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BIOLOGICAL MARKERS AND TREATMENT AS PROGNOSTIC FACTORS IN MULTIPLE MYELOMA

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Biological markers and treatment as prognostic factors
in multiple myeloma
THESIS FOR DOCTORAL DEGREE (Ph.D.)

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*“The more I study the sloth the more it reminds me of science:
The method might look slow and sometimes inefficient, but in fact
it optimises resources in an astonishing way and combined with
determination leads to result.”*

Horatio S. Darwin, British physician and naturalist

ABSTRACT

Multiple myeloma (MM) is an incurable disease with an increasing number of treatment options. The introduction of what are called novel drugs (bortezomib, lenalidomide and thalidomide) was an important step. These treatments have been well studied in clinical trials that involve selected patient groups and that focus on a specific treatment in a specific line of treatment. However, there is scarce information on the survival as a function of the entire treatment sequence. We wanted to clarify the effect of these treatments in a real-life setting. Furthermore, there are several well-known prognostic factors for MM, but the impact of those factors on survival in the era of novel treatment is not fully understood. In this thesis we aimed to: 1) understand in which order the treatments should be given, 2) define factors affecting prognosis, 3) increase the knowledge of cytogenetic abnormalities and their influence on prognosis and choice of treatment.

In **Paper I**, we retrospectively analysed the outcome in high-dose treated (HDT) patients. The patients were divided according to induction therapy. 142 patients had received conventional chemotherapy with either vincristine, doxorubicin, dexamethasone (VAD) or cyclophosphamide, betamethasone (CyBet) and 94 patients had received bortezomib, cyclophosphamide, betamethasone (VCB). We found that the VCB patients had a quicker and better response than the VAD/CyBet group as well as a longer time to progression.

In **Paper II**, we investigated 1 638 consecutive MM patients and compared their survival with that of a sex- and age-matched normal population. The use of novel agents as upfront treatment in non-HDT patients resulted in a significantly longer overall survival (OS) compared to those who received conventional chemotherapy. The OS was further improved by using novel agents in both first and second line of treatment and for these patients the OS approached the survival in the matched normal population. **Paper III** focused on MM patients with renal impairment at diagnosis. Previous studies have demonstrated the negative impact of renal impairment when conventional chemotherapy is used. We could confirm these findings. However, novel agents significantly improved the OS of non-HDT patients with renal impairment. Moreover, the difference in survival between those with and those without renal impairment vanished with the use of novel agents. Despite high response rates to novel treatment, approximately 20% of the patients do not respond to bortezomib therapy. In **Paper IV**, we demonstrated that changes associated with del(8)(p21) might be one explanation to bortezomib resistance. We found that MM cells without del(8)(p21) responded to bortezomib treatment by upregulating the pro-apoptotic TRAIL receptors, thus making the cells more sensitive to TRAIL/APO2L-mediated apoptosis. However, in cells with del(8)(p21) no upregulation was seen and the cells were largely resistant to TRAIL/APO2L-mediated apoptosis. These findings were also supported by clinical observations.

To summarize, with these studies we could confirm that the survival benefits with bortezomib, lenalidomide and thalidomide that have been demonstrated in clinical trials are also seen in real life. Furthermore, we demonstrate a possible resistance mechanism to bortezomib.

POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

Multipelt myelom är en cancersjukdom som utgår från benmärgens plasmaceller. Plasmaceller är en typ av vita blodkroppar (B-celler) som är viktiga för immunförsvaret. När plasmacellerna träffar på till exempel virus och bakterier börjar de tillverka antikroppar (immunglobuliner) som tar död på inkräktarna. Vid myelom har plasmacellerna blivit sjuka och börjat föröka sig onormalt mycket. Dessutom börjar de tillverka ett speciellt immunglobulin som kan mätas i blodet och/eller i urinen och som kallas M-komponent. Det är denna M-komponent man följer när man kontrollerar behandlingssvaret hos en patient. Dock finns det myelompatienter som inte utsöndrar någon M-komponent och hos dessa får man följa sjukdomen på annat sätt. Hos vissa patienter tillverkar myelomcellerna bara delar av immunglobulinet, de så kallade lätta kedjorna, och dessa kan man också mäta i blodet. Det finns även en liten grupp patienter som inte utsöndrar vare sig M-komponent eller lätta kedjor. Man blir då mer beroende av att ta upprepade prov från benmärgen för att kontrollera sjukdomsstatus.

I Sverige insjuknar varje år drygt 600 personer och de flesta av dessa är över 65 år. Myelom utgör cirka 2 % av alla dödsfall orsakade av cancer och 20 % av alla dödsfall orsakade av blodcancersjukdomar. Myelomcellerna kan producera ämnen som i sin tur stimulerar nedbrytning av skelettet, vilket ofta leder till smärta. Andra tecken på myelom är trötthet, infektions- och blödningsbenägenhet samt nedsatt njurfunktion. Ofta upptäcks dock sjukdomen via rutinprovtagning på vårdcentralen innan symptom hunnit uppkomma.

Behandling påbörjas först när sjukdomen börjar ge symptom, varför en del patienter kan vara behandlingsfria under lång tid. Yngre patienter, det vill säga de som är under 65 till 70 år, genomgår oftast så kallad högdosbehandling (HDT). Denna behandling blir effektivare om man först eliminerar så många myelomceller som möjligt. Därför får patienterna först 3 till 4 behandlingar med andra läkemedel (så kallad induktionsbehandling). Sedan samlas stamceller från patienten in och fryses ner och avslutningsvis får patienten en extra stark cytostatikakur, det vill säga HDT. För att påskynda benmärgens återhämning och minska risken för att patienten dör i komplikationer ger man tillbaka patientens egna stamceller (autolog stamcells-transplantation). Äldre patienter och multisjuka patienter klarar dock inte av denna behandling utan får istället olika kombinationer av läkemedel.

De senaste 10 till 15 åren har det skett en snabb utveckling av läkemedel. Framförallt har de tre läkemedlen bortezomib, talidomid och lenalidomid spelat en stor roll för prognosen. Trots detta är myelom fortfarande en obotlig sjukdom. Det beror på att en del cancerceller överlever behandlingen och orsakar återfall. Därför krävs det upprepade behandlingar med olika läkemedelskombinationer.

Informationen om hur effektivt ett nytt läkemedel är kommer från kliniska prövningar. Dessa prövningar inkluderar oftast endast en utvald grupp av patienter som inte alltid speglar den grupp av patienter som i slutändan kommer att få behandlingen. Dessutom fokuserar

dessas studier på en specifik behandling i ett visst skede av sjukdomen och tar inte hänsyn till vilka behandlingar som givits före och efter. Vi ville därför studera hur väl de nyare läkemedlen fungerar för de patienter som behandlas i den kliniska vardagen. Dessutom ville vi ta reda på om det spelar roll i vilken ordning de olika läkemedlen ges.

Förutom behandlingen finns det flera andra faktorer som påverkar prognosen.

Dessa inkluderar njurfunktion, blodvärde och skelettpåverkan. Det är dock inte helt klarlagt vilken effekt dessa faktorer har på prognos och överlevnad sedan de nyare läkemedlen, det vill säga bortezomib, lenalidomid och talidomid, introducerades på marknaden.

Dessutom har studier funnit olika kromosomavvikelser som påverkar prognosen vid myelom.

Vår grupp har tidigare demonstrerat att patienter med avsaknad av den korta armen på

kromosom 8 (del(8)(p21)) har en sämre prognos, men orsaken till detta är inte klarlagt.

Vi ville därför studera prognosfaktorer för att få en tydligare bild av deras betydelse i skenet av de behandlingsalternativ som finns tillgängliga. Likaså ville vi förstå varför del(8)(p21) påverkar prognosen och svaren på olika behandlingar.

I de tre första studierna gick vi igenom patientjournaler för att samla in data kring behandling samt svar på behandling. Den första studien innefattade 236 patienter som man hade för avsikt att ge HDT. Tidigare gav man endast konventionell cytostatika som induktionsbehandling inför HDT. Sedan de nyare läkemedlen introducerats har dessa dock tillfogats behandlingen. I Sverige använder man huvudsakligen en kombination där bortezomib ingår. Vi var därför intresserade av huruvida denna behandling gav lika god effekt i verkligheten som man sett i kliniska studier. Vi fann att patienter som fått bortezomib fick ett både snabbare och bättre svar på induktionsbehandlingen, och behandlingssvaret varade dessutom längre hos bortezomibpatienterna.

I andra och tredje delarbetena analyserade vi data från 1638 myelompatienter som behandlats på femton olika sjukhus i Sverige. Patienterna delades in i de som genomgått HDT (HDT-gruppen) och de som inte genomgått HDT (icke-HDT-gruppen). Den viktigaste slutsatsen från den andra studien var att icke-HDT-patienter som fått bortezomib, talidomid eller lenalidomid i första linjens behandling hade en tydligt förlängd överlevnad jämfört med dem som endast fått konventionell cytostatika. Vi kunde alltså bekräfta vad man tidigare sett i kliniska prövningar. Dessutom fann vi att överlevnaden förlängdes ytterligare om man använde dessa nyare läkemedel även när patienten fick återfall. I HDT-gruppen kunde vi dock inte se samma positiva effekt på överlevnaden.

Med tanke på det senaste årtiondets framsteg inom myelombehandling var vi nyfikna på överlevnaden hos myelompatienter i relation till överlevnaden i normalbefolkningen och jämförde därför dessa två (studie II). Vi kunde konstatera att myelompatienter tyvärr fortfarande har en klart sämre överlevnad än den svenska normalbefolkningen. Dock fann vi att äldre myelompatienter som erhållit nyare läkemedel både i första och andra behandlingsomgången började närma sig överlevnaden i normalbefolkningen.

Tredje delarbetet fokuserade på de patienter som hade njursvikt vid diagnostillfället. Flera tidigare studier hade visat att detta var en negativ faktor och att dessa patienter hade sämre överlevnad än de som hade normal njurfunktion vid behandling med konventionell cytostatika. Detta bekräftades också i vår studie. Dock fann vi att den prognostiska betydelsen av njursvikt för icke-HDT-patienter försvann om patienterna fick behandling med bortezomib, talidomid eller lenalidomid. Efter behandling med dessa nyare läkemedel hade patienterna med njursvikt samma överlevnad som de med normal njurfunktion.

Trots den goda effekten av bortezomibbehandling fungerar i dagsläget inte denna hos cirka 20% av myelompatienterna.

I den fjärde och sista studien visade vi att förändringar kopplade till del(8)(p21) kan vara en av orsakerna till bortezomib-resistens. Bortezomibs myelomdödande funktion beror delvis på att detta läkemedel får myelomceller att öka antalet ”dödsreceptorer” på sin cellyta, det vill säga uppreglerar receptorerna, så att immunförsvaret i sin tur kan döda cancercellerna. Vi fann att denna uppreglering av receptorer inte skedde i myelomceller med del(8)(p21), vilket resulterade i att färre myelomceller dog. Vi kunde också se att dessa fynd stämde överens med kliniska observationer.

LIST OF SCIENTIFIC PAPERS

- I. **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.
(*contributed equally)

- II. **Improved survival in myeloma patients: starting to close in on the gap between elderly patients and a matched normal population**
Liwing J*, UTTERVALL K*, Lund J, Aldrin A, Blimark C, Carlson K, Enestig J, Flogegård M, Forsberg K, Gruber A, Haglöf K, Kivle H, Johansson P, Lauri B, Mellqvist UH, Swedin A, Svensson M, Näsman P, Alici E, Gahrton G, Aschan J, Nahi H
Br J Haematol. 2014;164(5):684-93.
(*contributed equally)

- III. **The use of novel drugs can effectively improve response, delay relapse and enhance overall survival in multiple myeloma patients with renal impairment**
UTTERVALL K, Duru AD, Lund J, Liwing J, Gahrton G, Holmberg E, Aschan J, Alici E, Nahi H
PLoS ONE [Internet]. 2014; 9(7):[e101819 p.].

- IV. **Deletion of chromosome 8p21 confers resistance to bortezomib and is associated with upregulated decoy TRAIL receptor expression in patients with multiple myeloma**
Duru AD, Sutlu T, Wallblom A, UTTERVALL K, Lund J, Stellan B, Gahrton G, Nahi H, Alici E
Manuscript

OTHER RELEVANT PUBLICATIONS

- I. **Autologous hematopoietic stem cell transplantation in multiple myeloma and lymphoma: an analysis of factors influencing stem cell collection and hematological recovery**

Ungerstedt JS, Watz E, UTTERVALL K, Johansson B-M, Wahlin BE, Näsman P, Ljungman P, Gruber A, Axdorph Nygell U, Nahi H
Med Oncol. 2012;29(3):2191-9.

- II. **Addition of thalidomide to melphalan and prednisone treatment prolongs survival in multiple myeloma--a retrospective population based study of 1162 patients**

Lund J, K. UTTERVALL K, Liwing J, Gahrton G, Alici E, Aschan J, Holmberg E, Nahi H
Eur J Haematol. 2014;92(1):19-25.

