that prior invasive malignancies increased the risk of developing subsequent malignancies.[61,125] In contrast, Engelhardt *et al.* reported in a registry study including 774 MM patients, that prior malignancy did not increase the risk of a subsequent malignancy.[47] These studies have small numbers of patients who developed second malignancies, 49 and 58 patients, respectively, compared to 1,473 patients in our study. The conflicting results could be due to few number of patients and/or differences in statistical methods in data anlalyses, where Rifkin *et al.* used the Cox

proportional hazard regression model, the same model used in our analyses, and Engelhardt *et al.* used competing risk regression model, where death was modeled as a competing risk factor for developing subseqent malignancy. In sensitivity analyses, we performed a competing risk analysis with no change in our overall findings. Our study therefore supports the findings of Rifkin *et al*.

The underlying explanation for our findings, that a prior malignancy increases the risk of second malignancies in MM patients, could include genetic susceptibilities,[92-96,126-128] immunosuppression,[129-131] and therapy-related cancers,[63,70,132] Genetic factors that have been implicated in the development of second malignancies include polymorphisims in genes encoding drug-metabolizing enzymes and DNA repair pathways.[99,133] Interestingly, in a real-word dataset including 1,769 MM patients, one study reported that MM patients with amp(1q) positivity (a poor prognostic marker in MM) were more likely to have a history of prior maligancy compared to patients without amp(1q).[134] The authors also found that patients with two or more prior cancers had even greater odds of having amp(1q). In our study we did not have available molecular data to explore this relationship.

In a subgroup analysis, we found that MM patients with a prior cancer diagnosis had an increased risk of developing hematological malignancy, melanoma, non-melanoma skin cancer, and respiratory malignancy. It has been reported that patients with MM have an increased risk of developing both non-melanoma skin cancer and melanoma,[51,71] suggesting a possible biological relationship from genetic susceptibility. Another potential etiology would be immunosuppression, as it has been shown that melanoma and non-melanoma skin cancers are increased in other immunocompromised patient groups.[129-131] In support of the genetic susceptibility hypothesis, familial studies on MM patients and their relatives have shown a significant excess of melanoma and prostate cancer in these families, suggesting that in some cases the genetic related cause of MM might overlap with other cancers.[97] In addition, studies have found the same oncogenic mutations in both MM and melanoma, further strengthening the relationship.[93,126,128,135] We also found an association between prior malignancy and the development of respiratory malignancies in MM patients. To our knowledge, MM patients have not been found to have an increased risk of developing respiratory malignancies; on the contrary, data from the SEER database suggests a reduced incidence in MM patients.[51] The most common prior malignancy diagnosis in MM patients with subsequent respiratory tract cancer was female reproductive cancer (40%) (more specifically cervical cancer). The reason for this is unclear.

Smoking is a risk factor for both cervical cancer and respiratory tract malignancies[136] although it is not a risk factor for MM.

Taken together, a prior cancer diagnosis increased the risk of second malignancy development in MM patients and might suggest inherent genetic susceptibility in these patients, although further research is needed to confirm this. These patients are often excluded from clinical trials[22,98] and therefore future registry and population-based studies will be important to build upon our findings.

5.2.3 Impact of prior malignancy on survival

We found that prior malignancy negatively impacts survival in MM patients. Additionally, we showed that this relationship was dose-dependent as patients with two or more prior malignancy diagnoses had a two-fold increased risk of death compared to patients with one prior malignancy diagnosis. To our knowledge this is the first population-based study reporting on negative impact of prior malignancies on surivival in MM patients. In other hematological malignancies, such as follicular lymphoma, a similar pattern has been observed and prior malignancy has been found to be an independent predictor of mortality.[137] Interestingly, Toro *et al.* found that patients with chronic lymphocytic leukemia (CLL) had a reduced survival if they had a prior history of non-melanoma skin cancer.[138] The pathologic mechanism behind these findings is unknown but Toro *et al*. suggested that patients with prior history of non-melanoma skin cancer might have more aggressive disease. The reduced survival in MM patients with prior malignancies is likely multifactorial and could include reduced dose intensity of chemo- and radiation therapy, detrimental effects of previous chemo-radiation or surgical treatments on physical condition, or that MM that develops after another malignancy might be biologically different. Taken together, the impact of prior malignancies is clinically relevant for the individual patient and warrants future research and attention.

5.3 Study III - Risk factors for AML/MDS development

5.3.1 Treatment-related factors

5.3.1.1 Alkylating treatment with melphalan

We found that higher cumulative doses of melphalan increased the risk of AML/MDS development in MM patients. It is important to recognize that the majority of patients in this study were treated before the introduction of modern MM drugs and more than 95% received treatment with melphalan,

either long-term, as oral treatment, as part of HDM-ASCT, or as a combination.[139] Our results support previous data attributing alkylating treatment to the development of AML/MDS in MM patients.[48,63,70] In addition to prior literature, we show that higher cumulative doses of melphalan increase the risk of AML/MDS in patients with MM. Another study with limited numbers of patients did not find an association between cumulative doses of melphalan and development of AML/MDS in this population.[64] In Hodgkin's lymphoma survivors, cumulative doses of alkylating chemotherapy have been associated with increased risk of therapy related AML/MDS.[140,141]

Therapy-related AML/MDS usually develops with a latency period of months to years from treatment of the primary disease.[142] It has been shown that therapy-related AML/MDS has molecular and cytogenetic features that are different from *de novo* AML/MDS.[143] The mechanisms for development of AML/MDS after alkylating treatment have been thought to be related to direct DNA damage inducing mutations in hematopoietic progenitor cells.[68] More recently, researchers have found that clonal hematopoiesis could play a role as patients who develop AML/MDS have been found to have evidence of driver mutations and clonal hematopoiesis before they were treated with chemotherapy, suggesting a pre-existing clone that could have expanded during treatment.[144,145] In our study, the median time from MM diagnosis to development of AML/MDS was 3.8 years. This needs to be examined in association with the OS in MM patients in Sweden, which was poor during the majority of the time period we studied, with a median OS of <3 years.[13,146] Indeed, studies in breast cancer have shown that 50% of patients who develop subseqent AML/MDS after combined alkylating and topoisomerase chemotherapy do so after >5 years.[147] With significantly improved overall survival in MM patients observed over the past decade,[25] future population-based studies with MM patients exposed to melphalan chemotherapy in the era of modern MM treatment will be important to better define long-term risks of AML/MDS development.

In Europe, melphalan-based therapy with MVP (prednisone, bortezomib and melphalan) is widely used for MM patients who are ineligible for HDM-ASCT.[148] Additionally, melphalan flufenamide (Melflufen) was recently approved by the US Food and Drug Administration in combination with dexamethasone for patients with relapsed or refractory multiple myeloma.[149] The need for upfront HDM-ASCT in the era of modern MM treatment and MRD testing has been questioned,[150,151] and recent studies have shown an increased risk of AML/MDS following HDM-ASCT specifically.[72,152] Additionally, high-dose melphalan exposure has been shown to cause

significant increase in somatic mutations (on average a ~20% increase).[153-155] Population-based registry studies have both shown an increase[156] and no change in risk of developing AML/MDS after the introduction of ASCT-HDM in MM patients.[51,52] Interestingly, our finding of fewer AML/MDS during the last decade could indicate more widespread use of other newer agents, shorter follow-up time, or a combination of both.

5.3.1.2 Other alklyating agents and topoisomerase inhibitors

Although chemotherapy with vincristine, doxorubicine and dexamethasone is less freqently used today after introduction of modern MM treatment, alkylating treatment with cyclophospamide remains important for treatment of MM in combination with other agents.[157,158] We found that there was no difference in cumulative doses of cyclophosphamide, vincristine (antimicrotubular agent) or the topoisomerase inhibitor doxirubicin between patients who developed AML/MDS compared to those who did not, although these agents were used much less frequently in the patients in our study. In patients with breast cancer it has been shown that higher cumulative doses of cyclophosphamide and topoisomerase inhibitors increase the risk of developing subseqent AML/MDS.[159] In patients with MM specifically, Chuzick *et al.* suggested that cyclophosphamide might be less leukemogenic than melphalan as in their study no increased risk was observed for cyclophosphamide combinations whereas there was a duration-dependent increase in AML/MDS with melphalan.[63] Additionally, in a meta-analysis by Palumbo *et al*., the combination of lenalidomide plus cyclophosphamide was not associated with increased risk of hematological second malignancies compared to melphalan alone. In the same study, lenalidomide plus oral melphalan significantly increased the risk of hematological second malignancies compared to melphalan alone.[74]

5.3.1.3 Radiotherapy

Ionizing radiation is a well known carcinogen.[79] In our study, the frequency and cumulative doses of radiation treatment received was not associated with an increased risk of developing AML/MDS. Registry studies focusing on long-term cancer surviors and risk of second malignancies have shown a dose-response relationship between risk of second malignancy and radiation dose to the surrounding tissues.[81] Additionally, it has been reported that the increased risk can persist for >30 years.[160] To our knowledge, there is limited information on the association between radiotherapy and risk of subsequent AML/MDS in patients with MM. Our results support previous findings from the US Connect MM registry where no relationship was observed between radiation therapy and second primary malignancy incidence in MM patients.[61]

A possible reason could be that low-dose radiation therapy with limited fields is administered to patients with MM, or that the patients in our study did not live long enough to experience the increased risk from the radiotherapy.

5.3.2 Disease-related factors

It has been suggested that patients with plasma cell dyscrasias might have an increased inherent risk of developing AML/MDS. This is supported by increased risk of AML/MDS observed in patients with MGUS.[52,85] Additionally, it has been reported that AML/MDS develops in MM patients either concomittantly or prior to MM patients receiving chemotherapy.[161] We found that risk of AML/MDS was not affected by baseline MM factors, including M protein size, M protein isotype, number of plasma cells in the bone marrow, or CRAB criteria. Interestingly, 7.5% of patients in the AML/MDS group had light chain disease only compared to none of the matched controls. In contrast to our findings, the authors of a population-based study conducted on 5,652 patients with MGUS, where an 8-fold increased risk of developing AML/MDS was observed, reported that the risk varied between M protein isotypes and size of the M protein.[52] Another study found increased cumulative incidence for second malignancies in IgG myeloma.[47] Our results suggest that at diagnosis we do not have identifiable factors to predict which MM patients are at increased risk of developing AML/MDS. Whether light chain disease carries a different risk profile for development AML/MDS is unknown and further studies are needed to build upon our observation.

