# Towards personalized treatment in multiple myeloma based on molecular characteristics

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## Abstract

To date, the choice of therapy for an individual multiple myeloma patient has been based on clinical factors such as age and co-morbidities. The widespread evolution, validation and clinical utilization of molecular technologies, such as fluorescent *in-situ* hybridization and next generation sequencing has enabled the identification of a number of prognostic and predictive biomarkers for progression free, overall survival and treatment response. In this review we argue that in order to continue to improve myeloma patient outcomes incorporating such biomarkers into the routine diagnostic workup of patients will allow for the use of personalized, biologically based treatments.

## Introduction

Myeloma develops as the result of an evolutionary process during which a normal plasma cell moves through the pre-malignant state monoclonal gammopathy of uncertain significance (MGUS), to smoldering myeloma and myeloma that requires treatment.[1] Advances in therapy over the last two decades have improved patient outcomes whilst the use of new technology has increased our understanding of the molecular drivers that underlie disease initiation and progression. Due to underlying molecular variation, the clinical disease course is very heterogeneous.[2] Whilst some patients experience long remission periods, or functional cures, others relapse early or are refractory to therapy. In order to continue to improve outcomes, information regarding the molecular abnormalities driving these differences in outcomes needs to be incorporated into clinical care. These features may relate to mRNA, DNA or protein changes, but the aim is to identify aberrations that help inform the diagnosis, outcome or treatment relevant to a specific patient or subgroup of patients. Such molecular features or 'biomarkers' are defined by the NIH Biomarkers Definitions Working Group as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention'.[3]

The purpose of using biomarker driven, personalized, treatment approaches is to maximize benefit and reduce toxicity. In order to achieve this goal, the biomarker must be measurable in a robust and reproducible manner. Advances in technology have helped the identification and validation of myeloma biomarkers relevant to treatment such as those that can predict outcome for patients based on differences in survival (prognostic biomarkers) or target treatment to subsets of patients based on specific molecular pathology (predictive biomarkers). Some biomarkers can clearly be both prognostic and predictive and approaches to target these are likely to have the greatest impact on outcomes.

In this review we describe the current use of prognostic and predictive biomarkers in myeloma and speculate on advances that may enable further improvement in patient outcomes by employing these biomarkers to define personalized treatment strategies.

## Advances in molecular profiling technologies enabling the identification of biomarkers

The technologies enabling molecular profile analysis have evolved significantly over the last few decades contributing to an increased understanding of myeloma pathogenesis (**Figure 1**). Initial studies were performed using G-banding cytogenetics that identified translocations involving the immunoglobulin heavy chain (IgH) gene locus and hyperdiploidy as initiating events[4,5]. Translocations including t(4;14), t(6;14), t(11;14), t(14;16), and t(14;20) place oncogenes, *MMSET/FGFR3*, *CCND3*, *CCND1*, *MAF* and *MAFB* respectively, under the control of the *IgH* gene enhancer.[6,7] The downstream effect of upregulation of these genes converges on the increased expression of cyclin D protein family members, ultimately driving G1/S checkpoint dysregulation.[8] Hyperdiploidy, characterized by trisomies of odd numbered chromosomes also affects this checkpoint although the mechanism of its acquisition and downstream effect is less well understood. Subsequent studies have shown that secondary acquired lesions compound the cell cycle dysregulation, driving further proliferation and disease progression.[9]

The use of fluorescence *in-situ* hybridization (FISH), gene expression profiling (GEP) and single nucleotide polymorphism (SNP) array technologies, have expanded the knowledge of the myeloma genome and enabled its further classification into subgroups.[8,10,11] Cases cluster mainly based on the underlying structural genetic event (translocations and hyperdiploidy) with two classification systems surviving the test of time, the TC classification[8] and University of Arkansas for Medical Sciences (UAMS)[10] subgroups.

In more recent years, the introduction of next generation sequencing (NGS) technologies has allowed the identification of single nucleotide variants as well as larger structural changes including translocations and copy number abnormalities more quickly and cheaply.[12-15] Dozens of myeloma driver genes have been identified. The most common occur in the RAS and NF-kB families,[12,14,16] with many mutations associated with the primary myeloma molecular subgroups suggesting the underlying background initiating event drives the acquisition of subsequent molecular abberations.[13] A number of these new technologies are now CLIA certified and available for diagnostic use whilst additional techniques such as DNA methylation analysis remain confined to research laboratories.

## Advances in prognostic biomarkers

Prognostic biomarkers are used to identify the likelihood of disease relapse and/or predict overall survival. Classically they predict outcome irrespective of what therapy is given and enable more personalized outcome advice in the context of current treatment regimens. Several lesions have been identified as carrying an adverse outcome in myeloma. Some of these are clonal initiating lesions such as t(4;14), t(14;16) and t(14;20), whilst others are structural chromosomal changes or mutation events which tend to happen later in the evolutionary process.

### *t(4;14) – incidence 10-15% at diagnosis*

The t(4;14) results in the histone methyltransferase, *MMSET* and tyrosine kinase, *FGFR3* genes being placed downstream of IgH gene enhancers.[17] The spiked expression of *MMSET* is likely responsible for the adverse outcome, as in the subset of patients with concomitant loss of FGFR3 expression the outcome is equally as poor.[17,18] MMSET results in epigenetic reprogramming leading to a cascade of downstream effects including altered adhesion, enhanced growth and increased survival.[19] This reprogramming also leads to genetic instability including gain(1q), del(12p), del(13q), del(22q) and *BIRC2/3* homozygous deletion that may contribute to mediate the adverse outcomes.[20] In comparison to other risk groups the t(4;14) is particularly heterogeneous in terms of outcome, potentially influenced by these additional lesions and/or co-occurrence of del(17p).[21]

### *t(14;16)/t(14;20) – incidence 2-4% at diagnosis*

Similar genetic instability is seen in t(14;16) and t(14;20) cases that results in the upregulation of *MAF* and *MAFB* respectively and are associated with gain(1q) and del(17p). These subgroups are also associated with a mutational signature (a characteristic combination of mutation types) associated with the activity of the mRNA editing enzyme APOBEC and have an increased number of mutations.[16] MAF and MAFb protein have been demonstrated to mediate resistance to proteasome inhibitors[22,23], perhaps contributing to this subroup's adverse outcome seen in most [2,24,25], though not all [26], studies.