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LIST OF ABBREVIATIONS

ASCT	Autologous stem cell transplantation
BM	Bone marrow
BMSCs	Bone marrow stromal cells
CKD-EPI	Chronic kidney disease epidemiology collaboration
CR	Complete response
CTD	Cyclophosphamide, thalidomide, dexamethasone
CyBet	Cyclophosphamide, betamethasone
ECM	Extracellular matrix
GFR	Glomerular filtration rate
HDT	High-dose treatment
Ig	Immunoglobulin
IL-6	Interleukin 6
JAK-STAT	Janus kinase-signal transducer and activator of transcription
IGH	immunoglobulin heavy
IMiDs	Immunomodulatory drugs
IMWG	International Myeloma Working Group
ISS	International staging system
MAPK	Mitogen-activated protein kinase
MGUS	Monoclonal gammopathy of undetermined significance
MM	Multiple myeloma
MPT	Melphalan, prednisolone, thalidomide
MP	Melphalan, prednisolone
MPV	Melphalan, prednisolone, bortezomib
MRD	Minimal residual disease
MDRD	Modification of diet in renal disease
nCR	Near complete response
NF- κ B	Nuclear factor κ B
Novel agents	Bortezomib, thalidomide and lenalidomide
OPG	Osteoprotegerin
OS	Overall survival

PFS	Progression free survival
PI3K	Phosphatidylinositol-3 kinase
PR	Partial response
RANKL	Receptor activator of nuclear factor kappa B ligand
RIC	Reduced intensity non-myeloablative conditioning
RRMM	Relapse/refractory multiple myeloma
sCR	Stringent complete response
TRAIL	Tumour necrosis factor-related apoptosis inducing ligand
TTNT	Time to next treatment
TTP	Time to progression
VAD	Vincristine, doxorubicin, dexamethasone
VCB	Bortezomib, cyclophosphamide, betamethasone
Vel-Dex	Bortezomib, dexamethasone
VTD	Bortezomib, thalidomide, dexamethasone
VEGF	Vascular endothelial growth factor
VGPR	Very good partial response

1 BACKGROUND

1.1 EARLY HISTORY

The first well documented case of multiple myeloma (MM) was published in 1844 by Dr. Solly who described a 39-year-old woman with fatigue, severe bone pain and repeated fractures. She died four years after the onset of symptoms and the post-mortem examination revealed that a red substance had replaced the cancellous portion of the sternum as well as both femurs. The bone marrow (BM) cells were found to be very clear and oval-like with one or sometimes two bright nucleoli [1]. In 1850 Dr. Macintyre described one of his patients, a man who, at the age of 45, had consulted Dr. Macintyre five years earlier due to excruciating pain and fatigue. Because of oedema, Dr. Macintyre examined the urine from the patient and found it to “abound in animal matter” [2]. A sample of the urine was also sent to Dr. Bence Jones who described that the urine contained large quantities of a substance that resembled albumin, but differed from albumin in many ways [3] and this urinary protein was later named after Dr. Bence Jones. When the patient died in 1846 the autopsy showed that similarly to Dr. Solly’s patient a “red geletiniform substance” consisting mainly of large nucleated round or oval-shaped cells with a bright nucleolus filled the cancellous cavities [4]. The cells described in both these cases were most likely malignant plasma cells. Several more cases followed [5] and in 1873 the term “multiple myeloma” was introduced by von Rustizky who, during autopsy of a 47-year-old man, found eight separate tumours of BM that he called multiple myeloma [6] and in 1900 Wright described that MM consisted of plasma cells [7].

1.2 PATHOGENESIS

1.2.1 Normal B cell development

The immature B cells express cell-surface immunoglobulin (Ig) M with kappa or lambda light chains (IgM-kappa or -lambda) and develop in the BM from a lymphoid progenitor cell [8]. The immature B lymphocytes can leave the BM and migrate to the spleen where they mature and become IgM- and IgD-expressing B cells [8-10]. The majority of these cells become circulating naïve follicular B cells, but a fraction of the cells stay in the spleen as non-circulating marginal-zone B cells, Figure 1 [9].

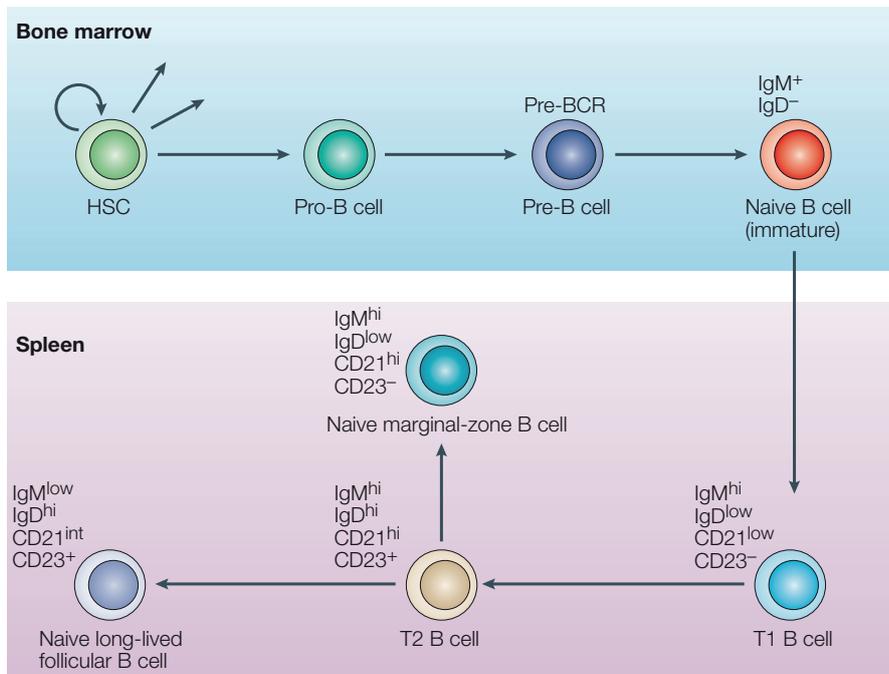


Figure 1. The development of B cells. HSC, haematopoietic stem cell; BCR, B cell receptor. Reprinted by permission from Macmillan Publishers Ltd: NATURE REVIEWS IMMUNOLOGY [9], copyright 2005.

On encounter with antigen, the marginal-zone B cells respond rapidly by proliferating and differentiating to short-lived plasma cells, Figure 2. Short-lived plasma cells can also be produced from circulating naïve follicular B lymphocytes. Thus, both B cells in the marginal zones and in the follicles are involved in the early extrafollicular response in which plasma cells that lack somatically mutated Ig genes are produced. These plasma cells are important for the initial response to pathogens, but they only secrete low-affinity IgM antibodies and are short-lived [8].

However, activated follicular B cells can also enter the follicles in the lymph node and give rise to germinal centres. In the germinal centres, the B cells proliferate and differentiate, resulting in long-lived plasma cells and memory B cells with high-affinity B cell receptors [8,9]. The long-lived plasma cells are terminally differentiated and can no longer proliferate. They move to the BM in order to find a survival niche where they can live for many months [8,11,12].

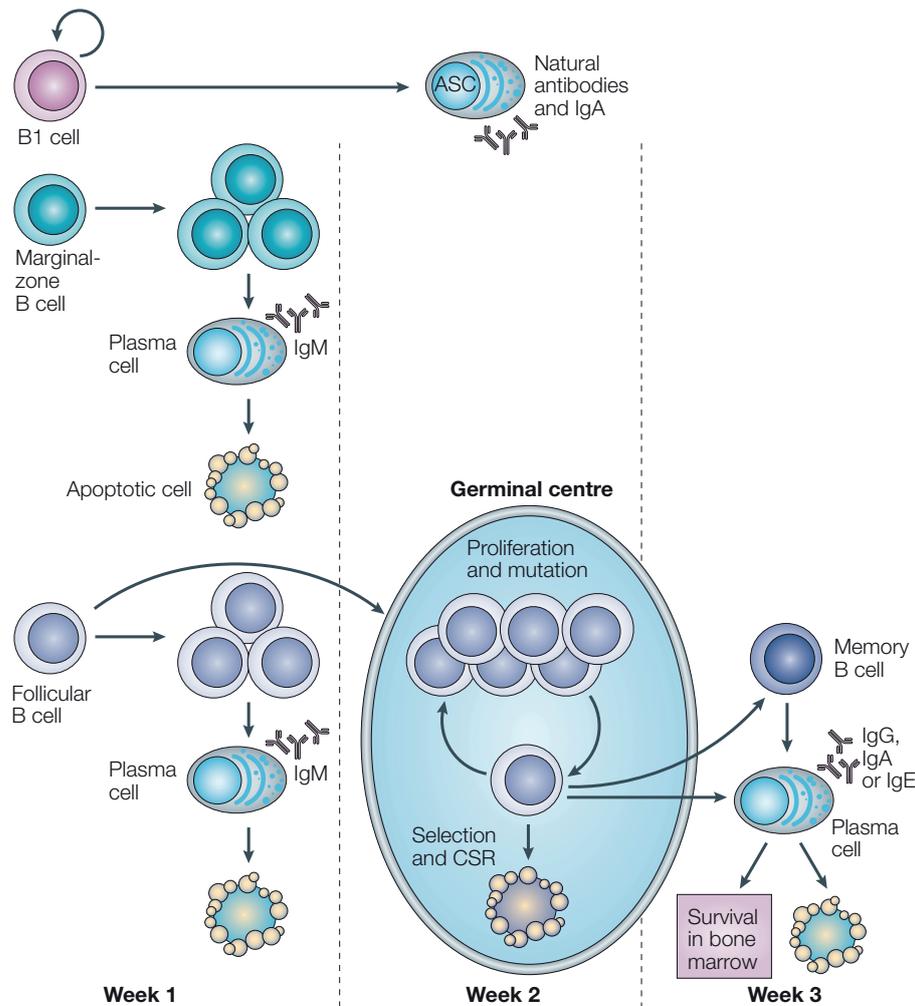


Figure 2 Formation of plasma cells. CSR, class-switch recombination. Reprinted by permission from Macmillan Publishers Ltd: NATURE REVIEWS IMMUNOLOGY [9], copyright 2005.

1.2.2 Origin of the multiple myeloma cells

MM cells are clonally expanded plasma cells but the exact origin of the MM cells is not fully understood. Studies have suggested that plasma cells lack proliferative capacity. Instead, there is evidence that the MM cells arise from a population of myeloma cancer stem cells that resemble memory B cells. These cells are thought to initiate disease, and to be the cause of progression and relapse [12,13]. However, it should be noted that some studies indicate that the malignant plasma cells themselves are tumorigenic and that they can proliferate [13].

1.2.3 Bone marrow microenvironment

The MM microenvironment consists of a cellular component, extracellular matrix (ECM), adhesion molecules, cytokines and growth factors, Figure 3. This is crucial for the growth, spread and survival of plasma cells and affects drug resistance and relapse [14,15].

The homing of MM cells to the BM is achieved with the help of cytokines that bind to MM cells, thus inducing motility and cytoskeletal rearrangements. In the BM, various adhesion molecules take part in the binding of MM cells to ECM and to BM stromal cells (BMSCs) [12,16].

1.2.3.1 Extracellular matrix

The ECM is composed of proteins, proteoglycans and glycosaminoglycans [15]. Myeloma cells adhere to the ECM, which leads to adhesion, migration and spread of the disease. In addition, these interactions can also give rise to what is called cell adhesion-mediated drug resistance. It is a spontaneous drug resistance, which occurs in untreated patients [17].

1.2.3.2 Cellular component

Bone marrow stromal cells

The BMSCs are essential for both normal plasma cells and MM plasma cells. However, differences are seen between normal BMSCs and MM BMSCs; for instance MM BMSCs produce high amounts of pro-inflammatory cytokines that stimulate MM growth. One explanation of the differences is that the MM cells select the population of BMSCs that best support the malignant cells [17]. BMSCs and MM cells interact through adhesion molecules leading to the production and secretion of MM-stimulating molecules such as interleukin 6 (IL-6) which stimulate MM cell growth, survival, drug resistance and migration [12,17].

Osteoclasts and osteoblasts

Bone lesions are common in MM patients. This is the result of an increased osteolysis (mediated by osteoclasts) and decreased osteogenesis (mediated by osteoblasts).

The MM BMSCs stimulate the osteoclastogenesis mainly through the expression of the receptor activator of nuclear factor kappa B ligand (RANKL) [12,15,17]. In normal BM, osteoprotegerin (OPG), secreted by osteoblasts and BMSCs, inhibits the maturation and activation of osteoclasts, thus protecting the bone against inadequate osteoclast activation. However, in MM BM the expression of OPG is downregulated [12,17].

In healthy individuals, bone resorption is followed by increased bone formation by osteoblasts, thus preventing the formation of osteolytic lesions. In MM, the activity and number of osteoblasts is decreased due to several factors including dysregulation of signalling molecules [12,15].

Endothelial cells

The MM cells stimulate the endothelial cells in the BM to proliferate and to form microvessels leading to an increased angiogenesis which is especially notable in patients with active disease [12,15,17]. Thus, the availability of nutrients and oxygen as well as the removal of catabolites increase [12,17]. The endothelial cells also support the growth and progression of MM by secreting growth and invasive factors [12,17].

Other cells

Adipocytes seem to be important for proliferation and migration of MM cells in the initial stage of the disease, but as the disease progresses the adipocytes disappear from the BM [17]. Among the hematopoietic cells, macrophages have been shown to protect the cells from apoptosis. They also seem to support survival and stimulate proliferation of MM cells *in vitro* due to their secretion of IL-6 and vascular endothelial growth factor (VEGF). Eosinophils are another example of hematopoietic cells that stimulate proliferation through a contact-independent manner [15].

1.2.4 Signalling pathways

Several signalling pathways are involved in myeloma proliferation, survival, drug resistance and migration, Figure 3. The signalling pathways are activated by cytokines secreted from both MM cells and BMSCs. Anti-apoptotic proteins, cytokines and cell-cycle modulators are some of their downstream targets [18,19].

Activation of the nuclear factor κ B (NF- κ B) pathway results in upregulation of adhesion molecules and increased secretion of cytokines that influence MM cell growth, survival and migration [18]. In at least half of the MM patients the NF- κ B pathway is constitutively active in both plasma cells and the BMSCs [19].

The mitogen-activated protein kinase (MAPK) pathway also influences cell differentiation, proliferation and survival. It is activated by RAS which are membrane-associated GTPases. NRAS and KRAS, two members of the RAS-family, are often mutated in MM and the mutation rate increases with disease progression. However, RAS mutations are rare in monoclonal gammopathy of undetermined significance (MGUS) and therefore RAS mutations are believed to be important for progression of MGUS to MM [18-20].

The Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway is constitutively activated in 50% of the MM patients leading to decreased apoptosis [19,20].

The phosphatidylinositol-3 kinase (PI3K) pathway is activated in about half of the MM cases, but mutations of the pathway are unusual. Via downstream targets such as mTOR, the pathway affects cell proliferation and survival [19,20].