5.3.3 Host-related factors

We showed that prior malignancy is a risk factor for developing subsequent hematological malignancy in patients with MM, suggesting a possible role for susceptibility genes. As previously discussed, other possible etiologies include immunosuppression or effect from previous therapies. Interestingly, Landgren *et al.* reported in 2012 on a genotype that they found was associated with the development of MDS in MM patients specifically,[94] supporting the susceptibility gene hypothesis. Furthermore, in a cohort of patients with therapy-related myeloid neoplasms, the incidence of germline mutations in the DNA damage response pathway was 20%.[162]

Increasing age, male sex and African American ethnic group are proposed host-related risk factors[47,54,58] for AML/MDS development in MM patients. Because of the nested case-control matched study design where MM patients who developed AML/MDS were matched to MM patients who did not develop AML/MDS by age and sex, these factors could not be

analysed in our study. The Swedish population during our study period was homogenous, with the majority of people being Caucasian and therefore the effect of ethnicity could not be assessed.

It has been reported that clonal hematopoesis at the time of ASCT in patients with lymphoma is associated with inferior survival and increased risk of therapy-related myeloid neoplasms.[163] In a recent study investingating the effect of clonal hematopoesis on clinical outcomes in MM patients, the authors found that clonal hematopoesis was associated with decreased OS and PFS, however no increased risk of therapy-related myeloid neoplasms was found.[164] In our study, due to lack of molecular data, we could not investigate the effect of clonal hematopoesis on AML/MDS development.

5.3.4 Lifestyle- and environmental factors

Several lifestyle- and environmental factors have been suggested to increase the risk of second malignancies in MM patients, including smoking and obesity.[67] In our study we did not find an association between history of smoking and AML/MDS development in MM patients. Tobacco smoking is known to be carcinogenic and has been found to be associated with development of AML/MDS but not MM.[165] It is possible that information bias or lack of power affected our results.

5.4 Strengths and limitations

Our studies have several strengths, including the very large sample size of patients diagnosed with MM and treated over a long time period as well as application of high-quality population-based data from Sweden. By using the nationwide register-based design, where data is gathered prospectively, we were able to account for recall bias and ensure the generalizability of our results. Underreporting of tumors should not affect our results to any extent as the overall completeness of the Swedish Cancer Registry has been reported to be >95%.[106] In a validation study, the diagnostic accuracy was almost 98% for hematopoietic lymphoproliferative malignancies in Sweden.[105]

In study III we were able to include detailed clinical and treatment data of all study patients who were treated at more than 50 hospitals distributed all over Sweden. This resulted in the possibility of manual review of each patient record and verification of MM diagnosis and subsequent AML/MDS via review of pathology reports and exclusion of patients incorrectly diagnosed. The nested case-control design allowed for determing rare outcomes like AML/MDS after MM, as randomized controlled clinical trials, considered the

gold standard in evidence-based medicine, often do not have enough follow-up time to allow for this development or a large enough population. The nested case-control design also allowed for control of potential confounding variables such as age, sex and calendar period.

A limitation of observational cohort studies, as conducted in this thesis, is that causality for association between exposure and outcome cannot be proven and there is an inherent risk of unrecognized biases and confounding factors. Limitations in studies I and II include lack of detailed clinical and treatment data for the patient population, including information on cause of death and pathological stage of the prior cancers, MM, and subsequent cancers. In addition, we did not have information on genetic profiles. Several of the subtype analyses had limited numbers of patients and should be interpreted with caution.

Before 1987, plasmacytomas and MM were registered with the same international classification of disease code in the Swedish Cancer Registry. This could affect our results since solitary plasmacytoma is not as aggressive a disease as MM.[166] However, in a sensitivity analysis for study I where we excluded all patients diagnosed before the year 1987 as well as those who had been diagnosed with plasmcytomas from 1987-2011, the overall findings were the same. Another potential limitation involves change in diagnostic techniques and criteria during the study period, especially in the earlier calendar periods. We tried to address this in a sensitivity analysis where we excluded all patients diagnosed before the year 1980 and the results were the same as our main results. In additional sensitivity analyses, we included patients who had been diagnosed at autopsy with no change in our overall results.

In study II, a possible bias from left-censoring of the data cannot be excluded. To minimize this effect, we allowed for a 15-year lead time for prior malignancies to develop as the Swedish Cancer Registry started in 1958 and patient enrollment started in 1973. In addition, we stratified our data according to decade of MM diagnosis and age category. In light of reduced survival in patients with prior malignancies it is possible that this group could have had less time to develop a subsequent malignancy compared to patients without a prior malignancy, although we did see that patients with prior malignancy developed subsequent malignancy significantly earlier than patients without a prior malignancy. A potential confounding factor in our analysis is carcinogen-induced malignancies including from smoking or sun exposure (UV light), however in sensitivity analyses where we excluded UV-

related malignancies (non-melanoma skin cancer and melanoma) and respiratory malignancies (surrogate for smoking-related malignancies), there was no change in our overall findings. As patients could be diagnosed with a prior and second malignancy one day before and after MM diagnosis, we performed a sensitivity analysis excluding all patients diagnosed with prior or subsequent malignancy within a month of the MM diagnosis with no change in our results.

Limitations in study III include lack of detailed molecular studies at MM diagnosis and at diagnosis of AML/MDS, which can be explained by the fact that the majority of the patients in the study were treated before molecular studies in MM and AML/MDS became available. Underreporting of AML/MDS have been found in studies assessing the completeness of the Swedish Cancer Registry especially during its early years,[106,167,168] although this should not affect the results of our study to any extent due to its nested case-control design. Too few patients in the study underwent HDM-ASCT to make assessments regarding risk of AML/MDS in this population specifically, or whether short or prolonged exposure to melphalan is associated with the same risk.

6 Summary and Conclusion

In summary, we found that:

- The development of a second malignancy had a negative impact on survival in patients with MM. Patients with the combination of MM and subsequent AML/MDS had an especially dismal outcome and higher risk of dying compared to patients with *de novo* AML/MDS. Survival in MM patients with and without a second malignancy improved during the calendar periods from 1958-2000 to 2001-2011. Patients diagnosed with a second malignancy from 2001-2011 had a higher risk of dying compared to patients without a second malignancy diagnosis before the introduction of modern MM treatment (1958-2000).

- Prior malignancy diagnosis was associated with increased risk of developing second malignancy in MM patients and negatively affected survival.

- Higher cumulative doses of melphalan were associated with increased risk of developing AML/MDS in MM patients. In contrast, baseline MM characteristics did not significantly alter the risk of subsequent AML/MDS development.

In conclusion, we confirmed and expanded on prior findings regarding surivival in MM patients with second malignancies. We showed that a second malignancy development is associated with inferior survival and made new observations regarding survival patterns after introduction of modern MM therapy. Additionally, we found that prior malignancy is a risk factor for second malignancy development in these patients and negatively affects survival, suggesting a possible role of inherent genetic susceptibility. Our results support previous data attributing alkylating treatment to the development of AML/MDS in patients with MM. Adding to prior literature, we showed that higher cumulative doses of melphalan increase the risk of AML/MDS, further emphasizing the role of treatment-related factors in this development which is likely a multifactorial process. Importantly, we also

showed that at diagnosis, baseline disease-related factors are not helpful to predict which patients are at an increased risk of developing AML/MDS.

This thesis highlights the importance of clinician and patient awareness of second malignancies in MM and the need for ongoing research to accurately identify risks of different types of second malignancies as well as causative biological mechanisms with the aim of determining preventive strageties and more effective treatments.

7 Future Studies

After decades of minimal improvement and absence of effective anti-MM treatments, the turn of the 21st century heralded a transformation within the MM field, reflected in several new and effective therapies becoming available for MM patients. This remarkable progress has, in turn, resulted in dramatically improved outcomes. Given that the overall survival for patients with MM is approaching 10 years, strategies to prevent secondary complications are becoming more important as one of the most serious event experienced by cancer survivors is the diagnosis of a new cancer.

With improved survival in MM patients, second malignancies are expected to increase in the future and could become part of decision-making regarding disease management as seen in other malignancies with favorable prognosis.

The pathogenesis of second malignancies in MM patients is thought to be a multifactorial process. Future studies where biological samples are analysed for comprehensive genetic and molecular testing at diagnosis and throughout the MM treatment course will likely provide valuable insight into the effects of various genetic- and treatment-related factors in second malignancy development.

Studies to date, including those presented in this thesis, have assessed melphalan exposure and risk of AML/MDS development conducted before the era of modern MM treatment when patients had significantly worse survival. It is possible that many patients in these studies might not have lived long enough to develop this complication. It is therefore reasonable to hypothesize that long-term risks could be currently under-estimated and future population-based studies with MM patients exposed to melphalan chemotherapy in the era of modern MM treatment and with longer follow-up will be critical to better define the long-term risks.

With multiple new treatment options in sight for MM management, including CAR-T cell therapies, bispecific antibodies, and pathway active small molecules, it is important that second malignancy risk continues to be evaluated in both population-based and registry studies which include individual treament data and long-term follow-up, and that clinical trials are designed to provide well-defined endpoints for secondary malignancies.

The ability to stratify patients with increased risk of second malignancies might affect treatment decisions as well as surveillance strategies in the future, including possible screening guidelines. In this regard indentifying patients with possible genetic susceptibility will be vital.

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Paper I

Survival in multiple myeloma patients who develop second malignancies: a population-based cohort study

Survival in multiple myeloma (MM) has improved significantly during recent decades both in younger and older patients.[1,2] The improved survival is considered to be primarily due to new treatment options in MM, including high-dose melphalan with autologous stem cell transplantation,[3] the immunomodulatory drugs and proteasome inhibitors.[4,5] Recently, second malignancies have gained great clinical and scientific attention in MM as three randomized clinical trials reported an increase in second malignancies associated with lenalidomide maintenance treatment.[6] In a newly published meta-analysis, exposure to lenalidomide plus oral melphalan was found to significantly increase hematologic second malignancies.[7] Previously we showed that MM patients had a 26% increased risk of developing any second malignancy when compared to the general population, and an 11-fold increased risk of developing acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS).[8] In the United States, second or higher-order malignancies are the third most common cancer diagnoses.[9] With improved survival in MM patients, second malignancies are expected to increase in the near future and possibly contribute to problems of disease management. Importantly, it has been shown that the cumulative risk of death from MM outweighs the risk of death due to second malignancies.[6] For the individual patient who develops a second malignancy, however, the outcome is of great importance. We conducted a large population-based cohort study, including all patients diagnosed with MM in Sweden, over a period of more than 50 years. This study aimed to investigate the effects of second malignancies on survival and assess changes following the introduction of modern myeloma therapy. Furthermore, as AML/MDS is over-represented in MM patients, we assessed patterns of survival specifically in these patients.

