From Department of Medicine, Huddinge, Division of Hematology
Karolinska Institutet, Stockholm, Sweden

CLINICAL STUDIES IN MULTIPLE MYELOMA

Johan Lund

Karolinska Institutet

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Clinical Studies in Multiple Myeloma

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By

Johan Lund

Principal Supervisor:
Assoc. Prof. Hareth Nahi
Karolinska Institutet
Department of Medicine, Huddinge
Division of Hematology

Co-supervisor(s):
Assoc. Prof. Evren Alici
Karolinska Institute
Department of Medicine, Huddinge
Division of Hematology

Assoc. Prof. Henrik Green
Linköping University
Department of Medical and Health Sciences
Division of Drug Research

Assoc. Prof. Maciej Machazcka
Karolinska Institute
Department of Medicine, Huddinge
Division of Hematology

Opponent:
Prof. Frits van Rhee
University of Arkansnas for Medicial Sciences
Myeloma Institute for Research and Therapy

Examination Board:
Dr. Caroline Heckman
University of Helsinki
Institute for Molecular Medicine Finland

Prof. Jan Palmblad
Karolinska Institute
Department Medicine, Huddinge
Division of Hematology

Assoc. Prof Fredrik Celsing
Karolinska Institute
Department of Medicine, Solna
Division of Hematology

To Adrian and Klara

ABSTRACT

Multiple Myeloma (MM) is an incurably disease of the bone marrow. It is characterized by the uncontrolled growth of clonal plasma cells (PCs) and leads to production of non-functional gammaglobulin. Clinical features include loss of normal bone marrow function, defect bone structure and kidney failure.

The first historical cases were described as "mollities ossium" in the 1840s. Atypical urine samples were described already in the 1840s but the specific pattern on electrophoresis of serum from MM patients was described in 1939.

PCs are highly specialized cells derived from B-lymphocytes. Every single PC produces a single class of antibody - one heavy chain (IGH) of IgG, IgA, IgD or IgE class and one light chain (IgL) κ or λ.

Current evidence suggests MM evolves from a non-malignant state – MGUS, Monoclonal Gammopathy of Undetermined Significance.

Assessment of chromosomal abnormalities is powerful in predicting outcome and there are also data suggesting that different treatment modalities are more efficient in treating MM with certain abnormalities.

The first modern treatment attempts were performed in the 1940s with urethane. Combination therapy of melphalan and prednisone (MP) was invented in 1969 and remained standard therapy until early 2000s when Thalidomide and Bortezomib was introduced. Stem cell transplant as treatment for younger patients were evolved in the 1980s and is still standard therapy.

Paper I is based upon a retrospectively collected database of all 1837 MM patients diagnosed at 15 Swedish between the years 2000 to 2011. From this material, we selected all patients treated with melphalan and prednisone (MP) or MP with added thalidomide (MPT) in 1st 2nd 3rd and 4th lines of treatment, a total number of 888. A meta-analysis of six previous clinical studies comparing MP to MPT in previously untreated MM could show a 6 months benefit to MPT. In our study median OS from beginning of 1st line of treatment was 2.2/4.2 years after MP/MPT respectively, and in 2nd, 3rd and 4th line of treatment 1.8/2.9, 1.4/1.6 and 1.1/1.9 years ($P < 0.0001$, 0.003, 0.74 and 0.235). The benefit of MPT over MP was bigger in our study compared to the randomized clinical studies. Minor differences in patient characteristics could partly explain the difference, though the difference still remained after adjusting for these markers.

In paper II, we show, in a pan-Nordic collaborative study, with patients from Sweden, Norway and Denmark, the impact of chromosomal abnormality of gain 1q21. From a cohort of in total 930 patients, 347 patients, with known 1q21 status, were studied and divided into 3 groups; gain 1q21, other chromosomal abnormalities (del (13q), del (17p), t(4;14) and/or t(14;16)) (OA) and no chromosomal abnormalities (NA). We observed the most dismal outcome from the gain 1q21 group and best outcome in NA with OA in between, treating with conventional cytostatic drugs. Adding Thal, Bor or Len to treatment could overcome poor prognosis in the NA group, but not for patients with gain 1q21.

Paper III and IV were both based on a prospective clinical study on Lenalidomide (Len) naïve relapsed or refractory MM patients, starting at the second line of treatment, in two parts, studying Len in combination with Dexamethasone (Dex). The first part, an observational study on LenDex in standard dosing up to 9 cycles with 133 participating patients showed a good response rate (79% ≥PR)

and a median time to progression (TTP) of 19 months. At response, PR or better, and two more consolidating cycles, patients were offered to enter a randomized phase II study, randomizing between continuous LenDex treatment and Len as single drug. There was a statistically insignificant trend to better progression free survival (PFS) in the LenDex group. No difference in overall survival (OS) could be shown.

In the fourth study we looked upon whether different single nucleotide polymorphisms (SNPs) in the ATP-binding cassette sub-family B member ABCB1 gene, encoding P-glycoprotein (P-gp) could have clinically effect on response rate and survival in the same patient cohort. P-glycoprotein is a transmembrane transport protein that is responsible for the extrusion of several drugs over the cell membrane. It is localized, among others, in the intestinal mucosa and the kidney tubules and this protein could both affect uptake and excretion of several drugs. ABCB1 is known as a marker for resistance to different chemotherapeutic agents. In our study we could show no significant differs in response or survival data between groups with different SNPs in the ABCB1 gene in the whole population, but in the low risk group according to cytogenetics, there was a significant difference in time to progression from difference in SNP in 1199G>A genotype, favoring patients with G/A over G/G genotype.

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LIST OF SCIENTIFIC PAPERS

I. **Addition of thalidomide to melphalan and prednisone treatment prolongs survival in multiple myeloma – a retrospective population based study of 1162 patients**
JOHAN LUND, Katarina Uttervall, Johan Liwing, Gösta Gahrton, Evren Alici, Johan Aschan, Erik Holmberg, Hareth Nahi
European Journal of Haematology, 2014, 92(1), 19-25

II. **Proteasome inhibitors and IMiDs can overcome some high-risk cytogenetics in multiple myeloma but not gain 1q21**
Hareth Nahi, Thea Kristin Våtsveen, JOHAN LUND, Bart M.S. Heeg, Birgitte Preiss, Evren Alici, Michael Boe Møller, Karin Fahl Wader, Hanne E.H. Møller, Lill Anny Grøseth, Brian Østergaard, Hong Yan Dai, Erik Holmberg, Gösta Gahrton, Anders Waage, Niels Abildgaard
European Journal of Haematology, 2016, 96(1), 46-54

III. **Lenalidomide vs. lenalidomide/dexamethasone maintenance after second-line lenalidomide/dexamethasone induction in Multiple Myeloma**
JOHAN LUND, Astrid Gruber, Birgitta Lauri, Sigrid Karstorp, Adil Duru, Cecilie Blimark, Agneta Swedin, Markus Hansson, Karin Forsberg, Lucia Ahlberg, Conny Carlsson, Anders Waage, Peter Gimsing, Annette Juul Vangsted, Ulf Frølund, Erik Holmberg, Evren Alici, Mats Hardling, Ulf-Henrik Mellqvist, Hareth Nahi
Manuscript

IV. **Pharmacogenetic study of the impact of ABCB1 Single nucleotide polymorphisms on the outcome in lenalidomide treated multiple myeloma patients – results from a Phase IV and a Phase II randomized study**
Ingrid Jakobsen Falk*, JOHAN LUND*, Henrik Gréen, Astrid Gruber, Evren Alici, Birgitta Lauri, Cecilie Blimark, Ulf-Henrik Mellqvist, Agneta Swedin, Karin Forsberg, Conny Carlsson, Mats Hardling, Lucia Ahlberg, Kourosh Lofti, Hareth Nahi
Manuscript
(*Contributed equally)

OTHER PUBLICATIONS NOT INCLUDED IN THE THESIS

A combination regimen of Bortezomib, cyclophosphamide and betamethasone gives quicker, better and more durable response than VAD/CyBet regimens: results from a Swedish retrospective analysis.
Uttervall K, Admasie J, Alici E, LUND J, Liwing J, Aschan J, Barendse M, Deneberg S, Mellqvist UH, Carlson K, Nahi H.
Acta Haematol. 2013;130(1):7-15. doi: 10.1159/000345422. Epub 2013 Jan 25

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Re-challenging with anti-CD38 monotherapy in triple-refractory multiple myeloma patients is a feasible and safe approach.
Alici E, Chrobok M, LUND J, Ahmadi T, Khan I, Duru AD, Nahi H.
Br J Haematol. 2016 Aug;174(3):473-7. doi: 10.1111/bjh.13776. Epub 2015 Oct 12

CONTENTS

LIST OF ABBREVIATIONS

ABCB1	ATP-binding cassette sub-family B member
AE	Adverse Event
AML	Acute Myeloid Leukemia
BCR	B-cell receptor
Bor	Bortezomib
Carf	Carfilzomib
CCND1	Cyclin D1 gene
CD	Cluster of Differentiation
CDKN2A	Cyclin-dependent kinase inhibitor 2A
CDKN2C	Cyclin-dependent kinase 4 inhibitor C
CKS1B	Cyclin-dependent kinases regulatory subunit 1
Cox 2	Cyclooxygenase 2
CR	Complete Response
CRAB	elevated Calcium, Renal impairment, Anemia, Bone lesions
CUL4	Culin 4
Dara	Daratumumab
DDB1	Damaged DNA Binding Protein 1
DH	Diversity Domain
Dex	Dexamethasone
EBMT	European Bone Marrow Transplant
Elo	Elotuzumab
FGFR3	Fibroblast Growth Factor Receptor 3 gene
FISH	Fluorescence in situ hybridization
IBMTR	International Bone Marrow Transplant Registry
IgH	Heavy immunoglobin chain
IgLκ	Light Immunoglobin chain, kappa
IgLλ	Light Immunoglobin chain, lambda
IKZF	IKAROS Family Zink Finger Complex
IL	Interleukin
IMiD	Immunomodulatory drug
IMWG	International Myeloma Working Group
JH	Joining Domain