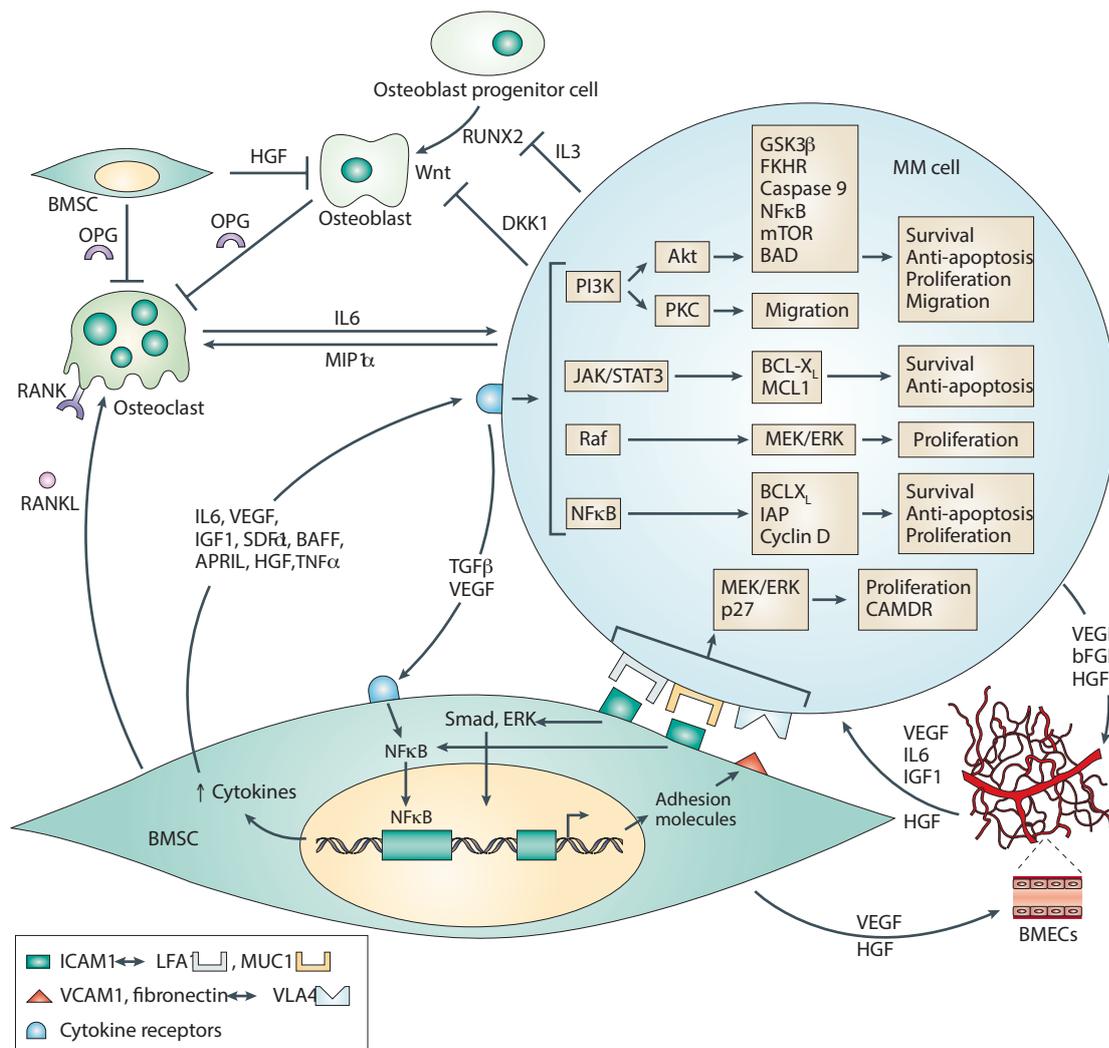


Figure 3 Interaction of multiple myeloma cells with the bone marrow environment. ERK, extracellular signal-regulated kinase; IGF1, insulin-like growth factor 1; IAPs, inhibitor of apoptosis proteins; MCL1, myeloid cell leukaemia sequence 1; ICAM1, intercellular adhesion molecule 1; VCAM1, vascular cell adhesion molecule 1; bFGF, basic fibroblast growth factor; HGF, hepatocyte growth factor; MIP1 α , macrophage inflammatory protein-1 α . Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Cancer [18], copyright 2007.

1.2.5 Genetic alterations

Chromosomal aberrations are common in MM and disease progression is probably the result of clonal evolution with accumulation of genetic alterations [21] [19]. Furthermore, studies have demonstrated the presence of several subclones with different chromosomal aberrations within the same patient. The subclones develop early in the disease process and are believed to be responsible for relapse [19,21,22].

The chromosomal aberrations can be divided into primary and secondary events. The primary events are important for plasma cell immortality and occur early in the evolution of the disease, as early as the MGUS stage [19,23]. The secondary events on the other hand are more common in smouldering MM, MM and plasma cell leukaemia and these events drive disease progression [19]. These secondary events are found only in subclones of the plasma cells and include secondary translocations, copy number variations, loss of heterozygosity, acquired mutations and epigenetic modifications [19,22].

According to the primary event, MM can be divided into hyperdiploidy and nonhyperdiploidy MM. Hyperdiploidy with trisomies involving the odd-numbered chromosomes 3, 5, 7, 9, 11, 15, 19 and 21 can be found in 50–60% of newly diagnosed MM. Patients with a hyperdiploid karyotype seem to have a better prognosis [12,19,22,23].

Nonhyperdiploidy is detected in 40–50% of MM. It is characterized by translocations involving the immunoglobulin heavy (IGH) alleles at 14q32 and these translocations can be found in almost all of the plasma cells. IGH are strong enhancers causing overexpression of the partner chromosome, most often an oncogene [12,19,22,23].

1.2.5.1 Translocations

t(11;14) is the most frequent translocation, found in 15–20% of the patients.

The translocation results in the overexpression of cyclin D1 and the prognostic impact is neutral or favourable [12,19,22-24].

t(4;14) has a negative impact on prognosis and is observed in approximately 15% of the MM patients making it the second most common IGH translocation [12,19,22,23].

The translocation upregulates two oncogenes, the fibroblast growth factor receptor 3 gene (FGFR3) and the multiple myeloma SET domain protein (MMSET). The latter of these two genes seems to be the most important molecular target for the translocation because FGFR3 is upregulated in only about 70% of patients with t(4;14) [12,22,24].

t(6; 14) is found in only a few per cent of the MM patients. It increases the expression of cyclin D3 but does not seem to have an impact on prognosis [12,19,22,23].

t(14;16) is observed in 5–10% of the MM patients and causes the dysregulation of the MAF oncogene and is associated with aggressive disease [12,19,22,23].

t(14;20) also results in the upregulation of the MAF oncogene but this translocation is found in only about 1–2% of the patients [12,19,22,23]. It is associated with poor prognosis in MM [23]. However, in MGUS and smouldering MM the translocation is linked to long-term stable disease [19].

1.2.5.2 Chromosomal gains and losses

Gain of 1q is associated with poor prognosis. It is observed in approximately 30–40% of the patients at diagnosis and in a greater portion of the patients at relapse, whilst it is uncommon in MGUS [19,22,23]. It is a marker of poor prognosis irrespectively of given treatment [12,19,23].

Loss of 1p is also linked to poor prognosis and is found in about 30% of patients with MM [12,19,23].

Loss of chromosome 13/13q, found in 50% of myeloma cases, was previously thought to be a marker of poor prognosis. However, later studies have demonstrated that del(13/13q) is not an independent prognostic factor and that the adverse prognosis is dependent on the correlation to other high-risk lesions [19,23].

Loss of 17p is linked to an aggressive disease with low overall survival (OS). It is identified in 10% of newly diagnosed myeloma cases and becomes more frequent in later stages of the disease. Loss of 17p results in a lower expression of the tumour suppressor gene TP53 which can explain the poor prognosis [19,23,25]. Mutated TP53, which is also associated with poor prognosis, is seen in a lower frequency among MM patients [19].

1.2.5.3 8p21

Dysregulations originating from changes in the 8p21 region have been linked to various malignancies including leukaemic mantle cell lymphoma [26] and B cell lymphoma [27], as well as to prostate cancer [28]. Del(8)(p21) is an independent predictor of poor prognosis in MM, and both progression free survival (PFS) and OS are adversely affected [29].

In **Paper IV**, we examine the consequences of del(8)(p21) with regards to treatment response and bortezomib resistance. Therefore, del(8)(p21) is discussed in more detail in later chapters.

1.3 EPIDEMIOLOGY

The incidence of MM increases with age and in Sweden the median age at diagnosis is 70 years for men and 73 years for women [30]. Worldwide, the incidence of MM is estimated at 86 000 cases per year [31] and in Sweden approximately 623 patients were diagnosed yearly between 2008 and 2011, which translates into an age-adjusted incidence rate of 6.5/100 000 [30]. The corresponding incidence rate in the USA is 6.3/100 000 [32].

The disease is more common among African Americans [32,33] and slightly more men than women are diagnosed with MM; in Sweden 56% of the 2 494 patients that were diagnosed between 2008 and 2011 were men. The difference is due to a higher incidence of MM in men younger than 75 years, whilst no difference is seen between men and women over the age of 75. However, the age-specific incidence rate is higher for men in all age groups with the highest rate among those aged 80–84 years (45 men per 100 000 compared to 30 women per 100 000) [30].

MGUS, a premalignant stage that precedes MM [34], is found in 1% of adults older than 25 years [12] and in 3–4% of adults over 50 years [35]. The risk of progression from MGUS to MM is 0.5–1% per year. The risk of progression is much higher for patients with the intermediate clinical stage called smouldering MM. The rate of progression in this group is 10% each year for the first 5 years. However, the prognosis for patients with smouldering MM varies; some develop end organ damage within two years whilst others have a very low rate of progression [35].

1.4 SYMPTOMS AND DIAGNOSIS

Common symptoms and clinical features of MM are bone pain (due to lytic bone lesions), infections, weakness, anaemia, weight loss, hypercalcemia and renal impairment [36].

The most common clinical features at diagnosis are shown in Table I [30]. However, in 10–40% of the patients the disease is diagnosed before symptoms appear [36].

These patients are classified as having smouldering MM [35].

Table I. The most common clinical features at diagnosis and the proportion of patients in Sweden with each feature.

Clinical feature	Proportion of patients (%)
Skeletal disease	76
Osteolytic lesions	61
Vertebral compression fractures	15
Anaemia	33
Renal failure	18
Hypercalcemia	15

1.4.1 Lytic bone lesions and hypercalcemia

Bone pain, a presenting symptom in 40–70% of the patients, is attributable to osteolytic destruction and pathological fractures. The osteolysis also leads to an increased release of calcium resulting in hypercalcemia, which can be further aggravated by renal dysfunction. The symptoms of hypercalcemia depend on the serum calcium level and on how fast the calcium level has risen. Patients with mild to moderate hypercalcemia (serum calcium >2.6–3.1 mmol/L) can demonstrate thirst, polyuria, nausea, muscular weakness, constipation and malaise. As the serum calcium increases, headache, confusion and dehydration can also appear and in the most severe cases acute renal failure, cardiac ventricular arrhythmia, shortening of the QT interval, coma and death may follow [36-38].

1.4.2 Infections

Bacterial infections are common among MM patients and infections are seen in 10–20% of the patients at diagnosis. It becomes more frequent in later disease and 15–60% of MM deaths are due to infections. MM patients have an impaired immune defence due to disease-induced immune defects (mainly because of low concentrations of polyclonal immunoglobulins). The susceptibility to infection is further increased by the immunosuppression caused by MM treatment. The respiratory tract is the most common site of infection and *Streptococcus pneumoniae* and *Haemophilus influenzae* are the most frequently found pathogens in non-neutropenic patients, whereas *Staphylococcus aureus* and Gram-negative bacteria are more common among neutropenic patients [36,37].

1.4.3 Anaemia

Anaemia is a rather common finding at diagnosis (20–60%), whilst significant thrombocytopenia and neutropenia are rare. There is also a correlation between disease stage and anaemia [36,37]. Factors such as lack of erythropoietin due to renal insufficiency, too few erythrocyte precursor cells, and impaired iron utilization cause anaemia either separately or combined. Furthermore, MM treatment can also contribute to anaemia [37].

1.4.4 Renal impairment

Renal impairment is a common finding in MM patients and has been associated with poor prognosis. Monoclonal light chains that are deposited in the renal tubules are the main cause of kidney damage. Although there are other factors that also cause renal failure in MM patients such as hypercalcemia, dehydration, infections and nephrotoxic drugs [36,37,39-41], only renal impairment caused by light chain cast nephropathy is considered a myeloma defining event according to a clarification from the International Myeloma Working Group (IMWG) [35].

Previously, the definition of renal impairment was a serum creatinine $>173 \mu\text{mol/L}$ [42]. According to this definition almost 20% of MM patients in Sweden suffer from renal impairment at diagnosis. Additionally, 35% have a serum creatinine $>110 \mu\text{mol/L}$ [30]. However, serum creatinine is not a true reflection of renal function. Therefore, in recently updated criteria IMWG recommend that glomerular filtration rate (GFR) should be used for assessing renal function. The GFR rate can either be measured or estimated using the modification of diet in renal disease (MDRD) formula or the chronic kidney disease epidemiology collaboration (CKD-EPI) formula and renal impairment is defined as $\text{GFR} <40 \text{ mL/min}$ [35].

1.4.5 Diagnostic criteria and staging

The diagnostic criteria according to the IMWG can be seen in Table II and Table III [35].

Table II. The diagnostic criteria according to International Myeloma Working Group.

<p>Monoclonal gammopathy of undetermined significance</p> <ul style="list-style-type: none"> • Serum M protein <30 g/L • Clonal bone marrow plasma cells <10% • Absence of myeloma defining events and amyloidosis
<p>Smouldering multiple myeloma</p> <ul style="list-style-type: none"> • Serum M protein (IgA or IgG) ≥30 g/L <i>or</i> urinary M protein ≥500 mg/24 h <i>and/or</i> clonal bone marrow plasma cells 10–60% • Absence of myeloma defining events and amyloidosis
<p>Multiple myeloma</p> <ul style="list-style-type: none"> • Clonal bone marrow plasma cells ≥10% <i>or</i> biopsy-proven plasmacytoma • ≥1 myeloma defining event

Table III. Myeloma defining events.

End organ damage	Biological markers of malignancy
<ul style="list-style-type: none"> • Hypercalcaemia: Serum calcium >0.25 mmol/L above normal or >2.75 mmol/L • Renal insufficiency: Creatinine clearance <40 mL/min or serum creatinine >177 µmol/L • Anaemia: Haemoglobin >20 g/L below normal or haemoglobin <100 g/L • Bone lesions: ≥1 osteolytic lesions on skeletal x-ray, CT or PET-CT 	<ul style="list-style-type: none"> • Clonal bone marrow plasma cells ≥60% • Serum free light chain ratio ≥100 • >1 focal lesions on MRI

CT, computed tomography; PET-CT, positron emission tomography-computed tomography; MRI, magnetic resonance imaging.

1.4.5.1 Staging

Several staging systems have been used over time, but the current standard is the International Staging System (ISS), Table IV. This staging system was published in 2005 based on data from over 10 000 patients in North America, Europe and Asia [43] and has been validated in a European study [44,45].

Table IV. International Staging System.

