LUND UNIVERSITY

Factors affecting prognosis of MGUS and multiple myeloma

Lemonakis, Konstantinos

2024

Document Version:
Publisher's PDF, also known as Version of record

Link to publication

Citation for published version (APA):
Lemonakis, K. (2024). *Factors affecting prognosis of MGUS and multiple myeloma*. [Doctoral Thesis (compilation), Department of Laboratory Medicine]. Lund University, Faculty of Medicine.

Total number of authors:
1

General rights
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

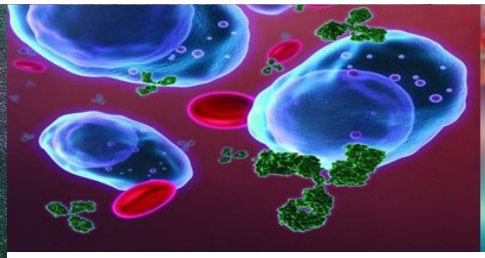
Read more about Creative commons licenses: https://creativecommons.org/licenses/

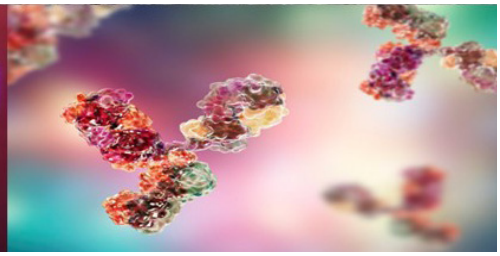
Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Download date: 18. Nov. 2026





Factors affecting prognosis of MGUS and multiple myeloma

KONSTANTINOS LEMONAKIS
DEPARTMENT OF HEMATOLOGY AND TRANSFUSION MEDICINE | LUND UNIVERSITY

Factors affecting prognosis of MGUS and multiple myeloma

Factors affecting prognosis of MGUS and multiple myeloma

Konstantinos Lemonakis

LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.

To be publicly defended on the 23rd of May at 09.00 in Belfragesalen, BMC D15, Sölvegatan 19, 223 62 Lund

Faculty opponent
Professor Beatrice Melin,
University of Umeå, Sweden

Organization: LUND UNIVERSITY

Document name: Doctoral dissertation **Date of issue** May 23rd 2024

Author: Konstantinos Lemonakis

Title: Factors affecting prognosis of MGUS and multiple myeloma

Abstract: Multiple myeloma (MM) is a malignant plasma cell disease preceded by an asymptomatic state called monoclonal gammopathy of undetermined significance (MGUS). Although extensively studied, the exact mechanisms of progression from MGUS to MM are still to be determined. The treatment of MM is continuously evolving, with several drugs being developed and incorporated in the management of the disease. A lot of research on MGUS and MM focuses on genetic factors, both inherited and acquired, with possible effect on MM prognosis. The aim of this thesis is to provide some insight on various aspects of the prognosis and treatment of MM.

Paper I investigates the impact of daratumumab based induction on stem cell mobilization parameters in newly diagnosed MM patients. Our analysis clearly shows that the use of daratumumab in induction leads to lower stem cell yield, more days of apheresis and more frequent use of plerixafor.

Paper II describes the association of extra copies of the long arm of chromosome 1 (1q) and the prognosis of MM. We were able to show that the presence of two or more extra copies of 1q carries a higher risk for shorter progression-free and overall survival in a large cohort of newly diagnosed MM patients.

In Paper III, we tested for association between two previously reported inborn genetic variants and MM survival in 871 newly diagnosed MM patients, without finding any association in this cohort.

Finally, Paper IV describes our attempt to investigate the role of latent infection as possible driver for the development of MGUS, by assessing the specificity of the monoclonal immunoglobulin characterizing the disorder against various infectious agents. We found that in a small subset of patients, M-component binds to epitopes in common viruses, suggesting that infection with the virus might act as an initiating event for MGUS.

Key words: multiple myeloma, MGUS, daratumumab, stem cell mobilization, gain(1q), amp(1q), myeloma survival, single nucleotide polymorphism, infections

Classification system and/or index terms (if any) Supplementary bibliographical information

Language English **ISSN and key title**: 1652-8220

Number of pages: 85 **ISBN:** 978-91-8021-568-8

Recipient's notes

Price Security classification

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature Date 2024-05-23

Factors affecting prognosis of MGUS and multiple myeloma

Konstantinos Lemonakis

LUND
UNIVERSITY

Copyright pp 1-85 Konstantinos Lemonakis
Paper 1 © 2023 Ferrata Storti Foundation
Paper 2 © 2023 The Authors. European Journal of Haematology published by John Wiley & Sons Ltd
Paper 3 © Springer Nature
Paper 4 © by the Authors (Manuscript unpublished)

Faculty of Medicine
Department of hematology and transfusion medicine

ISBN 978-91-8021-568-8
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University, Lund 2024

Media-Tryck is a Nordic Swan Ecolabel certified provider of printed material. Read more about our environmental work at www.mediatryck.lu.se
MADE IN SWEDEN

Table of Contents

Abstract 9
Populärvetenskaplig sammanfattning 11
List of Papers 14
Author's contribution to the papers 15
Abbreviations 16
1 Introduction 19
1.1 Plasma cells 19
1.2 MGUS 20
1.2.1 Definition 20
1.2.2 Epidemiology 20
1.2.3 Risk factors for MGUS 20
1.2.4 Pathogenesis of MGUS 22
1.2.5 From MGUS to myeloma 22
1.3 Multiple Myeloma 24
1.3.1 Definition and epidemiology 24
1.3.2 Prognosis of multiple myeloma 24
1.4 1q gains in myeloma 25
1.4.1 Prognostic implications of +1q 26
1.4.2 Gain(1q) vs amp(1q) 27
1.4.3 Double-hit myeloma 28
1.5 Genetic variations predisposing for MM 28
1.6 Treatment of newly diagnosed MM 30
1.6.1 Choice of induction therapy 30
1.6.2 Stem cell mobilization and collection 31
1.6.3 ASCT 32
1.6.4 Consolidation and maintenance 32
2 Aim of and rationale for the thesis 33
2.1 Overall aim of the thesis 33
2.2 Paper I 33
2.3 Paper II 34
2.4 Paper III 35

2.5 Paper IV 35
3 Methodology 37
3.1 Paper I. Daratumumab effect on stem cell collection 37
3.2 Paper II. Impact of +1q om NDMM survival 39
3.3 Paper III. Association of two specific SNVs with myeloma survival 42
3.4 Paper IV: Subclinical infections in MGUS 42
4 Ethical considerations 45
4.1 Paper I-III 45
4.2 Paper IV 45
5 Results and discussion 47
5.1 Paper I 47
5.1.1 Patient characteristics 47
5.1.2 Stem cell yield 48
5.1.3 Plerixafor use and days of apheresis 49
5.1.4 Discussion 50
5.1.5 Future directions 52
5.2 Paper II 53
5.2.1 FISH results 53
5.2.2 Patient characteristics 54
5.2.3 Response to treatment 54
5.2.4 Progression-free survival 57
5.2.5 Overall survival 58
5.2.6 Discussion 59
5.2.7 Future directions 64
5.3 Paper III 64
5.3.1 Patient characteristics 64
5.3.2 Association between SNPs and MM survival 65
5.3.3 Discussion 65
5.4 Paper IV 67
5.4.1 Patient characteristics and serology 67
5.4.2 M-component's specificity and treatment 67
5.4.3 Discussion 68
6 Concluding remarks 70
6.1.1 Paper I 70
6.1.2 Paper II 70
6.1.3 Paper III 71
6.1.4 Paper IV 71
7 Acknowledgements 72
8 References 73

Abstract

Multiple myeloma (MM) is a plasma cell malignancy preceded by a clinically asymptomatic clonal disorder called monoclonal gammopathy of undetermined significance (MGUS). Although MGUS has been associated with various predisposing factors such as infections and chronic inflammation, the exact role of latent infections in the pathogenesis of the disorder and progress to MM is poorly understood. The prognosis of MM is influenced by different genetic abnormalities, occurring mainly in clonal plasma cells, but some studies have implied that even inborn genetic variation might affect the course of the disease. Treatment of patients with MM has been rapidly evolving the last two decades, leading to a significant improvement in survival. Recently, daratumumab has been incorporated in the treatment of patients with newly diagnosed multiple myeloma (NDMM).

The aim of this thesis is to investigate the role of different aspects such as latent infections, cytogenetic abnormalities existing in myeloma or somatic cells, and modern treatment on MGUS and MM development and prognosis.

In **Paper I**, we investigated the effect of daratumumab introduction in first-line treatment of younger fit patients with newly diagnosed MM on stem cell mobilization and collection. Previous randomized studies had shown that the addition of daratumumab to standard of care leads to fewer stem cells collected prior to autologous stem cell transplantation. By examining 92 patients receiving daratumumab and comparing them with 125 patients not treated with the drug, we were able to show that addition of daratumumab independently affected stem cell collection, resulting in lower stem cell yield (5.14×10^6 vs 7.22×10^6 stem cells/kg, $p < 0.001$) and more median days of apheresis (2 vs 1, $p = 0.018$). This is the largest study investigating daratumumab effect on stem cell collection parameters in a real-world population to date.

Paper II provides an analysis of the significance of increased number of extra copies of the long arm of chromosome 1, known as +1q, within plasma cells of patients with newly diagnosed MM on their prognosis. +1q is the most common cytogenetic abnormality in MM detected by fluorescence in situ hybridization (FISH) and can be divided into gain(1q), meaning one extra 1q copy, or amp(1q), meaning ≥2 extra 1q copies. We collected data on 350 consecutive NDMM patients and we were able to show that those with amp(1q), when compared with cases with gain(1q) or without +1q, had worse progression-free (13.1 vs 36.1 vs 25.4 months, $p = 0.005$) and 3-year overall survival (56% vs 76% vs 80%, $p = 0.003$).

Besides cytogenetic abnormalities in myeloma cells, genome wide association studies (GWAS) have implied that single nucleotide polymorphisms (SNPs) in somatic cells might also affect MM prognosis. In **Paper III**, we tried to replicate the results of two such studies finding association between genetic variation in two different loci and myeloma survival, but we could not find any evidence for such an

association in a real-world Swedish patient population. This study highlights some of the drawbacks GWAS have and the importance of replication studies to avoid false positive results.

The **fourth project** included in the thesis is an ongoing prospective study attempting to investigate the association between latent infection and MGUS. Recently, it has been shown that the monoclonal antibody in the serum of MGUS and MM patients is targeted against different pathogens in a subset of cases. We aim to collect serum samples of 60 patients with MGUS and analyse the specificity of M-component against different infectious agents. As of today, we have obtained serum samples of 30 MGUS patients and found that in three of them, the M-component binds to herpes simplex virus (HSV), and in one case to Epstein-Barr virus (EBV).

Populärvetenskaplig sammanfattning

Multipelt myelom (MM) är en form av blodcancer som drabbar ca 600-700 patienter/år i Sverige. MM föregås av ett asymtomatiskt tillstånd som kallas MGUS (monoklonal gammopati av oklar signifikans) som innebär att man har hittat en onormal antikropp i blod och/eller urin, en s.k. M-komponent. M-komponenten tillverkas av en klon av plasmaceller som är en subtyp av vita blodkroppar och finns i benmärgen. I motsatsen till MM, är MGUS ett relativt vanligt tillstånd och de flesta patienterna förblir utan symtom livet ut. Det är dock oklart varför tillståndet uppstår och varför vissa patienter med MGUS utvecklar MM. Tidigare populationsbaserade studier har visat tydlig association mellan genomgång av olika infektioner och förekomst av MGUS och MM. Dessutom, nyligen publicerade data talar för att M-komponenten kan vara riktad mot vissa vanliga virus eller bakterier i ca 20-25% av MGUS och MM fall, något som antyder att en latent infektion kan vara involverad i initieringen av sjukdomen.

Prognosen av patienter med myelom påverkas av flera faktorer, såsom patientens ålder, övriga sjukdomar, allmäntillstånd med mera. De flesta patienter med myelom har förvärvade genetiska avvikelser i de maligna cellerna som påverkar prognosen. De analyseras vid diagnos och, till en viss del, kan påverka valet av behandling. En av de vanligaste kromosomavvikelserna är en eller flera extra kopior av kromosomarm 1q. En extra kopia benämns gain(1q) medan flera extra kopior benämns amp(1q) (amplification 1q). Flera studier antyder att extra 1q kopior är associerade med tidigt återfall och kortare överlevnad hos patienter med myelom. Den prognostiska skillnaden mellan gain(1q) och amp(1q) är däremot mindre studerad.

Under de senaste decennierna har det studerats hur medfödd genetisk variation, vilket innebär små skillnader i DNA-sekvens, kan öka risken för utveckling av olika sjukdomar eller påverka prognosen för en specifik sjukdom. Sådana medfödda variationer studeras via s.k. helgenomstudier där man går igenom medfödda genvarianter hos ett stort antal individer för att identifiera genvarianter som kan vara kopplade till ett specifikt drag eller sjukdom. Resultat av helgenomstudier tyder på att även utvecklingen av MGUS och MM samt MM prognos kan påverkas av sådan genetisk variation.

Även om MM i dagsläge är en obotbar sjukdom, stora framsteg har skett under de senaste två decennierna i behandlingen och prognosen för patienter med MM har blivit betydligt bättre. Bland annat har man senaste åren introducerat i primär behandling av patienter med nydiagnostiserat MM ett läkemedel som kallas daratumumab, eftersom forskningsresultat talar för att kombinationer som innehåller detta läkemedel ger högsta chans för en djup behandlingsrespons.

En viktig del av behandling för patienter med nydiagnostiserat myelom som är yngre än 70 år är autolog stamcellstransplantation eftersom den förlänger tiden till återfall

i sjukdomen. Flera steg ingår i den processen. Först behöver man samla patientens stamceller. För att kunna göra det, får patienten benmärgsstimulerande sprutor i magen i några dagar som gör att stamceller, som finns i benmärgen, kommer ut till blodet. Sedan räknar man stamceller i blodet och om de ligger på en bra nivå genomgår patienten en procedur som kallas aferes. Det innebär att blodet går genom en apparat som känner igen stamceller och behåller dem. Om stamceller inte ligger på en bra nivå brukar man ge vid ett tillfälle en annan spruta som kallas plerixafor för att få stamceller att lossna från benmärgen och komma ut i blodet. Nästa steg är cellgiftsbehandling som ges ca två veckor efter aferes. Slutligen, en dag efter cellgiftsbehandling, får patienten tillbaka sina stamceller, som sparats nedfrusna.

Det övergripande målet med denna avhandling är att undersöka effekten av olika genetiska egenskaper, som är specifika för myelomceller eller en del av medfödd genetisk variation, och införandet av modern terapi på myelom prognos. Dessutom syftar den till att ge insikt i vilken roll vissa infektioner har i etableringen av MGUS och dess progress mot MM. Avhandlingen består av fyra projekt.

I första projektet har vi utvärderat effekt av daratumumab innehållande behandlingskombinationer på olika parametrar av stamcellsinsamling. Sedan man började använda daratumumab, har man märkt att det har blivit svårare att samla patientens stamceller inför den autologa transplantationen. Inom den svenska myelomgruppen bestämdes att genomföra en studie för att göra en mer objektiv bedömning av detta. Studien godkändes av etikprövningsnämnden och har finansierats av svenska blodcancerförbundet. För att göra studien har vi samlat data på totalt 217 patienter och patienterna delades i två grupper: en grupp som hade fått behandling med daratumumab (92 patienter) och en kontrollgrupp som hade fått behandling före introduktion av daratumumab (127 patienter). Genom att använda lämpliga statistiska metoder har vi observerat att man i genomsnitt samlade färre stamceller hos patienter som fick daratumumab. Dessutom behövde patienterna som behandlades med daratumumab flera aferes dagar för att samla tillräckligt med stamceller (i genomsnitt 2 dagar mot 1 dag), och mer frekvent användning av plerixafor för att säkerställa att tillräckligt med stamceller finns i blodet för att kunna genomföra aferes. Således, vår studie har kunnat bevisa att introduktion av daratumumab i behandling av patienter med nydiagnostiserat myelom påverkar viktiga parametrar av stamcellsinsamling. Det innebär att man behöver utveckla nya strategier för att försäkra att autolog stamcellstransplantation, som är en viktig komponent i behandling, kan genomföras.

Andra projektet som ingår i avhandlingen är en studie som utvärderar vilken effekt förekomst av gain(1q) eller amp(1q) har på överlevnad av patienter med nydiagnostiserat myelom som har fått modern behandling i Sverige. Vi har samlat data från ca 350 patienter med myelom som har behandlats i Region Skåne och Region Halland mellan 2018-2021. Vi har dokumenterat vilken behandling patienterna har fått, grad av behandlingsrespons, datum för diagnos, återfall i myelom och död. Patienterna har delats i tre grupper: en med gain(1q), en med

amp(1q) och en med normalt antal 1q kopior. Vi har kunnat bevisa att patienter med amp(1q) får snabbare återfall och har klart kortare överlevnad jämfört med de två andra grupper. Prognosen av myelom skiljer sig inte mellan grupperna med gain(1q) och normalt antal 1q kopior. Denna studie utökar vår kunskap inom området och belyser behovet att tidigt identifiera patienter med nydiagnostiserat myelom som har amp(1q) och hitta effektiva läkemedelskombinationer för att förbättra deras prognos.

I tredje projektet har vi försökt återskapa resultat av två helgenomstudier som tidigare har publicerats och visat att två specifika genetiska varianter är associerade med sämre total överlevnad hos patienter med MM. Vi har inte kunnat hitta några bevis för ett sådant samband i en svensk patientpopulation bestående av 871 patienter. Denna studie belyser några av nackdelarna med helgenomstudier och vikten av replikationsstudier för att undvika falskt positiva resultat.

Fjärde delarbete i avhandlingen är en pågående prospektiv studie som inkluderar patienter med MGUS och undersöker om deras M-komponent binder mot vissa virus (såsom olika typer av herpesvirus) som de flesta människor har blivit exponerade för vid tillfälle och är asymtomatiska bärare. Vi planerar för att rekrytera sammanlagt 60 patienter i studie och har hittills inkluderat 30. Vi har identifierat tre patienter med M-komponent riktad mot herpesvirus och en mot Epstein-Barr virus. Behandlingsförsök mot herpesvirus har genomförts i dem tre patienterna utan någon effekt på M-komponentens storlek. Våra preliminära resultat talar för att bärarskap av lågvirulenta infektioner kan bidra och driva utveckling av MGUS i åtminstone en liten andel av patienterna. Mer riktad forskning behövs för att undersöka möjligheten att reversera processen genom framgångsrik behandling av infektioner.

List of Papers

Paper I

Impact of daratumumab-based induction on stem cell collection parameters in Swedish myeloma patients

Lemonakis K, Tätting L, Lisak M, Carlson K, Crafoord J, Blimark C, Santamaria A, Wichert S, Lenhoff S, Hansson M.

Haematologica. 2023;108(2):610-614. doi:10.3324/haematol.2022.281610

Paper II

Impact of 1q gains on treatment outcomes of patients with newly diagnosed multiple myeloma in a real-world Swedish population receiving modern treatment

Lemonakis K, Olsson-Arvidsson L, Karlsson C, Johansson B, Hansson M

Eur J Haematol. 2023 Sep;111(3):391-399. doi: 10.1111/ejh.14018

Paper III

Sequence variation at the MTHFD1L-AKAP12 and FOPNL loci does not influence multiple myeloma survival in Sweden

Ali M, Lemonakis K, Wihlborg AK, Veskovski L, Turesson I, Mellqvist UH, Gullberg U, Hansson M, Nilsson B

Blood Cancer J. 2019 Jul 30;9(8):57. doi: 10.1038/s41408-019-0222-8

Paper IV

Treatment of subclinical infections in MGUS patients

Lemonakis K, Hellmark T, Liljeqvist JA, Gunnarsson L, Hector M, Mellqvist UH, Axelsson P, Hansson M

This is an ongoing project and the manuscript has not yet been submitted to any peer-reviewed scientific journal

Author's contribution to the papers

Paper I

I have contributed to planning the project and collecting data. I have done the statistical analysis of the collected data and written the first draft of the manuscript. I have also been the corresponding author.

Paper II

I have co-planned the project, collected the data, conducted the statistical analysis, written the first draft of the manuscript and been the corresponding author.

Paper III

I have contributed to data collection, analysis of the clinical data and co-authored the manuscript.

Paper IV

I have co-planned the project and been contributing to patient recruitment and data collection.

Abbreviations

Amp(1q)	amplification 1q
ASCT	autologous stem cell transplantation
BCR	B-cell receptor
CI	confidence interval
CMV	cytomegalovirus
DVRd	daratumumab, bortezomib, lenalidomide, dexamethasone
DVTd	daratumumab, bortezomib, thalidomide, dexamethasone
EBV	Epstein-Barr virus
EMN	European Myeloma Network
FISH	fluorescens in-situ hybridization
FLC	free light chains
GC	germinal center
GWAS	genome-wide association study
HBV	hepatitis B virus
HCV	hepatitis C virus
HP	helicobacter pylori
HR	hazard ratio
HRCA	high risk cytogenetic abnormalities
HSV	herpes simplex virus
Imids	immunomodulators
IMWG	International Myeloma Working Group
ISS	International Staging System
LDH	lactate dehydrogenase
MGUS	monoclonal gammopathy of undetermined significance
MIAA	multiplex infectious agent array
MM	multiple myeloma
MRD	minimal residual disease
NDMM	newly diagnosed multiple myeloma
OR	odds ratio
ORR	overall response rate
OS	overall survival
PD	progressive disease
PFS	progression-free survival

Pis	proteasome inhibitors
R-ISS	revised international staging system
RR	relative risk
RRMM	relapsed or refractory multiple myeloma
SMM	smouldering multiple myeloma
SNP	single nucleotide polymorphism
SNV	single nucleotide variant
VGPR	very good partial response
VRd	bortezomib, lenalidomide, dexamethasone
VZV	varicella zoster virus

1 Introduction

1.1 Plasma cells

Plasma cells are terminally differentiated cells of B-lineage which are an essential component of humoral immunity, providing protection against infectious agents by producing and secreting antibodies targeted against these agents.