Len	Lenalidomide
LenDex	Lenalidomide and Dexamethasone
maf	musculoaponeurotic fibrosarcoma
MM	Multiple Myeloma
M-protein	Monoclonal protein in blood or in urine
MP	Melphalane and prednisolone
MPT	Melphalane, prednisolone and thalidomide
MRI	Magnetic Resonance Imaging
mTOR	mechanistic target of rapamycin
nCR	near Complete Response
OS	Overall Survival
PC	Plasma Cell
PFS	Progression Free Survival
P-Gp	P-glycoprotein
PI	Proteasome Inhibitor
PR	Partial Response
PTEN	Phophatase and tensin homolog gene
Roc1	Regulator of Culin 1
RAG	Recombination Activating Genes
SLAMF7	Signaling lymphocytic activating molecule family member 7
SNPs	Single Nucleotide Polymorphisms
Thal	Thalidomide
TNF-α	Tumor necrosis factor alpha
TP53	Tumor protein p53 gene
TTNT	Time to next treatment
VAD	Vincristine, Adriamycin (doxorubicin) and Dexamethasone
VEGF	Vascular Endothelial Growth Factor
VGPR	Very Good Partial Response
VH	Variability Domain

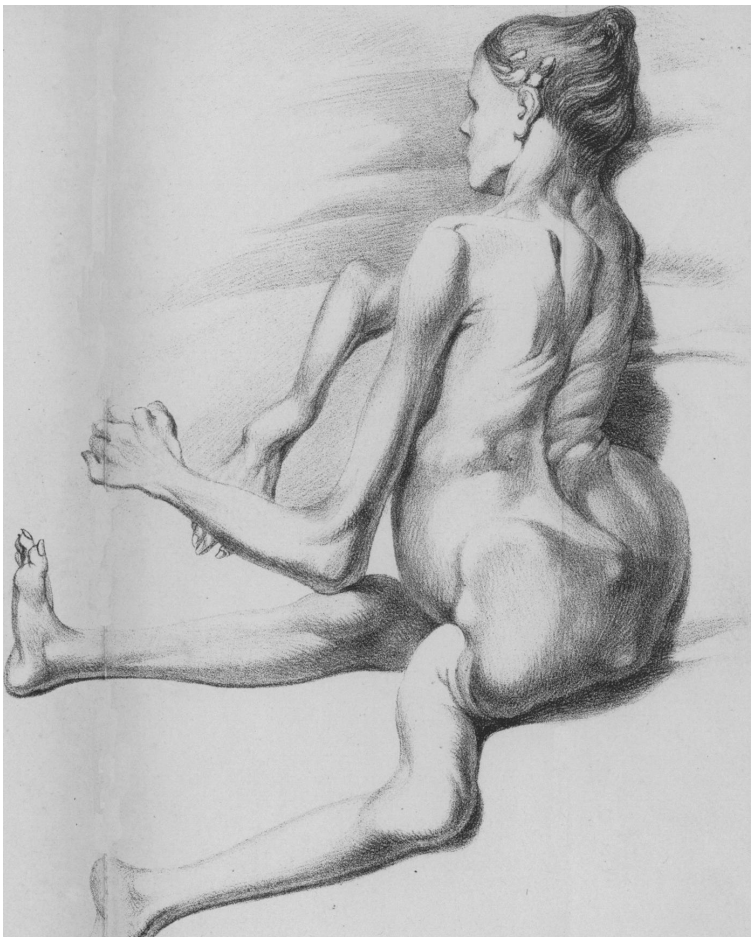
1 BACKGROUND

Introduction

Myeloma is currently an incurable malignant disease. It stands for about 2% of all cancer deaths and about 20% of all hematological malignancies(1). It is stated as a systemic malignant disease of the blood and the World Health Organization defines it as a lymphoproliferative B-cell disease. It is characterized as an uncontrolled proliferation of plasma cells (PC) in the bone marrow leading to extensive production of non-functional intact gammaglobulin or parts thereof. It can affect bone structure via enhancing osteoclasts, impair kidney function via hypercalcemia or have direct effect on tubulus of the defect gammaglobulins and cause loss of bone marrow function.

History

The first two recorded cases of what might have been multiple myeloma, but at that time was named “mollities ossium” were described by Samuel Solly in 1844 of two women, one of them Sarah Newbury, with severe and multiple fractures combined with fatigue. At the autopsies, the bones were particularly weak and under the microscope ”…the osseous structure of the bone was nearly absorbed, a mere shell being left. The interior was filled with a dark grumous matter, varying in colour from that of dark blood to a reddish light liver colour.”



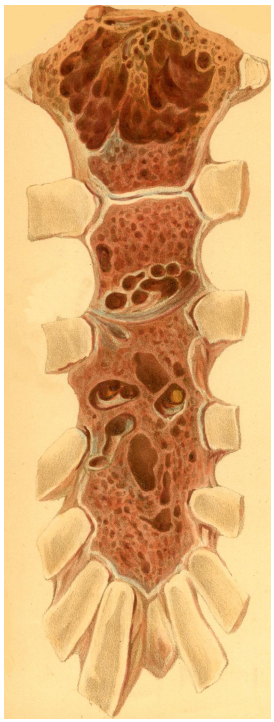


Figure 1. Sarah Newbury, the first known MM patient

In 1845, a 38-year-old London grocer, Thomas Alexander McBean, referred to Dr William Macintyre of Harley Street after having had severe chest pain and had noticed that his "body linen was stiffed by his urine". Dr Macintyre noticed severe body pain and peripheral oedema and precipitated a urine sample searching for albumin, finding somewhat odd results and sent a sample to Dr Henry Bence Jones, chemical pathologist at St George's Hospital, London. At the same time, the patient's GP, Dr Thomas Watson had sent another urine sample to Dr Bence Jones with the same question. Dr Bence Jones analysed the urine and determined the sediment to be different from albumin, mistaking it for being an oxide of albumin(3). Mr McBean passed away 1846 and during the autopsy, they noticed "…that all the ribs throughout their whole length were soft and brittle, so that they could be easily cut by the knife…", and "…their interior was charged with a soft gelatiniform substance of blood-red colour and unctuous feel…"(4). Histologically, they described large numbers of nucleated cells in the affected bones(3), but the term "plasma cells" were first used thirty years later by Waldeyer (what he probably described were Mast Cells(5), but he invented the actual term)(6). The term "Bence Jones protein" was never used by H Bence Jones himself, it was first used by a Dr Fleicher in Erlangen 1880(3). Bayne-Jones and Wilson first described two different types of Bence Jones proteins in 1922(6), and 1956 Leonard Korngold and his assistant Rose Lipari could identify two different types of Bence Jones proteins and could also show that they shared some of the antigenic determinants of homologous MM-globulin from serum(5). Later on the two different chains κ and λ were named in their honor(3). The specific pattern of MM in serum electrophoresis was shown in 1939(7). Prof Waldenström developed the concept of clonality in 1960(8) and in 1962 Edelman and Gally could show that Bence Jones protein and light chains of the serum shared the same characteristics(9).

Pathogenesis

Plasma Cell Development

Normal PCs are highly specialized cells derived from B-lymphocytes. Every PC producing a single type of antibody containing one class of immunoglobin heavy chain (IgG, IgA, IgE or IgD) and one class of light chain (κ or λ). The early B-cells develop from hematopoetic stem cells in the bone marrow, where they rearrange their heavy chain (IgH) gene segments and become precursor B-cells which express IgM and leave the bone marrow. They migrate to the spleen and become either marginal zone B-cells or follicular B-cells. The marginal zone B-cells are short lived and can react quickly on antigen stimulation, producing IgM and then die from apoptosis within one week. Follicular B-cells can also undergo the same quick maturation and become IgM-producing PCs, but can also undergo maturation in the germinal centers, where they would be co-stimulated with dendritic cells and T-cells and subsequently undergo somatic mutations of the immunoglobin genes.

Memory B-cells arise from germinal centers. B-cells with mutated immunoglobin genes, but still expressing BCR and not producing antibodies and mature and long-lived PCs. The memory B-cell circulates and supplies a rapid and strong response when stimulated with the same antigen that activated its parent B-cell. The PC circulates and eventually goes back to the bone marrow, producing high levels of antibodies, but do not proliferate. PCs, per definition, do not express BCR and can therefore not take up antigen and they lack MHCII and can no more act as antigen presenting cells. They live for some weeks, up to several months, but there is evidence that some of them might migrate to the bone marrow and live there for several years.(10)

During the development of early B-cells to PCs, they will rearrange their immunoglobin genes, both the heavy chain (IgH) genes and thereafter the genes coding for the light chains. The very large IgH gene is located on chromosome 14 and consists of 4 major domains. The variability domain (VH) consists of more than 100 DNA segments, the diversity domain (DH) of 27 DNA segments, the joining domain (JH) of 6 segments and constant domain of 6. The early rearrangement process is driven by specific enzymes, recombination activating genes (RAG) 1 and 2 of chromosome 11p, that first combine a fragment of the DH and a fragment of JH and then join this DH-JH with a VH fragment. If this combination is in frame the cell will go on activating the light κ chain gene (IgLκ) on chromosome 2 and the cell will become a mature IgM-κ producing cell. When the cell is unable to produce light κ-chains, it will in turn activate the light λ gene (IgLλ) on chromosome 20 and the cell will become an IgM-λ producing B-cell. This explains why there are double as much κ producing cells as λ producing ones. This process occurs stochastically and antigen independent.