Stage	Criteria	Median survival (months)
I	$\beta 2$ -microglobulin <3.5 mg/L <i>and</i> Serum albumin ≥ 3.5 g/dL	62
II	$\beta 2$ -microglobulin <3.5 mg/L and serum albumin <3.5 g/dL <i>or</i> $\beta 2$ -microglobulin 3.5–5.4 mg/L irrespective of serum albumin level	44
III	$\beta 2$ -microglobulin ≥ 5.5 mg/L	29

1.5 TREATMENT

There has been a huge increase in available treatments during the last fifteen to twenty years, as depicted in Figure 4. However, not all MM patients receive treatment since it is not initiated until the patient becomes symptomatic. This was previously defined as the presence of one or more of the so called CRAB criteria which comprise hyperCalcemia, Renal insufficiency, Anaemia and Bone lesion [42]. In the latest update from IMWG, the definition of disease demanding treatment has been expanded and now also involves MM defining events and biomarkers of malignancy, Table III [35].

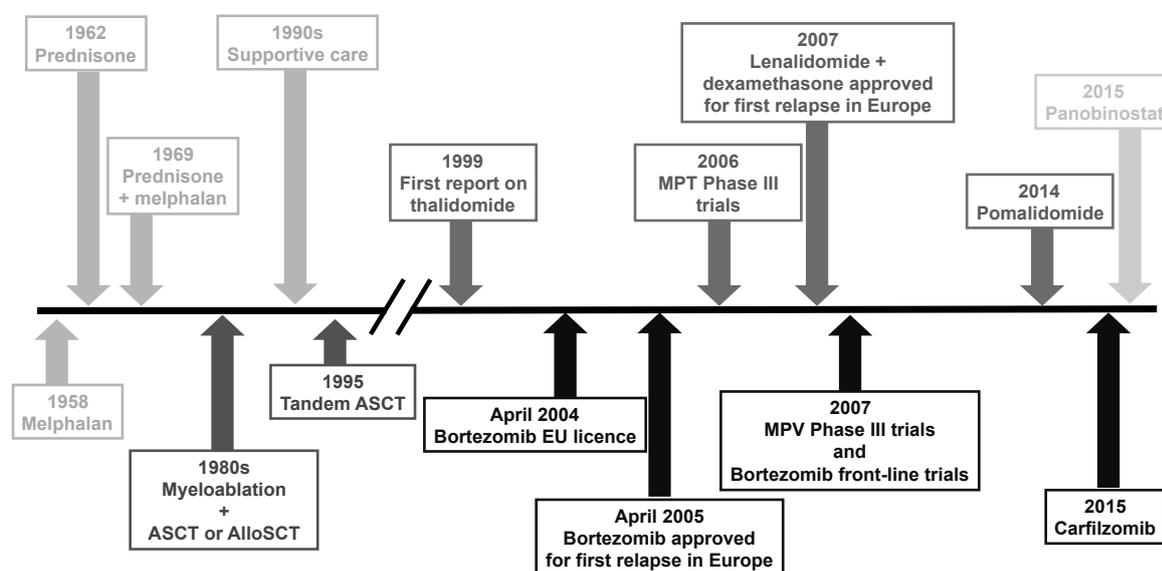


Figure 4. The development of myeloma treatments.

When treatment is initiated the choice of regimen is dependent on whether or not the patient is eligible for HDT. Patients with a biological age under 70 are usually candidates for HDT whilst those who are older or have extensive comorbidity are not. Before HDT, the patient receives induction treatment for 3–4 cycles in order to decrease the tumour burden. The current Swedish treatment recommendations are summarized in Table V and Table VI.

Table V. Initial treatment for HDT eligible patients.

Regimen	Interval	Studies
VCD <ul style="list-style-type: none"> • Bortezomib 1.3 mg/m² subcutaneous days 1, 4, 8 and 11 • Cyclophosphamide 1000 mg/m² intravenous day 1 • Dexamethasone 20 mg orally, days 1-2, 4-5, 8-9 and 11-12 	3 weeks	Bensinger et al. 2009 [46] Reeder et al. 2009 and 2010 [47,48] Kumar et al 2012 [49]
VTD <ul style="list-style-type: none"> • Bortezomib 1.3 mg/m² subcutaneous days 1, 4, 8 and 11 • Thalidomide 50–200 mg orally, daily • Dexamethasone 20 mg orally, days 1-2, 4-5, 8-9 and 11-12 	3 weeks	Cavo et al. 2010 [50] Rosiñol et al. 2010 and 2012 [51,52] Moreau et al. 2011 Ludwig et al 2012 and 2015 [53,54]
CTD <ul style="list-style-type: none"> • Cyclophosphamide 500 mg orally, days 1, 8 and 15 • Thalidomide 100–200 mg orally, daily • Dexamethasone 40 mg orally, days 1-4 and 15-18 	4 weeks	Yang et al. 2010 [55] Morgan et al. 2012 [56]

Table VI. Initial treatment for non-HDT eligible patients.

Regimen	Interval
MPT <ul style="list-style-type: none"> • Melphalan 0.25 mg/kg body weight, orally days 1-4 • Prednisolone 2 mg/kg body weight orally days 1-4 • Thalidomide 50–100 mg orally daily 	4-6 weeks
MPV <ul style="list-style-type: none"> • Melphalan 0.18 mg/kg body weight orally days 1-4 • Prednisolone 2 mg/kg body weight orally days 1-4 • Bortezomib 1.3 mg/m² subcutaneous once weekly for 4 weeks 	5 weeks
VTD <ul style="list-style-type: none"> • Bortezomib 1.3 mg/m² subcutaneous days 1, 4, 8 and 11 • Thalidomide 50–200 mg orally, daily • Dexamethasone 20 mg orally, days 1-2, 4-5, 8-9 and 11-12 	3 weeks
CTD <ul style="list-style-type: none"> • Cyclophosphamide 500 mg orally, days 1, 8 and 15 • Thalidomide 100–200 mg orally, daily • Dexamethasone 40 mg orally, days 1-4 and 15-18 	4 weeks
Vel-Dex twice weekly <ul style="list-style-type: none"> • Bortezomib 1.3 mg/m² subcutaneous days 1, 4, 8 and 11 • Dexamethasone 20 mg orally days 1-2, 4-5, 8-9 and 11-12 	3 weeks
Vel-Dex once weekly <ul style="list-style-type: none"> • Bortezomib 1.3 mg/m² subcutaneous days 1, 8, 15, 22 • Dexamethasone 20 mg orally days 1-2, 8-9, 15-16, 22-23 	5 weeks

1.5.1 Chemotherapy and corticosteroids

1.5.1.1 Alkylating agents

The alkylating agents cause cross-linkage of DNA strands by alkylation of DNA nucleotides, thereby inhibiting DNA replication.

Melphalan was introduced as MM treatment in the late 1950s and a decade later it was combined with prednisone with positive results; the combination of oral melphalan and prednisone (MP) resulted in a prolonged survival of 6 months when compared to melphalan as single treatment [57-62]. The combination MP was for many years the standard therapy for patients not eligible for HDT [63]. The most common side effect of melphalan is myelosuppression, especially thrombocytopenia, but it is otherwise well tolerated [60]. However, melphalan is partly eliminated via the kidneys and therefore dose adjustment is necessary in patients with renal impairment [64-67].

Cyclophosphamide can, like melphalan, be administered orally or intravenously. Studies have revealed that it is as effective as melphalan and that it also has an effect in melphalan-resistant patients [68-74]. The combination of cyclophosphamide and prednisolone has also been proven to be effective in late stages of the disease [75-77]. As for melphalan, myelosuppression is the dose-limiting toxicity, but neutrophils are more affected than thrombocytes [60]. In patients with severe renal insufficiency, the dose needs to be adjusted because even though the drug is metabolised in the liver, active metabolites are excreted via the kidneys [78].

Bendamustine is another alkylating agent. However, it differs from both melphalan and cyclophosphamide because it is also a purine analogue [79]. It was synthesized in East Germany as early as the 1960s [80], but it was not until the German reunification that it became available in other countries. However, at that time there was a lack of scientific evidence confirming its effect and therefore several clinical trials were initiated [81]. In Europe bendamustine was approved as upfront treatment for older MM patients in 2010 following a randomized trial comparing bendamustine plus prednisolone to MP. The study demonstrated equal overall response rates for the two therapies, but a higher rate of complete response (CR) as well as a longer time to treatment failure [82]. There are also several studies indicating an effect in relapse/refractory MM (RRMM) both with and without steroids [83] as well as in combination with novel agents [84-92]. Bendamustine also seems to be effective and well tolerated in patients with renal impairment [93-95].

Combinations

Several alkylator-based combinations have been studied and when compared to MP the response rate was found to be higher for many of the combinations [96-101]. However, other studies reported that there was no difference in survival in comparison to MP [102,103] and two meta-analyses determined that there was no significant survival benefit for combination chemotherapy in comparison to MP [104,105].

1.5.1.2 Other cytotoxic agents

Other cytotoxic agents worth mentioning are vincristine and doxorubicin. Vincristine belongs to the group of vinca alkaloids, whilst doxorubicin is an anthracycline. The vinca alkaloids' antitumour function is due to inhibition of mitosis [106,107], and the anthracyclines inhibit DNA, RNA and protein synthesis by binding to DNA-associated enzymes [108].

In the beginning of 1980s, the combination of vincristine, doxorubicin and dexamethasone (VAD) was shown to be effective in patients refractory to alkylating agents [109,110] and was for many years the standard induction therapy for patients eligible for HDT. However, the combination is no longer in use since it has been replaced by new and better treatment.

1.5.1.3 Glucocorticoids

There are a few different glucocorticoids that are being used in MM treatment: prednisone, prednisolone, dexamethasone and betamethasone. Prednisone was introduced in the 1960s when studies of prednisone as single agent could demonstrate an effect on the M protein level and on anaemia, but not on survival [111]. It is uncertain whether steroids in conventional doses have any effect on MM. However, high-doses of dexamethasone have been found to give a rapid response [109,112,113].

1.5.2 Novel agents

Until the late 1990s, MP was the standard treatment but in 1999 a major progress was seen with the introduction of the first novel agent, thalidomide.

1.5.2.1 Immunomodulatory drugs

The immunomodulatory drugs (IMiDs) function in various ways; they inhibit for instance the proliferation of malignant cells and they disrupt the interaction between myeloma cells and their environment. Furthermore, as the name suggests the IMiDs also affect the immune system with activation of T cells and NK cells [114].

Thalidomide was originally developed in the 1950s and sold as a sedative until the beginning of 1960s when it was withdrawn due to its severe teratogenic side effect [62,115].

In the late 1990s, it was tested on five MM patients with end-stage disease. One of the patients achieved a near complete response and a clinical trial was launched in which 30% of the patients, who all had advanced MM, responded [116]. When combined with dexamethasone the response rate in patients with RRMM increased to approximately 50% [117-125]. Furthermore, there are reports that thalidomide is effective in MM patients with involvement of the central nervous system [126]. The most common side effect of thalidomide is constipation, weakness and somnolence [116], but in combination with high-dose dexamethasone there is also an increased risk of venous thrombosis [125,127]. The elimination of thalidomide is not dependent on renal function and there is therefore no need for dose adjustment in patients with renal impairment [128].

Lenalidomide is an analogue of thalidomide that was approved in the USA in 2006 for the treatment of RRMM [129,130]. When it was combined with dexamethasone 90% of newly diagnosed patients responded [131] and the combination showed superior response in RRMM compared to dexamethasone alone [132,133]. The most frequent side effects are neutropenia, thrombocytopenia and venous thrombosis [132]. Lenalidomide is partly eliminated in urine and it is therefore necessary to adjust the dose depending on renal function [134].

Pomalidomide is a newer analogue of thalidomide. Studies have demonstrated the effectiveness of pomalidomide in RRMM patients both as a single agent [135,136] and in combination with low-dose dexamethasone, even in patients refractory to other IMiDs and/or bortezomib [137-140]. A phase III study compared pomalidomide plus low-dexamethasone to high-dose dexamethasone and found a longer PFS and a better response rate in the pomalidomide group [141]. The pharmacokinetics of pomalidomide does not seem to be affected by renal impairment, suggesting that it can be administered in full approved dose in patients with kidney failure [142].

1.5.2.2 Proteasome inhibitors

The proteasomes are important for the intracellular degradation of several proteins of which some are involved in the regulation of physiological and/or pathophysiological processes. In several cancers the proteasome pathway is important for the growth and spread of the malignant cells. Malignant cells often have an increased proteasome activity and the inhibition leads to growth arrest and apoptosis [143].

Bortezomib became available shortly after its efficacy in patients with RRMM was demonstrated in 2003 and 2004 in two phase II trials (SUMMIT and CREST) [144-146]. These studies were followed by a phase III trial comparing bortezomib to high-dose dexamethasone (the APEX trial) [147,148]. The studies demonstrated that bortezomib was effective as single agent in patients with RRMM, showing a response rate (partial response (PR) and CR) of 27–43%. Similar response rates were seen when using single-agent bortezomib as frontline treatment [149,150]. Bortezomib has also been shown to be effective in combination with various other agents, both in frontline and in later lines of treatment. For instance, when it was combined with dexamethasone 67–90% of newly diagnosed patients responded [151-154]. The VISTA trial examined the effect of adding bortezomib to MP for non-HDT eligible patients and could demonstrate a clear survival benefit with this combination compared to MP [155-157]. Peripheral neuropathy is an important dose-limiting side effect. However, a study in 2011 showed that administering bortezomib subcutaneously was just as effective as intravenous treatment, but with a lower incidence of peripheral neuropathy and since then subcutaneous treatment is preferred [158]. There is also a risk of herpes zoster infection due to reactivation of the virus. Therefore, prophylaxis should be prescribed during bortezomib treatment. Other common side effects include thrombocytopenia and neutropenia [159,160]. Bortezomib is a good alternative in patients with renal impairment as no dose adjustment is necessary in these patients [161,162].

Carfilzomib is a newer proteasome inhibitor and is expected to be approved in Sweden later this year for RRMM. The first reports from a phase II trials demonstrated a response rate of 24% and OS of 15.6 months in a group of heavily pre-treated patients of which the majority were refractory to bortezomib [163]. In bortezomib-naïve patients with RRMM response rates varied between 42 and 52% depending on dosage. Neither median duration of response nor time to progression (TTP) was reached for those patients who received the higher doses [164]. Phase III trials are being conducted and the preliminary results indicate high response rates using a combination of carfilzomib and low-dose dexamethasone [165] and both good response and improved PFS when carfilzomib was added to lenalidomide and dexamethasone [166].