### *Del(17p) – incidence 8-10% (using threshold of 20% positive cells)*

Deletion of the short arm of chromosome 17 has been associated with adverse outcome, thought to be related to the loss of expression of the tumor suppressor

*TP53*.[27] Whilst occasional studies suggest deletions in <20% of cells detected by FISH may have some clinical impact[28], most studies utilize a cut off of >20% to demonstrate a significant effect.[29,30] As the clone size increases the effect on outcome becomes more marked with some studies suggesting that clonal deletion in at least 60% of cells is required.[31] More recent data suggests that biallelic disruption either by two chromosomal deletions, by deletion in one and TP53 mutation in the other allele or biallelic mutation is what mediates the adverse outcome. [29,32]

### *Gain(1q) – incidence 30-35% at diagnosis*

Gain(1q) has been associated with adverse outcome although given the large number of genes situated on this chromosome, it is less clear which gene is responsible. Implicated genes at the most commonly gained locus (1q21) include *BCL9, MCL1, CKS1B* and *ANP32E*.[33-36] This locus is susceptible to gain due instability of the pericentromeric chromatin.[37] Other genes may also be important e.g. *CD45* at 1q32 when larger regions of the chromosome are gained.[38] There is an important distinction between gain, defined as one additional copy, and amplification, defined as >1 additional copy of 1q with amplified cases appearing to be associated with a more adverse outcome.[15,20,32]

### *Other translocations/copy number abnormalities*

Del(1p) (incidence 10% at diagnosis) frequently co-occurs with gain(1q) and has been shown to be associated with an adverse outcome in patients undergoing autologous stem cell transplant. This effect is potentially mediated by loss of *CDKN2C* and *FAF1* at 1p32 and/or *FAM46C* at 1p12 and/or *RPL5* and *EVI5* at 1p22.[24,39,40]

Myc aberrations (incidence 15-20% at diagnosis) are common and may be mediated by secondary translocations to the MYC locus at 8q22 or copy number change and are associated with adverse outcomes.[41,42]

t(11;14) and hyperdiploidy are usually considered standard-risk. Some studies suggest that individual trisomies may be able to overcome some of the adverse impact of other lesions such as t(4;14) and del(17p), with trisomy 3 appearing to have the greatest impact.[43,44] Another study looking at the impact of hyperdiploidy in this setting had conflicting findings.[45]

### *Mutations*

Mutations associated with adverse outcome that may function as prognostic biomarkers have also been identified and include those in *CCND1* and DNA repair pathway genes (*TP53*, *ATM*, *ATR* and *ZFHX4*).[15,42] Some mutations associated with a favorable outcome have also been identified eg *IRF4* and *EGR1*. Mutational analysis of genomic instability can also predict for adverse outcomes with increases in genome-wide loss of heterozygosity associated with adverse outcomes.[46]

### *RNA alterations*

Whilst DNA based assays are able to identify individual lesions and markers of global genomic instability, RNA and gene expression profiling (GEP) can be used to detect markers of increased proliferation and specific pathway expression changes.[47] The GEP scores of 70 genes, GEP70 (MyPRS)[48] or 92 genes, SKY92[49], have prognostic capabilities better than using any single lesion discussed above. They identify high-risk outcomes in around 15% of patients at diagnosis. Their perceived limitations lie in the lack of widespread availability and computational analysis required to interpret the results.

Novel fusion genes have also been identified in myeloma using RNA-seq data and some have been associated with adverse outcome, for example *CSNK1G2* and *CCND1* with shortened progression-free survival (PFS) and *MMSET* and *BCL2L11* with shortened overall survival (OS).[50]

### *Other disease features*

Other features of disease may also indicate high-risk outcomes for patients and hence act as prognostic biomarkers. The presence of plasma cells with blastic morphology, renal failure, extramedullary disease[51] and plasma cell leukemia at diagnosis all predict for worse outcomes. Circulating plasma cells, even at a lower level than meet the criteria for plasma cell leukemia, are also associated with adverse outcomes.[52,53] Recent studies have shown that the number and size of focal lesions on PET-CT and MRI imaging also predict for a poor outcome independent of molecular features.[54]

## Risk Stratification Systems – incorporating biomarkers

With the advances in technology and the increase in size of the datasets examined, the information concerning the clinical impact of the presence of these molecular lesions has changed. This has resulted in a shift from using a single lesion to define high-risk disease to the use of two or three collaborating lesions. In addition, as so called 'high-risk' lesions can occur in up to 30-50% of patients, the need to identify a smaller group (e.g. <15%) of patients who truly perform badly regardless of therapy has become apparent. Such patients can be considered 'ultra high-risk' and have been identified in the following ways (**Figure 2**):

### *i. Presence of more than one adverse cytogenetic lesion*

Translocations and copy number change associated with adverse outcome as describe above have been demonstrated to be cumulative such that the presence of more than one lesion predicts for a worse outcome than one lesion alone.[20,24] In the MRC Myeloma IX study (**Figure 2A**) patients with more than one adverse lesion were termed ultra high-risk and comprised 15% of patients.

### *ii. R-ISS*

The Revised International Staging System (R-ISS) score built on this concept and incorporates $B_2M$, albumin (from the previously used ISS) and LDH with structural lesions to more accurately predict risk. Risk is categorized into three groups, from low-risk R-ISS group I with ISS Stage I; no high-risk cytogenetic abnormality (CA) (del[17p] and/or t[4;14 and/or 14;16]) and normal LDH level; to high-risk R-ISS group III with ISS Stage III and high-risk CA or high LDH level (**Figure 2B**).[55] Between 10-18% of patients are classified as R-ISS III.[55-58] In this system each feature contributes equally to the risk group determination. As more data becomes available and the understanding of how these features reflect myeloma biology increases, it can be envisaged that staging systems will be refined and features will be weighted.

### *iii Double hit myeloma*

Building on the scores defined above the Myeloma Genome Project incorporated NGS and structural abnormalities to better define risk (**Figure 2C**).[32] The study defined the highest risk patients as 'double hit' myeloma, that is patients with two 'hits' to the same gene, either loss of both alleles of *TP53* (by mutation,

deletion or both) or with two extra copies of 1q resulting in amplification rather than a single gain. This group comprises 6-10% of patients and has a greater prognostic power than the R-ISS. A number of other groups have confirmed the importance of knowing both the copy number and *TP53* mutation status, and this lesion along with amp(1q) now represents the most recent refinement in myeloma risk prediction.

Interestingly, there remains a subset of patients carrying none of the molecular lesions discussed above who still relapse early. Such patients can be considered high-risk phenotypically as these patients in addition to having a short first PFS have a poor OS. Hence having become apparent, such patients may require therapy different to standard treatment at first relapse.[59] Ongoing molecular studies may help to identify the currently unrecognized drivers in this early relapse group, as altering up-front therapy remains likely to have the greatest benefit on long term survival.

### *Prognostic biomarkers at different disease time points*

The biomarkers described above were largely described in newly-diagnosed patient. Many adverse risk biomarkers become more frequent at later stages of the disease but still retain prognostic significance. Other biomarkers that can be incorporated later include response to therapy, especially when assessed by quantification of minimal residual disease (MRD) in the bone marrow (by next generation sequencing or next generation flow cytometry)[60-62] or by imaging techniques.[63]

## Risk-adapted therapy for high-risk groups

Aside from providing important prognostic information, the true benefit of identifying patients at high risk of early progression or death is to intervene and deliver different therapy to standard treatment. Such approaches are being investigated in several clinical trials. One of the challenges for these studies is that response rates generally do not differ between patients with high-risk and standard-risk disease as the natural history of high-risk patients is to respond well but relapse early. As such depth of response may not have the same prognostic importance in high-risk groups. In patients defined as del(17p) or >=2 cytogenetic abnormalities, stringent complete response (sCR) and MRD negativity did not translate into a

superior PFS or OS.[64] Early data suggests at deeper levels of MRD analysis this drawback may be overcome and confirmatory studies are awaited.[65]

Trials concentrating on high-risk disease can be performed in two ways. In all-comer trials the high-risk subgroup can be analyzed and reported separately and compared to non-high-risk patients, or trials can be specifically designed to optimize therapy for a prospectively recruited high-risk group (**Figure 3A-B**). The first approach provides reassurance on subgroup analysis that a given treatment shows as much benefit in the high-risk as standard-risk population but claims of efficacy in the smaller high-risk population will be limited by the statistical power and so large trials are required. The latter approach ensures studies are correctly powered to assess impact specifically in high-risk patients, however the definition of high-risk needs to be uniform and utilize reproducible biomarkers.