Plasma cells derive from mature germinal center B-cells.[1] After antigen recognition by the B-cell receptor (BCR), activated B-cells in lymphoid tissues start forming the germinal center (GC) upon interaction with helper T-cells.[2, 3] Those B-cells in the GC undergoing successful somatic hypermutation and positive selection to ensure the most appropriate antibody production differentiate to plasma cells.[1, 4] Several transcription factors play an important role in the positive selection of B-cells in the GC such as myc which triggers cell cycle activation and transition from the light to the dark zone of the GC, as well as mTORC1 which stimulates protein and lipid synthesis in B-cells.[5, 6, 7]

Plasma cell differentiation is restricted to high affinity GC B-cells. Decreased PAX5 expression in those B-cells allows overexpression of the transcriptional factor IRF4 which triggers plasma cell differentiation.[8, 9] Differentiated plasma cells exit then the lymphoid organs migrating with the help of chemokines such as CXCR4 and CXCL12 via the bloodstream to the bone marrow where they form multicellular niches comprising of stromal and other survival factors producing cells.[10, 11] In the bone marrow, plasma cells express high levels of CD38 and CD138 and lose B-cells markers such as CD19.[12]

Long-lived bone marrow residing plasma cells continuously produce and secrete large amounts of immunoglobulins. Inside the plasma cell, proteins are synthetized and folded within the expanded endoplasmic reticulum. Misfolded proteins are degraded by proteasome explaining why plasma cells are extremely dependent om proteasome function to avoid excess immunoglobulin in the cytoplasm which would lead to apoptosis.[1]

1.2 MGUS

1.2.1 Definition

Monoclonal gammopathy of undetermined significance (MGUS), first mentioned by Jan Waldenström back in 1960 as "essential hyperglobulinemia" or "benign monoclonal gammopathy",[13] is an asymptomatic premalignant plasma cell dyscrasia characterized by the presence of a monoclonal whole immunoglobulin or monoclonal free light chains in the serum produced by a plasma cell clone in the bone marrow. To meet the criteria of MGUS, the size of the monoclonal antibody should be <30 g/L and the percentage of clonal plasma cells in a bone marrow sample <10%. The most important criteria though is the absence of symptoms or organ damage due to the plasma cell clone or the monoclonal antibody/free light chains.[14] Depending on the type of antibody produced, MGUS can be divided into three different clinical subtypes: IgM MGUS, non-IgM MGUS or free light chain MGUS. This distinction is important as the two latter entities are associated with risk to progress to multiple myeloma (MM) or AL amyloidosis while IgM MGUS carries a risk of progression to lymphoproliferative disorders.[15, 16]

1.2.2 Epidemiology

First reports on the incidence and prevalence of MGUS already exist in the 1960s.[17] Several published large population-based studies and a systematic review has estimated the prevalence of MGUS in those older >50 to be 3.2%.[18, 19, 20, 21, 22, 23, 24] The risk of MGUS increases with age, with an estimated prevalence of 5.2% in individuals of 70 years or older.[19] MGUS is uncommon in younger individuals, with only 2% of MGUS patients being diagnosed under the age of 40 and one study showing a prevalence of 0.34% in persons between 10-49 years old.[25] The annual incidence of MGUS was estimated in a retrospective study to be 120 per 100.000 population at the age of 50 years in males, increasing to 530 per 100.000 population at the age of 90 years. In females, the same study estimated the incidence to be 60 per 100.000 population at the age of 50 years and 370 per 100.000 population at the age of 90 years.[26] The use of more sensitive methods to detect monoclonal immunoglobulins such as mass spectrometry could lead in the detection of MGUS in a larger proportion of the population.[27, 28]

1.2.3 Risk factors for MGUS

Besides age, other factors have been evaluated to clarify if they increase the risk for MGUS development.

- African-american origin: Reports that MGUS is more common in people of African-american descent exists already in the 1990s.[29] A study on 917 men

between 50-74 years old in Ghana revealed an age-adjusted prevalence of 5.84%.[30] A recent study using mass spectrometry to detect lower values of monoclonal immunoglobulins in the serum estimated that individuals of African-american origin had an increased relative risk to develop MGUS of 1.44 (95% confidence interval, CI 1.18 to 1.75).[28]

- Male sex: MGUS is more common in males than females as depicted in several epidemiological studies.[19, 26]
- Family history of hematological disorder: In a Swedish population-based study of 4.458 MGUS patients, 17.505 controls and their first degree relatives, a 2.8 fold risk of MGUS was observed in relatives of individuals with MGUS.[31] Similar results were shown in a smaller study from Mayo clinic.[32] There is even some evidence that common single-nucleotide polymorphisms in seven genes are independently associated with increased risk of MGUS, further supporting a genetic predisposition for MGUS.[33]
- Obesity: A meta-analysis of different studies evaluating the relationship between increased body mass index and MM revealed a significantly higher risk of developing MM in overweight (relative risk 1.12) and obese (relative risk 1.27) individuals.[34] Older reports indicated a similar risk even for MGUS[35] but a more comprehensive study from Iceland did not show any association between obesity and MGUS.[36]
- History of inflammatory conditions and autoimmune diseases: A population-based study from Sweden, which included 5403 patients with MGUS, 96.617 matched control subjects, and 262.931 first-degree relatives revealed a significantly increased risk for MGUS in patients with a personal history of inflammatory conditions (odds ratio, OR 1.4) and autoimmune diseases (OR 2.1) one year or more before the diagnosis of MGUS.[37] A meta-analysis of relevant studies estimated a 42% increased risk of MGUS in patients with prior history of any autoimmune disease.[38] In this meta-analysis, the association between history of disease and MGUS was stronger for pernicious anemia (relative risk, RR 1.67). Other systemic conditions linked with higher risk of MGUS development were polymyositis/dermatomyositis, rheumatoid arthritis, systemic sclerosis, and ankylosing spondylitis.
- History of infection: Similarly, the increased risk of MGUS in patients with prior history of infections is well established, with large epidemiological studies showing a 1.4-1.6-fold risk of MGUS in those patients.[37, 39]

1.2.4 Pathogenesis of MGUS

MGUS arises from post-germinal plasma cells which have undergone somatic hypermutation and class-switch recombination following antigenic exposure. MGUS cells can be distinguished from normal plasma cells by their abnormal immunophenotype. While both normal and MGUS plasma cells express CD138, in MGUS plasma cells usually do not express CD19 and are CD56 positive.[40]

Two different types of initiating genetic events have been identified in MGUS which are in most cases not overlapping which each other: 1) hyperdiploidy, meaning extra copies of usually odd-numbered chromosomes and 2) translocations involving immunoglobulin heavy-chain (IgH) gene on the long arm of chromosome 14 such as t(11;14), t(6;16), t(4;14), t(14;16) and t(14;20) involving oncogenes such as *CCND1, CCND3*, *MMSET/FGFR3*, *MAF*, and *MAFB*.[41, 42, 43] These translocations result in a relocation of oncogenes to the strong enhancer region of the immunoglobulin genes, making them susceptible to mutations and overexpression. Moreover, chromosome 13 deletion has been reported in about 25% of MGUS cases and dysregulation of cyclin D gene is commonly found in MGUS plasma cells.[44, 45]

1.2.5 From MGUS to myeloma

While MGUS is a common plasma cell dyscrasia, only a few patients progress to symptomatic disease, most commonly multiple myeloma (MM). To date, there is no way to predict which MGUS patients are going to develop myeloma. Secondary genetic events and interaction between bone marrow microenvironment and plasma cells have been implicated in the process of developing MM (Figure 1).

Secondary genetic events like somatic gene mutations and copy number abnormalities occur in the background of hyperdiploidy or IgH gene translocations. These occur only in a subset of clonal cells. Pre-clinical data in mouse models suggest that alterations in the *MYC* gene at chromosome 8q22 promote the evolution from MGUS to myeloma.[46] Indeed, t(8;14) (*IgH-MYC*) is present in only a few MGUS patients while its frequency significantly increases in newly diagnosed myeloma (NDMM).[47, 48, 49] Deletion of the short arm of chromosome 17 where the tumour suppressor gene *TP53* is located, is another genetic event which is rarely found in MGUS but exists in 5-10% of NDMM cases and is associated with poor prognosis.[50, 51, 52, 53] *NRAS* and *KRAS* are the two most commonly mutated genes in established myeloma whereas their alterations are rare in MGUS.[49, 50] Besides mutations in the aforementioned genes, analysis of NDMM cases revealed recurrent mutations in *BRAF, RB1* and *DIS3* genes.[50, 54] These mutations deregulate important cellular pathways such as NF-kB, RAS-ERK and G1/S cell cycle. Copy number abnormalities are often observed in myeloma and include amplification or deletion of a whole chromosome, a chromosome arm or a short region of an arm. They lead to accumulation of oncogenes promoting tumour cell survival. Extra copies of the

long arm of chromosome 1, gain(1q) is a common copy number abnormality, presenting in approximately 40% of NDMM cases.[55] Gene expression profiling studies have tried to identify subsets of MGUS patients with higher risk of developing MM.[56, 57]

Changes in the bone marrow microenvironment, both before and after the establishment of MGUS, could result in clonal selection and progression of MGUS to myeloma.[58] Already in MGUS state, the bone marrow niche is modified by fibroblasts resulting in upregulation of different extracellular matrix proteins and receptors. This remodelling becomes more pronounced in MM where it has been shown that two extracellular matrix affiliated proteins, ANXA2 and LGALS1, are more abundant.[59, 60] Mesenchymal stem and progenitor cells of the bone marrow niche have been found to regulate survival and proliferation of clonal plasma cells through the secretion of growth or anti-apoptotic factors like CXCL12 and interleukin-6.[58, 61] Furthermore, the bone marrow environment becomes more hypoxic in the presence of MM cells leading to increased expression of various cytokines such as interleukin-7, vascular endothelial growth factor and hypoxia-inducible factor 1, and promoting further dissemination of clonal cells.[62, 63]

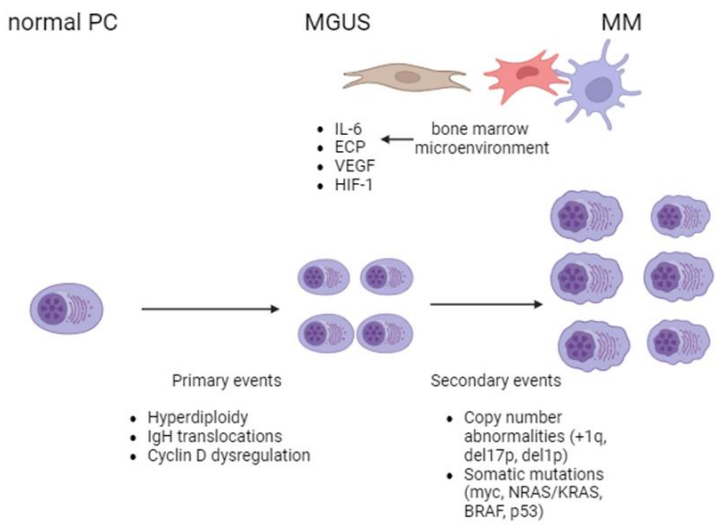


Figure 1 Primary genetic events such as hyperdiploidy or translocations involving IgH gene contribute to the establishment of MGUS while secondary events such as copy number alterations and somatic mutations might lead to progression to myeloma. Myeloid derived cells, fibroblasts and stromal cells in the bone marrow microenvironment play an important part in promoting survival and proliferation of clonal plasma cells. Figure created by biorender.

1.3 Multiple Myeloma

1.3.1 Definition and epidemiology

Multiple Myeloma (MM) is a malignant disease where the presence of a plasma cell clone causes - in contrary to MGUS - symptoms to the patient. According to International Myeloma Working Group (IMWG) criteria (Table 1), MM is diagnosed by the identification of a plasma cell clone in the bone marrow, the presence of a monoclonal paraprotein in the serum or urine and at least one of four symptoms/findings: 1) osteolytic bone lesions, 2) anemia, 3) renal failure and 4) hypercalcemia. In the absence of all four characteristics, MM can be diagnosed if any of the following three myeloma defining events exists: 1) ≥60% plasma cells in the bone marrow, 2) serum free light-chain (FLC) ratio of 100 or higher, provided involved FLC level is 100 mg/L or higher or 3) at least two focal lesions on magnetic resonance imaging.[14]

MM is the second most common hematological malignancy after lymphomas and accounts for approximately 1% of all cancers. Its annual incidence is about 5-6 cases/100.000 people.[64, 65, 66] Median age at diagnosis is about 70 years. In Sweden, according to the latest report from the national myeloma registry, around 700 people are diagnosed with MM per year, with the majority being men and 62% more than 70 years old.[67] As MM survival is getting better after the introduction of modern therapies, the prevalence of the disease is rapidly rising.[67, 68]

Table 1 Diagnostic criteria for MGUS, SMM and MM

	Non-IgM MGUS	Smouldering MM	MM
Monoclonal protein	M-component <30 g/L	M-component >30 g/L	any
Clonal bone marrow plasma cells	<10%	10-60%	any
Presence of bone disease, anemia, renal failure or hypercalcemia	No	No	Yes*

*In absence of these events, MM diagnosis can be made in the presence of ≥60% plasma cells in the bone marrow, serum free light-chain (FLC) ratio of 100 or higher, or at least two focal lesions on magnetic resonance imaging

1.3.2 Prognosis of multiple myeloma

Although the presenting features of myeloma are common between patients, the prognosis of the disease varies extensively with a subset of patients not responding to treatment or progressing rapidly while other stay alive and disease-free for many years. Attempts to identify patient groups with distinct prognosis have been made already since the 70s and knowledge on this matter has increased by the years.

The first clinical staging system was proposed by Durie and Salmon in 1975. They used clinical features such as the extent of anemia and bone disease, serum calcium levels and the concentration of monoclonal paraprotein in serum or urine to divide patients in three groups with low, intermediate, and high cell mass.[69] 30 years later, Greipp et al. published a simple prognostic system, the International Staging System (ISS), by analysing clinical and laboratory data of 10.705 patients with NDMM. They found that the combination of serum albumin and b2-microglobulin can reliably divide patients in three stages with distinct overall survival.[70]

These first staging systems did not consider cytogenetic abnormalities. With the increased use of fluorescens in situ hybridization (FISH),[71, 72, 73] it became apparent that the presence of structural abnormalities, such as translocations involving IgH locus and gains or losses of whole or parts of chromosomes, might predict survival of NDMM patients and the European Myeloma Network (EMN) published recommendations on the use of FISH in myeloma.[74] Several studies examined the impact of t(4;14) and del(17p) on myeloma prognosis and showed an inferior survival for patients carrying these abnormalities.[75, 76, 77, 78, 79] Lactate dehydrogenase (LDH) is a relevant biomarker in myeloma as high levels have been implicated with shorter survival.[80, 81] The IMWG proposed in 2015, after analysing data from 4.445 patients with NDMM enrolled onto 11 large clinical trials between 2005 to 2012, a revised international staging system (R-ISS) to incorporate cytogenetic abnormalities and LDH, thus better identifying patients with high risk disease.[82] Recently, another staging system named R2-ISS including gain(1q) has been proposed by the EMN.[83]

1.4 1q gains in myeloma

Extra copies of the long arm of chromosome 1 (+1q) is the most common abnormality detected by FISH in NDMM, using a specific probe covering 350 kb around the *CKS1B* gene in 1q21 region.[55, 84] Cases with +1q can be divided into those having only one extra copy, denoted gain(1q), or ≥2 extra copies, referred to as amplification of 1q or amp(1q).[85] Although +1q can be already detected in the premalignant stage of MGUS, its frequency increases through smouldering myeloma (SMM), NDMM and relapsed/refractory MM (RRMM).[84, 85, 86, 87] The presence of +1q in SMM has been associated with higher risk of progress to symptomatic MM.[88, 89]

+1q is a secondary copy number alteration and can arise through different events. Formation of isochromosome 1q is a known mechanism of +1q.[50] The University of Arkansas for Medical Sciences Myeloma group proposed the "jumping 1q syndrome" where patients with +1q have frequent breakpoints in the pericentromeric heterochromatin which leads to unstable chromatin resulting in a

repetitive pattern of segmental duplications that causes the same segment of DNA from 1q to "jump" around the genome, with increasing copy number.[90, 91]

Several genes located at 1q21 region are overexpressed in case of +1q and have been implicated in the pathogenesis of myeloma.[92] *CKS1B* is a small protein which acts as cell cycle regulator and its overexpression leads to ubiquitination and loss of expression of the tumor suppressor $p27^{kip1}$, thus activating cyclin-dependent kinases and promoting myeloma cell growth.[93, 94, 95] *CKS1B* may also, when overexpressed, lead to activation of the STAT3 and MEK/ERK pathways, contributing to myeloma drug resistance and further promoting its progression.[96]

MCL-1, an antiapoptotic protein and member of BCL2 family, plays a crucial role in the maintenance of long-lived plasma cells in the bone marrow and is also located in 1q21. +1q leads to higher expression of *MCL-1* whose function is enhanced by interaction with IL-6 in the bone marrow microenvironment.[97, 98, 99, 100]

Overexpression of *ADAR1*, an RNA editing protein located in 1q, might be involved in MM cell proliferation. In the CoMMpass study, *ADAR1* was associated with inferior survival and resistance to proteasome inhibitors, independent of 1q copy number.[101] Other genes located in 1q which might play a role in myeloma progression and resistance to therapy are *PDZK1* and *ANP32E*.[92, 102, 103] Recently, it has been implied that *ILF2*, a 1q21 amplification-specific cancer-relevant gene, induces MM resistance to DNA-damaging agents by interfering with RNA-binding proteins.[104]

1.4.1 Prognostic implications of +1q

The presence of +1q in NDMM has been associated with high-risk features such as increased frequency of ISS stadium 3, elevated LDH and incidence of high-risk cytogenetics, mainly t(4;14).[87, 105, 106, 107, 108] On the contrary, t(11;14) is less frequent in cases with +1q. Some studies showed that del(17p) is equally present in +1q cases as in cases without +1q but other have shown the opposite results. Clinical findings that are increased in NDMM patients with +1q are anemia, hypercalcemia, renal dysfunction and thrombocytopenia whereas the incidence of lytic bone lesions is the same in patients having or lacking +1q. [87, 105, 106, 107, 108]

The impact of +1q on the prognosis of NDMM has been an issue of extensive evaluation though the last two decades, in randomised clinical studies as well as in retrospective series. Hamamura et al published on 2006 the results of the Total Therapy study regarding patients with NDMM where the presence of +1q had inferior 5-year progression-free (PFS) and overall survival (OS). +1q was associated with shorter PFS and OS in multivariate analysis (hazard ratio, HR 1.86 for PFS and 1.78 for OS).[84] Similar results were reported by Neben et al from the HOVON-65/GMMG-HD4 trial. Patients with +1q, along with patients carrying del(17p) and t(4;14), had a significantly shorter PFS (HR 1.7) and OS (HR 1.9).[109] Longer follow-

up of a study comprising 520 patients from the French myeloma group showed inferior OS for patients with +1q in multivariate analysis (HR 1.58).[110] In this study, they could identify a subgroup of patients lacking del(17p), t(4;14) and +1q who had a very good prognosis with long OS after treatment with high dose melphalan and autologous stem cell transplantation (ASCT). Shah et al published a combined analysis of the Myeloma IX and XI studies, two randomized studies from the United Kingdom including 1905 NDMM patients, reporting that +1q cases had shorter OS (HR 1.68).[111]

The aforementioned studies were randomised clinical trials treating patients with older drug combinations. The role of NDMM therapy with modern drugs such as bortezomib or lenalidomide has not been evaluated in depth for patients with +1q in prospective randomised trials as most of them did not include +1q as a high-risk cytogenetic abnormality. Myeloma XI trial examined the role of lenalidomide maintenance, which is today the standard of care for transplant-eligible patients, and found that patients with high risk cytogenetic abnormalities (HRCA) benefit from lenalidomide with the exception of +1q.[112] In a recently published study by Mina et al., treatment with carfilzomib as induction prior to and consolidation after ASCT could partially mitigate the negative effect of the presence of one HRCA including patients with +1q.[113] Another prospective, but not randomised study, has used an intensive protocol of aggressive induction before and consolidation and maintenance after ASCT and showed promising results in patients with ultra-high risk myeloma, which includes the presence of at least two HRCA including gain or amp(1q).[114]

A plethora of retrospective studies have tried to address the prognostic significance of + 1q in the era of modern therapy. In a study conducted at Mayo clinic, 391 NDMM patients with +1q were compared with 985 patients with normal 1q and were found to have inferior OS in multivariate analysis (HR 1.5).[105] Similar results were published by Kastritis et al. on 912 patients with NDMM, where +1q was associated with worse PFS (HR 1.5) and OS (1.41).[108] A few other studies could show that treatment with bortezomib and/or lenalidomide at first line could not abrogate the negative impact of +1q.[106, 107, 115, 116] Even in the context of high dose melphalan and ASCT, +1q retains its negative effect on PFS and OS as shown by multiple studies.[117, 118, 119]

1.4.2 Gain(1q) vs amp(1q)

While there is a consensus that +1q negatively affects the prognosis in NDMM, it remains somewhat controversial if the number of extra 1q copies has an influence on survival. Several studies mentioned earlier investigated if there is a difference between gain(1q) and amp(1q). Most of them failed to show a significant difference,[84, 105, 107, 108, 111, 116, 120] including the randomised Total Therapy study and the two large retrospective series by the Mayo group and Kastritis at al. On the

contrary, other studies observed significantly worse prognosis of amp(1q) in comparison to gain(1q), leaving this matter open to further research. Scmidt et al could not find that gain(1q) had an adverse effect on survival in the absence of other HRCA when looking at 201 patients treated with a combination of bortezomib, lenalidomide and dexamethasone, whereas amp(1q) maintained its prognostic significance regardless of the presence of other HRCA.[106] Amp(1q) was more strongly associated with worse OS (HR 3.95) compared to gain(1q) (HR 1.66) in the randomised HOVON-65/GMMG-HD4 trial.[109] Similarly, patients with amp(1q) treated with ASCT had a dismal prognosis with a significantly shorter OS in multivariate analysis (HR 7.06).[119]

1.4.3 Double-hit myeloma

As +1q is a secondary genetic event in MM, it is often co-present with other cytogenetical events such as IgH translocations or del(17p). This has led to the proposal of double-hit myeloma, where the concomitant presence of two HRCA might lead to even worse prognosis, with the combination of +1q and t(4;14) being the most common one. Indeed, a subset of patients with ultrahigh-risk myeloma who does poorly despite modern treatment has been identified in several studies. In a retrospective analysis of patients with NDMM who have undergone ASCT at MD Anderson Cancer Center, 79 patients with ≥2 HRCA, mainly +1q combined with t(4;14) or del(17p), had a very short PFS of only 22.9 months.[121] Similarly, double-hit genetics were associated with a significantly shortened PFS (HR 4.27) and OS (HR 4.01) in a study of 139 patients receiving ASCT at the Royal Marsden Hospital NHS Foundation Trust.[122] Even in the randomised FORTE study by Mina et al., the use of potent modern regimens could not mitigate the negative impact of double-hit myeloma.[113] Interestingly, a study from Ohio State University assessed the clone sizes of patients simultaneously carrying +1q and t(4;14) and suggested that they can be divided in two groups where only patients with a +1q clone size of >20% and t(4;14) of >30% at the same time have a worse prognosis.[123] A few studies have investigated if the achievement of deep response to treatment such as MRD (minimal residual disease) negativity might improve the prognosis of double-hit myeloma with encouraging results, suggesting the need for the introduction of modern therapies with the capacity of reaching sustained MRD negativity in this patient population.[113, 120, 121]

1.5 Genetic variations predisposing for MM

As humans are identical in 99.5% of their genome, it is variation in the remaining 0.5% that makes each of us unique.[124] Single nucleotide variations (SNVs), which are single base-pair differences in the DNA sequence, are the most common

variations in the genome.[125] Due to the development of cost-effective, rapid genomic technologies, it is possible today to sequence large DNA amounts and identify genetic variants which predispose to certain diseases.