All patients diagnosed with MM from January 1 1958 to December 31 2011 were identified from the Swedish Cancer Register. Information was collected on sex, date of birth, and date of MM diagnosis. All subsequent second malignancy diagnoses were identified through cross-linkage within the Swedish Cancer Registry, and the type and date of the second malignancy documented. For each MM patient with a second malignancy, 1-3 patients without a second malignancy from the MM cohort were randomly selected and matched by age (+/- 3 years), sex, and date of MM diagnosis (+/- 1 year). The matching criteria also required that all the patients without a second malignancy had to be alive when the corresponding matched MM patients developed a second malignancy. Patients with non-identifiable match (5%) and those diagnosed with MM or a second malignancy at autopsy were excluded. Survival was estimated from the date of the second malignancy diagnosis and the same date for matched MM patients without second malignancy until death, emigration, or end of study (December 31 2012), whichever occurred first.

To analyze AML/MDS more thoroughly, we identified all patients with AML/MDS from the group of MM patients with a hematologic second malignancy. Each patient with MM and AML/MDS was matched by age (+/- 3 years), sex and year of AML/MDS diagnosis with 4 patients having *de novo* AML/MDS, and 4 patients diag-

Table 1. Risk of death in multiple myeloma patients with a second malignancy compared to patients without a second malignancy.

	HR	95%CI	*P*	1 year* (%)	5 year** (%)
Overall MM with a second malignancy (1547)	2.3	2.1-2.5	<0.001	52 *vs.* 81	18 *vs.* 30
Hematologic (n=200)	4.9	3.8-6.4	<0.001	27 *vs.* 82	9 *vs.* 33
Gastrointestinal (n=364)	3.4	2.8-4.1	<0.001	39 *vs.* 82	13 *vs.* 30
Male reproductive (n=220)	1.3	1.1-1.6	0.011	70 *vs.* 79	24 *vs.* 26
Female reproductive (n=60)	2.2	1.4-3.4	<0.001	57 *vs.* 87	29 *vs.* 39
Breast (n=95)	1.3	0.9-1.8	0.176	78 *vs.* 84	31 *vs.* 37
Kidney and urinary tract (n=112)	1.9	1.4-2.6	<0.001	55 *vs.* 77	12 *vs.* 30
Non-melanoma skin cancer (n=229)	1.4	1.2-1.8	<0.001	70 *vs.* 79	20 *vs.* 28
Melanoma (n=62)	1.3	0.9-1.9	0.236	81 *vs.* 83	23 *vs.* 36
Respiratory (n=68)	5.2	3.2-8.2	<0.001	25 *vs.* 81	8 *vs.* 32
Oral, nasal and pharyngeal (n=20)	2.9	1.4-6.3	0.006	55 *vs.* 85	15 *vs.* 34
Endocrine (n=25)	1.1	0.6-2.0	0.792	68 *vs.* 82	47 *vs.* 28
Nervous system (n=35)	5.1	2.7-9.8	<0.001	37 *vs.* 85	19 *vs.* 33
Bone and cartilage (n=5)	0.7	0.2-2.6	0.558	80 *vs.* 69	40 *vs.* 8
Soft tissue and mediastinal (n=15)	5.8	2.1-16.4	<0.001	53 *vs.* 89	8 *vs.* 34
Unspecified tumors (n=37)	14.2	6.0-33.9	<0.001	14 *vs.* 79	0 *vs.* 23

*MM: multiple myeloma; HR: hazard ratio; CI: confidence interval; n: number of MM patients diagnosed with each second malignancy type. Risk of death in multiple myeloma patients with a second malignancy (n=1547) compared to matched multiple myeloma patients without a second malignancy (n=4019). Survival was estimated from second malignancy diagnosis and the same date for matched MM patients without a second malignancy diagnosis. Patients with each second malignancy type are compared to matched MM patients without a second malignancy. Cox proportional hazard model for matched data was used to calculate hazard ratios (HRs) and 95% confidence intervals (CI). Kaplan-Meier method was used to estimate 1- and 5- year survival. Two-sided P<0.05 was considered statistically significant. *One-year survival is reported for second malignancy type* versus *matched MM patients without a second malignancy. **Five-year survival is reported for second malignancy type* versus *matched MM patients without a second malignancy.*

nosed with AML/MDS as a second malignancy (referred to as "secondary AML/MDS"), excluding patients with non-melanoma skin cancer and MM as the primary cancer diagnosis. Analyses were performed for each second malignancy type. In addition, survival in MM patients with AML/MDS (n=95) was compared to matched MM patients without a second malignancy. A separate analysis was performed for patients with MM and AML/MDS compared to matched patients with *de novo* AML/MDS (n=380) and to matched patients with secondary AML/MDS (n=380). To assess survival patterns before and after the introduction of modern myeloma therapy in Sweden, survival analyses were conducted for two different time periods, 1958-2000 and 2001-2011, including MM patients with and without a second malignancy in both calendar periods.

A total of 26,627 patients were diagnosed with MM in Sweden during the study period. Of these, 1547 (5.8%) patients developed a second malignancy and were matched to 4019 MM patients without a second malignancy. Median age at MM diagnosis was 70 years (74 years at second malignancy diagnosis). Median time to second malignancy diagnosis was 2.7 years.

Overall, MM patients with a second malignancy had a statistically significant 2.3-fold (95%CI: 2.1-2.5; P<0.001) increased risk of death in comparison to MM patients without a second malignancy (Table 1 and Figure 1A). Median survival was 1.1 years (95%CI: 1.0-1.2) after second malignancy diagnosis and 3.0 years (2.8-3.1) after corresponding date for MM patients without a second malignancy (P<0.001).

Multiple myeloma patients with AML/MDS had a 8.5-fold (5.5-13.2; P<0.001) increased risk of death compared to matched MM patients without a second malignancy. The median overall survival was 2.4 months (1.7-3.6) in MM patients with AML/MDS and one-year survival was 16%.

Patients with MM and AML/MDS had a statistically significant 1.7-fold (1.2-2.1; P<0.001) increased risk of death compared to matched patients with *de novo* AML/MDS. Patients with MM and AML/MDS did not have a statistically significant increased risk of death (1.2; 0.9-1.5; P=0.180) compared to matched patients with secondary AML/MDS (Figure 1B).

Risk of death for MM patients with and without second malignancy according to different time periods is presented in Table 2 and Figure 1C, and show that MM patients with second malignancies in 2001-2011 had a statistically significant 1.3-fold (1.1-1.5; P=0.005) increased risk of death compared to MM patients without second malignancies in the period 1958-2000.

Overall, we found that second malignancies negatively impact survival in MM patients. The inferior survival in MM patients with second malignancies is most likely multifactorial. One could argue that previous treatment with chemo- and radiotherapy leaves patients in a frail condition which could be the main culprit,[10] or that inherent factors specific to MM or the second malignancy are responsible. Furthermore, patients already treated for MM who develop a second malignancy might only be able to receive sub-optimal treatment due to toxicity problems. Taken together, the impact of second malignancies on survival is significant and clinically relevant for the individual patient, and warrants attention and further research.

Our findings, that survival in both MM patients with and without a second malignancy has been improving from 1958-2000 to 2001-2011, are extremely interesting. Possible explanations are that, overall, survival of patients with second malignancies has improved,[11] or it could be that new treatment options in MM are less toxic, thus leaving patients in a better condition to receive later treatment. Although survival in MM patients with a second malignancy has been improving, MM patients diagnosed with a second malignancy in 2001-2011 had a 30% higher risk of dying compared to

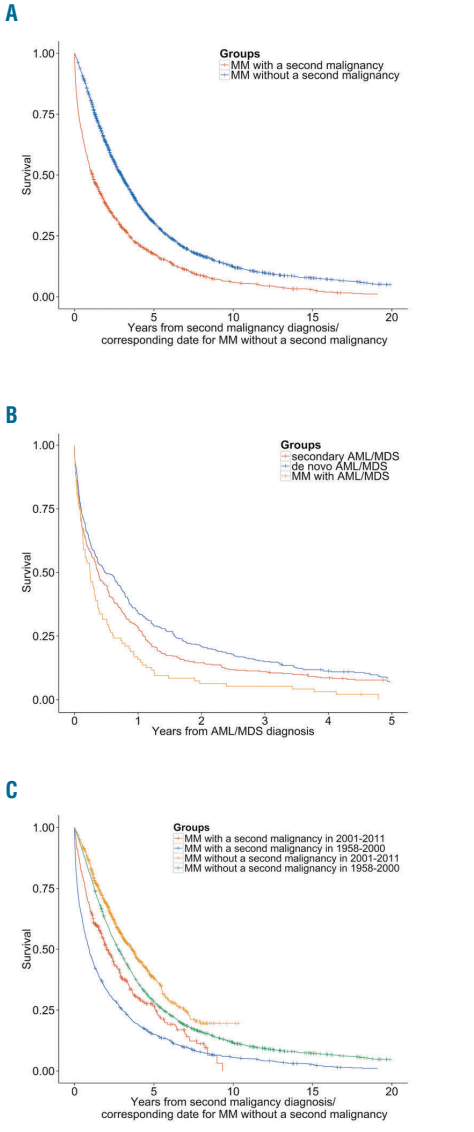

Figure 1. (A) **Kaplan-Meier curve of survival in multiple myeloma (MM) patients with and without a second malignancy.** (B) Kaplan-Meier curve of survival in patients with MM and acute myeloid leukemia (AML)/myelodysplastic syndrome (MDS), patients with secondary AML/MDS and patients with *de novo* AML/MDS. (C) Kaplan-Meier curve of survival in MM patients with and without a second malignancy, before and after the introduction of modern myeloma therapy.

Table 2. Comparison of risk of death in multiple myeloma patients with and without a second malignancy according to different time periods.