Subgroup analysis of previously reported clinical trials has led to several approaches being suggested for high-risk patients. This includes the observation that proteasome inhibitors overcome the some of adverse outcome associated with t(4;14) +/- del(17p).[66] This initial data was based on a small subgroup analysis of the VISTA study. Subsequent studies, and a meta-analysis, confirmed that bortezomib-based induction results in improved outcomes versus non bortezomib-based induction but does not fully overcome the adverse prognostic impact of these lesions.[67,68] A similar pattern is seen with lenalidomide maintenance post-autologous transplant (Jackson GJ et al, Lancet Oncology *in press*) suggesting novel agents can ameliorate but not abrogate adverse outcomes associated with high-risk disease. Studies in relapsed patients of the novel proteasome inhibitors carfilzomib and ixazomib also support this concept with a benefit over the control arm in high-risk patients but suboptimal outcomes compared to standard-risk patients.[69,70] More recently in newly-diagnosed patients tandem autologous transplant, post-transplant consolidation and maintenance have all proved effective for high-risk patients compared to standard of care[71] and may to some extent attenuate unfavorable outcomes, but no strategy to date is able to overcome the adverse effect of high-risk lesions completely. Prospective recruitment of high-risk patients to dedicated protocols is needed.

High-risk patients are currently being recruited into a number of ongoing trials (**Table 1**). One example is the Total Therapy series of studies that initially started as risk-agnostic (TT1–TT3a/b) and later moved to high-risk studies (TT5, TT5b and TT7). The phase II TT5 trial[72] recruited patients with GEP70 defined high-risk

disease and delivered dose dense chemotherapy, minimizing breaks between treatment phases by administering less intense therapy blocks aiming to prevent relapses that have been observed to occur during treatment breaks, for example during recovery from autologous transplant. Patients were compared to risk-matched patients in TT3 and no significant differences in survival were identified. However, the number of patients relapsing in the early treatment courses decreased with patients tending to relapse during the later maintenance phase. The latter iterations of the protocol (TT5b and TT7) have concentrated on this phase of treatment and are incorporating newer proteasome inhibitors and immune-based approaches.

Other ongoing studies are examining intensification of induction, the use of autologous and allogeneic transplantation approaches and immunotherapy approaches such as CAR-T cells. The first approach is exemplified by the UK MUK9b trial (NCT03188172), the US 2015-12 trial (NCT03004287) and the German GMMG-CONCEPT trial (NCT03104842) which all combine a CD38 antibody, proteasome inhibitor and lenalidomide as intensified upfront therapy along with prolonged courses of consolidation and maintenance.

The major difference between each of these studies is the definition of high-risk (e.g., GEP70, single or combinations of genetic lesions), which will make subsequent direct comparisons of PFS and OS challenging. However, there is little doubt that concentrating on this subgroup of myeloma will be a rewarding area for both patients and investigators. Given the long PFS and OS for standard-risk patients, trials designed for standard-risk require large numbers of patients and long follow up to demonstrate a statistical and meaningful clinical improvement of the intervention. The high-risk patient is an area of unmet clinical need and is also the ideal situation to demonstrate the clinical activity of a novel agent or novel approach and as such it is anticipated that other novel immunotherapy approaches such as CAR-NK cells, bispecific antibody therapy and antibody drug conjugates will move into first line studies for high-risk myeloma over the coming years.

An alternative approach to altering upfront therapy is to utilize the prognostic biomarker of MRD post-induction to alter treatment at this time-point. Studies addressing questions around intensification of therapy for MRD+ve patients or de-escalating therapy for MRD-ve patients are in development.

## Advances in predictive biomarkers

In contrast to prognostic biomarkers, predictive biomarkers forecast the likelihood of a favorable or unfavorable outcome with a specific agent. From mutations alone it can be estimated that two thirds of patients have actionable lesions with agents currently available or in development.[15] Other targetable lesions include primary translocation events and/or protein expression patterns. To date only a limited number, however, have been studied in clinical trials and these are discussed below.

### *Targeted therapeutics using predictive biomarkers currently in clinical trials*

#### *i. Venetoclax*

Venetoclax is an inhibitor of the anti-apoptotic protein BCL2. *In-vitro* data shows a higher sensitivity to venetoclax for cell lines and patient samples with a t(11;14). This is likely due to the higher BCL2 to MCL1 expression ratio that correlates with the presence of the translocation.[73,74] Two early phase clinical studies have been published (**Table 2**). The first studied single agent venetoclax in multiply relapsed/refractory patients and demonstrated an overall response rate (ORR) of 40% amongst t(11;14) patients.[75] The study also correlated BCL2:MCL1 and BCL2:BCL2L1 mRNA expression levels with responses and with t(11;14) status. The second study examined venetoclax in combination with bortezomib and dexamethasone. It demonstrated an ORR of 67% in all patients and 78% in t(11;14) patients. Similar to the single agent study, patients with higher BCL2 expression had deeper responses and longer PFS.[76] The high efficacy of the combination in patients without the t(11;14) or high BCL2 expression was speculated to be due to bortezomib upregulating NOXA, a pro-apoptotic factor that neutralizes MCL1 resulting in an increased ratio of BCL2:MCL1 and sensitivity to venetoclax.[74]

These findings suggest that moving forward venetoclax may not be limited to the t(11;14) subgroup and that when used in combination with a proteasome inhibitor, an assay measuring BCL2:MCL1 or BCL2:BCL2L1 mRNA expression ratios may be beneficial as a predictive biomarker. This biomarker driven strategy can be clearly seen with the trial combinations being examined in ongoing studies (**Table 2)**, where those with proteasome inhibitor combinations are open to all comers whereas single agent studies are restricted to t(11;14) patients.

### *ii. RAS pathway inhibitors*

Sequencing studies have identified mutations in RAS in approximately 50% of patients (25% *NRAS*, 25% *KRAS* and 4% *BRAF*) leading to the evaluation of RAS pathway inhibitors. These include BRAF inhibitors (e.g., vemurafenib and dabrafenib), and MEK inhibitors (e.g., trametinib and cobimetinib). The published experience to date is mostly limited to case reports and case series (**Table 3**) and provides encouraging evidence of activity in relapsed/refractory patients (e.g. with responses seen in 16/40 patients with measurable disease[77], although therapy was often in combination). Several prospective studies are now underway and should provide a more comprehensive analysis of efficacy (**Table 3**).