Genome-wide association studies (GWAS) aim to detect and associate genetic variants with a given trait or disease. To do so, a case-control approach is utilized, where a group having the disease is compared with a healthy group. The allele frequency of SNVs is examined through the genome aiming to find variants occurring more often in people affected by the disease than in the control group. For such a study to be carried out, one should have access to germline DNA from patients and healthy controls. Detected variants in a GWAS study need to be validated in separate cohorts to ensure that an association between the discovered variant and the disease exists. Meta-analysis of several independent studies is commonly used to increase statistical power and reduce false positive results. GWAS reaching high quality standards are being catalogued by the European Bioinformatics Institute (www.ebi.ac.uk/gwas).[126]

As some epidemiological studies showed increased risk for the disease in family members of MGUS and MM patients,[31, 32, 127] several GWAS have been conducted to identify SNVs with the possibility to predispose for MM. Twenty-four independent DNA sequence variants that associate with MM risk have been discovered by six large-scale, high-quality GWAS, with the results being replicated in independent samples (Figure 2).[128, 129, 130, 131, 132, 133, 134] Combined though, the 24 risk variants account only for about 16% of the estimated heritability of MM. Most of the association signals span a single candidate gene.

How SNVs in the affected genes functionally contribute to the development of MM is understudied. By taking a glance at the identified risk genes, they include genes implicated in plasma cell development and function (*TNFRSF13B*, *ATG5*, *ELL2*, *CBX7*, *KLF2*, and *HLA* region), autophagy (*WAC*, *ULK4*, *TOM1*), telomere maintenance (*POT1*, *TERC*), cell cycle regulation and DNA replication (*CDCA7L*, *CDKN2A*, *CCND1*, *RFWD3)*.[134]

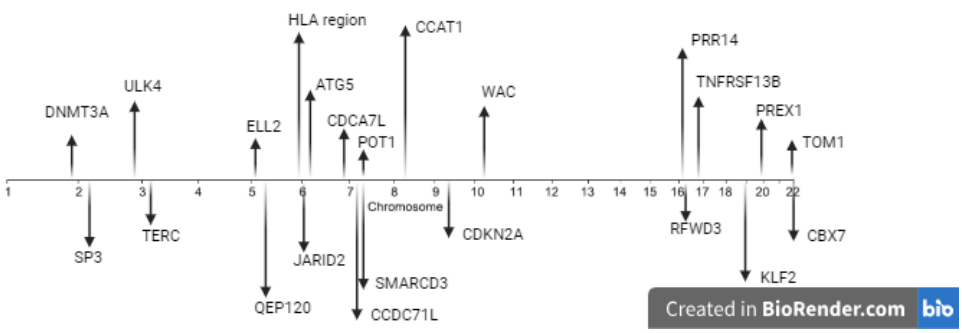


Figure 2 Genes associated with MM risk in GWAS studies

1.6 Treatment of newly diagnosed MM

The treatment landscape of MM has changed a great deal through the years. The introduction of high dose melphalan with autologous stem cell transplantation (ASCT) in the 90s was a crucial step towards the improvement in MM survival.[135, 136] The next important step was the development of proteasome inhibitors (Pis) such as bortezomib, and immunomodulators (Imids) such as thalidomide and lenalidomide during the 2000s.[137, 138, 139, 140] It is though during the last fifteen years that treatment options are rapidly evolving with the introduction of several drugs such as new generation Pis and Imids, different monoclonal antibodies and immune effector cell therapies.[141, 142, 143, 144, 145, 146]

There are several factors affecting the choice of first-line treatment, with age and comorbidities being the main ones. Younger, fit patients without significant comorbidities are considered to be eligible for intensive protocols where high dose melphalan and ASCT is still considered to be the standard of care.[147, 148] On the other hand, older or patients with comorbidities which makes them ineligible for intensive treatment based on ASCT, proceed to receive combinations of different classes of modern drugs.[149, 150, 151] There is no strictly defined upper age limit for ASCT, but 70 years is the common one in clinical praxis.

The treatment protocol for ASCT eligible patients can be divided in different phases:

- induction where the patient receives a combination of drugs to achieve a good response grade,
- stem cell mobilization and collection to assure the feasibility of ASCT,
- high dose melphalan and ASCT,
- consolidation to improve the response grade to previous treatment,
- maintenance to prolongate progression-free and overall survival

1.6.1 Choice of induction therapy

With the development of different drugs in the past decades, the choice of drug combination to be part of the induction therapy has changed significantly. Bortezomib has been the mainstay of induction treatment since the early 2010s, first in combination with cyclophosphamide and corticosteroids.[152] Due to clinical studies showing a significant benefit with Imids, cyclophosphamide was later substituted with thalidomide or lenalidomide.[147, 153]

Daratumumab is an antiCD38 antibody with proved activity against myeloma cells. Daratumumab binds to CD38 in plasma cells leading to their death by different mechanisms such as complement-dependent cytotoxicity, antibody-dependent cell-

mediated cytotoxicity, antibody-dependent cellular phagocytosis and direct apoptosis by crosslinking.[154] Daratumumab was first used in the treatment of relapsed disease, either as monotherapy or in combination with Pis or Imids.[155, 156, 157, 158, 159, 160] Recent studies though implied that the incorporation of daratumumab to first line treatment of both younger and older patients can improve their survival.[161, 162] More specifically, the addition of daratumumab to standard induction schedules for ASCT eligible patients improved progression-free survival in the setting of clinical trials which has led to quadruplet induction regimens including daratumumab, bortezomib, an Imid and dexamethasone being the new standard of care for this patient population.

1.6.2 Stem cell mobilization and collection

High dose melphalan is a myeloablative treatment which leads to a long-lasting myelosuppression. To shorten the period of cytopenia and susceptibility to life-threatening complications such as infections and bleeding, the patient's stem cells need to be collected prior to and reinfused after high dose melphalan. CD34 is used as a marker of stem cells.

There are two main strategies being used in order to mobilize the patient's stem cells, either steady-state with only cytokines, mainly filgrastim (G-CSF), or mobilization using chemotherapy prior to G-CSF.[163, 164] Steady-state strategy has the advantage of a more rapid and predictable rise in circulating stem cells while chemotherapy-based mobilization leads to higher stem cell yield.[165] In myeloma patients, cyclophosphamide has been widely used in a dose of 2-4 g/m^2 prior to G-CSF. To ensure adequate bone marrow regeneration after high dose melphalan, the goal is to collect and re-infuse a minimum of 2x10^6 stem cells/kg.[163] Plerixafor is a CXCR4 inhibitor that can be used as a rescue option to rapidly mobilize CD34+ cells in cases G-CSF fails to.[166, 167, 168, 169] At the planned day of apheresis after stem cell mobilization, the amount of CD34+ cells mobilized to the peripheral blood is measured to decide if the patient can proceed directly to apheresis or plerixafor needs to be utilized.

Several studies have evaluated and identified various risk factors for poor stem cell mobilization such as age >60, advanced stage of underlying disease, high number of prior treatment lines, prior therapy with melphalan or lenalidomide and low platelet count before mobilization.[163, 164, 170, 171, 172] Lenalidomide's negative effect on stem cell collection might be overcome by mobilization after fewer induction cycles and increased plerixafor use.[173, 174]

In the clinical studies which led to the approval of daratumumab-based quadruplet induction, it was noted that the addition of daratumumab had an impact on stem cell mobilization and collection, leading to increased use of plerixafor and lower yields of collected CD34+ cells/kg, though without impairing the feasibility of ASCT.[175]

Reported outcomes of small retrospective studies supported those data.[176, 177] This observation creates a clinical issue as adequate stem cell collection is an important part of the treatment of NDMM patients.

1.6.3 ASCT

During ASCT, the patient first receives myeloablative chemotherapy and then the collected by apheresis stem cells are infused. In myeloma, the role of high dose melphalan and ASCT as an effective and safe treatment has long been established. Even in the era of modern therapy and although there is growing evidence that it can be omitted at first line and utilized at first relapse without negatively affecting OS,[178, 179] ASCT seems to lead to longer PFS when compared with strategies using longer induction and consolidation schemes without ASCT.[147, 148] It needs to be pointed though that no randomized studies have evaluated ASCT in the era of quadruplet, daratumumab based induction.

1.6.4 Consolidation and maintenance

Consolidation with a limited number of cycles of the same or another combination of drugs after ASCT has been extensively studied with various results. In the studies leading to the approval of quadruplets as induction, two cycles of consolidation with the same four drug combination resulted to higher rates of MRD negativity.[161, 162] Thus, it is recommended today as standard of care.

Although consensus exists on the use of maintenance therapy with low dose of one or more drugs, there is still some controversy around the optimal duration of maintenance and drug combinations in different clinical settings.[180, 181, 182, 183, 184]

2 Aim of and rationale for the thesis

2.1 Overall aim of the thesis

The overall aim of the thesis is to assess the impact of different factors such as cytogenetic features specific to myeloma cells or existing in somatic cells, and the introduction of modern therapy on myeloma prognosis. Furthermore, it aims to provide insight in the role of certain infections on the establishment of MGUS and its progress towards multiple myeloma. The specific objectives of the individual papers and the rationale for the studies are described below.

2.2 Paper I

Goal of the study

To evaluate the effect of the introduction of daratumumab in induction therapy of NDMM patients on different stem cell collection parameters such as:

- Stem cell yield
- Days of apheresis
- Use of plerixafor

Rationale

Stem cell mobilization and collection is an important step in the treatment of patients with NDMM as it allows them to proceed to high dose melphalan and ASCT. CD34+ is an antigen on the surface of stem cells which is used to measure the amount collected by apheresis. To ensure adequate bone marrow engraftment, at least $2x10^6$ CD34+ cells/kg need to be reinfused. As many patients are going to be candidates for two ASCTs, either as part of a tandem ASCT approach at first line or a second ASCT in the relapse setting, the goal is to collect more than $4x10^6$ CD34+ cells/kg and, in some cases, $>6x10^6$ when a patient might be treated with three courses of high dose melphalan during the disease's course.

The clinical trials evaluating quadruplets, daratumumab-based regimens at induction as well as small retrospective series, highlighted the negative effect of this

treatment strategy on stem cell collection, as fewer CD34+ cells after more days of apheresis could be collected in patients getting treated with these regimens. Sweden has been one of the first countries incorporating the quadruplet regimens in the treatment of NDMM. As of January 2021, daratumumab in combination with bortezomib, dexamethasone and an Imid (thalidomide or lenalidomide at physician's choice) is recommended in the Swedish guidelines as induction treatment of patients with NDMM who are eligible for ASCT.

It was shortly after the utilization of this strategy that the effect on stem cell mobilization and collection was observed, and in meetings of the Swedish myeloma group it was decided that it is of value to conduct a study to measure and objectify this observation in a large real-world patient population as a first step in the process of developing strategies to mitigate this negative effect of the new therapy.

2.3 Paper II

Goal of the study

To investigate, in the era of modern therapy, if the prognosis of NDMM patients carrying gain(1q) or amp(1q) differ when compared to each other or with patients not having +1q in their myeloma cells.

Rationale

In any malignant disease, it is important to early recognize patient groups with a dismal prognosis and try to find strategies to mitigate that. In MM, there is a lot of data implicating different cytogenetical abnormalities as markers of worse survival. Extra copies of the long arm of chromosome 1 (+1q) is one of the most common abnormalities detected by FISH in NDMM, has extensively been investigated as a prognostic marker in prospective and retrospective studies, and consensus exists that it negatively affects survival. Though, there is much less data on differentiating between one (gain(1q)) or more (amp(1q)) extra copies of 1q as prognostic features of NDMM and the results are controversial. Moreover, these results come mainly from patient groups treated with outdated treatment regimens. This highlights the need for further research in the field. In our retrospective study, we aim to provide some insight in the prognostic significance of amp(1q) against gain(1q) in a real-world population treated upfront with modern drugs such as Pis and Imids, recognizing at the same time the limitations of a retrospective study.

2.4 Paper III

Goal of the study

To evaluate if inborn genetic variation in two individual loci affect the survival of patients with multiple myeloma.

Rationale

Several GWAS have tried to identify SNVs implicated in the establishment of MGUS and its progress to MM or influencing the survival of patients affected by the disease. Validating the associations implied by GWAS in different cohorts is a critical step in enhancing the quality of studies and avoiding false positive results.

Two studies have found associations between genetic variation in two different loci and myeloma survival. The first one was detected in a meta-analysis of 3256 cases from four clinical trials (two from the UK, one from the USA, and one from Germany) and was related to a single nucleotide polymorphism (SNP) located between the *MTHFD1L* and *AKAP12* genes at chromosome 6q25.1.[185] Though, the association was not uniformly present in all sample sets and no efforts to validate the results in an independent material were carried out.

The second study found an association between MM survival and SNP near the *FOPNL* gene locus at the short arm of chromosome 16.[186] The association was detected by two different meta-analyses: one of 545 cases from two clinical trials in USA and one of seven other data sets comprising in total 1087 patients. The positive replication in the second meta-analysis was mainly due to a large effect size in one small subset of patients, whereas the other six subsets did not show any evidence of association.

As the results of these discovery GWAS were not convincingly replicated and the possibility of false discovery remains, we aimed to replicate these associations in a series of MM patients in Sweden.

2.5 Paper IV

Goal of the study

- To assess if the M-component in patients with MGUS has a specificity for pathogens such as herpes virus I and II, varicella zoster virus, Epstein-Barr or cytomegalovirus.
- To assess if the plasma clone in MGUS will be decreased or eradicated after treatment of the pathogen in case of M-component's specificity against it.

Rationale

Numerous studies have established that viruses like Epstein–Barr (EBV), human herpes virus 8, hepatitis C virus (HCV) or bacteria can induce lymphoma and chronic lymphocytic leukemia. In contrast, the role of chronic infection in the pathogenesis of MGUS or MM is rarely investigated. Interestingly, recent data has shown that the monoclonal antibody in MGUS often has a specificity against common infectious agents such as Herpes simplex virus (HSV) type I or II, cytomegalovirus (CMV), Epstein–Barr virus (EBV), Varicella zoster virus (VZV), Helicobacter pylori (HP) or hepatitis C virus (HCV) indicating that these infectious agents may act as a driving force of the plasma cell expansion.[187, 188] Furthermore, treatment against HCV has resulted in the regress of M-component.[189] When, during the development from MGUS to MM, the MGUS clone becomes independent of antigen stimulation is unknown.

It would therefore be of interest to examine if eradication of the infectious agent in cases of MGUS with an M-component with a specificity against such an agent would lead to a decrease or disappearance of the M-component thus reducing or even eliminating the risk of progress to MM.

3 Methodology

3.1 Paper I. Daratumumab effect on stem cell collection

The idea for this project was born during a meeting of the Swedish myeloma group. Shortly after the introduction of daratumumab-based induction in the clinical praxis, its impact on stem cell collection was observed and discussed between the members of the group. The need to assess the extent of the negative impact was identified and the decision to conduct such a study was taken.

The group decided to conduct a retrospective study and all interested members collected data on relevant clinical and stem cell collection parameters on patients treated with daratumumab at induction and undergone apheresis. As a control group, data on the same number of patients who were treated before the era of daratumumab-based induction were collected. Sweden has seven stem cell transplantation centres: Umeå, Uppsala, Stockholm, Gothenburg, Linköping, Örebro and Lund. Representatives of all but Stockholm centre expressed interest in participating in the study and contributed with data.

The hypothesis of the study was that daratumumab-treated patients would have lower stem cell yield, would need more apheresis days and use more rescue-plerixafor to collect enough CD34+ stem cells. The primary endpoint was the mean CD34+ cells/kg collected by apheresis. Secondary endpoints were median days of apheresis, the use of plerixafor as rescue to mobilize stem cells and the proportion of patients failing to mobilize stem cells at all.

The study was approved by the Swedish ethical committee October 2021 and upon approval, data were collected as follows:

- Baseline characteristics such as age, gender, myeloma-related organ impairment and prognostic staging stadium
- Treatment related variables such as induction regimen, cycles of induction, the use of radiotherapy and the response grade according to IMWG criteria before stem cell collection
- Stem cell collection parameters such as the utilization of steady-state vs chemotherapy-based mobilization, days of apheresis, use of plerixafor as rescue and stem cell yield.

Emphasis was given to the collection of data on factors previously described to be relevant for the outcome of stem cell collection such as age, previous treatment with lenalidomide or alkylators, radiotherapy and time since diagnosis. The number of induction cycles served as a surrogate for time between the treatment start and mobilization.

It is of value to point out that, even if small differences exist between the transplantation centres involved in the study, all of them have similar policy regarding stem cell collection method and plerixafor use. Most importantly, this policy had not changed during the study's observation period, meaning it was the same for daratumumab and non-daratumumab treated patients. All centres in Sweden use Optia® continuous mononuclear collection system for apheresis. A standard washout period of two weeks from the end of the last induction cycle to the start of mobilization regimen is applied. Plerixafor was, during the period covered by this study, only used as rescue in case of low CD34+ cells/ml counts in the peripheral blood at the day of planned apheresis.

After excluding some patients due to missing data or switching from a non-daratumumab to a daratumumab containing induction regimen, 217 patients were included in the study analysis, as shown in Figure 3.

All statistical analyses were performed using IBM SPSS Statistics 25. Statistical comparisons between groups on baseline characteristics and response to treatment were tested with chi-square or Fisher's exact test for nominal variables, Mann-Whitney U test for ordinal variables and independent samples median test for continuous variables. To evaluate differences between variables with possible effect on mean value of collected CD34+ x 10^6 cells/kg, the independent-samples t-test was used. Multiple linear regression was used to determine independent impact of factors which showed statistically significant effect on mean value of collected CD34+ cells by the independent samples t-test. Comparison to test difference between study groups on the use of plerixafor was made by Fisher's exact test. The effect of addition of daratumumab-based induction on number of apheresis days was evaluated by the Mann-Whitney U test. A p-value <0.05 was considered as significant through all the above mentioned analyses.

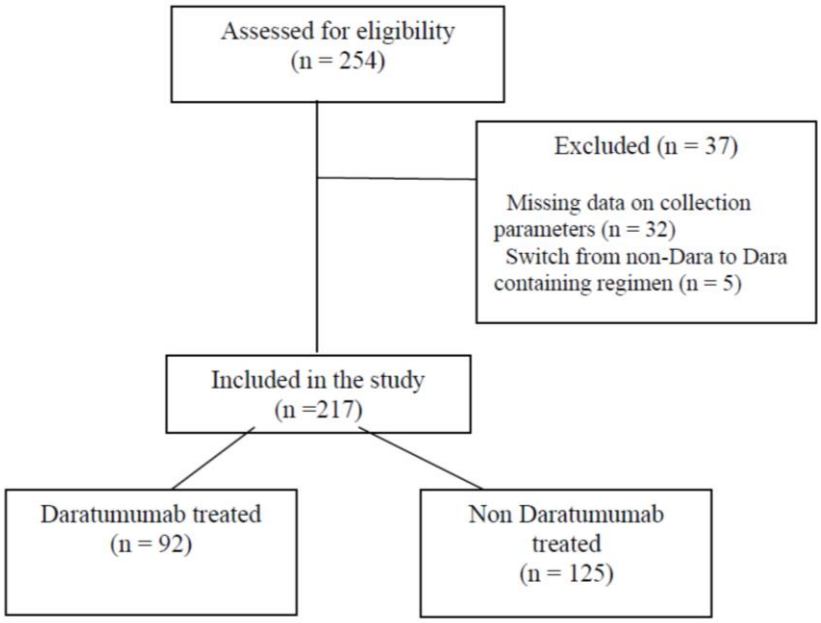


Figure 3 Patient disposition

3.2 Paper II. Impact of +1q om NDMM survival

Upon suspicion of MM or other plasma cell dyscrasias, patients are referred to the hematology department for work-up. Further investigation to establish a specific diagnosis consists of biochemistry, complete blood count, a skelettal computed tomography (CT scan) and a bone marrow sample. Analyses performed on bone marrow are microscopic assessment of the percentage of plasma cells and FISH analysis in isolated plasma cells. In the south region of Sweden (Region Skåne), there are five hematology departments and outpatient clinics who perform diagnostic evaluation of supected myeloma cases. Samples for FISH analyses from these departments are being sent to the department of Clinical Genetics, Pathology, and Molecular Diagnostics in Lund.