The cell then leaves the BM and finds its way into the secondary lymphoid organs. The second type of gene rearrangement occurs in the presence of antigen presenting cells and T-cells. New stochastic mutations will occur in the IgH DH-JH-VH complex and only cells producing antibodies more specific for the presented antigens will survive; the others will go into apoptosis. The last stage will be the class switch recombination, also in the secondary lymphoid organs, turning the B-cells into specific IgG, IgA or IgE-producing B-cells. These cells will finally mature to PCs or memory B-cells.(11)

Plasma Cells Development into Multiple Myeloma

MM develops from premalignant clonal PCs that have gone through the maturation process in germinal centers(1), but as PCs lack proliferative capacity, MM may evolve from memory B-cells(12). These cells would be the myeloma initiating cells and initiate disease but also serve as a reservoir of cells to induce disease relapse.

MM evolves in probably all cases from a non-malignant pre-stadium, MGUS - “monoclonal gammopathy of undetermined significance” (13). 3 % of all people older than 50 years have MGUS(14). The Annual follow up of MGUS patients have shown a MM development rate of 1 %(13). A subset of these patients is first diagnosed with smoldering myeloma – a condition that shares the diagnostic criteria with MM, but lack symptoms.

Chromosomal Abnormalities

Based on chromosomal analysis, the chromosome number pattern is a powerful prognostic factor in patients with MM. In general, loss of genetic material (such as hypodiploidy) in almost all malignancies is a marker of poor prognosis, whereas gains (such as hyperdiploidy) are usually associated with better outcome. Most probably because hypodiploidy cause loss of functions such as tumor suppressor functions, while hyperdiploidy causes gain of function that not always leads to interference with the cell cycle.

Hypodiploidy

The incidence of hypodiploidy is about 10% (15, 16). The importance of hypodiploidy for overall survival in MM is unclear. An adverse effect of hypodiploidy in general may be due to monosomy of specific chromosomes, where the general hypodiploidy might be a confounding factor. However correlations with adverse OS have been found in multivariate analysis independent of other aberrations (17, 18).

Hyperdiploidy

Chromosomal abnormalities are present in nearly all cases of MM. There are two described different pathways for abnormalities that drive the cells into MM. The first is represented by hyperdiploidy and involves the chromosomes 3, 5, 7, 9, 11, 15, 19 and 21and is seen in a bit more than 30% of patients(19). How the hyperdiploidy drives the MM evolution is today not fully understood. The other one is based upon IgH translocation, especially located to chromosomal locus 14q32(20).

Immunoglobin heavy chain (IgH) aberrations

Any gene that is translocated to be fused to the enhancer of the IGH gene in a PC will be highly expressed (1). Translocations to the IgH locus occurs in about 50% of MGUS, 60-65% of intramedullary MM and in 70-80% in extramedullary MM(21), indicating a gain of more mutations throughout the development of the disease.

t(11;14)(q13;q32)

The most frequent translocation including 14q32 is t(11;14)(q13;q32) occurring in approximately 30% of MM patients leading to dysregulating of CCND1, and consecutive overexpression of cyclin D1(22). MM patients with t(11;14) have a higher tendency of a non-secreting disease, morphology of a "lymphoplasmacytic" type and occur in high frequency in IgM MM(23, 24).

t(4;14), t(14;16) and t(14;20)

The second most common is t(4;14)(p16;q32), occurring in about 12-15% of MM patients leading to up-regulating of MMSET due to translocating this gene to the IgH-locus at 14q32 and, in turn, up-regulating of the fibroblast growth factor receptor 3 gene FGFR3(25). FGFR3 is a receptor tyrosine kinase(26) and is thus a possible target for therapy with receptor tyrosine kinase inhibitors (27). t(14;16)(q32;q23) also causes over-expression of c-*maf* (musculoaponeurotic fibrosarcoma) (28) which is a proto-oncogene(29). Another *maf* gene is the *mafB* which strongly correlates to t(14;20)(q32;q12), a translocation occurring in about 1-2% of all MM patients and strongly associated with poor prognosis (30), (31), (32). While patients with t(14;16) and t(14;20) have an equally aggressive disease as those with t(4;14) (33); due to the lower frequency of t(14;16), its significance is conclusive only in larger studies (33). So far, t(4;14) and t(14;16) translocations are considered to be specific for MM, no other known malignancy carries these aberrations.

Less frequent translocations are t(6;14)(6p21,q32), t(8;14)(q24;q32) and t(14;20)(q32;q11).

Structural aberrations

Chromosome 1

Structural aberrations of chromosome 1 are the most frequent ones in MM and are identified in 40-48% of all cases (19, 34). The most common aberration is gain of 1q21 which is seen in 40% of all newly diagnosed MM cases, and in about 70% of relapses (35) while it is quite rare in MGUS. Since gains of 1q are frequently seen at late stages of the disease, the locus is considered to play a pathogenic role in disease progression and gains are therefore associated with poor prognosis (35-37).

del (8p21)

Previous studies have linked molecular dysregulations originating from changes in the 8p21 region to various malignancies including leukemic mantle cell lymphoma (38) and B-cell lymphoma (39), and the loss of this region has been shown to have a negative effect on survival in head and neck cancers. Our group has previously reported that del(8p21), is an independent poor prognosis factor in MM and both progression free survival (PFS) and OS are adversely affected (40).

del (8q24)

8q24 encodes the c-myc gene. Gene expression profiling studies have pointed out the MYC pathway as a key player in the evolution of normal PCs to MM (41). The most well-known is t(8;14)(q24;q32) which is associated with Burkitt's lymphoma and NHL, and also described in MM.

Chromosome 13

Deletion of 13q or the whole chromosome 13, identified by FISH in less than 50% of the patients, has for many years been considered to be an adverse prognostic factor (42). However, in the earlier studies the association with poor prognosis was based on conventional cytogenetics and in almost all new studies using FISH and multivariate analysis including other chromosomal abnormalities the loss of 13q is not an independent prognostic factor (43-47).

del (17p) (p53)

This abnormality is a strong predictor of extremely low OS and the lowest rate of achieving CR in comparison to other or no abnormalities (48, 49). Deletion of 17p is probably one of the most predictive molecular markers for resistance to therapy and short OS in MM identified so far (36, 48). Neither high dose therapy nor allogeneic transplantation seems to overcome the dismal prognosis for patients with *TP53* deletions/mutations (45, 48).

Genomic Aberration	Incidence (%)
del(13q)	~50
14q32 Translocations	~50–60
Hyperdiploidy	~50
t(4;14)	~15
t(11;14)	~15
t(14;16)	~5
t(14;20)	~1
del(17p)	~10
gain(1q21)	~30–43
del(1p21)	~20

Table 1. Incidence of Chromosomal Abnormalities in MM

Data from van de Donk et al (50)

Other dysregulations

A number of gene dysregulations, such as activation of N- and K-Ras(51), or the tumor suppressors TP53, phosphatase and tensin homolog gene (PTEN), Cyclin-dependent kinase inhibitor 2A (CDKN2A) and Cyclin-dependent kinase 4 inhibitor C (CDKN2C) are known to have prognostic impact(1). The latter are believed to be secondary events in the MM-cell evolution. The IgH translocations are often seen in all of the PCs of a patient, however in these dysregulations only in certain subclones(11).

Clinical Features of Multiple Myeloma

Epidemiology

MGUS, the premalignant stage that may precede MM is found in about 1% of the population older than 25 years and in 3-4% in people older than 50(26, 52). About 1% of the patients with MGUS progress to MM every year(13). The progress into symptomatic MM is much higher of patients with smoldering MM, about 5% at a yearly rate(52).
The incidence in US is 5.6 per 100 000 person-years with a higher incidence in males than women (7.1 vs. 4.5/100 000 person years)(53). The Swedish incidence is 6.6/100 000. Due to better treatment modalities and longer survival the prevalence of the disease has risen. In 1980s' Sweden, a bit more than 1 000 patients lived with the disease and in 2012 the number had more than tripled (3 107). Some of the rise in prevalence can be due to shift in diagnostic criteria and the population number has also increased from 8 million inhabitants to nearly 10 million.(54)

Diagnosis

MM diagnosis is based on the presence of at least 10% clonal PCs in the bone marrow combined with at least one of four CRAB criteria (increased serum calcium level, renal dysfunction, anemia, and destructive bone lesions). (i.e. calcium level >2.75mmol/L, creatinine clearance <40 ml/minute, hemoglobin value of >20g/L below lowest limit of normal or below 100 g/L and one or more bone lesion on skeletal X-ray or CT-scan). In the last edition of International Myeloma Working Group's diagnostic criteria there has been added three more Myeloma Defining Events (MDEs) of the asymptomatic patient that also calls for treatment; 60% or more clonal PCs on bone marrow examination, serum free light chain ratio of 100 or greater, provided the involved chain level is at least 100 g/L, more than one lesion on Magnet Resonance Imaging (MRI), greater than 5mm in diameter.
MGUS is diagnosed from less than 10% clonal PCs of the bone marrow and an M-component in plasma less than 30 g/L and no CRAB-criterias up filled. Smoldering myeloma is diagnosed from 10-60% PCs in bone marrow or an M-component of >30 g/L.(55)

Risk Stratification

The standard MM risk estimation of MM has been based upon two risk scores. The Durie Salmon system(56) is based upon hemoglobin level, calcium level, number of lytic bone lesions and level of M-component in a way of estimating tumor mass and kinetics and transform it into prognosis. The ISS score was evaluated from univariate and multivariate analyses of

several potential predictors of survival in MM and form these markers a combination of β2-microglobulin and albumin provided "…the simplest, most powerful and reproducible three-stage classification."(57).