Ixazomib is an oral proteasome inhibitor that is under investigation. Preliminary data indicate effect in RRMM when used as a single agent, even in patients refractory to bortezomib [167,168]. Combined with lenalidomide and dexamethasone it has been studied as up-front treatment [169].

1.5.3 High-dose treatment with stem cell support

High-dose treatment (HDT) with melphalan followed by autologous stem cell transplantation (ASCT) has, since the late 1990s, been the standard treatment for patients under the age of 65 years. The effect of HDT has been studied in several clinical trials of which the majority demonstrated an improved response rate and PFS [170-175] and three of the studies also found a better OS compared to conventional therapy [170,172,173]. Nowadays, patients often receive a second HDT at relapse, provided that they had a good response to their previous HDT [176].

Despite positive results and long-term outcomes that are probably better than for HDT [177-179], the role of allogeneic stem cell transplantation is more controversial due to the high transplant-related mortality rate that is seen when using myeloablative conditioning. Reduced intensity non-myeloablative conditioning (RIC) decreases the transplant-related mortality [180]. However, the risk of late relapses increases after RIC transplantations [181].

1.5.4 Response evaluation

Treatment response is evaluated according to IMWG criteria, Table VII, [182]. In the last few years, data has emerged showing the importance of checking for minimal residual disease (MRD) when a patient is in stringent complete response (sCR), since MRD-negative patients have a markedly better OS than MRD-positive patients [183,184]. However, the optimal method for detection of MRD in MM patients is not yet clear [185].

Table VII. Response criteria.

Response	Serum M protein	Urine M protein	Bone marrow	Other
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 partial response (VGPR)	≥90% reduction	<100mg/24 hours		
Partial response (PR)	≥50% reduction	<200mg/24 hours		
No response (NR)	<50% reduction			
Progressive disease (PD)	25% increase Absolute increases of ≥5 g/L	25% increase ≥200 mg/24 hours		

1.6 PROGNOSIS

There is a large variation in duration of survival among individual MM patients, some only surviving a few months, whilst others live for many years [46]. However, with the development of new treatments the survival has gradually increased. In Sweden, the 1-year survival rate has increased each decade between 1973 and 2003 and for patients younger than 60–70 years the 5-year and 10-year survival rates have also increased [47]. Most likely, the improved survival rates seen since the 1980s are largely due to the introduction of HDT followed by ASCT. This was demonstrated in a Nordic population-based study in which the survival rate among HDT patients was compared to that of a historical control. The median OS for the historical control was found to be 3.7 whilst the median OS of the HDT had not been reached at the end of the study and the difference was found to be significant [48]. Nevertheless, since the introduction of novel agents the OS has increased even more [49-51]. For instance, the median OS among HDT patients relapsing before the introduction of novel agent was found to be 2.7 years whilst those relapsing when novel agents were available had a median OS of 6 years and a post relapse survival of 1.3 years and 3.6 years respectively [52].

2 AIMS

Multiple myeloma is an incurable disease and the majority of patients receive several treatment lines during the course of the disease. In the last decades, many new treatment alternatives have emerged and the treating physician now has numerous treatments to choose from. These different treatment options have been well studied in clinical trials. However, clinical studies focus on a specific treatment in a specific line of treatment and there is very little data on the effectiveness of re-treatment or on the survival as a function of the entire treatment sequence.

Beside treatment, there are other factors that predict survival in MM, including beta-2-microglobulin, creatinine, haemoglobin, calcium, bone lesions as well as various chromosomal aberrations. However, in the era of novel treatment the impact of these factors on survival is not fully understood.

Therefore, the aims of this thesis were:

1. To achieve a better understanding of the order in which the treatments should be given.
2. To define factors affecting prognosis.
3. To increase the knowledge of cytogenetic abnormalities and their influence on prognosis and choice of treatment, with special focus on del(8)(p21) and bortezomib treatment.

3 MATERIALS AND METHODS

3.1 STUDY POPULATION

3.1.1 Paper I, II and III

In **Paper I**, HDT eligible patients were studied whilst **Papers II** and **III** included newly diagnosed patients demanding treatment. Data including age, sex, type of myeloma and extent of bone disease as well as laboratory measurements at diagnosis were collected retrospectively from the hospitals' electronic medical records. Serum M protein and urine M protein values were collected at baseline and every significant change in the M protein level was noted. For **Papers II** and **III**, the MM drugs given in each treatment line were noted, with specific start and stop dates for each drug or drug combination. **Paper III** focused on MM patients with renal impairment at diagnosis, defined as eGFR <60 mL/min/1.73 m².

The patients were divided according to Figure 5, Figure 6 and Figure 7.

3.1.2 Paper IV

Bone marrow aspirates from newly diagnosed MM patients were collected and the CD138⁺ plasma cells from these samples were selected for further analysis.

For 140 consecutive patients clinical data, including information about given treatment and response to treatment, were collected. The patients were divided according to Figure 8.

3.2 DEFINITION OF ENDPOINTS

In all papers, response to treatment was assessed according to IMWG criteria, Table VII [182]. However, there was one exception: we used the term near complete response (nCR) instead of CR, since the clinical practice did not require that an undetectable M protein be confirmed by immunofixation.

In **Papers I–III** we used the terms:

- Time to next treatment (TTNT): the time between the start date of the administered drugs in the current treatment line and the start date of the administered drugs in the next treatment line
- Time to progression (TTP) or progression free survival (PFS): the time between the start date of the administered drugs in the current treatment line until progressive disease
- Overall survival (OS): the time from start of treatment to death or last follow-up.

For a portion of the patients in **Paper III**, renal response was also assessed according to the criteria suggested by Ludwig et al and the IMWG, Table VIII, [186,187].

Table VIII. Definition of renal response.

Renal response	Baseline GFR (mL/min)	Best GFR response (mL/min)
Complete response (CR _{renal})	<50	≥60
Partial response (PR _{renal})	<15	30-59
Minimal response (MR _{renal})	<15 15-29	15-29 30-59

3.3 LABORATORY METHODS

3.3.1 Fluorescent *in situ* hybridization

Fluorescent *in situ* hybridization (FISH) can be used to detect chromosomal abnormalities. It utilises specific probes that are labelled with fluorochrome and bind to single-stranded DNA and can thus identify either a specific or multiple chromosomal structures or a unique DNA sequence. The bound probes are then visualized by fluorescence microscopy [188].

In **Paper III**, FISH with a 13q14.3 deletion probe was performed on CD138⁺ BM cells. Normal peripheral blood mononuclear cells from donor were used as control.

FISH analysis was also performed in **Paper IV** to find other chromosomal abnormalities in addition to del(8)(p21), that are commonly seen in MM patients.

3.3.2 Real-time polymerase chain reaction

Polymerase chain reaction (PCR) makes it possible to detect small amounts of DNA in a sample by amplifying it. In standard PCR the reaction product is not detected until the end of the reaction, whilst in real time PCR the product is detected as it is amplified [189].

In **Paper IV**, quantitative real-time PCR was used to identify differences in gene expression levels between patients with and without del(8)(p21). The mRNA levels of genes located on the 8p21 region as well as of genes with a functional link to genes located in this region were measured.

3.3.3 Flow cytometry

Flow cytometry is a method to identify and characterize different cell types within a population. In the flow cytometer one cell at a time passes a laser beam. Depending on the size of the cell and its cytoplasmic granularity the light will be scatter in different ways. Furthermore, specific molecules on the surface of the cells or inside the cells can be marked with fluorescently labelled antibodies and the emitted fluorescence can also be measured [190].

Flow cytometry was used in **Paper IV** to examine the changes in cell surface expression of tumour necrosis factor-related apoptosis inducing ligand (TRAIL) receptors due to *in vitro* bortezomib treatment. To study the receptor expression, CD138⁺ plasma cells and

CD138⁺ BM mononuclear cells with and without del(8)(p21) were cultured in the presence or absence of bortezomib and stained with fluorescently labelled anti-TRAIL receptor antibodies, anti-CD38 and anti-CD138. The CD138⁺ cells were also stained with anti-CD45 before analysis.

Flow cytometry was also used to assess the sensitivity of myeloma cells with and without del(8)(p21) to bortezomib and soluble TRAIL/APO2L. Plasma cells were cultured with or without bortezomib and incubated with soluble TRAIL/APO2L before they were stained with anti-CD138 and anti-CD38 antibodies followed by Annexin V and propidium iodide staining and the amount of viable, apoptotic and dead cells could then be measured.

3.4 STATISTICAL METHODS

In **Papers I–III**, descriptive statistics were used to characterize the data and Cox regression analyses were used to identify predictive factors. In all papers, life table curves were calculated according to Kaplan-Meier and compared using log-rank tests.

In **Paper II**, multiple comparisons of continuous data were performed by analysis of variance, ANOVA. In the case of a statistically significant result in the ANOVA, statistical comparisons were made by use of the post-hoc test proposed by Fisher as a control for multiplicity. **Paper II** also employed multiple hypothesis testing. This means that each hypothesis was analysed separately and the existence of patterns in and the consistency of the results were considered in the analysis. In **Paper III**, the Mann-Whitney test for uncorrelated means was performed to investigate possible differences between two independent groups. In **Paper IV**, one-way ANOVA and paired/unpaired t-tests were used to analyse the relative expression of certain genes in MM cells with and without del(8)(p21).

In all papers, a probability value (P) of <0.05 was considered as significant.

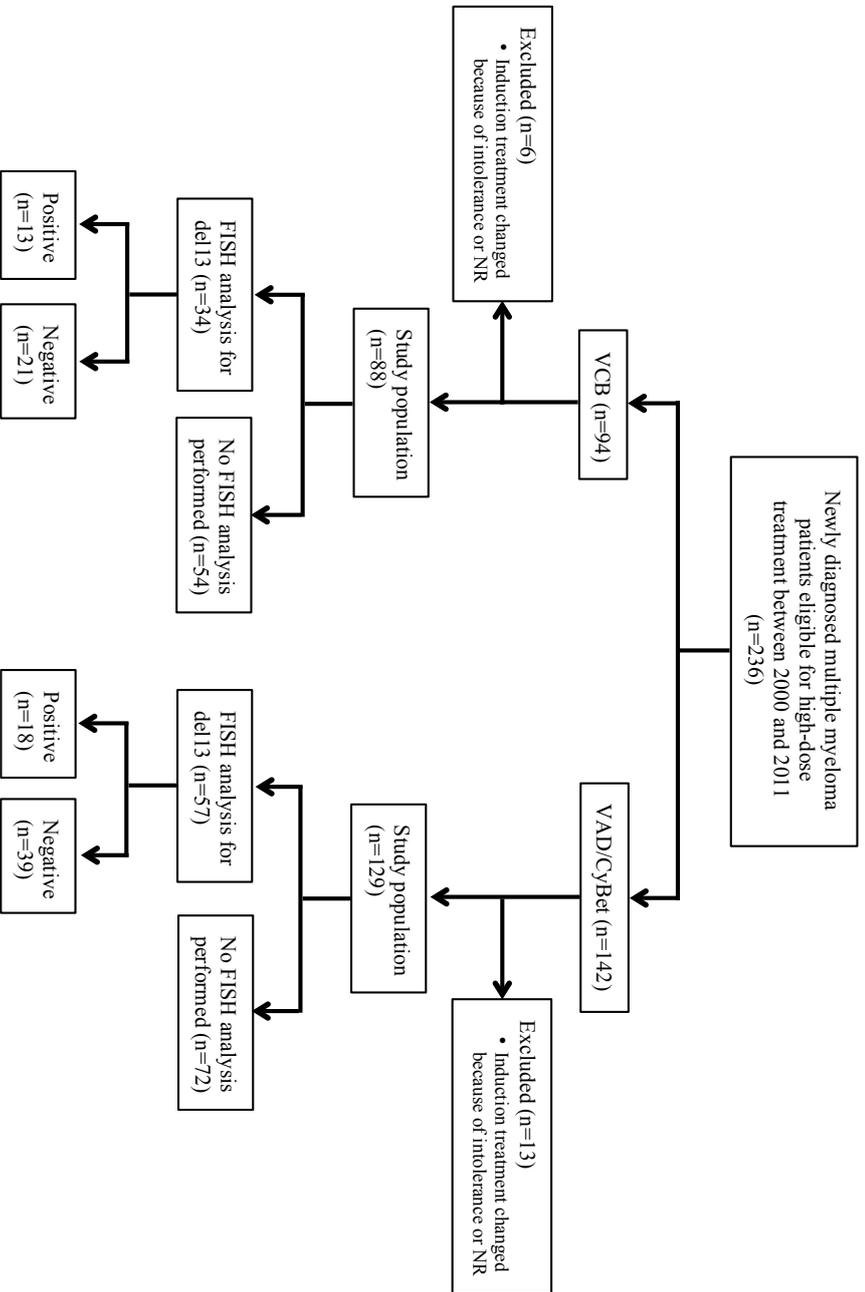


Figure 5. Patients in Paper I.