	HR	95%CI	P
MM without second malignancy 1958-2000 (n=2968) *versus* MM without second malignancy 2001-2011 (n=1051).	1.3	1.1-1.5	<0.001
MM with second malignancy 1958-2000 (n=1164) *versus* MM with second malignancy 2001-2011 (n=383).	1.5	1.3-1.8	<0.001
MM with second malignancy 2001-2011 (n=383) *versus* MM without second malignancy 1958-2000 (n=2968).	1.3	1.1-1.5	0.005

MM: multiple myeloma; HR: hazard ratio; CI: confidence interval; n: number of patients are compared in each analysis. Cox proportional hazard model was used to calculate HR and 95%CI, adjusting for age, sex and year of MM diagnosis.

patients without a second malignancy diagnosed before the introduction of modern myeloma therapy (1958-2000). This is an important observation given the expected increase in the number of patients with a second malignancy due to improving survival rates.[1,2]

Multiple myeloma patients who developed AML/MDS had a median survival of only 2.4 months and a 16% one-year survival. These are worse outcomes than reported in a recent case series where a median overall survival of six months was observed.[12] In an analysis comparing survival between MM patients with AML/MDS and patients with *de novo* AML/MDS, we found that they had a 70% higher risk of dying. However, a comparison of MM patients with AML/MDS to patients with secondary AML/MDS showed no difference in mortality. To our knowledge, this is the first time that such a comparison has been made in MM patients. Previous studies have reported that patients with therapy-related/secondary AML/MDS in general have a worse prognosis than patients with *de novo* AML/MDS,[13] with the cytogenetic profile thought to be one of the most important prognostic factors for survival.[14] Engelhardt *et al.* recently reported that 8 patients with MM and AML/MDS had complex chromosomal aberrations at AML/MDS diagnosis.[15] Further research is needed to build upon these findings.

Our study has several strengths, including a large sample size, long study period, and application of high quality population-based data from Sweden. By using the nationwide register-based design, where data are gathered prospectively, we were able to account for recall bias and ensure the generalizability of our results.

Limitations include the lack of detailed clinical and treatment data, as well as information on the molecular subtype of MM and the second malignancy. In the analyses where MM patients with and without second malignancies are compared between calendar periods, the selection of MM patients without second malignancies was not matched; however, in these analyses we adjusted for age, sex, and date of the MM diagnosis.

Taken together, in this large population-based cohort study including almost 27,000 MM patients diagnosed during five decades, we confirmed and expanded on prior findings regarding survival in patients with MM and second malignancies. We showed that a second malignancy is associated with a poor outcome and made important new observations regarding survival patterns after the introduction of modern myeloma therapy. Furthermore, we showed that the diagnosis of AML/MDS in MM patients is dismal, yielding a worse outcome than matched patients with *de novo* AML/MDS. These results emphasize the importance of identifying risk factors for second malignancies in MM patients.

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Paper II

REGULAR ARTICLE

blood advances

The impact of prior malignancies on second malignancies and survival in MM patients: a population-based study

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Key Points

- Prior malignancy negatively impacts survival in patients with MM and >1 prior malignancy reduces survival even further.
- A prior malignancy diagnosis increases the risk of developing a second malignancy in patients with MM.

In the present study, we aimed to evaluate 2 hypotheses. First, we hypothesize that prior malignancy is a proxy for genetic susceptibility that could be a risk factor for subsequent malignancy development in multiple myeloma (MM) patients. Second, we hypothesize that survival after MM is influenced by a prior malignancy. All patients diagnosed with MM from 1 January 1973 to 31 December 2010 were identified from the Swedish Cancer Register. Cox regression model was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) where prior malignancy was compared in MM patients who developed a subsequent malignancy and MM patients who did not. In another Cox regression model, survival was compared in MM patients with and without a prior malignancy diagnosis. A total of 19 791 patients were diagnosed with MM. Patients with a prior malignancy diagnosis had a significantly increased risk of developing a subsequent malignancy compared with MM patients without (HR 1.42, 95% CI 1.23-1.65, $P < .001$). MM patients with a prior malignancy diagnosis had a significant 1.21-fold increased risk of death (95% CI 1.115-1.26, $P < .001$) compared with MM patients without. MM patients with 2 or more prior malignancy diagnoses had a 1.34-fold increased risk of death (95% CI 1.19-1.52, $P < .001$). In this large population-based study, we report that prior malignancy increases the risk of subsequent malignancy development in MM patients. Furthermore, we found that prior malignancy negatively impacts survival and that >1 prior malignancy reduces survival even further.

Introduction

With improved survival in patients with multiple myeloma (MM), awareness of second malignancies has increased during recent years.[1-5] We have previously shown in a large population-based study that the risk of developing any second malignancy is 26% higher in MM patients compared with the general population; most importantly, they had an 11-fold increased risk of developing acute myeloid leukemia and myelodysplastic syndromes (MDS) and a twofold increased risk of developing nonmelanoma skin cancer.[6] Other studies have found MM patients to have an increased risk of developing certain types of second cancers, such as melanoma, nervous system tumors, and kidney and urinary tract tumors, although mechanisms and risk factors are not well understood.[7-10] Suggested risk factors for second malignancies include treatment-, disease-, environmental-, behavioral-, and host-related factors.[4,11-13] Host-related factors include both genetic and nongenetic; reported nongenetic factors include age, male sex, and obesity.[1,8,14] Genetic factors implicated in the development of second malignancies include polymorphisms in genes

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encoding drug-metabolizing enzymes and DNA repair pathways.[15,16] In addition, inherited genetic susceptibility for developing MM has been supported by genome-wide association studies that have identified single-nucleotide polymorphisms localized to several genomic regions that are robustly associated with MM risk.[17] Furthermore, familial studies on MM patients and their first-, second-, and third-degree relatives have shown a significant excess of cases of prostate cancer and melanoma in all types of relatives.[18] This might suggest that the genetic cause of MM overlaps with the causes of other cancers.[18-23] Information regarding prior malignancies and their impact on MM patients is limited. For instance, patients with prior malignancies are often excluded from clinical trials,[24,25] thus making it difficult to generalize the current literature to this group. In addition, previously published results on the effect of prior malignancies have been conflicting.[1,26] In the present study, we aimed to evaluate 2 hypotheses. First, we hypothesize that prior malignancy is a proxy for inherent genetic susceptibility that could be a risk factor for subsequent malignancy development in MM patients. Second, we hypothesize that survival after MM is influenced by a prior malignancy.

Methods

Central registry

All residents in Sweden have equal access to health care under a largely decentralized, taxpayer-funded system. All malignancy diagnoses are reported to the centralized nationwide Swedish Cancer Registry, which was established in 1958.[27] The diagnostic accuracy and overall completeness of the Swedish Cancer Registry is high (>95%).[28,29] Pathologists and physicians in Sweden are obliged by law to report each malignancy diagnosis to this register. Within the register, information on sex, date of birth, date of malignancy diagnosis, malignancy type, and date of death is registered.

Patient cohort

All patients diagnosed with MM from 1 January 1973 to 31 December 2010 were identified from the Swedish Cancer Registry. Information was gathered on sex, date of birth, date of MM diagnosis, and date of death. All cancer diagnoses prior to and after MM diagnosis were identified through cross-linkage within the Swedish Cancer Registry, and the type and date of the cancer were documented. Prior and subsequent malignancies were classified according to the International Classification of Disease-7 into the following subgroups: (1) breast cancer; (2) bone and cartilage; (3) ear, nose, and throat; (4) endocrine; (5) female reproductive; (6) gastrointestinal; (7) hematological; (8) kidney and urinary tract; (9) male reproductive; (10) melanoma; (11) nervous system; (12) respiratory tract; (13) soft tissue and mediastinal; and (14) unspecified tumors.

Approval was obtained from the Regional Ethical Review Board in Stockholm for this study. Informed consent was waived because we had no contact with study patients.

Data analysis

Risk factor analysis, assessing the effect of prior malignancies on the development of second malignancies in MM. The exposure was the binary categorical variable of a first malignancy diagnosis before MM diagnosis, and the outcome was the binary categorical variable of a primary malignancy diagnosis after MM diagnosis. Demographic characteristics were compared between groups using frequency measures and percentages as well as median values. A Cox regression model was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) where prior malignancy was compared between MM patients who developed a subsequent malignancy and those who did not, adjusted for age at MM diagnosis, date of MM diagnosis, and sex. Two-sided $P < .05$ was considered statistically significant. All MM patients in the study were censored either at date of death, at the time of first subsequent malignancy diagnosis, or at the end of study (31 December 2013), whichever occurred first. All malignancy diagnoses from autopsies were excluded. Malignancies in the same patient with exactly the same International Classification of Disease diagnosis code were both included if they were registered with more than a 5-year interval (recurrent disease). Time from first prior malignancy diagnosis to MM diagnosis (T1) was compared between MM patients who developed a subsequent primary malignancy and those who did not with Mann-Whitney-Wilcox test. Time from MM diagnosis to subsequent malignancy diagnosis (T2) was compared between those who had a prior malignancy diagnosis and those who did not with the Mood's median test.

A subgroup analysis was conducted with the same Cox regression model as described above to assess the risk of developing a specific subsequent malignancy subtype in MM patients with a prior malignancy diagnosis compared with those without.

Survival analysis. Survival was estimated from the date of the MM diagnosis until death, emigration, or end of study (31 December 2013), whichever occurred first. The Kaplan-Meier method was used to estimate survival in MM patients with and without a prior malignancy diagnosis. A Cox proportional hazard model was used to calculate HRs and 95% CIs. Two-sided $P < .05$ was considered statistically significant. A dose-dependent relationship, analyzing the effect of increasing number of malignancies on survival, was estimated using the same method, adjusting for age at MM diagnosis, date of MM diagnosis, and sex.

All statistical analyses were done with R version 3.1.1. (R foundation for Statistical Computing, Vienna, Austria).

Results

A total of 19 791 patients were diagnosed with MM in Sweden from 1 January 1973 to 31 December 2010. Of these, 2469 (12.5%) patients had 1 or more prior malignancy diagnoses at the time of MM diagnosis, and 17 322 (87.5%) patients had no prior history of malignancy. A total of 216 (8.8%) MM patients with a prior malignancy developed subsequent malignancies. The number of MM patients without a prior malignancy that developed a subsequent malignancy was 1257 (7.3%). Baseline patient characteristics of these groups are compared in Table 1. Types of both prior and subsequent malignancies are seen in Figure 1.