However, these risk scores take no account of chromosomal abnormalities, differential gene expressions nor other factors such as age and performance status. Numerous further studies have validated the influence of those biological factors' influences on MM-risk(58).
t(4;14) is considered as associated with a shorter PFS and OS(59). t(14;16) with up-regulated cyclin D1, a driving mutation in mantle cell lymphoma, was supposed to be a negative prognostic marker in MM, but has proven to have no impact on prognosis and is now considered as a standard risk marker(58-60).
del (17p) is repetitively shown to be a negative prognostic marker(43, 61, 62). TP53 is located on the short arm of chromosome 17 and is probably the cause of the negative impact on survival of this deletion. TP53 as a tumor suppressor gene and its role in survival of tumors cells after treatment is well described(63).
del (13q) can be observed in half of the patients with MM. Its role as a prognostic marker has been controversial. It has initially been proposed as a negative prognostic marker, but further studies have shown this to be mainly related to its frequent association to t(4;14) or del 17p(43, 62, 64). It is no longer considered as an independent prognostic marker in the absence of t(4;14) or del (17p) when evaluated by FISH(23, 24, 43, 62). When it is detected by conventional cytogenetics it comes out as a negative prognostic marker(65), probably due to that cytogenetics is a less sensitive method than FISH in detecting del (17p) and t(4;14) and del (13q) therefore seem to be a surrogate marker for those.
Also dysregulation of genes on chromosome 1 affects outcome. Gain 1q21 is linked to a poor prognosis(66-68), probably by dysregulation of CKS1B, a cell cycle regulator. It is also shown the more copies of 1q21, the worse outcome(69). Del 1p is also a negative prognostic marker, especially when linked to gain 1q21(70).
Del 8p21 has also by two groups been linked to a poor prognosis, but the results must be confirmed by other studies(40, 71).

Several attempts have been made to show the possibility of influencing the impact of prognostic markers by choosing the right treatment. It is shown that addition of Bortezomib (Bor) can partly overcome the negative impact of t(4;14)(72) and that use of Bor both in induction and thereafter as maintenance therapy can partly overcome the negative impact of del (17p)(69). Our own retrospective study could show that use of Thal, Bor or Len could overcome the negative prognosis of t(4;14), t(14;16) del13q and del17p compared to conventional cytostatic drugs, but not the negative impact of gain1q21(67).

Treatment Development

MM responds to both classical cytotoxic, immunomodulatory and other targeted drugs, as well as to autologous- and allogeneic stem cell transplantation(73)

The reigning assumption is that the correct combination of available treatment modalities will improve survival and even cure a fraction of the patients. Assessing prognostic parameters, in particular chromosome aberrations, up-and down-regulation of certain gene products, evaluation of different drug effect on different patient populations and analyses of other cell population of the patient e.g. natural killer (NK)–cell function or osteoclast activity will be helpful in selecting the best treatment regimen for the patients.

Still, MM is considered to be an incurable disease, due to the persistence of residual tumor cells(74, 75), but there are several new drugs and new combinations oncoming.

Early treatment attempts of MM were rhubarb pills and infusion of orange peel which was given to Sarah Newbury in 1844(2). Mr. McBean was given steel and quinine but was also subjected to phlebotomy and application of leeches(4). The first modern attempt to treat MM was in 1947 when the nephrologist Alwall in Lund (Sweden) started treatment with urethane(76, 77). This became the standard therapy for 15 years until Holland et al in 1966 randomized 83 patients to receive either urethane or placebo, showing no survival difference between the groups(6, 78).

In 1958, Blokhin et al reported the first data on treating MM with melphalan(79), followed by studies by Bergsagel and Hoogstraten in 1962, and 1967(80, 81). In 1962, Mass could show effect in lowering the serum globulin and rise in hematocrit on giving prednisone to MM patients, but could show no survival benefits compared to placebo(82).

The first efficient therapy against MM was the combination of melphalan and prednisone (MP), described by Alexanian and colleagues in 1969(83). This was standard therapy for decades.

The first attempts to perform bone marrow transplant were reported by Thomas et al in a series of six case studies publicized in 1957(84). All patients died from their disease, but no acute reactions from the transplanted bone marrow could be shown. It was not until the 80s, that studies on bone marrow transplant on MM with successful results were published. The first results from syngenic (twins) transplant were published in 1982 and 1986(85, 86) and in 1987 Garhton et al published a series of 14 patients with MM, transplanted with HLA-identical graft from siblings. 10 patients survived 6-34 months after transplantation with a median OS of 12 months(87). Research continued on

allogeneic transplant of MM, but in 1997 Bensinger et al published data from all patient registers of allogeneic stem cell transplant, including European Bone Marrow Transplant (EBMT) registry and the International Bone Marrow Transplant Registry (IBMTR)(88). They reported that 28%, of the patients lived 7 years after allogeneic transplant and possibly were cured, but a very high rate of transplant-related mortality, 41%, made the median OS short compared to that of autologous stem cell transplant, 18 vs. 43 months and the recommendation was to spare allogeneic transplant for selected patients.

Autologous stem cell transplant of MM with melphalan 140mg/m^2 was first reported in 1983 by Mcelwain and Powles(89). Out of 9 patients reported, one previously untreated with plasma cell leukemia, 4 previously untreated with MM and 4 previously treated with MM all responded to treatment and 3 out of the 5 untreated responded with CR. In 1987 Barlogie et al(90) reported on 7 patients that had relapsed or were refractory to both MP and VAD. They were given melphalan 140 mg/m^2 and TBI of 8.5 Gy followed by the infusion of autologous stem cells. All patients responded to treatment, two died after 2.5 and 3 months from pneumonia and osteomyelitis, but the rest were treatment free 2, 6, 11, 15 and 21 months after treatment. During the upcoming 10 years, several other studies showed survival benefit for high dose treatment with melphalan followed by the administration of peripherally harvested blood stem cells(91).

For the elderly, non-transplant eligible patients, however, there still were no better alternative treatment modalities than the combination of MP. An overview of 27 randomized trials of melphalan and prednisone versus different newer combination chemotherapies could show no benefit for the latter(92).

Introduction of IMiDs as MM drug

30 years after the introduction of MP, Barlogie and his colleagues could show that, in a group of 84 patients, refractory to standard treatment, 32% responded to treatment with thalidomide as a single agent(93).

Thalidomide was initially approved as a sedative and a treatment modality of pregnancy related morning sickness and nausea. Due to the company's explicit claim of safety it was approved as an over-the-counter drug in West Germany 1957 and soon after it was approved in more than 40 countries.

It was the work of the chief scientist of Chemie Grünenthal, a small West German pharmaceutical company. They were searching for new antibiotics and produced α –phthalimidoglutarimide, which they called thalidomide. They had no idea about this new molecule's pharmacological properties and started searching for certain pharmacological activity. The drug showed no antibiotic activity, and in fact, no other pharmacological action at all in rats and mice, and this made up the thought that the drug was completely harmless. Encouraged of

its presumed non-toxic properties the company thought, “a nonlethal sedative would have enormous market potential.” The problem with sedatives at this time, mostly barbiturates, was that they were easy to commit suicide with, but this drug was later advertised as “a completely safe solution to the mounting toll of barbiturate deaths.”

Reports from Australia and Germany could in November 1961 link the use of thalidomide to the rise of infants born with severe birth defects, which led to a publication in a newspaper in December the same year and thereby a rapid withdrawal of the drug from all markets. About 8 000-12 000 children were born with birth defects and out of them 5 000 survived beyond childhood.

Some years later, in 1965, Jacob Sheskin, a physician at a French hospital tested thalidomide in the treatment of a terminally ill patient with erythema nodosum leprosum (ENL) - a complication of leprosy. Sheskin found a bottle of 20 tablets with thalidomide, though he knew the drug was banned; he also remembered that the drug was used as a sleeping pill. The patient was dying and in extreme pain so Sheskin felt he had nothing to lose and gave the patient two pills. The patient slept for 20 hours, woke up and could leave the bed without assistance. Several trials on thalidomide treatment on ENL were made and one randomized study showed up with favorable results. During the coming years use of the drug was tested for several immune defect disorders.(94)

Several studies have tried to determine the mechanisms of thalidomide action. In 1991, it was shown that thalidomide inhibits tumor necrosis factor alpha (TNF-α) in an *in vitro* study(95). Two years later the same group demonstrated that thalidomide could decrease levels of TNF-α in patients with ENL(96). Later, *in vitro* studies could show thalidomide’s effects on T-cells, shifting from a Th1 to a Th2 phenotype by lowering of IL2 and IFN-gamma(97). 1994 D’Amato et al could show anti-angiogenic effect due to down-regulation of basic fibroblast growth factor(98) and inhibition of angiogenesis could later be shown as an effect of down-regulation of VEGF due to thalidomide, in a study of corneal vascularization on rabbits(99).