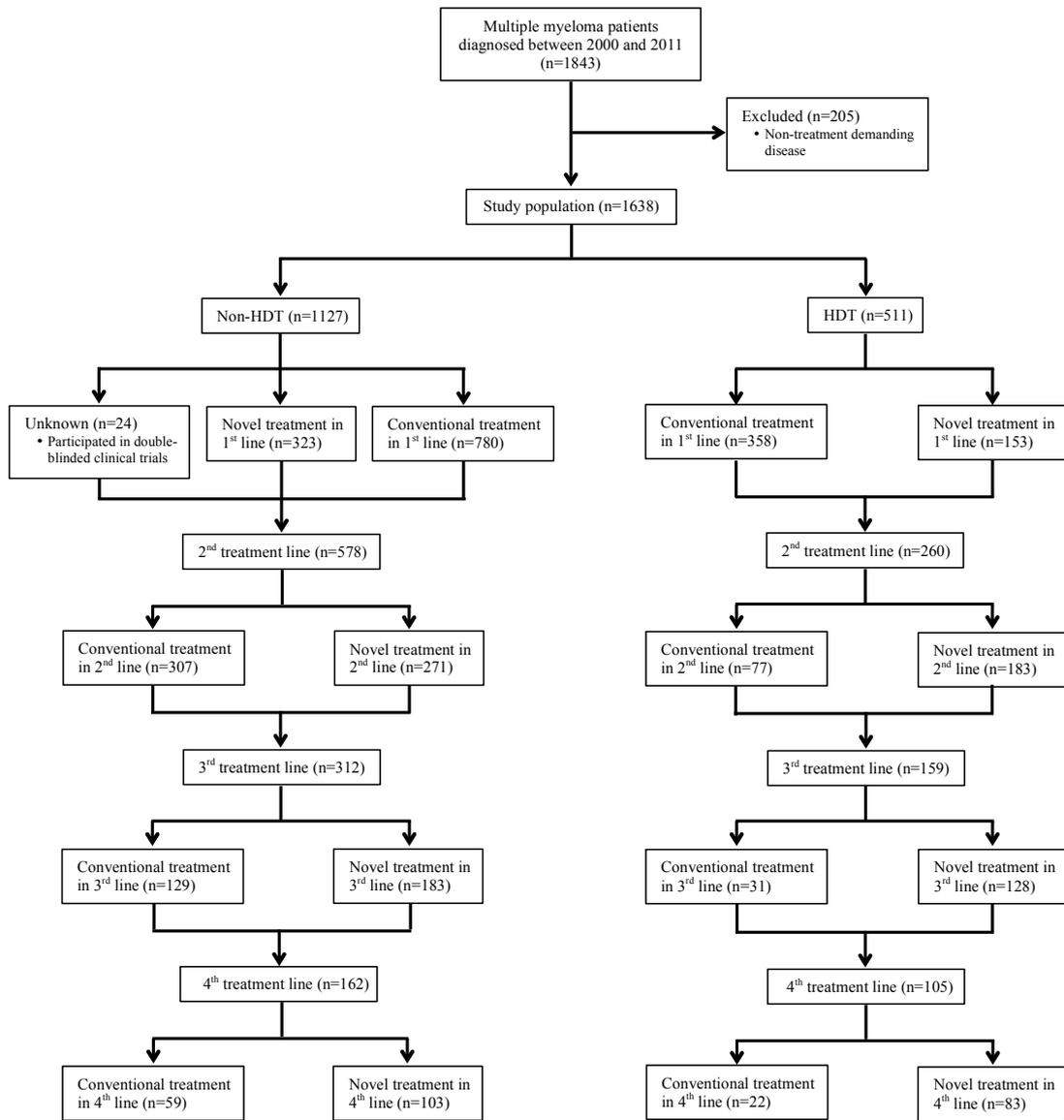


Figure 6. Patients in Paper II.

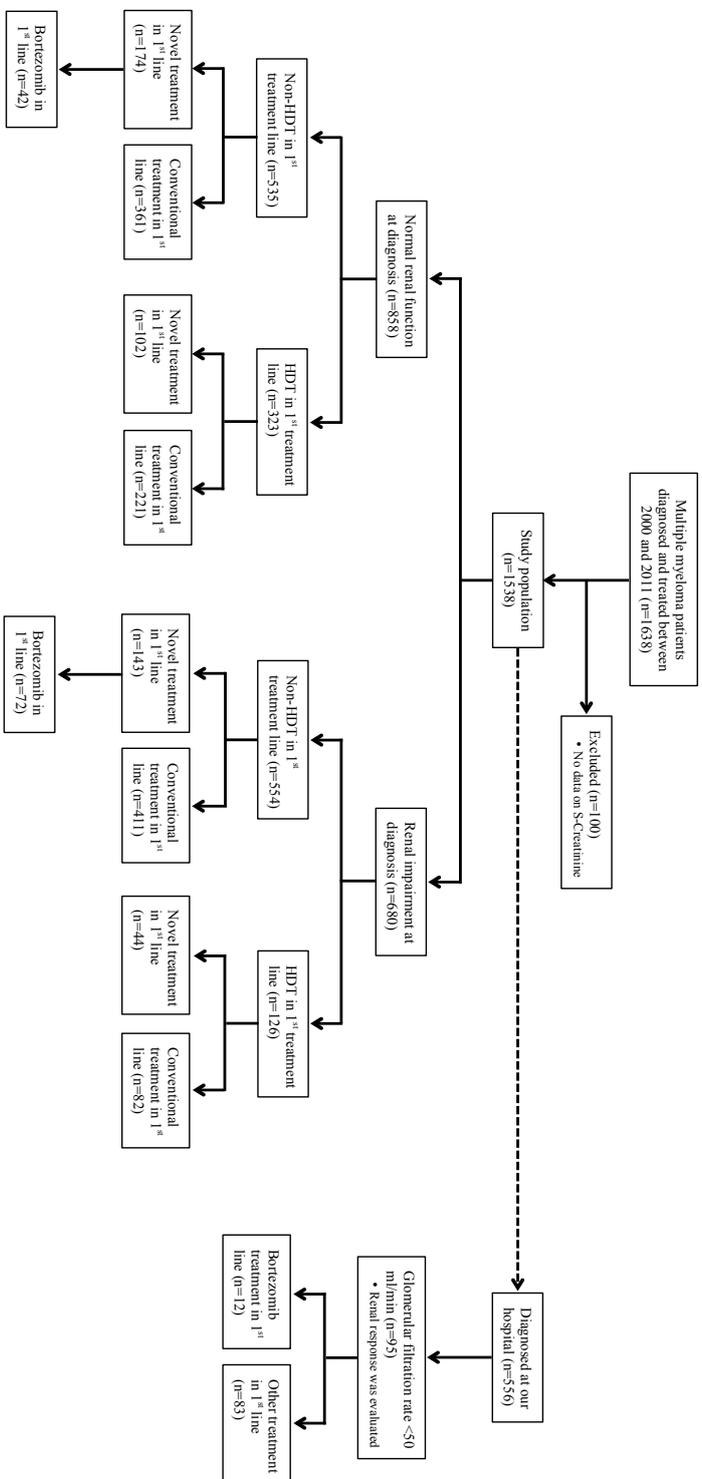


Figure 7. Patients in Paper III.

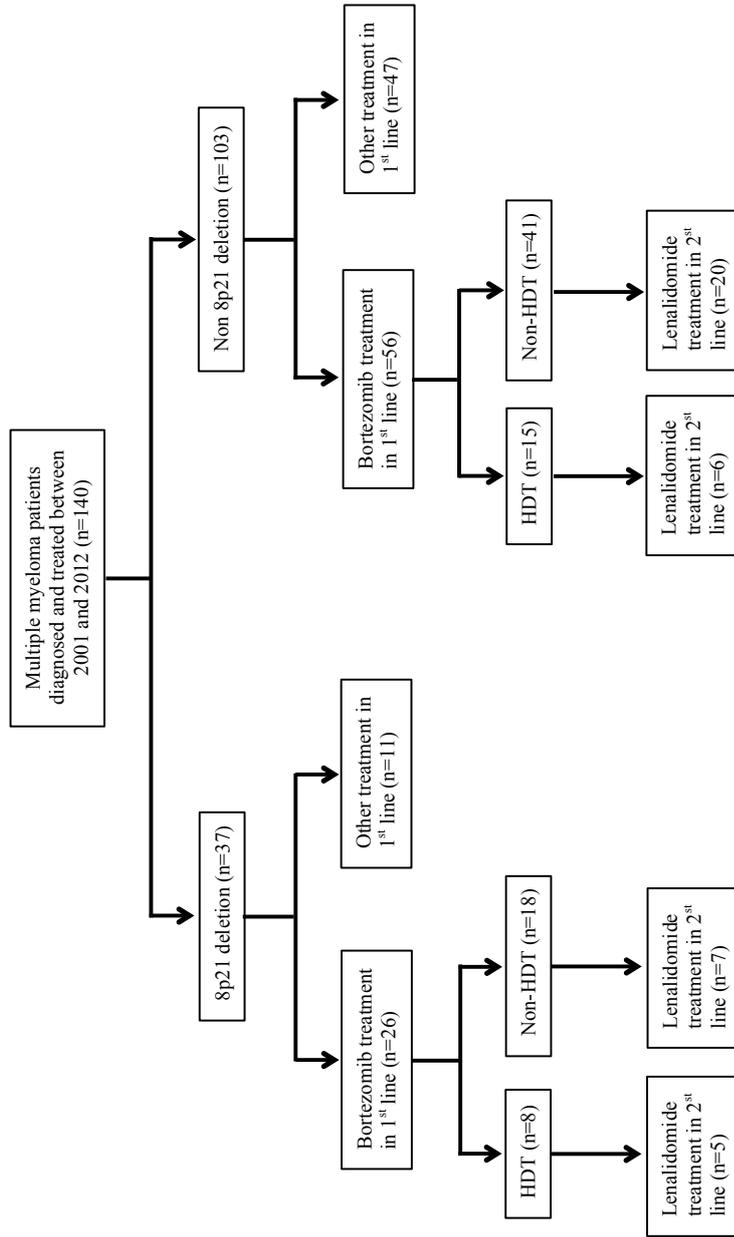


Figure 8. Patients in Paper IV.

4 RESULTS AND DISCUSSION

4.1 PROS AND CONS OF REAL-LIFE

Treatment guidelines are mostly dependent on the results of randomized clinical trials or meta-analysis of these trials. However, the study population in prospective clinical trials usually consists of highly selected patients and does not necessarily reflect the group of patients that will receive the treatment. Furthermore, randomized clinical trials are designed to address specific questions, such as the effect of a specific drug or drug combination in the first or second line of treatment on response and/or survival. The question of response and survival in relation to entire treatment sequences is nearly impossible to answer in randomized clinical trials, since this would require such a large number of patients as well as a very long follow-up time. For these reasons it is necessary to conduct population-based studies that will give more and better information about overall outcome for all patients in a given population.

In **Papers I–III**, we studied a large cohort of MM patients that were diagnosed and treated at up to 15 different hospitals in Sweden. The patients were obtained from the Swedish Cancer Registry, which covers almost 94% of all individuals with malignant disease [191].

In **Paper I**, we analysed response data from 88 patients treated with VCB and 129 patients treated with VAD or CyBet prior to HDT. Prior studies have demonstrated that combinations with novel agents lead to improved response rates and survival after HDT compared to conventional chemotherapy [154,192]. Therefore, we wanted to find out whether the positive results seen in clinical trials were also achieved in clinical reality.

Inspired by encouraging results in **Paper I**, we continued to look at a larger population of MM patients (n=1638) (**Papers II and III**). These patients had been treated at different hospitals in Sweden, some at university hospitals and others at regional or local hospitals. We therefore believe that our population represents the full spectrum of different MM patients and that our results are representative for the whole Swedish MM population.

One possible drawback with the study set-up is that we did not collect data on comorbidity. However, we tried to cover possible differences between the studied groups by adjusting for age, sex, type of MM and important laboratory values at diagnosis. Moreover, since the data were collected from different hospitals it is influenced by centre-specific clinical routines such as the intervals between response evaluation. Most often, response was measured every month, but sometimes 6–8 weeks passed between the controls. This might affect TTP and PFS, but to a lesser extent TTNT. Therefore, we believe that TTNT is more reliable in this setting. Furthermore, an undetectable M protein by standard electrophoresis was not always confirmed by immunofixation since it was not required in clinical practice. Consequently, the patients could not meet the criteria for CR. Therefore, we chose to analyse nCR instead.

4.2 TRICK OR TREAT? THE EXPERIENCE OF NOVEL DRUGS FOR NON-HDT PATIENTS

In **Paper II**, we could confirm that the survival benefits with novel drugs, which have been demonstrated in clinical trials [155,156,193-199], are also seen in real life. Nevertheless, even though the OS in the elderly MM population is approaching the survival in a matched Swedish population, MM can still not be considered a chronic disease and therefore there is still room for improvement.

Approximately two thirds of the patients in **Paper II** did not receive HDT. Patients treated with novel drugs were compared to those who received conventional treatment, Figure 6. Among non-HDT patients there were small, yet statistically significant differences in age, haemoglobin and albumin between those treated with novel drug and those treated with conventional ones, Table IX. However, the differences in laboratory values were small and of little clinical importance and as mentioned above we adjusted for these differences in multivariate analysis.

Table IX. Population characteristics at diagnosis for patients in Paper I.

	HDT	Non-HDT	Non-HDT 1 st line treatment		P
			Novel agents	Conventional agents	
No. of patients	511	1127	323	780	
Male, %	63	51	50	55	0.117
Median age at diagnosis, years (range)	58 (32–71)	75 (35–97)	72 (41–90)	76 (35–97)	<0.001
Skeleton destruction, %					
0	24	37	36	37	
1	10	9	6	9	0.206
More than 1	66	55	58	54	
Laboratory values at diagnosis, median					
Creatinine, mmol/L	86	99	95	101	0.102
Ca, mmol/L	2.4	2.4	2.4	2.4	0.395
B2 μ , mg/L	3.1	4.0	3.9	4.0	0.535
Hb, g/L	110	107	109	106	0.002
Albumin, g/L	35	33	34	33	0.032

Patients not receiving HDT had a clear survival benefit from novel treatment. For these, upfront treatment with novel drugs led to a median OS of 4.9 years compared to 2.3 years for those treated with conventional chemotherapy (Figure 9A) and the survival benefit remained in the multivariate analysis. These results are in line with those seen in clinical trials [157,199,200]. Interestingly, patients treated with novel agents in both first and second line had not reached the median OS at the end of the study (Figure 9B), whilst patients receiving

conventional treatment in first line and novel drugs in second line had a median OS of 3.3 years and patients receiving conventional treatment in both first and second line had a median OS of 3.0 years. This indicates that novel agents should be used upfront instead of being saved until relapse and that the survival can be prolonged with optimal use of existing drugs.

We were also interested in finding out whether the recent development of MM treatments meant that the OS among MM patients would be comparable to that in the normal population. Hence, in **Paper II** we used the Swedish population to select a sex- and age-matched cohort based on death rates between 2008 and 2010. Among non-HDT patients that received novel agents in both first and second lines, 69% were alive after three years and 63% after five years whilst the corresponding figures in the matched cohort were 88% and 79% respectively, Figure 9B. The non-HDT group is thus approaching the survival in the normal population.