### *iii. Therapies in development*

Further targets have been identified that may predict response to therapeutic agents although these are at earlier stages of development. Examples include *IDH1*/*IDH2* mutations and IDH inhibitors[78,79], loss of heterozygosity or *ATM/ATR* mutations and PARP inhibitors[46] and *FGFR3* mutations and FGFR3 inhibitors[80]. Several are being studied in large umbrella studies such as the Multiple Myeloma Research Foundation (MMRF) MyDRUG study and the Canadian "CAPTUR" study. These incorporate agents targeted to a large number of molecular drivers with a solid preclinical rationale, often repurposed from other diseases. Ongoing analysis is identifying further targets that can be incorporated into such studies. For example, fusion genes have been identified in myeloma and although rare (1%)[50,81], the majority contain a kinase domain suggesting kinase inhibitors may have a potential role.

Recent studies in solid tumor studies have shown that patients with a high mutation burden respond exceptionally well to PD1/PDL1 inhibitors. Generally the mutation burden in myeloma is lower than solid tumors but a percentage of cases with a t(14;16) MAF translocation have an APOBEC signature and high mutational burden providing a biological rationale to explore checkpoint inhibitors in this small group.

### *Limitations of targeted therapeutic approach*

An important caveat of targeted agents used on the basis of predictive biomarkers is the presence of clonal heterogeneity as not all cells may contain the genetic lesion. Biopsies from distinct sites of disease within the same patient at one time point, or from the same site at different time points have been shown to be

molecularly diverse.[82] These findings suggest that initiating events, present in 100% of clonal cells, may make good predictive biomarkers whereas secondary, usually subclonal, events would need to be in a high percentage of cells to be clinically useful. However, it could be hypothesized that where the targeted lesion is also of some prognostic importance, targeted therapy may still be clinically beneficial, for example by eliminating a high-risk clone and allowing a standard-risk clone to predominate may prove an effective therapeutic strategy. Using this logic, the best predictive lesions to target, but for which targeted agents unfortunately do not yet exist, are those that are present in a high proportion of clonal cells and are associated with adverse outcomes. For instance targeting MMSET, the oncogenic driver in t(4;14), fulfills both these criteria but has proved difficult for drug design to date. Other options include MAF targeted approaches and the identification and targeting of lesions associated with gain or amp(1q) and del(17p).

Another limitation of mutation targeted therapy is the lack of integration of RNA and protein level data into the decision making process. Since drugs mostly act on proteins the effect of mutations at the protein level is important. For example mutations in recurrent sites known to cause pathway activation may be acted upon clinically but variants of as yet unknown significance should be treated with caution. As more integrative molecular models become available this potential limitation may be overcome.

Finally targeting individual lesions that occur in low frequencies in patient populations poses a logistical problem for clinical trial design and requires novel trial approaches. For instance for t(11;14) and RAS pathway mutations, present in 15 and 50% of patients respectively, it is still possible to run lesion specific trials. In contrast, attempting to target lesions such as *IDH* mutations, present in <2% of patients, will require multi-center and potentially multi-disease collaborations. Such concepts are employed in umbrella studies (**Figure 3C**) or Basket studies (**Figure 3D**) where patients with different tumors are enrolled in the same protocol based on a molecular lesion identified. In addition, given the scarcity of some lesions in specific disease there is an argument that more single patient experiences also warrant publication.

## Conclusions

The improvement in survival for myeloma patients over the last decade has mainly benefited low-risk patients and now that PFS for this group of patients is in

excess of 8 years a new approach to improving outcomes is required. Current approaches to personalize myeloma therapy take into account age and co-morbidities but rarely consider molecular information. However, as the information concerning genetic analysis has become stronger, it can be postulated that one way to quickly improve outcomes further would be to incorporate such information into clinical algorithms. We have described two possible approaches by which this might be achieved. The first targets a cohort of patients with high-risk markers using intensified therapeutic approaches agnostic to molecular lesions. Such approaches include combinations of quadruplet or even quintuplet regimens and/or novel immunotherapy approaches such as bi-specific antibodies, antibody drug conjugates and CAR-T cells. This approach has the benefit of targeting patients with the worst outcomes and highest unmet clinical need. The challenge, however, is the lack of understanding about whether this is best achieved by incorporating an ever increasing numbers of additional agents, novel immunotherapy agents or whether the focus should be on designing more optimal treatment delivery approaches, such as different schedules and sequencing approaches using currently available agents.

The alternative strategy is to aim to use molecularly targeted agents that target lesions specific to an individual patient's disease and therefore have a higher likelihood of efficacy whilst avoiding unnecessary toxicity. The knowledge of the molecular basis of myeloma is ever expanding and so we can use this to define rationale drug targets as well as to utilize drugs already available for known targets. With respect to such predictive biomarkers utilizing therapies targeted to either initiating lesions or lesions with a high cancer clonal fraction seem most likely to be effective. In addition it seems likely that molecularly targeted agents will not be used alone, instead, these agents will be combined in specific subsets of disease with other agents that target more general plasma cell biological functions such as proteasome inhibitors, immunomodulatory drugs and monoclonal antibodies.

In closing, it is important to note that work to date concerning prognostic and predictive biomarkers has concentrated on genetic lesions within the plasma cell. Advances in protein technologies are occurring rapidly with the advent of tabletop analyzers,[11] mass spectrometry,[12] next generation flow cytometry, mass cytometry (CyTOF) and whole proteome analysis. These technologies will allow the study the myeloma proteome as well as components of the microenvironment and immune environment. With the increasing use of immune therapies it seems likely that

biomarkers related to these areas will be identified and will need to be incorporated into current models and treatment decisions.

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## Figures:

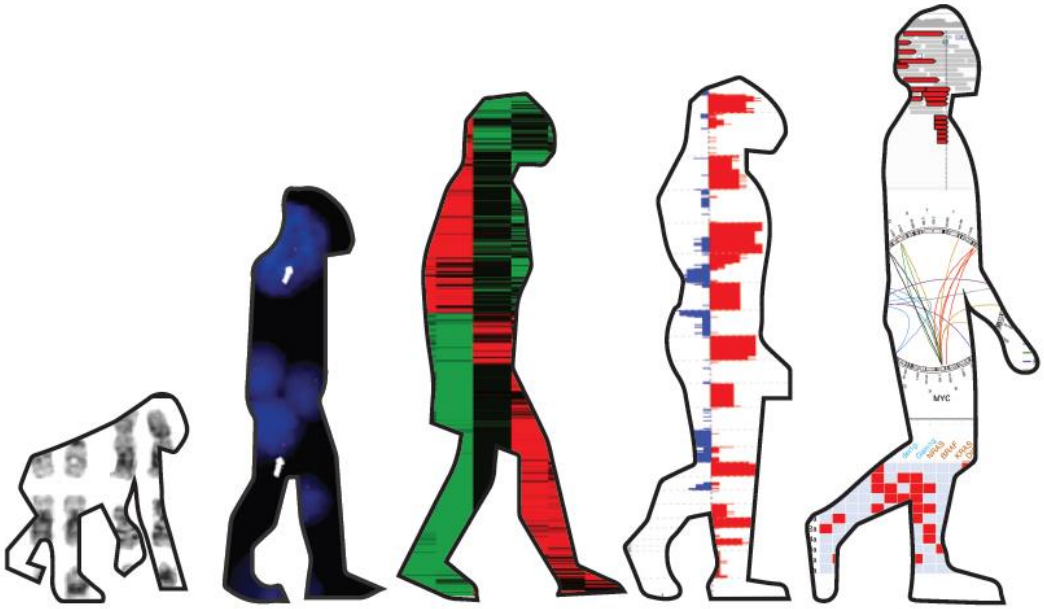


**Figure 1 - The Evolution of Molecular Analysis Techniques in Myeloma.**
Images from left to right show G-band karyotyping, fluorescence in situ hybridization (FISH), gene expression profiling (GEP) data, single nucleotide polymorphism (SNP) array data, next generation sequencing (NGS) data.

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Karyotype CC-BY: Panagopoulos I. et al. (2018) RUNX1-PDCD6 fusion resulting from a novel t(5;21)(p15;q22) chromosome translocation in myelodysplastic syndrome secondary to chronic lymphocytic leukemia. PLOS ONE 13(4): e0196181.
FISH CC-BY: Fernando RC. et al. (2015) Multiple myeloma cell lines and primary tumors proteoma: protein biosynthesis and immune system as potential therapeutic targets. Genes & Cancer 6:11-12.
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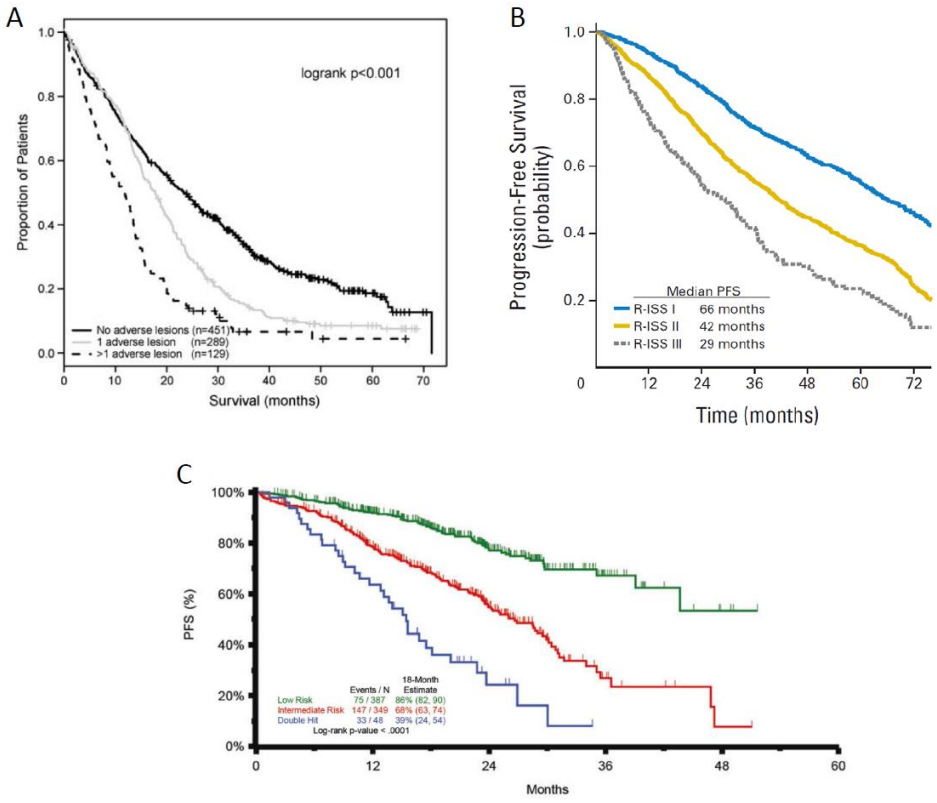
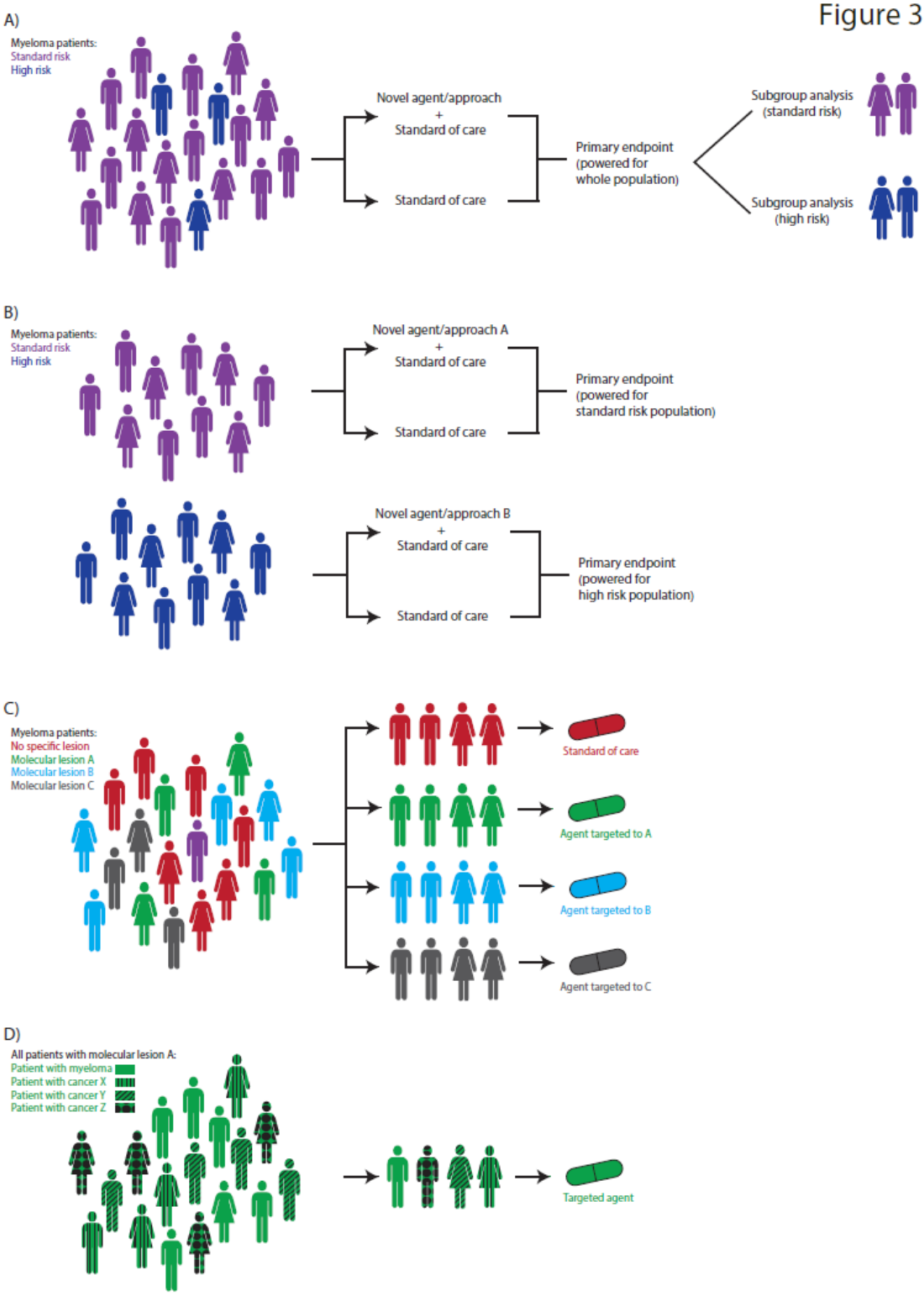
SNP array and NGS CC-BY: Bolli N. et al. (2018) Genomic patterns of progression in smoldering multiple myeloma. Nature Comms (9) 3363.