During the 90s, one of the main theories on searching for a cure against cancer was based upon finding ways to inhibit the tumors’ ability to induce angiogenesis: thus making up for Barlogies study, where the hypothesis was based upon the anti-angiogenic properties of Thal and the prominent bone marrow vascularization seen in MM.

6 randomized clinical studies were later on performed to compare the effect of adding thalidomide to MP (MPT) compared to that of MP(100-105), and a meta-analysis of those studies could show an overall survival benefit of 6 months for MPT(106), thus consolidating thalidomide’s status as an efficient MM drug.

Second Generation IMiDs

Later on, attempts were made to find new IMiDs in search for drugs with less side effects and higher potency. Lenalidomide (Len) was approved in 2006 and Pomalidomide in 2012 and today IMiDs are the backbone for various treatment regimens of MM.

Len was first presented as a potentially more potent analogue to thalidomide, but lacking side effects as somnolence, obstipation and neuropathy(107). Two randomized clinical studies presented in 2007 could show its potency in treating relapsed MM(108, 109) and upon the results from these two studies both the US Federal Drug Agency (FDA) and the European Medicines Agency (EMA) 2006 granted approval for the use of Len in combination with dexamethasone for the use in second line of MM-therapy(110). Results from the FIRST trial(111), where continuous use of LenDex compared with LenDex for 72 weeks and MPT for 72 weeks, could show better PFS for continuous use of LenDex was the basis for the approval for use of Len in first line treatment in patients ineligible for autologous stem cell transplantation both from FDA and EMA.

Pomalidomide was approved upon the MM-003-study comparing pomalidomide in combination with low dose Dex to high dose Dex as single drug in patients that had received at least two previous lines of therapy including Len and Bor. PFS for combination of pomalidomide and dexamethasone in this heavily pretreated group was 4.0 months compared to 1.9 months for high dose dexamethasone(112).

IMiDs and their Mechanism of Action

Though used as standard treatment for MM, the primary target for IMiDs anti-MM effect was unknown until 2010 when dr. Ito in a study of the teratogenic effects of Thalidomide could show that Thalidomide binds to a protein called cereblon(113). A study from 2014 could show that lack of cereblon expression in bone marrow samples of MM-patients correlated with inferior survival in patients treated with a Len- or Thal-based treatment regimen, but not with a regimen based upon Bor(114), thus making it clear that cereblon is essential for anti-MM activity.

Cereblon forms an ubiquitin ligase complex with DDB1, CUL4A and ROC1. When activated by IMiDs it ubiquitinates the two transcription factors IKZF1 and IKZF3, also named Ikaros and Aiolos, which in turn will be degraded in the proteasome(115). The absence of those two factors will in turn cause down-regulation of the two transcription factors IRF4 and MYC. These two are essential for the survival of the MM cell(116), and their absence will lead to the death of the MM cell. Interestingly IRF4 stimulates the production of MYC,

which in turn stimulates the production of IRF4, making an auto regulatory circuit in MM-cells(116).

IMiDs have numerous effects at the immune system as mentioned above. It has a large anti-inflammatory property as decreasing TNF-α, however TNF-α levels of the bone marrow of MM-patients are low. IMiDs also lower the levels of the pro-inflammatory factors IL1, IL6, IL12 and Cox 2 and raises levels of the anti-inflammatory factor IL10. Whether it is the IKZF1/IKZF3-pathway that leads to those effects of IMiDs or if there are more targets is unknown. It is shown, that IKZF3 binds to the IL2 gene promoter and represses the IL2 production in $CD4^+$ T-cells, and that addition of Len induces IL2 production. The same study could also show abrogated Len effect on IL2 in cells with their Cereblon gene knocked down(115). One theory of MM development is set upon its ability to avoid immune response upon down-regulation of NK-cells. NK-cells have the capacity of directly target tumor cells and kill them, and are therefore considered to be a keystone in the organism's defense against malignancies(117). NK cells are directly up-regulated by IL2(118), and so is Len effect on IL2 a possible mechanism in MM cell killing beside the effect on IRF4 and MYC.

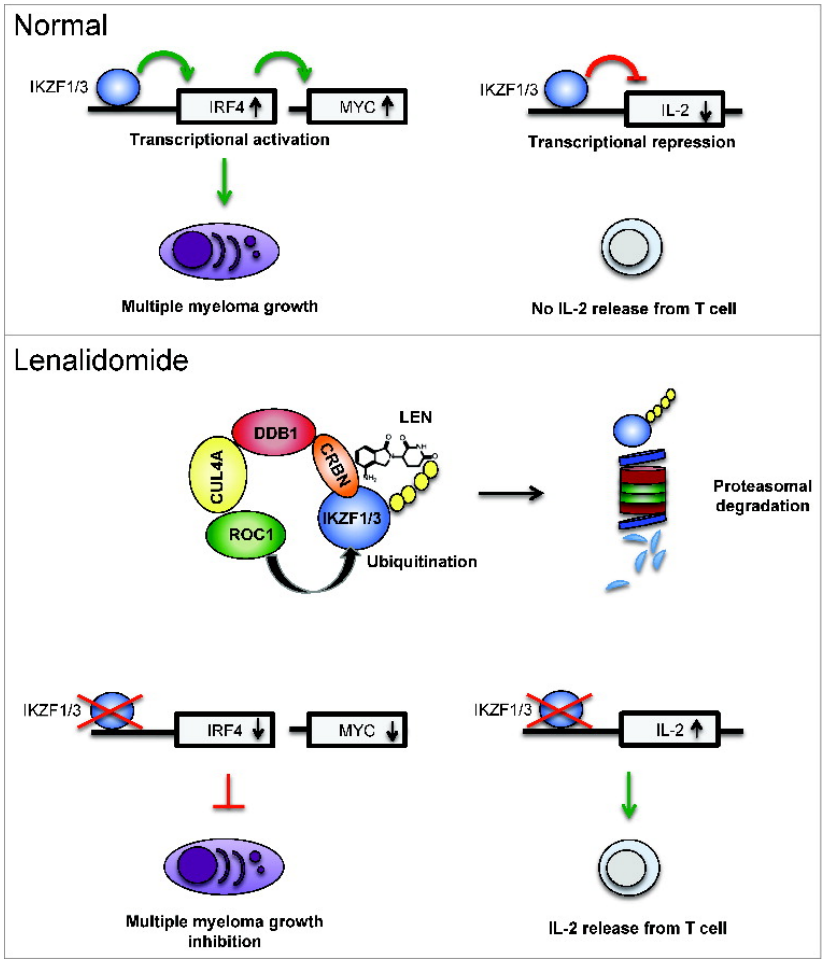


Figure 2. Lenalidomide action on Cereblon. Reprinted from Oncoimmunology **3**(7): e941742.(119) Open Acces.

Other modern treatment modalities

Proteasome inhibitors

The first proteasome inhibitor (PI), Bortezomib (Bor) became available in the clinic in 2003 and 2004 after demonstration of efficacy in two phase II trials(120, 121). These studies were followed up by a randomized phase III study comparing Bor with high dose Dex, showing benefit in response rate and OS for Bor, despite substantial cross over from Dex to Bor(122, 123). Later on it was (as Thal) added to the old MP-regimen in a comparison MP to MP with added Bor (MPV) showing benefit for MPV with acceptable increase in side effects(124-126) and from that the drug was totally accepted as an efficient MM agent.

Carfilzomib (Carf) is a newer PI, approved in US 2012 and in Europe in 2016. It has shown good effect as single drug(127), but has shown best effect in combination with Len and Dex in the ASPIRE study with an overall response rate (≥PR) of 87.1% in patients with relapsed MM(128).

Ixazomib is an oral PI, but yet not approved in Europe. It has shown promising result, even in patients previously treated with Bor(129) and in combination with LenDex(130), showing slightly less overall response rate (78%) than the combination Carf with LenDex.