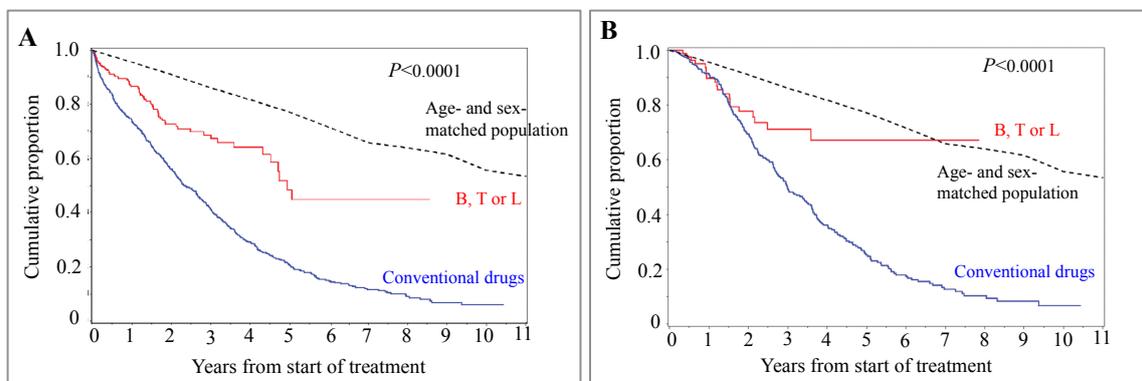


Figure 9. Overall survival in non-HDT patients. (A) Patients treated with novel agents in 1st line are compared to those treated with conventional agents in 1st line as well as to a case-matched population. (B) Patients treated with novel agents in 1st and 2nd lines are compared to those treated with conventional agents in 1st and 2nd lines as well as to a case-matched population. B, bortezomib; T, thalidomide; L, lenalidomide.

Seeing such good results for the MM population as a whole, we wondered if the survival benefits linked to novel treatment also were evident for patients with renal failure at diagnosis. As mentioned previously, renal failure is a frequent complication of MM. Furthermore, when using conventional chemotherapy renal impairment has been linked to increased morbidity and inferior survival [40,41]. From the study population used in **Paper II** we identified 1538 patients with known serum creatinine at diagnosis. Of these, 680 patients had renal impairment at diagnosis (i.e. eGFR <60 mL/min), Figure 7, and they were the main focus of **Paper III**.

We showed that patients with renal impairment at diagnosis had a worse median OS than those with normal renal function (33 versus 52 months, P < 0.001) and that the degree of renal impairment was correlated to OS (Figure 10), thus seemingly confirming that renal impairment is a risk factor.

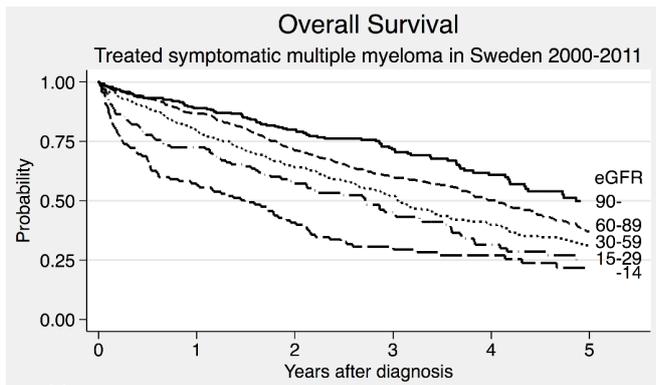


Figure 10. Overall survival. The patients are divided according to the chronic kidney disease classification.

However, with the use of novel agents as upfront treatment of patients not eligible for HDT the median OS was almost tripled compared to that seen after conventional chemotherapy (Figure 11A) and the same improvement was seen when analysing only the bortezomib treated patients. Multivariate analysis revealed that the difference remained highly significant after correction for age, calcium, haemoglobin and albumin. Moreover, with the use of novel agents we could no longer detect any difference in OS between non-HDT patients with and without RI, Figure 11B. These findings support earlier studies showing that the survival among patients with RI has improved since the introduction of novel agents [201] and that renal impairment is not an independent prognostic factor among newly diagnosed patients treated with novel agents [202].

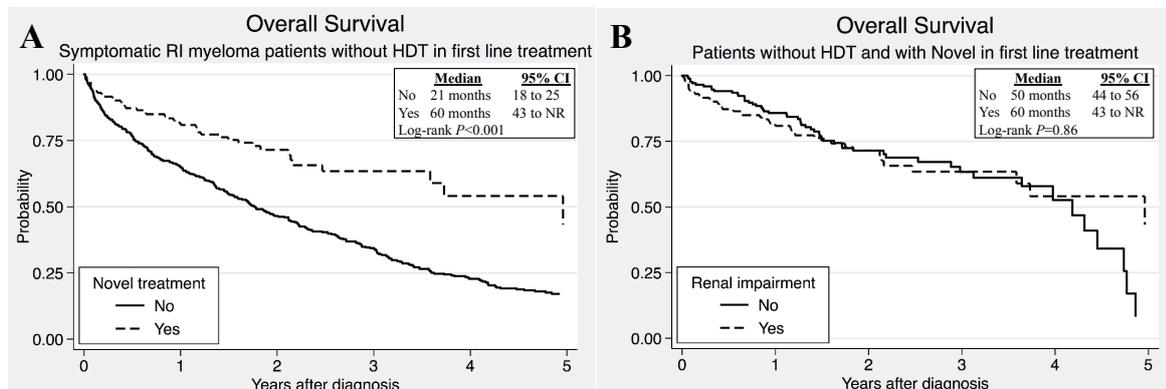


Figure 11. Overall survival in non-HDT patients. (A) Patients with renal impairment treated with novel agents compared to conventional agents in 1st treatment line. (B) Patients with and without renal impairment treated with novel agents in 1st treatment line.

The increased survival as well as prolonged TTNT might partly be explained by improved response rates after novel treatment compared to conventional chemotherapy, since a better disease control correlates to improved survival and PFS [201,203].

In **Papers II** and **III**, we found better response rates after treatment with novel agents, which in **Paper II** was consistent through treatment lines 1–4. Moreover, in **Paper II** we could demonstrate that a good response in one treatment line predicted a good response in the following treatment line, at least until the fourth treatment line. We also found that TTNT was dependent on the depth of response (Table X), which correlates with results from previous studies [204].

Table X. Median TTNT (months) in non-HDT patients depending on depth of response. The 95% CI is also shown. $P < 0.001$ in treatment line 1–3 and 0.085 in treatment line 4.

	1 st line	2 nd line	3 rd line	4 th line
All	17 (15–18)	12 (10–14)	9 (8–11)	7 (5–10)
No response	13 (11–14)	8 (7–9)	8 (7–10)	5 (4–7)
Partial response	18 (16–20)	16 (13–19)	11 (8–14)	10 (7–16)
Very good partial response	21 (20–32)	15 (11–18)	12 (8–16)	15 (9–23)
Near complete response	22 (16–29)	29 (13–32)	15 (7–26)	10 (6–20)

Renal response is another factor that correlates to improved survival [205] as well as to myeloma response [206] and when renal response and myeloma response coincide the outcome improves compared to when they occur separately [205]. In **Paper III**, renal response was evaluated in 95 patients diagnosed and treated at Karolinska University Hospital, Figure 7. We found that a greater portion of the bortezomib treated patients improved their GFR compared to conventionally treated (92% versus 69%, $P = 0.049$), which corresponds with earlier studies [206,207].

4.3 HDT PATIENTS: NOVEL TREATMENT – A SHORT STORY

The HDT population was the main focus of **Paper I** and was also part of **Papers II** and **III**. In **Paper I**, we could show that the VCB patients had a deeper response both before and after HDT (Figure 12) as well as a quicker response, Table XI. Our results are in line with those of other studies showing high response rates after induction with VCB [47,208]. However, in **Paper II** we could not quite confirm these findings, because even though patients treated with novel agents had a deeper response after the second and third treatment lines compared to those treated with conventional drugs, there was no significant difference after the first line of therapy. One explanation might be that the treatment in the HDT population in **Paper II** varied greatly. For instance, tandem-HDT as well as maintenance were more common among conventionally treated patients.

The previously demonstrated high response rates after induction with VCB seem to persist even when the dose of bortezomib is decreased [48]. This is of course interesting since lowering the dose of a drug is one way of decreasing the risk of side effects, thus making it more likely that at larger proportion of the patients will tolerate the treatment. In this case it might mean that more patients will be able to proceed to HDT. In our study we unfortunately did not have any information on adverse events but only a few patients discontinued induction treatment, indicating limited toxicity.

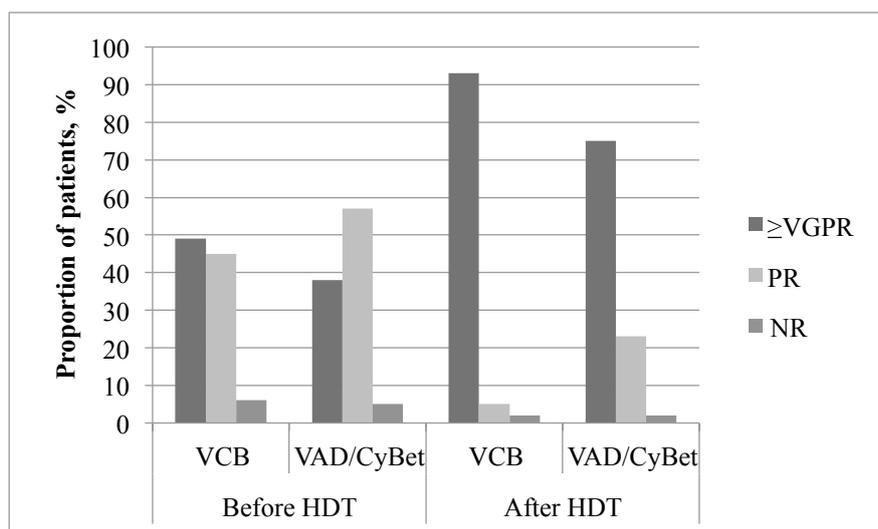


Figure 12. Response rates before and after HDT.

Table XI. Time to response (days) before HDT. There was a significant difference in both median time to first response and median time to best response between the two treatment groups, $P < 0.001$.

	VCB	VAD/CyBet
Median time to first response	32	57
Median time to best response	43	65
Range	8–133	9–195

As mentioned above, the quality of response is an important factor that affects long-term outcomes such as PFS and OS. This finding is also supported by our data in **Paper I** because we found a significantly prolonged TTP in the VCB group compared to the VAD/CyBet group, Figure 13A. ISS stage, age and bone lesions were other factors that affected TTP according to univariate analysis. However, the multivariate analysis revealed that bone lesions were not an independent factor, whilst type of treatment, ISS stage and age were still significant. Likewise, there was a tendency towards a longer TTNT for the VCB treated patients for whom the median TTNT was not reached compared to 2.9 years in the VAD/CyBet group but this was not statistically significant. However, in **Paper II** we could demonstrate that TTNT was dependent on the depth of response (Table XII) and as for the non-HDT patients a good response in one treatment line predicted a good response in the following treatment line. However, the survival benefits from novel treatment seen in non-HDT patients was not found among the HDT population, perhaps because the follow-up time was too short, Figure 13B.

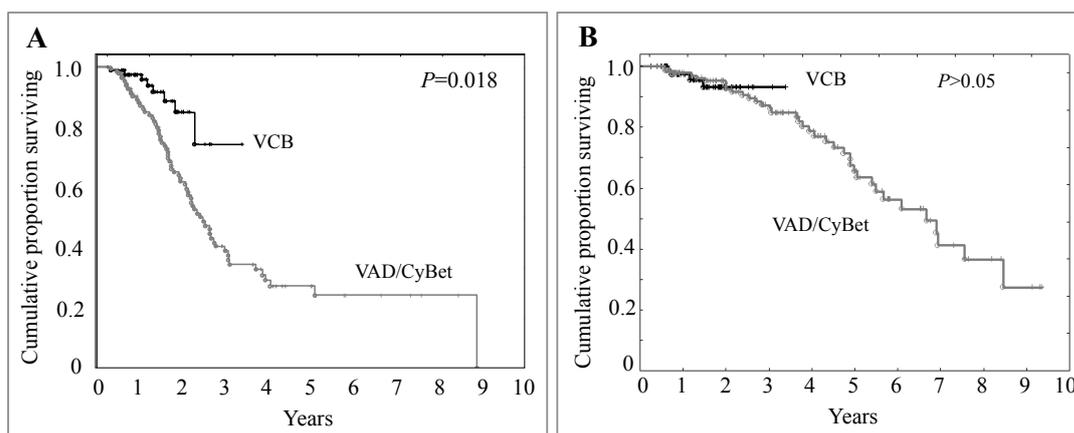


Figure 13. Effect of VCB versus VAD or CyBet as induction treatment. (A) Time to progression. (B) Overall survival

Table XII. Median TTNT (months) in HDT patients depending on depth of response. The 95% CI is also shown. $P < 0.05$ in treatment line 1–4.

	1st line	2nd line	3rd line	4th line
All	33 (29–36)	10 (9–13)	8 (7–9)	7 (5–10)
No response	18 (16–25)	6 (4–9)	5 (4–6)	3 (2–5)
Partial response	25 (22–30)	10 (8–13)	10 (7–11)	8 (7–10)
Very good partial response	29(26–42)	15 (8–17)	22 (8–23)	13 (5–18)
Near complete response	45 (38–50)	18 (11–31)	13 (11–21)	32 (6–32)

4.4 THE TRAIL TO RESISTANCE

As discussed in **Papers I–III**, the use of novel agents, such as bortezomib, has led to an improved outcome in MM patients. Nevertheless, around 20% of the patients do not respond to bortezomib [155]. We believe that this is partly due to del(8)(p21).

Our group has previously demonstrated that del(8)(p21), which is found in approximately 20% of newly diagnosed MM patients, is an independent factor associated with poor PFS and OS in MM patients receiving HDT [29,209]. In later published studies, del(8)(p21) has been further examined [210,211].

Other studies have revealed that one of the effects of bortezomib is the upregulation of the pro-apoptotic TRAIL receptors 1 and 2 on the MM cells, thus making them more sensitive to apoptosis induced by TRAIL/APO2L [212]. The TRAIL receptor gene cluster is located on the short arm of chromosome 8. Alterations of the expression of TRAIL receptors on the cell surface due to del(8)(p21) could therefore cause decreased sensitivity of tumour cells to TRAIL-mediated apoptosis. However, since the deletion is usually monoallelic the cells still carry one copy of each TRAIL receptor gene. Hence, bortezomib might still upregulate the receptors and eradicate the MM cells. Furthermore, there are several other genes located on 8p that might be involved in MM progression [213,214]. The aim of this study was therefore

to identify the consequences of del(8)(p21), especially in relationship to bortezomib treatment and TRAIL/APO2L-mediated killing.