## Figure 2 – Risk Stratification Systems and Outcome

Progression-free survival as defined by the different risk stratification systems.
A) Ultra high-risk defined by the presence of more than one adverse lesion [t(4;14), t(14;16), t(14;20), del(17p) and gain(1q)] in the analysis of 869 cases from the MRC Myeloma IX trial, published 2011.
B) Ultra high-risk defined by the R-ISS [low-risk R-ISS group I with ISS Stage I; no high-risk cytogenetic abnormality (CA) (del[17p] and/or t[4;14 and/or 14;16]) and normal LDH level; to high-risk R-ISS group III with ISS Stage III and high-risk CA or high LDH level] in a pooled study of 4,445 patients with NDMM from 11 clinical studies. Published 2016
C) Ultra high-risk defined as "double-hit" myeloma [either loss of both alleles of TP53 (by mutation, deletion or both) or with two extra copies of 1q resulting in amplification rather than a single gain] by incorporating next generation sequencing data in the Myeloma Genome Project analysis of 784 patients, published 2018.

Figure 3




## Figure 3 – Clinical Trial Design Strategies for Personalized Treatment in Myeloma

A) Current standard approach with all patients recruited and treated as part of a clinical trial with subsequent subgroup analysis that may, or may not, be adequately powered to examine the effect of the novel strategy in high-risk patients.

B) Trial design for high-risk patients which are identified upfront and entered into dedicated protocols. These may be phase II or III randomized studies (as shown) or earlier phase single arm studies.
C) Umbrella trial design with patient molecular lesions identified up front and entered into an arm examining a therapy appropriate to that lesion.
D) Basket trial design with patients with different cancers but with a shared molecular lesion entered into a study with an agent targeted to that lesion.

## Tables:

## Table 1 – Clinical Studies Specifically targeting high-risk disease in newly diagnosed myeloma patients

As per clinical trials.gov search for "high-risk myeloma" and including studies where the high-risk definition was included.

| | NCT number | Status | Location | Ph. | Treatment schema | Definition of high-risk used in the study |
|---|---|---|---|---|---|---|
| **Reported studies** | | | | | | |
| TT5 | NCT00869232 | Active, not recruiting | US | 2 | | • GEP70 defined high-risk gene expression profiling |
| **Ongoing studies** | | | | | | |
| ***Novel intensified combinations*** | | | | | | |
| TT5b | NCT02128230 | Recruiting | US | 2 | | • GEP70 defined high-risk gene expression profiling |
| MUK9b OPTIMUM Treatment Protocol | NCT03188172 | Recruiting | UK | 2 | Dara-CVRD | • Two of: (4;14), t(14;16), t(14;20), del(17p), gain(1q), del(1p)<br>• SKY92 defined high-risk gene expression profiling<br>• Plasma cell leukemia |
| 2015-12: A Study Exploring the Use of Early and Late Consolidation/Maintenance Therapy | NCT03004287 | Recruiting | US | 2 | Dara, carfilzomib or bortezomib, thalidomide, lenalidomide, dexamethasone, cisplatin, adriamycin, cyclophosphamide, etoposide, lenalidomide and dexamethasone. | • Myeloma Prognostic Risk Signature (MyPRS) risk score ≥ 50.4<br>• Lactate Dehydrogenase (LDH) ≥ 360 U/L<br>• Plasma cell leukemia. |
| Evaluation Induction, Consolidation and Maintenance Treatment With Isatuximab , Carfilzomib, Lenalidomide and Dexamethasone | NCT03104842 | Recruiting | DE | 2 | Isatuximab-KRDx6<br>Isa-KRDx4<br>Isa-KR maintenance<br><br>TE and TNE | • Presence of one or more of the following cytogenetic abnormalities (determined by FISH):<br>○ Del(17p) in ≥ 10% of purified cells<br>○ t(4;14)<br>○ > 3 copies +1q21<br>• ISS Stage II or III (all patients) |
| S1211 Bortezomib, Dexamethasone, and Lenalidomide With or Without Elotuzumab in Treating Patients With Newly Diagnosed High-Risk Multiple Myeloma | NCT01668719 | Recruiting | US SWOG | 1/2 | Elo-VRD vs VRD | • GEP70 or SKY92 defined high-risk gene expression profiling<br>• Translocation (14;16), and/or translocation (14;20), and/or deletion (17p) by FISH or cytogenetics<br>• Plasma cell leukemia<br>• Lactate dehydrogenase (LDH) >= 2 x institutional upper limit of normal (IULN)<br>• 1q21 amplification by FISH analysis |
| An Intensive Program With Quadruplet Induction and Consolidation Plus Tandem Autologous Stem Cell Transplantation in Newly Diagnosed High Risk Multiple Myeloma Patients: a Phase II Study of the Intergroupe | NCT03606577 | Not yet recruiting | FR | 2 | Dara-KRd induction and consolidation and tandem ASCT, Dara-R maintenance | • FISH analysis: del(17p), or t(14;16) or t(4;14). The FISH-positivity cut-off value for defining the presence of del(17p) in this study is 50% |