Antibodies

A number of monoclonal antibodies against MM are under development and have been developed during the past years. The first one was daratumumab (Dara), an antibody against CD38, a transmembrane glycoprotein expressed on myeloid and lymphoid cells, but also on non-hematological tissues(131). It is especially expressed on MM cells(132). Dara has in a series of clinical studies shown severe toxic effects on MM cells and good clinical outcome on MM-patients, both as single agent and in combinations with other drugs(133, 134). Elotuzumab, an antibody against signaling lymphocytic activating molecule family member 7 (SLAMF7), has no single drug effect on MM, but has shown promising results in combination with other anti MM agents. It has now singe agent effect on MM cells but in combination with other anti MM agents it has shown promising results. It works by binding both NK-cells and MM cells, triggering NK-cells and tagging the MM cells to make them more susceptible(135). It’s tested in clinical trials comparing Elotuzumab combined with LenDex to LenDex showing survival benefit for the combination(136)

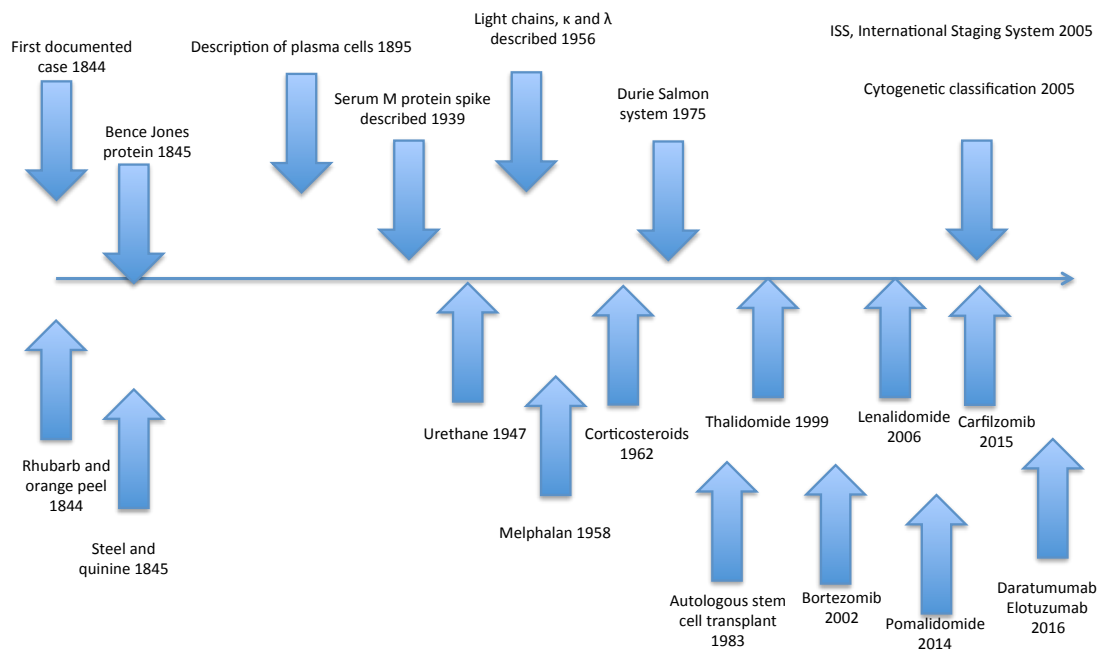


Figure 3. Timeline of MM history

Pharmacokinetics of Lenalidomide

Len is mainly excreted by the kidneys and is to a very less extent a subject for metabolism. *In vitro* it is shown to have a half time due to hydrolyzes of approximately 8 hours(137), but as its halftime in plasma due to renal excretion is about 3 hours(138), the metabolism stands for a minimal part of the elimination. More than 80% of Len is found un-metabolized in the urine within 24 hours after oral administration(139). In patients with renal impairment excretion is significantly prolonged. In patients with mild and severe renal impairment (CrCl <50 ml/min) less Len was excreted un-metabolized and half time was prolonged till 8-9 h(140).

P-glycoprotein

P-glycoprotein (P-gp) is a protein of the cell membrane and pumps foreign substances out of cells. It is distributed in intestinal epithelium, liver cells and in proximal tubules of the kidney. It is coded by the ABCB1-gene on chromosome 7. It was initially discovered as it was interacting with several anti cancer drugs(141) and dysregulation of it is a well-characterized mechanism by which cancer cells *in vitro* avoid action of chemotherapeutic agents. Due to its distribution in both the intestinal epithelium and kidney tubules, it could both interact with Len uptake and excretion. An *in vitro* study showed that Len is a weak substrate for P-gp(142) and another small phase I clinical study, combining Len with the mechanistic target of rapamycin (mTOR)-inhibitor

CCI-779, also a substrate for P-gp, could show possible drug to drug interaction with elevated Len concentrations upon increased doses of CCI-779(143), this suggesting a clinically significant effect on uptake and excretion of Len from P-gp inhibition.

Single-Nucleotide Polymorphisms (SNPs) in the gene, ABCB1, have been associated with altered expression and phenotype in P-gp(144). In two previous studies(145, 146), colleagues have shown impact on survival in AML from variations in ABCB1 SNPs.

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2 AIMS

As MM is an incurable disease and patients during their disease history receives several lines of treatment there is still a great need for new therapies; but there is also still a great need for getting better knowledge of the nature of the disease and to improve the knowledge of the existing treatment modalities to optimize the use of already existing drugs.

All new drugs are today tested in randomized trials with patients selected due to several inclusion and exclusion criterias, thus making up a study population that always not correspond exactly to the normal, "real life" population of MM patients found outside the studies. A defined study population, followed from start of treatment and given a defined drug combination in defined doses and under a defined time is of course easier to study than patients treated in the clinic. But to know the outcome of different drugs and combinations in the clinical setting it is also of great value to do retrospective studies, indeed to prove that the results in clinical studies are accurate in the clinic.

Aside the drugs we use there are several characteristics of MM that affect the prognosis of the disease. We know that the patients' performance status, staging according to ISS, creatinin and calcium levels and chromosomal abnormalities affect the efficacy of a specific drug and survival of the patient. However, we do not today know much how to use these data when we choose which treatment to use in a specific patient.

The specific aims of this thesis are:

1. To identify the importance of existing drugs combinations, and to validate phase III studies with large population based cohorts.
2. An attempt to identify/re-invistigate the impact of different chromosomal markers and attempt to identify new markers, gain 1q21, and their outcome for patients with MM.
3. To investigate the importance of maintenance of Len in relapsed and refractory MM and evaluate whether there is an inevitable need for combining Rev with Dex upon achieving respons.
4. To study clinical outcome in MM and compare it to different aspects of polymorphisms in DNA coding for a transport protein, P-glycoprotein, regarding efficacy and side effects in patients treated with Len.

3 Materials and Methods

Study population

Paper I and II

In paper 1 and 2 we retrospectively collected data from patient journals.

In the first paper, data were collected from a database of all patients diagnosed with MM at 15 Swedish centers from January 2000 until June 2011. Data including sex, age, type of MM, extent of bone disease, as well as laboratory measurements at diagnosis were collected. Serum M-protein and Urine M-protein were collected at baseline and then followed until patients' death, lost to follow up or until data cut-off. MM treatment data were collected with specific start- and stop-date for each drug or combination of drugs. Death dates, after the data collection, were obtained from the Swedish National Death Register. The study was focused on patients receiving MP or MPT in any of 1^{st}, 2^{nd}, 3^{rd} or 4^{th} line of treatment. From the data base cohort of 1843 patients, 1162 complied with the inclusion criteria for the study.

In the second paper, we used retrospectively collected data from patient journals from centers in Sweden, Norway and Denmark from patients diagnosed in the years 2006 to 2011. The patient cohort from Sweden was the same as in the first study, but limited to the years from 2006. The study was based upon 930 MM-patients, but was limited to 347 patients with known FISH data of gain of 1q21. Data were collected on sex, age, type of MM, bone lesions, and laboratory measurements including M-protein of serum and urine. As in the first paper response data and treatment data were followed up during the study to define responses and survival data. Death data outside the study were obtained from the central registers of the different countries.

Paper III and IV

Paper 3 and 4 are based upon two connected studies conducted in Sweden, Denmark and Norway. Patients were included from 9 Swedish, 2 Danish and 1 Norwegian center. The first study was an observational, non-interventional phase IV study of Len naïve MM patients, refractory to or relapsed after their first line of treatment. Inclusion criteria included age ≥18 years and measurable disease defined as a serum M-protein of >0.5 g/dl or Bence Jones protein >20mg/24h. Patients with plasma cell leukemia, amyloidosis or non-hematological malignancies were excluded. 133 patients were included and received standard dosing of lenalidomid

and dexamethasone - LenDex in 28 days cycles; Len 25 mg on days 1-21 and Dex 40 mg on day 1, 8, 15 and 22. A maximum of 9 cycles were administered to each patient. After achieving at least partial remission (PR) and two additional treatment cycles patients were invited to join the second study. The second study was a prospective, randomized, open-label phase II trial. 62 patients were randomized in a ratio of 1:1 to receive either single agent Len or to go on with combination treatment LenDex for up till 24 cycles or progression.

Definition of Endpoints

Treatment response was assessed according to IMWG criteria(55) with the exception of our own definition of nCR – (near Complete Remission). nCR was used as a complement to CR when the clinical praxis was to not perform a bone marrow sample in situations of immeasurable M-protein, but to distinguish the response from VGPR.

In the papers we used the terms:

TTNT – Time to next treatment, the time between treatment start in the current line of treatment and treatment start in the next line.

PFS – Progression free survival or TTP – Time to progression, the time from start of a treatment line till progression or death.

OS – Overall Survival, the time from start of a treatment line until death or last follow up.

Statistical Methods

Paper I

To test differences between two independent groups we used Mann-Whitney test. To evaluate differences in contingency tables Fisher´s exact test was used. Life time curves were calculated in accordance to Kaplan Meier and those were compared using log rank test.

Paper II

To test differences in patient characteristics and difference in response between subgroups, one way-ANOVA was used. To evaluate differences in contingency tables, Fisher´s exact test was used. To test for differences in OS and TTP between groups, log rank test was used. To evaluate predictive factors for survival, univariate and multivariate Cox proportional hazard regressions were made.

Paper III

Differences in patient characteristics were tested by ANOVA. For differences in contingency tables chi square test was used, or, for low frequencies, Fisher's exact test. ANOVA with Kaplan Meier methods were used to compare survival data.

Paper IV

Survival data were calculated with log rank test for significance and assessed with Kaplan Meier graphs. Cox regression models were used for multivariate analyses to adjust for patient characteristics. Differences in AE between groups were compared with Chi^2 test. The distribution of patient baseline characteristics were compared between genotype groups using Mann Whitney or Kruskal Wallis test for continuous variables, and Chi^2 or Fisher´s exact test for categorical variables.