MM patients with a prior malignancy diagnosis had an increased risk of developing a subsequent malignancy after MM diagnosis compared with MM patients without a prior malignancy (HR 1.42; 95% CI 1.23-1.65, $P < .001$) (Table 2). In a subgroup analysis, any prior malignancy in MM patients was associated with an increased risk of developing hematological (1.59; 95% CI 1.04-2.42, $P = .032$), malignant melanoma (HR 2.67; 95% CI 1.43-5.00, $P = .002$), nonmelanoma skin cancer (HR 1.99; 1.47-2.71, $P < .001$), and malignancies of the respiratory tract (HR 3.24; 1.79-5.88, $P < .001$) (Table 2).

The median time from the first prior malignancy diagnosis to MM diagnosis was 7.1 years both in the group that did not develop a

Table 1. Patient characteristics

	MM with prior malignancies	MM without prior malignancies
Patients, n (%)	2469 (12.5)	17 322 (87.5)
Age at MM diagnosis, median (range), y	75 (34-98)	71 (19-99)
Male sex, n (%)	1220 (49.4)	9 483 (54.5)
Year of MM diagnosis by category, n		
1973-1980	197	3 188
1981-1990	463	4 657
1991-2000	720	4 644
2001-2010	1089	4 833
Year of MM diagnosis, median (range)	1999 (1973-2010)	1992 (1973-2010)
Follow-up, median (range), y	2.0 (0.003-29.4)	2.6 (0.003-40.9)
Time to subsequent malignancy, median (range), y	2.3 (0.02-21.5)	3.2 (0.003-37.5)
Prior malignancy type, no. of patients (%)		
Hematologic	163 (6.6)	—
Gastrointestinal	323 (13.1)	—
Male reproductive	427 (17.3)	—
Female reproductive	401 (16.2)	—
Breast	329 (13.3)	—
Kidney and urinary tract	186 (7.5)	—
Melanoma	125 (5.1)	—
Nonmelanoma skin cancer	235 (9.5)	—
Respiratory	43 (1.7)	—
Ear, nose, and throat	46 (1.8)	—
Endocrine	93 (3.8)	—
Nervous system	61 (2.4)	—
Bone and cartilage	6 (0.2)	—
Soft tissue and mediastinal	17 (0.7)	—
Unspecified tumors	14 (0.6)	—
Number of prior malignancy diagnoses		
1	2158	—
≥2	311	—
Patients with subsequent malignancies, n (%)	216 (8.8)	1 257 (7.3)

—, Not applicable; n, number of patients.

subsequent malignancy and in the group that did develop a subsequent malignancy ($P = .732$). The median time to first subsequent malignancy diagnosis was 2.3 years (range 0.02-21.5) among patients who had a prior malignancy diagnosis compared with 3.2 years (range 0.003-37.5) in patients who did not have a prior malignancy diagnosis ($P = .003$).

MM patients with a prior malignancy diagnosis had a statistically significant 21% increased risk of death (HR = 1.21, 95% CI 1.15-1.26, $P < .001$) compared with MM patients without a prior malignancy diagnosis (Figure 2). In a dose-response analysis, MM patients with ≥2 malignancy diagnoses had a 34% increased risk of death (HR = 1.34, 95% CI 1.19-1.52, $P < .001$) compared with MM patients without a prior malignancy diagnosis (Figure 2).

Discussion

In our large population-based study, we found that prior malignancy diagnosis increased the risk of developing hematologic malignancies, melanoma, nonmelanoma skin cancer, and respiratory tract malignancies in patients with MM. In addition, we show that prior malignancy negatively impacts survival in patients with MM and that >1 prior malignancy reduces survival even further. We confirmed prior reports of solid tumors being more common than hematologic malignancies in MM patients, both prior and subsequent to the MM diagnosis.[2,26,30]

We found that 12.5% of patients with MM had prior malignancy and 8.1% developed a second malignancy. These results are similar to the findings of a recent registry analysis on 744 MM patients by Engelhardt et al.[1] Although another study by Hasskarl et al reported lower rates, 7% and 3%, respectively,[2] we also found that 6.6% of prior malignancies and 12.5% of subsequent malignancies were hematological. This rate of subsequent hematological malignancies has been observed in hematological malignancies other than MM, such as Hodgkin lymphoma.[31] The most frequently diagnosed malignancies prior to MM were gastrointestinal and female- and male-reproductive cancers. This is in line with Engelhardt et al's study,[1] although we found breast cancers to be more common compared with their study.

A prior malignancy diagnosis in MM patients was associated with a 40% increased risk of developing a subsequent malignancy, and these patients developed their subsequent malignancy almost a year sooner than those without a history of prior malignancy. There are limited published data regarding this association, and 2 recent registry studies reported conflicting results.[1,26] The Connect MM registry study by Rifkin et al comprised 1430 MM patients treated with lenalidomide and reported that prior invasive malignancies increased the risk of developing subsequent malignancies.[26] In contrast to this, Engelhardt et al reported in a registry study including 774 MM patients that prior malignancy did not increase the risk of a subsequent malignancy.[1] These studies have a small number of patients that developed second malignancies, 49 and 59 patients, respectively. Underlying explanation for our findings, that a prior malignancy increases the risk of second malignancies in MM patients, could include genetic susceptibilities,[17-22,32-35] immunosuppression,[36-38] and therapy-related cancers.[12,13,39]

We found MM patients with a prior cancer diagnosis to have an increased risk of developing hematological malignancy, melanoma, nonmelanoma skin cancer, and respiratory malignancy compared with MM patients who did not. We have previously shown that both patients with the precursor condition monoclonal gammopathy of undetermined significance and MM have an increased risk of developing nonmelanoma skin cancer, suggesting a possible biological relationship.[6] Increased incidence of melanoma in MM patients has also been reported,[8,40] and it has been shown that both melanoma and nonmelanoma skin cancers are increased in other immunocompromised patient groups.[36-38] Interestingly, familial studies on MM patients and their relatives have shown a significant excess of cancers such as melanoma in these families, suggesting that in some cases the genetic cause of MM might overlap with that of melanoma. In addition, studies have found the same oncogenic mutations in both MM and melanoma.[19,32,33,41] We found an association between prior malignancy and the

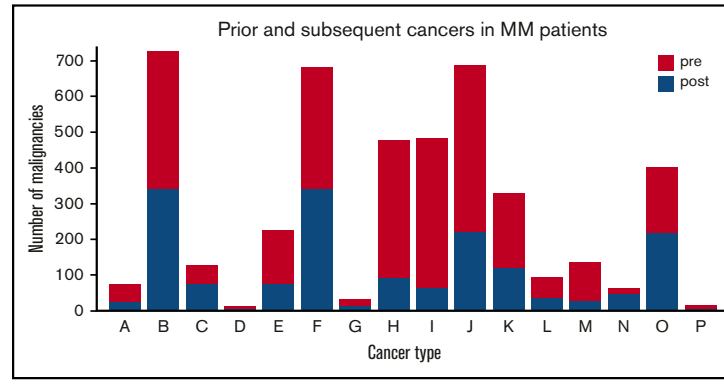

Figure 1. Number of prior and subsequent malignancies in MM patients according to malignancy types. Group letters: A, ear, nose, and throat; B, gastrointestinal; C, respiratory; D, bone and cartilage; E, melanoma; F, non-melanoma skin cancer; G, soft tissue and mediastinal; H, breast malignancy; I, female reproductive; J, male reproductive; K, kidney and urinary tract; L, nervous system; M, endocrine; N, unspecified tumors; O, hematological; and P, eye tumors.

development of respiratory malignancies in MM patients. To our knowledge, MM patients have not been found to have increased risk of developing respiratory malignancies; on the contrary, data from the Surveillance, Epidemiology, and End Results database suggest reduced incidence in MM patients.[40] The most common prior malignancy diagnosis in MM patients with subsequent respiratory tract cancer was female reproductive cancer (40%; more specifically, cervical cancer). The reason for this is unclear. Smoking is a risk the factor for both cervical cancer and respiratory tract malignancies,[42] although it is not a risk factor for MM. The increased incidence of hematological malignancies, specifically acute myeloid leukemia/MDS, is well documented in MM patients,[4,6,12,13] where alkylating agents have been considered to be 1 of the main contributing factors, although a role for non-treatment-related factors has also been reported,[4-6,8] and the recent discovery of a genotype associated with the development of MDS in MM[22] supports a role for susceptibility genes in this development.

Taken together, a prior cancer diagnosis increased the risk of second malignancy development in MM patients and might suggest inherent genetic susceptibility in these patients, although further research is needed to confirm this.

We found that prior malignancy negatively impacts survival in MM patients. We showed that this relationship was dose dependent because patients with 2 or more prior malignancy diagnoses had a significantly twofold increased risk of death compared with patients with 1 prior malignancy diagnosis. Interestingly, studies have found patients with other hematological malignancies, such as chronic lymphocytic leukemia, to have a reduced survival if they had a prior history of nonmelanoma skin cancer.[43] The pathologic mechanism behind these findings is unknown, but the authors suggested that patients with prior history of nonmelanoma skin cancer might have more aggressive disease. Our results confirm the findings that prior or synchronous malignancies increased the risk of death in MM patients.[1] Reduced survival in MM patients with prior malignancies is likely multifactorial and could include reduced dose intensity of chemo- and radiation therapy, detrimental effects of previous chemo-radiation or surgical treatments on physical condition of patients, or that MM that develops after another malignancy might be biologically different. Taken together, the impact of prior malignancies is clinically relevant for the individual patient and warrants future research and attention.