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|---|---|---|---|---|---|---|
| Francophone du Myélome "IFM 2018-04" (IFM 2018-04) | | | | | | |
| A Single Arm Study of Carfilzomib in Transplant Eligible High Risk Multiple Myeloma | NCT02217163 | Active, not recruiting | SG | 2 | Carfilzomib, cyclophosphamide and dexamethasone for up to 8 cycles prior to ASCT | • International Staging System (ISS) III<br>• del(17p), t(4;14), t(14;16), t(14;20), gain(1q) |
| ***Auto/Allo approaches*** | | | | | | |
| Autologous or Syngeneic Stem Cell Transplant Followed by Donor Stem Cell Transplant and Bortezomib in Treating Patients With Newly Diagnosed High-Risk, Relapsed, or Refractory Multiple Myeloma | NCT00793572 | Active, not recruiting | US | 2 | Auto/allo after VAD induction. Maintenance velcade. | • Any abnormal karyotype by metaphase analysis except for isolated t(11,14) and constitutional cytogenetic abnormality<br>• FISH detection of t(4;14), t(14;16) or deletion 17p<br>• Beta2-microglobulin > 5.5 mg/L<br>• Cytogenetic hypodiploidy<br>• Plasmablastic morphology (>= 2%) |
| Allogeneic Hematopoietic Stem Cell Transplantation With Ixazomib for High Risk Multiple Myeloma (BMT CTN 1302) | NCT02440464 | Recruiting | US | 2 | Ixazomib vs placebo post allogeneic transplant | • del(13), gain(1q), del(1p), t(4;14), t(14;16), t(14;20), del(17p) or high-risk criteria based on commercially available gene expression profiling (GEP) ; and elevated beta-2 microglobulin (≥ 5.5 mg/L at diagnosis).<br>• Plasma cell leukaemia<br>• Relapsed within 18 months of 1st line therapy |
| ECT-001 (UM171) Expanded Cord Blood Transplant to Treat High-risk Multiple Myeloma | NCT03441958 | Recruiting | CA | 1/2 | ECT-001 (UM171) expanded cord blood allogeneic transplant | • t(4;14), t(14;16), t(14;20), del(17p13), chromosome 1 abnormalities with ISS II or III<br>• Revised-ISS 3<br>• Plasma cell leukemia<br>• Refractory to first line triplet bortezomib-based induction treatment.<br>• ≥ 2 cytogenetics abnormalities as defined above regardless of ISS stage |
| Nonmyeloablative Allogeneic Stem Cell Transplant Followed by Bortezomib in High-risk Multiple Myeloma Patients | NCT02308280 | Recruiting | CA | 2 | Non myeloablative allogeneic transplantation followed by Bortezomib for 1 year after a Bortezomib-based induction and autologous stem cell transplantation.<br>Bortezomib: 1,3 mg/m2 subcutaneously every 2 weeks for 26 injections. | • International Staging System (ISS) III<br>• del(17p13), t(4;14) with ISS II or III, t(14;16), t(14;20) and chromosome 1 abnormalities by FISH<br>• Plasma cell leukemia<br>• Patients ≤ 50 years, regardless of cytogenetics or ISS stage |
| ***Immunotherapy approaches*** | | | | | | |
| Up-front CART-BCMA With or Without huCART19 in High-risk Multiple Myeloma | NCT03549442 | Recruiting | US | 1 | | • Beta-2-microglobulin ≥ 5.5 mg/L and LDH greater than upper limit of normal.<br>• High-risk FISH features: del(17p), t(14;16), t(14;20), t(4;14) in conjunction with Beta- 2-microglobulin ≥ 5.5 mg/L (i.e., revised ISS stage 3).<br>• Metaphase karyotype with >3 structural abnormalities except hyperdiploidy<br>• Plasma cell leukemia (>20% plasma cells in peripheral blood)<br>• Failure to achieve partial response or better to initial therapy with an |

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| | | | | | | "imid/PI" combination (thalidomide, lenalidomide, or pomalidomide in combination with bortezomib, ixazomib, or carfilzomib).<br>• Early progression on first-line therapy, defined as progression |
| CART-19 Post-ASCT for Multiple Myeloma | NCT02794246 | Active, not recruiting | US | 2 | CD19 CAR administered after ASCT | • Any of the following high-risk cytogenetic features, documented by FISH or metaphase karyotyping: del(17p), t(4;14), t(14;16), t(14;20).<br>• Standard-risk cytogenetics but elevated LDH and beta-2-microglobulin > 5.5 mg/L (i.e., R-ISS stage III). |
| Study of T Cells Targeting CD19/BCMA (CART-19/BCMA) for High Risk Multiple Myeloma Followed With Auto-HSCT | NCT03455972 | Recruiting | CN | 1 | Anti-CD19/BCMA CAR administed after ASCT | • not achieved VGPR before stem cell mobilization<br>• R-ISS III stage<br>• extramedullary disease<br>• del(17p), t(4;14), t(14;16) |
| Pembrolizumab + Lenalidomide Post Autologous Stem Cell Transplant (ASCT) in High-risk Multiple Myeloma (MM) | NCT02906332 | Active, not recruiting | US | 2 | Pembrolizumab and lenalidomide maintenance post-ASCT | • International Staging System (ISS) stage 3<br>• Deletion 13q by cytogenetics, and/or<br>• 1q amplification, 1p deletion, p53 deletions (17p deletions), t(4;14), t(14;16), t(14;20), hypodiploidy,<br>• High-risk gene expression profile (GEP) scores |
| 2015-10: Expanded Natural Killer Cells and Elotuzumab for High-Risk Myeloma Post- Autologous Stem Cell Transplant (ASCT) | NCT03003728 | Not yet recruiting | US | 2 | Elotuzumab and expanded natural killer cells post ASCT | • Gene Expression Profiling (GEP) 70 risk score of ≥ 0.66<br>• GEP 80 gene score of ≥ 2.48<br>• metaphase cytogenetic abnormalities<br>• lactate dehydrogenase (LDH) ≥ 360 U/L |
| **Completed, as yet unreported studies** | | | | | | |
| Bortezomib, Doxorubicin Hydrochloride Liposome, and Dexamethasone Followed by Thalidomide and Dexamethasone With or Without Bortezomib in Treating Patients With Multiple Myeloma | NCT00458705 | Completed | US | 2 | Bortezomib, liposomal doxorubicin and dexamethasone x 3<br>Thal/dex x 2 | • High-risk disease, defined as symptomatic International Staging System (ISS) stage 2 or 3<br>• Soft-tissue plasmacytoma<br>• Extension of a plasmacytoma into soft tissues<br>• Primary resistant myeloma, defined as unchanged or progressive diase despite two courses of standard treatment |
| Combination Bortezomib-containing Regimens in Newly Diagnosed Patients With t(4; 14) Positive Multiple Myeloma | NCT00570180 | Completed | CA | 2 | Vel, dex, liposomal dox x 4<br>ASCT<br>Cyclo, vel, pred x 8<br>+ dex maintenance | • t(4;14) |
| Celgene High Risk Multiple Myeloma (MM) Revlimid Induction and Maintenance Therapy | NCT00691704 | Completed | US | 2 | Induction:<br>Rd x4<br>Sequential maintenance:<br>- Velcade<br>- MP<br>- Len | • Deletion of chromosome 13 by cytogenetics<br>• Del(17p) by FISH or metaphase analysis<br>• FISH detection of t(4;14), t(14;16), t(8;14), or t(14;20)<br>• hypodiploidy detected by FISH or metaphase analysis<br>• any complex cytogenetic abnormality detected by cytogenetics , with the exception of hyperdiploidy |

| | | | | | | |
|---|---|---|---|---|---|---|
| Stem Cell Transplantation To Treat High Risk Multiple Myeloma With Reduced Toxicity Myeloablative Conditioning Regimen | NCT00615589 | Terminated, low accrual | US | 2 | Fludarabine/busulfan conditioned MUD allo | • Stage II/III, any of: t(4; 14), t(14; 16),(14:20) by FISH; 17p- by conventional cytogenetics or FISH; Δ13 by conventional cytogenetics; Hypodiploidy by conventional cytogenetics.<br>• Relapsed or persistent disease after ASCT.<br>• Persistent disease regardless of previous therapies.<br>• Plasma cell leukemia, regardless of previous therapies. |
| Vismodegib After Stem Cell Transplant in Treating Patients With High-Risk First Remission or Relapsed Multiple Myeloma | NCT01330173 | Completed | US | 1 | Vismodegib (hedgehog inhibitor) after ASCT | • Del(13), t(4;14), t(14;16) or del(17p)<br>• B2-M > 5.5 g/dL<br>• immunoglobulin A [IgA] phenotype |