At the gentics department in Lund, interphase FISH analyses in suspected plasma cell disease are performed on CD138+ isolated plasma cells. These cells are selected using the automated cell isolator RoboSepTM-S (Stemcell Technology, Cambridge, UK). Selected CD138+ cell get fixated, spread on to microscope slides and dehydrated with ethanol before hybridization of the FISH probes. One hundred informative cells on each slide are being analysed manually in an Axio Imager Z2 fluorescence microscope.

Positive FISH results are detected by the following probe sets:

- +1q by 1q21 *CKS1B*/1p32 *CDKN2C* FISH Probe Kit
- del(17p) by Vysis *TP53/CEP* 17 FISH Probe Kit
- t(4;14) by Vysis *IGH/FGFR3* DF FISH Probe Kit
- t(14;16) by Vysis LSI *IGH/MAF* DF Probe Kit
- t(11;14) by Vysis *IGH/CCND1* XT DF FISH probe Kit
- t(14;20) by Zytolight SPEC *MAFB/IGH* Dual color Dual fusion Probe

Analysis of +1q in the genetics department in Lund began february 2018. The clinical cut-off value used for +1q is >2% interphase nuclei with gains of 1q.

To investigate the impact of gain(1q) and amp(1q) om myeloma survival, we aimed to calculate PFS and OS in a retrospective study of consecutive patients with myeloma diagnosed between february 2018 and october 2021 in hematology departments of Region Skåne and the department of medicine in Halmstad. As stated above, all these departments send bone marrow samples for FISH analysis to Lund.

This project was conducted in collaboration with the department of Clinical Genetics, Pathology, and Molecular Diagnostics in Lund and a hematologist in Halmstad. It was approved by the Research Ethics Committee of Lund University, Sweden. Access was granted to a database at the genetics department comprising all cases in which FISH analyses had been performed on suspected MM cases and the results on +1q were known during the time period specified above. Review of the electronic medical records was performed to first identify patients who fulfilled the criteria of NDMM according to IMWG and then gather relevant data for the purpose of the study.

The database included 985 separate FISH analyses. Medical records were not accessible in 283 cases. After exclusion of cases that the work-up resulted in the diagnosis of MGUS, SMM, primary AL amyloidosis, plasma cell leukemia or other disorder, 346 patients with NDMM were identified and included in the study. These patients were divided into three groups: gain(1q), amp(1q) and no1q, depending on the presence of +1q and the number of extra copies of 1q (Figure 4). Patients were then further divided into two cohorts depending on being treated with ASCT or not.

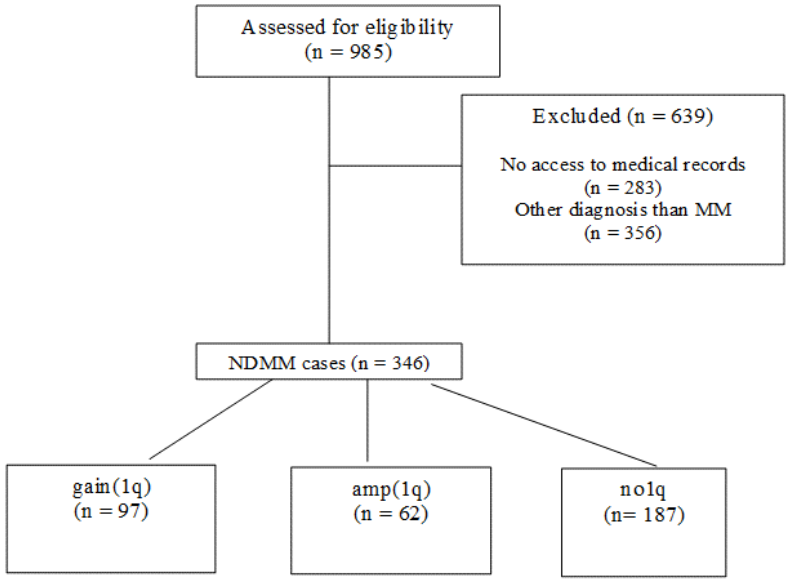


Figure 4 Patient disposition

The hypothesis of the study was that patients with amp(1q) have a worse prognosis than patients with gain(1q). The primary endpoint was PFS, and secondary endpoints were OS and overall response rate (ORR) after first line treatment.

To test our hypothesis, data were collected on:

- baseline characteristics such as age, gender, myeloma-related organ impairment, percentage of bone marrow plasma cells, LDH and FISH results
- first line treatment and response to it
- date of progressive disease (PD) as defined by IMWG and date of death.

PFS was defined as time from MM diagnosis to PD or death by any cause and OS as time from diagnosis to death by any cause. PFS and OS were analysed for the whole cohort and separately for patients receiving ASCT or not. Statistical analyses were performed using IBM SPSS Statistics 25. For the study's primary endpoint, the Kaplan-Meier method was used to generate PFS and OS curves and statistical significance was tested by the log-rank test. When median PFS or OS were not reached, 3-years PFS and OS were used as surrogates. Univariate Cox regression analysis was applied to evaluate the impact of gain(1q) and amp(1q), as well as known factors affecting MM prognosis, such as age and HRCA, on survival and export hazard ratios (HR). Multivariate Cox regression was performed to assess if relevant variables had an independent effect on survival. Differences in ORR and

grades of response among the three study groups were evaluated in the crosstab function of SPSS, using the chi-square and the Kruskal-Wallis tests to assess for statistical significance. *P* values <0.05 were considered statistically significant for all analyses.

3.3 Paper III. Association of two specific SNVs with myeloma survival

Some associations found in GWAS fail to be replicated in subsequent studies, highlighting the need for replication of positive results in control population. The associations between MM survival and two specific SNPs, rs12374648 at chromosome 6q25 described by Johnson et al.,[185] and rs72773978 at 16p13 reported by Ziv et al.,[186] were based on relatively small samples. Thus, our group was compelled to conduct a GWAS to try and replicate these relationships.

The patient population used in this study was 871 NDMM cases diagnosed between 2005 and 2015. Clinical data were recovered by the Swedish Myeloma Registry and the study was approved by Lunds University's ethical committee. These patients were previously genotyped in another GWAS which was conducted in collaboration with two other groups from Norway and Iceland.[131] The Swedish sample set was obtained by the Swedish National Myeloma biobank in Lund. The samples were genotyped using Illumina OmniExpress-Exome chips and imputed with reference haplotypes from 1000 Genomes.

The study's hypothesis was that the two SNPs in the candidate genes negatively affect MM OS. To test this hypothesis, log rank test implemented in R (v.2.8) with adjustment for age, sex, and ISS stadium was used. Overall survival was defined as time from treatment start to death by any cause. Variants with minor allele frequency (MAF) >5% located within 1Mb of the candidate genes got also tested.

3.4 Paper IV: Subclinical infections in MGUS

Chronic antigenic stimulation has been proposed as a pathogenic mechanism leading to MGUS and MM. Although monoclonal antibodies in MGUS and MM are believed not to be active against infectious agents, there is growing evidence that in a subset of cases, M-component is targeted against pathogens such as hepatitis, herpes viruses or helicobacter pylori. Treatment against hepatitis C has in some reports led to regression of the plasma cell clone in MGUS. Yet, the possible effect of treatment of latent infections in the plasma cell clone has not been extensively studied.

We hypothesised that successful treatment of the pathogen in cases of MGUS with specificity of the monoclonal immunoglobulin against the pathogen would lead to decrease or even eradication of the plasma cell clone measured by the M-component.

To test our hypothesis, we planned a prospective study including patients with MGUS referred to the hematology departments of the hospitals of Lund, Helsingborg and Borås. The study is ongoing. To be considered eligible to participate in the study a patient must meet all the following inclusion criteria:

- diagnosis of MGUS according to IMWG criteria
- male or female older than 18 years
- ability to understand and willingness to sign an informed consent form
- measurable disease defined as a serum M-component of IgG or IgA type between 1-20 g/L.

The diagnosis of MGUS is confirmed by appropriate tests including bone marrow aspiration, skeletal x-ray when appropriate, serum and urine protein electrophoresis, complete blood count, measurement of serum albumin, calcium and creatinine following local routines. The need for skeletal x-ray is decided by the hematologist evaluating the patient.

After signing informed consent all patients are tested for infectious agents as follows:

- faecal antigen test as well as serological testing for HP
- serological testing for the detection of antibodies against HSV-1 and -2 as well as VZV
- serological testing for HCV, Hepatitis B virus (HBV) and Human immunodeficiency virus (HIV)
- serological testing for EBV and cytomegalovirus CMV

A serum sample is obtained to identify and purify the M-component by chromatography, thus separating it from polyclonal immunoglobulins. The fraction containing the specific M-component is serologically analyzed for specific antibody reactivity against HSV 1 and 2, CMV, EBV and VZV at the Department of Microbiology, Sahlgrenska University Hospital, using their routine analysis.

Patients who have tested positive for HP are treated with a combination of esomeprazole 20mg, amoxicillin 1000mg and clarithromycin 500mg twice daily for a week. Eradication of HP is confirmed two months after completion of treatment by faecal antigen test. Before faecal antigen test is conducted, patients are told to

withhold treatment with proton pump inhibitors for two weeks as this can lead to false negative results.

Patients with M-component specific against HSV-1 or -2 or VZV are treated with valacyclovir 500mg twice daily for a week for HSV or 1000mg three times/day for a week for VZV followed by maintenance treatment with Valacyclovir 500mg twice daily for 6 months.

All patients having received treatment with an antimicrobial or antiviral agent are being followed up with measurement of M-component every month for a year. All the other patients are followed up with measurement of M-component every 3 months for a year. After the first year, all the patients are going to be followed up at the hematology outpatient clinic according to local guidelines.

We plan to include around 60 patients in the project. No formal statistical analysis is planned, and the results will be descriptively reported.

4 Ethical considerations

4.1 Paper I-III

In these retrospective studies, no new investigations were done to any of the participants and their treatment or follow-up was not affected by the studies. Data were collected by reviewing medical records (paper I and II) or retrieved by the Swedish Myeloma Registry (paper III) and were stored in electronic files. Every patient included in the studies was given a study number and could not be directly identified in the electronic data file. A patient log is kept for all patients in case the clinical data needs to be reviewed. Furthermore, data were presented in a group and not individual level. Thus, no ethical problems could be identified, and patients included in the studies were not asked to sign any informed consent.

All studies were approved by an ethical committee (Dnr 2021-05205 for paper I, Dnr 2021-04155 for paper II and Dnr 2013/540 for paper III).

4.2 Paper IV

Patients accepting to participate in this study might be treated or followed-up in a different manner than clinical practice. Thus, it is important that they get thorough information according to good clinical practice about the purpose and the procedures of the study as well as the alternatives. To assure that, an informed consent from with relevant information has been produced and the patients with MGUS have a screening visit to be informed and ask questions. All study procedures are being conducted after signing informed consent. Of course, all patients reserve the right to withdraw consent at any time and for any reason.

Medication used in the study are well tolerated and serious complications are rare. All the procedures included in the study are safe and widely used in clinical routine.

Spontaneous regress of M-component in patients with MGUS is extremely rare. On the contrary there is an annual risk of progression to MM which is an incurable disease. Thus, we believe that the eventual benefit of the eradication of MGUS clone outweighs the possible risk of treatment with the medication used in the study.

The collection and processing of personal data from patients enrolled are limited to those necessary to investigate the purpose of this study. These data are collected and processed with adequate precautions to ensure confidentiality according to the requirements of the national Data Protection Agencies.

This study is approved by the regional ethical committee of Lund, Dnr 2016/240 as well as the Swedish Medical Products Agency, Dnr 2016-003770-40.

5 Results and discussion

5.1 Paper I

5.1.1 Patient characteristics

Amongst the six transplantation centers participating in the study, ninety-two patients were treated with daratumumab in induction. Daratumumab in combination with bortezomib, thalidomide and dexamethasone (DVTd) was the most common induction regimen, used in 57% of the patients while 37% of this group received the quadruplet daratumumab, bortezomib, lenalidomide and dexamethasone (DVRd). The control group consisted of 125 patients, most of whom (72%) were treated with the combination of bortezomib, lenalidomide and dexamethason (VRd) which was the previous standard of care in NDMM in Sweden.

Regarding baseline characteristics, there was no difference in patients' demographics between the two groups, as shown in Table 2. Most of the patients were male and the median age was 61 in daratumumab treated compared to 63 in non-daratumumab treated patients. Myeloma related organ damage was comparable in both groups, with a slightly higher percentage of patients in the daratumumab treated group having renal failure at diagnosis. The presence of high-risk cytogenetics and higher ISS or R-ISS stages was not significantly different between the two study groups.

When looking at important treatment parameters (Table 3), there were more patients receiving lenalidomide in the control group and thalidomide in the daratumumab treated group. A slightly higher proportion of patients in the control group were treated with alkylators as part of induction, but the use of them was limited to only 15 patients in that group compared to four patients in the daratumumab group. Similar number of patients (14% in daratumumab and 16% in control group) needed radiation therapy against myeloma lesions in both groups. Median cycles of induction were four in both groups. An important difference between the groups was the achievement of significantly deeper response grade in the daratumumab treated group. Seventy-eight (86%) of patients getting a quadruplet regimen reached ≥VGPR (very good partial response) compared to 66% of patients who did not receive daratumumab in induction ($p = 0.001$).

Table 2 Baseline characteristics

	Daratumumab treated (n=92)	**Non-dararumumab treated** (n=125)
Age		
median (range)	61 (30-72)	63 (46-74)
>60 years	50 (54%)	80 (64%)
Gender		
Female	34 (37%)	47 (38%)
MM organ impairment		
anemia	24/73 (33%)	25/102 (24%)
bone disease	76/88 (86%)	100/123 (80%)
renal failure	16/76 (21%)	10/102 (10%)
hypercalcemia	15/74 (20%)	10/99 (10%)
ISS stadium III	24/77 (31%)	20/96 (21%)
High risk cytogenetics	24/85 (28%)	37/105 (35%)

5.1.2 Stem cell yield

The study could confirm the hypothesis that fewer CD34+ stem cells would be collected in patients treated with daratumumab in induction. By using the independent samples t-test, we could show that the mean stem cell yield was significantly lower in daratumumab treated patients (5.14×10^6 vs 7.22×10^6 cells/kg, $p < 0.001$) (Figure 5). More patients in the control group (86% vs 76%, $p = 0.051$) were able to collect $>4 \times 10^6$ stem cells/kg, while five patients in daratumumab group could not mobilize stem cells at all. The use of thalidomide or lenalidomide as part of the quadruplet did not influence the outcome of stem cell collection.

Previously described risk factors for poor mobilization were evaluated by independent samples t-test to assess their effect on stem cell yield. In univariate analysis, age >60, thalidomide and radiation negatively affected stem cell yield, while the use of lenalidomide led to higher levels of collected CD34+ cells. These factors, together with daratumumab, were inserted in the multiple linear regression model. In that, only daratumumab, radiation and age>60 could maintain their significant importance on stem cell yield (Table 4).

Table 3 Treatment characteristics and response

	Daratumumab treated (n= 92)	**Non-daratumumab treated** (n=125)	***p***
Induction drugs			
Lenalidomide	34 (37%)	107 (86%)	<0.001
Thalidomide	53 (58%)	12 (10%)	<0.001
alkylators	4 (4%)	15 (12%)	0.055
Radiation	13(14%)	20(16%)	*ns*
Median induction cycles	4 (range 3-6)	4 (range 3-10)	*ns*
≥ VGPR after induction	78 (86%)	79 (66%)	0.001

5.1.3 Plerixafor use and days of apheresis

Plerixafor use as rescue was significantly higher in daratumumab treated patients, 37% vs 6% (p <0.001) (Figure 6).

Median and mean days of apheresis were significantly more in daratumumab group (2 vs 1 in median, p = 0.018 and 1.65 vs 1.42 in mean, p = 0.031). Fourteen daratumumab treated patients (15%) needed >2 days of apheresis to collect the desired amount of CD34+ cells/kg compared with only nine (7%) non-daratumumab treated (p = 0.074).

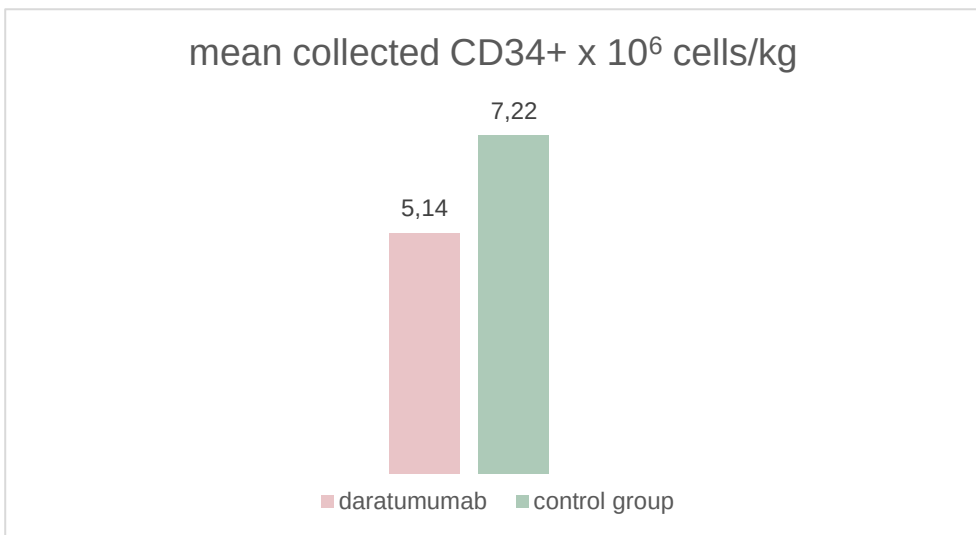


Figure 5 Stem cell yield

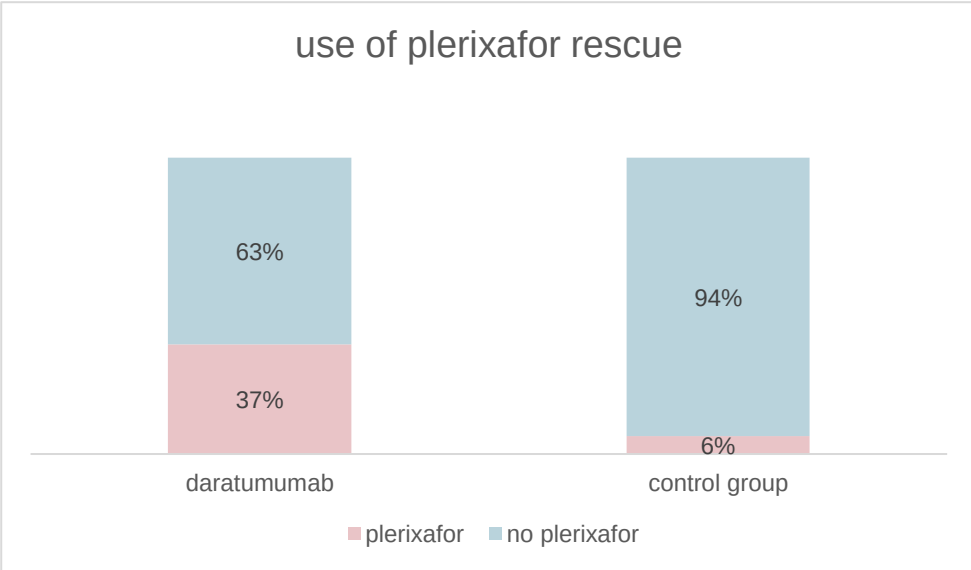


Figure 6 Plerixafor use

5.1.4 Discussion

The introduction of daratumumab in the induction regimen of younger fit patients with NDMM has shown to lead to higher rates of deep responses and longer PFS and has become the current standard of care for the treatment of this patient population.[161, 162]

Our study evaluated the impact of addition of daratumumab in standard induction regimen on important stem cell collection parameters in a large, country-wide real-world patient population with NDMM. We were able to show that adding daratumumab in induction results in statistically significant lower stem cell yield, more days of apheresis and increased rescue use of plerixafor. Daratumumab was the only factor affecting stem cell yield besides age>60 and radiation in a multiple regression analysis while other factors such as use of immunomodulators or alkylators at induction, depth of response at the time of mobilization and the number of induction cycles had no significant impact (Table 4). Off note, patients receiving daratumumab based induction had deeper responses prior to stem cell collection and ASCT, depicting the high efficacy of quadruplet regimens.

The main point of this study is the confirmation in a real-world setting of the results of the randomized phase 3 CASSIOPEIA study which compared DVTd to VTd and led to the regulatory approval of the quadruplet. In that study, the mean number of collected CD34+ x 10^6/kg was significantly lower in the DVTd arm (6.7 vs 10, p <0.0001).[175] The use of plerixafor was higher in DVTd group (22 vs 8%, p <0.0001) and patients in the DVTd group needed more days of apheresis to collect the desired

amount of stem cells (mean 1.9 vs 1.4 days). It is worth noting that patients included in the CASSIOPEIA study were in average more fit than real-world patients who undergo ASCT as they had to be <65 years old, have an eGFR>40 and adequate bone marrow function at diagnosis while these criteria do not exclude patients from ASCT outside the setting of clinical studies.

Similar results on stem cell collection parameters in real world setting have been presented by other groups. Papaiakovou et al. reported on 40 NDMM patients treated with daratumumab based induction at a single institution in Athens who had significantly lower mean values of collected stem cells and needed more often plerixafor rescue than 160 patients treated with a non-daratumumab based regimen during the same period.[176] Manjappa et al. reported a retrospective comparison of 16 patients receiving daratumumab as part of induction prior to ASCT to 92 control patients in a single institution between 2017-2020.[177] In that study there was a trend towards lower mean value of collected stem cells but not statistically significant, probably because of the low number of patients included.