Our study has several strengths, including a large sample size, long study period, and application of high-quality population-based data from Sweden. By using the nationwide register-based design, where data are gathered prospectively, we were able to account for recall bias and ensure the generalizability of our results. Underreporting of tumors should not affect our results to any extent because the overall completeness of the Swedish Cancer Registry has been reported to be >95%, although substantial underreporting has been noted for leukemia.[28] In a recent validation study, the diagnostic accuracy was ~98% for hematopoietic lymphoproliferative malignancies in Sweden.[29]

Limitations include the lack of detailed clinical and treatment data for the patient population in our study, including information on cause of death, pathological stage of the prior cancers, MM, and subsequent cancers. In addition, we did not have information on genetic profiles. A possible bias in cohort selection due to left censoring cannot be excluded, but we designed the study period to allow prior malignancies to be recorded, because the Swedish Cancer Registry started in 1958 and patient enrollment started in

Table 2. The risk of developing a certain subsequent malignancy subtype in MM patients with a prior malignancy diagnosis compared with those without

	HR	95% CI	*P*
Overall	1.42	1.23-1.65	**<.001**
Hematologic (n = 193)	1.59	1.04-2.42	**.032**
Gastrointestinal (n = 318)	1.13	0.81-1.58	.475
Male reproductive (n = 204)	0.74	0.44-1.26	.276
Female reproductive (n = 58)	0.71	0.28-1.79	.468
Breast (n = 83)	1.16	0.61-2.22	.643
Kidney and urinary tract (n = 105)	1.40	0.80-2.41	.234
Melanoma (n = 58)	2.67	1.43-5.00	**.002**
Nonmelanoma skin cancer (n = 256)	1.99	1.47-2.71	**<.001**
Respiratory (n = 64)	3.24	1.79-5.88	**<.001**
Oral, nasal, and pharyngeal (n = 18)	1.30	0.29-5.86	.731
Endocrine (n = 24)	0.78	0.18-3.37	.736
Nervous system (n = 35)	0.86	0.26-2.87	.808
Bone and cartilage (n = 5)	—	—	—
Soft tissue and mediastinal (n = 13)	1.91	0.40-9.03	.415
Unspecified tumors (n = 39)	1.28	0.49-3.33	.618

Two-sided $P < .05$ was considered statistically significant, shown in boldface.

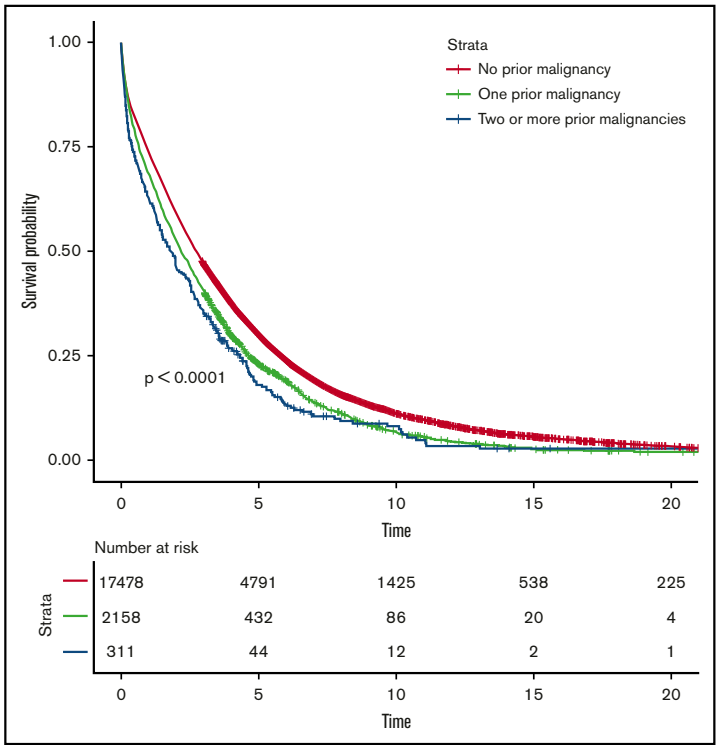

Figure 2. Survival in MM patients with and without prior malignancies. Survival was compared between patients with no prior malignancy, 1 prior malignancy, and 2 or more prior malignancies.

1973, allowing for a 15-year lead time. In addition, we stratified our data according to decade of MM diagnosis and age category. Regarding the risk factor analysis, it should be considered that because survival is significantly reduced in patients with prior malignancies, this group could possibly have had less time to develop a subsequent malignancy compared with patients without a prior malignancy. On the contrary, the patients with prior malignancy developed their subsequent malignancy significantly earlier than patients without a prior malignancy. To minimize this effect, we adjusted for age at MM diagnosis. Because patients could be diagnosed with a prior and second malignancy 1 day before and after MM diagnosis, we performed a sensitivity analysis excluding all patients diagnosed with prior or subsequent malignancy within a month of the MM diagnosis with no effect to our overall findings (data not shown). Several of the subtype analyses had a limited number of patients and should be interpreted with caution.

In this large population-based study, including ~20 000 patients with MM, we report that prior malignancy increases the risk of subsequent malignancy development in MM patients. Furthermore, we found that prior malignancy negatively impacts survival and that >1 prior malignancy reduces survival even further. The underlying explanation for our findings could suggest a role for susceptibility genes in the development of second malignancies; other possible etiologies include immune dysfunction in these patients or side effects from treatment. Given the increase of malignancy survivors in general, our findings are of importance both for the individual patients and their families and for the treating physician.

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G.J. is a PhD candidate at University of Iceland, and this work is submitted in partial fulfillment of the requirement for a PhD.

Authorship

Contribution: S.Y.K., G.J., and S.H.L. designed the study; G.J. and S.Y.K. obtained data; G.J. and S.H.L. performed the analyses; and G.J., S.Y.K., S.H.L., M.B., I.T., M.H., A.P., Y.S.J., and O.L. were involved in the analyses and the interpretation of the results, read, gave comments, and approved the final version of the manuscript, had full access to the data in the study, and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Paper III

Cumulative exposure to melphalan chemotherapy and subsequent risk of developing acute myeloid leukemia and myelodysplastic syndromes in patients with multiple myeloma

Running title: Risk factors for AML/MDS in MM patients

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Summary statement:
In this large nationwide population-based study including almost 27,000 multiple myeloma (MM) patients, we performed a detailed analysis focusing on risk factors for developing acute myeloid leukemia and myelodysplastic syndromes (AML/MDS). Among MM patient who developed AML/MDS the cumulative melphalan exposure was 3-fold (OR=2.8, 95% CI 1.7-5.2; p<0.001) higher compared to MM patients without a subsequent AML/MDS diagnosis. With increasingly improved overall survival in patients with MM driven by access to modern, effective therapies, strategies to avoid secondary complications are becoming more important. Future population-based studies with MM patients exposed to melphalan chemotherapy and with longer follow-up will be critical to better define the long-term risks.

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Abstract

Objectives: The aim of this study was to determine risk factors for development of acute myeloid leukemia/myelodysplastic syndromes (AML/MDS) in patients with multiple myeloma (MM). **Methods:** We identified all patients diagnosed with MM in Sweden from January 1st, 1958 to December 31st, 2011. A total of 26,627 patients were diagnosed with MM with during the study period. Of these, 124 patients (0.5%) developed subsequent AML/MDS. For each patient with MM and a subsequent AML/MDS diagnosis, we randomly selected a matched (age, sex, and date of MM diagnosis) MM patient without a subsequent second malignancy diagnosis. **Results:** The cumulative melphalan exposure was significantly higher (OR=2.8, 95% CI 1.7-5.2; p<0.001) among cases (median 988 mg; IQR 644-1,640) compared to controls (median 578 mg; IQR 360-967). Median time to AML/MDS development was 3.8 years (IQR 2.8 - 5.8). Risk of AML/MDS was not statistically altered by M protein isotype, anemia, renal failure, hypercalcemia, lytic bone lesions, or radiation therapy. **Conclusion**: In this nationwide population-based study, we show that increased cumulative doses of alkylating therapy with melphalan increases the subsequent risk of developing AML/MDS in patients with MM. Given improved survival in MM patients over the last decade future studies will be important to better define long-term risks.

Keywords: Multiple myeloma, AML/MDS, second malignancies, melphalan, alkylating therapy,

Introduction

Over the past decades, increased access to modern effective therapies has continued to improve overall survival for patients with multiple myeloma.[1-5] Indeed, in the U.S. the median overall survival for otherwise healthy younger individuals diagnosed with multiple myeloma is over 10 years.[6] Given that patients in these analyses were diagnosed at least 10 years ago, and in light of all new multiple myeloma drugs in development (including 15-20 bispecific monoclonal antibodies, several CAR T cell therapies, and a range of small molecules), it seems reasonable to conjecture that otherwise healthy individuals who are diagnosed with multiple myeloma in 2021 will have a projected median overall survival of 10-20 years.

In the early 1960s, alkylating treatment with melphalan was found to have an anti-MM effect and was until early 2000s the mainstay of treatment for MM in combination with prednisone (MP).[7] In an attempt to improve outcomes from 1960s and onwards, various combination chemotherapy regimens including vincristine, melphalan, cyclophosphamide and prednisone (VMCP), and vincristine, carmustine, doxorubicin and prednisone (VBAP) were used; however, these were not found to improve survival compared to treatment with MP.[8] In the 1980s, high dose melphalan with autologous stem cell transplantation (HDM-ASCT) was first introduced in the multiple myeloma field,[9] and in the 1990s it became widely implemented. Eventually it became part of standard of care for the treatment of multiple myeloma. Indeed, in the time of its introduction, HDM-ASCT was a major step forward for the field and it significantly improved the rate of response to therapy beyond available drugs.[10-13] Because of its superiority compared to available drugs, early clinical trials were designed to compare single versus tandem HDM-ASCT in multiple myeloma.[14,15] As the treatment field has continued to emerge and the access to modern, effective therapies has resulted in high rates of deep treatment

responses (minimal residual disease (MRD) negativity) in the absence of HDM-ASCT, more recent clinical trials have been designed to investigate the role of HDM-ASCT in the era of modern, effective therapies.[16,17] In 2017, a large randomized clinical trial for newly diagnosed multiple myeloma patients compared the median progression-free survival in patients treated with 5 cycles of bortezomib/lenalidomide/dexamethasone (VRd) including HDM-ASCT and followed by 1 year of lenalidomide maintenance therapy *versus* 8 cycles of VRd without upfront HDM-ASCT (i.e so-called "delayed transplant") and followed by 1 year of lenalidomide maintenance. The investigators found 30% and 20% MRD negativity rates in the two study arms, respectively; this translated into 14 months longer median progression-free survival in the treatment arm including upfront HDM-ASCT; however, there was no difference in overall survival.[17] At the ASH 2020 meeting, updated results from the same study with a median follow-up of 8 years continued to show no overall survival difference between the two treatment arms.[4] In 2021, the utility of upfront *versus* delayed HDM-ASCT remains an area of controversy in the myeloma field.[18-21] In transplant ineligible patients the newly published EHA-ESMO clinical practice guidelines recommend DaraMVP (Daratumumab, Melphalan, Bortezomib and Prednisone) as first line treatment.[22]

In 1970, the first case study of AML/MDS in patients with multiple myeloma was published,[23] and, in 1979 the first randomized study showing patterns of AML/MDS among myeloma patients treated with alkylator therapies was introduced.[24] Subsequent retrospective and prospective studies have been published and confirmed an increased risk of AML/MDS in patients with multiple myeloma,[10,25-32] although the exact biological mechanisms have not been well established. Based on small numbers, an increased risk of AML/MDS has been proposed to be associated with higher cumulative melphalan dose and treatment duration (i.e. treatment

related factors) in some,[24,33,34] but not other studies.[35,36] A prior population-based study investigating the risk of AML/MDS in 5,652 individuals diagnosed with the myeloma precursor condition monoclonal gammopathy of undetermined significance (MGUS) showed excess risk compared to the general population, supporting the fact that patients with plasma cell disorders are at an increased risk (i.e. disease related factors).[27] We were motivated to conduct the first large population-based study designed to determine patterns of cumulative exposure to alkylating chemotherapy and subsequent risk of developing AML/MDS in patients with multiple myeloma. In our analysis we included a comprehensive evaluation of disease related factors.