In all studies a p-value of 0.05 was considered significant.

Response	Serum M-protein	Urine M-protein	Bone Marrow	Free Light Chain
Stringent Complete Response (sCR)	Undetectable by immunofixation	Undetectable by immunofixation	Absence of clonal cells	Normal free light chain ratio
Complete Response (CR)	Undetectable by immunofixation	Undetectable by immunofixation	≤5% plasma cells	
Very good Complete Response (VGPR)	≥90% reduction	<100 mg /24h		
Partial Response (PR)	≥50% reduction	≥90% reduction and <200 mg /24h		
No Response (NR)	≤50% reduction			
Progressive Disease (PD)	25% increase, ≥0,5 g/dl	25% increase, or increase of ≥200 mg/24h		

Table 2 IMWG response criteria

4 Results and Discussion

The recent two decades have presented an astonishing development in diagnosing and treating MM. Stem cell transplant has provided great benefit for younger patients and the evolvement of the "novel" drugs has since Barlogie's first attempt with thalidomide revolutionized treatment in both young and elderly patients. Diagnostics has also improved. New methods in characterizing malignant diseases from conventional cytogenetics via FISH to molecular genetics as Next Generation of Sequencing gives us enormous potential in classification, risk estimation and determination of which drug or combination of drug and in which order to give to each patient. We have in four papers looked upon some aspects of this.

Pros and cons Retrospective studies vs. Prospective ones

We have in two retrospective studies analyzed outcome in "real life patients" in a way to try to translate results in multiple prospective studies to the clinical praxis. It is well known that the prospective clinical trials provide us with controlled results with a certain level of statistical significance. From the statistical significance of the studies we also could draw conclusions about similar patients to them participating in the certain study. However, the study populations in clinical studies are highly selective. Certain exclusion and inclusion criteria provide studies with a manageable group of study objects, often lacking patients from a certain age, with certain performance status etc. Furthermore, specific studies are often designed to answer specific questions; e.g. a certain drug in a certain line of therapy and takes not much account of what happened before the study and what happened after. Here, I think, wide retrospective studies have a role.

In a retrospective population based study you have all patients and not a selected group that you in some way have to "interpret" when to draw conclusions to your own "real life patients". You have also information from the beginning to the very end. In clinical studies you often do not have the time to follow up patients for many years, results must be published, and survival endpoints are presented as PFS and TTP instead of OS.

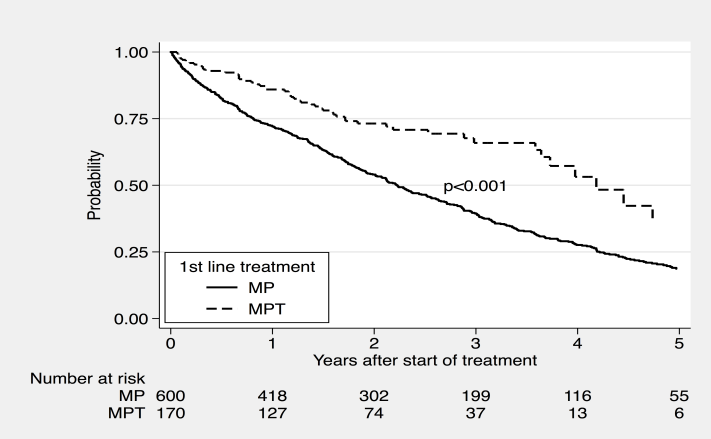
The great problem with the retrospective setting is that there is no control of the groups that are studied. If the study is to study differences in outcome between two groups of patients that were given different medication it is likely that patient characteristics as age, performance status, lab data influences the clinician's choice and there makes the studied groups different from the beginning, interfering with the results. There are statistical methods, at least partially, to overcome this problem, but the problem still is there, making data from population based studies graded lower when it comes to evidence grading.

Thalidomide in clinical practice

After the introduction of thalidomide in 1999(93), six prospective randomized studies were performed comparing the benefits of the combination of melphalan and prednisone (MP) with melphalan prednisone and thalidomide (MPT)(102, 103, 147-150) showing better outcome for the latter. A meta analysis of these studies showed increased progression free survival and overall survival of 6 months(151).

From our retrospective register where we collected data from all 1843 patients diagnosed with MM from January 2000 to June 2011 at 15 different Swedish hospitals, we sorted out all patients treated with MP or MPT, providing us with a complete cohort of all patients from a defined population given those drug combinations. From the study group, we selected all patients given MP or MPT in first, second, third and fourth line of therapy, a total of 888 patients. We could show differences in overall survival favoring MPT in all studied lines of therapy, but the results were not significant in line three and four, maybe due to too small groups, but also maybe due to lack benefit from intense treatment in heavy pretreated patients.

The results in first line were a bit surprising; as mentioned above, meta analysis of previous randomized studies showed a median OS-benefit of 6 months favoring MPT and we could show a significant difference of 2 years (2,2 vs. 4.2 years for MP vs. MPT respectively)(152). Of course there has to have been problems with selection basis; as a physician you probably do not choose to add a likely more poisonous drug to a fragile and elderly patient, but still, after adjusting for differences in prognostic factors as hemoglobin, creatinine and albumin levels the results were still significant and the difference in age between the group was minor (median age 77 vs. 75 years). The question was also raised whether there were differences in supportive care due to that MP and MPT were given in different time periods since we started collection of patient data before thalidomide got its permission and that it took a while until it was fully introduced in the clinical practice. To solve that issue we also compared patient treated with MP in the same time period as patients treated with MPT and the differences in survival were still significant.



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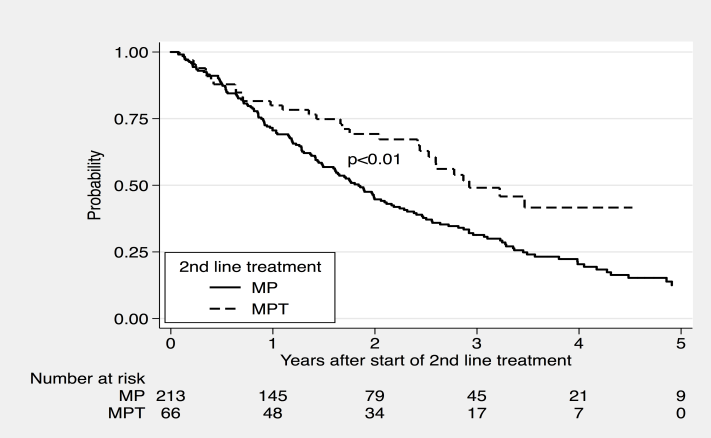

B

Figure 3. Overall survival for patients treated with MP compared to MPT. 1st line A, 2nd line B

Gain of 1q21

In paper II(67) another retrospectively collected patient cohort was analyzed. In this study patients came from three Nordic countries - Sweden, Denmark and Norway. The Swedish patients were partly the same as in paper II, but limited to a shorter time period, as mentioned above. We knew that gain 1q21 is one of the most common aberrations in MM and that it is linked to an overexpression in Cyclin-dependent kinases regulatory subunit 1 (CKS1B) which is associated to a worse prognosis in MM(153). The question here was whether we could show, in our retrospectively studied patients, a difference in outcome based on FISH data on the chromosomal abnormality of gain of1q21 and if it was possible to overcome this assumed worse prognosis by treating with novel agents vs. conventional cytostatics. We had data from 930 patients, but had to limit the study to 347 patients with known FISH data on gain of 1q21.

The cohort was divided into 3 groups: gain of 1q21 (n=119), other chromosomal abnormalities (OA) (defined as del(13q), del(17p), t(4;14), and/or t(14;16)) (n=105) and a group with no abnormalities or other than the ones listed above (NO) (n=123).

Results on survival between the groups treated with conventional cytostatics were in line with our assumption. OS was better in the NO group compared to the two groups with chromosomal abnormality. No difference could be seen between the gain 1q21 and the OA groups. We could show, in line with previous randomized studies(69, 72), that treating with novel agents (Thal, Bor and Len) could overcome the negative impact of chromosomal aberrations. However, in our material, the negative impact of gain 1q21 still existed, despite use of novel agents.