Table 4 Uni- and multivariate analysis of factors affecting stem cell yield

	Mean CD34 x 10^6 cells/kg	**Univariate analysis**	**Multivariate analysis**	
		p	Coefficient B	*p*
Age >60 years				
Yes	5.87	**0.007**	-1.535	**<0.001**
No	7.03		0	
Induction Daratumumab				
Yes	5.14	**<0.001**	-2.099	**<0.001**
No	7.22		0	
Lenalidomide				
Yes	6.70	**0.025**	0	0.483
No	5.66		-0.576	
Thalidomide				
Yes	5.51	**0.014**	-0.928	0.282
No	6.69		0	
Alkylators				
Yes	7.51	0.070		
No	6.23			
Radiation				
Yes	4.66	**<0.001**	-2.207	**<0.001**
No	6.64		0	
Cyclophoshpamide mobilization				
Yes	6.32	0.796		
No	6.57			
≥4 induction cycles				
Yes	7.31	0.154		
No	6.30			

Previous studies have identified various risk factors for poor stem cell mobilization for patients with myeloma or other hematologic malignancies such as age >60, advanced stage of underlying disease, high number of prior treatment lines, prior therapy with melphalan or lenalidomide and low platelet count before mobilization.[163, 164, 170, 171, 172] In our study, lenalidomide was associated in univariate analysis with higher stem cell yield. This could probably be explained by the significantly higher percentage of patients treated with lenalidomide in the non-daratumumab group (86% vs 37%, Table 3). Lenalidomide did not significantly impact stem cell yield in multivariate analysis (Table 4).

Our study has some limitations due to the real-world setting. There are small differences between the different transplantation centres regarding dose and duration of G-CSF treatment, the decision to use plerixafor as rescue in poor mobilizers and the goal of collected stem cells due to different use of tandem transplantation or the intention to store stem cells for a second ASCT at relapse. These differences could affect to a certain extent the results of primary and secondary outcomes but, as they have not changed over time, are expected to equally influence both groups. Data on some factors that have previously been reported to affect stem cell yield, such as platelets before mobilization and time from the start of treatment to start of mobilization, have not been collected and therefore could not be evaluated in uni- and multivariate analysis. Cycles of treatment could be considered a surrogate for time from treatment start to mobilization.

In this study, we have not investigated if the lower number of collected stem cells influenced safety of ASCT. Other studies have described that a decrease in stem cell yield could lead to an increase in antibiotics use but no increase in mortality.[175, 176]

The mechanism by which daratumumab affects stem cell mobilization are poorly understood. CD34+ stem cells express CD38 to a lesser extent than plasma cells.[190] Thus, stem cell reduction after daratumumab treatment could be a possible explanation. On the other hand, a study showed that daratumumab was not toxic in vitro to mobilized CD34+ progenitor cells from myeloma patients.[191] An alternative explanation could be that daratumumab disrupts the interaction between CD38+ cells and bone marrow microenvironment, possibly impacting their capacity to effectively mobilize from the bone marrow into circulation.[192, 193] This theory is further supported by a small study showing that several genes involved in adhesive/homing stem cell function were altered after treatment with daratumumab, including downregulation of the main homing molecule CXCR4.[194]

5.1.5 Future directions

Considering the results of this study as well as other studies being conducted afterwards, there is adequate data on the negative impact of daratumumab based induction on stem cell collection. Attempts should be made to find ways to abrogate

this effect. In some transplantation centres in Sweden, there has been a shift in using mainly steady-state mobilization and planning for more frequent use of plerixafor to secure adequate stem cell collection and achieve a more predictable apheresis course. Indeed, promising results with this strategy have been recently published.[195]

It would be of importance to continue this study to assess the safety of ASCT in the era of quadruplet induction, by evaluating the time to bone marrow recovery and the rate and severity of infections in the cytopenic period following ASCT.

5.2 Paper II

5.2.1 FISH results

The most common abnormality in the 346 patients with NDMM was +1q which was detected in 159 cases (45.9%). A hundred forty eight patients (39.9%) had no abnormalities detected by FISH but it is worth pointing out that the analysis of t(11;14) was missing in 63 patients as this translocation was not a part of the FISH panel used during the first months of the period covered by this study. The frequencies of abnormalities detected is shown on Figure 7.

Gain(1q) was more frequent than amp(1q). Gain(1q) was the sole abnormality in 68% of positive cases while in the remaining cases it was co-present with del(17p), t(4;14), t(14;16) or t(11;14). Amp(1q) was accompanied by another HRCA, defined as del(17p), t(4;14) or t(14;16) in 32% of positive cases. Interestingly, no patient with amp(1q) had a concomitant t(11;14) while around 10% of gain(1q) cases had this translocation. In patients with amp(1q), del(17p) and t(14;16) were more frequently observed compared with gain(1q) positive patients (16.1% vs 7.2%, p = 0.067 for del(17p) and 11.3% vs 3.2%, p = 0.047 for t(14;16)) whereas there was no difference in the co-presence of t(4;14).

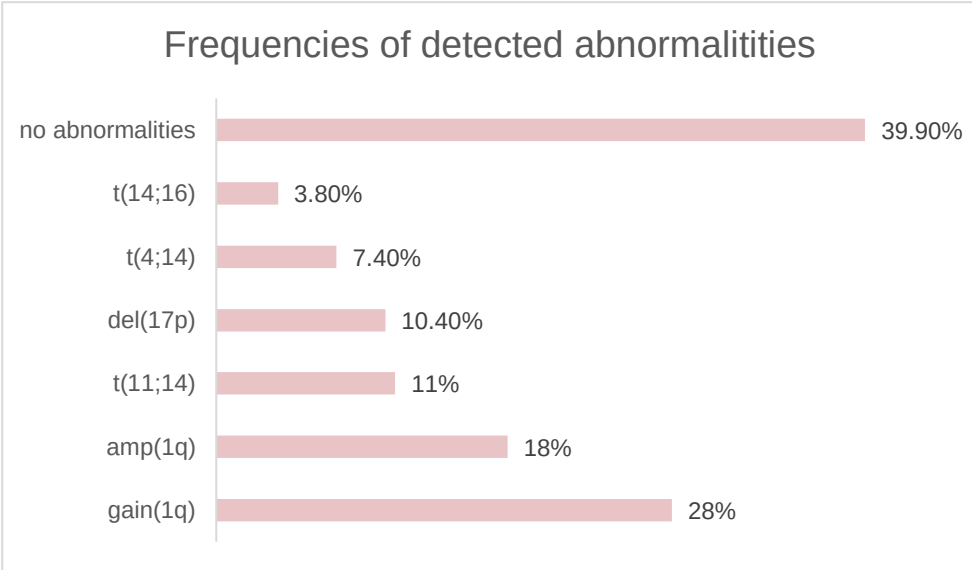


Figure 7 Frequency of abnormalities detected by FISH in the study population

5.2.2 Patient characteristics

Besides the presence of HRCAs already described, the only other statistically significant difference in baseline characteristics (Table 5) between the three study groups was higher ISS and R-ISS stages in patients with +1q. Median age and gender distribution was the same amongst groups. More frequent presence of anemia, bone disease or hypercalcemia was not observed in any of the groups whereas there was a marginal difference in the presence of renal disease which was more seen in patients with amp(1q).

5.2.3 Response to treatment

As this was a retrospective study, the choice of treatment was not guided by the presence of +1q. Ninety-eight (28.3%) patients were eligible for intensive treatment with high dose melphalan followed by ASCT. The most common induction regimen was bortezomib, lenalidomide and dexamethasone (VRd) used in 55% of patients and equally distributed between the three study groups. Patients in the non-ASCT eligible cohort were treated by a variety of regimens, mainly based on a combination of bortezomib with steroids and Imids or alkylators. There were no statistically significant differences amongst study groups, but a tendency to more frequent use of Pi+Imid combination (mainly VRd) was noted in patients with +1q.

Table 5 Baseline characteristics. Ns = not significant

	gain(1q) (n=97)	**amp(1q)** (n=62)	**no(1q)** (n=187)	***p***
Median age (range)	72 (43–89)	73 (50–88	72 (38–92)	ns
Female gender, n (%)	38 (39%)	28 (45%)	82 (44%)	ns
Myeloma-related organ impairment, n(%) Anemia Bone disease Renal disease hyperkalcemia	 34 (35%) 70 (72%) 26 (27%) 19 (20%)	 22 (36%) 45 (73%) 23 (37%) 13 (21%)	 59 (32%) 143 (77%) 36 (19%) 31 (17%)	 ns ns 0.015 ns
ISS stage III, n(%)	35 (44%)	20 (43%)	43 (32%)	0.026
HRCA, n(%)	20 (21%)	20 (32%)	24 (13%)	0.002
R-ISS stage III, n(%)	16 (21%)	8 (19%)	13 (10%)	0.015

Overall response rate (ORR) in the whole study cohort was slightly higher in patients with gain(1q) (93.8% vs 88.3 in patients with amp(1q) vs 84.2% in patients with no1q, $p = 0.068$). Looking at the groups depending on ASCT eligibility (Figures 8 and 9), no differences in response rates were seen in the ASCT cohort where, as expected, the vast majority of patients responded very well to treatment reaching ≥VGPR (93.5% in gain(1q) vs 100% in amp(1q) vs 88.7% in no1q patients, $p = 0.355$). On the contrary, patients with +1q responded better to treatment in the non-ASCT cohort, where 63.1% of gain(1q) and 58.7% of amp(1q) patients reached ≥VGPR compared to 44.6% of patients with no1q ($p = 0.032$).

In the non-ASCT cohort, higher ORR and rate of ≥VGPR were seen in patients treated with a combination of Pi + Imid compared to those treated with only Pi or Imid. When looking separately at response rates depending on 1q status, this was true for patients in the no1q group, while patients with gain(1q) had no statistically significant benefit when treated with Pi + Imid combination. Patients with amp(1q), in contrast to those with gain(1q), reached deeper responses when treated with Pi + Imid.

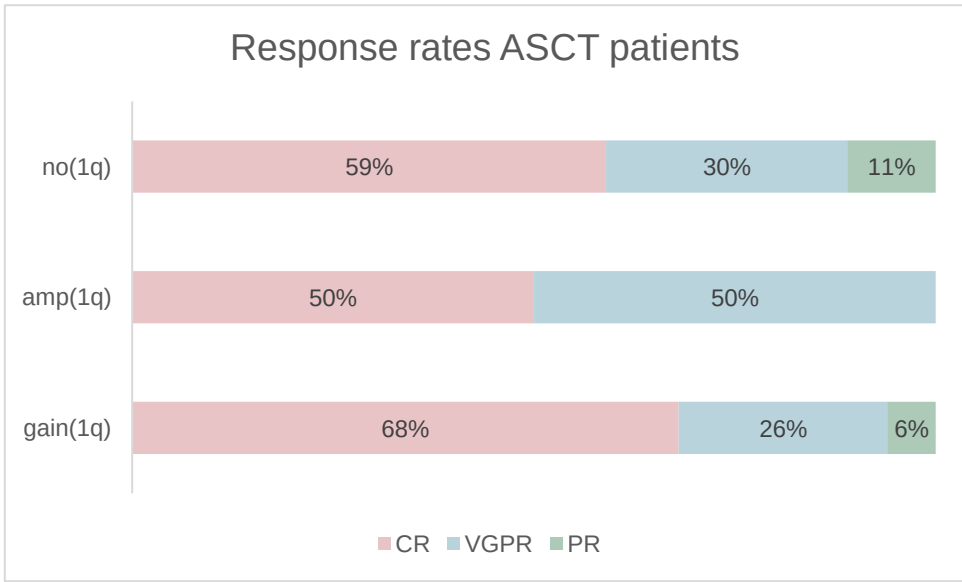


Figure 8 grade of response in ASCT patients

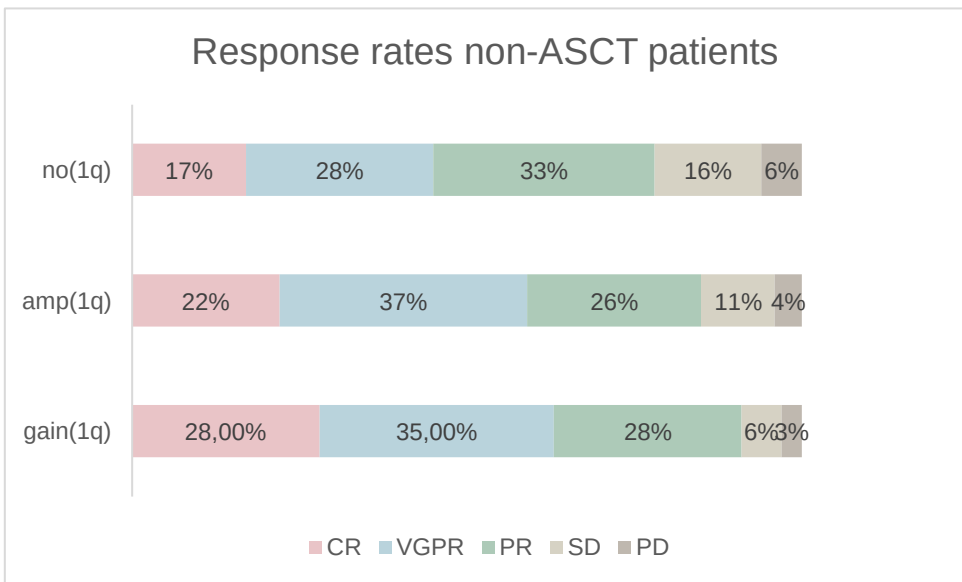


Figure 9 grade of response in non-ASCT patients

5.2.4 Progression-free survival

All patients included in the study were eligible for survival analysis. At data cut off (1st of March 2022), the median follow-up time was 22 months (range 0.1–49 months). In total, 40 (41%) patients with gain(1q), 42 (68%) with amp(1q) and 89 (48%) with no1q had either progressive disease or died.

Considering the whole cohort, patients with amp(1q) had a shorter median PFS (13.1 months, 95% confidence interval (CI) 8.2–18.1 months vs. 36.1 months, 95% CI 23.1–49.1 months for gain(1q) vs. 25.4 months, 95% CI 19.8– 31.1 months for no1q, $p = 0.005$).

As expected, due to the relatively short follow-up time, only a few patients in each group in the ASCT cohort had progressed at data cut-off. The median PFS was not reached in any of these groups. The 3-year PFS was 82% for gain(1q), 59% for amp(1q) and 64% for no1q, without any statistically significant difference (p =0.182) (Figure 10).

On the contrary, more patients had an event of progression or death in the non-ASCT group, making it possible to see some significant differences based on 1q status. Specifically, patients with amp(1q) had a shorter median PFS than those with gain(1q) or no1q (9.1 months, 95% CI 5.7–12.5 months vs. 19.5 months, 95% CI 10.6–28.4 months vs. 18.7 months, 95% CI 14.3–23 months, p =0.062) (Figure 10).

In the non-ASCT cohort, no statistically significant differences in PFS were observed in any of the 1q groups depending on different treatment combinations, although a tendency to shorter median PFS could be suspected in amp(1q) patients treated with only Pi without Imid (8.6 months with only Pi vs 15.3 months with Pi + Imid combination). When treated with Pi + Imid, patients with amp(1q) had shorter PFS than those with gain(1q) and no(1q), but this was not statistically significant (15.3 months, 95% CI 4.9–25.6 months vs. 22.0 months, 95% CI 10.2–33.8 months vs. 19.6 months, 95% CI 11.9–27.3 months, p =0.448).

Cox regression was utilized to evaluate the impact on PFS of different variables with known prognostic significance such as age, presence of HRCAs, ISS and R-ISS stage. In univariate analysis, age, amp(1q) and higher ISS or R-ISS stages were associated with a shorter PFS in the whole cohort. These parameters were then inserted in the multivariate model. Amp(1q) did not show to independently affect PFS in this model. Older age (HR 1.04, p <0.001) and higher ISS (HR 1.57, p = 0.017) or R-ISS (HR 1.61, p =0.049) stages were the only factors to significantly predict shorter PFS in the whole cohort.

In the ASCT patients, amp(1q) was strongly associated with worse PFS (HR 3.52, p =0.045) in multivariate analysis. Besides amp(1q), higher ISS or R-ISS stages were the other factors associated with shorter PFS in this cohort.

When looking at the non-ASCT group, amp(1q) was associated with a shorter PFS only in univariate Cox regression analysis. In this cohort, only the presence of HRCAs was independently associated with a shorter PFS (HR 1.80, p =0.028).

Gain(1q) did not seem to have any impact in PFS in any of the cohorts.

Patients with double-hit myeloma, defined as the co-presence of amp(1q) and another HRCA such as del(17p), t(4;14) or t(14;16), comprised a group with extremely short median PFS (8.1 months, 95% CI 6.2– 10.0 months), which was significantly shorter when compared with patients with just amp(1q) (15.6 months, 95% CI 6.2–9.9 months, p =0.01).

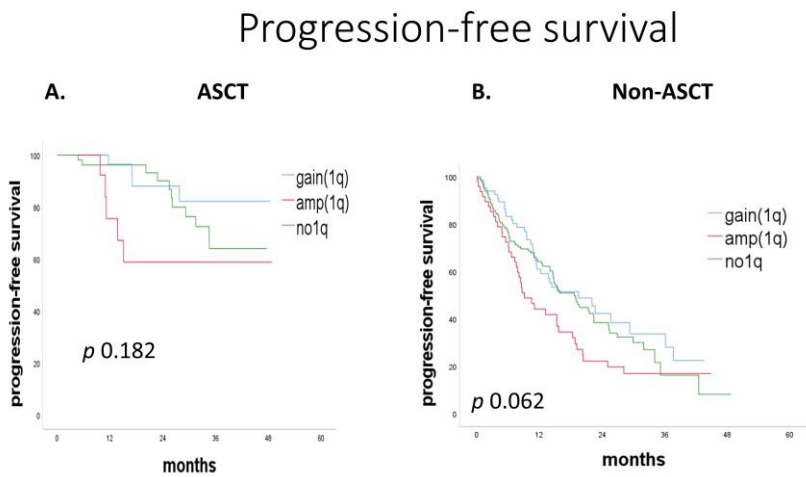


Figure 10 Progression-free surival in the ASCT (A.) and non-ASCT (B.) cohorts by 1q status

5.2.5 Overall survival

At data cut-off, 72 patients in the whole cohort had died. Median OS was not reached in any of the 1q groups. 3-year OS was though significantly shorter for amp(1q) (56% vs 76% for gain(1q) vs 80% for no1q, p = 0.003). The presence of amp(1q) could independently predict in multivariate Cox regression analysis a worse OS (HR 1.99, p = 0.039). Other factors with independent effect on OS were older age (HR 1.05, p = 0.06), ISS stage III (HR 3.02, p <0.001) and R-ISS III (HR 2.04, p = 0.030).

Similar results were observed in the ASCT cohort, where amp(1q) was strongly associated with shorter OS in multivariate analysis (HR 6.35, p =0.039). 3-year OS

was 61% for patients with amp(1q) compared to 100% for those with gain(1q) and 96% for those with no1q (p =0.006) (Figure 11).

The impact of amp(1q) on OS was less in non-ASCT patients than in the ASCT cohort. 3-year OS in this group was 54% for amp(1q), 64% for gain(1q) and 73% for no1q (p =0.042) (Figure 11). In multivariate analysis though, only ISS stage III was independently associated with shorter OS (HR 2.24, p =0.039). Treatment with a combination of Pi + Imid did not show any benefit in OS (HR 0.65, p =0.273).

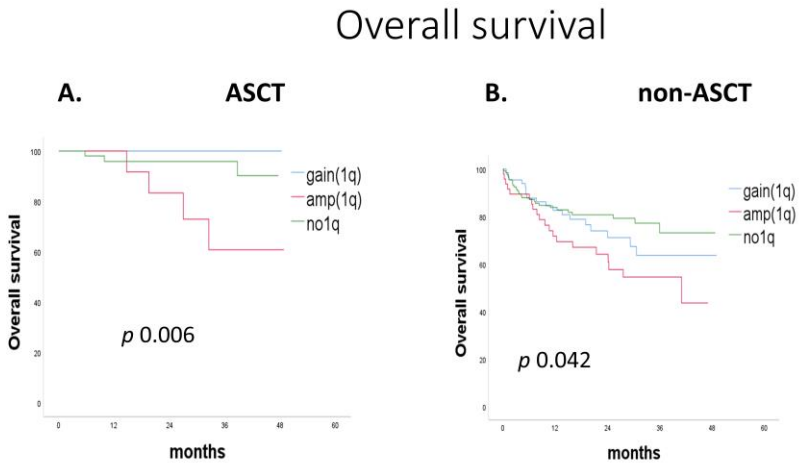


Figure 11 Overall survival in ASCT (A.) and non-ASCT (B.) cohorts by 1q status

5.2.6 Discussion

Following the rapidly evolving treatment landscape with the development of drugs with novel mechanisms of action and drug combinations, the prognosis of MM is becoming more and more favourable. However, there is still a subset of patients who progress rapidly after first-line treatment and have an inferior overall survival. Thus, it is important to try and identify these patients, preferably already at diagnosis, and develop strategies to improve their outcomes.

As described previously in this thesis, the presence of extra copies of 1q is the most common cytogenetic abnormality detected by FISH in NDMM. Although it has been extensively investigated to assess its effect on prognosis, it remains a somewhat controversial issue, especially regarding the difference in prognosis between gain(1q) and amp(1q). Furthermore, the recommended first-line treatment for NDMM patients has changed several times over the last two decades, trying to

catch-up with the results of studies investigating different treatment protocols. This means that some older studies evaluating the prognostic significance of various cytogenetic abnormalities may become outdated and the need to confirm the results in the era of newer treatment combinations evolves. In that context, we assumed that our study tries to answer an important question regarding the difference in prognostic significance between MM cases with gain(1q) and amp(1q).

The most important point of this study is that it clearly shows that, in a moderately large, homogenous, real-world NDMM population treated with modern therapy, amp(1q), but not gain(1q) can predict an inferior progression-free and overall survival (Figure 12).