The mRNA levels of certain genes were analysed and compared between patients with (n=19) and without (n=6) del(8)(p21). Several differences were seen for genes located on or near the 8p21 region (Figure 14A) as well as for genes located elsewhere but with a functional link to genes located in this region (Figure 14B). Of these, some are of greater interest with regards to MM survival, for instance the overexpressed MYC oncogene which has been linked to MM cell survival [215-217] as well as the upregulation of PTK2B which promotes tumour progression [214]. The low expression of TP53 is of course also of interest [218]. We expected that the TRAIL receptor gene cluster would be affected, but we found similar expression levels of both the pro-apoptotic receptors TRAIL-R1, -R2 and the anti-apoptotic decoy receptor TRAIL-R3. The only significant difference in expression at mRNA level was for the anti-apoptotic decoy receptor TRAIL-R4 which was upregulated in patients with del(8)(p21), Figure 15. When cell surface expression levels of the TRAIL receptors were analysed using flow cytometry similar results were seen. However, the upregulation of TRAIL-R4 in 8p21 deleted cells did not reach statistical significance, which might be explained by the fact that a smaller sample size was used for flow cytometric phenotyping.

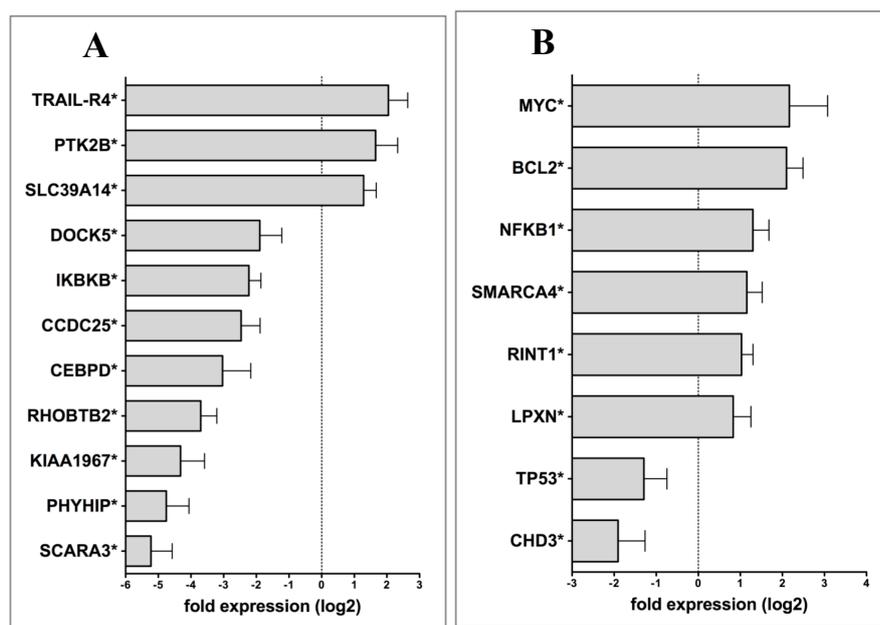


Figure 14. The relative mRNA level expression of genes in multiple myeloma cells with del(8)(p21) compared to those without the deletion. (A) Genes located on or near 8p21. (B) Genes located elsewhere on the chromosome but with a functional link to genes located on 8p21.

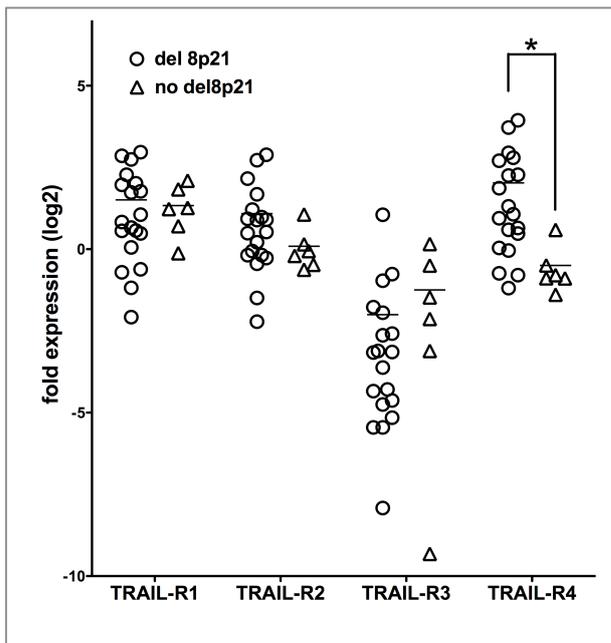


Figure 15. The relative mRNA level expression of TRAIL receptors in multiple myeloma cells with and without del(8)(p21).

The MM cells were then treated *in vitro* with bortezomib for 24 hours and the TRAIL receptor expression was again analysed using flow cytometry. In MM cells from seven patients without del(8)(p21), bortezomib upregulated the expression of TRAIL-R1, thus making them more susceptible to TRAIL-mediated apoptosis. However, this was not seen in cells with del(8)(p21), Figure 16A. The expression of TRAIL-R2 and -R3 was not affected in MM cells with del(8)(p21), whilst the TRAIL-R3 was slightly upregulated in cells not carrying del(8)(p21). In MM cells with del(8)(p21), bortezomib tended to decrease the expression of TRAIL-R4 to the same levels as in cells without del(8)(p21), Figure 16B. We believe these changes seen in TRAIL receptor expression, due to bortezomib treatment, are myeloma specific because the expression of TRAIL receptors in the CD138⁻/CD45⁺ compartment of the BM was not affected by bortezomib treatment.

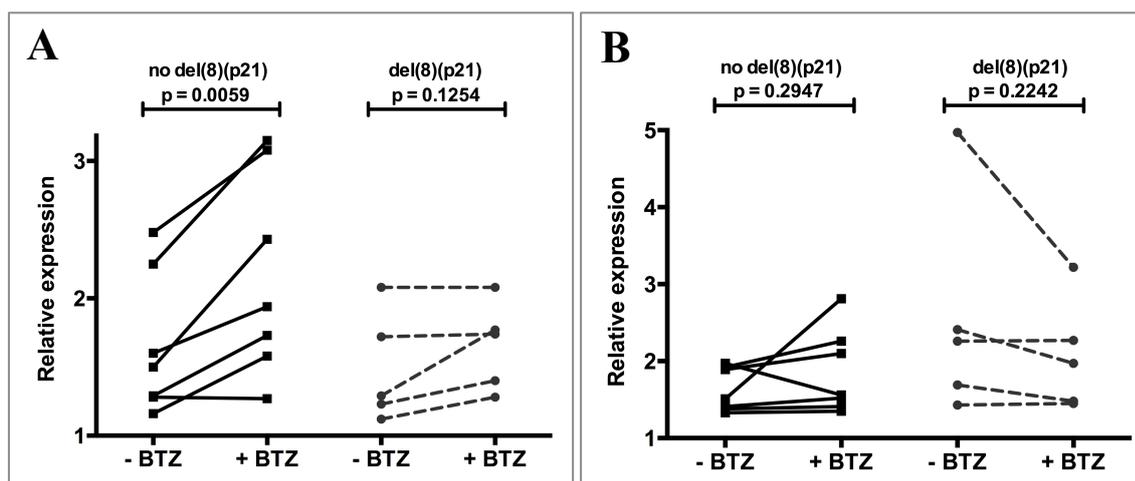


Figure 16. Relative cell surface expression of TRAIL receptors in MM cells from 6 patients with del(8)(p21) and 7 patients without the deletion. The expression levels are normalized to corresponding isotype controls. (A) The pro-apoptotic receptor TRAIL-R1. (B) The anti-apoptotic receptor TRAIL-R4. BTZ, bortezomib.

Since bortezomib treatment failed to upregulate the pro-apoptotic TRAIL receptors in MM cells with del(8)(p21) we wondered whether this also made the cells less sensitive to TRAIL-mediated apoptosis. MM cells with and without del(8)(p21) were therefore treated with bortezomib and/or soluble TRAIL/APO2L. We found that bortezomib treatment alone and in combination with TRAIL/APO2L failed to induce apoptosis in MM cells with del(8)(p21) even though a slight downregulation of CD138 expression was seen. Downregulation of CD138 can be an early marker of apoptosis in MM cells [219] but in cells with del(8)(p21) it seems that the downregulation of CD138 expression does not trigger apoptotic pathways, thus indicating a possible apoptotic resistance mechanism. Conversely, in MM cells without del(8)(p21) apoptosis was seen after treatment with bortezomib and TRAIL/APO2L. The effect was seen when the two treatments were given separately and was significantly increased when bortezomib and TRAIL/APO2L were combined.

Furthermore, we examined the clinical response of patients with and without del(8)(p21) and could confirm that patients with del(8)(p21) have a significantly shorter TTP and OS, Figure 17. In order to evaluate the effect of bortezomib we analysed non-HDT eligible patients that received bortezomib as upfront treatment (n=82). Patients carrying del(8)(p21) showed a very poor response to bortezomib with half of the patients not responding and only 10% reaching VGPR, Figure 18A. On the other hand, patients without del(8)(p21) responded very well and the figures were reversed; 50% reached VGPR and only 10% did not respond, Figure 18A. However, despite their poor response to bortezomib treatment, non-HDT patients with del(8)(p21) had a good response to lenalidomide as second line treatment (Figure 18B), indicating that this treatment could be preferred for this group of patients.

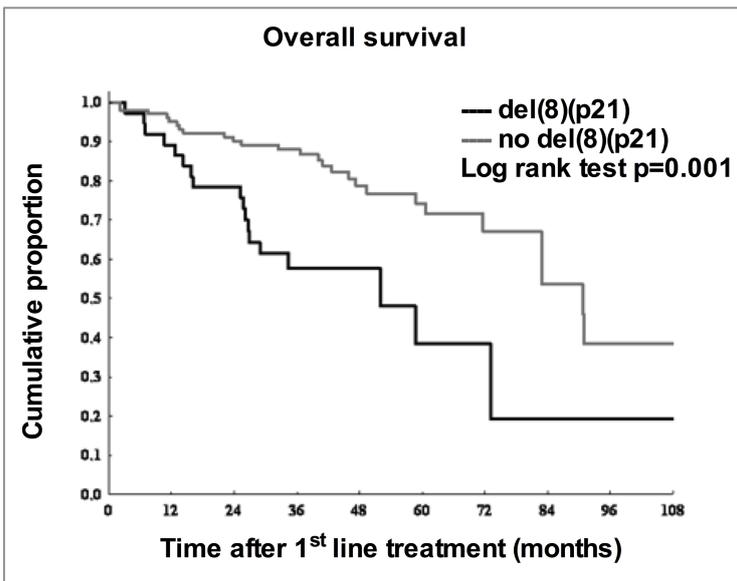


Figure 17. Overall survival from start of 1st treatment line in patients with (n= 37) and without (n=103) del(8)(p21).

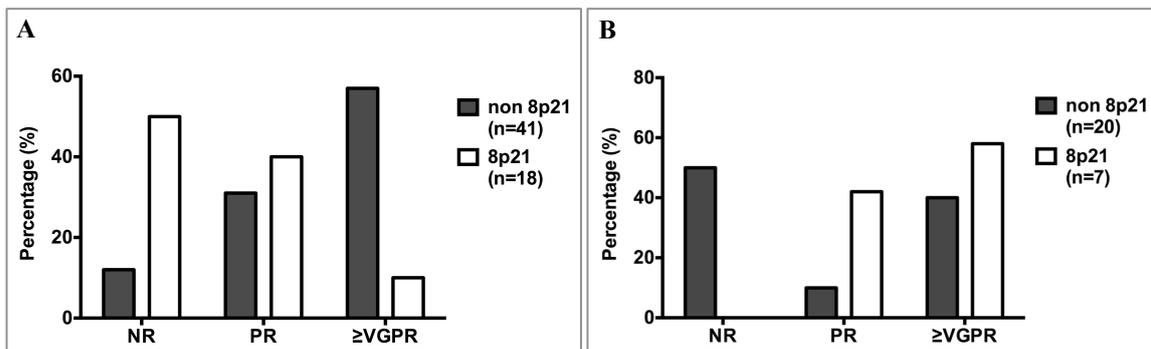


Figure 18. Response rate among non-HDT MM patients. (A) Response to upfront treatment with bortezomib. (B) Response to 2nd treatment line among patients that received bortezomib in 1st treatment line and lenalidomide in 2nd treatment line.

5 CONCLUSIONS

In the last 15 years, there has been a great development of treatment for MM.

We demonstrate that the good results seen in clinical trials are also found in a real-life setting.

More specifically we demonstrate that:

- The combination of bortezomib, cyclophosphamide and betamethasone is a highly effective induction regimen with a rapid and deep response as well as prolonged TTP and post-HDT advantages compared to conventional chemotherapy.
- The use of bortezomib, lenalidomide and thalidomide results in a higher response rate, increased TTNT and, most importantly, a longer OS compared to conventional agents in non-HDT patients.
- The OS in the best non-HDT outcome group is closing the gap to the matched Swedish cohort but MM is not a chronic disease yet, especially not in younger patients.

Concerning prognostic markers, we demonstrate that renal impairment is still an important prognostic marker in patients treated with conventional agents but we also show that novel agents can overcome the negative impact of renal impairment with improved OS survival in non-HDT patients.

Twenty per cent of MM patients do not respond to bortezomib treatment. We demonstrate that changes associated with del(8)(p21) might be the foundation of resistance to bortezomib treatment and resistance against bortezomib-mediated sensitization to TRAIL/APO2L killing. These results are also supported by clinical data.

6 FUTURE PERSPECTIVES

The future holds challenges and promises.

There is a continuous and exciting development of new treatments for MM, for instance the new monoclonal antibodies targeting SLAMF7 and CD38 as well as the histone deacetylase inhibitors. Hopefully, this development of more efficient drugs will also make it possible to individualize the treatment for each patient. This might well be the saviour for those with myeloma as well as for an economic environment that has to take account of an aging population and therefore reduced margins of hospital care.

The demands of efficiency will also inhibit the testing of expensive treatments with a small chance of succeeding, thus increasing the importance of knowing, in advance, who will benefit from a certain treatment and who will not. One example, which is given in this thesis, is the resistance to bortezomib seen in patients with del(8)(p21). It would be interesting to see if this could be generalised to other proteasome inhibitors such as carfilzomib. Our results also indicate that patients with del(8)(p21) benefit greatly from lenalidomide. This would be interesting to investigate further.

Furthermore, with the ongoing discussion about starting MM treatment earlier it will become even more essential to know in which order treatments should be given. For this, large population-based studies are needed. Big Data, Data Mining and Data Warehousing will be crucial in the attempts to retrieve information from patients and control groups, amplified by the growing amounts of digital medical records and registered information of large groups in our society. Here, international cooperation will be of utmost importance and the possibility to combine data from different countries will most probably continue to be a growing field in basic research.

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