## Table 2 – Clinical Studies of Venetoclax

As per clinical trials.gov and Pubmed searches for "myeloma" and "venetoclax".

| **Published studies** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Phase | Year published | Patient population | Combination | Administration | No. | No. t(11;14) | ORR in all | ORR in t(11;14) |
| 1 | 2017[75] | Relapsed/refractory<br>61% bortezomib and lenalidomide double refractory | Venetoclax | Intrapatient escalation to max 300, 600, 900 and 1200mg cohorts, expansion of 1200mg cohort. Dexamethasone could be added at progression on venetoclax. | 66 | 30 | 21% | 40% |
| 1b | 2017[76] | Relapsed/refractory<br>39% bortezomib refractory<br>53% lenalidomide refractory | Venetoclax, bortezomib and dexamethasone | Intrapatient escalation to max 100, 200, 300, 400, 500, 600, 800, 1000 and 1200mg cohort, expansion of 800mg cohort. In combination with bortezomib and dexamethasone | 66 | 9 | 67% | 78% |

| **Ongoing studies** | | | | | |
|---|---|---|---|---|---|
| Phase | Location | Patient population | Combination/Administration | NCT | Estimated completion |
| 1b | US, Australia, France | Relapsed after at least 1 prior line of therapy | Venetoclax, bortezomib and dexamethasone | NCT01794507 | 2018 |
| 1/2 | US, Australia, Canada, Europe | Part 1: t(11;14) relapsed/refractory<br><br>Part 2: relapsed/refractory | Dose escalation of venetoclax with fixed doses of daratumumab and dexamethasone (plus bortezomib for part 2) | NCT03314181 | 2023 |
| 1/2 | US and Europe | Relapsed after at least one prior line of therapy, t(11;14) | Venetoclax +/- dexamethasone | NCT01794520 | 2021 |
| 1b/2 | Europe | Relapsed/refractory<br>3-5 prior lines of therapy | Arm A: cobimetinib<br>Arm B: cobimetinib plus venetoclax<br>Arm C: cobimetinib, venetoclax plus atezolizumab | NCT03312530 | 2020 |
| 2 | US | Relapsed/refractory 1-3 prior lines of therapy | Venetoclax, carfilzomib and dexamethasone | NCT02899052 | 2020 |
| 2 | US, Europe | Relapsed after at least one prior line of therapy, cohorts for t(11;14) positive and negative | Venetoclax, pomalidomide and dexamethasone | NCT03567616 | 2020 |
| 3 | World-wide | Considered sensitive or naïve to proteasome inhibitors and received 1 to 3 prior lines of therapy | Venetoclax/placebo plus bortezomib and dexmathasone | NCT02755597 | 2020 |
| 3 | World-wide | t(11;14)<br>Relapsed/ refractory | Venetoclax and dexamethasone vs pomalidomide and dexamethasone | NCT03539744 | 2022 |

## Table 3 - Clinical Studies of RAS Pathway Targeted Therapies

As per clinical trials.gov and Pubmed search for “myeloma” and the following terms “vemurafenib”, “dabrafenib”, “trametinib”, “cobimetinib”, “RAS”, “BRAF”, “MEK”.

| **Retrospective cohorts and case reports** | | | | | |
|---|---|---|---|---|---|
| Type | Year published | Patient population | Combination/Administration | Number of patients | Overall response rate |
| Retrospective | 2016[77] | Oncogenic mutations of NRAS, KRAS or BRAF or GEP pathway activation in relapsed/refractory patients | Trametinib as single agent or in combination with other agents | 58, 40 with measurable disease at time of commencing trametinib | 16/40 (40%) |
| Case report | 2017[83] | Relapsed/refractory patient with extramedullary disease and BRAF V600E mutation | Vemurafenib and cobimetinib | 1 | Patient responded |
| Case report | 2014[84] | Relapsed/refractory patient with extramedullary disease and BRAF V600E mutation | Vemurafenib | 1 | Patient progressed through treatment |
| Case report | 2014[85] | Relapsed/refractory patients with BRAF V600E mutations | Vemurafenib | 2 | Both patients responded |
| Case report | 2013[86] | Relapsed/refractory patient with extramedullary disease and BRAF V600E mutation | Vemurafenib | 1 | Patient responded |

| **Ongoing studies** | | | | | |
|---|---|---|---|---|---|
| Phase | Location | Patient population | Combination/Administration | NCT | Estimated completion |
| 1 | UK | Relapsed/refractory<br>BRAF, NRAS or KRAS mutated | RO5126766 twice weekly or Mon/Wed/Fri dosing schedule | NCT02407509 | 2016<br>(but ongoing) |
| 1 | Boston, US | Relapsed/refractory<br>BRAF, NRAS or KRAS mutated | Cohort 1 BRAF V600 mutated: Dabrafenib<br>Cohort 2 BRAF mutated or BRAF plus KRAS/NRAS mutated: trametinib<br>Cohort 3: KRAS or NRAS mutated: trametinib | NCT03091257 | 2021 |
| 2 | Canada | Relapsed/refractory<br>BRAF, NRAS or KRAS mutated | Trametinib initially with AKT inhibitor GSK2141795 added at progression<br>Cohorts of biomarker positive and negative patients | NCT01989598 | 2018 |
| 2 | Germany | Relapsed/refractory<br>BRAF V600E/K mutated | Encorafenib and binimetinib combination | NCT02834364 | 2021 |
| 1b/2 | Europe | Relapsed/refractory<br>3-5 prior lines of therapy | Arm A: cobimetinib<br>Arm B: cobimetinib plus venetoclax<br>Arm C: cobimetinib, venetoclax plus atezolizumab | NCT03312530 | 2020 |
| **Umbrella studies** | | | | | |
| 2 | US | MATCH study:<br>Multiple diseases and multiple treatments | Guided by molecular characterization including BRAF, RAS, PIKC3A mutations, CCND1, CDK4, CDK6 amplification | NCT02465060 | 2022 |
| 2 | US | TAPUR study:<br>Multiple diseases and multiple treatments | Guided by genomic variant identification including BRAF, KRAS, NRAS | NCT02693535 | 2019 |
| 2 | Canada | CAPTUR study:<br>Multiple diseases and multiple treatments | Guided by genomic variant identification including BRAF | NCT03297606 | 2021 |