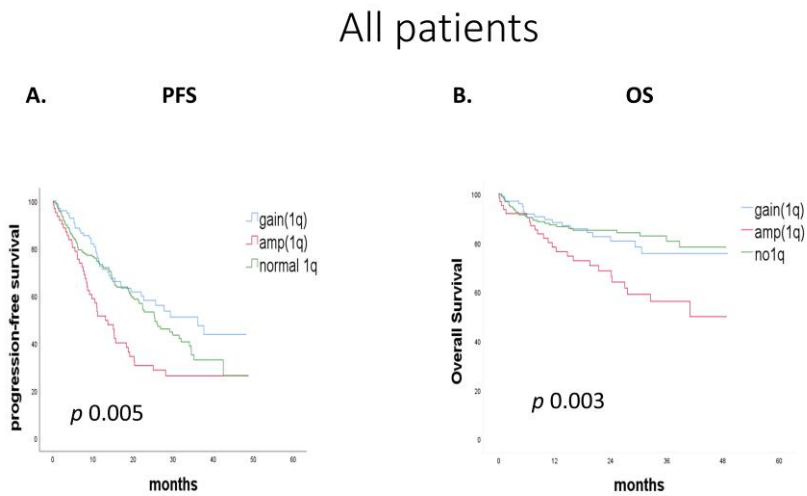


Figure 12 Progression-free (A.) and overall survival (B.) by 1q status in the whole cohort

We tried to investigate this further in patient subgroups. We found that the negative prognostic impact of amp(1q) is mainly seen in younger, fit patients receiving intensive treatment protocols including ASCT. Indeed, the presence of amp(1q) at diagnosis was associated in this study with a 3.5-fold greater risk for progression and 6.5-fold greater risk for death, regardless of other HRCAs or ISS stage.

In patients not eligible for ASCT, the impact of amp(1q) was not that prominent. Although it was associated with higher risk for progress or death in univariate analysis, it could not maintain its significance when adjusted for other factors. This could be, at least partially, attributed to the relatively short follow-up time of the study.

It is of importance to note that patients with amp(1q) responded at least equally well to first-line treatment compared with patients without +1q, reaching deep response grade. All ASCT treated patients with amp(1q) reached ≥VGPR, suggesting that it is not lack of response to treatment that accounts for the negative prognosis but probably the ability of MM cells carrying amp(1q) to rapidly develop resistance to treatment.

Gain(1q) did not impact survival in any of the study cohorts. These results come in conflict with some other published studies which reported equally adverse prognosis of +1q irrespectively of the number of extra copies.[105, 107, 108] A possible explanation for that could be the different time frame of the studies, indicating that more patients in our study received modern therapy and suggesting that current treatment protocols might have improved outcomes in gain(1q) but not in amp(1q) cases. This could further be supported by the findings of another study in a cohort treated with VRd, in which patients with gain(1q) lacking other HRCAs had a similar PFS as those with no1q, in contrary to patients with amp(1q) who had inferior PFS.[106]

The two probably most well-known cytogenetical aberrations with negative prognostic significance are del(17p) and t(4;14). In our cohort though, we could not find any statistically significant association between these abnormalities and myeloma survival, although a tendency to shorter PFS in ASCT patients carrying t(4;14) and non-ASCT patients bearing del(17p) was implied (Figures 13 and 14). The fact that, according to Swedish guidelines, special measures are taken to abrogate their previously known deleterious effect, such as tandem ASCT and Pi containing maintenance treatment in younger patients or the combination of Pi + Imid in elderly patients, might have played a crucial role in that.

When looking separately at PFS of patients with HRCAs (del(17p), t(4;14) or t(14;16)), it was clearly shown that only the co-occurrence of amp(1q), but not gain(1q), was associated with a specifically high risk of short PFS (HR 5.05, p <0.001). In the ASCT cohort, all three patients with amp(1q) and another HRCA had progressed within a year after ASCT (Figure 15). Even if three patients are not a sufficient sample size, this could indicate the extremely dismal prognosis of patients with double-hit myeloma.

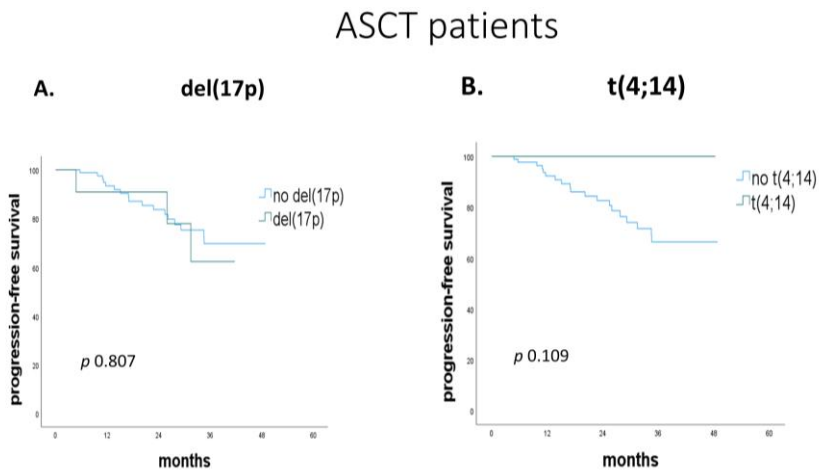


Figure 13 Progression-free survival in patients with high-risk cytogenetics treated with ASCT

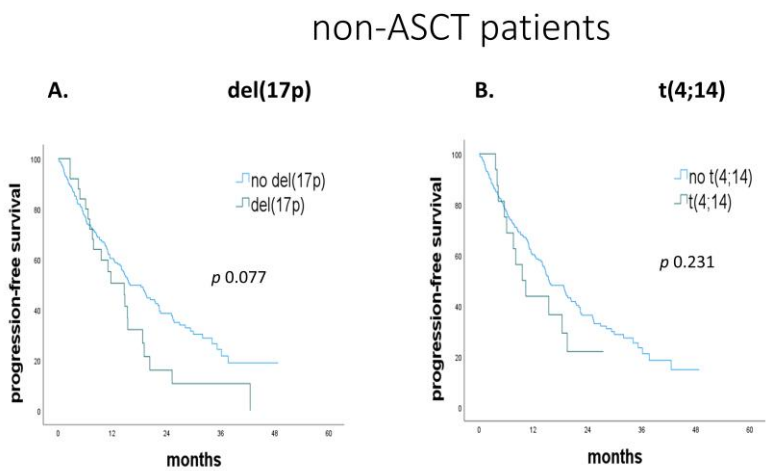


Figure 14 Progression-free survival in non-ASCT patients with high-risk cytogenetics

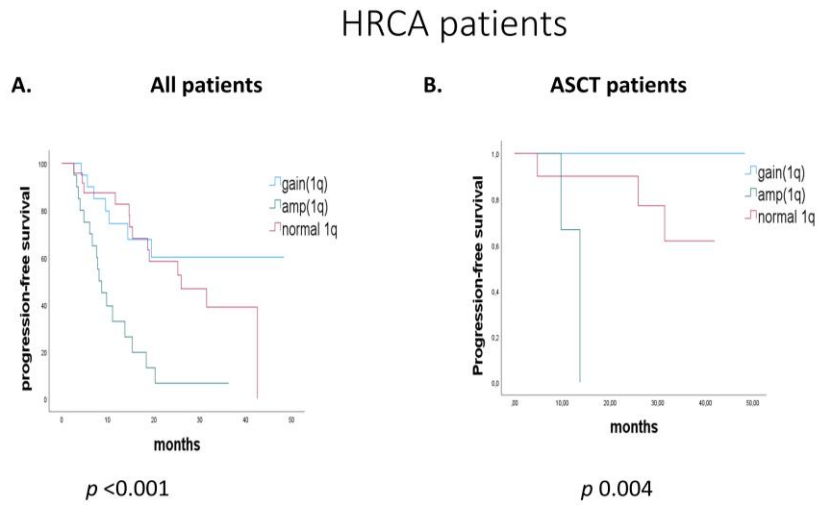


Figure 15 Progression-free survival in patients with high risk cytogenetics by 1q status. A. all patients, B. ASCT cohort

Our study has some limitations. Firstly, the follow-up time is relatively short to allow definitive conclusions on overall survival. Secondly, as it is a retrospective study, there was a great variety of different treatment regimens used, making it very difficult to assess the effect of different medications on myeloma survival and investigate if any particular drug combination can abrogate the negative prognostic significance of amp(1q). On the other hand, +1q does not influence the choice of therapy for patients included in the study. Another drawback of the study is that we did not evaluate the impact of clone size, meaning the percentage of plasma cells carrying +1q, on treatment outcomes. Furthermore, the cut-off used in the genetic department of Lund to declare NDMM patients positive for FISH abnormalities is 2%, while it is recommended by the European Myeloma network to use more conservative positive cut-off levels of 10% for fusion probes and 20% for numerical abnormalities. However, other studies addressing this issue did not find any difference depending on +1q clone size.[108]

Our study adds some more information in the existing body of literature on the importance of extra copies of 1q in NDMM patients. It points out that amp(1q) carries a significantly high risk for shorter PFS and OS and patients carrying this abnormality should be considered a distinct group with dismal prognosis and an unmet need for more efficient therapy. This highlights the necessity of clinical trials addressing this issue by investigating if novel therapies such as antiCD38 antibodies or immune effector cell therapy have the ability to improve outcomes in this patient population. On the contrary, gain(1q) does not shorten myeloma survival with current treatment protocols. As an immediate consequence of this study, the local myeloma group has come to agreement with the genetics department to separate in FISH reports of NDMM patients the presence of gain(1q) and amp(1q).

5.2.7 Future directions

Based on the results of this study, our group has come in contact with other members of the Swedish myeloma group who have expressed interest to expand the project. We are planning to retrospectively investigate the effect of different HRCA detected by FISH with focus on amp(1q) in a larger, nation-wide population during a longer period. As the antiCD38 antibody daratumumab has become an important part of first-line treatment since January 2021, we are going to evaluate if this addition can, at least partially, overcome the dismal impact of amp(1q). Of course, larger randomized trials are needed to address this question and explore novel efficient treatment combinations for this patient group.

5.3 Paper III

5.3.1 Patient characteristics

Johnson et al. found in their study that a single nucleotide polymorphism (SNP) labelled rs12374648 and located between the *MTHFD1L* and *AKAP12* genes at chromosome 6q25.1, was associated with inferior MM overall survival.[185] Similarly, Ziv et al. conducted a meta-analysis of GWAS and found a negative impact of rs72773978 near *FOPNL* at 16p13 on survival of NDMM patients.[186] To try and validate these results, the genome of 871 Swedish patients with NDMM was analysed. These patients were diagnosed between 2005 – 2015 in different regions covering the whole country. Their baseline characteristics and data on first-line treatment were retrieved by the Swedish Myeloma registry where more than 90% of myeloma patients diagnosed in the county are included. They had received treatment according to current Swedish guidelines at the time of diagnosis. Median age for the cohort was 68 years. About one third of the patients were treated with intensive treatment protocol based on ASCT. About half of the patients received a bortezomib-based regimen at first-line, while the use of Imids was limited to 26% of the patients (Table 6).

Table 6 Patient characteristics and treatment patterns

Patient number	871
Female gender, n (%)	340 (39%)
Median age	68
ISS stage III, n(%)	234 (27%)
First-line treatment, n (%)	
ASCT	283 (32.5%)
Bortezomib	427 (49%)
Immunomodulators	228 (26.2%)

5.3.2 Association between SNPs and MM survival

At data cut-off on April the 5th of 2016, median follow-up time was 39.5 months. No evidence of association between OS and either rs12374648 (HR 0.97, 95% CI 0.81–1.2, p =0.84) or rs72773978 (HR 0.98, 95% CI 0.7–1.4, p =0.93) was found (Figure 16).

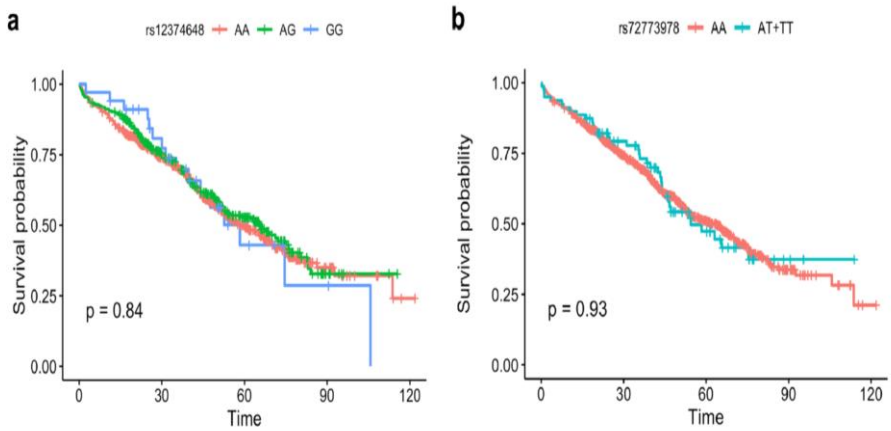


Figure 16 Overall survival by SNP in rs12374648 (a) and rs72773978 (b)

5.3.3 Discussion

Genome-wide association studies trying to find associations between SNPs and disease appearance, or outcome, need a large number of subjects in order to achieve that. Positive results need to be replicated in subsequent studies to confirm true associations. Six large-scale, high-quality GWAS have identified 24 independent DNA sequence variants predisposing for MM,[134] but the association of inborn genetic variation to MM prognosis in terms of overall survival is not well studied, as is the case in several other malignancies.[196, 197, 198]

Our study tried to replicate the association of sequence variation at two different loci to MM survival in a real-world population. We could not find any association between the reported loci and overall survival.

Single nucleotide polymorphisms on chromosome 6q25.1 were shown to negatively affect myeloma prognosis in a pooled analysis of 3256 patients being treated in four different clinical trials, reported by Johnson et al.[185] The strongest association was seen for a SNP marked by rs12748648, with a combined HR of 1.34 (95% CI 1.22–1.48, p = 4.69 x 10^{-9}). This SNP could predict inferior survival independently of age, ISS stage and first-line treatment. The authors conducted even a separate

analysis on a subset of patients with known HRCAs in myeloma cells and could show that the association was still present. Although the association in the combined analysis had a high statistical significance, it was mostly apparent in one of the four studies and no attempts to duplicate the results has been made afterwards. Rs12374648 is located between the *MTHFD1L* and *AKAP12* genes. The authors of the discovery study suggested hypomethylation at this genomic region as a possible mechanism for the impact on survival.

Possible reasons for the discrepancy between the discovery study for rs12748648 and our study could be the differences in patient characteristics. Johnson's study comprised patients included in clinical trials while our study is a population-based series. Thus, our study probably includes patients with comorbidities who are usually excluded from clinical trials. Indeed, the median age in our study is 68 years compared to 57-66 in the four studies included in the pooled analysis. The most important difference though can be seen in the treatment of the patients. As the four studies were planned to incorporate ASCT in the treatment, in total 68% of patient in Johnson's analysis received ASCT compared to only 32.5% in our real-world study. Almost double the patients in our series received bortezomib at first-line compared to the pooled analysis (49% vs 27%). Population size in our study is significantly smaller (871 vs 3256 MM cases), but comparable to the British study showing the strongest association in the pooled analysis (1163 patients).

A meta-analysis of two GWAS from USA, comprising 545 patients, indicated a strong association between a SNP located at the *FOPNL* locus on chromosome 16p.13, labelled rs72773978.[186] Patients with the minor allele had a reduction in OS of 2.7 years (HR 2.65, 95% CI 1.94 – 3.58, $p = 6 \times 10^{-10}$). The association was then replicated by the authors in 1090 MM cases, although somewhat weaker (HR 1.34, 95% CI 1.01-1.78, $p = 0.044$). Rs72773978 might induce higher expression of *FOPNL* leading to increased centrosome function, implicating this gene as the causal one for the impact on MM prognosis, as centrosome amplification has been linked to poor outcomes in MM.[199] In our study though, we could not see the same association. Differences in patient population between our real-world study and the discovery cohort which was a meta-analysis of patients included in clinical trials, as well as the low patient number in the discovery study, could potentially explain the discrepancy. Indeed, the positive result described by the authors of the discovery study when they tried to replicate the original association, was observed only in a small subset of patients (109 of totally 1090) with a very large HR, while no association could be seen in the other cohorts included in the replication attempt.

In conclusion, our study highlights the uncertainties GWAS have in trying to identify SNPs associated with MM prognosis. It indicates that the results previously published on rs12748648 and rs72773978 might be false positive, but the differences between the studies in baseline characteristics and treatment do not allow for definitive conclusions. Larger patient cohorts are probably needed to further investigate these associations.

5.4 Paper IV

5.4.1 Patient characteristics and serology

As of today, 30 patients with MGUS have been screened in the study. One case was screen failure as serology testing failed to be acquired. The median age of patients included is 63 years. Six patients (21%) have M-component of IgA type while the remaining patients have IgG M-component. The mean size of M-component is 10.3 g/l.

All patients had IgG antibodies against EBV and all but one against VZV. Nineteen (65%) patients had a previous infection with HSV and 21 (72%) with CMV. Only four tested positive for HP (Table 7).

Table 7 Baseline characteristics and results of serology testing

n	29
Age, median (range)	63 (40-86)
M-component type, n (%)	
IgG	23 (79%)
IgA	6 (21%)
M-component size, mean g/l (range)	10.3 (1-23)
Serology, n (%)	
HSV	19 (65%)
VZV	28 (97%)
EBV	29 (100%)
CMV	21 (72%)
HP	4 (14%)

5.4.2 M-component's specificity and treatment

After purification, the M-component's specificity against different viruses was analysed in 18 patients with IgG isotype. We were able to identify three cases with an M-component binding to epitope in HSV and one case binding to EBV. In one case the antibody could bind to both EBV and HSV, probably suggesting insufficient purification and contamination with polyclonal IgG. No cases with M-component targeted against VZV or CMV could be detected.

According to study protocol, two of four patients received successful HP eradication treatment, confirmed by serological assay for HP after end of treatment, but no change in M-component was seen (Table 8). The other two HP positive patients did not get eradication treatment. In the first case, the antibody titer was too low and deemed as likely false positive, while in the other case the M-component showed specificity against HSV, and the patient got treated with valacyclovir.

All three patients with an M-component targeted against HSV received treatment with valacyclovir but this did not affect the size of the M-component (Table 8).

5.4.3 Discussion

Although the theory of chronic antigen stimulation as a driver for the development of lymphoid malignancies and the association of infections and inflammatory conditions with plasma cell disorders has been investigated, there is not much evidence on their implication in the development of MGUS. In the last decade though, some studies have looked at a possible antibody function of the monoclonal antibody in patients with MGUS, SMM and MM and revealed that it might be targeted against different pathogens in a significant subset of patients. Indeed, by using multiplex infectious-antigen array (MIAA), one study found that the IgG M-component targeted a specific pathogen in 23.4% of 244 patients with plasma cell disease,[187] while another study reported even that monoclonal antibodies of IgA isotype targeted EBV in a small number of MM patients.[188] The most common pathogen targeted by the monoclonal antibody in those studies was EBV, followed by HSV, enterovirus, VZV, H. pylori, CMV, HCV and HBV. Before testing for its specificity against these agents, purification of the M-component was conducted. In cases of MGUS or MM with an M-component targeted against a specific pathogen, eradication of the infection could be an attractive approach to remove the driving force, inducing a possible decline in or disappearance of the plasma cell clone, thus improving the prognosis of the disease. This hypothesis is supported by previously reported cases of regression of M-component after successful treatment of HCV.[189]

Table 8 Effect of treatment on M-component size

	M-component size (g/l)	
	Treatment start	End of follow-up
HP eradication treatment		
Patient 1	12	12
Patient 2	13	12
Antiviral treatment		
Patient 1	3	3
Patient 2	12	14
Patient 3	14	17

In our study, purification of an IgG M-component and analysis of its specificity against viruses was successful in 18 patients. We were able to identify that the purified antibody was targeted against HSV in three patients and EBV in one patient. In one case, we could see that the M-component was binding to both EBV and HSV, most probably due to contamination with polyclonal antibodies during the purification attempt.

Four of the included patients tested positive for H. pylori and two of them received eradication treatment without any impact on M-component's size. Treatment with valacyclovir in the three patients with a specificity of the monoclonal antibody against HSV did not affect its size either. This could probably be explained by the fact that HSV establishes latency inside the host's cells where it is protected by antiviral medication and cannot be eradicated.[200]

Patient recruitment proved to be more difficult than expected. Although MGUS is a common disorder, patients with small M-components are usually being followed by primary care physicians and not being referred to a hematology department. Inclusion was also stopped during the covid19 pandemic to avoid the risk of patients being infected with the virus during visits to the hospital.

The study is ongoing with a plan to recruit up to 60 patients with MGUS.

6 Concluding remarks

In this thesis, different factors affecting the prognosis of patients with newly diagnosed multiple myeloma or MGUS have been investigated, including cytogenetic variants in NDMM cases as well as modern medication against the disease and the role of antigenic stimulation in the establishment of MGUS.

The main conclusions from the projects comprising the thesis are as follows:

6.1.1 Paper I

- Incorporation of daratumumab in the induction therapy of patients with NDMM leads to significantly worse outcomes of stem cell collection. This drawback can though be compensated by the improved outcomes of patients receiving daratumumab-based first-line treatment in terms of response grade, as shown even in this paper, as well as longer progression-free survival, as depicted in randomized trials.
- Physicians treating patients with intention to transplant need to take into consideration daratumumab's effect on stem cell mobilization and develop strategies to ensure adequate stem cell collection. These strategies could include more extended use of effective mobilization factors such as plerixafor.

6.1.2 Paper II

- The number of extra copies of the long arm of chromosome 1 in myeloma cells is an important prognostic factor in patients with NDMM. Amplification of 1q leads to significantly shorter survival in this patient population, which is more prominent in younger fit patients eligible for autologous stem cell transplantation. Outcomes tend to be worse when amp(1q) is co-present with other high-risk cytogenetic abnormalities such as t(4;14) or del(17p). In contrast, only one extra 1q copy does not seem to affect prognosis in NDMM, at least in the era of modern therapy.
- The presence of extra copies of 1q, and specifically of gain or amp(1q), should be investigated in all cases of NDMM, and taken into account when assessing the prognosis of an individual patient.

- Treatment protocols should be explored to attempt to improve the prognosis of patients with amp(1q), probably incorporating modern agents with different mechanism of action, preferably in the setting of randomized clinical trials.

6.1.3 Paper III

- As MM is a relatively rare disease, GWAS studies investigating associations between single nucleotide polymorphisms and MM prognosis face the difficulty of including enough patients to draw definitive conclusions.
- Validation of results presented in such studies is critical to ensure that these are not false-positive. The validation itself is though also a task with certain limitations, as patient cohorts might quite differ in terms of baseline characteristics or treatment.
- Our study could not confirm previously reported data, highlighting the above mentioned limitations.