Methods

Swedish Cancer Registry

All residents in Sweden have equal access to health care under a largely centralized, taxpayer-funded universal healthcare system. All malignancy diagnoses are reported to the centralized nationwide Swedish Cancer Registry, which was established in 1958.[37] The diagnostic accuracy and overall completeness of the Swedish Cancer Registry is very high (>95%).[38,39] Pathologists and physicians in Sweden are obliged by law to report each malignancy diagnosis to this register. Within the register, information on sex, date of birth, date of malignancy diagnosis, and type of malignancy is stored. Date of death was obtained from the Cause of Death Registry.

Patient cohort

We identified all patients diagnosed with multiple myeloma in Sweden from January 1st, 1958 to December 31st, 2011 reported to the Swedish Cancer registry. Information was collected on sex, date of birth and date of multiple myeloma diagnosis. A record linkage was performed with the

cancer registry to obtain information on all subsequent AML/MDS diagnoses within the multiple myeloma cohort. For each patient with multiple myeloma and subsequent AML/MDS diagnosis (cases), one patient without a subsequent second malignancy diagnosis was randomly selected from the multiple myeloma cohort and matched by age (+/- 3 years), sex, and date of multiple myeloma diagnosis (+/- 1 year) (controls). Additionally, it was required that all controls had to be alive when the corresponding matched multiple myeloma patient developed AML/MDS. For both cases and controls, we obtained detailed clinical and treatment information from medical records. These included history of smoking as well as laboratory variables at diagnosis which included complete blood counts, chemistry panel, and beta-2-microglobulin values. Serum protein electrophoresis, serum immunofixation, urine electrophoresis, and urine immunofixation findings were also recorded. Skeletal X-ray results were reviewed, and bone involvement was considered if patient had lytic lesions, pathological compression fractures or severe osteopenia. Furthermore, bone marrow examination (at multiple myeloma diagnosis and at AML/MDS development for cases), type of therapy received, and cumulative doses of each chemotherapy agent were obtained as well as cumulative doses of radiation. Cumulative treatment doses were calculated from date of diagnosis until AML/MDS diagnosis and corresponding date for controls. Response to treatment was categorized as follows: complete response (CR), stringent complete response (sCR), very good partial response (VGPR), partial response (PR), stable disease (SD), and progressive disease (PD) according to the international uniform response criteria for multiple myeloma.[40] The study period was divided into the following five calendar periods: 1958-1970, 1971-1980, 1981-1990, 1991-2000 and, 2001-2011. End of follow-up was December 31st, 2012. Approval was obtained from the Regional Ethical Committee in Stockholm for this study. Informed consent was waived because we had no contact with study patients.

The patients in this study had received treatment at more than 50 hospitals and medical records were found for 97 of 124 (78%) AML/MDS patients. After review of pathology results, a total of ten patients were excluded, six patients had a primary diagnosis of solitary plasmacytoma and four were excluded as their secondary diagnosis was not consistent with AML/MDS. Of the 87 matched controls, medical records were found for a total of 69 patients (79%). Medical records were not found for similar number of cases (22%) and controls (21%). The total number of patients with a diagnosis of multiple myeloma during each calendar period that later developed AML/MDS was: 1958-1970; 3 (3%), 1971-1980; 15 (17%), 1981-1990; 31 (36%), 1991-2000; 24 (28%) and, 2001-2011; 14 (16%).

Data analysis

Demographic characteristics at multiple myeloma diagnosis, disease related factors, frequency of radiation therapy received, cumulative radiation doses, HDM-ASCT, cumulative chemotherapy doses and response to treatment were compared between groups using frequency measures and percentages as well as median and mean values. Mean results were accompanied with standard deviation (SD) and median values with interquartile range (IQR). One-way ANOVA, Chi-square and Kruskal-Wallis tests were used to perform statistical analyses. Statistical analysis for treatment comparison were performed when ten or more patients in each group received the respective treatment. Additionally, we further investigated cumulative melphalan exposure between cases and controls, reported as odds ratios (OR) and 95% confidence intervals (CI) derived from logistic regression. Two-sided P values <0.05 were considered statistically significant. Follow-up was estimated from date of multiple myeloma diagnosis until date of

death, or end of study period, whichever occurred first. (All statistical analyses were done with R version 3.6.1. (R foundation for Statistical Computing, Vienna, Austria).

Results

A total of 26,627 patients with newly diagnosed multiple myeloma were reported to the Swedish Cancer Registry during the study period. Of these 124 (0.5%) patients developed subsequent AML/MDS (cases). The median age at multiple myeloma diagnosis was 73 (IQR 63-78) years for cases and 70 years (IQR 60-77) for controls (Table 1). The median follow-up time for cases was 4.1 years (IQR 3.1-6.3) and 5.8 years (IQR 4.1-8.5) for controls. The median time from multiple myeloma diagnosis to AML/MDS diagnosis was 3.8 years (IQR 2.8 - 5.8).

Treatment exposure and risk of AML/MDS

The cumulative melphalan exposure was 3-fold significantly higher (OR=2.8, 95% CI 1.7-5.2; p<0.001) among MM patients who subsequently developed AML/MDS (median 988 mg; IQR 644-1,640) compared to MM patients who did not develop AML/MDS (median 578 mg; IQR 360-967). The median cumulative cyclophosphamide dose was 6900 mg (IQR 3650-9900) for cases and 4000 mg (IQR 2250-9375) for controls (p=0.26). The median cumulative doxorubicin dose was 140 mg (IQR 80-240) for cases and 243 mg (IQR 194-345) for controls (p=0.17). The median cumulative vincristine dose was 18 mg (IQR 8-48) for cases and 6 mg (IQR 5-21) for controls (p=0.10). A total of five patients (6%) in the case group underwent HDM-ASCT and six in the control group (9%). Radiation treatment was administered to 24% of patients in the case group and 23% of patients in the control group, respectively. The median cumulative radiation dose administered in the case group was 33 Gy (IQR 30-51) and 38 Gy (IQR 30-50) in the

control group (p=0.28). A PR response or better was observed in 62% of patients in the case group and 54% of patients in the control group (p=0.36) (Table 2).

Disease related and other factors

A total of 52% of cases and 46% of controls reported previous or current smoking history (p=0.68). Serum calcium, serum creatinine, hemoglobin concentration, and bone involvement (CRAB criteria) at multiple myeloma diagnosis were not statistically different between cases and controls (Table 1). The median percentage of bone marrow plasma cells at diagnosis was 23% in the case group and 22% in the control group (p=0.87). In the case group 58% of patients were found to have IgG M protein at diagnosis, 34% had IgA, 2% IgM and 7% had light chain disease. In the control group 66% of patients had IgG M protein at diagnosis, 32% had IgA and 2% had IgM. No patients had light chain disease in the control group. The median serum M protein concentration in the case group was 31 g/L (IQR 18-45) and 32 g/L (IQR 18-45) in the control group. Patients in the case group had a median urinary protein concentration of 1,050 mg (IQR 193-3,000) and 587 mg (IQR 150-1,300) in the control group and this difference was not statistically significant (p=0.15). A total of three patients (3%) in the case group and two patients (3%) in the control group had evidence of amyloidosis at diagnosis.

Discussion

In this large nationwide population-based study, including almost 27,000 multiple myeloma patients diagnosed during five decades in over 50 hospitals in Sweden, we found that higher cumulative dose of melphalan was associated with increased risk of developing AML/MDS. In contrast, baseline multiple myeloma characteristics did not significantly alter the risk of

subsequent AML/MDS development. After decades of no improvement and absence of effective anti-myeloma drugs, since the turn of the 21th Century the myeloma field has undergone a transformation reflected in several new, effective therapies becoming available for multiple myeloma patients.[41] This, in turn, has resulted in dramatically improved overall survival.[5,42] Given that recent studies show that high-dose melphalan exposure causes significant increase in somatic mutations (on average a ~20% increase),[43-45] it seems reasonable to hypothesize that the long-term risks are currently under-estimated.

The majority of patients in this study were treated before the introduction of modern myeloma drugs and more than 95% received treatment with melphalan, either long term, as oral treatment, HDM-ASCT, or a combination.[46] Our results support previous data attributing alkylating treatment to development of AML/MDS in multiple myeloma patients.[24,33,34] In addition to prior literature, we show that cumulative doses of melphalan increase the risk of AML/MDS in patients with multiple myeloma. Therapy-related AML/MDS usually develops with a latency period of months to years of treatment from primary disease.[47] It has been shown that therapy-related AML/MDS has molecular and cytogenetic features that are different from de-novo AML/MDS.[48] The mechanisms for development of AML/MDS after alkylating treatment have been thought to be related to direct DNA damage inducing mutations in hematopoietic progenitor cells.[49] More recently, researchers have found that clonal hematopoiesis could play a role as patients who develop AML/MDS have been found to have evidence of driver mutations and clonal hematopoiesis before they were treated with chemotherapy suggesting a pre-existing clone that could have expanded during treatment.[50,51] In our study the median time to development of AML/MDS was 3.8 years. During the majority of the study period survival in multiple myeloma patients in Sweden was poor.[42,52,53] With

significantly improved overall survival in multiple myeloma patients observed over the past decade,[5] future population-based studies with multiple myeloma patients exposed to melphalan chemotherapy in the era of modern myeloma treatment will be important to better define long-term risks.