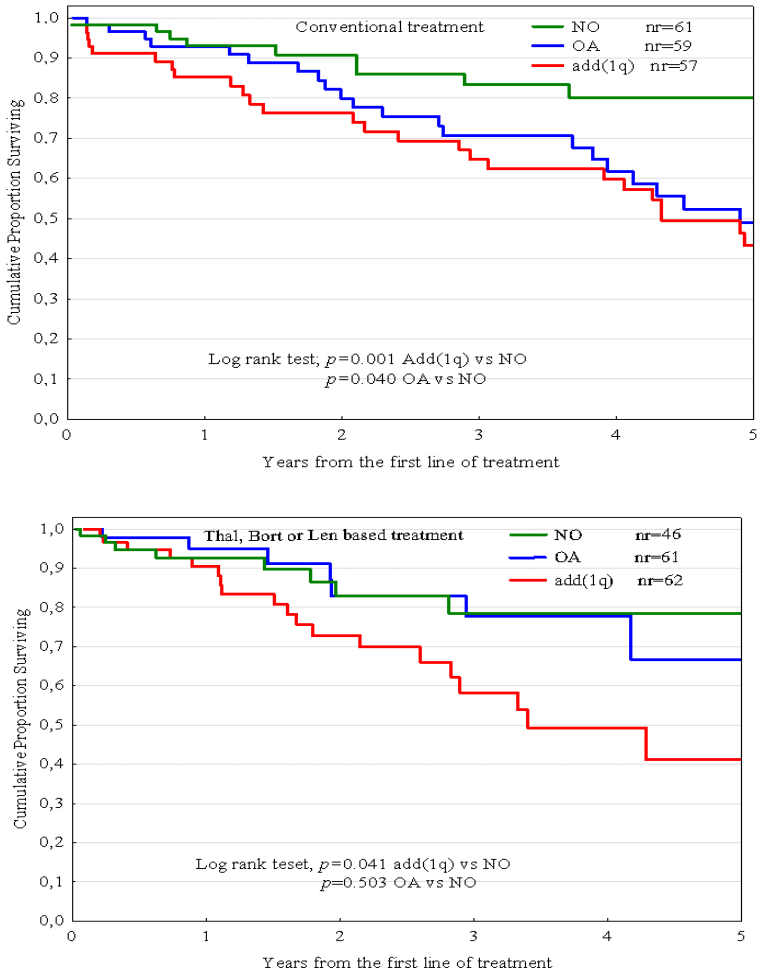

Figure 4. OS comparing patients with gain 1q21, other abnormalities (del(13q), del(17p), t(4;14) or t(14;16) (OA) and no abnormalities (NO) treated with A; conventional cytostatic agents B; Thal, Bort or Len

Need for different kind of studies?

Thus, in paper I and II, we could in our retrospective setting show up with similar results as in previously performed clinical studies and in some way prove that their results are possible to transform into the clinical

praxis. As discussed before, clinical trials have their disadvantages (and so do retrospective studies!), and we find it important to assess the results from studies and see if they still are valid when it comes to clinic.

From our data in paper II, we could also show new results on gain 1q21. No clinical study has put up the question whether negative impact on survival from gain 1q21 could be overcome by use of PIs or IMiDs. This question will remain unanswered without a prospective clinical trial; however, well-designed retrospective studies can hint the answer.

Lenalidomide

In paper III and IV we studied lenalidomide as second line treatment in relapsed or refractory MM. The patient groups are discussed above. At the time of the study the indication for use of lenalidomide was in second or later lines of therapy base upon two studies by Dimopoulos and by Weber(108, 109). The study design was to treat a patient group with the standard treatment of lenalidomide and dexamethasone (LenDex), and upon response, to randomize into two groups – one with continuous standard treatment of, LenDex and the other with Len as single drug.

Prolonged treating with corticosteroids has lots of side effects and to exclude it from MM could possibly give benefit to patients. *In vitro* studies showed that Thal and other IMiDs could enhance interleukin 2 (IL2) and interferon gamma (IFN-gamma) production and enhance NK-cell mediated MM cell killing(154) and that this effect from Len was depleted by adding corticosteroids(118). Thus we designed the study to first get a response from LenDex and then to see whether it was possible to withdraw Dex.

The response rate (PR or better) in our study was better than in the studies by Dimopoulos and Weber (79% vs. 60% and 61% respectively), and so was TTP (19 months vs. 11.3 and 11.1 months), but our study was restricted to patients in second line of treatment and theirs to second or later lines.

We could see no difference in OS between the groups in the observational part of the study (Len vs. LenDex). There was a trend towards better TTP, favoring LenDex, but the result was not significant. We could not, however, see any phenotype differences among circulating lymphocytes including NK-cells. The conclusion would be that LenDex is a safe treatment regimen and that prolonged treatment with Len of LenDex after achieving at least a PR provides sustained and clinically relevant responses.

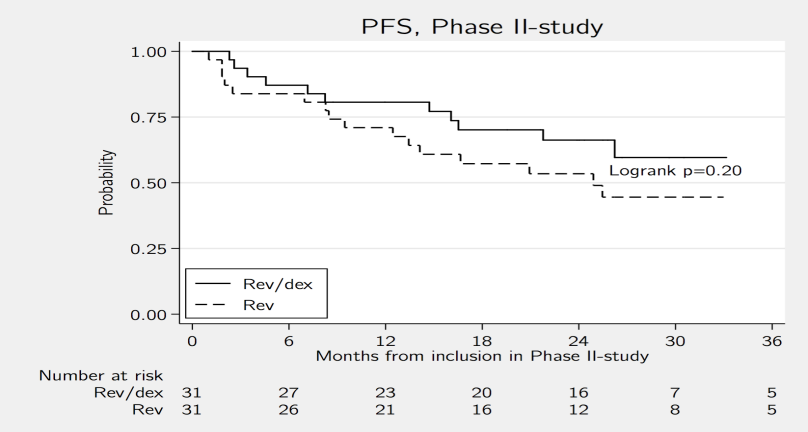

Figure 5. PFS in phase II study comparing Len vs. LenDex

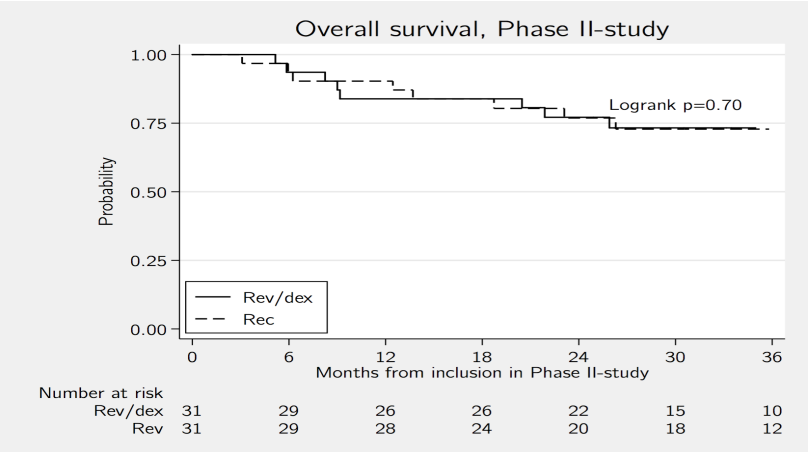

Figure 6. OS in phase II study comparing Len vs. LenDex

The pharmacokinetic studies of paper IV could show no significant effect of any ABCB1 variant on Len treatment response, OS, TTP or risk of hematological AE. We could see a trend to that the variant 1199G>A could show a little longer TTP. The effect of P-gp on the excretion and uptake of Len is probably minor and our study is small. To answer the question whether there is an actual effect or not, we will have to perform a bigger study and also include measurement of Len concentration in serum and maybe also urine to link the knowledge on genotype with clinical outcome in a more certain way.

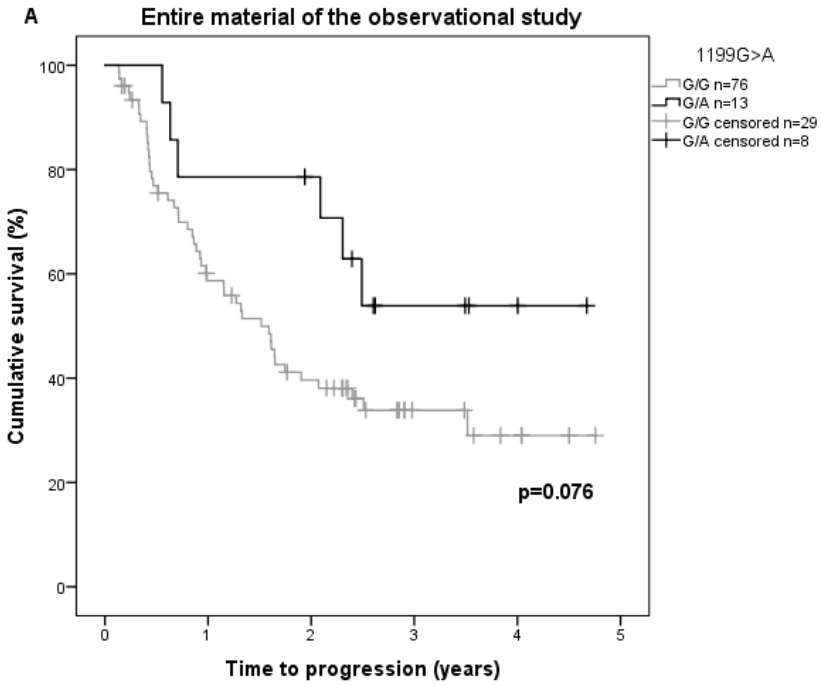

Figure 7. TTP in relation to 1199G>A genotype.

5 Future Perspectives

Although there has been several progress in understanding biology of MM, and its implication both to the nature of the disease, and to improve treatment modalities, there is still much to do. MM is still classified as an incurable disease, and most patients diagnosed with MM, except the very elderly, will die from MM.

Today, we choose treatments from factors relating to the patient, e.g. performance status, age etc. We don't consider disease specific factors, as chromosomal abnormalities and different gene expression, in some significant extent, when to choose treatment modalities.

It would be great to improve the knowledge on chromosomal abnormalities and see if MM with certain modalities were more susceptible to certain treatments. We have seen, when we changed from therapy with classical cytostatics into what was called "novel agents", that certain "high risk" abnormalities could be overcome by treating with Thal, Bor of Len. Now, it would be interesting if there were differences between today's treatment modalities, when it comes to different prognostic markers.

Further studies on pharmacogenetics, a still not much explored research field when it comes to MM, would also possibly improve treatment of MM. It would be of great interest to set up a study to compare serum levels of certain drugs, to compare it to genetic markers on uptake and excretion, and to clinical outcome.

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