6.1.4 Paper IV

- Although a clear association between infections and chronic inflammation with MGUS has been reported, the role of pathogens as drivers for the establishment of the disorder has not been thoroughly investigated, especially regarding M-component's function as an ineffective antibody against them.
- Purification of M-component is a demanding process, making such efforts quite challenging.
- We were able to show in a small MGUS series that the M-component might be targeted against different infectious agents, implying their importance on the development of the disease. Further studies in larger cohorts are needed to shed more light in this matter.
- Eradication of latent viruses is not possible in many cases, thus making it difficult to evaluate possible impact of treating a latent infection on the size of M-component.

7 Acknowledgements

I would like to express my gratitude to anyone contributing to the research and supporting me through this work. Special thanks to:

Markus Hansson, my main supervisor, for giving me the opportunity to become a Phd student and guiding me through the process of research by providing valuable advice.

Stig Lenhoff, my co-supervisor, for sharing his extensive knowledge in the field of myeloma.

Love Tätting, Mikael Lisak, Kristina Carlson, Jacob Crafoord, Cecilie Blimark, Antonio Santamaria, all members of the Swedish myeloma group, for contributing with data and giving me the chance to present my projects at the regular meetings of the group and provide insightful comments on them.

Linda Olsson-Arvidsson and **Bertil Johansson**, colleagues at the Genetics department of Lund, for providing the data on FISH abnormalities in the 1q project and coming up with valuable ideas to improve the project.

Conny Karlsson, hematologist in Halmstad's hospital, for helping with chart review of patients for the 1q project.

Mina Ali, Anna-Karin Wihlborg, Ingemar Turesson, Urban Gullberg and Björn Nilsson, for the co-operation in Paper III.

Per Axelsson, Ulf-Henrik Mellqvist and Ljupco Veskovski, hematologists contributing to patient recruitment in the study evaluating the role of latent infections in the pathogenesis of MGUS. **Maria Hector,** for her valuable help planning this study. **Lena Gunnarsson, Jan-Åke Liljeqvist** and **Thomas Hellmark,** for conducting the laboratory analyses included in the project.

All my colleagues and staff at the hematology departments of Lund and Malmö for creating a friendly and inspiring environment to work in.

The Swedish blood cancer patient association (**Blodcancerförbundet**), for assisting with financial grants to my research.

My wife, **Elpida**, and my children, **Nikolas** and **Emma**, for providing me with love and support through all those years.

8 References

1. Bonaud, A., Khamyath, M. & Espeli, M. The cellular biology of plasma cells: Unmet challenges and opportunities. *Immunol Lett* **254**, 6-12 (2023).
2. Young, C. & Brink, R. The unique biology of germinal center B cells. *Immunity* **54**, 1652-1664 (2021).
3. Okada, T. *et al.* Antigen-engaged B cells undergo chemotaxis toward the T zone and form motile conjugates with helper T cells. *PLoS Biol* **3**, e150 (2005).
4. Mesin, L., Ersching, J. & Victora, G.D. Germinal Center B Cell Dynamics. *Immunity* **45**, 471-482 (2016).
5. Finkin, S., Hartweger, H., Oliveira, T.Y., Kara, E.E. & Nussenzweig, M.C. Protein Amounts of the MYC Transcription Factor Determine Germinal Center B Cell Division Capacity. *Immunity* **51**, 324-336 e325 (2019).
6. Calado, D.P. *et al.* The cell-cycle regulator c-Myc is essential for the formation and maintenance of germinal centers. *Nat Immunol* **13**, 1092-1100 (2012).
7. Ersching, J. *et al.* Germinal Center Selection and Affinity Maturation Require Dynamic Regulation of mTORC1 Kinase. *Immunity* **46**, 1045-1058 e1046 (2017).
8. Nera, K.P. & Lassila, O. Pax5--a critical inhibitor of plasma cell fate. *Scand J Immunol* **64**, 190-199 (2006).
9. Klein, U. *et al.* Transcription factor IRF4 controls plasma cell differentiation and class-switch recombination. *Nat Immunol* **7**, 773-782 (2006).
10. Lindquist, R.L., Niesner, R.A. & Hauser, A.E. In the Right Place, at the Right Time: Spatiotemporal Conditions Determining Plasma Cell Survival and Function. *Front Immunol* **10**, 788 (2019).
11. Hargreaves, D.C. *et al.* A coordinated change in chemokine responsiveness guides plasma cell movements. *J Exp Med* **194**, 45-56 (2001).
12. Halliley, J.L. *et al.* Long-Lived Plasma Cells Are Contained within the CD19(-)CD38(hi)CD138(+) Subset in Human Bone Marrow. *Immunity* **43**, 132-145 (2015).
13. Waldenstrom, J. Studies on conditions associated with disturbed gamma globulin formation (gammopathies). *Harvey Lect* **56**, 211-231 (1960).
14. Rajkumar, S.V. Updated Diagnostic Criteria and Staging System for Multiple Myeloma. *Am Soc Clin Oncol Educ Book* **35**, e418-423 (2016).
15. Turesson, I. *et al.* Monoclonal gammopathy of undetermined significance and risk of lymphoid and myeloid malignancies: 728 cases followed up to 30 years in Sweden. *Blood* **123**, 338-345 (2014).

16. Kyle, R.A. *et al.* Long-Term Follow-up of Monoclonal Gammopathy of Undetermined Significance. *N Engl J Med* **378**, 241-249 (2018).
17. Kyle, R.A., Finkelstein, S., Elveback, L.R. & Kurland, L.T. Incidence of monoclonal proteins in a Minnesota community with a cluster of multiple myeloma. *Blood* **40**, 719-724 (1972).
18. Saleun, J.P., Vicariot, M., Deroff, P. & Morin, J.F. Monoclonal gammopathies in the adult population of Finistère, France. *J Clin Pathol* **35**, 63-68 (1982).
19. Kyle, R.A. *et al.* Prevalence of monoclonal gammopathy of undetermined significance. *N Engl J Med* **354**, 1362-1369 (2006).
20. Iwanaga, M., Tagawa, M., Tsukasaki, K., Kamihira, S. & Tomonaga, M. Prevalence of monoclonal gammopathy of undetermined significance: study of 52,802 persons in Nagasaki City, Japan. *Mayo Clin Proc* **82**, 1474-1479 (2007).
21. Aguzzi, F., Bergami, M.R., Gasparro, C., Bellotti, V. & Merlini, G. Occurrence of monoclonal components in general practice: clinical implications. *Eur J Haematol* **48**, 192-195 (1992).
22. Landgren, O. *et al.* Risk of monoclonal gammopathy of undetermined significance (MGUS) and subsequent multiple myeloma among African American and white veterans in the United States. *Blood* **107**, 904-906 (2006).
23. Malacrida, V., De Francesco, D., Banfi, G., Porta, F.A. & Riches, P.G. Laboratory investigation of monoclonal gammopathy during 10 years of screening in a general hospital. *J Clin Pathol* **40**, 793-797 (1987).
24. Wadhera, R.K. & Rajkumar, S.V. Prevalence of monoclonal gammopathy of undetermined significance: a systematic review. *Mayo Clin Proc* **85**, 933-942 (2010).
25. Landgren, O. *et al.* Prevalence of myeloma precursor state monoclonal gammopathy of undetermined significance in 12372 individuals 10-49 years old: a population-based study from the National Health and Nutrition Examination Survey. *Blood Cancer J* **7**, e618 (2017).
26. Therneau, T.M. *et al.* Incidence of monoclonal gammopathy of undetermined significance and estimation of duration before first clinical recognition. *Mayo Clin Proc* **87**, 1071-1079 (2012).
27. Murray, D. *et al.* Detection and prevalence of monoclonal gammopathy of undetermined significance: a study utilizing mass spectrometry-based monoclonal immunoglobulin rapid accurate mass measurement. *Blood Cancer J* **9**, 102 (2019).
28. El-Khoury, H. *et al.* Prevalence of monoclonal gammopathies and clinical outcomes in a high-risk US population screened by mass spectrometry: a multicentre cohort study. *Lancet Haematol* **9**, e340-e349 (2022).
29. Cohen, H.J., Crawford, J., Rao, M.K., Pieper, C.F. & Currie, M.S. Racial differences in the prevalence of monoclonal gammopathy in a community-based sample of the elderly. *Am J Med* **104**, 439-444 (1998).
30. Landgren, O. *et al.* Prevalence of monoclonal gammopathy of undetermined significance among men in Ghana. *Mayo Clin Proc* **82**, 1468-1473 (2007).

31. Landgren, O. *et al.* Risk of plasma cell and lymphoproliferative disorders among 14621 first-degree relatives of 4458 patients with monoclonal gammopathy of undetermined significance in Sweden. *Blood* **114**, 791-795 (2009).
32. Vachon, C.M. *et al.* Increased risk of monoclonal gammopathy in first-degree relatives of patients with multiple myeloma or monoclonal gammopathy of undetermined significance. *Blood* **114**, 785-790 (2009).
33. Weinhold, N. *et al.* Inherited genetic susceptibility to monoclonal gammopathy of unknown significance. *Blood* **123**, 2513-2517; quiz 2593 (2014).
34. Larsson, S.C. & Wolk, A. Body mass index and risk of multiple myeloma: a meta-analysis. *Int J Cancer* **121**, 2512-2516 (2007).
35. Landgren, O. *et al.* Obesity is associated with an increased risk of monoclonal gammopathy of undetermined significance among black and white women. *Blood* **116**, 1056-1059 (2010).
36. Thordardottir, M. *et al.* Obesity and risk of monoclonal gammopathy of undetermined significance and progression to multiple myeloma: a population-based study. *Blood Adv* **1**, 2186-2192 (2017).
37. Lindqvist, E.K. *et al.* Personal and family history of immune-related conditions increase the risk of plasma cell disorders: a population-based study. *Blood* **118**, 6284-6291 (2011).
38. McShane, C.M. *et al.* Prior autoimmune disease and risk of monoclonal gammopathy of undetermined significance and multiple myeloma: a systematic review. *Cancer Epidemiol Biomarkers Prev* **23**, 332-342 (2014).
39. Brown, L.M., Gridley, G., Check, D. & Landgren, O. Risk of multiple myeloma and monoclonal gammopathy of undetermined significance among white and black male United States veterans with prior autoimmune, infectious, inflammatory, and allergic disorders. *Blood* **111**, 3388-3394 (2008).
40. Raja, K.R., Kovarova, L. & Hajek, R. Review of phenotypic markers used in flow cytometric analysis of MGUS and MM, and applicability of flow cytometry in other plasma cell disorders. *Br J Haematol* **149**, 334-351 (2010).
41. Zingone, A. & Kuehl, W.M. Pathogenesis of monoclonal gammopathy of undetermined significance and progression to multiple myeloma. *Semin Hematol* **48**, 4-12 (2011).
42. Mikulasova, A. *et al.* Genomewide profiling of copy-number alteration in monoclonal gammopathy of undetermined significance. *Eur J Haematol* **97**, 568-575 (2016).
43. van Nieuwenhuijzen, N., Spaan, I., Raymakers, R. & Peperzak, V. From MGUS to Multiple Myeloma, a Paradigm for Clonal Evolution of Premalignant Cells. *Cancer Res* **78**, 2449-2456 (2018).
44. Chiecchio, L. *et al.* Timing of acquisition of deletion 13 in plasma cell dyscrasias is dependent on genetic context. *Haematologica* **94**, 1708-1713 (2009).
45. Bergsagel, P.L. *et al.* Cyclin D dysregulation: an early and unifying pathogenic event in multiple myeloma. *Blood* **106**, 296-303 (2005).

46. Chesi, M. *et al.* AID-dependent activation of a MYC transgene induces multiple myeloma in a conditional mouse model of post-germinal center malignancies. *Cancer Cell* **13**, 167-180 (2008).
47. Shou, Y. *et al.* Diverse karyotypic abnormalities of the c-myc locus associated with c-myc dysregulation and tumor progression in multiple myeloma. *Proc Natl Acad Sci U S A* **97**, 228-233 (2000).
48. Walker, B.A. *et al.* Translocations at 8q24 juxtapose MYC with genes that harbor superenhancers resulting in overexpression and poor prognosis in myeloma patients. *Blood Cancer J* **4**, e191 (2014).
49. Barwick, B.G., Gupta, V.A., Vertino, P.M. & Boise, L.H. Cell of Origin and Genetic Alterations in the Pathogenesis of Multiple Myeloma. *Front Immunol* **10**, 1121 (2019).
50. Pawlyn, C. & Morgan, G.J. Evolutionary biology of high-risk multiple myeloma. *Nat Rev Cancer* **17**, 543-556 (2017).
51. Lionetti, M. *et al.* Molecular spectrum of TP53 mutations in plasma cell dyscrasias by next generation sequencing: an Italian cohort study and overview of the literature. *Oncotarget* **7**, 21353-21361 (2016).
52. Jovanovic, K.K. *et al.* Deregulation and Targeting of TP53 Pathway in Multiple Myeloma. *Front Oncol* **8**, 665 (2018).
53. Liu, P. *et al.* Activating mutations of N- and K-ras in multiple myeloma show different clinical associations: analysis of the Eastern Cooperative Oncology Group Phase III Trial. *Blood* **88**, 2699-2706 (1996).
54. Walker, B.A. *et al.* Mutational Spectrum, Copy Number Changes, and Outcome: Results of a Sequencing Study of Patients With Newly Diagnosed Myeloma. *J Clin Oncol* **33**, 3911-3920 (2015).
55. Bisht, K. *et al.* Chromosomal 1q21 abnormalities in multiple myeloma: a review of translational, clinical research, and therapeutic strategies. *Expert Rev Hematol* **14**, 1099-1114 (2021).
56. Zhan, F. *et al.* Gene-expression signature of benign monoclonal gammopathy evident in multiple myeloma is linked to good prognosis. *Blood* **109**, 1692-1700 (2007).
57. Dhodapkar, M.V. *et al.* Clinical, genomic, and imaging predictors of myeloma progression from asymptomatic monoclonal gammopathies (SWOG S0120). *Blood* **123**, 78-85 (2014).
58. Mouhieddine, T.H., Weeks, L.D. & Ghobrial, I.M. Monoclonal gammopathy of undetermined significance. *Blood* **133**, 2484-2494 (2019).
59. Slany, A. *et al.* Extracellular matrix remodeling by bone marrow fibroblast-like cells correlates with disease progression in multiple myeloma. *J Proteome Res* **13**, 844-854 (2014).
60. Glavey, S.V. *et al.* Proteomic characterization of human multiple myeloma bone marrow extracellular matrix. *Leukemia* **31**, 2426-2434 (2017).
61. Podar, K., Richardson, P.G., Hideshima, T., Chauhan, D. & Anderson, K.C. The malignant clone and the bone-marrow environment. *Best Pract Res Clin Haematol* **20**, 597-612 (2007).

62. Colla, S. *et al.* Low bone marrow oxygen tension and hypoxia-inducible factor-1alpha overexpression characterize patients with multiple myeloma: role on the transcriptional and proangiogenic profiles of CD138(+) cells. *Leukemia* **24**, 1967-1970 (2010).
63. Azab, A.K. *et al.* Hypoxia promotes dissemination of multiple myeloma through acquisition of epithelial to mesenchymal transition-like features. *Blood* **119**, 5782-5794 (2012).
64. Kazandjian, D. Multiple myeloma epidemiology and survival: A unique malignancy. *Semin Oncol* **43**, 676-681 (2016).
65. Sant, M. *et al.* Incidence of hematologic malignancies in Europe by morphologic subtype: results of the HAEMACARE project. *Blood* **116**, 3724-3734 (2010).
66. Siegel, R.L., Miller, K.D. & Jemal, A. Cancer statistics, 2016. *CA Cancer J Clin* **66**, 7-30 (2016).
67. group, s.m. Myelom, kvalitetsregisterrapport: Regionalt cancercentrum väst; 2023.
68. Cid Ruzafa, J. *et al.* Patient population with multiple myeloma and transitions across different lines of therapy in the USA: an epidemiologic model. *Pharmacoepidemiol Drug Saf* **25**, 871-879 (2016).
69. Durie, B.G. & Salmon, S.E. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. *Cancer* **36**, 842-854 (1975).
70. Greipp, P.R. *et al.* International staging system for multiple myeloma. *J Clin Oncol* **23**, 3412-3420 (2005).
71. Cui, C., Shu, W. & Li, P. Fluorescence In situ Hybridization: Cell-Based Genetic Diagnostic and Research Applications. *Front Cell Dev Biol* **4**, 89 (2016).
72. Cook, J.R., Hartke, M., Pettay, J. & Tubbs, R.R. Fluorescence in situ hybridization analysis of immunoglobulin heavy chain translocations in plasma cell myeloma using intact paraffin sections and simultaneous CD138 immunofluorescence. *J Mol Diagn* **8**, 459-465 (2006).
73. Smadja, N.V. *et al.* Further cytogenetic characterization of multiple myeloma confirms that 14q32 translocations are a very rare event in hyperdiploid cases. *Genes Chromosomes Cancer* **38**, 234-239 (2003).
74. Ross, F.M. *et al.* Report from the European Myeloma Network on interphase FISH in multiple myeloma and related disorders. *Haematologica* **97**, 1272-1277 (2012).
75. Sato, S., Kamata, W., Okada, S. & Tamai, Y. Clinical and prognostic significance of t(4;14) translocation in multiple myeloma in the era of novel agents. *Int J Hematol* **113**, 207-213 (2021).
76. Nemec, P. *et al.* Complex karyotype and translocation t(4;14) define patients with high-risk newly diagnosed multiple myeloma: results of CMG2002 trial. *Leuk Lymphoma* **53**, 920-927 (2012).
77. Kalff, A. & Spencer, A. The t(4;14) translocation and FGFR3 overexpression in multiple myeloma: prognostic implications and current clinical strategies. *Blood Cancer J* **2**, e89 (2012).

78. Thakurta, A. *et al.* High subclonal fraction of 17p deletion is associated with poor prognosis in multiple myeloma. *Blood* **133**, 1217-1221 (2019).
79. Corre, J. *et al.* del(17p) without TP53 mutation confers a poor prognosis in intensively treated newly diagnosed patients with multiple myeloma. *Blood* **137**, 1192-1195 (2021).
80. Dimopoulos, M.A., Barlogie, B., Smith, T.L. & Alexanian, R. High serum lactate dehydrogenase level as a marker for drug resistance and short survival in multiple myeloma. *Ann Intern Med* **115**, 931-935 (1991).
81. Terpos, E. *et al.* High serum lactate dehydrogenase adds prognostic value to the international myeloma staging system even in the era of novel agents. *Eur J Haematol* **85**, 114-119 (2010).
82. Palumbo, A. *et al.* Revised International Staging System for Multiple Myeloma: A Report From International Myeloma Working Group. *J Clin Oncol* **33**, 2863-2869 (2015).
83. D'Agostino, M. *et al.* Second Revision of the International Staging System (R2-ISS) for Overall Survival in Multiple Myeloma: A European Myeloma Network (EMN) Report Within the HARMONY Project. *J Clin Oncol* **40**, 3406-3418 (2022).
84. Hanamura, I. *et al.* Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantation. *Blood* **108**, 1724-1732 (2006).
85. Schmidt, T.M., Fonseca, R. & Usmani, S.Z. Chromosome 1q21 abnormalities in multiple myeloma. *Blood Cancer J* **11**, 83 (2021).
86. Merz, M. *et al.* Cytogenetic abnormalities in monoclonal gammopathy of undetermined significance. *Leukemia* **32**, 2717-2719 (2018).
87. Hanamura, I. Gain/Amplification of Chromosome Arm 1q21 in Multiple Myeloma. *Cancers (Basel)* **13** (2021).
88. Neben, K. *et al.* Progression in smoldering myeloma is independently determined by the chromosomal abnormalities del(17p), t(4;14), gain 1q, hyperdiploidy, and tumor load. *J Clin Oncol* **31**, 4325-4332 (2013).
89. Bustoros, M. *et al.* Genomic Profiling of Smoldering Multiple Myeloma Identifies Patients at a High Risk of Disease Progression. *J Clin Oncol* **38**, 2380-2389 (2020).
90. Sawyer, J.R. *et al.* An acquired high-risk chromosome instability phenotype in multiple myeloma: Jumping 1q Syndrome. *Blood Cancer J* **9**, 62 (2019).
91. Sawyer, J.R. *et al.* Evidence for a novel mechanism for gene amplification in multiple myeloma: 1q12 pericentromeric heterochromatin mediates breakage-fusion-bridge cycles of a 1q12 approximately 23 amplicon. *Br J Haematol* **147**, 484-494 (2009).
92. Chakraborty, R. & Gertz, M.A. +1q: amplifying the bad genes in myeloma. *Leuk Lymphoma* **58**, 1771-1773 (2017).
93. Chang, H. *et al.* CKS1B nuclear expression is inversely correlated with p27Kip1 expression and is predictive of an adverse survival in patients with multiple myeloma. *Haematologica* **95**, 1542-1547 (2010).