We found that there was no difference in cumulative doses of other alkylating agents used or topoisomerase inhibitor use between the two groups, however these were used much less frequently than melphalan. In Europe, melphalan-based therapy with MVP is widely used for multiple myeloma patients who are ineligible for HDM-ASCT.[22,54] Additionally, melphalan flufenamide (Melflufen) was recently approved by the FDA in combination with dexamethasone for patients with relapsed or refractory multiple myeloma.[55] The need for upfront HDM-ASCT in the era of modern myeloma treatment and MRD testing has been questioned,[18,19] and recent studies have shown an increased risk of AML/MDS following HDM-ASCT specifically.[30,56] Interestingly, our finding of fewer AML/MDS during the last decade could indicate more widespread use of other newer agents, shorter follow-up time, or a combination of both.

In our study the frequency and cumulative doses of radiation treatment received as well as smoking history, both well-known carcinogens[57,58] were not associated with increased risk of developing AML/MDS. Our results support previous findings from the U.S. Connect MM registry where no relationship was observed between radiation therapy and second primary malignancy incidence in MM patients.[59] A possible reason could be that low dose radiation therapy and limited fields is administered to patients with MM.

We found that risk of AML/MDS was not affected by baseline multiple myeloma factors, including M protein size, M protein isotype, number of plasma cells in the bone marrow and the CRAB criteria. We are not aware of other studies analyzing risk factors in this detail before. In

contrast to our findings, the authors of a population-based study conducted on 5,652 patients with MGUS, where an 8-fold increased risk of developing AML/MDS was observed, reported that the risk varied between M protein isotypes and size of the M protein.[27] Our results suggest that at diagnosis we do not have identifiable factors to predict which multiple myeloma patients are at increased risk of developing AML/MDS.

Our study has several strengths, including the very large study sample of patients diagnosed and treated over a long time period, the availability of detailed clinical and treatment data of all the study patients who were treated at more than 50 hospitals distributed all over Sweden. Additionally, the matched nested case-control design of the study allows for control of potential confounding variables such as age, gender and calendar period.

Limitations include lack of detailed molecular studies at myeloma diagnosis and at diagnosis of AML/MDS. Under reporting of AML/MDS have been found in studies assessing the completeness of the Swedish Cancer Registry specially during its early years,[38,60,61] although this should not affect the results of our study to any extent due to its nested case-control design and large number of patients. Too few patients in the study underwent HDM-ASCT to make assessments regarding risk of AML/MDS in this population specifically, or whether short or prolonged exposure to melphalan is associated with the same risk.

In summary, in this large nationwide population-based study, we confirm and expand on previous findings that alkylating therapy with melphalan increases the risk of AML/MDS development in multiple myeloma. Specifically, we show that an increased cumulative dose of melphalan is associated with a higher risk of developing AML/MDS in patients with multiple myeloma. Given that the overall survival for patients with multiple myeloma is approaching 10-

20 years, future population-based studies with multiple myeloma patients exposed to melphalan chemotherapy and with longer follow-up will be critical to better define the long-term risks.

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Authorship

Contribution: S.Y.K and G.J designed the study and obtained data. G.J performed the analyses. S.Y.K, G.J, O.L, A.P, I.T. M.B, B.D and M.H were involved in the analyses and the interpretation of the results, read, gave comments, and approved the final version of the manuscript.

Conflict of interest disclosure: O.L has received honoraria for scientific talks and/or served on advisory boards for Adaptive, Amgen, Celgene, Janssen, BMS, and Takeda; and has served as a member of independent data monitoring committees for Janssen, Takeda, Merck and Theradex. All the other authors declare no conflicts of interest. M.B reports the following, all outside of the submitted work: all outside of the submitted work: research funding from Takeda; member of independent data monitoring committee for Mundipharma; Member of grant committee for Incyte; lecture honoraria from Astra-Zeneca, Roche, Pfizer and BMS.

Data Sharing: The data that support the findings of this study are not available online due to restrictions from the ethical approval.

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Variable	Cases (multiple myeloma patients who developed AML/MDS); n=87	Controls (multiple myeloma patients who did not develop AML/MDS); n=69	P values
Age at MM diagnosis, median (IQR)	73 (63-78)	70 (60-77)	0.19
Gender (M), n (%)	49 (56)	38 (55)	1.00
History of smoking, n (%)	32 (52)	18 (46)	0.68
Year of MM diagnosis, n (%)			
1958-1970	3 (3)	1 (1)	
1971-1980	15 (17)	9 (13)	
1981-1990	31 (36)	22 (32)	
1991-2000	24 (28)	23 (33)	
2001-2011	14 (16)	14 (20)	
Time to secondary malignancy, median years (IQR)	3.8 (2.8-5.9)	*NA*	
Diagnostic factors & Laboratory tests			
Hemoglobin (g/L), mean (SD)	112 (+/-20)	109 (+/-16)	0.37
Calcium (mmol/L), mean (SD)	2.6 (+/-0.5)	2.4 (+/-0.4)	0.10
Creatinine (µmol/L), mean (SD)	140 (+/-131)	125 (+/-75)	0.51
Bone involvement, n (%)	57 (66)	50 (73)	0.45
Albumin (g/L), median (IQR)	34 (30-39)	34 (29-39)	0.47
B2M concentration (mg/L), median (IQR)	4.1 (2.8-5.3)	5.1 (2.3-7.6)	0.64
Plasma cells in BM (%), median (IQR)	23 (13-30)	22 (10-35)	0.87
Serum M protein (g/L), median (IQR)	31 (18-45)	32 (22-49)	0.54
Type of M spike, n (%)			
IgG (κ or λ)	39 (57)	42 (67)	0.16
IgA (κ or λ)	23 (34)	20 (32)	
IgM (κ or λ)	1 (2)	1 (1)	

Light chain disease	5 (7)	0 (0)	
Urine M protein (mg/L), median (IQR)	1,050 (193-3,000)	587 (150-1,300)	0.151
Kappa, n (%)	26 (60)	19 (65)	
Lambda, n (%)	18 (40)	10 (35)	
Amyloidosis, n (%)	3 (4)	2 (3)	1.00

Abbreviations: MM: Multiple myeloma; IQR (Inter Quartile Range); SD (Standard Deviation); MM-AML/MDS; All patients with Multiple myeloma who developed AML/MDS. Controls; Patients with Multiple myeloma who did not develop AML/MDS and were matched by year of birth (± 3 years), sex, and date of MM diagnosis (± 1 year) to MM patients who developed AML/MDS. B2M= beta-2-microglobulin.

Table 1. Demographics and clinical characteristics of patients with multiple myeloma who developed AML/MDS compared to patients who did not

Treatment	MM-AML/MDS n=87		Control n=69		P values
Chemotherapy, cumulative dose (po and/or IV)	**Median (IQR)**	**n (%)**	**Median (IQR)**	**n (%)**	
Melphalan, mg	988 (644-1640)	86 (98)	578 (360-967)	66 (96)	<0.001
Cyclophosphamide, mg	6900 (3650-9900)	27 (31)	4000 (2250-9375)	23 (33)	0.26
Carmustine, mg	190 (125-345)	11 (12)	360 (NA)	1 (1)	-
Doxorubicin, mg	140 (80-240)	18 (20)	243 (194-345)	14 (20)	0.17
Vincristine, mg	18 (8-48)	19 (22)	6 (5-21)	20 (29)	0.10
Interferon, million units	691 (246-1005)	11 (13)	137 (97-291)	6 (9)	-
Etoposide, mg	1320 (872-2350)	4 (5)	4140 (NA)	1 (1)	-
Lomustine, mg	540 (410-670)	2 (2)	100 (NA)	1 (1)	-
Thalidomide, mg	59400 (19600-125750)	7 (8)	4500 (2475-8675)	4 (6)	-
Bortezomib, mg	-	-	21 (NA)	1 (1)	-
Lenalidomide, mg	-	-	945 (NA)	1 (1)	-
Other types of therapy					
Radiation therapy received, n (%)	21 (24)		16 (23)		0.70
Cumulative dose of radiation (Gy), median IQR	33 (30-51)		38 (30-50)		0.28
HDM-ASCT, n (%)	5 (6)		6 (9)		-
Response to treatment, PR or better, n (%)					
Yes	54 (62)		37 (54)		0.36
No	8 (9)		10 (14)		
Unknown	25 (29)		22 (32)		

Abbreviations: MM: Multiple myeloma; SD (Standard Deviation); Po: per os administration; IV: intra venous administration; MM-AML/MDS; All patients with Multiple myeloma who developed AML/MDS. Controls; Patients with Multiple myeloma who did not develop AML/MDS and were matched by year of birth (± 3 years), sex, and date of MM diagnosis (± 1 year) to MM patients who developed AML/MDS.

Table 2. Treatment administered and response to therapy in patients with multiple myeloma who developed AML/MDS compared to patients who did not

Appendix A

Appendix A: ICD- 7 Codes for Cancer Subtypes used in Study I-III

Cancer subtype	ICD-7 code
Hematologic	200-209
Gastrointestinal	150-157.0, 199.3
Male reproductive	177-179.0
Female reproductive	171-176.9
Breast	170-170.9
Kidney and urinary tract	180-181.9
Non-melanoma skin cancer	191-191.9
Melanoma	190-190.9
Respiratory	161-163.0
Oral, nasal, and pharyngeal	140-148.0, 160.0-160.9
Endocrine	194-195.1, 195.3-195.9
Nervous system	193-193.9, 1921
Bone and cartilage	196-196.9
Soft tissue and mediastinal	197-197.9, 164.0, 195.2, 158.0
Unspecified tumors	192, 192.2-192.9,199,199.1-199.2, 199.4-199.9

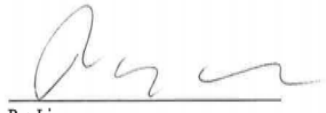
Appendix B

Appendix B

2013-01-09

Angående etikansökan för studien: *Sekundära maligniteter hos patienter med myelom - en retrospektiv studie avseende kliniska karakteristika, risk faktorer och prognos.*

Härmed intygas att Sigurdur Y Kristinsson är anställd vid Hematologiskt Centrum, Karolinska Universitetssjukhuset, Solna och att dom resurser som behövs för projektets genomförande är tillgängliga för de sökande.



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