94. Shaughnessy, J. Amplification and overexpression of CKS1B at chromosome band 1q21 is associated with reduced levels of p27Kip1 and an aggressive clinical course in multiple myeloma. *Hematology* **10 Suppl 1**, 117-126 (2005).
95. Chang, H. *et al.* Multiple myeloma patients with CKS1B gene amplification have a shorter progression-free survival post-autologous stem cell transplantation. *Br J Haematol* **135**, 486-491 (2006).
96. Shi, L. *et al.* Over-expression of CKS1B activates both MEK/ERK and JAK/STAT3 signaling pathways and promotes myeloma cell drug-resistance. *Oncotarget* **1**, 22-33 (2010).
97. Zhang, B., Gojo, I. & Fenton, R.G. Myeloid cell factor-1 is a critical survival factor for multiple myeloma. *Blood* **99**, 1885-1893 (2002).
98. Samo, A.A. *et al.* MCL1 gene co-expression module stratifies multiple myeloma and predicts response to proteasome inhibitor-based therapy. *Genes Chromosomes Cancer* **57**, 420-429 (2018).
99. Peperzak, V. *et al.* Mcl-1 is essential for the survival of plasma cells. *Nat Immunol* **14**, 290-297 (2013).
100. Gupta, V.A. *et al.* Bone marrow microenvironment-derived signals induce Mcl-1 dependence in multiple myeloma. *Blood* **129**, 1969-1979 (2017).
101. Teoh, P.J. *et al.* Aberrant hyperediting of the myeloma transcriptome by ADAR1 confers oncogenicity and is a marker of poor prognosis. *Blood* **132**, 1304-1317 (2018).
102. Inoue, J. *et al.* Overexpression of PDZK1 within the 1q12-q22 amplicon is likely to be associated with drug-resistance phenotype in multiple myeloma. *Am J Pathol* **165**, 71-81 (2004).
103. Ota, A. *et al.* Novel Interleukin-6 Inducible Gene PDZ-Binding Kinase Promotes Tumor Growth of Multiple Myeloma Cells. *J Interferon Cytokine Res* **40**, 389-405 (2020).
104. Marchesini, M. *et al.* ILF2 Is a Regulator of RNA Splicing and DNA Damage Response in 1q21-Amplified Multiple Myeloma. *Cancer Cell* **32**, 88-100 e106 (2017).
105. Abdallah, N. *et al.* Clinical characteristics and treatment outcomes of newly diagnosed multiple myeloma with chromosome 1q abnormalities. *Blood Adv* **4**, 3509-3519 (2020).
106. Schmidt, T.M. *et al.* Gain of Chromosome 1q is associated with early progression in multiple myeloma patients treated with lenalidomide, bortezomib, and dexamethasone. *Blood Cancer J* **9**, 94 (2019).
107. Xu, J. *et al.* The paradoxical prognostic role of 1q21 Gain/Amplification in multiple myeloma: every coin has two sides. *Leuk Lymphoma* **61**, 2351-2364 (2020).
108. Kastritis, E. *et al.* Chromosome 1q21 aberrations identify ultra high-risk myeloma with prognostic and clinical implications. *Am J Hematol* **97**, 1142-1149 (2022).
109. Neben, K. *et al.* Administration of bortezomib before and after autologous stem cell transplantation improves outcome in multiple myeloma patients with deletion 17p. *Blood* **119**, 940-948 (2012).

110. Avet-Loiseau, H. *et al.* Long-term analysis of the IFM 99 trials for myeloma: cytogenetic abnormalities [t(4;14), del(17p), 1q gains] play a major role in defining long-term survival. *J Clin Oncol* **30**, 1949-1952 (2012).
111. Shah, V. *et al.* Prediction of outcome in newly diagnosed myeloma: a meta-analysis of the molecular profiles of 1905 trial patients. *Leukemia* **32**, 102-110 (2018).
112. Panopoulou, A. *et al.* Optimizing the value of lenalidomide maintenance by extended genetic profiling: an analysis of 556 patients in the Myeloma XI trial. *Blood* **141**, 1666-1674 (2023).
113. Mina, R. *et al.* Carfilzomib induction, consolidation, and maintenance with or without autologous stem-cell transplantation in patients with newly diagnosed multiple myeloma: pre-planned cytogenetic subgroup analysis of the randomised, phase 2 FORTE trial. *Lancet Oncol* **24**, 64-76 (2023).
114. Kaiser, M.F. *et al.* Daratumumab, Cyclophosphamide, Bortezomib, Lenalidomide, and Dexamethasone as Induction and Extended Consolidation Improves Outcome in Ultra-High-Risk Multiple Myeloma. *J Clin Oncol* **41**, 3945-3955 (2023).
115. Nahi, H. *et al.* Proteasome inhibitors and IMiDs can overcome some high-risk cytogenetics in multiple myeloma but not gain 1q21. *Eur J Haematol* **96**, 46-54 (2016).
116. Tang, H.K.K. *et al.* The impact of bortezomib-based induction in newly diagnosed multiple myeloma with chromosome 1q21 gain. *Ther Adv Hematol* **13**, 20406207221082043 (2022).
117. Varma, A. *et al.* Outcome of Multiple Myeloma with Chromosome 1q Gain and 1p Deletion after Autologous Hematopoietic Stem Cell Transplantation: Propensity Score Matched Analysis. *Biol Blood Marrow Transplant* **26**, 665-671 (2020).
118. Shah, G.L. *et al.* Gain of chromosome 1q portends worse prognosis in multiple myeloma despite novel agent-based induction regimens and autologous transplantation. *Leuk Lymphoma* **58**, 1823-1831 (2017).
119. Skerget, M., Skopec, B., Zver, S. & Podgornik, H. Amplification of Chromosome 1q Predicts Poor Overall Survival in Newly Diagnosed Multiple Myeloma Patients. *J Hematol* **12**, 109-113 (2023).
120. Wang, Y. *et al.* The prognostic role of 1q21 gain/amplification in newly diagnosed multiple myeloma: The faster, the worse. *Cancer* **129**, 1005-1016 (2023).
121. Pasvolsky, O. *et al.* Outcomes of Autologous Stem Cell Transplantation in Patients with Ultra-High-Risk Multiple Myeloma. *Transplant Cell Ther* **29**, 757-762 (2023).
122. Panopoulou, A. *et al.* Impact of Ultra High-risk Genetics on Real-world Outcomes of Transplant-eligible Multiple Myeloma Patients. *Hemasphere* **7**, e831 (2023).
123. Ozga, M. *et al.* Concomitant 1q+ and t(4;14) influences disease characteristics, immune system, and prognosis in double-hit multiple myeloma. *Blood Cancer J* **13**, 167 (2023).
124. Gonzaga-Jauregui, C., Lupski, J.R. & Gibbs, R.A. Human genome sequencing in health and disease. *Annu Rev Med* **63**, 35-61 (2012).
125. Genomes Project, C. *et al.* A global reference for human genetic variation. *Nature* **526**, 68-74 (2015).

126. Pearson, T.A. & Manolio, T.A. How to interpret a genome-wide association study. *Jama* **299**, 1335-1344 (2008).
127. Altieri, A., Chen, B., Bermejo, J.L., Castro, F. & Hemminki, K. Familial risks and temporal incidence trends of multiple myeloma. *Eur J Cancer* **42**, 1661-1670 (2006).
128. Broderick, P. *et al.* Common variation at 3p22.1 and 7p15.3 influences multiple myeloma risk. *Nat Genet* **44**, 58-61 (2011).
129. Chubb, D. *et al.* Common variation at 3q26.2, 6p21.33, 17p11.2 and 22q13.1 influences multiple myeloma risk. *Nat Genet* **45**, 1221-1225 (2013).
130. Weinhold, N. *et al.* The CCND1 c.870G>A polymorphism is a risk factor for t(11;14)(q13;q32) multiple myeloma. *Nat Genet* **45**, 522-525 (2013).
131. Swaminathan, B. *et al.* Variants in ELL2 influencing immunoglobulin levels associate with multiple myeloma. *Nat Commun* **6**, 7213 (2015).
132. Mitchell, J.S. *et al.* Genome-wide association study identifies multiple susceptibility loci for multiple myeloma. *Nat Commun* **7**, 12050 (2016).
133. Went, M. *et al.* Identification of multiple risk loci and regulatory mechanisms influencing susceptibility to multiple myeloma. *Nat Commun* **9**, 3707 (2018).
134. Pertesi, M. *et al.* Genetic predisposition for multiple myeloma. *Leukemia* **34**, 697-708 (2020).
135. Attal, M. *et al.* A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Français du Myélome. *N Engl J Med* **335**, 91-97 (1996).
136. Child, J.A. *et al.* High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. *N Engl J Med* **348**, 1875-1883 (2003).
137. Richardson, P.G. *et al.* A phase 2 study of bortezomib in relapsed, refractory myeloma. *N Engl J Med* **348**, 2609-2617 (2003).
138. Jagannath, S. *et al.* A phase 2 study of two doses of bortezomib in relapsed or refractory myeloma. *Br J Haematol* **127**, 165-172 (2004).
139. Dimopoulos, M. *et al.* Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. *N Engl J Med* **357**, 2123-2132 (2007).
140. Singhal, S. *et al.* Antitumor activity of thalidomide in refractory multiple myeloma. *N Engl J Med* **341**, 1565-1571 (1999).
141. Dimopoulos, M.A. *et al.* Carfilzomib or bortezomib in relapsed or refractory multiple myeloma (ENDEAVOR): an interim overall survival analysis of an open-label, randomised, phase 3 trial. *Lancet Oncol* **18**, 1327-1337 (2017).
142. Leleu, X. *et al.* Pomalidomide plus low-dose dexamethasone is active and well tolerated in bortezomib and lenalidomide-refractory multiple myeloma: Intergroupe Francophone du Myelome 2009-02. *Blood* **121**, 1968-1975 (2013).
143. Petrucci, M.T. & Vozella, F. The Anti-CD38 Antibody Therapy in Multiple Myeloma. *Cells* **8** (2019).
144. Lonial, S. *et al.* Elotuzumab Therapy for Relapsed or Refractory Multiple Myeloma. *N Engl J Med* **373**, 621-631 (2015).

145. Moreau, P. *et al.* Teclistamab in Relapsed or Refractory Multiple Myeloma. *N Engl J Med* **387**, 495-505 (2022).
146. San-Miguel, J. *et al.* Cilta-cel or Standard Care in Lenalidomide-Refractory Multiple Myeloma. *N Engl J Med* **389**, 335-347 (2023).
147. Attal, M. *et al.* Lenalidomide, Bortezomib, and Dexamethasone with Transplantation for Myeloma. *N Engl J Med* **376**, 1311-1320 (2017).
148. Cavo, M. *et al.* Autologous haematopoietic stem-cell transplantation versus bortezomib-melphalan-prednisone, with or without bortezomib-lenalidomide-dexamethasone consolidation therapy, and lenalidomide maintenance for newly diagnosed multiple myeloma (EMN02/HO95): a multicentre, randomised, open-label, phase 3 study. *Lancet Haematol* **7**, e456-e468 (2020).
149. Durie, B.G.M. *et al.* Bortezomib with lenalidomide and dexamethasone versus lenalidomide and dexamethasone alone in patients with newly diagnosed myeloma without intent for immediate autologous stem-cell transplant (SWOG S0777): a randomised, open-label, phase 3 trial. *Lancet* **389**, 519-527 (2017).
150. Mateos, M.V. *et al.* Overall survival with daratumumab, bortezomib, melphalan, and prednisone in newly diagnosed multiple myeloma (ALCYONE): a randomised, open-label, phase 3 trial. *Lancet* **395**, 132-141 (2020).
151. Facon, T. *et al.* Daratumumab, lenalidomide, and dexamethasone versus lenalidomide and dexamethasone alone in newly diagnosed multiple myeloma (MAIA): overall survival results from a randomised, open-label, phase 3 trial. *Lancet Oncol* **22**, 1582-1596 (2021).
152. Reeder, C.B. *et al.* Cyclophosphamide, bortezomib and dexamethasone induction for newly diagnosed multiple myeloma: high response rates in a phase II clinical trial. *Leukemia* **23**, 1337-1341 (2009).
153. Moreau, P. *et al.* VTD is superior to VCD prior to intensive therapy in multiple myeloma: results of the prospective IFM2013-04 trial. *Blood* **127**, 2569-2574 (2016).
154. Sanchez, L., Wang, Y., Siegel, D.S. & Wang, M.L. Daratumumab: a first-in-class CD38 monoclonal antibody for the treatment of multiple myeloma. *J Hematol Oncol* **9**, 51 (2016).
155. Lonial, S. *et al.* Daratumumab monotherapy in patients with treatment-refractory multiple myeloma (SIRIUS): an open-label, randomised, phase 2 trial. *Lancet* **387**, 1551-1560 (2016).
156. Mateos, M.V. *et al.* Subcutaneous versus intravenous daratumumab in patients with relapsed or refractory multiple myeloma (COLUMBA): a multicentre, open-label, non-inferiority, randomised, phase 3 trial. *Lancet Haematol* **7**, e370-e380 (2020).
157. Dimopoulos, M.A. *et al.* Daratumumab, Lenalidomide, and Dexamethasone for Multiple Myeloma. *N Engl J Med* **375**, 1319-1331 (2016).
158. Palumbo, A. *et al.* Daratumumab, Bortezomib, and Dexamethasone for Multiple Myeloma. *N Engl J Med* **375**, 754-766 (2016).
159. Dimopoulos, M. *et al.* Carfilzomib, dexamethasone, and daratumumab versus carfilzomib and dexamethasone for patients with relapsed or refractory multiple myeloma (CANDOR): results from a randomised, multicentre, open-label, phase 3 study. *Lancet* **396**, 186-197 (2020).

160. Dimopoulos, M.A. *et al.* Daratumumab plus pomalidomide and dexamethasone versus pomalidomide and dexamethasone alone in previously treated multiple myeloma (APOLLO): an open-label, randomised, phase 3 trial. *Lancet Oncol* **22**, 801-812 (2021).
161. Moreau, P. *et al.* Bortezomib, thalidomide, and dexamethasone with or without daratumumab before and after autologous stem-cell transplantation for newly diagnosed multiple myeloma (CASSIOPEIA): a randomised, open-label, phase 3 study. *Lancet* **394**, 29-38 (2019).
162. Voorhees, P.M. *et al.* Addition of daratumumab to lenalidomide, bortezomib, and dexamethasone for transplantation-eligible patients with newly diagnosed multiple myeloma (GRIFFIN): final analysis of an open-label, randomised, phase 2 trial. *Lancet Haematol* **10**, e825-e837 (2023).
163. Giralt, S. *et al.* Optimizing autologous stem cell mobilization strategies to improve patient outcomes: consensus guidelines and recommendations. *Biol Blood Marrow Transplant* **20**, 295-308 (2014).
164. In: Carreras, E., Dufour, C., Mohty, M. & Kröger, N. (eds). *The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies*. Springer
Copyright 2019, The Editor(s) (if applicable) and The Author(s). Cham (CH), 2019.
165. Sheppard, D., Bredeson, C., Allan, D. & Tay, J. Systematic review of randomized controlled trials of hematopoietic stem cell mobilization strategies for autologous transplantation for hematologic malignancies. *Biol Blood Marrow Transplant* **18**, 1191-1203 (2012).
166. Li, J. *et al.* Effectiveness and cost analysis of "just-in-time" salvage plerixafor administration in autologous transplant patients with poor stem cell mobilization kinetics. *Transfusion* **51**, 2175-2182 (2011).
167. Spoerl, S. *et al.* Patients' outcome after rescue plerixafor administration for autologous stem cell mobilization: a single-center retrospective analysis. *Transfusion* **57**, 115-121 (2017).
168. Harvey, R.D. *et al.* Temporal changes in plerixafor administration and hematopoietic stem cell mobilization efficacy: results of a prospective clinical trial in multiple myeloma. *Biol Blood Marrow Transplant* **19**, 1393-1395 (2013).
169. Cheng, J. *et al.* Plerixafor is effective given either preemptively or as a rescue strategy in poor stem cell mobilizing patients with multiple myeloma. *Transfusion* **55**, 275-283 (2015).
170. Ungerstedt, J.S. *et al.* Autologous hematopoietic stem cell transplantation in multiple myeloma and lymphoma: an analysis of factors influencing stem cell collection and hematological recovery. *Med Oncol* **29**, 2191-2199 (2012).
171. Pozotrigo, M. *et al.* Factors impacting stem cell mobilization failure rate and efficiency in multiple myeloma in the era of novel therapies: experience at Memorial Sloan Kettering Cancer Center. *Bone Marrow Transplant* **48**, 1033-1039 (2013).
172. Mohty, M. *et al.* Autologous haematopoietic stem cell mobilisation in multiple myeloma and lymphoma patients: a position statement from the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant* **49**, 865-872 (2014).

173. Popat, U. *et al.* Impairment of filgrastim-induced stem cell mobilization after prior lenalidomide in patients with multiple myeloma. *Biol Blood Marrow Transplant* **15**, 718-723 (2009).
174. Cavallo, F. *et al.* Stem cell mobilization in patients with newly diagnosed multiple myeloma after lenalidomide induction therapy. *Leukemia* **25**, 1627-1631 (2011).
175. Hulin, C. *et al.* Stem cell yield and transplantation in transplant-eligible newly diagnosed multiple myeloma patients receiving daratumumab + bortezomib/thalidomide/dexamethasone in the phase 3 CASSIOPEIA study. *Haematologica* **106**, 2257-2260 (2021).
176. Eleutherakis Papaiakovou, E. *et al.* Impact of Daratumumab-Containing Induction on Stem Cell Mobilization and Collection, Engraftment and Hospitalization Parameters Among Multiple Myeloma Patients Undergoing Autologous Stem Cell Transplantation. *Blood* **138**, 3886-3886 (2021).
177. Manjappa, S. *et al.* Impact of Daratumumab on Stem Cell Collection, Graft Composition and Engraftment Among Multiple Myeloma Patients Undergoing Autologous Stem Cell Transplant. *Blood* **136**, 35-37 (2020).
178. Perrot, A. *et al.* Early Versus Late Autologous Stem Cell Transplant in Newly Diagnosed Multiple Myeloma: Long-Term Follow-up Analysis of the IFM 2009 Trial. *Blood* **136**, 39-39 (2020).
179. Hari, P. *et al.* Long-term follow-up of BMT CTN 0702 (STaMINA) of postautologous hematopoietic cell transplantation (autoHCT) strategies in the upfront treatment of multiple myeloma (MM). *Journal of Clinical Oncology* **38**, 8506-8506 (2020).
180. McCarthy, P.L. *et al.* Lenalidomide Maintenance After Autologous Stem-Cell Transplantation in Newly Diagnosed Multiple Myeloma: A Meta-Analysis. *J Clin Oncol* **35**, 3279-3289 (2017).
181. Jackson, G.H. *et al.* Lenalidomide maintenance versus observation for patients with newly diagnosed multiple myeloma (Myeloma XI): a multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol* **20**, 57-73 (2019).
182. Goldschmidt, H. *et al.* Bortezomib before and after high-dose therapy in myeloma: long-term results from the phase III HOVON-65/GMMG-HD4 trial. *Leukemia* **32**, 383-390 (2018).
183. Moreau, P. *et al.* Maintenance with daratumumab or observation following treatment with bortezomib, thalidomide, and dexamethasone with or without daratumumab and autologous stem-cell transplant in patients with newly diagnosed multiple myeloma (CASSIOPEIA): an open-label, randomised, phase 3 trial. *Lancet Oncol* **22**, 1378-1390 (2021).
184. Ho, M. *et al.* The Effect of Duration of Lenalidomide Maintenance and Outcomes of Different Salvage Regimens in Patients with Multiple Myeloma (MM). *Blood Cancer J* **11**, 158 (2021).
185. Johnson, D.C. *et al.* Genome-wide association study identifies variation at 6q25.1 associated with survival in multiple myeloma. *Nat Commun* **7**, 10290 (2016).
186. Ziv, E. *et al.* Genome-wide association study identifies variants at 16p13 associated with survival in multiple myeloma patients. *Nat Commun* **6**, 7539 (2015).

187. Bosseboeuf, A. *et al.* Monoclonal IgG in MGUS and multiple myeloma targets infectious pathogens. *JCI Insight* **2** (2017).
188. Bosseboeuf, A. *et al.* Analysis of the Targets and Glycosylation of Monoclonal IgAs From MGUS and Myeloma Patients. *Front Immunol* **11**, 854 (2020).
189. Bigot-Corbel, E. *et al.* Hepatitis C virus (HCV) infection, monoclonal immunoglobulin specific for HCV core protein, and plasma-cell malignancy. *Blood* **112**, 4357-4358 (2008).
190. Albeniz, I., Turker-Sener, L., Bas, A., Kalelioglu, I. & Nurten, R. Isolation of hematopoietic stem cells and the effect of CD38 expression during the early erythroid progenitor cell development process. *Oncol Lett* **3**, 55-60 (2012).
191. Ma, X. *et al.* Daratumumab binds to mobilized CD34+ cells of myeloma patients in vitro without cytotoxicity or impaired progenitor cell growth. *Exp Hematol Oncol* **7**, 27 (2018).
192. Nijhof, I.S. *et al.* CD38 expression and complement inhibitors affect response and resistance to daratumumab therapy in myeloma. *Blood* **128**, 959-970 (2016).
193. Ghose, J. *et al.* Daratumumab induces CD38 internalization and impairs myeloma cell adhesion. *Oncoimmunology* **7**, e1486948 (2018).
194. Venglar, O. *et al.* Effect of Daratumumab-Containing Induction on CD34+ Hematopoietic Stem Cells before Autologous Stem Cell Transplantation in Multiple Myeloma. *Blood* **138**, 2764-2764 (2021).
195. Thurlapati, A. *et al.* Stem Cell Mobilization for Multiple Myeloma Patients Receiving Daratumumab-Based Induction Therapy: A Real- World Experience. *Transplant Cell Ther* **29**, 340 e341-340 e344 (2023).
196. Shu, X.O. *et al.* Novel genetic markers of breast cancer survival identified by a genome-wide association study. *Cancer Res* **72**, 1182-1189 (2012).
197. Wu, C. *et al.* Genome-wide association study identifies common variants in SLC39A6 associated with length of survival in esophageal squamous-cell carcinoma. *Nat Genet* **45**, 632-638 (2013).
198. Wu, C. *et al.* Genome-wide interrogation identifies YAP1 variants associated with survival of small-cell lung cancer patients. *Cancer Res* **70**, 9721-9729 (2010).
199. Chng, W.J. *et al.* Clinical implication of centrosome amplification in plasma cell neoplasm. *Blood* **107**, 3669-3675 (2006).
200. Cohen, J.I. Herpesvirus latency. *J Clin Invest* **130**, 3361-3369 (2020).

KONSTANTINOS LEMONAKIS is a hematologist working at the department of Hematology, Oncology and Radiation Physics, Skåne University Hospital, Sweden. This thesis is based on four projects investigating various factors affecting the prognosis of multiple myeloma.

Printed by Media-Tryck, Lund 2024 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of hematology and transfusion medicine

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2024:74
ISBN 978-91-8021-568-8
ISSN 1652-8220